A close-up photograph of fern fronds. The fronds are green with prominent veins, some showing signs of aging or damage. In the foreground, several fronds are a vibrant red color, likely indicating a different species or a specific growth stage.

Arnold van der Valk
Editor

Herbaceous Plant Ecology

Recent Advances in Plant Ecology



Springer

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A.G. Van der Valk
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Editor

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Plant species richness and diversity of the serpentine areas on the Witwatersrand

R. A. Reddy · K. Balkwill · T. McLellan

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Abstract Soil chemistry can play an important role in determining plant diversity. Serpentine soils are usually toxic to many plant taxa, which limits plant diversity compared to that on adjacent non-serpentine soils. The usually high concentrations of toxic metals in serpentine soils are considered to be the edaphic factors that cause low diversity and high endemism. This paper aimed primarily to determine whether there is a relationship between serpentine soil chemistry and species richness on the Witwatersrand and to compare species richness of the serpentine areas with that of adjacent non-serpentine areas as well as with the species richness of the serpentine areas in the Barberton Greenstone Belt. The alpha- and beta-diversity of the Witwatersrand serpentine and non-serpentine areas was also investigated. A secondary aim of this study was to determine which of the non-serpentine taxa were more common on the serpentine than off the serpentine, which taxa were more common off the serpentine than on the serpentine and which taxa were equally common on and off

serpentine soils. There was no significant difference in alpha-diversity between the serpentine and the adjacent non-serpentine areas, but beta-diversity is higher between serpentine plots than between non-serpentine plots. Although soil factors do affect species richness and diversity of plants on the Witwatersrand to a limited extent, the concentrations of soil chemicals in serpentine soils are not sufficiently different from those in non-serpentine soils to significantly influence the species richness and diversity of the serpentine soils. The high, but similar, diversity on serpentine and non-serpentine soils on the Witwatersrand indicates that soil factors do not play a significant role in determining diversity on potentially toxic soils in the area.

Keywords Alpha-diversity · Beta-diversity · Modified-Whittaker plots

Introduction

Southern Africa has the world's highest plant species density at the sub-continental level (Cowling et al. 1989). The main reasons for this high species richness are environmental heterogeneity, two different climatic regimes, i.e. summer and winter rainfall regions, recurrent climatic fluctuations since the mid-Pliocene and the number of relicts that survived in pockets along the coast (Goldblatt 1978).

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Biodiversity represents the complexity of life on earth (Wilsey et al. 2005). High species diversity means a complex community, as a greater variety of species means a greater array of interactions (Brower and Zar 1984). The stability of the ecosystem is also linked to diversity, since most stable communities have large numbers of species and an even distribution of individuals between species (Williams 1987; Tilman 1996). Humans, by overexploitation, habitat modification or deliberate destruction, reduce this influx (Leps 2004; Sodhi et al. 2004). Fragmentation, grazing, forestry and human influence are decreasing the biological diversity of earth's resources (Tilman 1996). The official estimate by the IUCN indicates that 13% of the world's plant species are under threat; however other scientists (Pitman and Jorgensen 2002) place this figure between 22% and 47%. Understanding and predicting species diversity is therefore essential in identifying areas vulnerable to species loss and thus providing a focus for conservation efforts (Cowling et al. 1989; Pearson 1996).

Three important components of species diversity are: alpha-diversity—the number of species in a homogenous community (Cowling et al. 1992); Beta-diversity—species turnover along an environmental or habitat gradient (Whittaker 1972); and gamma-diversity—species turnover for different ecosystems along geographic gradients (Cowling et al. 1992).

Alpha-diversity, in the strict sense, is species richness (Whittaker 1972). However, species richness is only one aspect of diversity (Gaston 1996) and on its own is not as important in an ecosystem as the combined effects of richness and evenness (Hooper and Vitousek 1997; Wilsey et al. 2005). Diversity indices, such as the Shannon–Weiner and Simpson indices, are therefore useful because they combine both species richness and evenness into a single value (Magurran 1988; Dörgeloh 1999) which can be used in environmental monitoring and conservation.

Beta-diversity is the extent of species replacement or biotic change along environmental gradients (community turnover) and depends on habitat diversity (Wilson and Shmida 1984). An accurate measure of beta-diversity is important because it indicates the degree to which habitats are partitioned by species, i.e. the degree of patchiness. Alpha- and beta-diversity together measure the overall diversity of an area (Peet 1974; Wilson and Shmida 1984).

Environmental factors, especially soil fertility and climate, play an important role in determining species richness (Stohlgren et al. 1999; Grace 2001). Edaphic factors account for much variation in species data and soil pH, and the Mg:Ca ratio plays an important role in species distribution patterns (Esler and Cowling 1993). In the semi-arid areas of southern Africa, soil factors play an important role in determining transitions between vegetation types and plant communities (Esler and Cowling 1993). High levels of local diversity have been considered characteristic of nutrient poor soils in the Cape (Goldblatt and Manning 2002).

Species numbers are controlled by biological interactions such as edaphic conditions and competition (Grime 1979; Bond 1983 in Cowling 1990). Serpentine soils are very diverse due to various factors such as parent material, climate, relief and biological activity (Brooks 1987). These soils generally have high concentrations of Ni, Cr, Co and Fe, high Mg:Ca ratios and low concentrations of N, P and K. All this makes for harsh environmental conditions which in turn result in a low diversity of plant species and unique, usually endemic flora (Kruckeberg 1954; Proctor et al. 1980). The serpentine soils on the Witwatersrand are not as toxic as the serpentine soils of other areas (Reddy et al. 2001). Thus it is expected that there will be a higher diversity on the serpentine soils of this area relative to other serpentine areas.

In 1934, Raunkiaer proposed a system to describe vegetation based on the position of buds or regenerating parts which he found to be related to climate (Rickleffs 1973). He proposed several life form categories the major ones being: phanerophytes—buds occur on the tips of branches, associated with tropical climates, i.e. trees and shrubs; chamaephytes—buds occur at ground level during unfavourable seasons, mainly associated with cold, dry climates, i.e. small shrubs and herbs; hemicryptophytes—dormant buds occur just beneath or at the soil surface; cryptophytes—buds are deeply buried and also store food; and therophytes—“annuals” that survive in seed form, associated mainly with deserts and some grasslands. Hemicryptophytes and cryptophytes are usually associated with cold, moist temperate areas; most dieback to ground level during winter and regenerate the following season (Rickleffs 1973). Fire plays an important role in the

maintenance of the grassland biome on the Witwatersrand (Kerfoot 1987) and thus it is expected that cryptophytes and hemicryptophytes, with their buds protected below the soil surface, will be the dominant life forms on the Witwatersrand.

The Witwatersrand region is geologically and topographically diverse (McCallum and Balkwill 1999). This provides a diversity of habitats and hence the potential for a high level of plant diversity in this region. There is no unique serpentine flora and there are no serpentine endemic taxa on the Witwatersrand (Reddy et al. 2001). On serpentine soils that have been exposed for long periods, prolonged evolution has led to a very high floral diversity at both the genus and species level (Berazain 1992; Batianoff and Specht 1992; Williamson 1995). So, with the potential high diversity of this region and the possible long exposure of the Witwatersrand serpentine soils, are the serpentine areas on the Witwatersrand as species diverse as the adjacent non-serpentine areas?

The aims of this paper are to: (1) compare the relationship between species richness of the flora and selected soil elements at serpentine sites and their adjacent non-serpentine sites, (2) determine how species rich the serpentine areas on the Witwatersrand are and how this richness compares to the adjacent, non-serpentine areas, (3) investigate the alpha- and beta-diversity of the Witwatersrand serpentine soils and the adjacent non-serpentine soils and to compare the beta-diversities of the all Witwatersrand serpentine areas with those of the sites studied in the Barberton Greenstone Belt and (4) determine which of the *bodenvag* taxa, i.e. taxa that are indifferent to edaphic conditions, are more common on the serpentine than off the serpentine, which taxa are more common off the serpentine than on the serpentine and which taxa are equally common on and off the serpentine.

The following predictions are made:

1. Because the serpentine soils on the Witwatersrand are not as toxic as the serpentine soils of other areas, e.g. the Barberton Greenstone Belt (Reddy et al. 2001), species richness of herbaceous taxa on serpentine soils on the Witwatersrand will be similar to species richness of herbaceous taxa of adjacent, non-serpentine areas,
2. Studies of other serpentine sites globally have shown that serpentine soils with high concentrations of Ni, Cr, Co and Fe, high Mg:Ca ratios and

low concentrations of N, P and K usually have a low diversity and a high degree of endemism (Kruckeberg 1954; Proctor et al. 1980). Thus, the lower concentrations of chromium and nickel or the magnesium:calcium ratio of serpentine sites would result in a higher number of tolerant species and few or no endemic taxa,

3. The similarity between the floras of the serpentine and non-serpentine soils on the Witwatersrand is greater than that between the serpentine flora of the Witwatersrand and that of the Barberton Greenstone Belt serpentines.

Study areas

Johannesburg is situated in an area known as the Highveld of South Africa. The topography of the region is flat grassland with irregular, undulating rocky hills and ridges. Altitude on the Witwatersrand ranges between 1450 and 1750 m. This region, including the Witwatersrand serpentine areas (Reddy et al. 2001), is within the grassland biome and for the most part the vegetation falls into the rocky Highveld grassland (Bredenkamp and van Rooyen 1996). This grassland type, formerly known as Bankenveld, is fire-maintained (Acocks 1988), with the vegetation adapted to fire and consisting mainly of resprouting forbs. Trees and shrubs are confined to the rocky ridges and outcrops which offer some protection from fire.

Precipitation ranges between 600 and 750 mm per annum and occurs mainly in summer. Temperatures range between 12°C (daily minimum) and 39°C (daily maximum) with an average of 16°C. Winters are very dry and severely frosty (Bredenkamp and van Rooyen 1996). Many of the trees found at the sites have high latex or resin contents in the sap (pers. obs.). This is possibly a mechanism to overcome the harsh, frosty Highveld winters and another reason why they are confined to the less fire prone, rocky areas (Balkwill and Botany II 1993, 1995). High latex or resin contents in the sap could also act as a deterrent to insect herbivory.

The serpentine areas on the Witwatersrand mainly occur around the south-western and south-eastern margins of the granite dome found to the north of Johannesburg, and at some localities within the dome. These sites are situated in a densely populated urban area and are subject to various forms of disturbance.

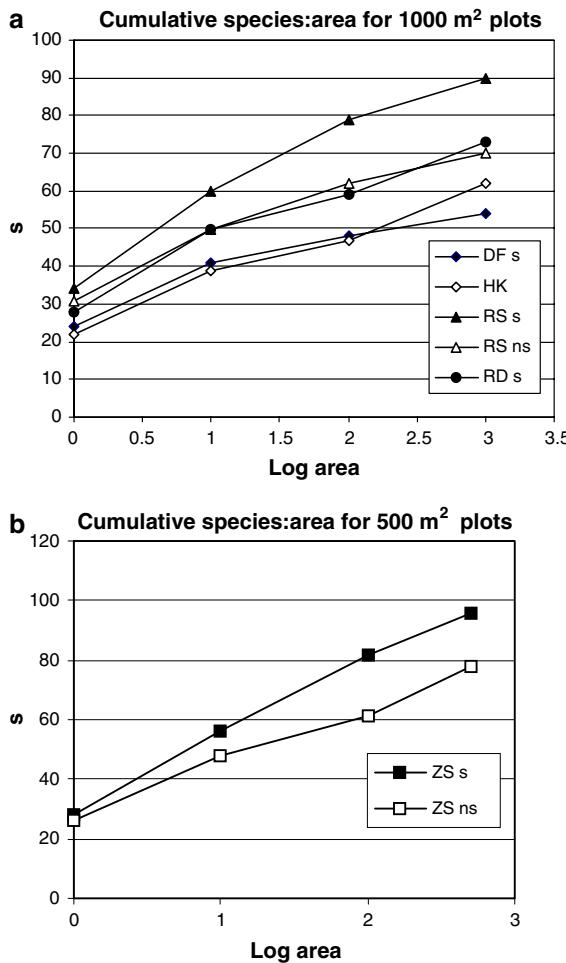


Fig. 1 (a) Cumulative species richness: log area curves for 1000 m² serpentine (s) and non-serpentine (ns) plots excluding the single non-serpentine plot at Robindale. DF: Diamond Four, HK: Honingklip, RD: Robindale, RS: Ruimsig. (b) Cumulative species richness: log area curves for 500 m² serpentine (s) and non-serpentine (ns) plots at Zandspruit (ZS)

Most of the large serpentine areas have been built upon and only small patches remain (Fig. 1 in Reddy et al. 2001).

Methods

Modified Whittaker plots (Stohlgren et al. 1995) were used to determine the species diversity of these sites. These plots usually are 0.1 ha, i.e. 1000 m² (50 m × 20 m); however, on the Witwatersrand, some serpentine bands were too narrow to accommodate full-size plots. In these cases (Zandspruit and Weltevreden) half-sized plots (50 m × 10 m) were used. Nested

within each plot (whether full- or half-sized) were ten 0.5 m × 2 m (1 m²) sub-plots systematically placed within the inside border, two 2 m × 5 m (10 m²) sub-plots in alternate corners and a 5 m × 20 m (100 m²) sub-plot in the centre (Stohlgren et al. 1999).

The number of plots per site varied depending on the size of the site. On five of the nine serpentine sites, namely Darrenwood, Diamond Four, Illiondale, Robindale and Ruimsig, three modified Whittaker plots were used. At the Dombeya site, because of its size, six plots were used; four plots were used at the Estherpark site for the same reason. Where possible, the four non-serpentine sites had the same number of plots as their adjacent or proximate serpentine sites; so Honingklip and Ruimsig had three plots and the Zandspruit site only two half-sized plots. The non-serpentine soils at Robindale consisted of only a narrow band on which only one full-sized plot could be fitted. The plot sizes on both the serpentine and the adjacent non-serpentine soils were of the same size. For sites that were on ridges, plots were randomly placed at the base and mid-slopes and top of the slope. On hills, plots were placed at the base and mid-slopes on either side of the hill.

The taxa present in each plot and its sub-plots were recorded. Soil was gathered to a depth of about 5 cm from the four corners and the centre of each plot 0.1 ha plot, and because of the cost of soil analysis, soils from all the plots of each site were bulked together, so soils analysis was done per site, not per plot. In the case of the interface sites, the serpentine and non-serpentine soils were analysed separately. Soil samples were sent to the Agricultural Research Council (ARC) for analysis of %C, %N, soil pH and exchangeable cations. Exchangeable cations were determined using the ammonium acetate method (Schollenberger and Simon 1945).

A paired *t*-test was used to compare selected soil chemicals at the sites that had both serpentine and adjacent non-serpentine soils. The Pearson's Correlation coefficient was used to determine whether or not there was a significant correlation between total species richness, at the 500 m²/1000 m² plot scale, and soil chemicals at each site.

Cover was determined using a modified line intercept method. A 50 m tape was placed lengthwise in the centre of the plot. The presence or absence and identity of taxa at every 10 cm interval were recorded

(Whittaker et al. 1979). Percentage cover was determined from the number of points along the 50 m tape at which taxa occurred.

Paired *t*-tests were used to test for species richness between serpentine and non-serpentine sites at the 1 m² (point) and 500 m²/1000 m² (plot) levels. Because of the differing sizes of the plots, the log of species richness was plotted against the log of area to give a linear graph for the serpentine and adjacent non-serpentine sites. The extrapolated species richness values were then read off at the 500 m² and 1000 m² points (Witkowski pers. comm.).

The Shannon–Weiner and the Simpson indices were used to measure alpha-diversity. The Shannon–Weiner index expressed heterogeneity whilst the Simpson index is sensitive to changes in common species and is determined by the relative concentration of dominance (Whittaker, 1972; Peet 1974; Kent and Coker 1994; Dörgeloh 1999).

The formula for the Shannon–Weiner index is given by: $H' = -\sum^s I = \sum p_i \ln p_i$, where s = number of species, p_i = the proportion of individuals or abundance of the i th species and \ln = log base e and expressed heterogeneity. In this instance abundance was used. The formula for the Simpson index is: $D = 1 - (\sum [n_i(n_i - 1)]/[N_i(N_i - 1)])$, where D = Simpson's Index, n_i = number of individuals of species i and N = the total number of individuals. The closer the D value to 1, the more even the distribution of species. A paired *t*-test was used to test for significance. The Shannon–Weiner index values were then used to calculate the Evenness Index using the formula $J = H'/\ln s$ where s is the number of species present.

The mean cumulative species number was calculated per site for both the serpentine and non-serpentine plots. These results were then plotted against the log of area, using Microsoft Excel (2003). Log of area was used so that one can more easily see details for small values.

Sørensen's similarity index ($Q_s = 2c/a + b$, where c = taxa in common, a = total taxa in plot 'a' and b = total taxa in plot 'b') was used to measure similarity between plots (Kent and Coker 1994). Beta-diversity is the dissimilarity between plots. Two identical plots would have a similarity value of 1, then dissimilarity (beta-diversity) is calculated as 1-Sørensen's similarity index (Kent and Coker 1994). An unpaired *t*-test was used to test for significance

between total similarity of serpentine sites against total similarity of the non-serpentine sites. A complete linkage cluster diagram, using Statistica version 6, was then constructed to show beta-diversity for all sites excluding the smaller Weltevreden, Zandspruit and Robindale sites. Beta-diversity (dissimilarity) was plotted against the log of area.

Sørensen's similarity index was used to compare the similarities between the Barberton Greenstone Belt serpentine and non-serpentine groups and the Witwatersrand serpentine and non-serpentine groups. It was also used to compare similarities between the different growth forms at each of the serpentine/non-serpentine sites on the Witwatersrand.

The *bodenvag* taxa were determined by listing all the taxa occurring in the plots on and off serpentine soils, and then counting the number of times they occurred in serpentine and adjacent non-serpentine plots. Student's *t*-test was used to determine which *bodenvag* taxa are significantly more common on the serpentine than off the serpentine, which taxa are more common off the serpentine than on the serpentine and which taxa are equally common on and off the serpentine.

Statistical analysis

The Epistat statistical programme (Gustafson 1986) was used to perform Student's *t*-tests and Pearson correlations. Microsoft Excel (2003) was used to construct the species:area graph (Fig. 1). STATISTICA (data analysis software system) version 6 (2001) was used to construct Figs. 2 and 3.

Results

There was significantly more total Cr and soluble N in the serpentine soils than in the adjacent non-serpentine soils (Table 1a). There was no significant correlation between species richness and the various soil parameters of the serpentine soils (Table 1b). There was a significant, positive correlation between species richness and the total Cr and soluble Ni in the non-serpentine soils (Table 1c).

A total of 449 taxa and 415 species occur on the Witwatersrand serpentine areas (Reddy et al. 2001). The intensive study using the modified Whittaker

Fig. 2 Graphs showing average percentage cover based on line intercept counts for serpentine and adjacent non-serpentine areas. DF: Diamond Four, HK: Honingklip, RS: Ruimsig, ZS: Zandspruit. s = serpentine, ns = non-serpentine

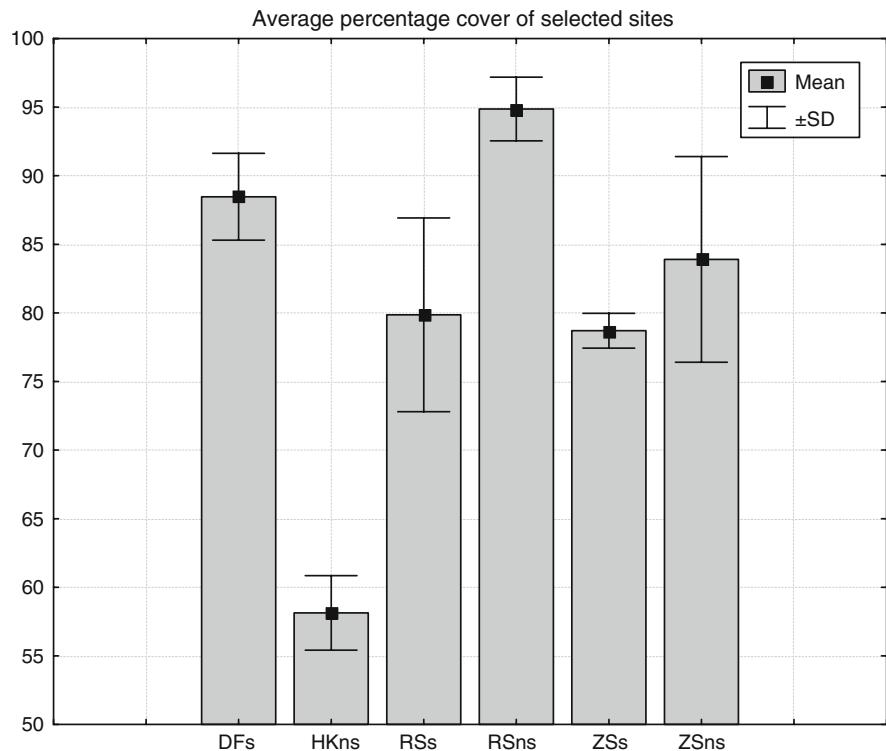
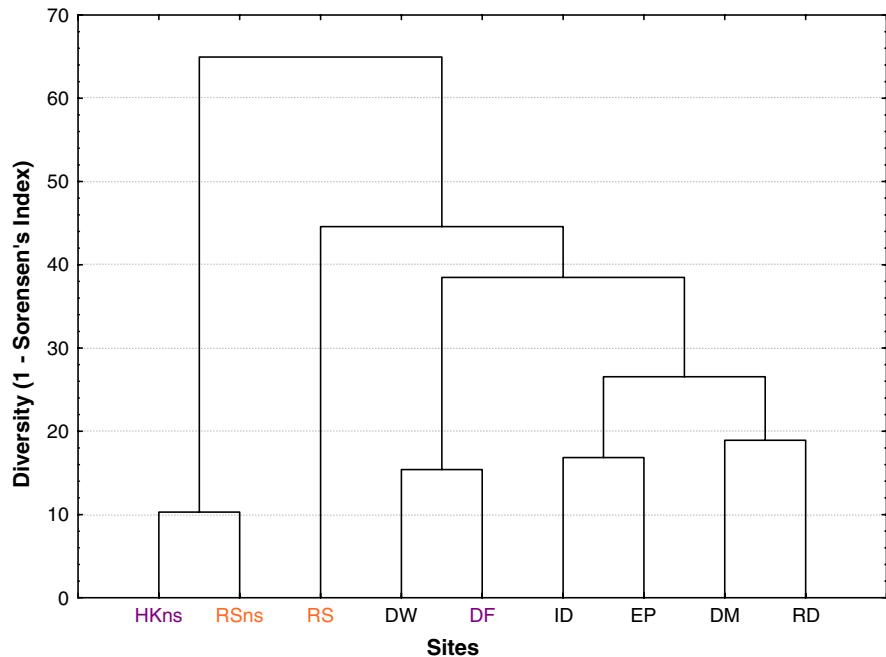


Fig. 3 Cluster diagram of beta-diversity for 9 sites. DF: Diamond Four, DM: Dombeya, DW: Darrenwood, EP: Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, s = serpentine, ns = non-serpentine



plots added a further 15 taxa. This brought the total number to 464 taxa and 413 species. According to McCallum and Balkwill (1999), there are 1478 plant species on the Witwatersrand. This means that 27.9%

of the total Witwatersrand species occur on serpentine soils.

The Robindale and Ruimsig sites showed the greatest point species richness among the serpentine

Table 1 (a) Comparison between soil chemicals for the four serpentine sites with adjacent non-serpentine areas. (b) Correlation between species richness and various soil properties for all serpentine plots combined, average total species

richness \pm sd: 74.0 ± 17.65 , $n = 9$, df = 7. (c) Correlation between species richness and soil properties for all for all non-serpentine plots combined, $n = 4$, df = 2. Average total species richness \pm sd: 82.8 ± 22.5

Soil chemical	Mean \pm sd		Paired <i>t</i> -test (df = 3)	
	Serpentine	Non-serpentine	<i>t</i>	<i>P</i>
Panel A				
Mg:Ca	1.78 ± 0.36	1.25 ± 0.24	2.5	0.09
pH	5.88 ± 0.47	5.80 ± 0.42	0.35	0.75
Total Cr ($\mu\text{g/g}$)	1152.62 ± 120.36	721.81 ± 213.30	4.76	0.02*
Sol. Cr ($\mu\text{g/g}$)	0.45 ± 0.07	0.37 ± 0.05	2.08	0.13
Total Ni ($\mu\text{g/g}$)	752.04 ± 404.55	257.08 ± 190.04	2.37	0.1
Sol. Ni ($\mu\text{g/g}$)	16.02 ± 5.13	6.31 ± 3.23	2.88	0.06
Total Fe ($\mu\text{g/g}$)	50722.5 ± 7063.55	38312.5 ± 9667.67	1.74	0.18
Sol. Fe ($\mu\text{g/g}$)	147.6 ± 22.28	110.08 ± 26.24	2.02	0.14
Sol. Mg ($\mu\text{g/g}$)	269.54 ± 22.61	211.43 ± 163.59	0.46	0.68
Sol. N (%)	0.29 ± 0.04	0.19 ± 0.08	5.09	0.02*
Parameter	Parameter values (mean \pm sd)		Correlation (r)	<i>t</i>
Panel B				
Mg:Ca	1.6 ± 0.59		-0.23	0.64
pH	6.04 ± 0.92		-0.01	0.03
Total Cr ($\mu\text{g/g}$)	1002.28 ± 293.23		0.17	0.46
Total Ni ($\mu\text{g/g}$)	685.94 ± 353.10		-0.41	1.19
Sol. Ni ($\mu\text{g/g}$)	12.45 ± 5.64		0.09	0.25
Total Fe ($\mu\text{g/g}$)	47278.9 ± 10746.2		-0.17	0.45
Sol. N (%)	0.26 ± 0.6		0.27	0.75
Panel C				
Mg:Ca	1.3 ± 0.2		0.89	2.73
pH	5.8 ± 0.4		0.62	1.11
Total Cr ($\mu\text{g/g}$)	721.8 ± 213.3		0.9998	63.7
Total Ni ($\mu\text{g/g}$)	257.1 ± 190.0		0.94	3.88
Sol. Ni ($\mu\text{g/g}$)	6.3 ± 3.2		0.96	5.02
Total Fe ($\mu\text{g/g}$)	38312.5 ± 9667.7		0.08	0.12
Sol. N (%)	0.18 ± 0.08		-0.71	1.44

* = significant, sol. = soluble

sites (Table 2a). The Weltevreden site, despite being the smallest serpentine site, also had a high point species richness. There were no significant differences in species richness between the paired serpentine and non-serpentine sites.

Extrapolating adjacent serpentine and non-serpentine plots down to 500 m^2 (Table 2b), all the serpentine plots, except Diamond Four, had more taxa than the non-serpentine plots. Weltevreden and Zandspruit, the 2 small sites, had the greatest species

richness (100 and 133 respectively). Extrapolating up to 1000 m^2 shows the same trend.

The cumulative species: area curves (Fig. 1a, b) show a steady increase in species as area increases for all sites. At the Zandspruit and Ruimsig sites, the serpentine areas showed a greater increase in species as the area increased than the non-serpentine areas. The Diamond Four serpentine area and Honingklip non-serpentine area, although further apart from each other than the other serpentine/non-serpentine paired

Table 2 (a) Point species richness (i.e. average number of species recorded for 1 m² sub-plots on and off serpentine soils). ! indicates half-sized (500 m²) plots. (b) Species

richness (i.e. number of species recorded per 1000 m²/500 m² plots) on and off serpentine soils, with extrapolations shown in italics

Sites	Serpentine mean ± sd (n)	Non-serpentine mean ± sd (n)	t (df)	P
Panel A				
DM	7.9 ± 2.9 (6)			
DW	6.2 ± 3.0 (3)			
EP	5.1 ± 1.9 (4)			
ID	6.6 ± 2.2 (3)			
!WV	9.4 ± 0.6 (2)			
RD	10.4 ± 2.7 (3)	13.3 ± 3.9 (1)		
DF/HK	8.7 ± 3.4 (3)	7.9 ± 2.3 (3)	1.04 (2)	0.41
RS	10.6 ± 2.7 (3)	11.6 ± 3.2 (3)	0.40 (2)	0.73
!ZS	7.9 ± 3.7 (2)	9.4 ± 3.7 (2)	0.28 (1)	0.83
Sites	Serpentine	Non-serpentine		
	Calculated mean ± sd (n) or <i>Extrapolated</i>	Calculated mean ± sd (n) or <i>Extrapolated</i>	t (df)	P
	1000 m ²	500 m ²		
Panel B				
DW	79.3 ± 4.5 (3)	59.6		
DM	58.8 ± 14.2 (6)	53.1		
EP	68.3 ± 10.3 (4)	37.6		
ID	57 ± 8.0 (3)	39.8		
RD	93.3 ± 29.9 (3)	70.8	115 (1)	59.6
!WV	100.0	73.5 ± 2.1 (2)		
DF/HK	56 ± 12.2 (3)	42.2	64.3 ± 7.2 (3)	53.1
RS	89.7 ± 10.1 (3)	84.1	70.3 ± 1.5 (3)	59.6
!ZS	133.4	96.0 ± 14.1 (2)	89.1	78 ± 9.9 (2)
				0.75 (2) 0.53
				3.52 (2) 0.1
				1.05 (1) 0.48

DF: Diamond Four, DM: Dombuya, DW: Darrenwood, EP, Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, WV: Weltevreden, ZS: Zandspruit. ! = half-sized (500 m²) plots

sites, were very similar to each other with regard to the number of species occurring in both these areas.

The serpentine plots of the Diamond Four/Honingklip site comprise the only site which consistently shows significantly more cover ($t = 12.6, P = 0.0002, 4 \text{ df}$) than the non-serpentine plots (Fig. 2). The cover of the non-serpentine plots at Ruimsig was significantly higher than that of the serpentine sites ($t = 3.5, P = 0.02, 4 \text{ df}$). The Zandspruit site gave variable results for both the serpentine and non-serpentine areas.

Darrenwood and Diamond Four were the most diverse serpentine sites, with the Weltevreden site being the least diverse. There was no significant difference in diversity between the serpentine and the adjacent non-serpentine areas, for both the

Shannon–Weiner and Simpson indices (Table 3a). With regard to evenness (Table 3b), only the half plots, at Weltevreden and Zandspruit, show evenness values of less than 0.50. Diamond Four, the least disturbed site (Reddy et al. 2001), showed the most even distribution of species (0.74) of all the serpentine sites and Weltevreden showed the least evenness (0.42). Estherpark and Illiondale, despite the high degree of disturbance (Reddy et al. 2001), show high evenness values, 0.71 and 0.61, respectively. However there was no significant difference in species richness between the serpentine and adjacent non-serpentine sites. All sites show a greater dominance than evenness; Weltevreden, which shows the least evenness, has the highest D value.

Table 3 (a) Shannon–Weiner (H') and Simpson (D) Indices of serpentine plots and adjacent non-serpentine plots. (b) Species evenness indices of serpentine plots and non-serpentine plots

Site (<i>n</i>)	HN (Mean \pm sd)		<i>t</i>	<i>P</i>	df	D (Mean \pm sd)		<i>t</i>	<i>P</i>	df
	s	ns				s	ns			
Panel A										
DF/HK (3)	1.31 \pm 0.41	1.05 \pm 0.33	0.8	0.5	2	0.79 \pm 0.10	0.96 \pm 0.01	2.81	0.11	2
RS (3)	1.06 \pm 0.17	0.97 \pm 0.03	0.8	0.5	2	0.80 \pm 0.05	0.90 \pm 0.07	1.16	0.25	2
ZS (2)	0.95 \pm 0.37	1.10 \pm 0.11	0.4	0.7	1	0.94 \pm 0.01	0.94 \pm 0.01	1	0	1
Site (<i>n</i>)	DF s (3)	HK ns (3)	RS s (3)	RS ns (3)	ZS s (2)	ZS ns (2)				
Panel B										
Evenness index (J): (H/log s)	0.74	0.58	0.54	0.54		0.48	0.58			

DF: Diamond Four, HK: Honingklip, RS: Ruimsig, ZS: Zandspruit. s = serpentine, ns = non-serpentine

The Barberton Greenstone Belt serpentines have 884 plant taxa while only 442 taxa occur on the Witwatersrand serpentine areas. There are 239 taxa that are common between these two areas. Thus there is only a 36% similarity (64% dissimilarity) between the total serpentine taxa of the Witwatersrand serpentine areas and the Barberton Greenstone Belt serpentine areas. A comparison of Sørensen's similarity values for the serpentine and non-serpentine plots of the Barberton Greenstone Belt (Williamson and Balkwill unpublished, Changwe and Balkwill 2003) with that of the Witwatersrand serpentine sites with adjacent non-serpentine plots shows a very high degree of similarity between the Witwatersrand plots (Table 4a).

Dissimilarities between serpentine plots ranged from 42 to 90% (Table 4b). Estherpark has the lowest similarity, or conversely, the greatest beta-diversity of all the serpentine sites. It has a large, very diverse site encompassing grassland, a seepage area and rocky outcrops and thus has a diverse flora compatible with the various micro-habitats within this site. Zandspruit and Weltvreden are small sites, the plots sampled are closer together, and hence they show a greater similarity. Non-serpentine scores range from 30 to 51% dissimilarity (Table 4b). Zandspruit non-serpentine plots showed the greatest beta diversity; this could perhaps be due to small sample error.

An unpaired *t*-test showed that there was a significant difference in the total similarity between serpentine and non-serpentine plots, with the serpentine plots being significantly more diverse than the non-serpentine plots. The dendrogram showing beta-diversity (Fig. 3) also shows this difference between the serpentine and non-serpentine sites.

There is a very high similarity between grass taxa found on and off serpentine soils, ranging from 73 to 86% using the Sørensen's Similarity Index (Table 4c). At all sites, grass and forb taxa showed the greatest similarities, the Zandspruit site having the greatest similarity. Diamond Four showed the next highest similarity to Honingklip despite these two sites not being adjacent to each other. With regard to trees and shrubs, the Robindale site was most similar and Zandspruit the least.

Hemicryptophytes are the dominant life form both on and off serpentine (Fig. 4a). There were more cryptophytes, hemicryptophytes and chamaephytes off serpentine soils and more phanerophytes and therophytes on serpentine (Fig. 4b). Using a paired *t*-test, there is no significant difference between life forms on and off serpentine (Table 5) with the exception of the number of phanerophytes that occur on and off serpentine at the Zandspruit site where there are significantly more phanerophytes (trees and shrubs) on serpentine than off serpentine.

Fifty-six *bodenvag* taxa were identified (Appendix 1); of these, 24 were more common on serpentine, 30 were more common on the adjacent non-serpentine areas and 2 taxa were equally common on and off serpentine. Hemicryptophytes made up 80.36% of these *bodenvag* taxa. Of these 56 taxa, only *Nidorella hottentottica* was significantly more common on serpentine soils and *Hermannia depressa* was significantly more common off serpentine.

The *bodenvag* taxa that are more common on and more common off serpentine are from eight and 12 families, respectively. Only four families, Gramineae, Asteraceae, Leguminosae and Liliaceae, are common

Table 4 (a) Sørensen's similarity indices between serpentine and non-serpentine plots, from the Barberton Greenstone Belt (BGB) and the Witwatersrand serpentine and non-serpentine plots (WWRS). (b) Comparison of average Sørensen's similarity values and beta-diversity within and between plots of four adjacent serpentine and non-serpentine sites on the

Witwatersrand Results of unpaired *t*-test to compare total similarity between serpentine and non-serpentine sites: df = 10, *t* = 2.5, *P* = 0.03 (significant) (c) Percentage Sørensen's similarity for different growth forms of each site with serpentine and non-serpentine plots

Site	BGB								WWRS		
	* RT	* SM	* KL	* GV	* MC	* MM	* CZ	+DV	RS	DF	ZS
Panel A											
% Similarity	46	44	43	40	39	36	31	31	70	69	49
Mean ± sd	38.75 ± 5.7								68.25 ± 11.87		
Site (<i>n</i>)	Similarity between serpentine plots		Beta-diversity (1 - Q _s)		Similarity between non-serpentine plots		Beta-diversity (1 - Q _s)				
	Mean ± sd		Mean ± sd		Mean ± sd		Mean ± sd				
Panel B											
RD (3)	0.42 ± 0.07	0.58	—								
RS (3)	0.45 ± 0.16	0.55	0.70 ± 0.02	0.3							
ZSs (2)/ZS ns (2)	0.58	0.42	0.49	0.51							
DFs (3)/HKns (3)	0.52 ± 0.08	0.48	0.69 ± 0.04	0.31							
DM (6)	0.40 ± 0.12	0.6	—								
DW (3)	0.51 ± 0.08	0.49	—								
EP (4)	0.31 ± 0.11	0.9	—								
ID (3)	0.43 ± 0.05	0.57	—								
WV (2)	0.58	0.42	—								
Mean (<i>n</i>) ± sd	0.47 (9) ± 0.09	0.53 ± 0.15	0.63 (3) ± 0.12	0.37 ± 0.22							
Site (<i>n</i>)	% Similarity of growth forms between serpentine and non-serpentine plots										
	Grasses			Forbs			Trees and Shrubs				
Panel C											
DF/HK (3)	80.9	63.9	23.5								
RD (3)	76	51.2	50								
RS (3)	79.2	63.4	27.3								
ZS (2)	88.5	64.5	10.8								

* = Williamson & Balkwill (unpublished data) and + = Changwe & Balkwill (2003). RT: Rosentuin, SM: Sawmill, KL: Kalkloof, GV: Groenvaly, MC: Mundt's Concession, MM: Magnesite, CZ: Core Zone, DV: Dunbar Valley, RS: Ruimsig, DF: Diamond Four, ZS: Zandspruit

DF: Diamond Four, DM: Dombeya, DW: Darrenwood, EP, Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, WV: Weltevreden, ZS: Zandspruit

to both. Thus there is only a 40% similarity (Sørensen's Index) between families more common on and more common off serpentine.

In total, 23 of the *bodenvag* taxa belong in the Graminae (12% of the Witwatersrand total for the family), nine (3.9% of the Witwatersrand total for the family) in the Asteraceae and four (3% of the Witwatersrand total for the family) in the

Leguminosae. There are three representatives each of the Liliaceae *s.l.* (4% of the Witwatersrand total for the family) and Rubiaceae (9% of the Witwatersrand total for the family) and two each from the Euphorbiaceae (7% of the Witwatersrand total for the family) and Acanthaceae (11% of the Witwatersrand total for the family). The remainder are single representatives from 10 other families.

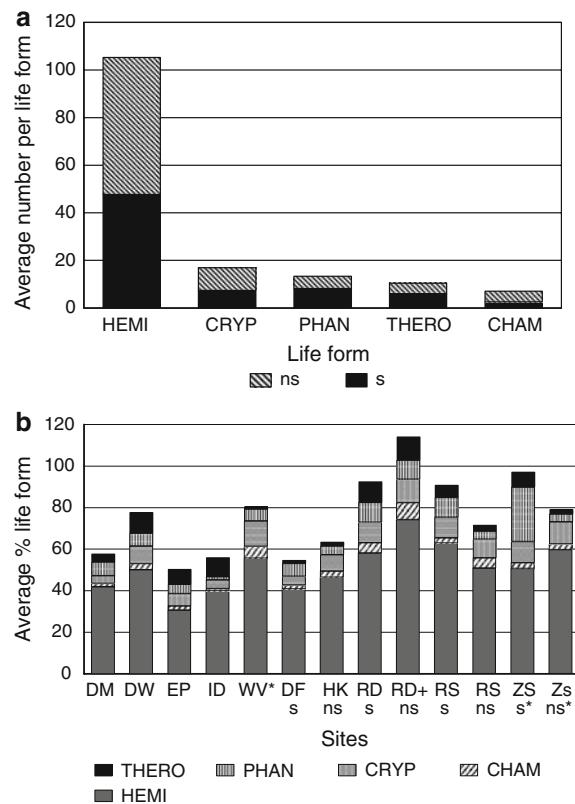


Fig. 4 (a) Average contribution of the various life forms on and off serpentine soils. HEMI = Hemicryptophyte, CHAM = Chamaephyte, CRYP = Cryptophyte, PHAN = Phanerophyte, THERO = Therophyte, s = serpentine, ns = non-serpentine. (b) Average percent of a particular Raunkiaer life form per 500 m²/1000 m² plots at each site. DF: Diamond Four, DM: Dombuya, DW: Darrenwood, EP, Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, WV: Weltevreden, ZS: Zandspruit. s = serpentine, ns = non-serpentine. HEMI = Hemicryptophyte, CHAM = Chamaephyte, CRYP = Cryptophyte, PHAN = Phanerophyte, THERO = Therophyte. ! indicates half-sized, (500 m²) plots. RD+ ns = single plot

The Gramineae are the best represented *bodenvag* taxa on and off the serpentine soils. There are more grasses common on serpentine (13) than off serpentine (11), but not significantly so.

Discussion

The grassland biome on the Witwatersrand, including the serpentine areas (Reddy et al. 2001), is under threat from urbanisation and development. The Zandspruit site currently has been fenced off and appears to be targeted for development, the

Estherpark and Darrenwood sites are now incorporated into office parks, and Ndabushe, once a private game reserve, is now being developed as a luxury housing estate; the koppies still remain, but are isolated from other natural habitats. Emma Park and Robindale are parks, but are home to vagrants. Diamond Four, a privately owned plot, and the Ruimsig Entomological Reserve appear to be safe, for now; however even the Ruimsig site is bordered by houses on two sides and by roads on the remaining two sides.

There is a significant, positive correlation between total Cr and species richness for the non-serpentine plots. Chromium concentrations in normal soils usually are in the order of 100 µg/g and range from 1000 to 25,000 µg/g on serpentiniferous soils, with an average of 5000 µg/g (Brooks 1987). So, although the concentration of chromium in the non-serpentine soils is higher than that given by Brooks (1987) for normal soils, it is still below toxic concentrations of chromium usually found in serpentine soils. Even the concentrations of chromium in the Witwatersrand serpentine soils, with an average of 1153 µg/g, are at the lower end of the range for chromium given by Brooks for serpentine soils (1987). The Cr concentrations of the Barberton Greenstone Belt serpentines range between 163 and 7329 µg/g (Balkwill et al. 1995).

However, even though chromium may be abundant in soils, it is not necessarily available to plants, as Cr forms highly insoluble complexes in the soil (Wild 1974b; Tilston and MacNair 2001). Tetyana (1998) found plants with between 50 and 10500 ppm of Cr in both above and below ground parts. It was also found that at high concentrations of chromium, the more toxic, less common, though more easily taken up Cr IV did inhibit growth (Tetyana 1998). As there is a higher than normal Cr concentration in both the serpentine and the adjacent non-serpentine areas, but no distinct floras, it is possible that the available Cr is in the less bioavailable form of Cr III. As Tetyana (1998) concluded, more work needs to be done in this area.

The positive correlation in the level of soluble N perhaps implies that there is sufficient soluble N to support that number of taxa. The converse is true for the non-serpentine plots. There is mainly a positive correlation between mean species richness and mean levels of soil chemicals.

Table 5 A comparison of life form classes between serpentine and adjacent non-serpentine sites, $n = 3$, df = 2 for HK/HN and RSs/RSns; $n = 2$, df = 1 for ZSs/ZSns

Raunkiaer life forms	Average occurrence																
	DF	HK		<i>t</i>	<i>P</i>	RS s		RS ns		<i>t</i>	<i>P</i>	ZS s		ZS ns		<i>t</i>	<i>P</i>
		Mean ± sd	Mean ± sd			Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd			Mean ± sd	Mean ± sd				
Hemicryptophytes	40.7 ± 8.6	46.3 ± 5.5	0.7	0.6	62.7 ± 13.3	50.7 ± 3.5	1.9	0.2	50.5 ± 13.4	59.5 ± 4.9	0.69	0.61					
Chamaephytes	1.7 ± 0.6	2.7 ± 1.5	0.9	0.5	2.3 ± 0.6	4.7 ± 2.1	1.8	0.2	2.5 ± 2.1	2.5 ± 0.7	0	1					
Cryptophytes	4.0 ± 2.6	7.7 ± 0.6	2.5	0.1	9.7 ± 5.5	8.7 ± 0.6	0.3	0.8	10.0 ± 1.4	10.5 ± 6.4	0.1	0.94					
Phanerophytes	6.0 ± 4.4	4.0 ± 1	0.7	0.6	9.3 ± 4.9	3.7 ± 1.2	2.6	0.1	26.0 ± 2.8	3.5 ± 0.7	15	0.04*					
Therophytes	1.3 ± 0.6	1.7 ± 0.6	1	0.4	5.7 ± 4.5	2.7 ± 1.5	0.9	0.5	7.0 ± 1.4	2.0 ± 1.4	2.5	0.24					
Total taxa	53.7 ± 11.6	62.3 ± 7.2	0.8	0.5	89.7 ± 10.1	70.3 ± 1.5	3.5	0	96.0 ± 14.1	78.0 ± 9.9	1.05	0.48					

DF: Diamond Four, HK: Honingklip, RS: Ruimsig, ZS: Zandspruit. s = serpentine, ns = non-serpentine. * = significant

Nickel toxicity is cited as one of the main reasons for the differences in species composition and richness between serpentine and non-serpentine soils (Wild 1974a; Morrey et al. 1989; Robertson 1992). While the soluble Ni concentrations of the serpentine soils are higher than the non-serpentine soils on the Witwatersrand (Table 1a), it is considerably lower than the serpentine soils of the Barberton Greenstone Belt where the Ni concentrations range from 85 to 314 µg/g (Balkwill et al. 1995).

Reddy et al. (2001) reported that the concentration of total chromium was 12 times greater and that of nickel 1.5 times greater in the Barberton Greenstone Belt serpentine soils than in the Witwatersrand serpentine soils, thus indicating that the serpentine soils on the Witwatersrand are not as toxic as that of the Barberton Greenstone Belt serpentine soils.

The alpha-diversity of southern African vegetation does not differ greatly from equivalent biomes elsewhere at 1000 m² (Cowling et al. 1989). By global alpha-diversity standards 80–100 species per 1000 m² indicates a rich flora (Whittaker et al. 1984; Scholes and Walker 1993; Dörgeloh 1999). South African grassland biomes have a higher average richness than Fynbos (Cowling et al. 1989). Species richness in the Cape Floristic Region ranges from 40 to 85 per 1000 m² (Goldblatt and Manning 2002); in the lowveld savanna Bushbuckridge area Shackleton (2000) counted 68 taxa per 1000 m² on conserved lands and 76 taxa per 1000 m² on communal lands; in the Barberton Greenstone Belt region, Changwe and Balkwill (2003) counted 31–84 taxa per 1000 m² on non-serpentine soils and 29–83 taxa per 1000 m² on serpentine soils. Cowling et al. (1989) found the South African grassland biome to contain between 58 and 98

taxa with an average of 82 taxa per 1000 m². On the Witwatersrand, the serpentine and adjacent non-serpentine plots that were within the global range of a rich flora were: the serpentine soil plots at Weltevreden (100 taxa—extrapolated), Robindale (93), Ruimsig (90) and Zandspruit (133—extrapolated). The only non-serpentine site within the globally species rich range was the Zandspruit site with 89 taxa (extrapolated). In contrast the California chaparral only had 34 species per 1000 m² whilst in Western Australia plots were found to have only 69 species for the same area (Goldblatt and Manning 2002). Thus several serpentine sites on the Witwatersrand have rich floras according to global standards.

More trees and shrubs occur in the serpentine plots and the serpentine areas as a whole because these areas are rocky and provide some protection from fires which occur frequently in this grassland biome. The high numbers of herbs in common implies that there is very little difference between the serpentine and adjacent non-serpentine areas. A greater tree/shrub cover does not imply a lower forb/grass cover (Stohlgren et al. 1999). This is true for the Witwatersrand serpentine areas where there are conspicuous woody elements due to the rockiness of these areas (mean number of rocks/m² of serpentine soil = 14; mean number of rocks/m² of adjacent non-serpentine soil = 2; $n = 13$), but the number of forb and grass taxa occurring in these areas is still high.

The tree and shrub cover is very dense on some serpentine areas at the Zandspruit site. The second serpentine plot was on one of these areas; thus shading effects could account for the low cover. Abundance of herbaceous plants tends to be associated with high soil fertility (Rothstein and Zak 2001).

Lower cover at Honingklip might have been because the non-serpentine site had been more recently burnt than the serpentine area and so many of the resprouters had not yet begun to grow or reached their full extent.

Non-serpentine plots are more similar to each other than to the serpentine plots. This could be because woody vegetation occurs in the rockier serpentine plots. There is very little turnover (beta-diversity) between both serpentine and non-serpentine sites. This is either because the serpentine soils are only mildly toxic or because the harsh climatic factors on the Witwatersrand override the serpentine condition resulting in a uniform Highveld grassland vegetation. In addition, if environmental gradients are gradual, then turnover (beta-diversity) with distance is relatively low (Scholes 1997).

Green plants tend to evolve towards a scattered arrangement of distribution within habitats or geographical areas in order to escape intraspecific competition at a given locality, as long as the species are located within reasonable dispersal distances (Whittaker 1965; Grace 2001). Stohlgren et al. (1999) found that the species composition overlap between each 1000 m² study plot significantly decreased with increasing distance apart. There is a closer similarity between non-serpentine areas than between serpentine areas (Fig. 3). This indicates that the serpentine patches are, to some extent, more isolated from one another than are the adjacent non-serpentine areas. A comparison of the total Witwatersrand serpentine flora with that of the total Barberton Greenstone Belt serpentine flora, using the Sørensen's Similarity index, indicated only a 34% similarity between these two serpentine areas.

Hemicryptophytes are the dominant life form among the grassland herbs and grasses. Herbaceous taxa flush during early spring (pre-rain flora) or summer. This life strategy ensures survival not only during fires but during the harsh winters when there is bright sunshine but very little rainfall. In grasslands, moisture (due to erratic rainfall), soil properties and temperature are responsible for variations in floristic composition, functional attributes of species, vegetation dynamics and ecosystem functioning (Roux 1969; O'Connor and Bredenkamp 1997; Ellery et al. 1991). According to Roux (1969) water is stored in underground structures during the rainy season. The rise in temperature after winter stimulates flowering utilising

the water and nutrients that were stored the previous season. Above ground dieback, together with burning, facilitates long-term maintenance of high levels of species richness (Grace 2001). This is because dieback opens up spaces and reduces shading and soil insulation (Dell et al. 2005), thus allowing for seed recruitment and the flushing of hemicryptophytes. Selection for hemicryptophytes in a fire prone environment reduces the probability of population fragmentation and this also reduces the rate of turnover (Cowling et al. 1989). Thus the predominance of phanerophytes and cryptophytes on the serpentine areas is likely to be because the Highveld grassland is a fire driven ecosystem and these rocky outcrops provide protection from fires. A greater number of therophytes, most of which are weeds, occur on serpentine soils because the phanerophytes provide a shady environment unsuitable for most species indigenous to the grassland habitat and these pioneer species are therefore able to exploit the shady niche.

Grasses are dominant both on and off serpentine on the Witwatersrand, but only a few of the 62 taxa that occur on and off serpentine occur per plot. This means that richness and diversity is due to the non-grass species, especially the hemicryptophytes. Growth and flowering of grasses peak in autumn, and during winter the tall, dried grass stalks provide a microclimate that protects the hemicryptophytic herbs from frost and low temperatures provided that there is no fire.

Humans have played a major role in the fragmentation of natural habitats. South African grasslands are facing their biggest threat as the human population grows and the demand for housing increases. Currently 40% of the grassland biome has been irreversibly transformed (O'Connor and Kuyler 2005). Even the serpentine areas, rocky and with shallow soils, are not exempt. According to Leps (2004), many plant sub-populations depend on a constant influx of diaspora from their surroundings. With the serpentine sites and indeed the grasslands becoming so fragmented, this influx is constantly being hampered and could lead to losses in species diversity.

We predicted that the lower levels of chrome and nickel and the lower magnesium to calcium ratio of serpentine sites on the Witwatersrand would have led to a higher number of tolerant species. As there is no significant difference in diversity between serpentine

soils and adjacent non-serpentine soils on the Witwatersrand and there are no serpentine endemic taxa, we conclude that on the Witwatersrand, soil factors do not significantly influence diversity.

There is a greater similarity between the floras of the serpentine and non-serpentine soils on the Witwatersrand than the similarity between the serpentine floras of the Witwatersrand and the Barberton Greenstone Belt. The serpentine soils on the Witwatersrand are not as toxic as those of the Barberton Greenstone Belt area. So although there is no distinct serpentine flora on the Witwatersrand, there do appear to be distinct serpentine communities.

In addition, the extreme seasonal variations in temperature and the availability of water on the Highveld could possibly override the effects of edaphic factors of the Witwatersrand serpentine areas (Kerfoot 1987). As a result there is no significant difference in species richness or diversity on and off serpentine soils on the Witwatersrand.

Environmental factors and/or climate are the reasons most often cited for high species diversity (Cowling et al. 1989; O'Connor and Bredenkamp 1997) in a region. For serpentine soils, edaphic factors, usually high concentrations of toxic metals in the soils, are the reasons given for a high level of floral diversity and endemism. The average concentration of total Fe in normal soils is 38,000 µg/g (Kamprath 2000). This is similar to the concentrations found in the non-serpentine soils. The total Fe concentration of the serpentine soils, however, is only slightly higher than the non-serpentine soils.

Climatic factors, e.g. rainfall, frost and temperature, together with fire are the reasons cited for the formation of grasslands (Ellery et al. 1991; Stock

et al. 1997; O'Connor and Bredenkamp 1997). Regarding the Witwatersrand serpentine soils, the high, but similar, diversity on serpentine and non-serpentine soils and the predominance of hemicryptophytes indicates that climate, rather than soil factors, is the main determinant of species diversity.

Conclusions

The lack of any significant difference in species richness and diversity between the serpentine and non-serpentine sites on the Witwatersrand indicates that soil factors do not significantly influence diversity. Despite this, there does appear to be a distinct serpentine community. This is possibly because the rocky serpentine ridges at the study sites, like most rocky ridges on the Witwatersrand, provide fire protection for phanerophytes. The similarities in species richness and diversity between the serpentine and non-serpentine areas on the Witwatersrand, together with the predominance of hemicryptophytes, would indicate that climate, rather than soil factors, is the determining factor in species diversity on the Witwatersrand.

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Appendix 1 Likely *bodenvag* taxa: Life forms and whether taxa are equally common or more common on/off serpentine and their levels of significance. Ch = Chamaephyte, C =

Cryptophyte, H = Hemicryptophyte, P = Phanerophyte, T = Therophyte, s = serpentine, ns = non-serpentine. 18df, n = 20,* = significant.

	Taxon	Life form	s mean ± sd	ns mean ± sd	t	P
More common on serpentine	<i>Indigofera hedyantha</i> Eckl. & Zeyh.	P	3.4 ± 5.7	0.4 ± 1.3	1.5	0.15
	<i>Phyllanthus maderasparensis</i> L.	H	1.2 ± 0.9	0.3 ± 1.0	0.9	0.41
	<i>Nidorella hottentotica</i> DC.	H	0.8 ± 0.6	4.9 ± 5.7	2.7	0.01*
	<i>Bewsia biflora</i> (Hack.) Goossens	H	3.6 ± 6.0	0.6 ± 1.1	1.5	0.16
	<i>Tephrosia capensis</i> (Jacq.) Pers. var. <i>capensis</i>	H	4.2 ± 7.9	0.6 ± 1.7	1.3	0.2
	<i>Ipomoea crassipes</i> Hook.	H	0.1 ± 0.3	0.6 ± 0.9	1.6	0.12

Appendix 1 continued

	Taxon	Life form	s mean ± sd	ns mean ± sd	t	P
	<i>Melinus nerviglumis</i> (Franch.) Zizka	H	29.9 ± 32.3	12.3 ± 15.4	1.5	0.15
	<i>Hypoxis rigidula</i> Bak. var. <i>pilosissima</i> Bak.	C	0.8 ± 1.5	1.0 ± 2.0	0.2	0.82
	<i>Aster harveyanus</i> Kuntze	H	1.7 ± 3.3	0.3 ± 0.7	1.3	0.23
	<i>Gazania krebsiana</i> Less. ssp. <i>serrulata</i> (DC.) Roessl.	H	0.3 ± 0.7	1.1 ± 1.6	1.6	0.13
	<i>Eragrostis chloromelas</i> Steud.	H	10.4 ± 22.1	2.8 ± 4.2	1	0.33
	<i>Cymbopogon excavatus</i> (Hochst.) Stapf ex Burtt Davy	H	10.5 ± 25.6	11.9 ± 6.4	0.2	0.87
	<i>Andropogon schirensis</i> A. Rich.	H	12.2 ± 22.0	5.3 ± 8.7	0.9	0.39
	<i>Eragrostis curvula</i> (Schrad.) Nees	H	3.2 ± 7.4	1.2 ± 2.7	0.8	0.46
	<i>Elionurus muticus</i> (Spreng.) Kunth	H	17.9 ± 22.9	15.7 ± 11.9	0.3	0.79
	<i>Commelina africana</i> L. var. <i>lancispatha</i> C.B. Cl.	H	4.1 ± 7.1	2.1 ± 6.3	0.7	0.52
	<i>Acalypha peduncularis</i> E. Mey. ex Meisn.	H	11.1 ± 24.5	5.8 ± 9.0	0.6	0.55
	<i>Melinus repens</i> (Willd.) Zizka ssp. <i>repens</i>	T	4.6 ± 7.9	1.4 ± 3.0	1.1	0.28
	<i>Diheteropogon amplexens</i> (Nees) Clayton	H	10.6 ± 14.6	7.4 ± 7.8	0.6	0.56
	<i>Schizachyrium sanguineum</i> (Retz.) Alst.	H	5.9 ± 12.8	6.0 ± 15.1	0	0.99
	<i>Asparagus sauveolens</i> Burch.	P	3.2 ± 6.2	1.1 ± 2.0	0.98	0.35
	<i>Cynodon dactylon</i> (L.) Pers.	Ch	2.6 ± 5.5	1.8 ± 2.9	0.4	0.68
	<i>Chlorophytum fasciculatum</i> (Bak.) Kativu	C	0.6 ± 0.8	0.4 ± 0.7	0.3	0.78
	<i>Setaria sphacelata</i> (Schumach.) Moss var. <i>sphacelata</i>	H	13.4 ± 19.2	12.1 ± 15.1	0.2	0.87
Equal	<i>Asclepias ascendens</i> (Schltr.) Schltr.	H	0.3 ± 0.7	0.3 ± 1.0	0.2	0.87
	<i>Chaetacanthus costatus</i> Nees	H	3.8 ± 4.2	4.1 ± 5.4	0.14	0.89
More common off serpentine	<i>Hermannia depressa</i> N.E. Br.	H	0.7 ± 1.7	8.7 ± 11.1	-2	0.02*
	<i>Senecio venosus</i> Harv.	H	1.0 ± 2.0	4.4 ± 5.1	-2.1	0.05
	<i>Kohautia amatymbica</i> Eckl. & Zeyh.	H	0.8 ± 1.8	1.7 ± 0.9	0	0.92
	<i>Eragrostis racemosa</i> (Thunb.) Steud.	H	1.6 ± 2.3	15.6 ± 27.2	-2	0.1
	<i>Helichrysum nudifolium</i> (L.) Less.	H	0.4 ± 0.8	3.4 ± 6.9	-2	0.2
	<i>Scabiosa columbaria</i> L.	H	0.6 ± 1.8	3.3 ± 5.8	-2	0.15
	<i>Thesium exile</i> N.E. Br.	H	0.6 ± 1.2	1.9 ± 3.8	-1	0.28
	<i>Ziziphus zeyheriana</i> Sond.	P	0.6 ± 1.5	5.3 ± 11.2	-1	0.17
	<i>Helichrysum coriaceum</i> Harv.	H	0.6 ± 1.0	1.7 ± 2.2	-2	0.15
	<i>Anthospermum rigidum</i> Eckl. & Zeyh. ssp. <i>pumilum</i> (Sond.) Puff	H	1.1 ± 2.2	2.2 ± 2.4	-1.4	0.17
	<i>Brachiaria serrata</i> (Thunb.) Stapf	H	10.9 ± 9.9	17.3 ± 13.1	-1	0.23
	<i>Panicum natalense</i> Hochst.	H	1.2 ± 1.8	2.7 ± 6.6	-1	0.48
	<i>Trachypogon spicatus</i> (L.f.) Kuntze	H	2.5 ± 6.4	6.0 ± 9.8	-1	0.34
	<i>Vernonia oligocephala</i> (DC.) Sch. Bip. ex Walp.	H	2.7 ± 3.6	4.0 ± 4.4	-1	0.48
	<i>Sonchus dregeanus</i> DC.	T	0.4 ± 0.9	1.8 ± 5.0	-1	0.36
	<i>Hyparrhenia hirta</i> (L.) Stapf	H	13.6 ± 15.1	21.3 ± 33.2	-1	0.49
	<i>Themeda triandra</i> Forssk.	H	37.8 ± 75.0	58.0 ± 27.2	-1	0.45
	<i>Pearsonia sessilifolia</i> (Harv.) Dümmer ssp. <i>sessilifolia</i>	H	0.5 ± 1.5	1.1 ± 2.0	-1	0.48
	<i>Crabea angustifolia</i> Nees	H	0.4 ± 0.9	0.6 ± 1.1	0	0.68
	<i>Urelytrum agropyroides</i> (Hack.) Hack.	H	5.1 ± 9.8	7.6 ± 18.7	0	0.71

Appendix 1 continued

Taxon	Life form	s mean ± sd	ns mean ± sd	t	P
<i>Rhynchosia totta</i> (Thunb.) DC. var. <i>totta</i>	H	0.6 ± 1.2	0.8 ± 1.4	-0.4	0.69
<i>Pentanisia angustifolia</i> (Hochst.) Hochst.	H	3.0 ± 6.4	3.3 ± 3.2	0	0.89
<i>Heteropogon contortus</i> (L.) Roem. & Schult.	H	9.9 ± 12.5	13.0 ± 17.2	-1	0.65
<i>Setaria nigrirostris</i> (Nees) Dur. & Schinz	H	25.2 ± 69.1	39.6 ± 70.2	-1	0.65
<i>Ocimum obovatum</i> E. Mey. ex Benth. ssp. <i>obovatum</i> var. <i>obovatum</i>	H	1.8 ± 3.4	2.3 ± 2.3	0	0.7
<i>Tristachya leucothrix</i> Nees	H	3.2 ± 10.6	6.0 ± 9.8	-1	0.55
<i>Gerbera viridifolia</i> (DC.) Sch. Bip. ssp. <i>viridifolia</i>	H	1.1 ± 1.6	1.3 ± 1.7	0	0.74
<i>Aristida congesta</i> Roem. & Schult. ssp. <i>barbicollis</i> (Trin. & Rupr.) De Winter	H	1.1 ± 1.9	6.2 ± 14.3	-1	0.25
<i>Ledebouria marginata</i> (Bak.) Jessop	C	0.6 ± 1.8	0.7 ± 1.1	0	1

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Temporal changes in species diversity and composition in abandoned fields in a *trans*-Himalayan landscape, Nepal

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Abstract Secondary succession is an increasing phenomenon due to global changes in agriculture policies and practices. The empirical findings are biased towards the temperate zone. Abandonment of agriculture fields is less frequent in the subtropical and tropical zones where agriculture areas are, in general, expanding. But there are exceptions; a rapid rate of abandonment of agricultural fields have taken place in the arid *trans*-Himalayan region, due to today's globalization of economy. We analysed agriculture fields that were abandoned between 1950 and 2003 in a large u-valley in central Nepal (3400 m a.s.l.). The potential forest vegetation is dominated by *Pinus wallichiana* and shrubs of junipers and cotoneaster species. We tested the intermediate richness hypothesis in relation to vegetation cover, soil development and whether old-field succession is convergent or divergent with species data from 242 1 m² plots in 5 age-classes. The main species compositional turnover expressed by Detrended

Correspondence Analyses (DCA) correlated, as expected, with time after abandonment. Fields that were abandoned a long time ago are closer to forest at the periphery of the agricultural landscape. Moisture of the soil significantly increased with age of abandonment, but total vegetation cover and pH were negatively related to age. Beta diversity expressed in DCA SD-units showed an increasing trend with age of abandonment, supporting the divergence pattern in old-field succession. The reason why the succession is not converging may be due to browsing by domestic animals that prevent a closed canopy of pines and juniper to develop. There was a significant hump-shaped pattern in species richness along the temporal gradient, which agrees with the intermediate species-richness hypothesis. There was a rapid increase in species richness in plots close to the villages that were used for haymaking which increased the seed input significantly.

Keywords GLM · Himalaya · Manang · Multivariate analyses · Old-field succession · Secondary succession · Species richness

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Introduction

Secondary succession has been a key issue in vegetation ecology (Drury and Nisbet 1973; Egler 1952; Horn 1974; Pickett et al. 1987), and its

importance is increasing due to accelerating land-use changes in most parts of the world (Bazzaz 1996). Old-field succession is one type of secondary succession, which has become more common due to global changes in agricultural policies and practices. Research on abandoned crop fields was started by Oosting (1942), in North Carolina, USA, and since has been used to elucidate processes in succession (e.g. Bard 1952; Bazzaz 1996; Odum 1960, 1969; Quarterman 1957). Several patterns in species richness and turnover are expected in the course of secondary succession, but these have been debated for a long time. The classical view was that species diversity increase during the course of succession and reach a maximum in the later phases (Clements 1936; Margalef 1968; Odum 1969, 1971; Slobodkin and Sanders 1969), but the current view is that diversity is higher in the intermediate phases than in the late phases of succession (Horn 1974; Bazzaz 1996 and others). However, diversity is a loose composite concept, and has to be more operational if one is to test the above predictions. Two types of diversity changes are found in succession; richness within a community (alpha) and difference in species composition between communities (beta). Since community is also a loose concept, this division has some limitations, especially as they are not scaled to unit area (Palmer 1990; Whittaker 1987). If they are scaled to unit area by substituting the community by sampling units, the concept is standardised and can be used to estimate different aspects of diversity along a temporal gradient.

In the context of successional changes, beta diversity implies divergence or convergence. Early ecologists considered succession as a convergent development, where different communities, or stages, replaced one another in a predictable pattern, and converged into a relatively stable long-term community, or climax. Studies in recent decades claim that succession is a multidirectional probabilistic process, which may have several different endpoints (Bazzaz 1996; Collins and Adams 1983; Glenn-Lewin 1980; Matthews 1979). This allows divergence and convergence, depending on site conditions, e.g., development of tree layer or not in the mature phases. Many studies have found that successional development into forest may result in convergence since the dominant canopy reduces the micro scale variability on the ground (Bard 1952;

Bazzaz 1996; Horn 1974; Odum 1969; Pickett 1989; Pickett and McDonnell 1989; Quarterman 1957; Vetaas 1997). In a recent study in the high Andes, Sarmiento et al. (2003) found that abandoned agricultural fields above the forest line had a divergent development.

A temporal change in the number of species per sampling unit (alpha diversity) is expected. Patterns of species richness have been justified both by long-term studies on fixed abandoned agricultural fields, for e.g. Tilman (1986); Pickett (1989); Tunnell et al. (2004) and by chronological studies based on research after certain years of abandonment, such as Bazzaz (1996); Bonet and Pausas (2004); Sarmiento et al. (2003); Ruprecht (2005), Arbelo et al. (2006) and Otto et al. (2006). A low number of species in the initial phase is an inevitable phenomenon in primary succession but also in old-field succession. The controversy is whether the maximum richness is found in the mature phases or in the intermediate phases. The former view is related to the classical view of convergent development into a stable climax with high species richness (Clements 1936; Margalef 1968; Odum 1969, 1971; Slobodkin and Sanders 1969). However, in recent decades several authors have found maximum richness in intermediate phases (Bazzaz 1996; Brown and Southwood 1987; Horn 1974). The intermediate phases may have a mixture of light-demanding early-phase species and shade-tolerant late-phase species. If one assumes that biomass and vegetation cover increase with time, then maximum richness in the intermediate phases could be deduced from Grime's model on species-richness and biomass. This model predicts higher richness at intermediate levels of biomass, which may correspond to the intermediate phases of succession. If disturbance is defined as removal of biomass (*sensu* Grime) then the intermediate disturbance model (Connell 1978) will also predict higher richness at intermediate biomass levels, i.e. intermediate succession phases. Thus even if the old-field succession does not develop into a forest, one may expect higher richness in the intermediate phases compared to the oldest fields. One may also expect decrease in soil pH as vegetation cover increases. This development will be enhanced by acidic litter from pine and junipers species. However, grazing may prevent this development and facilitate open calcareous

grassland, which in the temperate zone is known to be rich in species (Butaye et al. 2005; Piqueray et al. 2007 and references therein).

The variation in the total number of species within each succession phase will depend on variation in beta diversity and the species richness through the course of succession. Even with a very low alpha diversity in the mature phases, the total number of species may be high if beta diversity is high. The variation in total number of species in each phase must be seen in relation to convergence versus divergence and the trend in species richness.

We report from an old-field succession in abandoned sub-alpine wheat and buckwheat fields located in the arid *trans*-Himalayan (dry inner valleys) zone in Nepal. Here we attempt to test three hypotheses on species diversity. The study of old-field succession has mostly been developed in temperate lowland vegetation (Bazzaz 1996; and references therein); considerably less information is available for high altitude old-fields (Sarmiento et al. 2003).

We hypothesise that changes in species composition will have a divergent development. The area is used for grazing and it is not certain if the old-field will develop into *Pinus wallichiana* A. B. Jack. forest or *Juniperus* shrubs, which surround the agricultural landscape. We hypothesised that number of species would show a maximum at the mid-successional

phase; this is tested against the null hypothesis of no trend. If one uses the sampling plots as units, alpha diversity means species richness per plot and beta diversity means the species turnover between a set of plots.

The aims of the study are to (1) quantitatively describe the temporal changes in species composition, vegetation cover and soil properties by means of ordination, (2) evaluate if the succession is divergent or convergent and (3) test the hypothesis of enhanced species richness in intermediate phases.

Materials and methods

Study area

The study area is located in the Manang district, within Annapurna Conservation Area, in north-central Nepal, latitude 28°40' N and longitude 84°01' E (Fig. 1). It is situated between 3175 and 3475 meters above sea level (m a.s.l.) in the *trans*-Himalayan region between the Himalayan range and the Tibetan Plateau.

The area is north of the massive Annapurna range which has a maximum elevation above 7000 m a.s.l. Thus it receives little of the monsoon rain, which comes from the south-east. The mean annual precipitation during the year is ca. 400 mm (Anonymous

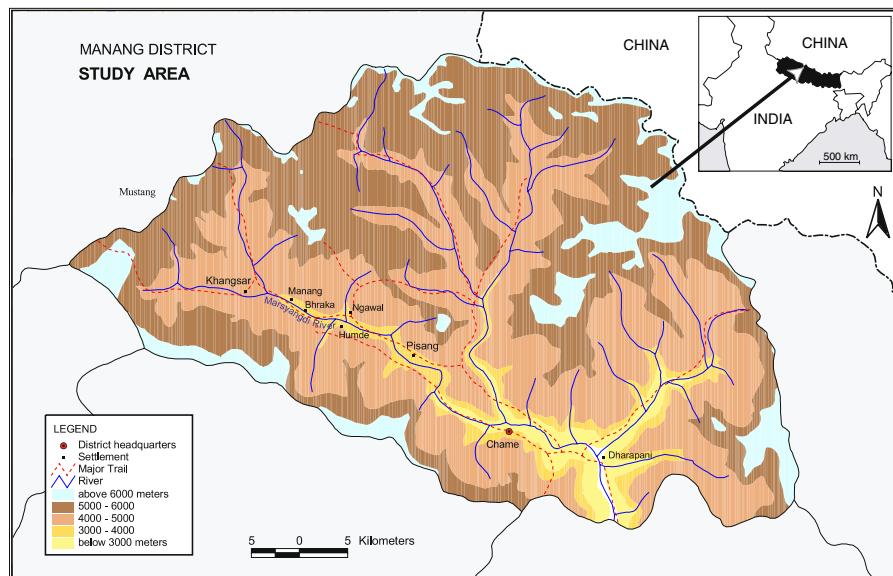


Fig. 1 Map of study area and the location of the two villages Bhraka and Pisang

1995). The mean maximum/minimum temperatures recorded at Jomsom (nearest comparable station) are 7.9°C/–1.75°C in winter and 22.6°C/14.15°C in summer during 1995 (Anonymous 1999). Snow is common during winter.

There is decreasing moisture from east to west in the upper Manang valley, and the south-facing slopes are significantly drier and warmer than those facing north. All agricultural fields are found on the south exposed slopes or at the valley bottom. This is also reflected in the vegetation and forest formation. At elevations above 3000 m a.s.l. there is forest of *Pinus wallichiana* A. B. Jack., *Betula utilis* D. Don and *Abies spectabilis* (D. Don) Mirb., on the north-facing slopes, and some forest of *P. wallichiana* on the dry south-facing slopes. Here shrubs of *Juniperus indica* Bertol., *Rosa* spp. and *Caragana* spp. dominate the nearby landscape. The valley bottom is in an intermediate situation, and forest of *P. wallichiana* is the mature vegetation where grazing and forest cutting is restricted. Above the timberline (4000–4300 m a.s.l.) there is a harsh continental climate, which allows only steppe vegetation similar to that of the Tibetan plateau (Miehe 1982).

The dominant land-use is agriculture and domestic animals with yak, horse, mule, sheep and goats graze in the alpine pastures above the timberline (4300 m a.s.l.) during the summer. In the winter the animals are brought down to graze the grasslands of the lower slopes and valley floor. All the abandoned fields and active fields are open for grazing after the harvest in late September.

Field methods

Two study sites, Pisang and Bhraka (Fig. 1), were selected as suitable sites for sampling. The total agricultural area in Pisang is 150 ha and almost 75% is abandoned. In Bhraka 60% of the agricultural land (total 185 ha) is abandoned. The spatial pattern of the abandonment is similar in the two sites (and all villages in the valley); the oldest abandoned fields are located in the periphery (0.5–2 km away from the village) and the active fields are closest to the concentrated village settlement. The two villages are located 12 km apart with an elevation range of 300 m (3175 to 3475 m a.s.l.) at Pisang and 50 m (3400 to 3450 m a.s.l.) at Bhraka. About 42 abandoned fields were selected to assure that different age classes (see

below) were represented as equally as possible. The selected fields represent ages from 1 to 50 years of abandonment. There was some uncertainty about the age of some old-fields, thus the fields were grouped into age-classes of 10-year intervals, except age-class I (1 to 5 years). The border of the oldest abandoned fields (>35 years, age-class V) were determined by fallen stonewalls, terraces and heaps of stones. The fields are by and large evenly grazed, but not mowed except for some cutting of hay during the week before harvesting of the active fields and the return of the animals from the summer farms (Aase and Vetaas 2007).

A regularly spaced systematic sampling method (Kershaw and Looney 1985) was used in each field and the longest diagonal was chosen as a transect line. Another line was made perpendicular to this, which crossed the midpoint of the longest diagonal. Plots of 1 × 1 m were regularly placed along both lines. The plot closest to the border of the old-field was always 10 m away from the edge, and the distance between plots was 10 m. Each plot was divided into four subplots and presence/absence of all rooted species inside the plot was noted. This gave a relative abundance estimate from 1 (i.e. present in 1 subplot) to 4 (present in all 4 subplots), which is a rough estimate suitable for ordination analyses. A total of 242 plots were sampled, and each age-class has between 44 and 52 plots.

In each plot, total vegetation and stone cover percentage (%) were estimated visually. Soil moisture and pH were measured with a soil pH and Moisture Tester (Model DM 15; Takemura Electric Works Ltd., Japan). In addition to elevation, a clinometer-compass was used to measure the slope and aspect that were then used to calculate the relative radiation index (RRI) for each plot (Oke 1987; Vetaas 1993). The adjacent vegetation of each sampled old-field was recorded, where '1' indicated presence of shrubs or trees and '0' indicated other fields. All the environmental variables, their abbreviations, and their units of measurement are given in Table 1. All plant species occurring inside each 1-m² plot (i.e. species richness) were identified following the nomenclature of Hara et al. (1978), Hara and Williams (1979), Hara et al. (1982), Press et al. (2000). The growth form of each plant recorded was categorised into five types; forbs, graminoid, fern, shrub and tree (Appendix Table A1) using the method of Sarmiento et al. (2003).

Table 1 List of abbreviated environmental variables, their full form, statistical summary and criteria of measurement followed

Short form	Full form	Minimum	Mean ± S.E.	Maximum	SD	Measurement criteria
Temgra	Temporal gradient	0.2	2.1 ± 0.1	4.5	1.0	Age constrained CCA first axis sample score
Elev	Elevation	3175.0	3370.0	3475.0		Altimeter value, meter above sea level (m a.s.l.)
Forest	Forest	0.0	0.6 ± 0.0	1.0	0.5	Closeness of each plot, '1' if close to forest, '0' other
Mois	Moisture	0.5	2.9 ± 0.1	9.5	1.9	Probe estimation (1 dry to 9 moist)
pH	% Of H ⁺ concentration	5.0	6.5 ± 0.0	7.7	0.3	Probe estimation
RRI	Relative radiation index	0.5	0.9 ± 0.0	1.0	0.1	Composit value of aspect, slope and latitude
Spp. no	Total species number	6.0	14.4 ± 0.2	25.0	3.5	Count
Ston cov	Stone cover %	0.0	37.9 ± 2.2	99.0	34.1	Visual estimate %
Agecl	Age class	1.0	2.9 ± 0.1	5.0	1.4	Temporal data with 10 years interval
Veg cov	Vegetation cover %	5.0	70.6 ± 1.4	99.0	22.2	Visual estimate %

SD stands for standard deviation, S.E. stands for standard error for mean, H⁺ is for hydrogen ion, and % is the percentage

Numerical data analysis

The relationship among the explanatory variables was explored by correlations. Detrended Correspondence Analysis (DCA) was used to explore the species composition of all plots. Default options, such as detrending by segments, Hill's scaling and downweighting of rare species were used. The first two axes were then correlated with the explanatory variables. DCA was used to measure beta diversity within each age class by estimating the length of the ordination axes, i.e. species turnover in standard deviation units (SD) (Hill and Gauch 1980). We used SD-units of axis-I and II, together with the total inertia (eigenvalue) to evaluate if there was divergent or convergent development pattern in the succession.

Species richness: alpha diversity

Age is defined here as the real observed age of abandonment of each old-field after interviewing local people. The age-constrained Canonical Correspondence Analysis (CCA) axis-I sample score from ordination is called the temporal gradient hereafter. Age is classified into 5 age-classes for simplicity. Species richness was related to the temporal gradient by means of a Generalised Linear

Model (GLM) (McCullagh and Nelder 1989; Nelder and Wedderburn 1972). When the response variable is expected to have a Poisson distribution, e.g., species count data, GLM is able to link the expected response to the explanatory variables with a log-link function. The main explanatory variable used here is the temporal gradient. This method of using age-constrained CCA-axis-I sample scores is similar to Lepš et al. (2001); Bartolome et al. (2004). Species richness was regressed against the temporal gradient using GLM. The temporal gradient is the main environmental variable and was changed into a continuous variable. This constrained continuous temporal variable is more reliable than the age interval variable, i.e. age class. A preliminary analysis shows the high correlation between the real age of the plots and the age constrained CCA-axis-I sample score which was higher than 0.88.

The age of abandonment increases with the distance to the village centre, thus the successional change in species composition is spatially structured where plots close to each other in space and time of abandonment have more similar species composition than those that are more distance in space and time. Thus autocorrelation is part of the phenomenon under study. We view this analyse as test of three different patterns; no relationships, linear (positive or

negative) and unimodal. It is not the temporal gradient as such that cause the pattern, thus this is not an explicit inferential statically test of causality. Due to this we have not used any spatial models to check for spatial autocorrelation.

To evaluate monotonic versus unimodal trend along the temporal gradient, we tested the significance of the additional deviance explained by a second order polynomial term against a linear term. Regression was done between all measured variables against species richness to evaluate the strength of the explanatory variable. We also checked the relation with % vegetation cover, which will indicate a potential sampling effect; i.e. high cover yields many individuals thus enhanced probabilities for more species (May 1975). The adequacy of the fitted models was confirmed by plotting standardised residuals against fitted values, and by the normal probability plots of the fitted values (Crawley 1993). An *F*-test statistics was used, as the deviance was under dispersed (Hastie and Pregibon 1993).

The CCA-axis-I species score was also used to measure the relative successional optimum for each plant species occurring in the dataset (Lepš et al. 2001). CANOCO Version 4.5 (ter Braak 2002) and its graphical program CANODRAW (Smilauer 2002) were used to analyse the relationship between variation in species composition and predictor variables. Regression analysis between species richness and different environmental variables of old abandoned fields was done with the statistical program S-PLUS (Anonymous 2002).

Results

Environmental correlation

There is a high significant correlation ($r = 0.875$) between the age class and temporal gradient (Table 2). Similarly, % vegetation cover, % stone cover and pH show significant negative correlation with age class and temporal gradient, while forest-proximity (plots that were close to forest or woody species) and soil moisture showed significant positive correlation with both age class and temporal gradient. Moisture showed a negative correlation with % stone cover, pH and elevation (Table 2).

Environmental correlation with DCA axes

The summary of DCA results (Table 3) showed that axis-I have a high eigenvalue (0.55) and is correlated with the temporal gradient. The length of the temporal gradient was 4.8 SD-units, which means that most species showed a complete turnover and a unimodal response with the measured environmental variables. Axis-I represents a complex gradient and correlates with most of the environmental variables (Table 3). Axis-II separated the two sites due to the significant correlation with relative radiation index, elevation and moisture (Fig. 2). On an average the elevation is higher at Bhraka, and the species at upper part of the ordination diagram are more common in this site. The successional changes along axis-I take place in parallel in the two sites. The most pronounced

Table 2 Environmental correlation coefficient matrix of explanatory variables ($n > 100$, $P \leq 0.05$, $r \geq |0.195|$)

Variables	Age class	Temgra	Veg cov	Ston cov	RRI	pH	Elev	Forest	Mois
Age class	1.0								
Temgra	0.8757	1.0							
Veg cov	-0.3232	-0.4129	1.0						
Ston cov	-0.214	-0.2202	0.188	1.0					
RRI	-0.1285	-0.2317	0.1316	-0.0806	1.0				
pH	-0.3079	-0.3035	0.028	0.4089	0.0359	1.0			
Elev	-0.0687	-0.2199	-0.0104	0.1496	-0.0598	0.1742	1.0		
Forest	0.3437	0.4144	-0.2661	-0.1309	-0.1802	0.0003	-0.0839	1.0	
Mois	0.214	0.2639	0.0418	-0.2729	0.0914	-0.5622	-0.2195	-0.0127	1.0

Bold entries are statistically significant coefficients

Full forms of the variables are given in the Table 1

Table 3 Environmental correlation (weighted) and summary of DCA in the major three axes

	I	II	III
<i>Environmental correlation with DCA axes</i>			
Temporal gradient	1.00	0.02	-0.50
Elevation	-0.26	0.48	0.10
Forest (closeness)	0.45	-0.10	-0.27
Moisture	0.24	-0.54	-0.49
pH	-0.25	0.30	0.45
Relative radiation index	-0.20	-0.57	-0.16
Stone cover (%)	-0.18	0.16	0.50
Age class	0.80	0.05	-0.30
Vegetation cover (%)	-0.38	-0.39	0.39
<i>DCA summary from dataset</i>			
Eigenvalues	0.55	0.23	0.15
Lengths of gradient	4.76	2.65	2.58
Species–environment correlations	1.00	0.61	0.40
Cumulative percentage variance of species data	14.30	20.20	24.10
Cumulative percentage variance of species–environment relation	48.80	56.80	0.00

Bold entries at the environmental correlation are statistically significant coefficients where, $n > 100$, $P \leq 0.05$, $r \geq |0.195|$

difference between the two sites is that plots in Bhraka are located in the valley bottom and do not have high radiation index on average such as the other site, Pisang.

Analyses of species composition and beta diversity

A total of 136 species was recorded in the study area (Appendix A1). They belonged to 40 different families among which Asteraceae was the most dominant, and grasses were the second largest family. Annual herbs, such as, *Malva neglecta* Wall. ex Sweet, *Capsella bursa-pastoris* (L.) Madik., *Chenopodium album* L., *Convolvulus arvensis* L., *Fagopyrum esculentum* Moench., *Medicago lupunia* L. and *Brassica rapa* Roxb., (Fig. 2) are dominant in the youngest fields, found towards the negative end of the DCA axis-I. Woody perennials such as *Rosa sericea* Lindl., *Lonicera obovata* Royle ex Hook. f. & Thom., *Berberis ceratophylla* G. Don, *Juniperus indica* Bertol. and *Pinus wallichiana* A. B. Jack. are more prominent in the oldest fields. They occurred towards the right end of the DCA axis-I (Fig. 2).

Trifolium pratense L., *Atremisia gmelinii* Weber ex Stechm., *Artemisia caruifolia* Buch.-Ham., *Cyanoglossum zeylanicum* (Vahl ex Hornem.) Thunb. ex Lehm. and *Taraxacum eriopodum* DC., are found at the middle part of the DCA space and are common species along the entire temporal gradient. Graminoids are quite dominant in some agriculture fields. *Phelum alpinum* L. is abundant in early pioneer stages, whereas *Brizia medica* L. and *Brachypodium sylvaticum* (Huds.) P. Beauv are dominant in later phases. The most common grass, *Pennisetum flaccidum* Griseb., has its optimum in the middle of the temporal gradient, but is found all along the temporal gradient.

The beta diversity (length of gradient in SD-units) along the temporal gradient is shown in Fig. 3. There is a clear increasing trend in the length of gradients with increasing age class of the abandoned fields along the DCA axis-I, but the trend is not so clear along axis-II. Total inertia, i.e. sum of all eigenvalues, shows an increasing trend with age class. This clearly says the degree of divergence and heterogeneity increased with the age of abandoned fields as indicated by the length of the gradients of the DCA axis-I and their eigenvalues.

Patterns of species richness in abandoned fields and their environmental characters

Percentage vegetation cover had a surprisingly negative correlation with age class (Table 2). There was high vegetation cover (almost 100%) in some recently abandoned fields (age-class I), and some of the oldest abandoned fields of age-class V had vegetation cover of only 5%. Plots that were in the oldest abandoned fields had some plots with maximum % stone cover but some recently abandoned plots also had almost 90% cover. pH of the plots was between 5 and 7.7, with the moisture gradient between 1 (driest) and 9.5 (wettest) (Table 1). These plots belonged to two sites almost 12 km apart (Fig. 1), at an elevation gradient of 3175 to 3475 m a.s.l. The relative radiation index (RRI) values are between 0.1 to 1 (Table 1). Total number of species present in each age class and their mean and maximum values obtained after summing in SPLUS are given in Table 4.

There is a clear unimodal response in species richness along the temporal gradient, with highest

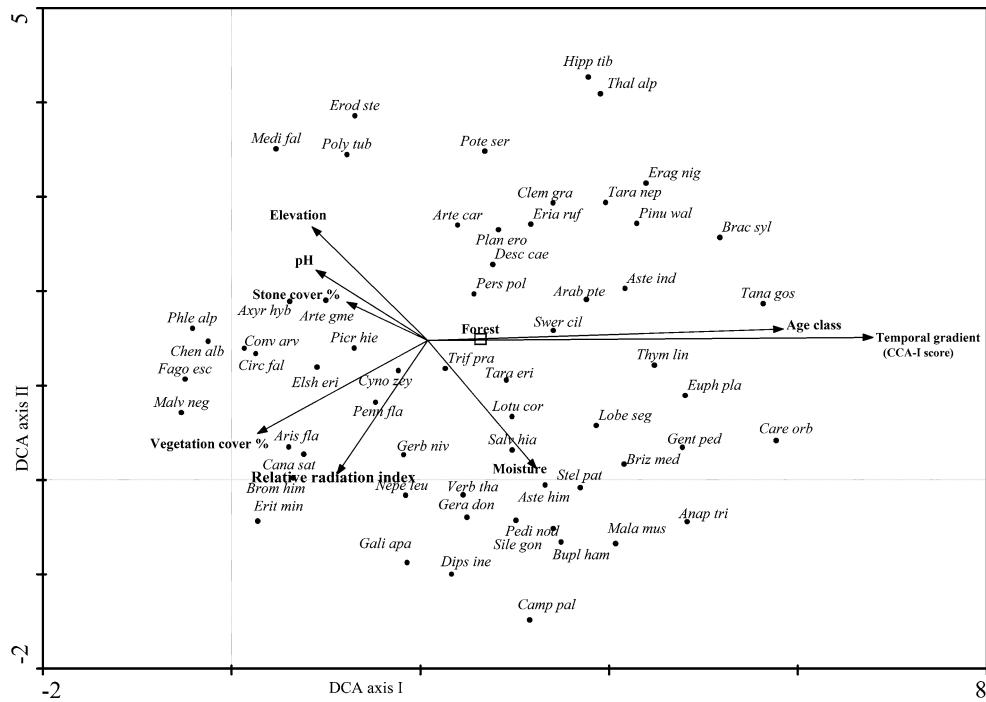


Fig. 2 DCA biplot of environmental variables and species (black dots). Species weight ranges between 1 and 100% in the inclusion rule passed by 60 species only. Full name and author

of each plant species is given in Appendix Table A1. Abbreviation of environmental variables represented by ‘bold arrow’ is given in Table 1

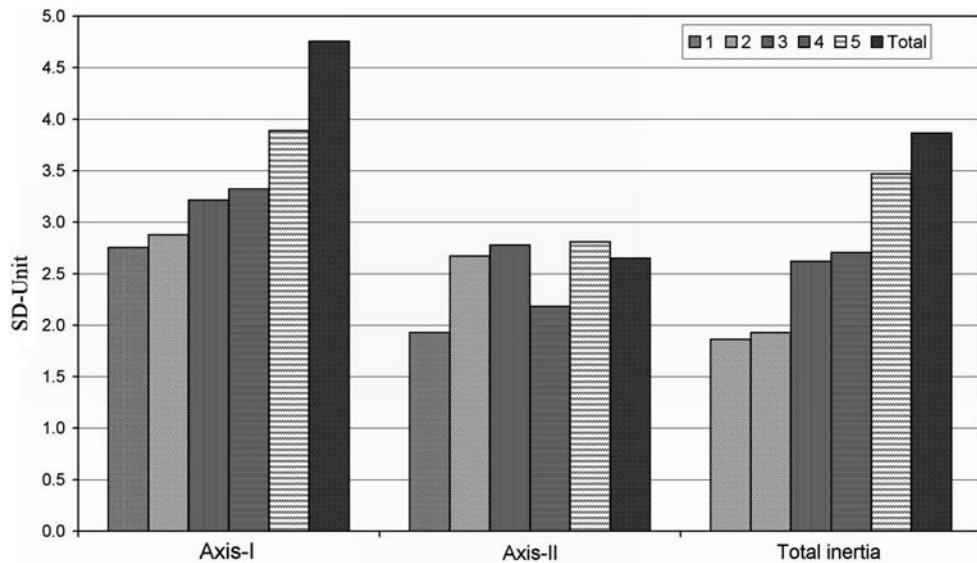


Fig. 3 Beta diversity patterns among age classes and total dataset estimated by DCA SD-units for axes I, II and total inertia. Symbols ‘1–5’ and ‘Total’ inside the figure box

represent age classes and total dataset. There is a clear pattern of increasing degree of divergence as well as heterogeneity in this old-field succession

species richness found at the mid successional phase (Table 5 and Fig. 4). After testing this significant model with the null model it showed a significant

result (Table 5). Among other environmental variables, age class and temporal gradient showed a significant non-linear dependence with species

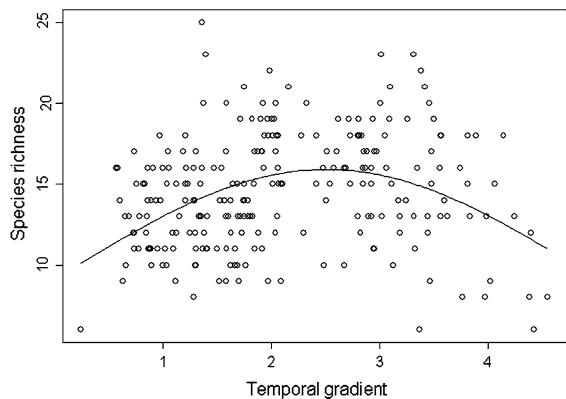
Table 4 Age classes, their interval, number of plots and range in species number per plot and mean value

Classes	Interval (years)	Plots number	Total no. of species in all plots	Minimum no. of species per plot	Maximum no. of species per plot	Mean no. of species per plot
1	1–5	52	68	6	25	13
2	6–15	52	66	9	23	15
3	16–25	48	64	7	22	14
4	26–35	44	87	8	20	15
5	>36	46	95	6	23	15
Total	1–50	242	136	6	25	14

Table 5 Regression statistics for different explanatory variables regressed against species richness using generalised linear models

Models	Resid. Df	Resid. Dev	Test Df	Expl. Dev	F-value	Pr(F)
Temporal gradient	240	209	1.0	3.6	4.3	<0.05
Age class	240	209	1.0	4.0	4.1	<0.05
Vegetation cover (%)	240	198	1.0	15.0	18.0	<0.001
Stone cover (%)	240	212	1.0	0.7	0.8	ns
RRI	240	213	1.0	0.1	0.1	ns
pH	240	209	1.0	4.0	4.2	<0.05
Elevation	240	209	1.0	3.4	4.0	<0.05
Forest	240	209	1.0	4.0	4.3	<0.05
Moisture	240	194	1.0	18.2	23.1	<0.001
<i>Second order polynomial</i>						
poly(Temporal gradient, 2)	239	184	2.0	29.0	19.0	<0.001
poly(Age class, 2)	239	206	2.0	7.0	4.0	<0.05
poly(Vegetation cover, 2)	239	198	2.0	15.0	9.0	<0.001
poly(Moisture, 2)	239	193	2.0	20.0	13.0	<0.001

Total degrees of freedom (Df) is 242 and null deviance (Dev) is 213. Resid. = residual, Expl. = explained, for other abbreviations see Table 1

**Fig. 4** Plant species-richness pattern along the continuous temporal gradient is modelled by canonical correspondence analyses. The unimodal response is fitted by generalised linear model, see Table 5

richness. However, vegetation cover, pH, elevation and moisture showed linear relations. Stone cover and RRI did not show any significant relationships (Table 5).

Discussion

Landscape and vegetation pattern

The main factor that explains the trends in species composition was the differences in time of abandonment, whereas secondary factors are difference in radiation index, moisture and pH. Thus the results confirm the expected pattern for a succession development found in the literature (Bazzaz 1996), however, some patterns have site-specific explanation

and some may relate to scale both in time and space. For instance, the positive correlation between fields close to forest and the temporal gradient (Table 2) is because fields that were abandoned a long time ago are closer to forest at the periphery of the agriculture landscape, whereas those fields still in practice are closer to the concentrated village settlement.

Elevation was not significantly correlated with age class (Table 2). Research that evaluates temporal patterns in species richness in steep terrace cultivation (average inclination of slope is 30°) (Dobremez 1976; Vetaas and Grytnes 2002; Vetaas 2002) has to take into account the effect of elevation, especially when a space-for-time substitution approach is used (Bazzaz 1996; Matthews 1979; Pickett 1989). Since elevation is rejected as a factor in this study we interpret the temporal pattern in species richness and in species composition in the two sites as one general pattern. There is a significant negative correlation between soil pH and moisture (Table 2). Positive correlations are found among the soil moisture, age class and temporal gradient, but pH decreases with abandonment. This pattern of soil pH matches with Arbelo et al. (2006) but not with Blatt et al. (2005), and many others such as Prach and Rehoukova (2006); Dickie et al. (2007). Most of them have found a positive correlation between pH and age of abandonment and, similarly, with soil moisture. Soil in the study area has a very high pH (Mong and Vetaas 2006) and a decrease in pH as the vegetation cover increased is to be expected due to more litter and humus.

Vegetation cover does not increase with the temporal gradient even though stone cover is higher in young old-fields. In fact, the temporal gradient is negatively correlated with vegetation cover (Table 2). This is similar to Bonet and Pausas (2004) but contrasts with the general pattern of development in secondary succession (Bazzaz 1996; Brown et al. 2006; Drury and Nisbet 1973; Horn 1974). However, old-field succession is sometimes different in this respect. Production in some old-field sites is found to increase drastically in the first years after abandonment (Odum 1960, 1969), because of heavy fertilization in the time period before abandonment. High cover (>80%) in abandoned fields of age-class I is not related to the use of chemical fertilizers in our study, but may be due to the decomposition of previously added farmyard manure.

High cover of herbs and annual grasses in the younger abandoned fields has also been found by other authors (Bartha et al. 2003; Bonet and Pausas 2004; Otto et al. 2006; Pickett 1982; Ruprecht 2005).

The temporal gradient is highly correlated to the first DCA axis (Table 3), as is expected in old-field succession studies and has been documented by Sarmiento et al. (2003) and Arbelo et al. (2006). The successional sequence along the temporal gradient (CCA axis-I) shows a pattern where herbs and grasses are common in the early phase, whereas shrubs, trees and perennial herbs dominate in the later phases (Appendix Table A1). This change in growth form dominance is a general pattern in secondary succession and old-field succession (Bazzaz 1996; Ruprecht 2005). Plants in the very early stages are often common weeds from the agricultural fields, which agrees with the findings of Teira and Peco (2003); Bazzaz (1996); Sarmiento et al. (2003); Bonet and Pausas (2004) and Ruprecht (2005).

Diversity pattern

We found a significant unimodal pattern between species richness and the temporal gradient (Fig. 4). A unimodal pattern of species richness during old-field succession was also found by Horn (1974); Bazzaz (1996); Pickett (1982); Brown and Southwood (1987); Teira and Peco (2003); Bonet (2003) and Bonet and Pausas (2004). However, there are also studies that show a linear increasing pattern (Carson and Barrett 1988; Collins et al. 1995; Odum 1969; Otto et al. 2006; Ruprecht 2005; Sarmiento et al. 2003; Tramer 1975). High species richness at mid succession is commonly explained by the overlap of pioneer species (light demanding) and the species in the mature phase (shade tolerant) (Bazzaz 1996). High species richness at the intermediate phase may also be due to intermixing of the different growth forms as explained above (annual herbs versus perennial woody plants). Bonet (2003) argued that the mid-successional phase has more functional groups such as annual, biennials forbs and grasses which give high species richness. According to Bazzaz (1996) species richness is positively correlated with structural heterogeneity created by irregular substrate (e.g., stones and pebbles) and the beta diversity of the surrounding vegetation. The increase of species richness in the first phase of

succession (age-class I) is inevitable as the biomass and number of individuals increase (sampling effect). This is enhanced by local factors such as high seed input from land use practices. Hay is collected every year (Aase and Vetaas 2007) from different places during late summer before the animals return from the summer grazing. This hay is dried for the winter fodder on the youngest abandoned fields close to the village. This will be supplying a heavy load of seeds to the ground, and may cause a very high variation in number of species in the plots from age-class I. High richness at early stages has also been found in other studies such as Sarmiento et al. (2003) and Teira and Peco (2003). Explanations for the high within-age-class variation of species richness could be local factors such as percent stone cover (used to prevent erosion when the field was active) and seed input from hay drying. The decrease in the oldest abandoned fields is more of a puzzle with respect to the local site factors. First, there is no proper forest cover with shady habitats and second, these sites are closer to the surrounding forest with a more diverse influx of seed; both factors would be expected to enhance the number of species. Grazing is presumably the main factor in inhibiting a forest cover to develop. The pH is thus decreasing and it does not develop into calcareous grassland known for its high richness in temperate zone of Europe (Butaye et al. 2005). However, the nutrient level may be lower in these fields since they have not been recently manured by compost.

The degree of beta diversity in different age classes (Fig. 3), indicate a divergent pattern of secondary succession. This pattern of succession matches with the findings of several other studies (del Moral 1995; Ferweda 1987; Herben et al. 1993; Rikhari et al. 1993; Sarmiento et al. 2003). All these studies indicated that surrounding vegetation plays an important role for the composition and structure of the mature vegetation. We argue that in addition to the surrounding vegetation, high grazing pressure and interactions between species and spatial heterogeneity among the fields will all contribute to a divergent pattern. The homogenization function by canopy cover is not present since pine trees and junipers are present only as scattered individuals. Lack of a closed canopy of trees or

shrubs is most likely due to grazing since both goats and sheep eat the seedlings of *Juniperus* spp. and *Pinus wallichiana* (Mong and Vetaas 2006). Shrubs such as *Berberis* spp., *Cotoneaster* spp. and *Rosa* spp. seem to represent different developmental stages on more arid locations with higher pH. Seedlings of both *Pinus wallichiana* and *Juniperus* spp. were found under bushes of *Berberis ceratophylla* G. Don, *Lonicera obovata* Royle ex Hook. f. & Thom., *Astragalus rhizanthus* Royle ex Benth., *Rosa sericea* L. and *Hippophae tibetana* Schlehd. in these locations. These bushes may act as nursery bushes for the *Pinus* spp. seedlings (cf. Bonet and Pausas 2004). A contrasting development is found in more moist and assumingly more nutrient-rich locations with lower pH, where herbs such as *Anaphalis trinervis*, *Aster himalaicus*, *Silene gono-sperma* and *Campanulatum pallidia* are dominant.

We conclude that there is a high number of species at the mid succession, related to an intermingling of different growth forms. Local factors such as hay-making enrich the seedbank which may give an enhanced amount of species richness in the early to mid successional phase. The reason for the decrease in the older phase is not related to competitive exclusion from canopy dominants. The divergent development seems to be related to differences in soil moisture and related pH-values. The area is semi-arid and the ability to retain soil moisture is an important factor behind differences in species composition. We expect a pine forest to develop if grazing is reduced, which may be likely since reduced agriculture production will reduce the demand for manure made from pine and animal dung.

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Appendix

Table A1 List of all plant species found on the studied plots, their family, abbreviation, growth form, CCA axis-I species score and frequency (Freq.) of occurrence of individual species

S. no.	Species name	Family	Abbreviation	Growth form	CCA-I score	Freq.
1	<i>Brassica rapa</i> Roxb.	Brassicaceae	Bras rap	Forb	-2.8	3
2	<i>Capsella bursa-pastoris</i> (L.) Madik.	Brassicaceae	Caps bur	Forb	-2.8	5
3	<i>Chesneya cuneata</i> (Benth.) Ali.	Fabaceae	Ches cun	Forb	-2.8	1
4	<i>Dactylis glomerata</i> L.	Poaceae	Dact glo	Grass ^a	-2.8	1
5	<i>Galium acutum</i> Edgew.	Rubiaceae	Gali acu	Forb	-2.8	3
6	<i>Oxytropis microphylla</i> (Pall.) DC.	Fabaceae	Oxyt mic	Forb	-2.8	1
7	<i>Poa annua</i> L.	Poaceae	Poa ann	Grass	-2.8	2
8	<i>Trigonella pubescens</i> Edgew. ex Baker	Fabaceae	Trig pub	Forb	-2.8	1
9	<i>Triticum aestivum</i> L.	Poaceae	Trit aes	Grass	-2.8	3
10	<i>Elymus thomsonii</i> (Hook. f.) Melderis.	Poaceae	Elym tho	Grass	-2.6	6
11	<i>Fagopyrum esculentum</i> Moench.	Polygonaceae	Fago esc	Forb	-2.5	31
12	<i>Malva neglecta</i> Wallr.	Malvaceae	Malv neg	Forb	-2.3	53
13	<i>Chenopodium ambrosioides</i> L.	Chenopodiaceae	Chen amb	Forb	-2.3	8
14	<i>Elsholtzia eriostachya</i> (Benth.) Benth.	Lamiaceae	Elsh eri	Grass	-2.1	14
15	<i>Arisaema flavum</i> (Forssk.) Schott	Araceae	Aris fla	Forb	-2.0	13
16	<i>Themeda triandra</i> Forsk.	Poaceae	Them tri	Grass	-2.0	2
17	<i>Phleum alpinum</i> L.	Poaceae	Phle alp	Grass	-2.0	66
18	<i>Chenopodium album</i> L.	Chenopodiaceae	Chen alb	Forb	-1.9	78
19	<i>Cirsium falconeri</i> (Hook. f.) Petr.	Asteraceae	Cirs fal	Forb	-1.9	41
20	<i>Cannabis sativa</i> L.	Cannabaceae	Cana sat	Forb	-1.8	14
21	<i>Erysimum benthamii</i> Monnet.	Brassicaceae	Erys ben	Forb	-1.7	3
22	<i>Medicago lupulina</i> L.	Fabaceae	Medi lup	Forb	-1.7	6
23	<i>Axyris hybrida</i> L.	Chenopodiaceae	Axyr hyb	Forb	-1.5	115
24	<i>Picris hieracioides</i> L.	Asteraceae	Picr hie	Forb	-1.5	44
25	<i>Fagopyrum dibotrys</i> (D. Don) H. Hara	Polygonaceae	Fago dib	Forb	-1.2	2
26	<i>Miscanthus nepalensis</i> (Trin.) Hack.	Poaceae	Misc nep	Grass	-1.2	1
27	<i>Solsola nepalensis</i> Grubov.	Chenopodiaceae	Sols nep	Forb	-1.2	6
28	<i>Convolvulus arvensis</i> L.	Convolvulaceae	Conv arv	Forb	-1.1	69
29	<i>Bromus himalaicus</i> Stapf.	Poaceae	Brom him	Grass	-1.1	86
30	<i>Eritrichium minimum</i> (Brand) H. Hara	Boraginaceae	Erit min	Forb	-1.0	15
31	<i>Artemisia gmelinii</i> Weber ex Stechm.	Asteraceae	Arte gme	Forb	-1.0	152
32	<i>Medicago falcata</i> L.	Fabaceae	Medi fal	Forb	-0.9	56
33	<i>Rumex nepalensis</i> Spreng.	Polygonaceae	Rume nep	Forb	-0.9	4
34	<i>Polygonum tubulosum</i> Boiss.	Polygonaceae	Poly tub	Forb	-0.7	58
35	<i>Gerbera nivea</i> (DC.) Sch.Bip.	Asteraceae	Gerb niv	Forb	-0.6	68
36	<i>Nepeta leucophylla</i> Benth.	Lamiaceae	Nepe leu	Forb	-0.6	45
37	<i>Equisetum arvense</i> L.	Equisetaceae	Equi arv	Fern	-0.5	5
38	<i>Erodium stephanianum</i> Willd.	Geraniaceae	Erod ste	Forb	-0.5	36
39	<i>Morina polyphylla</i> Wall. ex Dc.	Dipsacaceae	Mori pol	Forb	-0.4	9
40	<i>Cynoglossum zeylanicum</i> (Vahl ex Hornem.) Thunb. ex Lehm.	Boraginaceae	Cyno zey	Forb	-0.4	180

Table A1 continued

S. no.	Species name	Family	Abbreviation	Growth form	CCA-I score	Freq.
41	<i>Lotus corniculatus</i> L.	Fabaceae	Lotu cor	Forb	-0.2	14
42	<i>Geranium donianum</i> Sweet	Geraniaceae	Gera don	Forb	-0.2	21
43	<i>Verbascum thapsus</i> L.	Scrophulariaceae	Verb tha	Forb	-0.2	42
44	<i>Acronema nervosum</i> H. Woff.	Apiaceae	Acro ner	Forb	-0.1	10
45	<i>Pennisetum flaccidum</i> Griseb.	Poaceae	Penn fla	Grass	-0.1	115
46	<i>Trifolium pratense</i> L.	Fabaceae	Trif pra	Forb	0.0	201
47	<i>Artemisia caruifolia</i> Buch.-Ham.	Asteraceae	Arte car	Forb	0.0	132
48	<i>Galium aparine</i> L.	Rubiaceae	Gali apa	Forb	0.1	53
49	<i>Arabidopsis himalaica</i> (Edgew.) O. E. Schulz.	Brassicaceae	Arab him	Forb	0.3	9
50	<i>Crepis tibetica</i> Babc.	Asteraceae	Crep tib	Forb	0.3	1
51	<i>Gnaphalium affine</i> D. Don	Asteraceae	Gnap aff	Forb	0.3	9
52	<i>Rubus pungens</i> Cambess.	Rosaceae	Rubu pun	Forb	0.3	2
53	<i>Salvia hians</i> Royle ex Benth	Lamiaceae	Salv hia	Forb	0.3	28
54	<i>Plantago erosa</i> Wall.	Plantaginaceae	Plan ero	Forb	0.3	95
55	<i>Stellaria patens</i> D. Don	Caryophyllaceae	Stel pat	Forb	0.5	24
56	<i>Taraxacum eriopodum</i> DC.	Asteraceae	Tara eri	Forb	0.5	118
57	<i>Persicaria polystachya</i> (Wall. ex Meisn.) H. Gross	Polygonaceae	Pers pol	Forb	0.6	42
58	<i>Deschampsia caespitosa</i> (L.) P. Beauv.	Poaceae	Desc cae	Grass	0.6	110
59	<i>Pedicularis nodosa</i> Pennell	Scrophulariaceae	Pedi nod	Forb	0.7	18
60	<i>Dipsacus inermis</i> var. <i>mitis</i> Wall.	Dipsacaceae	Dips ine	Forb	0.7	25
61	<i>Swertia ciliata</i> (D. Don ex G. Don) B. L. Burtt.	Gentianaceae	Swer cil	Forb	0.7	73
62	<i>Erianthus rufipilus</i> (Stueud.) Griseb.	Poaceae	Eria ruf	Grass	0.8	84
63	<i>Halenia elliptica</i> D. Don.	Gentianaceae	Hale eli	Forb	0.8	5
64	<i>Lepidium apetalum</i> Willd.	Brassicaceae	Lepi ape	Forb	0.9	5
65	<i>Bupleurum hamiltonii</i> N. P. Balakr	Apiaceae	Bupl ham	Forb	0.9	36
66	<i>Cotoneaster integrifolius</i> (Roxb.) G. Klotz.	Rosaceae	Coto inr	Shrub	1.0	13
67	<i>Silene gonosperma</i> var. <i>himalayensis</i> (Rohrb.) Bocq.	Caryophyllaceae	Sile gon	Forb	1.1	40
68	<i>Potentilla sericea</i> L.	Rosaceae	Pote ser	Shrub	1.3	21
69	<i>Anemone rivularis</i> Buch.-Ham.ex DC.	Ranunculaceae	Anem riv	Forb	1.3	12
70	<i>Lonicera obovata</i> Royle ex Hook. f. & Thom.	Caprifoliaceae	Loni obo	Shrub	1.5	9
71	<i>Selinum wallichianum</i> (DC.) Raizada & Saxena	Apiaceae	Seli wal	Forb	1.5	5
72	<i>Epipactis royleana</i> Lindl.	Orchidaceae	Epip roy	Forb	1.5	9
73	<i>Aster himalaicus</i> C. B. Clarke	Asteraceae	Aste him	Forb	1.6	28
74	<i>Heracleum nepalense</i> D. Don	Apiaceae	Hera nep	Forb	1.7	6
75	<i>Campanula pallida</i> Wall.	Campanulaceae	Camp pal	Forb	1.7	15
76	<i>Artemisia biennis</i> Willd.	Asteraceae	Arte bie	Forb	1.7	8
77	<i>Clematis graveolens</i> Lindl.	Ranunculaceae	Clem gra	Forb	1.7	44
78	<i>Lobelia seguinii</i> var. <i>doniana</i> (Skottsb.) E. Wimm.	Campanulaceae	Lobe seg	Forb	1.8	46
79	<i>Gaultheria trichophylla</i> Royle	Ericaceae	Gaul tri	Forb	1.8	1
80	<i>Potentilla anseriana</i> L.	Rosaceae	Poti ans	Forb	1.8	1
81	<i>Potentilla cuneata</i> Wall. ex Lehm.	Rosaceae	Pote cun	Forb	1.8	2
82	<i>Juniperus indica</i> Bertol.	Cupressaceae	Juni ind	Shrub	1.9	11
83	<i>Euphrasia platyphyllea</i> Pennell	Scrophulariaceae	Euph pla	Forb	2.0	28

Table A1 continued

S. no.	Species name	Family	Abbreviation	Growth form	CCA-I score	Freq.
84	<i>Arabis pterosperma</i> Edgew.	Brassicaceae	Arab pte	Forb	2.0	43
85	<i>Persicaria nepalensis</i> (Meisn.) H. Gross	Polygonaceae	Pers nep	Forb	2.0	2
86	<i>Taraxacum nepalense</i> Soest.	Asteraceae	Tara nep	Forb	2.1	40
87	<i>Andropogon munroi</i> C. B. Clarke	Poaceae	Andr mun	Grass	2.1	11
88	<i>Briza media</i> L.	Poaceae	Briz med	Grass	2.1	57
89	<i>Erigeron uniflorus</i> L.	Asteraceae	Erig uni	Forb	2.1	12
90	<i>Dicranostigma lactucoides</i> Hook. f. & Thomson	Papaveraceae	Dicr lac	Forb	2.1	4
91	<i>Hedysarum campylocarpon</i> H. Ohashi	Fabaceae	Hedy cam	Forb	2.2	3
92	<i>Malaxis muscifera</i> (Lindl.) Kuntze	Orchidaceae	Mala mus	Forb	2.2	27
93	<i>Thymus linearis</i> Benth.	Lamiaceae	Thym lin	Forb	2.2	66
94	<i>Gentiana pedicellata</i> (D. Don.) Griseb.	Gentianaceae	Gent ped	Forb	2.3	15
95	<i>Oxytropis williamsii</i> Vassilcz.	Fabaceae	Oxyt wil	Shrub	2.3	7
96	<i>Gentiana crassuloides</i> Bureau & Franch.	Gentianaceae	Gent cra	Forb	2.3	9
97	<i>Aster indamellus</i> Grier.	Asteraceae	Aste ind	Forb	2.3	30
98	<i>Origanum vulgare</i> L.	Lamiaceae	Orig vul	Forb	2.3	2
99	<i>Danthonia cumminsii</i> Hook. f.	Poaceae	Dant cum	Grass	2.4	4
100	<i>Potentilla lineata</i> Trev.	Rosaceae	Pote lin	Forb	2.4	2
101	<i>Valeriana jatamansii</i> Jones	Valerianaceae	Vale jat	Forb	2.5	7
102	<i>Anaphalis triplinervis</i> (Sims.) C. B. Clarke	Asteraceae	Anap tri	Forb	2.5	15
103	<i>Ajuga bracteosa</i> Wall. ex Benth.	Lamiaceae	Ajug bra	Forb	2.6	4
104	<i>Leontopodium jacotianum</i> Beauv.	Asteraceae	Leon jac	Forb	2.6	2
105	<i>Rosa sericea</i> Lindl.	Rosaceae	Rosa ser	Shrub	2.6	8
106	<i>Pinus wallichiana</i> A. B. Jackson	Pinaceae	Pinu wal	Tree	2.6	18
107	<i>Caltha palustris</i> Tamura	Ranunculaceae	Calt pal	Forb	2.7	6
108	<i>Hippophae tibetana</i> Schlehd.	Elaeagnaceae	Hipp tib	Shrub	2.7	20
109	<i>Eragrostis nigra</i> Nees ex Steud.	Poaceae	Erag nig	Grass	2.7	27
110	<i>Potentilla fruticosa</i> L.	Rosaceae	Pote fru	Shrub	2.7	9
111	<i>Tanacetum gossypinum</i> Hook. f.	Asteraceae	Tana gos	Forb	2.8	31
112	<i>Brachypodium sylvaticum</i> (Huds.) P. Beauv.	Poaceae	Brac syl	Grass	2.9	33
113	<i>Carex orbicularis</i> Boott.	Cyperaceae	Care orb	Grass	2.9	13
114	<i>Salvia nubicola</i> Wall. ex Sweet	Lamiaceae	Salv nub	Forb	3.0	3
115	<i>Stellera chamaejasme</i> L.	Thymelaeaceae	Stel cha	Cushion	3.0	3
116	<i>Androsace globifera</i> Duby	Primulaceae	Andr glo	Cushion	3.0	2
117	<i>Berberis ceratophylla</i> G. Don	Berberidaceae	Berb cer	Shrub	3.1	9
118	<i>Thalictrum alpinum</i> L.	Ranunculaceae	Thal alp	Shrub	3.1	16
119	<i>Ephedra gerardiana</i> Wall ex. Stapf.	Ephedraceae	Ephe ger	Shrub	3.1	3
120	<i>Pterocephalus hookeri</i> (C. B. Clarke) Diels	Dipsacaceae	Pter hoo	Shrub	3.1	4
121	<i>Polygonatum verticillatum</i> (L.) All.	Polygonaceae	Poly ver	Forb	3.2	4
122	<i>Gentiana robusta</i> King ex Hook. F.	Gentianaceae	Gent rob	Forb	3.3	11
123	<i>Allium hypsistum</i> Stern.	Alliaceae	Alli hyp	Forb	3.4	2
124	<i>Astragalus rhizanthus</i> Royle ex Benth.	Fabaceae	Astr rhi	Shrub	3.4	5
125	<i>Chenopodium foliosum</i> (Moench) Asch.	Chenopodiaceae	Chen fol	Forb	3.4	1
126	<i>Cuscuta europaea</i> L.	Convolvulaceae	Cusc eur	Forb	3.4	1

Table A1 continued

S. no.	Species name	Family	Abbreviation	Growth form	CCA-I score	Freq.
127	<i>Delphinium williamsii</i> Munz.	Ranunculaceae	Delp wil	Forb	3.4	2
128	<i>Deyeuxia pulchella</i> (Griseb.) Hook. f.	Poaceae	Deye pul	Grass	3.4	3
129	<i>Elephantopus scaber</i> L.	Asteraceae	Elep sca	Forb	3.4	1
130	<i>Herminium macrophyllum</i> (D. Don) Dandy	Orchidaceae	Herm mac	Forb	3.4	2
131	<i>Juncus triglumis</i> L.	Juncaceae	Junc tri	Grass	3.4	5
132	<i>Krascheninnikovia ceratoides</i> (L.) Gueldenst.	Chenopodiaceae	Kras cer	Forb	3.4	1
133	<i>Lilium nepalense</i> D. Don	Liliaceae	Lili nep	Forb	3.4	2
134	<i>Saussurea stracheyana</i> (Kuntze) Lipsch.	Asteraceae	Saus str	Forb	3.4	2
135	<i>Sibbaldia cuneata</i> Hornem. ex Kuntze	Rosaceae	Siba cun	Forb	3.4	1
136	<i>Viola pilosa</i> Blume	Violaceae	Viol pil	Forb	3.4	4

Nomenclature follows Hara et al. (1978, 1982); Hara and Williams (1979) and Press et al. (2000). The table is arranged according to the values of successional optimum. This successional optimum is the CCA axis-I species score. Each value is on a relative scale, high negative values signify the species of the youngest (age-class I) abandoned field and the highest positive values signify the species of the oldest abandoned field (Lepš et al. 2001)

^a Grass means graminoid

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Role of desert annuals in nutrient flow in arid area of Northwestern China: a nutrient reservoir and provider

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Abstract Previous studies have tested the “vernal dam” hypothesis of spring ephemeral herbs in hardwood forests. The desert annual is a component of the desert ecosystem that takes advantage of water resources and temperature conditions during the rainy season to rapidly complete its life cycle within several months. To understand the role desert annual/ephemeral plants play in nutrient flow, we studied vegetation cover, nitrogen content and litter production of annual plants and litter decomposition rate in plant communities dominated by four shrubs (*Haloxylon ammodendron*, *Hedysarum scoparium*, *Calligonum mongolicum*, and *Nitraria tangutorum*) and two dominant annuals (*Agriophyllum squarrosum* and *Halopeplis arachnoidea* Moq) in Minqin, northwestern China. Results indicate that over half of the total vegetation cover was provided by annuals. Annuals also took up a large amount of nitrogen (0.46–3.78 g N m⁻²) along the

oasis–desert ecotone. Litter production and nutrient content were higher in areas dominated by annual plants than in areas dominated by shrubs. Furthermore, the litter decomposition rate of the annuals was higher than that of the shrubs, except for the shrub *H. ammodendron*, although almost all of the litter’s carbon (C) and nitrogen (N) remained after 6 months of decomposition. Without the annuals, more nutrients and rainwater might be lost through leaching or dust transfer caused by the wind erosion. In addition, green twigs of the annuals are the food for some animals, we found some green twigs and litter from annuals left in front of gerbil and rabbit burrows, sometimes even blocking these burrows. Thus, desert summer annuals, like nutrient reservoirs and providers, take up nutrients during the rainy season, providing some animals and microbes with food, and finally release these nutrients after death.

Keywords Annual plant · Ephemeral · Nitrogen · Nutrient · Vernal dam

Bao-Ming Chen and Gen-Xuan Wang contributed equally to this work.

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Introduction

Muller and Bormann (1976) reported that in a hardwood forest ecosystem, the spring ephemeral/annual herb *Erythronium americanum* could take up nitrogen (N) and potassium (K), and grow rapidly during the period between snowmelt and the leafing-out of the deciduous canopy. The amount of N and K

that was taken up was similar to that of the total system loss during that period. Once the canopy closed, the ephemerals senesced and released N and K while trees were clearly active in growth and nutrient uptake. Therefore, the ephemeral herb could act as a short-term sink or a “vernal dam,” leading to a reduction in nutrient loss through its nutrient uptake during an appropriate period and the preservation of the nutrient capital of the ecosystem. Many studies have tested this “vernal dam” hypothesis (Blank et al. 1980; Zak et al. 1990; Rothstein 2000).

Annual plants survive and finish their life cycle during short periods that are favorable for germination and growth. Generally, the phenological characteristics of annuals are strongly related to the climate (temperature and moisture) of desert ecosystem (Beatley 1974; Pan and Huang 1995; Weltzin and Tissue 2003). Desert annual plants are classified as “summer” annuals if they germinate after summer or early autumn rains and as “winter” annuals if they germinate during autumn or early winter rains (Beatley 1974). The initial “vernal dam” studies of annuals were conducted in hardwood forests, rather than desert. Desert ecosystems, with lower nutrient levels and quantity of water, are quite different from hardwood forests. Our previous study showed that desert ephemeral or annuals played an important role in improving soil nutrient content (Chen et al. 2007). We hypothesized that annuals in desert may play a more important role in nutrient conservation than those in temperate hardwood forests.

Most studies of desert annuals were limited to the Chihuahuan Desert (Gutierrez and Whitford 1987; Guo and Brown 1997; Fernández and Reynolds 2000), Mojave Desert (Beatley 1974; Brooks 2000; DeFalco et al. 2001), Sonoran Desert (Halvorson and Patten 1975; Werk et al. 1983; Forseth et al. 1984; Adondakis and Venable 2004), and Negev Desert in Israel (Gutterman 2000), where winter rainfall plays an important role in ephemeral/annual growth, and most of the annuals are spring or winter plants. Moreover, few studies have focused on the role of desert annuals (including both spring/winter annuals and summer annuals) in nutrient conservation (Parker et al. 1984), though annuals play a vital role in soil rehabilitation and production in desert ecosystems due to stabilization of dune surface, prevention of wind erosion, and stability maintenance of desert ecosystems (Wang et al. 2003; Li et al. 2004).

The annuals in the Minqin Desert ecosystem are referred to as summer annuals, since rainfall mainly occurs during the summer and autumn seasons with little or no rainfall during the winter and spring. When the temperature remains extremely high, summer annuals finish their entire life cycles during summer and autumn, which is quite different from the spring/winter annuals (Freas and Kemp 1983). In order to understand the role of summer annuals in nutrient conservation, we investigated vegetation cover, N uptake, litter production and decomposition of four dominant shrubs and two dominant annuals in Minqin, located on the west edge of the Tengger Desert in northwestern China.

Materials and methods

Study site

The study was conducted in Minqin County ($38^{\circ}05' - 39^{\circ}06'N$, $103^{\circ}02' - 104^{\circ}02'E$) of Gansu Province, northwestern China, where two large deserts—the Tengger and Badain Jaran—are joined. The county is surrounded by deserts to the east, west and north, and is the location of the Hongyashan Reservoir, which is the largest desert reservoir in Asia. Minqin is downstream of the Shiyang River in the Hexi Corridor, and is located in an inland river basin of China’s arid zone. This corridor experiences sporadic, light precipitation with an annual rainfall of 113 mm, which mainly occurs between May and October. The annual mean temperature is $7.6^{\circ}C$, and the annual evaporation is about 2603.4 mm (Han and Wang 2002). Figure 1 shows the precipitation distribution and monthly temperature in 2002. The soil was typical sand alkaline soil.

The sampling transect and quadrats are located between the Hongyashan Reservoir and the Tengger Desert (Fig. 2). Twenty-one quadrats (10×10 m) were established along the transect (from oasis to desert) at 50-m intervals. Quadrat No. 1 was closest to the Reservoir and quadrat No. 21 was closest to the desert.

Vegetation description

In order to protect the Hongyashan Reservoir, a man-made community of *Haloxylon ammodendron* was

Fig. 1 The precipitation and temperature of 2002 in Minqin. **a** The average monthly precipitation and temperature, “P” means precipitation, “T” means temperature; **b** the precipitation distribution

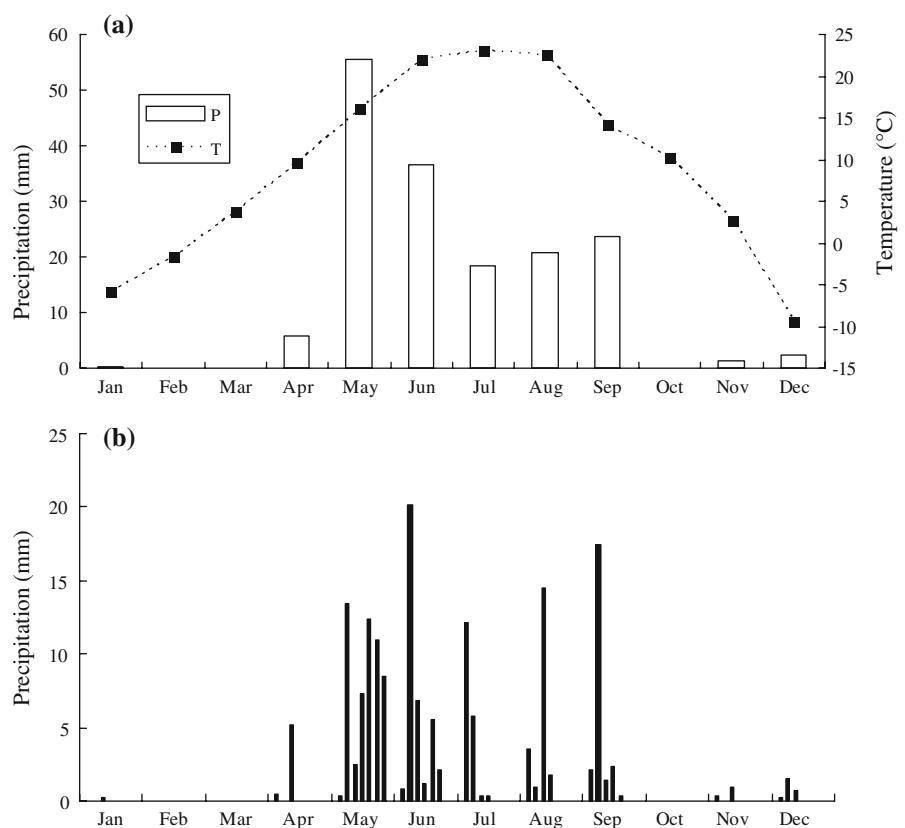
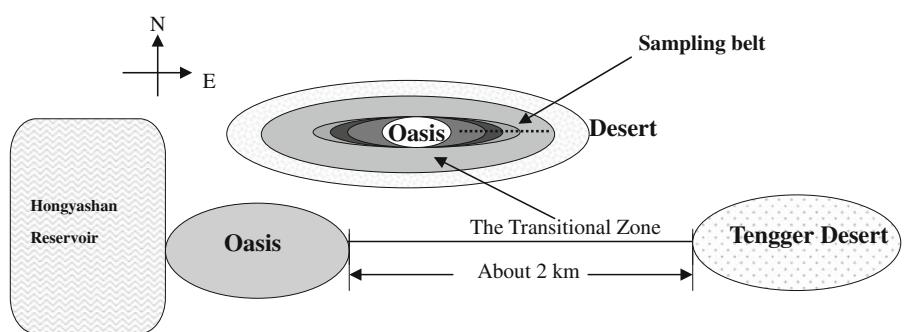


Fig. 2 Schematic illustration of the oasis–desert transitional zone and sampling belt



established to the east of the reservoir in 1960s. The area close to the reservoir was dominated by *Haloxylon ammodendron* with the inclusion of less abundant shrubs *Hedysarum scoparium* and *Calligonum mongolicum* and some annuals (*Agriophyllum squarrosum* and *Halopeplis arachnoidea Moq*) interspersed among the shrubs instead of under the shrub canopies. As the distance increased from oasis, more annuals encroached upon the shrubs. The area close to the desert was mainly dominated by the strong

xerophil—*Nitraria tangutorum* (a spiny shrub) which lives in the sand dunes. The annuals *Agriophyllum squarrosum* and *Halopeplis arachnoidea Moq* were interspersed among the dunes. Airborn sand and dust were intercepted by *N. tangutorum* and gathered around their stems. Roots emerged from the stems buried by sand, causing *N. tangutorum* sand dunes to form and develop year by year. Chen et al. (2007) provides a description of the vegetation along the oasis–desert transitional zone in northwestern China.

Vegetation cover and N taken up by annuals

In each quadrat along the sampling transect, vegetation cover was recorded by percentage for all species for July, August and September 2002 separately. Simultaneously, the number of each plant species was counted, and 6–10 individuals of each annual were randomly harvested to determine the mean biomass per plant of the two annuals. They were dried at 60°C to determine dry biomass and subsamples were ground into powder to measure total N concentration. Total nitrogen was measured using a Kjeltec auto 1030 analyser (Tecator, Höganäs, Sweden). Biomass was estimated by multiplying the mean biomass per plant. Then, the uptake of N by the annuals/ephemerals was estimated by multiplying the dry biomass by the concentration of N.

Plant litter

In each quadrat the litter of the six dominant species was collected from randomly selected plants (five plants for each shrub species and 10 for each annual species) in the middle of the transect (quadrats No. 7, 9, 11, and 13) from October to November. Then they were mixed and dried at 60°C to determine the average litter production per plant and N concentration. Most of the annual's litter was standing, while that of shrubs fell on the ground. The litter from the annuals was collected by hand. Shrub litter was collected using litter traps and plastic placed on the forest floor under the selected shrubs once a week when litter was actually falling. This study only investigated the aboveground litter. The input of nitrogen into soil by litter was calculated by multiplying the total N of the litter and the litter production of each plant. The total N input was the sum of all the dominant plants in each quadrat. Total N was measured with a Kjeltec auto 1030 analyser (Tecator, Höganäs, Sweden). Litter C content was determined using the $K_2Cr_2O_7$ method (Allen 1989).

The litterbag technique (Bocock and Gilbert 1957) was used to quantify the litter decomposition rate. Nylon-net bags (10×10 cm, 1-mm mesh) each containing 5 g of dried litter were randomly buried in interspace soil (at a depth of about 5 cm) in December. The mesh size (1 mm) was large enough to permit aerobic microbial activity and to allow free entry of small soil animals. After 6 months of decomposition, six replicated litterbags of each

species were recovered. Litter decomposition rate was expressed by the percentage of mass lost.

Nutrient contents of the litter after decomposition were calculated as follows:

$$\text{C remaining in litter after decomposition (\%)} = (C_{la} \times M_{la}) / (C_{lb} \times M_{lb}) \times 100 \quad (1)$$

$$\text{N remaining in litter after decomposition (\%)} = (N_{la} \times M_{la}) / (N_{lb} \times M_{lb}) \times 100 \quad (2)$$

where C_{lb} is the initial C content of the litter, C_{la} is the C content of the litter after decomposition, N_{lb} represents the initial N content of the litter, N_{la} is the N content of the litter after decomposition, M_{lb} is the mass of the litterbags before decomposition, and M_{la} is the litter mass remaining after decomposition.

Statistics

Statistical analyses were conducted using SPSS 11.0 for Windows. Correlation coefficients of litter quality with litter decomposition rate were analyzed. One-Way ANOVA was used to analyze the differences in plant litter decomposition rates and litter C and N concentrations between plants.

Results

Vegetation cover

During the rainy season, the annual cover was much higher than the shrub along the oasis–desert transitional zone in northwest China (Fig. 3). The annual

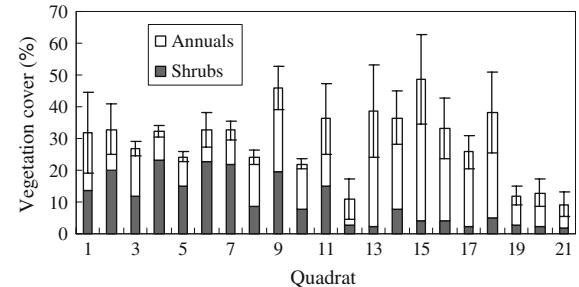


Fig. 3 Vegetation cover. Each value represents the mean of 3 months (July, August, and September), and the values are the sum of the shrubs and annuals. Error bars indicate SD of the ephemerals, while the SD of the shrubs is not shown since the changes of shrub cover are slight among the 3 months

plant cover ranged from 7.5 to 44.5% and accounted for 65.3% of the total vegetation cover along the transect on average. The shrub cover ranged from 1.7 to 23.4%. The total vegetation cover ranged from 9.2 to 48.7%, and showed no discernible trend in large variations along the transect (from quadrat No. 1 to 21). Shrubs dominated the areas close to the oasis, while annuals dominated the areas close to the desert. An analysis of the vegetation cover indicates that the annuals took up the largest proportion along the zone in Minqin.

Nitrogen taken up by annuals

In general, desert annuals complete their life cycle during a rainy season when temperature and moisture are favorable for growth (Mulroy and Rundel 1977). During their life cycles, they have a higher potential to absorb the nutrients needed to complete their lives (Eickmeier and Schussler 1993). Along the transect, the quantity of N taken up by annuals ranged from 0.46 to 3.78 g N m⁻², and N uptake was higher in the areas dominated by annuals than in the areas dominated by shrubs (Fig. 4). The highest N uptake 3.78 (g N m⁻²) appeared in quadrat No. 15, the lowest 0.46 (g N m⁻²) was found in quadrat No. 12. Additionally, N uptake was tightly related to the biomass of the plant.

Litter production and N return through litter

The litter production of the annuals was much higher than that of the shrubs (Fig. 5a). It was two to threefold greater in the quadrats dominated by the annuals (No. 13–18) than other quadrats. The highest

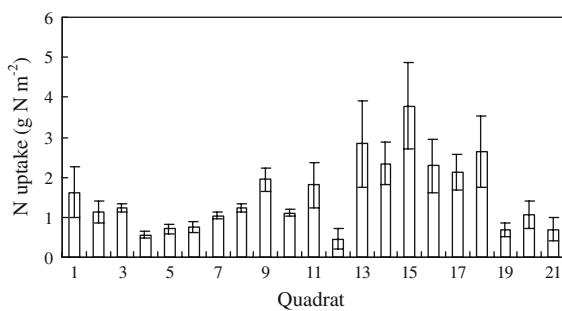


Fig. 4 Nitrogen taken up by annuals (expressed by the mean nitrogen concentration multiplying the mean biomass). Each value represents the mean of 3 months (July, August, and September). Error bars indicate SD

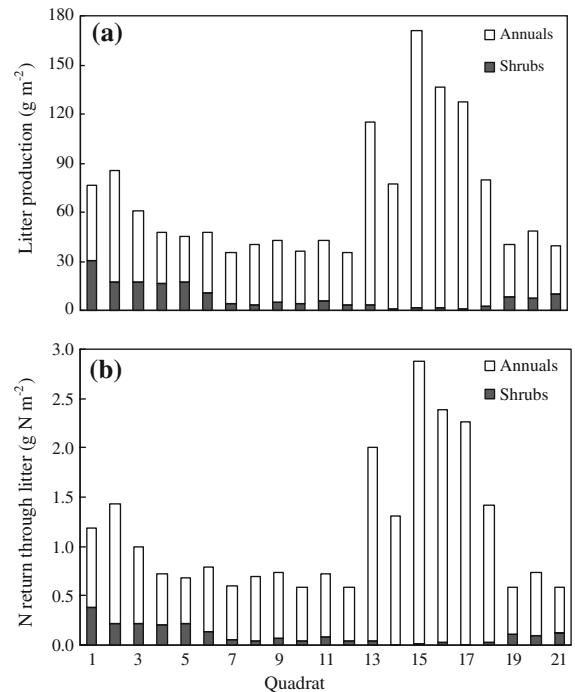


Fig. 5 Litter production and N return to soil through litter. **a** Litter production; **b** nitrogen return through litter. Nitrogen return expressed by nitrogen concentration of litter multiplying the mean biomass of plants

litter dry mass reached 170.7 g m⁻² (quadrat No. 15), the lowest was merely 35 g m⁻² (quadrat No. 7) (Fig. 5a). The quantity of N (litter total N × litter mass) returned through litter showed similar trends to litter production (Fig. 5b).

Litter decomposition

Litter decomposition largely reflects litter nutrient release and the nutrient cycle between plants and soil. Results showed significant differences in litter decomposition and C and N concentration among the six plants (Table 1). After decomposing for 6 months, the highest levels of remaining C and N were found in *C. alaschanicum*. The lowest levels of remaining C were found in *A. squarrosum* and the lowest remaining N was found in *N. tangutorumr* (Table 2). Nitrogen remains were much higher than C remains in the litter, which was likely due to the higher N concentration in litter after decomposition compared to levels before decomposition (Tables 1 and 2). This finding indicates that most nutrients remained in the litter after 6 months' decomposition

Table 1 Litter initial C, N content and their litter decomposition rate after 6 months decomposition

Species	Litter decom. rate %	Carbon concentr. %	N concentr. %	C/N
<i>H. ammodendron</i>	42.85 ± 1.09 a	25.70 ± 0.78 d	1.15 ± 0.043 c	22.43 ± 0.46 d
<i>H. scoparium</i>	26.90 ± 1.17 d	43.63 ± 0.63 a	1.43 ± 0.036 b	30.62 ± 0.48 a
<i>C. alaschanicum</i>	14.53 ± 0.96 e	34.70 ± 1.27 c	1.09 ± 0.073 c	31.76 ± 1.01 a
<i>N. tangutorumr</i>	35.39 ± 1.24 c	33.23 ± 1.05 c	1.37 ± 0.030 b	24.26 ± 0.90 b
<i>A. squarrosum</i>	38.06 ± 0.99 b	37.89 ± 1.28 b	1.85 ± 0.066 a	20.48 ± 0.82 c
<i>H. arachnoideus</i>	43.54 ± 1.52 a	24.15 ± 0.95 d	1.40 ± 0.022 b	17.22 ± 0.51 e

Data are arithmetic means ± SD. Litter C, N concentration of each plant is the mean of selected four quadrats (No. 7, 9, 11, and 13), the litter decomposition rate is the mean of six replicated litter bags. ANOVA One-Way test was used to analyze the differences in plant litter decomposition rate, litter C and N concentration between plants. In a column, means followed by different letter are significantly different at $P < 0.05$

Table 2 Litter C and N content after decomposition and their remains in litter after 6 months decomposition

Species	Carbon concentr. %	N concentr. %	C remains %	N remains %
<i>H. ammodendron</i>	21.48 ± 0.89	1.66 ± 0.071	47.77 ± 2.43	82.49 ± 2.46
<i>H. scoparium</i>	38.57 ± 0.73	1.70 ± 0.053	64.62 ± 1.16	86.70 ± 1.48
<i>C. alaschanicum</i>	34.03 ± 0.93	1.17 ± 0.043	83.82 ± 1.07	91.74 ± 1.01
<i>N. tangutorumr</i>	25.72 ± 1.14	1.63 ± 0.081	50.01 ± 1.38	76.87 ± 2.90
<i>A. squarrosum</i>	21.46 ± 1.39	2.34 ± 0.087	35.08 ± 3.43	78.35 ± 3.82
<i>H. arachnoideus</i>	19.73 ± 1.12	1.96 ± 0.059	46.13 ± 2.67	79.04 ± 2.51

Data are arithmetic means ± SD. Litter C, N concentration and their remains of each plant is the mean of the six replicated litter bags

(from December to May), which might be favorable for nutrient conservation in desert ecosystems.

Discussion

Generally speaking, desert soils are poorly developed and low in nutrients compared with soils of other ecosystems. Nitrogen is considered the second most limiting factor after water for arid land productivity (Ettershank et al. 1978; Fisher et al. 1988). Additionally, the canopy is unclosed in desert ecosystems. In the deserts of northwestern China, annuals germinate during summer and autumn since the constant and frequent rainfall provides them with a prerequisite to live, and a part will survive and complete their reproduction in autumn. Most summer annuals have a high-photosynthetic efficiency at increased temperatures when water is available (Mulroy and Rundel 1977).

In this study, the annuals uptake of N ranged from 0.46 to 3.78 g N m⁻², which is much higher than the net N uptake by the spring ephemeral/annual herb

Erythronium americanum (0.1 g m⁻², equal to 1.0 kg ha⁻¹) in hardwood forests. This finding suggests that desert annuals play a more important role in retaining nutrients than those in hardwood forests. Rothstein (2000) tested the “venal dam” hypothesis and found that in hardwood forests soil microorganisms, instead of annuals, played a key role in N cycling. In hardwood forests, summer-green tree species accounted for 94% of the aboveground net production, while the annuals accounted for less than 5% of the aboveground production (Muller and Bormann 1976). In the present study, the summer desert annuals accounted for over half of the vegetation cover along the transect (Fig. 3). It was reported that the mean coverage of ephemeral plants reached 13.9% during seasons that experience low rainfall (Spring), and 40.2% during rainy seasons (Summer: May to June) at Gurbantunggut Desert in Xinjiang. At this location, ephemeral plants acted as the major contributor to dune surface stabilization by reducing nutrient loss through wind erosion in the desert of northwestern China (Wang et al. 2003). Thus, it can be seen that desert annuals play a vital

role in N cycling and conservation, mainly due to the mass of annuals per square meter rather than their potential nutrient uptake.

At our experimental site, it was also observed that germination and death of the annuals occurred frequently, suggesting that incessant events of annual germination and death also changed the microhabitat. Our previous study showed that soil nutrients beneath the annual plants were higher than in the adjacent bare soil, suggesting that annuals have the potential to improve the nutrient level of the soil (Chen et al. 2007). Thus, without annuals, more bare soil might appear in the region, which may lead to higher nutrient loss through wind erosion (Prospero and Lamb 2003), and NH₃ volatilization, nitrification and denitrification (Peterjohn and Schlesinger 1990; Evans and Ehleringer 1993; Zaady et al. 1996; Evans and Belnap 1999; Schaeffer et al. 2003).

In our experimental site, several summer annuals were scattered among shrubs and large covered areas. It should be noted that the timing of the annuals' growth season was in the range of the shrubs' growth season. In general, shrubs leafed out in May, and senesced in October, while the annuals germinated between May and June and senesced in September. Nutrient competition might occur between them, although it would not be severe since most of the annuals grow among the shrubs rather than under them. Regardless of whether the shrubs competed for the nutrients against the summer annuals or not, the existing and living annuals in the desert per se fixed a part of the soil nutrient (especially N) and atmospheric C and N (Figs. 3 and 4), though the annuals might not be the dominant sink during that season. The annuals act as nutrient conservers or resource sinks and play an important role in desert ecosystems.

Water, a limiting resource for plant growth in the desert, mainly comes from rain. The annual plant community is, therefore, dependent on rainwater (Went 1949; Guterman 2000). The omnipresent annuals will absorb rainwater and grow after an appropriate rainfall, even if some of them die before completing their life cycles. During summer and autumn, which have large and frequent rainfall pulses (Fig. 1b), rainwater is not completely taken up within the short-time period, leading to water and nutrient loss caused by run off.

By studying American deserts, Walvoord et al. (2003) demonstrated that substantial quantities of N,

in the form of nitrate, were leached and accumulated beneath the soil zone over millennial periods, indicating that nitrate leaching occurred in deserts. However, the existence of annuals partly reduces such nutrient leaching loss. Moreover, annuals undoubtedly enhance the efficiency of rainwater use in desert ecosystems via reduction of evaporation and, consequently, reduction of nitrogen loss with rainwater since we observed nitrate and ammonium levels of 0.78 and 0.63 mg l⁻¹, respectively, in the rainwater at our study site. Therefore, the summer annuals could be considered as a "resource-reserve" coupled with water-pulse, acting as a nutrient container and a carbon assimilator. Meanwhile, they partly reduce nutrient loss and water flow in desert ecosystems.

Litter also played an important role as a nutrient regulator in the desert. When the annuals died, nutrients (C, N, etc.) retained in their stand litter and dead roots (Table 1) were transformed or released into the soil during decomposition by microbes and animals. Results showed that the rate of annual plant litter decomposition was higher than that of the shrubs, except *H. ammodendron*, due to the narrow C–N ratio in litter (Table 1). This result indicates that plants in a desert ecosystem have a high nutrient input because of the higher litter chemical quality, expressed as narrow C–N nutrient ratios (Parker et al. 1984). Furthermore, the lower C–N ratio in litter might contribute to the nutrient release from litter (Singh et al. 1999). However, our results indicated that after decomposing for 6 months most C and N remained in the litter (Table 2), and the remains might be used in the upcoming growth season.

In addition, desert annuals provide some small animals with food. We observed that some green twigs and annual litter was left in front of some gerbil and rabbit burrows, even blocking the burrows in some cases. The rapid growth of desert summer annuals represents an important pulse in soil nutrient supply (Mulroy and Rundel 1977; Parker et al. 1984), and plays an important role in desert productivity (Halvorson and Patten 1975). Hence, summer annuals provide some microbes and animals with high-quality food in desert ecosystems. Simultaneously, the released nutrients could be used for plant growth in the next season.

Without annuals, more nutrients and rainwater might be lost through leaching or dust transfer caused

by the wind erosion. In this case, the desert ecosystem would be an unimaginable landscape, resulting in waste of rainwater, loss of soil nutrients, and finally aggravated desertification (Martínez-mena et al. 2002). Desert annuals, like a nutrient dam and protector, take up nutrients during the rainy seasons, reduce nutrient loss caused by leaching and wind erosion, and provide some animals and microbes with food. In the future, more attention should be paid to the community function of desert annual plants.

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The effects of fire frequency and grazing on tallgrass prairie productivity and plant composition are mediated through bud bank demography

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Abstract Periodic fire, grazing, and a variable climate are considered the most important drivers of tallgrass prairie ecosystems, having large impacts on the component species and on ecosystem structure and function. We used long-term experiments at Konza Prairie Biological Station to explore the underlying demographic mechanisms responsible for tallgrass prairie responses to two key ecological drivers: fire and grazing. Our data indicate that belowground bud banks (populations of meristems associated with rhizomes or other perennating organs) mediate tallgrass prairie plant response. Fire and grazing altered rates of belowground bud natalinity, tiller emergence from the bud bank, and both short-term (fire cycle) and long-term (>15 year) changes in bud bank density. Annual burning increased grass bud banks by 25% and decreased forb bud banks by 125% compared to burning every 4 years. Grazing increased the rate of emergence from the grass bud bank resulting in increased grass stem densities while decreasing grass bud banks compared to ungrazed prairie. By contrast, grazing

increased both bud and stem density of forbs in annually burned prairie but grazing had no effect on forb bud or stem density in the 4-year burn frequency treatment. Lastly, the size of the reserve grass bud bank is an excellent predictor of long-term ANPP in tallgrass prairie and also of short-term interannual variation in ANPP associated with fire cycles, supporting our hypothesis that ANPP is strongly regulated by belowground demographic processes. Meristem limitation due to management practices such as different fire frequencies or grazing regimes may constrain tallgrass prairie responses to interannual changes in resource availability. An important consequence is that grasslands with a large bud bank may be the most responsive to future climatic change or other global change phenomena such as nutrient enrichment, and may be most resistant to exotic species invasions.

Keywords Bud bank · Fire · Grazing · Konza Prairie LTER · Meristem · Vegetative reproduction

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Introduction

Although the patterns of change in community composition and productivity in response to fire and grazing in grasslands have been well described, the underlying demographic mechanisms responsible are largely unexplored (Benson et al. 2004). The few

studies that have examined soil seed banks in tallgrass prairie have shown that the seed bank is a poor predictor of aboveground community composition (Abrams 1988; Rabinowitz and Rapp 1980). By contrast, in perennial grasslands and many other herbaceous communities, the belowground population of meristems associated with rhizomes or other perennating organs (the “bud bank” *sensu* Harper 1977) may play a fundamental role in local plant population persistence, structure, and dynamics. In fact, in North American tallgrass prairie, recent studies indicate that regeneration and maintenance of plant populations are regulated principally by vegetative reproduction and belowground bud bank dynamics, with seed reproduction and seed banks playing a minor role (Benson and Hartnett 2006; Benson et al. 2004).

In grasslands dominated by clonal plant growth forms, such as the tallgrass prairie, the size of the bud bank available for tiller (ramet) recruitment, the patterns of bud dormancy and activity, and the rate of tiller emergence from the bud bank may determine both the population dynamics of individual species as well as species composition changes in response to fire frequency, grazing, and climate variability (Olson and Richards 1988). In addition, population processes such as the demography of buds and tillers may strongly regulate temporal and spatial variability in ANPP seen in response to fire and grazing. Previous studies have examined aboveground tiller population dynamics in response to density, nutrient availability, and/or grazing intensity (Briske and Butler 1989; Coughenour et al. 1985; Hartnett 1993; Kays and Harper 1974; Noble et al. 1979; Olson and Richards 1988; Vinton and Hartnett 1992; Wikberg and Svensson 2003), but few studies have examined the consequences of these factors, and of tillering rates, on the ultimate recruitment source for tillers: the belowground bud bank (Benson et al. 2004).

Previous research by Benson et al. (2004) compared the effects of two extremes of fire frequency in tallgrass prairie (annually burned and burned at 20-year intervals) on belowground bud densities. Benson et al. (2004) found that annually burned prairie had larger grass bud banks and smaller forb bud banks compared to infrequently (20-year) burned prairie and that, unlike seed banks, the composition of the belowground bud population closely resembled the

aboveground plant community. We conducted our study at Konza Prairie Biological Station using the long-term, replicated fire-frequency and grazing experiment. Our objectives were to extend the research of Benson et al. (2004) to examine effects of fire frequency (annual and 4-year burning), large ungulate grazers (bison), and their interaction on belowground bud demography. An additional objective was to examine the relationship between bud demography and ANPP under different fire frequencies to assess the contribution of bud banks to both short-term (fire-cycle) and long-term (>15 year) variation in ANPP.

Plants can be studied at the level of populations of genets or populations of ramets (Harper 1977; Harper and White 1974). Our approach was to study the grassland as a population of parts, namely buds and stems. We hypothesized that the effects of fire frequency and grazing on plant species composition and on ANPP are mediated principally through demographic effects on the bud bank. Figure 1 presents a simplified conceptual model of how fire and grazing may act on bud and stem populations to affect patterns observed at the larger scales of community composition and productivity. If the established changes in community composition responses to fire frequency and grazing are mediated by altered demography of plant parts, particularly belowground buds, then we predict a decrease in grass buds and an increase in forb buds with decreasing fire frequency. Similarly, grazing should decrease grass buds and increase forb buds. If long-term plant productivity in tallgrass prairie is mediated by altered bud demography, then tallgrass prairie with lower reserve bud banks will have lower long-term productivity because the lower availability of meristems constrains aboveground stem population size and, thus, biomass production. Bud bank size should also track observed fluctuations in ANPP in 4-

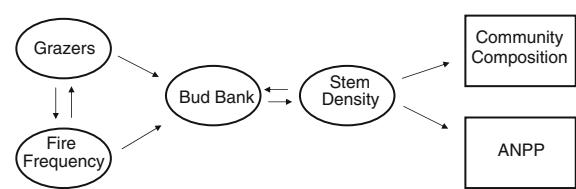


Fig. 1 Conceptual model illustrating a mechanistic hypothesis of how grazing and fire frequency influence community composition and productivity

year burn prairie, with the largest bud banks occurring just before the pulse of productivity after a fire.

Methods

Site description

Konza prairie biological station and long-term ecological research (LTER) site

The Konza Prairie Biological Station (KPBS) is 3,487 hectare tallgrass prairie preserve located in the Flint Hills of northeastern Kansas ($39^{\circ}05' N$, $96^{\circ}35' W$). The vegetation is dominated by perennial, warm-season grasses such as *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium*. Spring (April) burning treatments (burned at 1, 2, 4, 10, and 20 year intervals) have been maintained on replicate watershed units since 1971. Transects for this study were established in watershed units representing a factorial combination of grazer presence (bison present or absent) and fire frequency (1-year and 4-year average fire return intervals). Each treatment combination was replicated twice for a total of eight watersheds included in the study (1D, SpB, N1A, N1B, 4A, 4B, N4A, N4D). The bison population contained 217 animal units (AU) at the start of 2004, 210 AU at the start of 2005, and 208 AU at the beginning of 2006. Transects for the study were positioned on established grazing lawns (sites regularly utilized by bison and repeatedly grazed). Mean annual precipitation at KPBS is 834 mm/yr and mean January temperature is $-3^{\circ}C$ with a mean July temperature of $27^{\circ}C$. See www.konza.ksu.edu/konza for further description of the site and its biota.

Above and belowground sampling

In each watershed, two 50-m transects were placed near the established LTER plant species composition and ANPP study transects in the upland prairie vegetation growing on shallow, cherty, silty clay loam soil overlying limestone and shale layers (Udic Argiustolls, Florence series). Six random points along each transect were chosen for aboveground stem and belowground bud sampling. At each random point, all the live stems (defined as greater than 50% green, potentially photosynthetic tissue) within a 25×25 cm

sampling frame were counted, clipped at ground level and sorted as grass or forb. Because sedges are indistinguishable from grasses belowground and constitute a small fraction of graminoid biomass, all graminoids were combined as ‘grass’ both above and belowground. In the grazed watersheds, the grazing intensity was estimated at each sub-sample along a transect before stem harvesting began using a scale, (0 = none of the stems grazed, 1 = up to one-third of stems grazed, 2 = between one- and two-thirds, and 3 = a grazing lawn with almost all stems grazed). The soil within the frame was excavated to a 10-cm depth and all belowground perennating organs (rhizomes, etc.) were collected in a plastic bag and stored in a cooler for transport.

Stem and bud density measurements were taken in March (late dormant season), June (peak growing season), and September (end of growing season) of 2004 and 2005, and March and June of 2006. All March meristem numbers were adjusted to include the buds that had already broken dormancy from the bud bank and initiated new stems before sampling occurred. Due to a laboratory processing error, most of the meristem data from March and June 2005 were not usable. To estimate March 2005 grass meristem density on a single transect, the grass meristem density in September was added to the aboveground grass stem density from June. This method provides reliable estimates because more than 99% of aboveground stems are recruited from the bud bank as opposed to originating from seed (Benson and Hartnett 2006), and because the seasonal pattern of bud development is known (Dagleish and Hartnett 2006). Meristem data from three transects were available from March 2005 and were used to check the accuracy of the estimates, which were reasonable for grasses, but not for forbs. Therefore, grass meristem estimates are presented from all 3 years, whereas meristem data for forbs is only presented for 2004 and 2006. Probability of emergence from the bud bank was calculated by dividing the number of above ground stems present in June by the number of belowground buds present in March. Because the June stem data were used to estimate the March bud data for 2005, the probability of emergence was only calculated for 2004 and 2006. Long-term ANPP data for the uplands of the watersheds without grazers were obtained from the Konza Prairie LTER database (<http://www.konza.ksu.edu/konza>).

Sample processing

Belowground samples were washed free of soil and examined under a dissecting scope. Belowground buds (rhizome meristems) were counted and scored as either grass or forb based on bud morphology and the morphology of the attached root systems. Only developed meristems that formed a distinct stem tissue bud were counted. Different grass growth forms have different morphology and therefore require different counting techniques: rhizomatous grass buds can be found at the rhizome nodes, sometimes covered with a bud scale, but are quite visible without much dissection; caespitose (bunch or tufted growth form) grass buds can be found at the base of each stem and oftentimes partial dissection of the base of the tiller is required in order to count the buds. Many samples included both growth forms and the appropriate technique was used for each growth form within a sample.

Analysis

The mean of each response variable (stem and bud density of grasses and forbs) was calculated using the 12 sub-samples taken from each watershed at each sampling time. To test for differences in stem densities and March grass buds we used a two-way, repeated measures ANOVA with fire frequency and presence of grazers as fixed factors and year as the repeated measure using SAS 9.1 software (Proc Mixed, SAS 2003). We used an autoregressive covariance structure (ar(1) option in Proc Mixed) for the stem data and an unstructured model for the grass buds because these models had the lowest value of Akaike Information Criterion (AIC) of ten possible covariance structures fit to each data set. To test for differences in March forb meristem and June grass and forb meristem densities (only 2 years of data available), we used a three-way ANOVA design with fire frequency, presence of grazers, and year as fixed factors (Proc GLM, SAS 2003). When there were no significant differences in the response variables between years, a reduced model was fit using only fire frequency and presence of grazers as fixed effects. All the tests were two-tailed, based on Type III sums of squares and considered significant at the $\alpha = 0.05$ level.

Linear regression was used to explore the relationship between grazing intensity and the response

variables (Proc Reg, SAS 2003). These analyses were conducted using transects as the experimental units because an average grazing intensity was measured using the average of six sub-samples at the transect scale, rather than simply the presence or absence of grazers which is applied to the entire watershed. Transects, then, are independent measures of grazing intensity; but not independent measures of grazer presence. We had limitations in our ability to assess the effects of year and grazing intensity, however, because many of the grazing intensities were not measured in more than 1 year, including year as a factor resulted in an over-parameterized model. Model fit was evaluated by examining studentized residuals and Cook's distances calculated for each data point. Outliers were identified as points having studentized residuals that did not fall between -2 and +2 and Cook's distances >1 . A single data point met these criteria in the June grass meristems data set and was removed for all of the analyses described here. To examine the relationship between long-term average ANPP and the response variables, linear regression was again used with the watershed as the experimental unit.

Results

Fire, grazing, and grazing intensity all had significant effects on aboveground stem and belowground bud populations of both forbs and grasses.

Aboveground stems

The effect of fire frequency and the presence or absence of grazers on average peak grass stem density differed among the 3 years of the study (Fig. 2, fire*grazing*year: $F_{2,8} = 10.0$, $P = 0.007$). The three-way interaction indicated that there was no consistent, independent effect of either treatment on peak grass stem densities, and that the interaction between the presence of grazers and fire frequency was dependent upon the year. By contrast, forb stem densities responded to fire frequency and the presence of grazers similarly over the 3 years of the study. There was a significant interaction between fire frequency and the presence of grazers (Fig. 3, $F_{1,4} = 28.3$, $P = 0.006$). Grazing significantly increased the average forb stem density in the

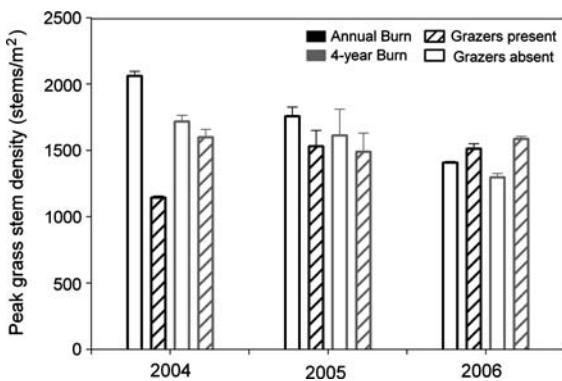


Fig. 2 The effects of fire frequency and grazing on peak (June) grass stem density. The effect was not consistent over the 3 years of the study (fire*grazing*year $P = 0.0007$). Grazing tended to reduce peak grass stem density in both fire frequency treatments in 2004 and 2005; however, this trend was reversed in 2006. Bars represent means with 1 SE

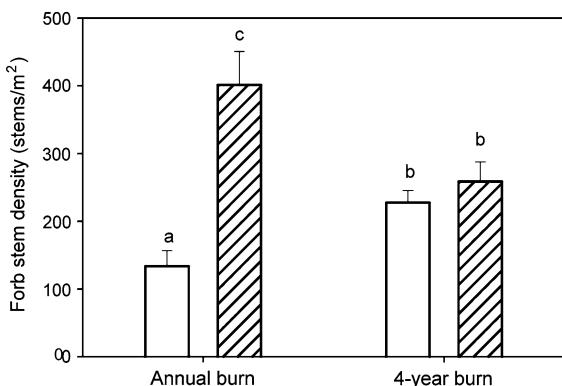


Fig. 3 The effect of fire frequency and grazing on peak (June) forb stem density. The effect was consistent among years (fire*grazing $P = 0.0005$). Bars represent means \pm 1 SE; letters represent significant differences at $\alpha = 0.05$

annually burned treatments from 134.0 ± 22.7 to 401.3 ± 49.3 (Mean \pm 1 SE, Fig. 3); however, the presence of grazers did not affect forb stem densities in the 4-year burn frequency (grazers absent: 227.3 ± 17.9 , grazers present: 258.7 ± 28.7). In addition, the 4-year burn frequency had a forb stem density intermediate to the annually burned, grazers present treatment and the annually burned, grazers absent treatment (Fig. 3).

Owing to the patchy and variable nature of bison grazing, the presence or absence of grazers on an entire watershed may not accurately reflect the amount of grazing pressure that each sampled area actually experienced. A closer assessment of the

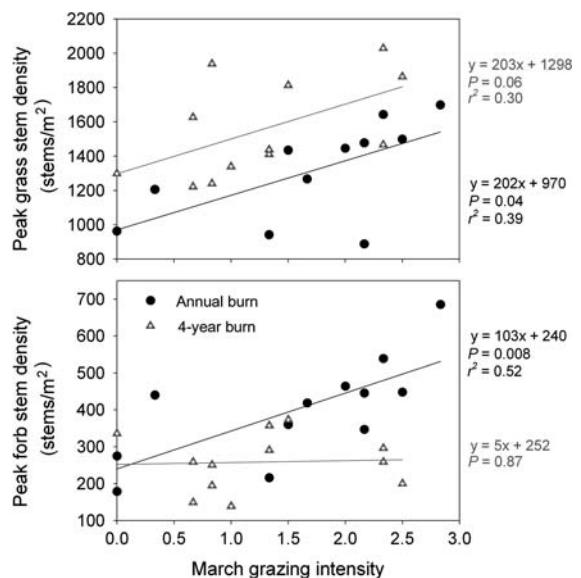


Fig. 4 Relationship between grazing intensity and stem density. Points represent averages on a single transect

relationship between grazing intensity and grass stem density revealed consistent patterns: peak grass stem density (June) increased linearly with grazing intensity in the annual burn frequency ($F_{1,10} = 5.80$, $P = 0.04$, $r^2 = 0.39$; Fig. 4). A similar trend was observed in the 4-year burn frequency, though the relationship was only marginally significant ($F_{1,10} = 4.32$, $P = 0.06$, $r^2 = 0.30$; Fig. 4). Whereas the slopes of both relationships were nearly identical (annual burn: 201.5, 4 year: 202.9), the values in the 4-year frequency tended to be higher as did the intercept value, though the intercept values were not significantly different (mean \pm SE: annual burn, 970 ± 160 ; 4-year burn: 1298 ± 144). Forb stem density also increased with grazing intensity in the annually burned treatment ($F_{1,10} = 10.7$, $P = 0.008$, $r^2 = 0.52$, Fig. 4) but there was no relationship between grazing intensity and forb stem density in the 4-year burn frequency ($F_{1,10} = 0.03$, $P = 0.874$).

Belowground buds

There was a significant interaction between fire frequency and grazer effects on peak belowground grass meristem density (fire*grazing: $F_{1,4} = 9.7$, $P = 0.04$). Grass meristem density was lower in the presence of grazers than in their absence in the annually burned treatment, while no such trend was

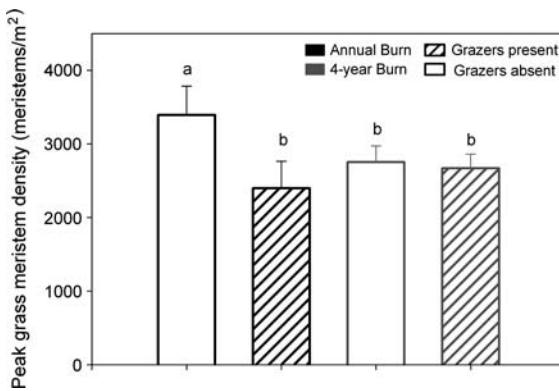


Fig. 5 The effects of fire frequency and grazing on peak grass meristem density. Bars represent means ± 1 SE; letters represent significant differences at $\alpha = 0.05$

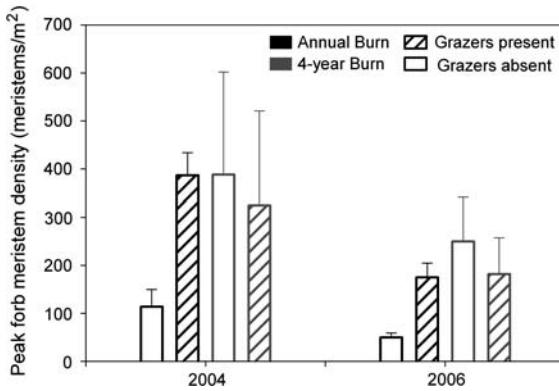


Fig. 6 The effects of fire frequency and grazing on forb meristem densities. The patterns belowground were similar to the stem patterns aboveground (fire*grazing $P = 0.002$). Bars represent means ± 1 SE

evident in the 4-year burn frequency (Fig. 5). A significant interaction between fire frequency and the presence of grazers was evident in the peak forb meristem density as well (fire*grazing: $F_{1,4} = 39.5$, $P = 0.0002$, Fig. 6). The presence of grazers increased mean peak forb meristem density in the annual burn frequency from 114.7 ± 36.0 to 386.3 ± 47.3 in 2004 (mean ± 1 SE). Decreasing the fire frequency from 1 to 4 years significantly increased average peak forb meristem density (Fig. 6). The presence of grazers, however, did not change average peak forb meristem density in the 4-year fire frequency treatment, and means were similar to the annually burned and grazed treatment (388.0 ± 213.8 without grazers, 325.0 ± 195.7 with grazers; mean ± 1 SE). There was also a significant

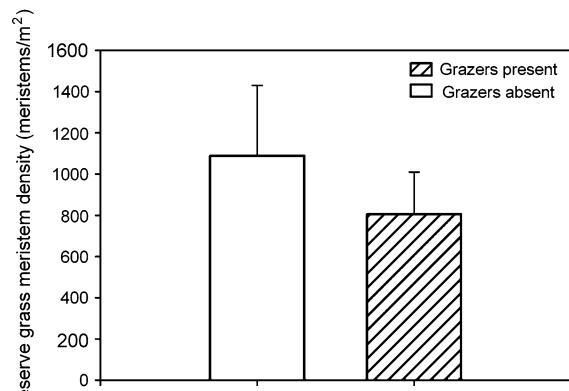


Fig. 7 The effects of grazing on reserve meristem densities. Grazing significantly reduced the reserve grass meristem densities in both fire treatments. ($P = 0.009$). Bars represent means ± 1 SE

interaction between grazing and year ($F_{1,4} = 12.02$, $P = 0.0085$); though the patterns of forb meristem density were similar among treatments, the densities decreased significantly from 2004 to 2006. There was no relationship between peak meristem density and grazing intensity (data not shown).

Our data indicate that grazing increased the rate of transition from bud to tiller as the decrease in buds from March to June was well matched to the increase in stems over the same time period. Grazing significantly reduced the reserve grass meristem density in June ($F_{1,8} = 11.97$, $P = 0.009$), with no significant main effect of, or interaction with, fire frequency (Fig. 7). Forb meristem density in June exhibited a similar response to fire frequency and grazing that is shown on Fig. 3 (fire*grazing in June $F_{1,15} = 8.38$, $P = 0.02$). No significant linear relationship was detected between June meristem density (either grasses or forbs) and March grazing intensity (data not shown).

Effects of year since fire

In the 4-year burn frequency, the probability of a grass tiller emerging from the bud bank peaked in the year after a spring fire (the second growing season after fire) in both the treatment with and without grazers (Fig. 8a). In the growing season following a fire, the probability of bud emergence and the number of buds per tiller were similar to annually burned prairie. However, in the second year following the fire, bud emergence increased and the number of buds

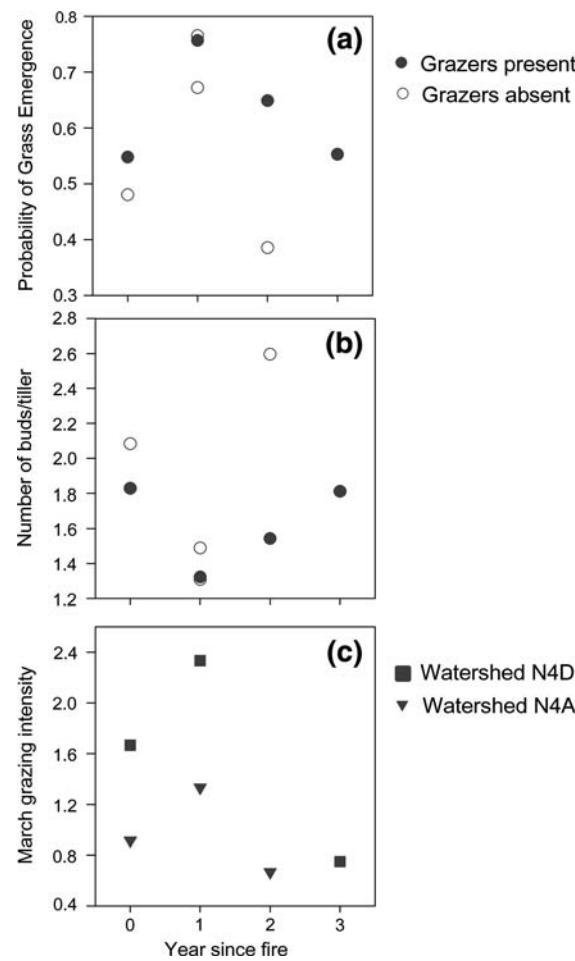


Fig. 8 The effects of year since fire on the probability of grass emergence from the bud bank (a), on the number of buds/tiller (b), and on grazing intensity (c)

per tiller decreased. In the treatment with grazers, the probability of emergence from the bud bank gradually declined with year since fire. By contrast, the number of buds produced per tiller was the lowest the year after fire (the second growing season). In the growing season immediately following the fire (year 0 on Fig. 8), the probability of grass emergence and the number of buds per tiller are both similar to those measured on annually burned watersheds. However, 4-year burned watersheds were more variable among years for both responses: the coefficient of variation (CV) for the probability of grass emergence was 0.30 and the CV for the number of buds per tiller was 0.36 compared with the annual burned watersheds which had CV values of 0.17 and 0.25, respectively. Grazing intensity showed a similar pattern, peaking

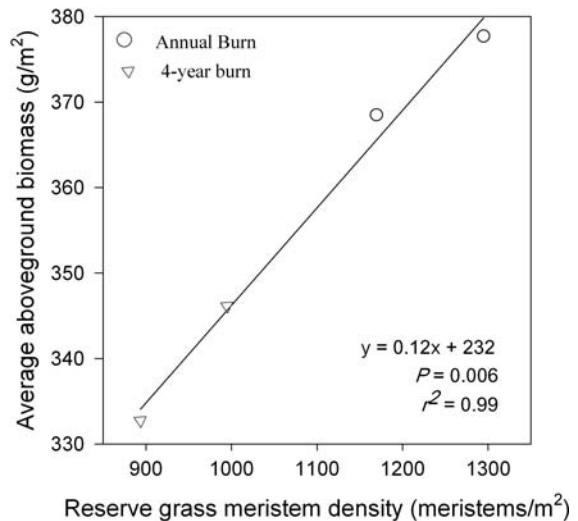


Fig. 9 Reserve bud banks are a very good predictor of long-term aboveground net primary productivity on annual burn and 4-year fire frequency watershed without grazers. Points represent averages in the uplands for an entire watershed

the second growing season after the fire and declining subsequently (Fig. 8c). The magnitude of the grazing intensity differed between the two watersheds that had both bison grazing and the 4-year fire frequency, but the pattern of change over time was consistent.

Relation to long-term productivity

The average belowground reserve grass bud bank was a very strong predictor of long-term ANPP in prairie protected from grazing (Fig. 9, $F_{1,2} = 12.81$, $P = 0.006$, $r^2 = 0.99$). Annually burned, ungrazed prairie had both higher meristem densities and higher average ANPP than ungrazed prairie burned every 4 years. Long-term average ANPP was not significantly, linearly related to peak stem density or to peak belowground meristem density ($P > 0.172$, data not shown).

Discussion

Our data indicate that tallgrass prairie plants are responding to key ecological drivers such as fire and grazing with altered rates of belowground bud natalinity, tiller emergence from the bud bank, and both short-term (fire cycle) and long-term changes in bud bank density. This study provides a mechanistic

explanation for previously described patterns of the effects of fire and grazing in tallgrass prairie, demonstrating a strong link between ecosystem processes (e.g., productivity patterns) and the modular demography of belowground plant parts.

Complete exclusion of fire in tallgrass prairie has been shown to significantly decrease the grass bud bank and increase the forb bud bank (Benson et al. 2004). The results of our study show the same trends in the bud bank after decreasing the fire frequency from 1 to 4 years. In addition, prairie that is burned at an intermediate fire frequency showed greater year-to-year variability in grass bud bank size and in the probability of emergence from the bud bank than annually burned prairie. Population processes, such as the demography of buds presented here, have the capacity to explain temporal and spatial variability in ANPP. For example, long-term studies at KPBS have shown higher temporal variability in ANPP in the 4-year burn frequency treatments, with maximum productivity in response to a fire that follows several years without fire (Knapp et al. 1998). Such pulses of productivity in response to fire after several years of no fire can be understood in light of our results. Our data show a gradual increase in reserve bud bank densities over a 2–3-year period in the absence of fire that allows a larger tillering response in the season immediately following fire. Annually burned sites do not experience a 2–3-year period of reserve bud accumulation, and, therefore, we do not observe similar temporal variability in ANPP with high postfire pulses in productivity. However, long-term averages of ANPP on annual and 4-year burned sites show that 4-year burned sites have lower productivity overall, creating spatial variability in ANPP at KPBS in response to fire frequency. Our results show that reserve bud density, or the size of the bud bank, is a very good predictor of long-term average ANPP. In addition to the promising results of this study for the local scale, a similar relationship between bud bank size and productivity has been observed at larger scales incorporating grasslands across the Great Plains of the central United States (Dagleish and Hartnett 2006). At both the local and regional scales, understanding the demography of bud banks is key for understanding temporal and spatial variability in ANPP.

While annual burning increased bud banks, persistent grazing over the long-term (>10 years of

grazing pressure) resulted in depleted bud banks. Our study effectively connects observed increases in aboveground tillering due to ungulate grazing to changes in belowground bud demography that are consistent with previously reported decreases in belowground allocation to roots, rhizomes, and buds (Albertson et al. 1953; Archer and Detling 1984; Biswell and Weaver 1933; Hartnett 1989; Weaver and Hougen 1939). Whereas our study suggests that an increase in tiller density is an important mechanism of compensatory regrowth following herbivory in tallgrass prairie, previous studies examining the effects of grazing on tiller density have found contrasting results for different grass species (Hartnett 1989; Olson and Richards 1988; Vinton and Hartnett 1992). The current study extends these previous species-specific results by demonstrating that for the grass guild as a whole grazing stimulates the release of buds from dormancy and results in an overall increase in tillering in response to grazing intensity. One explanation for the long-term decline in bud banks in the presence of grazers may be an increased risk of tiller mortality, a response reported previously for tallgrass prairie graminoids (Archer and Detling 1984; Vinton and Hartnett 1992). Because tiller vegetative fecundity (number of new rhizome buds produced per tiller) does not peak until the following spring (Dagleish and Hartnett 2006), tillers that die the previous fall are unable to contribute to replenishing the bud bank. Even if the grazed tillers survive the growing season through to the next spring, a reduction in biomass accumulation may reduce bud production. Both the death of tillers before they produce buds as well as lowered bud production due to decreased growth rates and biomass accumulation could explain the observed gradual decreases in the bud bank with grazing over time. Depletion of bud banks has several potential consequences such as decreased ability to respond to resource pulses, decreased rates of stem population recovery after disturbance or stress, decreased productivity of the grassland, and decreased potential for compensatory re-growth following herbivory.

The increased heterogeneity in the bud bank created by decreased fire frequency and grazing may also contribute to the increased plant community heterogeneity observed on 4-year, compared to annually, burned prairie (Collins and Steinauer 1998). Grasses may have increased abundance following fire

due to their large reserve bud bank and their enhanced emergence rates from the bud bank, but then lose any competitive benefits of that fire event without continued burning. There may also be differences in tiller survival with the enhanced emergence rates due to density dependence. Species-specific studies have demonstrated that decreased fire frequency stimulated bud production and increased ramet densities in perennial forbs such as *Solidago canadensis* and *Ratibida columnifera* (Elder 2001; Hartnett 1991). Grazing increased recruitment from belowground bud banks in some forb species examined by Damhoureyeh and Hartnett (1997), even though these species were not directly consumed by bison. In a 10-year study comparing the effects of bison and cattle grazing on the tallgrass prairie, Towne et al. (2005) found that increases in just two perennial forb species, *Sympyotrichum ericoides* and *Solidago missouriensis*, were the primary cause for the observed increase in total forb cover. Both these perennial forbs reproduce vegetatively through belowground buds and much of their spread is likely due to recruitment from the bud bank. Increasing the number of species-specific studies would broaden our understanding of the demographic mechanisms and potential positive feedbacks between stem and bud demography that drive changes in community composition in response to fire and grazing.

The effects of fire frequency and grazing on the patterns of plant community composition and productivity in tallgrass prairie have been previously described. This study provides a mechanistic explanation for previously described patterns, demonstrating a strong link between ecosystem processes (e.g., productivity patterns) and the modular demography of belowground plant parts. Although our data provide greater insight into the broad patterns of bud and tiller responses to fire and grazing, further study is needed to fully tease apart the effects of fire and grazing on resource availability (light, nitrogen) and meristem availability. However, we demonstrate that bud banks play an important role in plant community composition shifts and temporal and spatial variability in ANPP in response to ecological drivers such as fire and grazing and represent a crucial link between organismal, community, and ecosystem level processes.

Management activities that deplete bud banks over the long term, such as decreased fire frequencies and

persistent grazing in tallgrass prairie, can potentially result in decreased ability of the grassland to capitalize on new resource pulses or respond to other environmental changes. Decreased ability to capitalize on available resources may result in increased invasibility if the propagule pressure of non-natives is sufficient to capitalize on new resource pulses. Preliminary results from this study at KPBS indicate that a minimum threshold bud density may be an important factor that allows residents to rapidly preempt resources and resist invasion by exotic species (Hartnett and Dagleish, in prep.). Lastly, decreased bud populations could lead to decreased productivity under increasingly variable precipitation regimes. An important consequence of our research is that prairie with a large reserve bud bank may be the most responsive to future climatic change or other global change phenomena such as nutrient enrichment and may be the most resistant to phenomena such as exotic species invasions.

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Do plant functional types based on leaf dry matter content allow characterizing native grass species and grasslands for herbage growth pattern?

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Abstract Few studies have focused on vegetation characteristics of importance to feeding domestic herbivores, mainly the seasonal pattern of herbage growth at spring. Our objective is to establish and to evaluate a simple method of ranking grassland communities for these characteristics. We combined approaches at plant species level (comparison of grass species growing in a pure stand) and plant community level (comparison of grasslands differing mainly in their nutrient availability). Firstly, we ask if the ranking of species by leaf dry matter content (LDMC), a functional parameter used to assess the plant strategy for resource acquisition and use, is consistent with a classification of the species using three plant features that determine plant growth pattern at spring (beginning and ending of stem elongation, leaf lifespan). Secondly, for three networks of natural grasslands, we test whether there is consistency when ranking them by their dominant

plant functional type (PFT A, B or C) established previously at species level, and by the three agronomic characteristics. For species growing in pure stands, there was a significant effect of PFT for the three plant features. For species having a low LDMC (A and B PFT), there were earlier stem elongation in the season, earlier flowering and shorter leaf lifespan. The opposite was observed for species having a high LDMC (C and D PFT). For grassland communities dominated by A-PFT, the ceiling yield for leaves and stems occurred earlier in spring than for those dominated by C-PFT. Results were consistent at plant and community levels. Scaling up from plant to community was well mediated by PFT. Plant features which characterize species for resource acquisition and use are consistent with herbage growth patterns at plant community level. These results show that herbage growth pattern and composition depend on PFTs and that knowing the PFT dominance is of great importance to plan the use of grasslands. We can expect to use the PFT approach to perform vegetation diagnosis at field level when the objective is to rank grassland communities for their agronomic characteristics.

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Flowering · Leaf dry matter content · Leaf lifespan ·
Phosphorus · Defoliation

Introduction

Many studies describe the effect of land management on ecosystem productivity and nutrient cycling (see for example, Lavorel and Garnier 2002), but few of them focus on vegetation characteristics of importance to feeding domestic herbivores (seasonal pattern of herbage growth, suitability for deferred grazing or late cutting). Other than productivity, some plant characteristics, like the time at which stems begin to elongate and the time at which ceiling yield occurs for leaves and stems are of value for organizing management practices, particularly the defoliation regimes (Duru et al. 2005). Relying on the literature, we hypothesized that leaf ceiling yield depends on leaf lifespan (LLS) (Parsons 1988; Lemaire 1999), and that in spring, stem ceiling yield depends on flowering time (Robson et al. 1988).

The challenge for prediction of community or ecosystem response to changes in land management has prompted a search for key community functional parameters (formerly trait Violle et al. 2007) that take account of: (i) the capacity to exploit resource-rich or -poor environments; (ii) the capacity for competitive dominance; (iii) the response to disturbance (Westoby 1998; Wilson et al. 1999). It has long been recognized that this objective requires a functional classification of species (Grime et al. 1988) based on functional parameters directly linked to the functions of growth and development of plants (hard traits), or strongly correlated to other variables which are indirectly related to these functions (soft traits, Weiher et al. 1999). A short list of key plant functional parameters has been identified (Diaz and Cabido 1997), leading to the development of the concept of plant functional type (PFT). PFTs are defined as non-phylogenetic groups of species displaying common biological parameters that enable them to fulfil specific functions in a similar way within an ecosystem (Gitay and Noble 1997). PFTs are regarded as practical tools to reduce a wide diversity of species to a small number of entities, and as a concept enabling the identification of general principles for the functioning of organisms which can be used for making predictions. The basis of PFT rests on an analysis of the functional parameters developed by plants growing in communities. One distinguishes traits of response, which are those whose values change in response to factors applied to

the community, and traits of effect that act on the processes of the ecosystem (productivity and nutrient cycling among others) (Lavorel and Garnier 2002).

We intend to examine whether a species classification based on a single plant functional parameters (leaf dry matter content: LDMC or specific leaf area: SLA) can provide the three plant characteristics which are needed for modelling herbage growth of species-rich grasslands (Duru et al. 2005). In this article, we examine the possibility of using PFTs based on leaf functional parameters to characterize native grass species and grasslands for herbage growth pattern. To this end, we address two questions:

- (1) Are PFTs based on leaf functional parameters (that assess plant strategy of resource capture, usage and availability (Wilson et al. 1999) also able to rank grass species for key plant features (like LLS, the beginning of stem elongation and the flowering time), that determine agronomic properties?
- (2) Does the characterization of grassland communities by PFT allows them to be ranked for characteristics of agronomic interest, i.e. herbage growth patterns?

For the first question, the literature shows that LLS is significantly correlated to LDMC and SLA that are functional marker of plant strategy for resource use (Ryser and Urbas 2000; Garnier et al. 2001a, b; Al Haj Khaled et al. 2005). On the other hand, answering both questions, the dates at which plants reach determined phenological stages are related to their growth rate, capture and use of nutrients (Sosebee and Weibe 1973) and also to leaf functional parameters (Cruz et al. 2002). For the second question, we hypothesize that plant functional parameters can be used to link processes from individual plant response to community diversity and ecosystem functioning, following the mass ratio theory (Grime 1998), and as suggested by Garnier et al. (2004).

To answer the two questions, we used two sets of experiments. One consisted of pure stand grass crops for which part of the results examining correlations between leaf plant parameters were published earlier (Al Haj Khaled et al. 2005). In this article, the new approach is to focus on the relationship between leaf parameters and plant features, especially for different levels of N availability. The second consists of a

network of grassland communities, and three sets of fertilizer experiments studied at grassland community level. We thoroughly examined the herbage growth pattern in the light of the plant functional approach; the grassland productivity for some of these experiments being analysed previously (Duru et al. 1994; Calvière and Duru 1999).

Materials and methods

Experiment at species level

Experimental site

The study was carried out at INRA, Toulouse Research Centre, France ($1^{\circ}43'E$, $43^{\circ}5'N$). The altitude is 150 m above sea level; annual mean precipitation is 700 mm and average daily temperature $13^{\circ}C$. Nineteen grasses (Table 1) were sown in pure stand on October 2000 following a randomized block design with three replicates. Each plot consisted of eight rows each 1.2 m long and 15 cm apart. The species were chosen according to their

contribution to the biomass of native grasslands in the Pyrenean Mountains. The seeds of most of the species were collected in their native habitat in Ariège, Central Pyrenees ($1^{\circ}17'E$, $42^{\circ}51'N$, 600–900-m asl). Each species was cultivated with two levels of nitrogen supply, limiting and non-limiting for growth (noted N+ and N– respectively). The nitrogen applications were 120 and 40 kg ha $^{-1}$ for N+ and N– for 2001 summer growth, 150 and 0 for the 2002 spring growth and 120 and 0 for the 2002 autumn growth. All the experimental area was irrigated with a sprinkler system designed to maintain the soil moisture content close to field capacity.

Plant measurements

LDMC and SLA were measured over three growing seasons (Al Haj Khaled et al. 2005). Only average measurements were considered here. The LLS, the time elapsed between the lamina appearance and its senescence, was determined in two seasons and expressed in degree-days; base $0^{\circ}C$). Detailed methods of sampling were explained in Garnier et al. 2001a, b, and Al Haj Khaled et al. 2005.

Table 1 Ranking grass species by pre-established plant functional groups (PFT) based on LDMC and species characteristics by groups

Species code	Latin name	PFT	Core (X) vs. singular species (specificity for the indicated plant feature) ^a
Hl	<i>Holcus lanatus</i>	A	X
Lp	<i>Lolium perenne</i> L.	A	X
Ao	<i>Anthoxanthum odoratum</i> L.	B	Flowering–
Ae	<i>Arrhenatherum elatius</i> (L.) Beauv.	B	X
Dg	<i>Dactylis glomerata</i> L.	B	X
Pt	<i>Poa trivialis</i> L.	B	X
Fa	<i>Festuca arundinacea</i> L.	B	LLS+
Ac	<i>Agrostis capillaris</i> L.	C	Flowering+
Pp	<i>Phleum pratense</i> L.	C	Flowering+
Tf	<i>Trisetum flavescens</i> (L.) Beauv.	C	Flowering+
Cc	<i>Cynosurus cristatus</i> L.	C	X
Fr	<i>Festuca rubra</i> L.	C	X
Ap	<i>Bromus erectus</i> L.	D	X
Bp	<i>Brachypodium pinnatum</i> (L.) P. Beauv.	D	X
Bs	<i>Brachypodium sylvaticum</i> (Huds.) Beauv.	D	X
Bm	<i>Briza media</i> L.	D	X
Fo	<i>Festuca ovina</i> L.	D	X
Dc	<i>Deschampsia cespitosa</i> (L.) P. Beauv.	D	LLS+; stem 10+
Mc	<i>Molinia caerulea</i> L. (Moench)	D	LLS+; stem 10+

^a It was indicated those with species characteristic that made a species core (X) or singular (+ or –) within a PFT (+ means that the found value was greater than those found for the core species); LLS leaf life span, stem 10: date at which stem reach 10-cm height

We have considered two phenological stages, the “10-cm long stem” (beginning of stem elongation) and the flowering time. Twice a week, five tillers per species per replicate and per level of N were harvested by cutting at ground level. After dissection of the tiller, the height of the apex was measured from the cutting level to the bottom of the reproductive apex. The stage was considered reached when 50% of apices of the harvested tillers reached 10-cm height or had flowered. The time scale is expressed in Julian days.

Data processing

On experiment 1, ANOVA was done to examine whether or not exist significant effect of the four PFTs (A, B, C, D) previously established on a LDMC basis through a hierarchical cluster analysis (Ansquer et al. 2004, Table 1) on the three key plant features indicated above. PFTs and N treatments were considered as factors. Regression analysis between LDMC and each of the three plant features were done pooling together data of both N treatments, and considering all of the species or only core species as described in Table 1.

On experiment 2, ANOVA was done to examine whether or not significant effects of the PFT exist upon the growth pattern of the plant component; i.e. the dates at which the ceiling yield occurred for leaf and stem masses. Regression analysis was done to compare grass and dicotyledon species for the dates at which ceiling yield occur and leaf digestibility.

Network of grassland communities

Experimental sites and designs

Three experiments were carried out in the central Pyrenees (Table 2): Portets 1 and 2, (altitude 1,300 m above sea level, 2°E, 47°30' N), Ercé (600–900 m asl, 1°17'E, 42°51'N).

Portet 1 consisted of four pastures (H1, H2, H3, H4) differing in their fertilizer management and defoliation regime (Balent and Duru 1984). The pastures were labelled according to their original soil fertility level: H1 (high), H2 (medium), H3 (low) and H4 (very poor). In the years before the experiment, the pastures were grazed by ewes from early

November to early May, and cut for hay either twice (H1 and 2) or once (H3 and 4) in summer. In 1986, two fertilizer treatments were applied with four replications on each of the pastures: one with 120 kg N, 50 kg P and 200 kg K per ha (H' plots), the second with only 120 kg N (H plots, excepted on H1). All fertilizers were applied at the beginning of March. Measurements used in this article were done in 1988, the fourth year of fertilizer application. For the pastures H3 and H4 (those having the lowest fertility level at the beginning of the experiment), there was a significant effect of the fertilizer treatment on the PFT composition (Table 2).

Portet 2 was located close to the H4 pasture (see above). From 1987, two fertilizer treatments with four replications were compared with a control without fertilization. Measurements used in this article were done in 1991 (labelled “I”) and in 1992 (labelled “J”). One fertilizer treatment consisted in annual NPK fertilization (same rate as for Portet 1) from 1987 to 1992, whereas the other consisted in N fertilization only during the year of herbage measurements were done, i.e. 1991 or 1992. There was a significant effect of the NPK fertilizer on the PFT composition (Table 2).

Ercé consisted of eight grassland communities studied in 2002 that differed in their defoliation regime and fertilizer management. They were paired according to the proportion of A- and C-PFT to obtain four grassland community types with two replications. The four types differed significantly for the PFT composition or the plant nutrient status (Table 2).

For each experiment and treatment, two common characterizations were established. Firstly, we computed an average sward nutrient status on the basis of plant analysis. Sward nutrient indices, computed from N concentration and herbage mass (N index), and from P and N (or K and N) concentrations for P and K indices (Duru and Ducrocq 1997) provide a clear ranking of fields for their nutrient limitation for herbage production (Lemaire and Gastal 1997). A global index was computed putting together the three indexes (Duru and Ducrocq 1997). When the global index is equal to 100, it means that the considered nutrient is not limiting for herbage growth. Secondly, using botanical records (Daget and Poissonnet 1971) we computed the abundance of grasses and the proportion of the different PFTs (Table 2).

Table 2 Characterization of the three experiments (Portets 1 and 2, Ercé) for studying grassland communities

Location & year	Defoliation management	Fertilizer management ^a	Design for statistical analysis	Number of replications ^b	Sward nutrient index	Grasses	
						Abundance %	PFT ^c A-B-C
Portet-1 1988	Early cutting	N PK	H'1 (high fertility)	4 F	97	47 ^a	38-62-0
	Early cutting	N1	H2 (medium fertility)	4 F	75 ^b	50 ^a	20-43-37 ^a
		N1 PK3	H2'	4 F	97 ^a	51 ^a	20-40-40 ^a
	Late cutting	N1	H3 (low fertility)	4 F	70 ^b	50 ^a	0-42-58 ^b
		N1 PK3	H3'	4 F	90 ^a	57 ^a	0-25-75 ^a
	Late cutting	N1	H4 (very low fertility)	4 F	66 ^b	30	0-35-65 ^b
		N1 PK3	H4'	4 F	96 ^a	42 ^a	0-14-86 ^a
Portet-2 1991	Late cutting	Control	I	4 F	62 ^b	52 ^b	0-4-96 ^a
		N1	I'	4 F	75 ^b	68 ^a	0-10-90 ^a
		N1 PK5	I''	4 F	100 ^a	67 ^a	6-54-40 ^b
Portet-2 1992	Late cutting	Control	J	4 F	66 ^b	59 ^a	0-4-96 ^a
		N1 PK2	J'	4 F	82 ^a	64 ^a	0-13-87 ^a
		N1 PK6	J''	4 F	89 ^a	62 ^a	2-54-44 ^b
Ercé 2002	Early cutting	High manure rate	K	2 G	80 ^a	55 ^a	58-34-8 ^a
	Early cutting	Medium manure rate	K'	2 G	69 ^b	48 ^a	42-44-4 ^a
	Late cutting or grazing	Medium manure rate	K''	2 G	73 ^b	54 ^a	28-27-45 ^b
	Late cutting or grazing	No fertilizer	K'''	2 G	56 ^c	49 ^a	10-12-78 ^c

^a The indices indicate the number of consecutive year of application of a given nutrient

^b Grassland communities (G) or fertilizer treatments (F)

^c Abundance of each group was established from botanical records (no species belonging to the D-PFT)

For a given experiment (one location-year), data following a different letter on a given column are significantly different ($P < 0.05$); comparisons were done only for PFT A and C in the last column

Plant measurements

In order to determine the growth curves and the herbage composition, 5–8 cuts were made every 2 weeks from April to July at different places for each date. Sampling was made at 2-cm height on a frame of 0.25 m² with a handy mower. Grasses were separated from dicotyledonous species at Portet 2 and Ercé. Leaves were separated from stems on all the species (Portets-1 and 2) or only on grasses (Ercé). Each component was dried for 48 h at 70°C, weighed and then milled for N, P and K analysis.

Data processing

For each treatment, ANOVA was performed between two successive sampling dates to determine the ceiling yield in spring (maximum above-ground

biomass) for the whole plant community and for each specific and organ component (grass vs. dicotyledons, leaf vs. stem) on the basis of the four replications and taking into account the available data. The beginning of stem elongation was assessed by the date at which stem mass reached 20% of the standing herbage mass (Duru, unpublished data).

To find out whether grasses and dicotyledonous species have the same behaviour, correlations were made for the different criteria (ceiling yield, etc.) between the life-forms in all the plant communities in each experiment. ANOVA was also performed to assess whether there was a fertilizer effect (Portets 1 and 2) or a PFT effect (Ercé) on herbage growth and composition. There were 3, 2 and 1 sets of data at Portet 1, 2 and Ercé respectively.

To examine whether a given PFT is a good functional parameter to characterize plant community

for their herbage growth pattern, regression analysis was performed between the content of the A or C PFT and the same stand characteristics as above.

Results

Linking grass LDMC and plant features at species level

There were very significant effects ($P < 0.001$) of PFT on each of the three plant features. N fertilizer significantly decreases the LDMC and brings forward the 10 cm stem height stage. Indeed, LLS was shortened and the flowering time was brought forward, though not significantly (Table 3). There was no significant N effect within each of the functional groups. There was no interaction between N and PFT. Expressed in percent of the criteria, the N effect was the greatest for LDMC and LLS. The relative difference in LDMC between PFTs (>21%) was greater than those resulting from N depletion (<17%). Species were ranked in a similar order for the N+ and N- treatments whatever the plant features (Spearman correlations >0.90). Same analysis done with SLA instead of LDMC did not give a significant relationship, except for flowering time (not shown). In summary, N fertilizer tends to enhance the plant features which are typical of

species having a resource capture strategy assessed through LDMC.

Considering LDMC as the ranking reference, species ranked in A- and B-PFTs were core species, except *F. arundinacea*, due to its long LLS (Fig. 1). Species ranked in C-PFT were core for *F. rubra* and *C. cristatus*, but *A. capillaris*, *P. pratense* and *T. flavescens* were singular for their early flowering (Table 1). Finally, almost all species belonging to the D-PFT were core species. However, *D. cespitosa* and *M. caerulea* were singular because they have longer LLS, and their 10-cm stem height occurred later than for core species.

Correlations were always significant ($P \leq 0.01$), and they were higher when considering only core species (Table 3). Slopes and intercepts of the regressions were not significantly different when done for whole and core species. This means that these are sound relationships, so that LDMC can be considered as the best plant functional parameter for assessing the studied plant features.

Equations allowed the magnitude of plant feature variation to be computed as a function of LDMC. We observed that an increase in LDMC of 100 (mg g^{-1}) due to a change in PFT (from A to C or D) or use of low N fertilizer could be accompanied by major changes in plant features. Considering all species and N treatments together, there was a significant

Table 3 ANOVA for leaf lifespan (LLS), stem 10-cm height and flowering date for PFT and N treatments (19 grasses), and regression analysis between leaf dry matter content (LDMC) and plant features

LDMC and plant features	ANOVA			Regression analysis between LDMC and plant features			
	Plant strategies (PFT)	N ^a	N effect (%)	r r'	Comparison slope/ intercept ^b	Equations	
LDMC (mg g^{-1})	***	***	15				
LLS (DD)	***	§	15	0.64*** 0.67***	ns/§	$y = 6.6x - 660$	$y' = 4.9x - 322$
10 cm stem height (day)	***	*	8	0.67*** 0.70***	ns/ns	$y = 0.36x + 21$	$y' = 0.27x + 41$
Flowering time (day)	***	ns	3	0.46** 0.48**	ns/ns	$y = 0.27x + 78$	$y' = 0.23x + 91$

§ $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, non significative

N effect = |N+ to N-|/N+

^a There is no interaction between PFT and N treatment

^b Comparisons were done pooling PFTs A and B, and C and D

Correlations taking account of the whole species (r) or the core species (r')

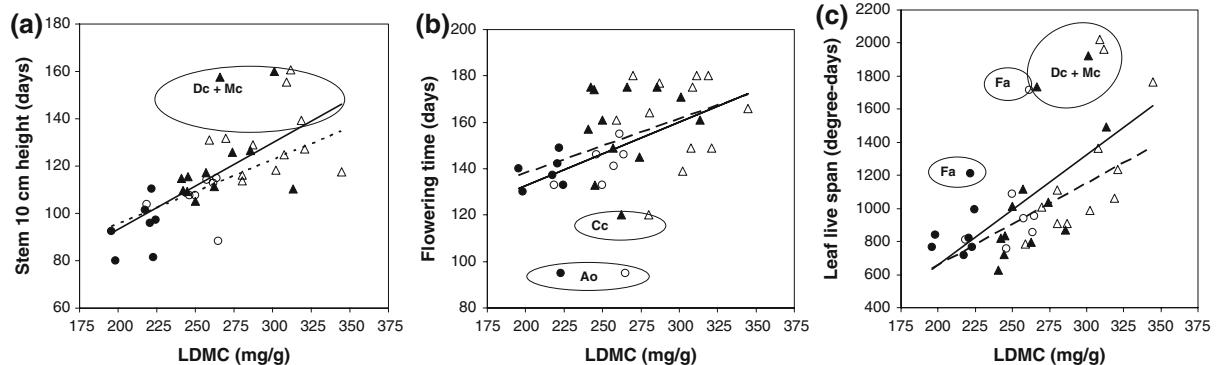


Fig. 1 Relationships between LDMC (X-axis) and dates at which stem-10 cm height (a) flowering (b) occurred, and leaf live span (c). Species belonging of A and B- PFT (●○) and C and D- PFT (▲△) for N+ treatments (closed symbols) and N-

treatments (open symbols); singular species are indicated by a circle (see Table 1 for species code). Straight line regression are drawn for whole species (full line) or without the singular species (dotted line)

correlation with LDMC. Increasing LDMC from 200 to 300 mg g⁻¹ leads to (see Fig. 1):

- a delay in the date at which the reproductive apex reaches 10-cm height by 36 days (whole data set) or by 27 days when *M. caerulea* and *D. cespitosa*, which flowered much later, were excluded (Table 1);
- a delay of 27 or 23 days in the flowering date according to whether or not *A. odoratum* was included;
- an increase in LLS by 660 or 490 degree-days according to whether or not *F. arundinacea*, *M. caerulea* and *D. cespitosa* were included;

Utilization of grass PFTs for ranking grassland communities by their growth patterns

Comparison of grass and dicotyledonous components within two sets of grassland communities

For the two sets of grassland communities for which the grasses and dicotyledons were separated, we

compared their weights (Table 4). In both experiments, ceiling yield of grasses occurred significantly ($P < 0.05$) later than for dicotyledons. The same was observed to a lesser extent for the ceiling yield of stems, but the opposite was found for that of leaves. On the other hand, for each experiment, there were significant correlations ($P < 0.05$) between the dates at which ceiling yield occurred for grasses and dicotyledons. Finally, it appears that the difference between grasses and dicotyledons for the date at which the ceiling yield occurred was observed whatever the grassland community, i.e. its management and its PFT composition.

Growth patterns of grassland communities with different grass PFTs

ANOVA was performed to see whether a significant change in PFT (Table 2) leads to a change in herbage growth patterns of plant components (Table 5).

Table 4 Comparison between grasses (G) and dicotyledons (D) components within two set of grassland communities for dates at which ceiling yield occur

§ $P \leq 0.10$; ** $P \leq 0.01$;
*** $P \leq 0.001$

Sward characteristics	Comparison between grasses and dicotyledons for dates at which ceiling yield occur and leaf organic matter digestibility	Ercé (n = 8)	Portet 2 (n = 6)
Standing green herbage mass	Correlation	0.88**	0.76§
	Rank and average differences (days)	G > D 21	G > D 14
Standing green leaf mass	Correlation	0.99***	
	Rank and average differences (days)	G < D 11	
Standing stem mass	Correlation	0.75§	
	Rank and average differences (days)	G > D 12	

At Portet 1, where the C-PFT proportion was significantly the greatest (H3' and H4' plots), the date at which stem ceiling yields occurred was significantly delayed in two cases out of three, as was the date at which stem content reached 20% of the above-ground herbage mass. When there was no significant change in PFT composition (H2 plots), the date at which stem content reached 20% of the above-ground herbage mass was delayed for the plot having the lower sward nutrient status.

At Portet 2, when the C-PFT proportion was significantly the greatest (I and I', J and J'), the date at which the leaf ceiling yields occurred was significantly delayed for grasses as well as for the entire herbage. It was also true at one date for the stem ceiling yield. In other cases, the sampling stopped too early to examine whether there was any difference. The date at which the stem content reached 20% of the above herbage mass was always significantly the latest for these plots.

At Ercé, when the C-PFT proportion was significantly the greatest (K'' and K''' plots), the same trends as above were observed for the grass component.

Finally, there was no contrary example where a change in PFT composition (from A or B to C) led to an earlier date at which ceiling yield for leaves or stems occurred. When there were no significant differences in PFT composition between plant communities, differences in plant features could be related to sward nutrient status. For example, for H2 and H2' plots (Portet 1), or for K and K' plots (Ercé), there was a significant change in the date at which the stem content reached 20% of the herbage mass or the date at which the ceiling yield occurred respectively, which could be related to sward nutrient status.

Regression analysis shows significant negative correlations between the A-PFT proportion (or positive with the C-PFT proportion) and the dates at which leaf and stem ceiling yields occurred or the stem content reached 20% of the standing herbage mass (Table 6). Considering the whole herbage (Portet 1) or only the grass component (Ercé), correlations were significant ($P < 0.05$) respectively in eight cases out of 12, or in 10 cases ($P < 0.1$) out of 12.

Table 5 Growth pattern of plant component

Experiment	Treatment	Dominant PFT	Dates (julian days) at which					
			Ceiling yield of green leaf mass occurred		stem mass equal 20% of herbage mass		Ceiling yield of stem mass occurred	
			G	W	G	W	G	W
Portet 1 (1988)	H2	BC	—	152 ^a	—	123 ^a	—	175 ^a
	H2'	BC	—	154 ^a	—	115 ^b	—	175 ^a
	H3	BC	—	159 ^a	—	123 ^a	—	185 ^b
	H3'	C	—	154 ^a	—	115 ^b	—	175 ^a
	H4	C	—	171 ^b	—	135 ^a	—	185 ^b
	H4'	BC	—	163 ^a	—	128 ^b	—	175 ^a
Portet 2 (1991)	I	C	203 ^b	175 ^b	161 ^b	142 ^b	203 ^b	200 ^b
	I'	C	203 ^b	175 ^b	161 ^b	140 ^b	203 ^b	203 ^b
	I''	BC	161 ^a	161 ^b	147 ^a	128 ^a	184 ^b	184 ^a
Portet 2 (1992)	J	C	203 ^c	190 ^b	140 ^a	135 ^b	>202	>202
	J'	C	188 ^b	156 ^a	130 ^b	131 ^b	>202	>202
	J''	BC	161 ^a	161 ^a	122 ^c	120 ^a	>202	>202
Ercé (2002)	K	AB	145 ^a	—	110 ^a	—	154 ^a	—
	K'	AB	148 ^a	—	112 ^a	—	170 ^b	—
	K''	BC	164 ^b	—	133 ^b	—	184 ^c	—
	K'''	C	158 ^b	—	147 ^c	—	189 ^c	—

G grasses, W whole herbage

For each experiment, data having a different letter in the same column were significantly different at 5% level

Table 6 Correlation between PFT composition (%A or %C) and dates at which some sward characteristics occurred for the grass component (G) or the whole herbage (W)

Experiment	PFT composition (%A or %C)	Dates at which					
		Ceiling yield of green leaf mass occurred		Stem mass equal 20% of herbage mass		Ceiling yield of stem mass occurred	
		G	W	G	W	G	W
Portet 1	A		-0.87**		-0.60 ns		-0.70§
	C		0.94**		0.84*		0.70§
Ercé	A	-0.58 ns		-0.98***		-0.74*	
	C	0.74*		0.94***		0.84**	

§ $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Discussion

Relevance of leaf LDMC for capturing plant features of agronomic interest

Data from the pure stand of grasses show that LDMC was the plant functional parameter which is best correlated, albeit only roughly, with the three plant features of agronomic value for modelling herbage growth pattern (McCall and Bishop-Hurley 2003). The SLA, mainly correlated flowering time, indicating an ability to compete better for light (Tilman 1985), was not significantly correlated with the two other plant features.

These findings are more or less supported by the literature. It is well known that LDMC is correlated with LLS, another leaf functional parameter that is an indicator of plant strategy for resource capture and use (Garnier et al. 2004). This is also true for relationships between tissue composition or density (Ryser and Lambers 1995). Less obvious was the relationship between LDMC and the dates on which species reach their phenological stages. However, it was shown that these plant characteristics are related to plant growth rates and capture and use of nutrients (Sosebee and Weibe 1973).

Fertilizer N always significantly decreased LDMC, and, to a lesser extent, changed the three plant agronomic features. Nevertheless they varied in the expected direction, giving consistent correlations between the plants' agronomic features and LDMC. This effect of N fertilizer is well known for LLS (Fichtner and Schulze 1992; van der Werf et al. 1993; Ackerly and Bazzaz 1995; Ryser and Lambers 1995; Knops and Reinhart 2000; Schippers and Olff 2000)

and 10-cm stem height; the latter depending both on growth and development processes.

Considering plant functional parameter values for species growing in their ideal habitats (i.e. low N availability for species having high LDMC) would probably improve these correlations. Furthermore, for given climatic conditions, the species ranking for plant leaf functional parameters seems not to be environment dependent (Poozesh et al. 2005), so a database built on species strategies can be used to rank plant communities in a given area (Coleno et al. 2005) for agronomic characteristics. However, as species are sensitive to N, using a PFT database would probably minimize differences between plant communities.

For most of the studied species, the relationships between plant features are relatively consistent, meaning that it is the result of long co-evolution between the plants and their environment, including management practices (Ackerly 2003). On the other hand, improving grass by breeding aims to "de-correlate" such plant functional parameters (Hazard et al. 2006). For example, the search for perennial ryegrass genotypes well suited to grazing maintains LDMC and LLS, but delays the reproductive phase (Al Haj Khaled, unpublished data).

Relevance of PFT for assessing herbage growth pattern at plant community level

We wish to evaluate the consistency between species characteristics and grassland community properties. This represents a double jump, from individual plant features to their aggregation at plant community level through PFTs, and from PFTs to grassland community properties.

From individual plant features to the plant community

Passing from individual plant functional parameter to their aggregation at plant community level is based on the “biomass ratio hypothesis” proposed by Grime (1998). It postulates that ecosystem properties should depend on species functional parameter and on species contribution to the whole above-ground biomass of the community (Garnier et al. 2004). Our decision to examine links between plant features established on species growing in pure stands and some agronomic properties at plant community level is based on this hypothesis.

On the other hand, grass and dicotyledonous species associated in the same grassland community tend to have the same behaviour. This was observed previously for leaf functional parameters measured at field level (Ansquer et al. 2005; Louault et al. 2005) as well for leaf stem ratio (Calvière and Duru 1999).

From plant features to grassland community properties

Passing from plant features to grassland community properties is based on the fact that herbage growth pattern and composition are driven by the former. This is supported by findings in grazing ecology, mainly for perennial rye grass. Indeed, it was shown that LLS is a key functional parameter determining the herbage senescence rate (Parsons 1988), and consequently the moment at which the ceiling yield occurred. Our finding is that this relationship applies for different PFTs. It allows one to evaluate up to what point there were differences in dates on which leaf ceiling yield occurred between grassland communities composed of different PFTs. These results were consistent with those of previous research studies that have shown that LLS provides a good estimate of the leaf ceiling yield (Reich 1993).

Thus, for the set of grassland communities which differ the most in terms of PFTs (Portet 2 and Ercé), differences in dates at which leaf ceiling yield occurred varied from 13 to 42 days for the grass component. Similar differences could be due to nutrients which lengthen the LLS in case of N deficiency (Duru and Ducrocq 2002). Nevertheless the observed differences are of great importance from an agronomic point of view (Lemaire 1999), and

consistent with the differences in LLS observed between a A- and a C-PFT. It is about 350 degree-days, that is to say around 30 days for the climatic conditions considered.

The fact that there are consistent results between dates for phenological stages and stem ceiling yield was to be expected, although not recorded in the literature. Differences in dates of ceiling yield for stems between grassland communities varied from 20 to 35 days, which is in agreement with the interval observed for flowering times (Al Haj Khaled, unpublished data). Unfortunately, there are few data in the literature about this process.

To sum up, there are consistent results between plant features assessed at species level, mediated through PFTs, and grassland characteristics at plant community level. This means that:

- ranking grassland communities for the date at which the ceiling yield of plant components occurred, as well as for stem elongation and tissue composition, can be well predicted by plant features mediated through LDMC. Thus we observed the same ranking for the dates at which key agronomic stages occurred at plant community level (stem20% < ceiling leaf < ceiling stem), and the time at which key plant features are observed for each of the PFTs (date of beginning stem elongation < LLS < flowering date);
- PFTs based on LDMC are appropriate for plant and grassland classifications.

Application for modelling and management of grassland communities

In this article, we focus on the effect-PFT by comparing plant communities that differed in their PFT compositions—fertilizer treatments being used to create them. Indeed, some conclusions can be drawn considering the response of PFT (response-PFT, Lavorel and Garnier 2002) mainly to fertilizer treatments (Table 1). For fertilizer treatments (i.e. those having the highest plant nutrient status), our results confirm that fertilizer application decreased the proportion of PFT having a strategy of resource conservation (Knops and Reinhart 2000).

On the other hand, we focus on the comparison of grassland communities for their herbage growth

pattern. We do not present data on their productivity, especially herbage accumulation rate, because it has been shown that the sward nutrient status is the main factor determining the herbage growth rate, even for species-rich grasslands differing in species and PFTs (Duru and Calvière 1996). Indeed, in agricultural situations where there is wide variability in defoliation regimes, whatever the nutrient availability, growth processes such as radiation use efficiency and light interception are more sensitive to N depletion than to PFT (Duru et al. 2005). Consequently, knowing the plant functional group or measuring LDMC is of little value for ranking species or grassland communities for plant growth rate. Indeed PFT compositions of the community respond both to nutrient availability that is well known (Wilson et al. 1999 for example) and to defoliation management that can have complex effects due its four dimensions (frequency, intensity, timing and extent), (Grubb 1998). Other criteria such as sward nutrient index will perform much better (Duru et al. 1994; Jouven et al. 2006). However, plant functional approach allows the identification of key plant features such as phenological stages that are essential for modelling the differences in herbage growth patterns between plant communities as pointed out previously (McCall and Bishop-Hurley 2003).

Ranking grasslands for the date at which ceiling yield occurs through PFT determination is a direct application of this study. It could be done at farm and landscape levels. Indeed, grassland managers aim at optimizing vegetation growth over their entire grazing or cutting area, but also over time. For example, White et al. (2004) insist on the need for greater vegetation diversity as spatial and temporal scales increase (from field to farmland; from a season to a year) particularly where there is considerable variability in animal requirements over animal types and growing seasons. Knowing the dominant grassland PFT should allow harvesting dates to be chosen to maximize yield. This means that grassland managers do not cut (Robson et al. 1988) or graze (Parsons 1988) swards later than when ceiling yield is reached, which occurs at different dates for reproductive or vegetative growth according to the dominant PFT (McCall and Bishop-Hurley 2003, and this study).

Conclusion

We have shown that LDMC was the plant functional parameter that best describe the species for plant features useful to rank grassland communities for their herbage growth pattern. Grass species can be gathered in types (PFTs) that differed for most of the species by three plant features (stem 10-cm height, flowering date and LLS). Scaling up from plant to community was well mediated by PFT. Grassland communities which differed by their PFT compositions and differed too by the dates at which ceiling yield occurred (stem, leaf, whole herbage) even when there were no differences in the plant nutrient status. Ceiling yields occurred from 1 to 3 weeks later for grassland communities dominated by PFT having a strategy of resource conservation (high LDMC), which is of great importance to plan the use of grasslands. In sum, plant features which characterize species for resource acquisition and use at plant level are consistent with plant communities' properties for characterizing herbage growth pattern at plant community level.

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Clonal growth strategies in simultaneously persistent and expanding *Trifolium repens* patches

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Abstract Plants with clonal growth can generate patches dominated by a single species. In time, patches can change and may fragment, form a ring, dissolve or both persist and expand. For patches to maintain their original habitat and simultaneously increase in size, ramets or clonal fragments must both promote local persistence inside the patch and grow out of the patch into new habitats. This study analyses simultaneously expanding and persistent *Trifolium* patches in a nutrient-poor lawn that is frequently cut, and where the *Trifolium* is competitively superior to the grass species. *Trifolium* primary stolon growth strategies were analyzed in relation to their location (border, middle, and center) inside the patch, and according to patch size (small, medium, and large). It was hypothesized that different growth strategies inside a patch can explain both persistent and expanding patch of *Trifolium*, and that growth strategies were different between patch sizes. Primary *Trifolium* stolons had two different growth strategies inside and at the border of patches: (i) stolons at the border were long, grew fast, had few lateral stolons, and grew out of the patch, while (ii) stolons inside the patch were smaller, grew slowly, and had more lateral stolons and a wide range of growth directions. Growth strategies were not different between patch

sizes. The directional growth and the high growth rate at the border will increase the patch size with time, while the growth strategy near the center consolidates the patch in space and time, by placing ramets inside the patch. Different growth strategies near the center and on the border result in *Trifolium* patches that are simultaneously persistent and on the increase. The results also indicate a division of labor among primary *Trifolium* stolons in a patch.

Keywords Clonal growth · Growth angles · Growth form · *Trifolium repens* patches · Patch dynamic

Introduction

Under natural conditions plants often live in a mixture of species. Plants and habitats are heterogeneously distributed in ecosystems. This is often true for clonal plants, which frequently show a clumped distribution in plant communities (Hutchings and Mogie 1990; Herben and Hara 2003). Clonal plants have a finite average dispersal range because of clonal growth, and consequently interspecific competition is local. This can generate intraspecific aggregation and reduces contact with other species, resulting in interspecific segregation. According to the “spatial segregation hypothesis,” single species patches can arise in this manner (Pacala 1997). Patches dominated by single species can also arise as

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a consequence of external factors that may alter the habitat, such as herbivory (Harris 1987) animal excrement (Marriott et al. 1987; Schwinning and Parsons 1996a), and edaphic factors (Snaydon 1962; Simpson et al. 1987) or simply clonal demography per se (Bell 1984).

The inside of a patch offers a spatial refuge from interspecific competition, so that only ramets or clonal fragments on the patch border compete with other species (Pacala 1997; Silvertown and Charlesworth 2001). In this situation, ramets near the center versus those on the border experience quite different growth conditions. Clonal plants can respond to habitat conditions by plastic production of ramets and branches (de Kroon and Hutchings 1995) and growth direction (Sampaio et al. 2004). Therefore, clonal fragments inside a patch and on the border are expected to respond differently to growth conditions, and to exhibit different growth strategies.

Trifolium repens L. is a clonal species that can be distributed patchily in a mixed pasture of grass species (Kershaw 1959; Baker and Williams 1987; Cain et al. 1995; Edwards et al. 1996). The degree of competition with grasses will affect the demography of *Trifolium* and hence also the clonal structure. In pastures that have been fertilized (nitrogen rich) and that are infrequently cut, grasses create shade and are competitively superior *Trifolium*. At the other extreme, in a lawn that is unfertilized (nitrogen poor) and frequently cut, grasses have low production and *Trifolium*, with its ability to fix nitrogen, is the superior competitor (Schwinning and Parsons 1996a). Here I have studied *Trifolium* patches in a lawn that was unfertilized and frequently cut, where *Trifolium* is the superior competitor. The lawn is a suitable environment for *Trifolium* to establish and grow. Plants must be dominant competitors to maintain patches, otherwise grass species will outcompete *Trifolium*, and the patches are not formed, or they dissolve.

Over time, single species patches can change and may develop a ring form (Wikberg and Svensson 2003; Lanta et al. 2008), fragment, dissolve (Cain et al. 1995), or increase in size (Marriot et al. 1997). Patches increase in size when fragments grow out of the patch into new habitat, i.e. when patch expansion is promoted by a high growth rate and ramets are placed outside of or on the patch border. A superior competitor is expected to grow out of the patch with 180° away from

the center as the most effective direction when occupying new space. However, expansion itself is not sufficient for patch persistence. With just expansion, patches will dissolve in the center and develop a ring form (Wikberg and Svensson 2003; Lanta, et al. 2008). For patches to be maintained in space, fragments must also promote local persistence (de Kroon and Schieving 1990). This may be done by placing ramets inside the patch in tight aggregates. Both processes of expansion and persistence must occur in a patch that is increasing in size while simultaneously maintaining its original habitat. These two processes are achieved through the use of two different growth strategies inside a patch: a consolidating strategy for persistence in the center and an opportunistic strategy of rapid growth on the border. Therefore, patches dominated by a single species require more than one growth strategy to be able to simultaneously expand and persist in space and time.

Patches can be of various sizes, from single plants forming one small patch (Bell 1984) to large patches created from merging patches. A large patch requires more consolidating growth strategies than a small patch to persist in space, due to the geometric reality that the area/circumference ratio increases linearly with the radius of a circle. Marriott et al. (1987) suggested that the fastest spread of *Trifolium* would occur from small patches. Thus, the size of patches can affect the demography of *Trifolium* stolons.

The present study analyses the growth strategies of *Trifolium* primary stolons inside *Trifolium* patches. I hypothesize that (i) there are different growth strategies inside and on the border of *Trifolium* patches and (ii) there are different growth strategies among *Trifolium* patch sizes. The aim is to quantify growth strategies that can explain both a persistent and expanding patch of *Trifolium* in a habitat where *Trifolium* is competitively superior to surrounding grasses, and to determine if growth strategies differ between patch sizes.

Materials and methods

Study species

Trifolium is a clonal perennial legume with a creeping stem (stolon). The stolon consists of a series of nodes separated by internodes. Each node has one

leaf, two root primordia, and an axillary bud. The axillary bud can develop into a lateral stolon or an inflorescence. A fully developed individual consist of a primary stolon with lateral stolons. Each node is a potential independent ramet, and all interconnected ramets form a clonal fragment. As the plant grows horizontally old parts die while young shoots capture new space (Baker and Williams 1987). Reproduction by seed is rare in established *Trifolium* populations (Turkington et al. 1979). *Trifolium* has a symbiotic relationship with the nitrogen-fixing bacteria *Rhizobium trifolii*, which result in its presence as a very common pasture plant in many temperate regions of the world (Baker and Williams 1987).

Data sampling

Six patches of *Trifolium* were selected from a lawn at the Norwegian Institute of Agricultural and Environmental Research, Grassland and Landscape Division, Kvithamar, Norway ($63^{\circ}26' N$, $10^{\circ}53' E$, 30 m a.s.l.). The lawn had been sown in 1999 with only the grass species *Lolium perenne*, *Festuca trachyphylla*, *Deschampsia cespitosa*, *Agrostis capillaris*, *Festuca rubra*, *Poa pratensis*, and *Agrostis stolonifera*. *Trifolium* plants had immigrated since then and established distinct patches. The patches were persistent and had also increased in size over the years (personal observation). Between the patches there were no *Trifolium* plants. The lawn was cut twice a month from May until September and no fertilizer was applied from 2003 to 2006. The soil is a silt loam overlying silty clay loam, classified as a Typic Cryaquoll (Soil Survey Staff 1998). When the lawn was established in 1999, the upper 80 cm of the original soil was partly replaced by layers of coarse sand and gravel and a mixture of finer sand and soil. This was to ensure free and fast water drainage. The climate is humid continental with cool summers and long, cold winters (Critchfield 1996). The average precipitation (1961–1990) is 890 mm per year and the average annual temperature is $5.3^{\circ}C$. Meteorologic data were provided by the Norwegian Institute of Agricultural and Environmental Research, Grassland and Landscape Division, Kvithamar.

Two large (dm: 470 cm, 580 cm), two medium (dm: 340 cm, 260 cm), and two small (dm: 155 cm, 143 cm) sized patches were selected in the spring of 2006. Inside each patch, *Trifolium* stolons were

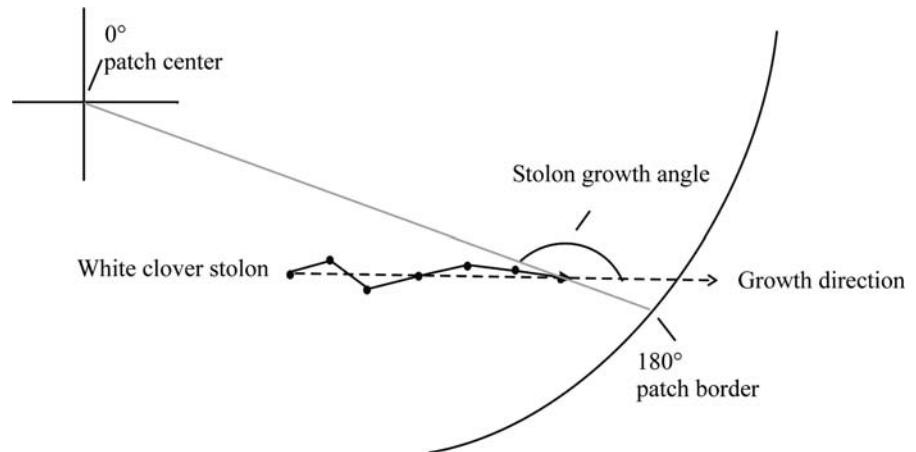
randomly selected from three locations: the patch border with a 20 cm radius, the center with a radius 1/3 of the total patch, and between the border and center. These locations or zone types are referred to as border zone, center zone, and middle zone, respectively, while a particular location in a patch is referred to as a zone. The location of the apex determined to which zone a stolon belonged. To be able to measure stolon growth, the stolons were marked on June 13 and 14, 2006, with a number and a small stick at the apical tip. In the large and medium patch size classes, 10 primary stolons were marked in all zones, i.e. 30 stolons per patch, and in small patches, 5 primary stolons were marked in each zone, i.e. 15 stolons per patch. A total of 210 primary stolons were marked. A primary stolon refers to the first order stolon (main stolon) within a fragment.

The stolons were retrieved from August 21 to 24, 2006. The following variables were measured on each marked stolon: stolon survival, apical tip damage, stolon length (mm), stolon growth (mm) since June, growth direction ($^{\circ}$), number of and growth direction of rooted lateral stolons. The number of lateral stolons on the primary stolon was recorded only up to 150 mm behind the apex in order to reduce the influence on the measurements of lateral stolons growing in a zone other than the primary apex.

The intention was to include only primary stolons when they were marked in June. It is sometimes necessary to remove surrounding vegetation to determine if a stolon is a primary or a lateral stolon. However, it was undesirable to damage vegetation in June. Therefore, I also recorded if the marked stolon was a primary or a lateral stolon in August. A few lateral stolons had been marked by mistake and these were not included in the dataset. Primary stolons with damaged apical tips were excluded from analyses of stolon growth and length. Dead stolons were also excluded from all analysis. The design therefore became unbalanced.

I defined the growth direction of a stolon as the line between the apex and a node that was 150 mm proximal. Growth angles were measured as the angle between the stolon growth direction and a reference line from the patch center to the stolon apex (Fig. 1) (Sampaio et al. 2004). A growth direction toward the center of the patch was defined as a growth angle of zero. Thus, a growth direction toward the patch border corresponded to a growth angle of 180° .

Fig. 1 Measurement of the growth angle of *Trifolium* stolons inside *Trifolium* patches, with the growth direction toward the patch center defined as corresponding to a growth angle of 0°. The stolon is composed of several nodes (black points) and internodes (black lines). The stolon growth direction (dashed line and arrow) is the line between the apex and a node 150 mm behind



Statistical analysis

Only primary stolons were included in the analysis. Differences in stolon growth and length were tested in a nested ANOVA according to the following linear model:

$$X_{ijkl} = \mu + A_i + B_j + AB_{ij} + c(A)_{k(i)} + c(A)B_{jk(i)} + D(c(A)B)_{l(ijk)} \quad (1)$$

where X_{ijkl} is the measured response of stolon l from zone type j and patch k of patch size class i , μ is the mean response, A_i is the main effect of patch size class i , B_j is the main effect of zone type j , AB_{ij} is the interaction effect of patch size class i and zone type j , $c(A)_{k(i)}$ is the nested effect of patch k of patch size class i , and $D(c(A)B)_{l(ijk)}$ is the residual. $c(A)B_{jk(i)}$ is the interaction between patch k and zone type j . The interaction between patch and zone type in this design is confounded by effects from zones. Capital letters in the model represent fixed effects while small letters represent random effects. F -test statistics were calculated as described in Underwood (1999, pp. 364–369). To try to increase the power in test of patch size class and zone type, post hoc pooling was performed with respect to $c(A)_{k(i)}$ and $c(A)B_{k(i)j}$ following procedures described in Underwood (1999, pp. 268–273). Normality and homoscedasticity were evaluated using residual plots, and response variables were transformed (log) if necessary before ANOVA analyses were performed. When relevant, ANOVA analyses were followed by the Tukey multiple comparisons procedure and family-wise correction of significant level using the Duun–Sidak procedure (Quinn and Keough 2002). Stolon growth

was also analyzed in an ANCOVA-model with the June start length as covariate.

There were low frequencies of lateral stolons on primary stolons. Branching was therefore analyzed as a binary response variable (presence/absence) with log-linear models in GLM. The presence of lateral stolons was different among the 18 zones (combinations of zone type and patch) ($P < 0.01$, $df = 125$). The data were therefore separated with respect to patch size class, to test the variation between zone type and patches within the three patch size classes. These P -values were combined in a Fisher method meta-analysis (Sokal and Rohlf 1995, p. 795), and only patch was different ($P < 0.01$, $df = 6$). Thus, the data were aggregated at a patch level when testing the effect of patch size class. The presence of dead apex and dead stolons were also analyzed as a binary response variable with log-linear models in GLM.

Angular data were analyzed with circular statistics (Zar 1999; Jammalamadaka Rao and SenGupta 2001). It was of interest to uncover any systematic variation in both mean growth angle and angular dispersion among patch size classes and zone types. However, to my knowledge there are no standard statistical methods for analyzing a circular response variable based on a hierarchical design like the present one. I therefore used an ad hoc approach when analyzing the angular measurements. The strategy was first to find out whether the nested effects of model (1) could be ignored so that standard tests for circular data could be used. The 18 sampled populations (zone) had unimodal rather than uniform circular distributions (Rayleigh test on angular distances from sample mean angles, $P < 0.001$,

$n = 135$). Thus, the analyses could be based on the existence of a mean angular direction in each population.

I first used permutation tests to evaluate whether there were differences in mean growth angles among patches within patch size class and zones (i.e., presence of $c(A)_{k(i)}$ and $c(A)B_{jk(i)}$ effects). Tests for differences in mean angles were based on permutations of angular distances (Zar 1999, p. 635) calculated as the distance from each measured growth angle to the mean angle for the corresponding combination of zone type and patch size class. In this way, any effect of patch size class or zone type was removed from the calculated angular distances. I used two permutation strategies in tests for differences among patches within patch size class; these were free permutations and permutations among patches within zone type. When testing for effects of zones I also used permutations among zones within patches. In all five tests, 999 permuted datasets (unrestricted permutations) were used to simulate the distribution of the Kruskal–Wallis chi-square statistic (Zar 1999, p. 637), which was used as a test statistic. All test supported the null hypotheses, so I concluded that there was no effect from patches and zones for mean growth angle. These results allowed me to pool observations from the same combination of patch size class and zone type and use the non-parametric Rao's test of homogeneity-test for equality of polar vectors in order to test for differences among these nine pooled populations. The non-parametric version was used because of different concentration parameters ($P = 0.003$) among the populations (Jammalamadaka Rao and SenGupta 2001).

Initial tests for differences in angular dispersion among patches and zones were based on permutations of absolute values of angular distances. I used the same procedure and test statistic as described for mean growth angle above. However, these tests were interpreted differently than previous tests because the absolute values of angular distances were not corrected for any effect of patch size class or zone type. However, the dispersion in growth angle was not different among patches (unrestricted permutations: $P = 0.98$; permutations among patches within zone type: $P = 0.981$). I found an effect of zones on dispersion in two of the tests (unrestricted permutations: $P = 0.015$; permutations within patches: $P < 0.001$), but no effect was found when

permutations were among patches within zone type ($P = 0.386$). These results suggest that there is an effect of zone type on dispersion, but no nested effects of zones within zone type. I therefore pooled observations from the same combination of patch size class and zone type and used the non-parametric Rao's test of homogeneity-test for dispersion of angular data in order to test for differences among these nine pooled populations. A family-wise correction of significant level was used according to the Dunn–Sidak procedure (Quinn and Keough 2002).

The growth angles of lateral stolons were tested in a parametric paired-sample test with angles (Zar 1999, p. 645).

All analyses were performed using S-plus version 6.2 (Insightful Corporation, Seattle, USA).

Results

Dead apical apex and stolons

The number of primary stolons with dead apices ($n = 16$) and dead primary stolons ($n = 5$) was low and did not vary among the 18 zones ($df = 149$, $P = 0.440$). This suggests that the lawn experienced low disturbance.

Stolon length and growth

Stolon length and stolon growth during 9 weeks differed among zone types, but not among patch size classes (Table 1). Both length and growth were largest in the border zone (Figs. 2 and 3). ANCOVA with the start length in June as a covariate for stolon growth revealed no new results compared to those presented in Table 1 (main effect of June length $F = 31.99$, $P < 0.001$).

Lateral stolons on primary stolons

Overall, there was a low frequency of lateral stolons on the youngest part of the primary stolons. There was no difference among patch size classes ($df = 2$, $F = 0.664$, $P = 0.577$). However, there was a difference in the presence of lateral stolons among zone types ($df = 2$, $F = 3.53$, $P = 0.03$), with more primary stolons with lateral stolons in the center and middle parts of the patch (Fig. 4). The growth

Table 1 ANOVA table for stolon growth (over 9 weeks) and stolon length (log transformed) of *Trifolium* primary stolons

	df	Stolon growth			Stolon length		
		SS	F	P	SS	F	P
Patch size class	2	3198.4	0.630 ^a	0.535	0.596	1.361 ^a	0.262
Zone type	2	136336.0	26.845 ^a	<0.001	9.439	21.533 ^a	<0.001
Patch size class: zone type	4	17171.1	1.691 ^a	0.159	0.551	0.629 ^a	0.643
Patch (patch size class)	3	5529.9	0.698	0.555	0.616	0.893	0.448
Patch (patch size class): zone type	6	9025.0	0.570	0.753	0.456	0.331	0.919
Residuals	84	221603.4			19.310		

^a Test statistic calculated with residuals as error term after effects of patch (patch size class) and patch (patch size class): zone were eliminated from the model by post hoc pooling

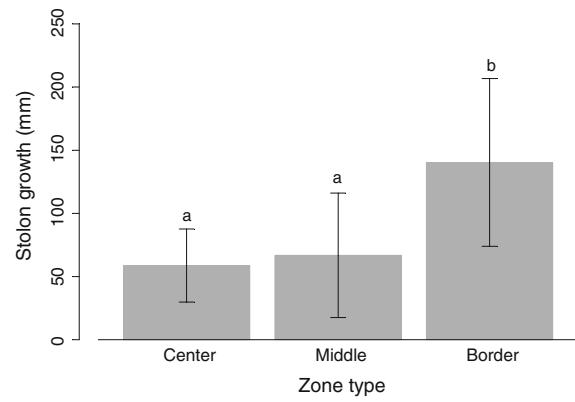


Fig. 2 Mean and SD for stolon growth (mm) during 9 weeks, June 13–August 24, of *Trifolium* primary stolons in different patch zone types. Bars with different letters are significantly different ($P > 0.017$) in a Tukey multiple comparison test

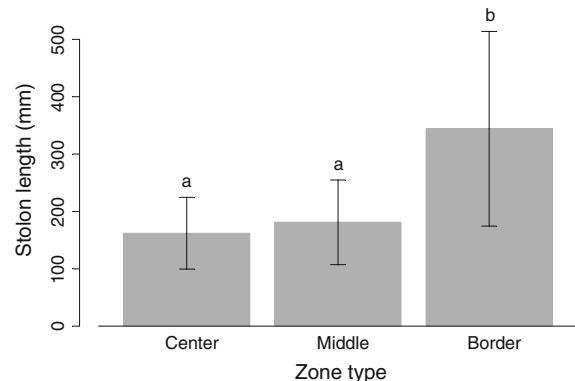


Fig. 3 Mean and SD for stolon length (mm) of *Trifolium* primary stolons in different patch zone types, August 24. Bars with different letters are significantly different ($P > 0.017$) in a Tukey multiple comparison test

directions of lateral stolons and of their appurtenant primary stolons did not differ significantly in a paired test ($n = 47$, $F = 0.605$), thus the growth direction of

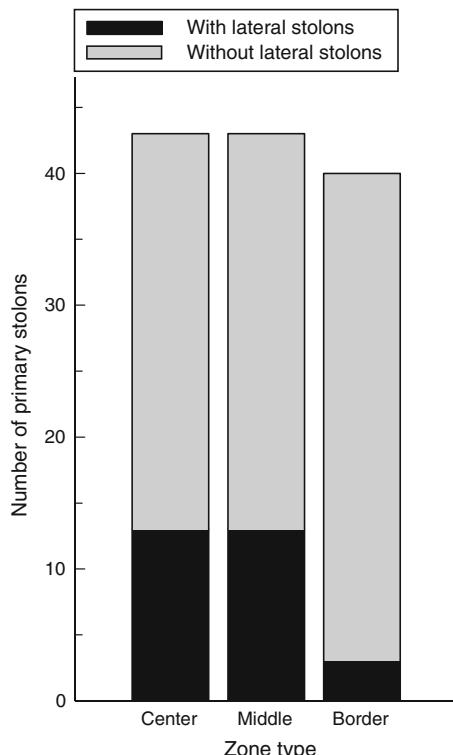


Fig. 4 Number of primary stolons with and without lateral stolons on the youngest 150 mm in different patch zone types

primary stolon and their youngest lateral stolons was similar.

Growth angles

There were differences in the dispersion of growth angles, but not for polar angles, among combinations of zone types and patch size classes (Table 2). The total mean growth angle was $171.8^\circ \pm 88^\circ$. The data were therefore split both with respect to zone type

and patch size class for further analyses of dispersion of growth angles.

There was an effect of patch size class on dispersion of growth angles in the middle zone, as well as an effect of zone type in small and medium patches (Table 2). In the middle zone, medium and large patches had a larger range of growth angles than in small patches. Small- and medium-size patches both had a small range of dispersion in the border zone compared with other zones (Fig. 5).

Discussion

The growth of primary stolons was higher in patch borders than in the middle and center of patches. These results are in accordance with those obtained in artificially created *Trifolium* patches (Marriot et al. 1997) and in a 30-year-old pasture (Kemball et al. 1996). Stolons in the border zone were also the largest and had fewer lateral stolons in the youngest 150 mm. Moreover, in small- and medium-sized patches, most stolons in the border zone were growing out of the patch. The growth direction of stolons can give an estimate as to where the fragments are placing their ramets, and whether the stolons are growing out of the patch or toward the center. Stolons growing out of the patch will promote patch expansion, while growth in another direction

will promote consolidation. These results suggest an expansion of the patch from long stolons at the border, which grow out of the patch with a high growth rate. However if all primary stolons grew out of the patch, one would expect an open area in the center without any stolons, and a ring form instead of a patch (Wikberg and Svensson 2006; Lanta et al. 2008). This was not the case in this study. For a patch to maintain its space, fragments must consolidate their dominance inside the patch, by aggregating ramets and placing ramets inside the patch. In the center patch zone, stolons grew in a greater number of different directions compared to the border. In addition, stolons in the center and middle were small and slow growing as compared to those on the edge, and had more lateral stolons. All these responses contributed to consolidating the patch.

The results indicate two distinct growth strategies: (i) primary stolons on the patch border that are long, fast growing with few lateral stolons and that grow out of the patch and (ii) primary stolons in the patch center and middle zone that are shorter, grow more slowly and with more lateral stolons than the border, and that exhibit a wide range of growth directions. The combination of these two strategies is essential in a single *Trifolium* patch to maintain the patch in space and time, while at the same time contributing to increase the patch size. The species in this way consolidated its dominance within patches and expands at the border. If the variation in stolon length and bud activity is adaptive, it will promote both patch consolidation and clonal expansion (de Kroon and Schieving 1990).

The variation in growth forms can be characterized as a continuum, with two different growth forms on separate ends, the phalanx and guerrilla types. The phalanx form evolves tightly aggregated ramets with high intraspecific contact, whereas the guerrilla form results in more loosely aggregated ramets and high interspecific contact. The guerrilla growth form has a opportunistic strategy of rapid growth and infiltrate the surrounding vegetation, while the phalanx growth form has a conservative strategy of consolidation and slower growth (Doust 1981). The guerrilla and phalanx growth forms have mainly been defined by the mean internode length between ramets (Doust 1981). The phalanx growth form with its short internodes typically produces dense small patches of ramets, as in tussock-forming grasses. However,

Table 2 Degree of freedom, test statistic, and *P*-value for Rao's test for homogeneity of polar vectors and dispersion of growth directions of primary *Trifolium* stolons inside patches

	Polar vectors		Dispersion		
	df	H	df	H	P
Zone type* patch size class	8	5.939	0.654	8	113.625 <0.001
Effect of patch size class for different zone types					
Border		2		7.047	0.030
Middle		2		13.541	0.001
Center		2		2.377	0.305
Effect of zone type for different patch size classes					
Large		2		5.939	0.051
Medium		2		12.531	0.002
Small		2		18.066	<0.001

Note: Zone type* patch size class is a variable with all combinations of zone type and patch size class, α (0.017) is adjusted according to the Dunn–Sidak procedure

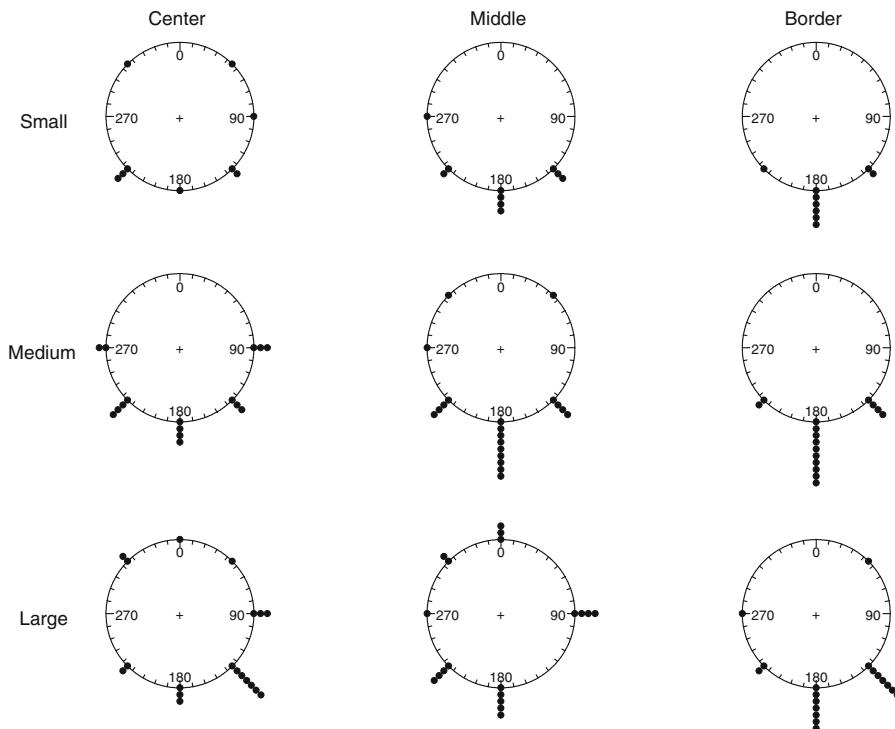


Fig. 5 Circular histogram of growth angles ($^{\circ}$) of primary *Trifolium* stolons inside *Trifolium* patches for all combinations of patch size classes (row) and patch zones (column). 0° is

growth toward the center and 180° toward the border of the patch. Each dot represents one primary stolon

this study indicates that branching angles and branching rates are also important in forming patches. Therefore, in this study, stolons localized on the border expressed a guerrilla growth form and an opportunistic strategy, whereas stolons near the patch center featured a phalanx growth form with a consolidating strategy. *Trifolium* is often classified as a typical guerrilla species because of the considerably long internodes between ramets on a stolon (McLellan et al. 1997), with long stolons wandering in space. However, these results suggest that this developmental growth of a single fragment is counteracted by growth strategies at the patch level. Branching patterns may alter this growth form and the growth strategy according to environmental conditions. These are plastic traits and different growth strategies can therefore prevail in different habitats (Stocklin 1992). For instance, one single clone can have different growth strategies under changed environmental conditions and can adjust its growth form from phalanx to guerrilla (Doust and Doust 1982; Slade and Hutchings 1987; Ye et al. 2006).

The environmental conditions important for growth strategies can be present in the field or created by the clonal plants themselves. The two most important environmental factors affecting the spatial distribution of ramets and their growth strategy are competition and distribution of resources (Hutchings and Mogie 1990). Gradients of environmental factors such as light (Solangaarachchi and Harper 1987; Weischede et al. 2006), density (Cain et al. 1995), and substrate (Welham et al. 2002) can induce a plastic response in *Trifolium*. Resources and competitors are often patchily distributed. Generally this distribution is described as induced by other species or the environment independent of the species in question. This is however not the case for clonal species. Clonal growth can per se generate a patchy distribution of resources and competitors. For example, Matthew et al. (1995) found different edaphic soil factors inside and outside of *Trifolium* patches. Single species aggregation will also affect competition (Pacala 1997). Inside a patch the neighbors of a single ramet are more likely to be of the same species than at the border, and the intensity of intraspecific

competition is higher while that of interspecific competition lower than on the very edge of the patch. In order to hold its dominant position in a patch, a species has to be competitively superior, like *Trifolium* in this unfertilized lawn. Otherwise one could expect an oscillation between *Trifolium* and grass species (Schwinning and Parsons 1996a, b).

Patches dominated by a single species are also typical for tussock-forming grasses with a typical phalanx grow form. It is suggested that dense tussocks are stable when ramets are interconnected inside a clonal fragment. Interconnected ramets can allocate fewer resources to structures involving competition, and more to reproduction. This self/non-self discrimination decreases the interspecific competition and stabilizes the tussock in space and time (Herben and Novoplansky 2008). Hence, growth strategies are also important for persistent patches in tussocks. Not all clonal plants have growth strategies that promote a patchy distribution; for example, *Gelchoma hederacea* grows in heterogeneous and fertile habitats and moves continuous through the habitat. This is effective in spatially and temporally variable environments and in escaping competition (Stuefer and Hutchings 1994). This is in contrast to the stable lawn used in this experiment, which featured low interspecific competition.

The results showed the use of different growth forms and strategies inside a patch, but not among patches of different size. In contrast, Marriot et al. (1997) recorded the greatest stolon growth in small patches as compared with larger patches, but they suggested further research to elucidate the cause. However, Marriot et al. (1997) included smaller patches in their study as compared to the present study. Therefore, patch size-dependent growth may occur in the smallest patches, but not in the larger. Additionally, the patches in Marriot et al.'s study were created by over sowing clover in a sward, as opposed to the naturally established patches in this study. This can also explain the unequal results.

In large patches, the dispersion of growth angles was not different among zone types, in contrast to the medium- and small-sized patches, where stolons at the border were growing out of the patch. If large patches are a result of the merging of smaller patches, then stolons at the border of an original patch will be located in the center or middle in the new larger patch. This can result in a different distribution of

growth angles among zone types in the large patches as compared to the small and medium patches. Therefore it is reasonable to assume that the largest patches are most likely a result of the merging of smaller patches, but this hypothesis has not been tested.

These results indicate a division of labor (Alpert and Stuefer 1997) among *Trifolium* primary stolons in a patch, including two different growth strategies that result in expansion and consolidation of the patch. In a habitat where *Trifolium* is the superior competitor, this division of labor among stolons implies a genet with a patch form, as in this study, in contrast to a ring form.

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California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence

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Abstract Early emergence of plant seedlings can offer strong competitive advantages over later-germinating neighbors through the preemption of limiting resources. This phenomenon may have contributed to the persistent dominance of European annual grasses over native perennial grasses in California grasslands, since the former species typically germinate earlier in the growing season than the latter and grow rapidly after establishing. Recently, European perennial grasses have been spreading into both non-native annual and native perennial coastal grass stands in

California. These exotic perennials appear to be less affected by the priority effects arising from earlier germination by European annual grasses. In addition, these species interactions in California grasslands may be mediated by increasing anthropogenic or natural soil nitrogen inputs. We conducted a greenhouse experiment to test the effects of order of emergence and annual grass seedling density on native and exotic perennial grass seedling performance across different levels of nitrogen availability. We manipulated the order of emergence and density of an exotic annual grass (*Bromus diandrus*) grown with either *Nassella pulchra* (native perennial grass), *Festuca rubra* (native perennial grass), or *Holcus lanatus* (exotic perennial grass), with and without added nitrogen. Earlier *B. diandrus* emergence and higher *B. diandrus* density resulted in greater reduction in the above-ground productivity of the perennial grasses. However, *B. diandrus* suppressed both native perennials to a greater extent than it did *H. lanatus*. Nitrogen addition had no effect on the productivity of native perennials, but greatly increased the growth of the exotic perennial *H. lanatus*, grown with *B. diandrus*. These results suggest that the order of emergence of exotic annual versus native perennial grass seedlings could play an important role in the continued dominance of exotic annual grasses in California. The expansion of the exotic perennial grass *H. lanatus* in coastal California may be linked to its higher tolerance of earlier-emerging annual grasses and its ability to access soil resources amidst high densities of annual grasses.

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Introduction

The order of emergence among competitors affects the growth, survival, and fecundity of plants across a wide range of species, especially in situations with well-defined growth windows and high densities of competitors (Miller et al. 1994; Verdú and Traveset 2005). Early emergence in relation to competitors can allow preemption of available resources by the early emerging species, yielding disproportionate advantages (Ross and Harper 1972). Once established, the early-emerging species may maintain dominance even if it is a relatively poor competitor during later life-stages.

Priority effects have been well documented in grasses (Harper 1961; Ross and Harper 1972; Miller 1987; D'Antonio et al. 2001), and may be of particular importance in maintaining the current structure of California grasslands. These grasslands have experienced extensive invasion by exotic annual grasses and steep declines in native perennial bunchgrasses over the last two centuries (Heady 1988; D'Antonio et al. 2007). The continued success of exotic annual grasses suggests some competitive advantage over native perennial grasses (Dyer and Rice 1997; Hamilton et al. 1999; Corbin et al. 2007), although the two groups coexist in some coastal prairie sites (Heady et al. 1988; Stromberg et al. 2001). Several studies have shown that mature native perennial grasses can successfully compete with exotic annuals (e.g. Peart 1989b; Seabloom et al. 2003; Corbin and D'Antonio 2004b; Lulow 2006), but the natives may be sensitive to competition with annual plants at the seedling stage, particularly because exotic annual grasses tend to emerge at much higher densities and earlier than native perennial grasses (Dyer and Rice 1997; Hamilton et al. 1999; Humphrey and Schupp 2004; DiVittorio et al. 2007). Exotic annual grasses germinate days after the first fall or winter rains, while native perennial grasses are typically slower to germinate (e.g., Reynolds et al. 2001). Subsequent aboveground production of exotic annual grasses outpaces that of native perennial grasses, potentially reducing light, water, and nutrients available for

emerging perennial seedlings (Jackson and Roy 1986; Dyer and Rice 1999). While some native bunchgrass adults are thought to be long-lived (Hamilton et al. 1999), they can eventually succumb to drought, burrowing animals, or other mortality sources. If they cannot successfully recruit due to seedling competition from exotic annual grasses, then populations should eventually decline.

Efforts to reintroduce native perennial bunchgrasses have had limited success when annuals are present at their typical high densities (Wilson and Gerry 1995; Clausnitzer et al. 1999; Dyer and Rice 1999; Brown and Rice 2000). Management strategies that negate priority effects and reduce the strength of annual grass competition via reduced performance or density should improve the survival of perennial grass seedlings and increase the success of restoration efforts (Corbin et al. 2004). For example, supplementing native seed supply (Seabloom et al. 2003), killing annual grasses or their seeds (Stromberg and Kephart 1996; Moyes et al. 2005), or the planting of native perennial grasses as seedling plugs rather than seeds (Dyer et al. 1996; Buisson et al. 2006) increases native grass establishment in restoration sites, demonstrating the strong role exotic annual seedling density plays in the suppression of native perennial grass establishment.

Beyond the competitive advantage gained by exotic annual grasses via earlier and abundant germination, grassland restoration efforts in California also need to deal with large increases in soil nitrogen availability due to atmospheric nitrogen deposition and colonization by nitrogen-fixing shrubs (Maron and Connors 1996; Vitousek et al. 1997; Weiss 1999, 2006; Fenn et al. 2003; Haubensak et al. 2004; Dise and Stevens 2005). Increases in soil nitrogen availability are hypothesized to confer a competitive advantage to fast-growing exotic species (Vitousek et al. 1987; Huenneke et al. 1990; Burke and Grime 1996), and appear to disproportionately benefit exotic annual grasses in California (e.g., Huenneke et al. 1990; Maron and Connors 1996; Kolb et al. 2002; Lowe et al. 2003). For example, encroachment by non-native annual grasses onto serpentine-derived soils, long a refuge from exotic annual grass competition for many California native grassland species, occur when soil nitrogen levels become elevated (Huenneke et al. 1990; Weiss 1999). Continued increases in nitrogen deposition predicted through the next century (Phoenix et al. 2006; Weiss

2006) could have widespread consequences for patterns of exotic species invasion and spread. Restoration strategies in California coastal grasslands will need to include the direct and indirect effects of soil nitrogen enrichment on native perennial grass versus exotic annual grass performance.

Even as restoration efforts to reestablish native perennial grasses continue, exotic perennial grasses such as *Holcus lanatus*, *Festuca arundinaceae*, and *Phalaris aquatica* have begun a second transformation of some areas in western grasslands (Elliott and Wehausen 1974; Foin and Hektner 1986; Peart 1989a; Ewing 2002). The ability of exotic perennial grasses to colonize the same annual-dominated stands that have excluded native perennial reestablishment (Foin and Hektner 1986; Peart 1989c) suggests that at least some European perennial grass species are less susceptible to priority effects and competition from annual grasses. Given the shared life-history of California and European perennial bunchgrasses, the differential success of exotic versus native perennials may be linked to subtle differences in reproductive output, germination timing, or seedling growth traits, or responses to soil nitrogen enrichment. For example, *Holcus lanatus*, a common exotic perennial invader in mesic coastal grassland, producing copious seed (Peart 1989a), has higher relative growth rates than some California native perennial grass species (Thomsen et al. 2006a; Corbin and D'Antonio, unpublished data), and appears to be highly responsive to nitrogen enrichment (Schippers et al. 1999; Thomsen et al. 2006a). This species may thus be less hindered than native perennial grasses by rapidly germinating exotic annuals or high soil nitrogen availability at the seedling stage. Comparisons of the performance of native versus exotic perennial grass seedlings when faced with soil nitrogen enrichment or competition with exotic annual grasses may help explain the disparity in their ability to colonize annual dominated grasslands.

Our objective in this study was to determine the importance of annual grass emergence time and density to the growth of native and exotic perennial grass seedlings with and without nitrogen addition. We hypothesized that:

- (1) Reversing the typical “annual-followed-by-perennial grass” order of emergence should increase perennial (native and exotic) grass seedling growth by negating the priority effect.

- (2) Performance of the native perennial grass seedlings should be reduced to a greater extent than that of exotic perennial grass seedlings when grown with high densities of exotic annual grasses.
- (3) The negative impact of exotic annual grasses on native perennial grasses should be greater with added nitrogen because of the greater ability of annuals to respond rapidly to increased resource availability. In contrast, N addition should either have no effect or benefit exotic perennials grown with exotic annuals.

We tested these hypotheses in a greenhouse using grass seedlings grown from seed. We experimentally manipulated annual grass emergence time, annual grass density, and soil nitrogen availability and tracked the performance of native and non-native perennial grasses.

Materials and methods

Study species and source population description

We chose *Nassella pulchra* A. Hitchcock (Barkworth), *Festuca rubra* L., and *Holcus lanatus* L. as the target perennial species, and *Bromus diandrus* Roth as the annual competitor. Henceforward, the grasses will be referred to by their generic names; all nomenclature follows Hickman (1993). These grass species co-occur in their ranges in California (Hickman 1993). *Nassella* is a native perennial grass that was historically abundant throughout California (Heady 1988), is among the most common native bunchgrasses today in the California Floristic Province (Bartolome and Gemmill 1981), and is a widely used species in grassland restoration. *Festuca* is a coastally restricted species that remains abundant in some remnant prairies (Stromberg et al. 2001). *Holcus* is an exotic perennial invader in mesic coastal and inland California grasslands, encroaching on both non-native annual dominated and remnant native perennial patches (Peart 1989a; Corbin et al. 2007). *Bromus* is a widespread Eurasian species that appears to be a strong competitor in California annual grasslands (Wilken and Painter 1993), particularly in more nitrogen-rich sites (Maron and Jefferies 1999; Rice and Nagy 2000; Hoopes and Hall 2002).

We collected seeds of all of the species at Tom's Point Preserve ($38^{\circ}13' N$, $122^{\circ}57' W$), a private nature reserve near Tomales, CA, USA. The site has been the location for a number of field studies focused on competition and resource use of native perennial, exotic perennial, and exotic annual grasses (e.g. Corbin and D'Antonio 2004a, b; Corbin et al. 2005; DiVittorio et al. 2007). The vegetation is a mosaic of exotic annual, native perennial, or exotic perennial species with large patches of *Holcus* and *Bromus*.

Experimental design

We conducted the experiment in the Oxford Tract Greenhouse at the University of California, Berkeley from March 8, 2002 to June 4, 2002 under ambient temperature conditions. We arranged the experiment in a randomized full factorial design, with the following fixed factors: focal perennial species (*Nassella*, *Festuca*, *Holcus*), *Bromus* density (low, high), nitrogen availability (low, high), and *Bromus* emergence time (simultaneous, delayed). All pots were mixed species in that they had *Bromus* with three individuals of one of the perennial grass species. In addition to the mixed species pots, we planted perennial grasses in monoculture (three individuals) and crossed the monoculture treatments with nitrogen availability to measure perennial grass response without interspecific competitors. The monoculture pots with ambient nitrogen treatment for each perennial species served as a baseline of growth for that species (see below). We replicated each treatment eight times.

Using 50% fine sand and 50% peat moss soil mix (UC mix), we planted seeds of a given focal species (*Nassella*, *Festuca*, or *Holcus*) in each perennial treatment pot (10 l, 20-cm diameter). After emergence, we thinned the germinated plants in each pot to three seedlings spaced a minimum of 5 cm from each other and the side of the pot. We transplanted additional seedlings to pots with fewer than three emerged perennials.

We planted *Bromus* seeds either concurrently with the planting of the perennial seeds ("simultaneous") or 14 days afterward ("delayed"). This design allowed us to evaluate how variation in early seedling growth timing interacts with exotic annual density and identity of the competitor (perennial species).

We planted *Bromus* at two densities: "high" ($1,592 \text{ seeds m}^{-2}$, 50 seeds/pot) and "low" (637 seeds m^{-2} ,

20 seeds/pot). We based our high-density treatment on the density of annual seeds found in an equivalent area of *Bromus* dominated stands at Tom's Point preserve (DiVittorio et al., unpublished data) and elsewhere in the state (Young et al. 1981). The low-density treatment was meant to approximate annual grass seed densities reduced by management, but is not based on field measurements.

In order to create the "high" soil nitrogen treatment, we added 0.93 g of blood meal (Green All 100% Organic Blood Meal; 13-0-0) to the wet soil surface in three doses (0, 30, 60 days after perennial planting). This "high" level of nitrogen addition (10 g N m^{-2} over the course of the experiment) is consistent with the estimates of soil nitrogen input by nitrogen fixing shrubs in some coastal grasslands (Maron and Jefferies 1999; Haubensak et al. 2004) and falls within the range of nitrogen fertilization used in previous grassland studies (e.g., Inouye and Tilman 1988). No nitrogen was added to the "low" soil nitrogen treatment.

We positioned pots randomly with respect to treatment in the greenhouse, and rotated within and between benches every 2 weeks. We watered plants to soil saturation three or four times per week. At the first appearance of a developing *Bromus* flowering culm, we destructively sampled all plants by cutting at the soil surface and separated material by species. After drying the aboveground plant material in an oven at 70°C for 2 days, we recorded the mean individual dry biomass of each species by pot.

Statistical analysis

We converted biomass measurements into an index of relative shoot yield per plant (RYP), calculated as the perennial biomass in an experimental treatment divided by the mean biomass of the conspecific monoculture treatment plants in ambient nitrogen (hereafter referred to as control): $\text{RYP} = \text{Biomass}_{\text{experimental}} / \text{Mean Biomass}_{\text{control}}$. This index allowed us to compare across the perennial grasses in evaluating the relative effect of the treatments on their growth (Johansson and Keddy 1991).

We performed all statistical tests with SYSTAT 11.0 (Systat Software, Inc.). Our analysis began with a general assessment of the impact of *Bromus* on perennial grass performance using a series of one-sample *t*-tests. We conducted one *t*-test for each

species to compare the actual growth in competition with *Bromus* to growth in a hypothetical null model (RYP = 1). Competitive treatments were pooled across *Bromus* density, *Bromus* emergence time, and nitrogen addition. We then determined the effect of nitrogen availability on perennial species growth in monoculture with a two-factor ANOVA, using species identity and nitrogen availability as independent fixed factors. We evaluated the independent and interactive effects of *Bromus* density, emergence timing, nitrogen availability, and perennial species identity with a separate four-factor analysis of variance (ANOVA). We used Tukey HSD post-hoc tests ($\alpha = 0.05$) to make any appropriate pair-wise comparisons in the four and two-factor ANOVA tests.

The RYP data used in the *t*-tests were log transformed to improve normality before analysis. Prior to application of the ANOVA tests, we applied Cochran's test for unequal variances to the raw data to assess its heteroscedacity. The subset of biomass data used in the two-way ANOVA passed Cochran's test, and so was left untransformed. The RYP data used in the four-factor ANOVA failed Cochran's test. We again performed a log transformation to both normalize the data and reduce heteroscedacity. The transformed RYP data did not pass the test, but was closer to the given critical value. Because a balanced design ANOVA, such as that employed in this study, is robust to minor departures from homogeneity of variances (Underwood 1997; McGuinness 2002), we proceeded with the four-factor ANOVA on the transformed data. We present back-transformed data and errors in figures when data were transformed for analysis.

Results

Perennial response to *Bromus* presence

Growth of all three perennial species was suppressed in the presence of *Bromus*. When compared to the hypothetical control RYP value of 1, the aboveground production of *Holcus* ($t = -9.983$, $df = 58$, $P < 0.0001$), *Nassella* ($t = -17.381$, $df = 63$, $P < 0.0001$), and *Festuca* ($t = -23.150$, $df = 63$, $P < 0.0001$) was significantly reduced when grown with *Bromus*. However, the degree to which *Bromus*

affected perennial growth differed among species. Competitive treatments reduced *Holcus* growth by nearly 75%. The two native perennials experienced even greater suppression: *Nassella* growth was reduced by 85%, while *Festuca* growth was reduced by 95% (Fig. 1).

Perennial response to nitrogen addition in monoculture

Nitrogen availability significantly interacted with perennial grass species identity in the monoculture treatments (Table 1a). *Holcus* and *Festuca*, but not *Nassella*, responded to nitrogen addition with increased aboveground growth (Fig. 2). *Holcus* grew significantly larger than the other two perennial species: in both the ambient and added nitrogen treatments, *Holcus* growth surpassed *Festuca* and *Nassella*, while the aboveground production of the two native grasses was statistically indistinguishable (Fig. 2).

Perennial response to varying *Bromus* density, nitrogen addition, and order of emergence

There were two significant three-way interactions in the four-factor ANOVA: (1) Perennial sensitivity to *Bromus* density varied with nitrogen addition and order of emergence and (2) Perennial species identity interacted with *Bromus* density and nitrogen addition

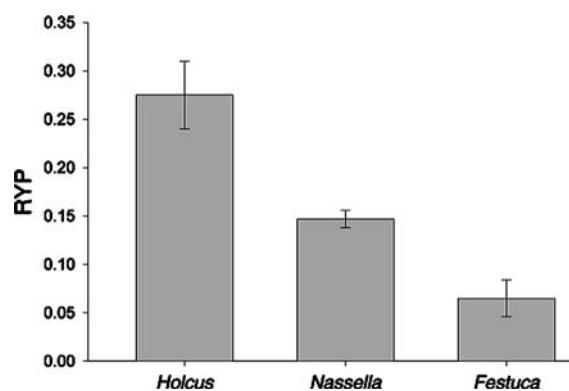


Fig. 1 Relative shoot yield of perennials (RYP) in response to competition with *Bromus*, averaged across all *Bromus* density, nitrogen, and *Bromus* emergence time treatments (mean \pm 1 SE). An RYP below 1 suggests a reduction in growth with *Bromus* competitors relative to growth in monoculture

Table 1 Summary of two-factor and four-factor ANOVA tests
(a) Two-factor ANOVA: effect of perennial species identity (PS) and nitrogen availability (N) on aboveground production.
(b) Four-factor ANOVA: effects of perennial species identity (PS), *Bromus* emergence time (BET), *Bromus* density (BD), and nitrogen availability (N) on relative shoot yield per plant

Treatment	df	Mean-square	F	P
<i>(a) Two-factor ANOVA</i>				
Perennial species	2	47.808	161.19	<0.001
Nitrogen	1	6.695	22.573	<0.001
PS × N	2	1.841	6.209	0.004
Error	40	0.297		
<i>(b) Four-factor ANOVA</i>				
Perennial species	2	45.797	32.823	<0.001
<i>Bromus</i> emergence time	1	296.475	212.490	<0.001
<i>Bromus</i> density	1	26.100	18.707	<0.001
Nitrogen	1	7.133	5.113	0.025
PS × BET	2	4.573	3.278	0.040
PS × BD	2	0.160	0.115	0.892
PS × N	2	15.665	11.228	<0.001
BET × N	1	0.211	0.151	0.698
BET × BD	1	0.841	0.603	0.439
BD × N	1	5.412	3.879	0.051
PS × BET × BD	2	0.467	0.335	0.716
PS × BET × N	2	3.722	2.668	0.072
PS × BD × N	2	5.012	3.592	0.030
BET × BD × N	1	7.240	5.189	0.024
PS × BET × BD × N	2	1.463	1.049	0.353
Error	162	1.395		

Significant results are in bold

(Table 1b). These interactions are discussed in detail below.

Bromus density × nitrogen addition × order of emergence

Delayed *Bromus* emergence significantly increased the productivity of the perennial grasses (Table 1b). Positive production responses by the perennials in delayed *Bromus* emergence treatments occurred at both *Bromus* densities although the “release” was stronger at low *Bromus* density (Fig. 3). Nitrogen addition had no effect on this pattern. High *Bromus* density generally resulted in lower perennial grass growth, although the effect was negated in the simultaneous *Bromus* emergence pots with added nitrogen (Fig. 3).

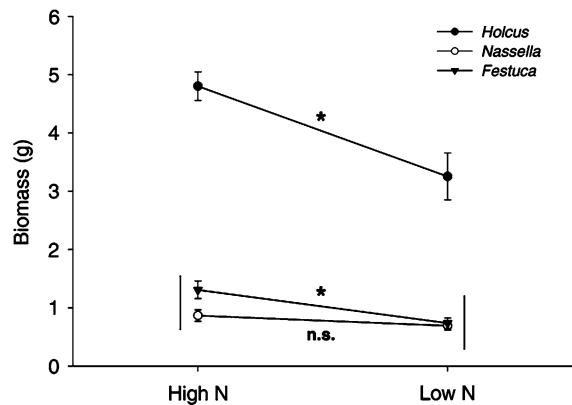


Fig. 2 Perennial aboveground growth in monoculture with and without nitrogen addition (mean ± 1 SE). Vertical lines indicate non-significant differences within a given nitrogen treatment. Asterisks indicate differences within a single species across nitrogen treatments; “n.s.” indicates non-significance

Perennial species identity × Bromus density × nitrogen addition

The three perennials differed greatly in their responses to reduced *Bromus* density and increased nitrogen availability (Table 1b). In the high nitrogen treatment, *Holcus* RYP was significantly greater than that of *Nassella* or *Festuca* at both high and low *Bromus* density (Fig. 4). *Nassella* and *Festuca*, the two native species, responded similarly to each other in the high nitrogen treatment. In contrast, in the low nitrogen treatment, *Nassella* outperformed *Festuca*, and at the high *Bromus* density, outperformed *Holcus*. In the low *Bromus* density treatment, *Holcus* and *Nassella* RYP were similar, while *Festuca* RYP was significantly lower (Fig. 4).

Overall, *Holcus* experienced the least growth in the high *Bromus* density, low nitrogen level treatment (Fig. 4). Reduced *Bromus* density and increased soil nitrogen availability tended to increase *Holcus* performance (Fig. 4). Like *Holcus*, *Festuca* exhibited the least growth in the high *Bromus* density treatment at the lower nitrogen level. Although *Festuca* growth increased in the low *Bromus* density treatment, overall, *Festuca* experienced the most consistent suppression by *Bromus* of the three perennial grasses (Fig. 4). In contrast to *Holcus* and *Festuca*, *Nassella* experienced its lowest level of growth in high *Bromus* density with high soil nitrogen. *Nassella* RYP was significantly increased with reductions in either nitrogen availability or *Bromus* density.

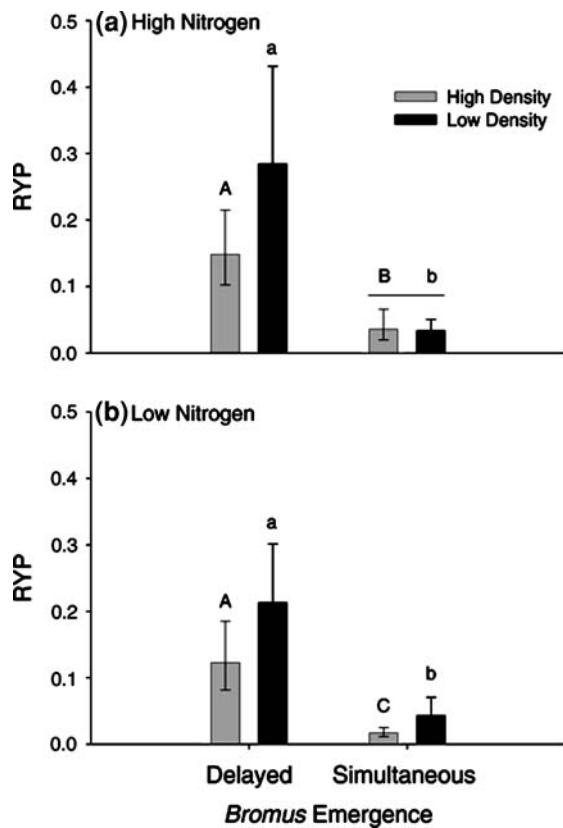


Fig. 3 Relative shoot yield of perennials (RYP) in high and low *Bromus* density treatments at simultaneous and late *Bromus* emergence times, separated by nitrogen addition treatment (mean \pm 1 SE). Horizontal lines indicate non-significant differences within a given nitrogen and emergence time treatment combination. Shared letters indicate non-significant differences within a given density of *Bromus* across nitrogen addition and emergence time treatment combinations

Discussion

This study gauged the importance of density and timing of emergence of an abundant annual grass on potentially coexisting perennial grasses under different levels of soil fertility. The annual grass competitor *Bromus* suppressed growth of all three perennial species relative to their growth in monoculture. As predicted, the level of suppression tended to increase with *Bromus* density and decrease when perennial grasses emerged before *Bromus*. Nitrogen addition increased the suppression of one native perennial by *Bromus*, while the other native perennial showed no response. Suppression of the exotic perennial by *Bromus* decreased with nitrogen addition.

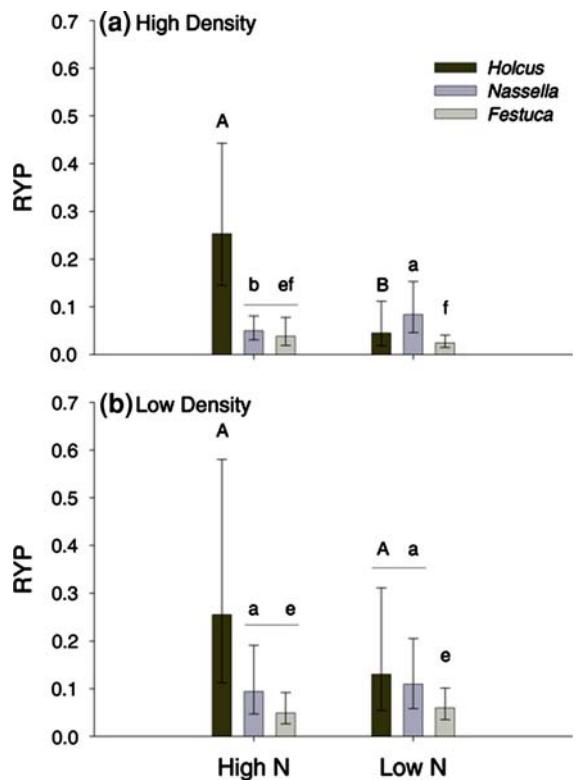


Fig. 4 Relative shoot yield of perennials (RYP) with and without nitrogen addition, separated by *Bromus* density treatment (mean \pm 1 SE). Horizontal lines indicate non-significant differences within a given density and nitrogen treatment combination. Shared letters indicate non-significant differences within a single species across all density and nitrogen treatment combinations

Order of emergence and density impacts on native perennial grass seedling growth

Both the native and exotic perennial grasses responded strongly to delays in *Bromus* emergence, increasing relative yield per plant (RYP) up to eight-fold (Fig. 3). The reduced *Bromus* density treatment similarly increased perennial grass RYP. The strength of these responses demonstrates the potential importance of the timing of emergence for perennial grass seedlings and supports the findings of previous studies in California grasslands and other semi-arid grassland systems that highlight the sensitivity of the seedling stage in perennial grasses (Bartolome and Gemmill 1981; Dyer et al. 1996; Dyer and Rice 1997; Clausnitzer et al. 1999; Hamilton et al. 1999; Brown and Rice 2000; Humphrey and Schupp 2004; Lenz and Facelli 2005). California native perennial grasses

must survive the summer drought, and accordingly, invest less in their aboveground growth or reproductive efforts in the first year than do exotic annual grasses (Jackson and Roy 1986; Holmes and Rice 1996). Annual grasses, on the other hand, must complete their life cycle before California's annual summer drought and so are among the earliest plants to germinate following the onset of the rainy season. The high seedling densities and rapid aboveground growth exhibited by exotic annual grasses create an intense competitive environment for later emerging seedlings (Jackson and Roy 1986; Corbin et al. 2007).

Priority effects have been reported in numerous systems across a broad range of taxonomic groups (e.g., Harper 1961; Lawler and Morin 1993; Shorrocks and Bingley 1994; D'Antonio et al. 2001; Kennedy and Bruns 2005). In California grasslands, established native perennial stands are generally resistant to invasion by exotic annual grasses (Seabloom et al. 2003; Corbin and D'Antonio 2004b; Lulow 2006), representing a priority effect manifested through arrival in a previous growing season. However, priority effects for exotic annual grasses competing with perennial seedlings occur over much shorter time scales. Previous work has shown that differences in emergence timing of even a few days can be enough to reverse competitive hierarchies between similar grass types (Harper 1961; Ross and Harper 1972; Rice and Dyer 2001). Some grasses, including *Nassella*, may even accelerate emergence in response to annual grass neighbors, presumably to minimize the impacts of later emergence (Dyer et al. 2000). Here, we demonstrate the strong priority effects that annual grasses can have on the seedlings of potentially coexisting perennial grasses; reversing the order of emergence of the grass types had a large effect on perennial grass seedling productivity, even when greatly outnumbered by an exotic annual grass.

Early emergence and high densities of exotic annual seedlings are obstacles that must be overcome for the restoration and maintenance of native-dominated grasslands (Stromberg et al. 2007). Successful restoration of native perennial grasslands often employs at least one of three strategies that improve perennial seedling performance: increasing native seed input (e.g., Seabloom et al. 2003), decreasing annual grass seed input/germination (e.g., Moyes et al. 2005), or planting plugs of native perennial grasses rather than starting them from seed (e.g.,

Dyer et al. 1996; Huddleston and Young 2004). The first two strategies reduce the relative abundance of exotic annual grasses, while the last, in effect, reverses the order of emergence of exotic annual and native perennial grasses.

Once established, some stands of native perennials are able to exclude annual grass invasion (Corbin and D'Antonio 2004b), and some studies suggest that native perennial grasses are stronger per-capita competitors than exotic annual grasses (e.g., Seabloom et al. 2003; DiVittorio et al. 2007). However, the high rate of soil turnover in California grasslands due to gopher and feral pig disturbance will create numerous opportunities for exotic annual grasses to compete with native perennial seedlings within restoration projects over the long-term (Hobbs and Mooney 1985; Kotanen 1995; Stromberg and Griffin 1996). Given that exotic annual propagule supply grossly exceeds that of native perennials, asymmetry in competition through emergence timing and density will likely limit the long-term ability of native perennial grasses to reestablish dominance in restored areas unless disturbance is minimized.

Nitrogen addition impacts on native perennial grass seedling growth

Differential responses to nitrogen addition between the two native perennial grass species point toward differences in their ability to access this resource amidst abundant exotic annual grass cover. *Nassella* in the monoculture treatment did not increase aboveground growth following nitrogen addition, which suggests that it was not nitrogen limited in this study. Alternatively, the response could have been reflected by reduced investment in belowground growth (Chapin 1980), which we did not measure in this study. However, *Nassella* root:shoot did not decrease with nitrogen fertilization in another study (Thomsen et al. 2006a). When nitrogen was added to *Nassella* grown with a high density of *Bromus*, *Nassella* experienced a relative decrease in aboveground growth. *Festuca*, on the other hand, did exhibit a positive growth response to nitrogen addition in monoculture, demonstrating that it was nitrogen limited. However, *Festuca* showed no response to nitrogen addition when grown with *Bromus*, suggesting that *Festuca* was unable to benefit from added nitrogen with neighboring annuals

present. The responses of both *Nassella* and *Festuca* are consistent with an indirect negative relationship between soil nitrogen and native perennial grass performance due to increased productivity of exotic annual grasses.

Our findings complement a number of studies in California and other semi-arid grasslands which found that exotic annual grasses benefit disproportionately from soil nitrogen supplementation relative to native perennial grasses (e.g., Huenneke et al. 1990; Maron and Connors 1996; Claassen and Marler 1998; Prober et al. 2002; Lowe et al. 2003; Abraham et al., unpublished data; but see Kolb and Alpert 2003). This suggests that native perennial grass seedlings are less likely to establish when faced with both exotic annual grass competition and persistent or increasing soil nitrogen enrichment. While this does not bode well for grassland restoration efforts, Seabloom et al. (2003) found that, with seed supplementation, perennial grass seedlings were capable of establishing in stands of exotic annual grasses across a gradient of nitrate availability. However, their exotic annual grass stands were not dominated by *Bromus diandrus*; it is possible that the identity of the annual grass competitor plays a role in determining the outcome of perennial grass seedlings. Corbin and D'Antonio (2004a) showed a first year reduction in annual grass suppression of native perennial seedlings after soil amendment with sawdust (to reduce available soil nitrogen), but found that there was no long-term benefit to the native grasses. Active management of soil nitrogen levels may prove to be less important for maintenance of native perennial dominated grasslands than reducing the density of exotic annual grass competitors (DiVittorio et al. 2007).

Comparison of exotic and native perennial grass seedling response

Productivity, tolerance of annual grass competition, and response to resource pulses differed significantly between native and exotic perennial grasses in this study. While each perennial species responded to the inclusion of *Bromus* with marked reductions in growth, *Holcus* tended to be less affected than either native perennial. Relative to *Festuca*, *Holcus* suffered a smaller overall reduction in growth in every treatment combination. In low nitrogen conditions,

Nassella RYP was equal to or greater than *Holcus* RYP. However, *Nassella* RYP was lower than that of *Holcus* at a higher soil nitrogen level. Additionally, *Holcus* productivity was much greater than that of either native perennial in monoculture (Fig. 2).

A field study of *Holcus* establishment in exotic annual grasslands found that *Holcus* seedling growth was not affected by competition with annual grasses (Thomsen et al. 2006b). Thomsen et al. (2006a) also demonstrated that *Holcus* has much higher initial relative growth rates than native perennial grasses, under both high and low nitrogen conditions. Individual size in grasses is often positively correlated with survival likelihood and competitive ability (Davies et al. 1999; Ewing 2002; Page and Bork 2005). As such, even if exotic annual grasses suppressed *Holcus*, *Festuca*, and *Nassella* equally, *Holcus* would likely be better able to compete with other grassland species due to its faster growth rate and larger size. Given the results of this study and that of Thomsen et al. (2006b), it is likely that *Holcus* seedlings better establish in exotic annual grass dominated stands than do native perennials.

Unlike the two native perennials, *Holcus* growth increased with additions of nitrogen both in monoculture and in combination with *Bromus*, suggesting that *Holcus* is a relatively strong competitor for soil resources with *Bromus*, at least when water is not limiting as in this study. This response was mirrored in a field study; elevated light and nutrient availability due to gopher disturbance increased *Holcus* colonization relative to an exotic annual, *Vulpia bromoides* (Pearl 1989c). *Holcus* also showed increased productivity with higher nitrogen availability while in competition with native perennial grasses (Schippers et al. 1999; Ewing 2002; Thomsen et al. 2006a); therefore, it appears to be not only highly responsive to nitrogen enrichment, but also able to access that nitrogen alongside a range of potential competitors.

Implications for management

California's annual-dominated grasslands have excluded native perennial grasses for decades, but in the coastal zone, they are often colonized by a number of exotic perennial bunchgrass species; this suggests that there are critical differences between traits of these native and exotic perennial grasses. In

this study, the native and exotic perennial grasses we investigated responded differently to both soil fertilization and competition with exotic annual grasses. Currently, common strategies for native grass management are based on mitigating the emergence of exotic annual grasses, and the associated negative effects of nitrogen enrichment that accompany their high density and rapid growth. However, management tools to specifically limit the encroachment of exotic perennial grass are largely undeveloped, and the efficacy of current management techniques on limiting their spread is not well known (Stromberg et al. 2007). Management practices that reduce annual grass seedling density could inadvertently increase the abundance of *Holcus*, particularly in areas with high soil nitrogen availability. As European perennial grasses become increasingly abundant and widespread in California coastal grasslands, land management based on generalizable life history differences among native perennial and exotic annual grasses will lose efficacy. Successful management of native grassland species will require continued research into the traits of a wider range of native and exotic bunchgrasses and their species-specific responses to current restoration practices and future environmental conditions.

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Do seed and microsite limitation interact with seed size in determining invasion patterns in flooding Pampa grasslands?

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Abstract Additive influences of the invasion ability of species (invasiveness) and the characteristics of the habitat which make it invasible (invisibility) cannot fully explain grassland invasion patterns. We tested the hypothesis that different species assemblages of grassland communities may partly result from interactive influences between the relative invasiveness of available species and community invisibility. During 10 months, we evaluated seed and microsite limitation of seedling emergence, survival, and recruitment of plants belonging to species with different seed size (large-seeded species vs. small-seeded species), with in a two-phase community mosaic typical of semi-natural grasslands in the southern flooding Pampa of Argentina. Seeds of large versus small-seeded species were sown either in species-poor patches dominated by a tall tussock grass (“pajonal”) or in species-rich patches dominated by short grasses (“matrix”), subjected to different levels of canopy disturbance (cut vs. uncut). Seed addition promoted seedling emergence for 7 out of the 10 species sown, and this

effect was higher for large than for small-seeded species. After seed limitations were removed, interactive effects among seed size, community state and canopy disturbance reflected a strong positive influence of seed size on plant recruitment only in cut pajonal patches. Therefore, according to the stage of invasion process, relative species success may depend on non-interactive (seed-size effect on seed limitation to seedling emergence) or interactive influences among species invisibility and community invasiveness (from seedling emergence to plant recruitment). As a general conclusion, different assemblages of species are expected to successfully colonize spatially close grassland patches, according to both the available invasible species (seed size) and the community state and stage (species composition and canopy disturbance).

Keywords *Paspalum quadrifarium* · Ground cover · Disturbance · Recruitment · Litter

Introduction

Research on colonization of plant communities by native or exotic species has commonly focused on species traits related with invasion ability (or invasiveness) (Kolar and Lodge 2001; Richardson and Pyšek 2006), the characteristics of the resident community that determine its susceptibility to invasion (invisibility) (Davis et al. 2000; Keane and Crawley

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2002; Paiaro et al. 2007), or the relative role of propagule versus microsite availability as key components of species invasiveness and community invasibility, respectively (e.g., Oesterheld and Sala 1990; Burke and Grime 1996; Lonsdale 1999; Moles and Westoby 2002). Two untested assumptions can be inferred from those studies: (a) species invasiveness and hence the key traits for defining invasiveness do not depend on resident community traits; and (b) plant community invasibility is independent of specific invader traits. However, additive influences of species invasiveness and community invasibility may not fully explain why different plant species assemblages are preferentially invaded by different species (Tilman 1997). Despite extensive efforts, identification of general traits providing for invasive capacity has been an elusive task, probably, because it results from different traits in different habitats (Alpert et al. 2000).

Despite of colonization and invasion terms are typically applied in reference to the occupation of areas by species already present (natives) or absent (non-natives or exotics) in the region, respectively (Alpert et al. 2000; Davis and Thompson 2000), and despite of all the species we studied here are natives or naturalized in the region, we will use the invasion term and its derivatives in all cases (native, exotic, and naturalized species), for simplicity.

Propagule availability is a key factor for plant invasion and species composition (Turnbull et al. 2000). Several authors have shown that local population density can be often increased by addition of seeds (Houle and Phillips 1989; Primack and Miao 1992; Eriksson 1998; Stampfli and Zeiter 1999). However, these studies did not specifically address changes in community-level parameters like species richness or canopy disturbance. Oesterheld and Sala (1990), Tilman (1997), and Zobel et al. (2000) not only analyzed the influence of seed availability on seedling emergence but also that of microsite conditions (i.e., species richness or canopy disturbance). They concluded that local limitations in both seed and microsite availability for plant recruitment are important forces generating small-scale community patterns in grasslands (Eriksson and Ehrlén 1992).

Seed mass is an extremely important trait for the establishment strategy of a plant species (Jakobsson and Eriksson 2000; Dalling and Hubbell 2002; Turnbull et al. 2004). The number of seeds a plant

can produce for a given amount of energy devoted to reproduction is inversely related to the mass of the seeds produced (Jakobsson and Eriksson 2000; Henery and Westoby 2001). The low seed output of the larger-seeded species may be compensated during seedling establishment, as seedlings from large seeds are generally better at tolerating stresses such as drought, defoliation, shade, and competition with other plants (reviewed in Leishman et al. 2000). Ground cover (living plants plus litter) is an important environmental factor that influences seedling emergence (Carson and Peterson 1990; Facelli and Pickett 1991; Isselstein et al. 2002) as well as survival (Isselstein et al. 2002; Emery and Gross 2006) and may control the existence of “safe sites” for plant recruitment (Harper 1977; Xiong et al. 2003). Therefore, the reduction of seedling emergence by ground cover should be more severe for species with small seeds than for species with large seeds. Small seeds are more likely to be shaded or covered by ground cover than large seeds, and shading may inhibit seed germination (Rice 1985; reviewed in Leishman et al. 2000). Besides, large seeds could be more easily detected by seed eaters than small seeds, so that granivores may differentially affect emergence patterns according with species seed size (Reader 1993). Overall, small-seeded species are therefore expected to be constrained by microsite rather than seed availability (since they can only colonize suitable patches not reached by a larger-seeded species), whereas large-seeded species would be more strongly limited by seed availability (Turnbull et al. 1999; Moles and Westoby 2002).

In this paper, we tested the hypothesis that different species assemblages in grasslands may partly result from interactive influences between relative invasiveness of available species and community attributes. Specifically, we evaluated the prediction that once recruitment limitations derived from seed availability are removed, different components of relative species invasiveness (emergence precocity, emergence success, and seedling survival) change with community structure and canopy disturbance.

We analyzed for 10 months different components of species invasiveness (emergence precocity, emergence success and survival, and recruitment) for 10 species differing in seed size in a semi-natural grassland of the southern flooding Pampa in Argentina. Temperate Pampa grasslands are considered to

be particularly susceptible to exotic forb invasions as a result of grazing by domestic ungulates (Mack 1989). Grassland disturbance by large herbivores has a significant impact on vegetation structure in the Pampas (Sala et al. 1986; Facelli 1988; Insausti et al. 1999; Altesor et al. 2005). Little is known, however, about how plant community structure, canopy disturbance, and seed availability interact to determine invasion processes in these grasslands. Moreover, mechanisms responsible for differential recruitment of different grassland species are still poorly known (but see Chaneton et al. 2002).

Methods

Site description

The experiment was carried out in natural grasslands of the southeast Pampa of Argentina (37° S, 58° W). The study site was used for cattle grazing under a continuous stocking rate and was subjected to winter burnings but was never ploughed or fertilized. The climate is humid temperate, with a mean annual precipitation of 1,000 mm and a mean annual temperature of 14°C , ranging from 6.8°C in July to 21.8°C in January (Soriano et al. 1991). Soils are characterized by moderate to high alkalinity levels, phosphorous deficiency, and poor drainage (Berasategui and Barberis 1982).

Vegetation consisted of a two-phase mosaic comprising two community states ranging 5–50 m in diameter. Tall-tussock grass patches (hereafter, “pajonal”) co-dominated by the native grasses *Paspalum quadrifarium* and *Paspalum exaltatum* were characterized by a low species number and were scattered within a species-rich matrix of short grasses (up to 0.4 m tall). The latter community state have been variously classified as humid prairies (dominated by *Leersia hexandra*, *Mentha pullegium*, and *Solanum malacoxylon* among other species), or halophytic steppes (dominated by *Distichlis spicata*), reflecting variation in local soil properties (Vervoorst 1967; Perelman et al. 2003). *P. quadrifarium* and *P. exaltatum* are perennial bunch grasses that form dense and compact tussocks characterized by a tall canopy (up to 1.5 m) and flowering stalks up to 2 m tall, high ratio of dead to alive standing biomass, and a thick litter layer (Laterra et al. 2003). The pajonal is

a fire-prone grassland and current grazing management requires occasional burnings to improve forage production and quality. Grazing is concentrated in the short-grass matrix; cattle feed on pajonal patches only for a short period during post-fire resprout of *Paspalum* tillers (Laterra et al. 2003). However, several fire and crop cycles can remove the pajonal and then gave way to short-grass matrix alternative stable state (Laterra et al. 1998). Other important sources of disturbance at the study site are winter floods and small-mammal burrowing (Insausti et al. 1999, 2005; Chaneton 2006).

Study species

In mid summer (February) 2001, ripe seeds from 19 of the most common grassland species were collected within 2 km from the study site. The germination ability of all species was tested under controlled conditions (150 seeds/species in three replicates, $20\text{--}30^{\circ}\text{C}$, 16 h photoperiod), and 10 species were finally chosen for study, based on their high germinability (Table 1). The chosen species were representative of different functional groups in the local species pool. They offered the largest amount of ripe and germinable seeds during the period when most species were still dispersing seeds (Faggi and Collantes 1978). Nomenclature follows “Catálogo de Plantas Vasculares de la Argentina” (<http://www.darwin.edu.ar>).

Experimental design

A split-split-plot design was applied on six experimental areas (blocks), each comprising two adjacent (paired) pajonal and matrix patches 100 m apart from each other. Within each community patch, a 4×5 m plot (main plots) was fenced (1.50 m height) to prevent cattle trampling but still allowing defoliation. In order to reduce soil and topographic differences, the distance between plots within each block varied between 2 and 5 m. Two sub-plots (2×5 m each one) were defined within each main plot on December 9, 2001, the canopy within one sub-plot was cut to simulate cattle grazing at modal height (4–5 cm and 6–7 cm above soil level, for matrix and pajonal patches, respectively; hereafter, cut treatment), whereas the other one was left intact (hereafter, uncut treatment). It is important to note

Table 1 Species included in the recruitment experiment, some aspects of their biology, and number of seeds sown per experimental plot

Species	Family	Longevity	Origin	Phenology	Seed mass ^a (mg)	Germination test (%)	Seeds/plot
<i>Aster squamatus</i>	Asteraceae	Perennial	Native	Warm-season	0.058	72.50	125
<i>Bromus catharticus</i>	Poaceae	Perennial	Native	Cold-season	9.999	87.33	100
<i>Bromus mollis</i>	Poaceae	Annual	Exotic	Cold-season	1.840	99.33	100
<i>Eragrostis retinens</i>	Poaceae	Perennial	Native	Warm-season	0.082	85.33	100
<i>Eryngium eburneum</i>	Umbelliferae	Perennial	Native	Warm-season	0.810	42.00	200
<i>Hordeum pusillum</i>	Poaceae	Annual	Native	Cold-season	2.189	47.33	200
<i>Lotus glaber</i>	Fabaceae	Perennial	Exotic	Warm-season	1.014	87.33	125
<i>Poa lanigera</i>	Poaceae	Perennial	Native	Cold-season	0.240	44.67	200
<i>Rumex crispus</i>	Asteraceae	Perennial	Exotic	Cold-season	0.880	93.00	100
<i>Sporobolus indicus</i>	Poaceae	Perennial	Native	Warm-season	0.259	30.00	60+

^a Correspond to the seed mass. Seeds/plot: total sown seeds by sub-sub-plot

+ Because of the lack of seeds only 60 seeds of *Sporobolus indicus* were sown. Nomenclature follows the Catálogo de Plantas Vasculares (<http://www.darwin.edu.ar>)

that cattle avoid mature *Paspalum* spp. tussocks, although after the pajonal is burned young tillers become intensely grazed. We intended to simulate an intense disturbance of the pajonal canopy so as to remove most of the old aboveground tissues, thus promoting new tillers. Our cutting treatment produced large bare-ground gaps (70–80% of total area) in-between the base of tussocks.

Within each sub-plot, 20 sowing areas (sub-sub-plots) were defined by using a 10 cm diameter PVC pipe inserted 2 cm deep into the soil, leaving a 3 cm tall barrier above the soil surface. These barriers precluded seed loss from secondary dispersion (by wind or water), while allowing access to seed predators. Sub-sub-plots were regularly placed on a 1 × 4 m grid. In the matrix patches (basal cover, ca. 90%), sub-sub-plots always included rooted plants, whereas in the pajonal patches (basal area, 20–30% covered by *Paspalum* spp.), sub-sub-plots were always located in bare ground. Immediately after cutting (December 10–11, 2001), seeds of the 10 study species were randomly sown in the 10 sub-sub-plots, one species per sub-sub-plot. Sowing densities were varied among species to adjust for species-specific differences in germinability (Table 1). Sown seeds were put in contact with the soil surface. The remaining 10 sub-sub-plots within each sub-plot were left as unsown controls. Since the fences of two blocks were broken and the short-grass matrix severely disturbed by livestock, only the remaining four blocks were included in this study.

The sub-plots were characterized in terms of canopy height, which was measured in each sub-plot by vertically introducing a tape measure through the canopy at five random points. Although cows were allowed to graze the plots through the fences, vegetation height within uncut sub-plots remained higher than in cut sub-plots throughout the experiment: December: cut pajonal = 4.0 ± 0.6 cm, uncut pajonal = 90.2 ± 5.5 cm, cut matrix = 2.3 ± 0.3 cm, uncut matrix = 35 ± 6.2 cm; April: cut pajonal = 43.3 ± 6.1 cm, uncut pajonal = 88.7 ± 4.1 cm, cut matrix = 11.7 ± 1.3 cm, uncut matrix = 28.6 ± 2.9 cm; August: cut pajonal = 63.7 ± 4.2 cm, uncut pajonal = 93.9 ± 6.2 cm, cut matrix = 11.0 ± 1.6 cm, uncut matrix = 17.7 ± 2.1 cm. The interception of photosynthetically active radiation (PAR) was also measured at five random points in each sub-plot, by placing a photon flux sensor (LI-COR Li-188 B) below the canopy at ground level: February: cut pajonal = 30.2 ± 1.1%, uncut pajonal = 99.6 ± 0.1%, cut matrix = 4.1 ± 2.0%, uncut matrix = 38.0 ± 6.9%; October: cut pajonal = 30.9 ± 9.4%, uncut pajonal = 99.8 ± 0.03%, cut matrix = 9.3 ± 1.2%, uncut matrix = 56.7 ± 5.2%.

Monitoring seedling emergence and survival

All emerging seedlings and established plants were counted within sown and unsown sub-sub-plots at four dates through a 10-month study period (December 20 and 26, 2001; 23 and 24 January; 10, 11 and

17 April, 9 May; 16 and 27 September, 2002). By frequent monitoring of experimental plots, we were able to confirm that seedling emergence and mortality for the study species were sharply segregated in time. No newly emerged seedlings were recorded after mid January; dead seedlings were only found after that time. None of the study species reached reproductive maturity within the experimental plots. The third count extended over two months due to flooding conditions, which did not allow access to some blocks. However, differences in sampling dates were controlled for using the block effect in statistical analyses. Flooding conditions did not directly influence the sown plots and thus had no bearing on seedling dynamics. With the aim of homogenizing the competitive environment, and because of the high variability in seedling emergence observed in the first sampling date, the number of seedlings per sub-subplot was set to 20 by thinning the excess seedlings after each count. Thinning focused on the smallest (subordinate) seedlings found in each sub-sub-plot having a seedling surplus, and performed by cutting seedlings at soil level to avoid soil disturbance. Seedling monitoring in the pajonal patches was done by temporarily removing the thick litter layer present in-between the grass tussocks.

Although individual seedlings were not marked, the temporal segregation observed between emergence and mortality periods allowed us to estimate demographic process rates on the basis of the total number of seedlings counted on each sampling date, as described below. Nevertheless, it is quite possible that this procedure somewhat underestimated the seedling emergence or mortality due to complete disappearance of preyed seedlings before the second counting date. A non-random distribution of this error source among study species cannot be discarded.

Cumulative seedling emergence (e) in both sown and unsown treatments was calculated for each sub-sub-plot as:

$$e = e_0 + \sum (e_{i+1} - e_i), \quad (1)$$

where e_0 is the number of seedlings in the first count, e_i is the number of seedlings in the count i , and the difference between successive counts was only taken into account when it was greater than 0.

Emergence success (E) from sown seeds was calculated for each sub-sub-plot as:

$$E = (e - e_c) * s^{-1} * g^{-1}, \quad (2)$$

where e and e_c are the mean cumulative seedling emergence for sown and unsown sub-sub-plots within a given sub-plot, respectively, s is the number of sown seeds, and g is the germinable fraction of sown seeds (Table 1).

Emergence precocity (P) was estimated through the relative contribution of seedlings that emerged during the first observation period of the cumulative seedling emergence (e , Eq. 1):

$$P = e_0/(e). \quad (3)$$

Seedling survival (S) data were calculated as the percentage of plants (seedlings and/or later development stages) that survived by the end of the experiment:

$$S = (e_f/(e - e_r)), \quad (4)$$

where e (Eq. 1) is the cumulative emergence, e_f is the number of plants in the last record, and e_r is the cumulative number of seedlings removed after the first three counts.

The transition probability from seed to established plant for added seeds, hereafter referred to as “recruitment” (R), was calculated by multiplying E (Eq. 2) times S (Eq. 4).

Data analysis

A split-split-plot analysis of variance (ANOVA) was performed to test the effects of seed addition, community state, canopy disturbance, species, and seed size on the cumulative emergence (e), with main plots corresponding to community states (pajonal vs. matrix), sub-plots corresponding to disturbance treatment (cut vs. uncut), sub-sub-plots corresponding to seed addition (sown vs. unsown), and sub-sub-sub-plots corresponding to individual species (as listed in Table 1) or species grouped by seed size (small vs. large seed size). Species were classified into small or large seed size categories according with their mean seed mass (small seeded: 0.06–0.259 mg/seed vs. large seeded: 0.81–9.99 mg/seed). We selected the four species with smaller seed size corresponding to the 30th percentile of the seed size distribution. To test for the effect of community state, disturbance, and species or seed size species group on seedling

emergence precocity (P), success (E), survival (S), and recruitment (R), only data from germinable sown seeds in the seed addition treatment were analyzed, so that species and species grouped by seed size were considered as sub-sub-plots. Multiple post hoc comparisons were performed using LSD (least significant difference) tests ($\alpha = 0.05$). Data on emergence success were log transformed, and data on emergence precocity and survival were angularly transformed to approximate normality. Statistical analyses were performed with the SYSTAT 5.0 statistical package (SYSTAT 1992).

Results

Species responses to seed addition

Seed addition increased seedling emergence (e) for 7 out of the 10 species sown ($F_{9,216} = 14.08$, $P_{\text{seed addition} \times \text{species}} < 0.001$, Table 2). While species showed different e in sown treatments depending on the community state and disturbance treatment, cumulative emergence of study species in the unsown treatment was extremely low or nil (Table 3). Overall, the mean response to seed addition of e was four times greater in pajonal than in matrix patches, but did not significantly differ between disturbance treatments (seed addition \times disturbance) (Table 4). The response to seed addition of large-seeded species was greater than that of small-seeded species (seedlings emerged in sown plots, mean \pm SE, large-seeded species:

Table 2 Cumulative seedling emergence (e) (means \pm 1SE, $n = 16$ plots) of the 10 studied species in sown and unsown plots in a tall-tussock grassland of the flooding Pampa of Argentina

Species	Sown	Unsown
<i>Aster squamatus</i>	2.00 ± 1.64	0.01 ± 0.004
<i>Bromus catharticus</i>	18.07 ± 6.50	0.00 ± 0.00
<i>Bromus mollis</i>	13.56 ± 4.39	0.00 ± 0.00
<i>Eragrostis retinens</i>	0.31 ± 0.31	0.00 ± 0.00
<i>Eryngium eburbeum</i>	7.31 ± 2.41	0.03 ± 0.02
<i>Hordeum pusillum</i>	18.00 ± 5.46	0.01 ± 0.001
<i>Lotus glaber</i>	17.25 ± 3.63	0.66 ± 0.29
<i>Poa lanigera</i>	3.00 ± 1.27	0.00 ± 0.00
<i>Rumex crispus</i>	7.83 ± 2.63	0.02 ± 0.02
<i>Sporobolus indicus</i>	0.00 ± 0.00	0.00 ± 0.00

13.7 ± 1.8 , small-seeded species: 1.3 ± 0.5), as reflected by a significant interaction between seed addition and species seed size (Table 4). However, there were no significant higher-order interactions between seed size and seed addition with either community state or disturbance treatment (Table 4).

Community state and disturbance effects

Species responses to community state and canopy disturbance varied according with the different component of species invasiveness being considered. While emergence precocity (P) was three times higher in uncut than in disturbed pajonal patches, it was nil in the short-grass matrix for both disturbance treatments (Fig. 1a, Table 5). Community state, but not disturbance treatment, affected emergence success (E), with E being higher in pajonal than in matrix patches (Fig. 1b, Table 5). Overall seedling survival (S) in the pajonal was three times higher in cut than in uncut patches, whereas cutting treatment did not affect S in matrix patches (Fig. 1c, Table 5). Although a large number of seedlings emerged in the uncut pajonal, none survived after the second count. Overall recruitment (R) was higher in disturbed pajonal than in the other treatment combinations (Fig. 1d, Table 5).

Large-seeded species emerged more precociously (P) and were more successful (E) than small-seeded species, and this advantage was higher in pajonal than in matrix patches (Fig. 2a, b), regardless of canopy disturbance (Table 5). Cutting had a positive influence on seedling survival for large-seeded but not for small-seeded species (Fig. 2c, Table 5), irrespective of community state. Species with large seeds (particularly *Rumex crispus* and *Hordeum pusillum*) recruited more successfully than small-seeded species, and this difference was most pronounced in disturbed pajonal patches (Fig. 2c, Table 5). As a result of the patterns described, the ranking of species as reflected by R varied depending on community state and disturbance treatment (Fig. 3).

Discussion

In agreement with our prediction, once seed limitations are removed (a) the relative invasiveness (plant recruitment) of study species and species groups varied depending both on the community state and

Table 3 Cumulative seedling emergence (e) (means \pm 1SE, $n = 4$ plots) of the 10 studied species in sown and unsown plots of tall grass “pajonal” and short-grass matrix community patches in the flooding Pampa of Argentina

Species		Cut pajonal	Uncut pajonal	Cut matrix	Uncut matrix
<i>Aster squamatus</i>	Sown	0.00 \pm 0.00	8.00 \pm 6.16	0.00 \pm 0.00	0.00 \pm 0.00
	Unsown	0.00 \pm 0.00	0.00 \pm 0.00	0.02 \pm 0.012	0.00 \pm 0.00
<i>Bromus catharticus</i>	Sown	27.00 \pm 14.15	43.05 \pm 14.78	2.00 \pm 1.22	0.25 \pm 0.25
	Unsown	0.00 \pm 0.00	0.03 \pm 0.03	0.00 \pm 0.00	0.00 \pm 0.00
<i>Bromus mollis</i>	Sown	21.00 \pm 10.50	32.75 \pm 6.07	0.50 \pm 0.50	0.00 \pm 0.00
	Unsown	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
<i>Eragrostis retinens</i>	Sown	1.75 \pm 1.25	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	Unsown	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
<i>Eryngium eburbeum</i>	Sown	6.75 \pm 4.50	0.00 \pm 0.00	7.50 \pm 2.06	15.00 \pm 7.38
	Unsown	0.16 \pm 0.08	0.00 \pm 0.00	0.01 \pm 0.01	0.01 \pm 0.01
<i>Hordeum pusillum</i>	Sown	22.25 \pm 13.73	43.50 \pm 4.79	2.50 \pm 1.26	3.75 \pm 3.75
	Unsown	0.00 \pm 0.00	0.00 \pm 0.00	0.02 \pm 0.012	0.01 \pm 0.01
<i>Lotus glaber</i>	Sown	21.25 \pm 6.61	22.25 \pm 5.09	11.75 \pm 6.50	13.75 \pm 5.44
	Unsown	0.08 \pm 0.04	0.00 \pm 0.00	0.86 \pm 0.36	1.56 \pm 0.69
<i>Poa lanigera</i>	Sown	5.00 \pm 3.00	7.00 \pm 3.24	0.00 \pm 0.00	0.00 \pm 0.00
	Unsown	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
<i>Rumex crispus</i>	Sown	21.75 \pm 6.61	0.00 \pm 0.00	4.75 \pm 1.03	4.75 \pm 1.44
	Unsown	0.08 \pm 0.08	0.00 \pm 0.00	0.06 \pm 0.05	0.01 \pm 0.01
<i>Sporobolus indicus</i>	Sown	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	Unsown	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

Table 4 ANOVA test of different factors and their interaction on cumulated emergence (e) of large and small-seed size species in response to seed addition and in different combinations of community patches and disturbance treatments in a tall-tussock grassland of the flooding Pampa of Argentina

Source of variation	Df	F	P
Community state (C)	1.280	1.41	0.234
Disturbance (D)	1.6	9.68	0.021
Seed addition (SA)	1.12	143.07	<0.001
Seed size (SS)	1.280	0.15	0.373
C \times D	1.6	0.02	0.881
C \times SA	1.6	11.08	0.016
C \times SS	1.280	0.15	0.702
D \times SA	1.12	0.01	0.926
D \times SS	1.280	0.797	0.373
SA \times SS	1.280	57.56	0.001
C \times D \times SS	1.280	0.39	0.531
C \times D \times SA	1.12	0.10	0.755
C \times SA \times SS	1.280	1.09	0.298
D \times SA \times SS	1.280	0.71	0.401
C \times D \times SA \times SS	1.280	0.19	0.657

P-value-level of significance ($P < 0.05$ printed in bold)

canopy disturbance, and (b) the invasibility responses to community states and disturbance stages was species and functional group (according to seed size) specific. Except for four species that failed to recruit (Fig. 3), different components of plant recruitment including seed availability, emergence success, and seedling survival contributed differentially to species invasiveness, according with species identity, seed size, and resident community structure.

Overall species responses to seed addition

In accordance with previous studies in grasslands where seed addition increased plant diversity (Tilman 1997; Zobel et al. 2000; Foster and Tilman 2003), recruitment of several study species was strongly seed limited. Limitations in seed availability are common in many plant communities (Turnbull et al. 2000). This pattern is relevant to conservation and restoration efforts because processes that currently affect plant population abundance (e.g., habitat fragmentation) also reduce soil seed banks (Bakker and Berendse 1999; Cox and Allen 2008).

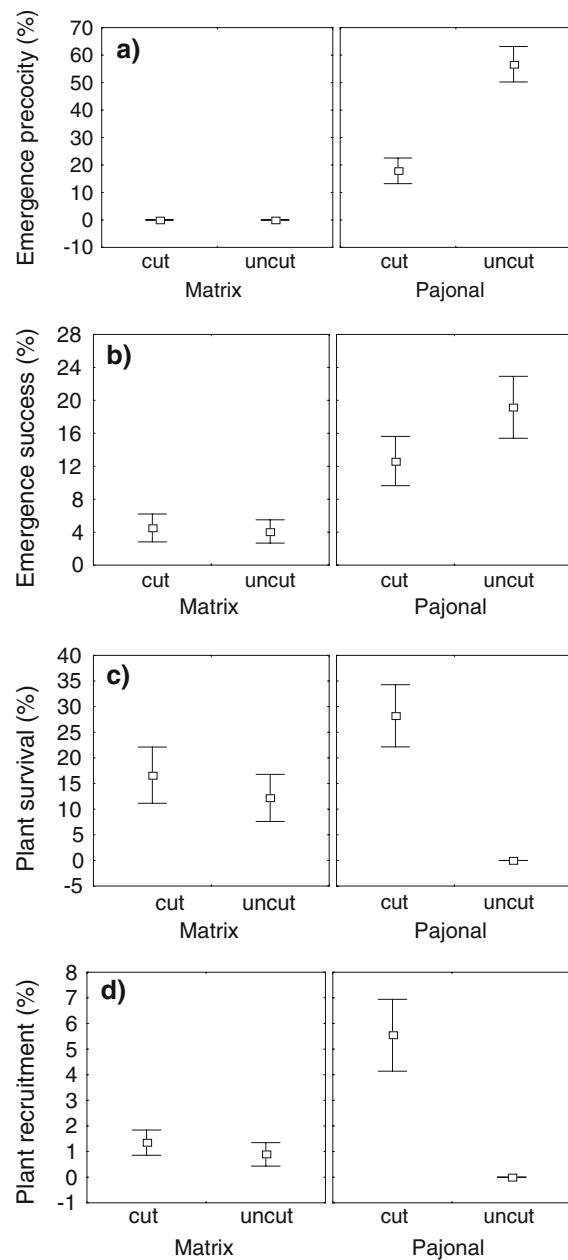


Fig. 1 Cumulative seedling emergence (emergence precocity) (a) emergence success expressed as percentage of sown seeds (b) plant survival, the percentage of emerged seedlings that survived until the last count (c), and plant recruitment expressed as percentage of sown seeds (d) according to community state (pajonal and matrix) and disturbance treatment (cut and uncut). Points represent the mean and bars indicate 1 standard error ($n = 40$)

Community patches that are initially poor in species may remain poor if there is insufficient arrival of new diaspores from their close neighborhood, while

species-rich patches may be maintained by dispersal from a diverse local species pool (Loreau and Mouquet 1999). Whereas seed addition to the species-rich matrix patches represented an “augmentation” experiment, as sown species were already present in the target community, seed addition to the species-poor pajonal can be considered an “introduction” experiment since, unless disturbed, sown species are not normally members of pajonal patches (see Turnbull et al. 2000). Assuming equilibrium conditions, biotic and abiotic conditions are not expected to represent a fundamental barrier for the recruitment of resident species but for the recruitment of non-resident species (Turnbull et al. 2000). In the present study, when equilibrium conditions were altered by removed canopy disturbance, species sown in the species-poor pajonal patches resulted more limited by seed availability than species sown in the matrix.

Colonization strategy theory predicts that recruitment limitation by seed availability should be more common among large-seeded species than among small-seeded ones (Turnbull et al. 1999). Our results agree with this prediction and other empirical evidences (Moles and Westoby 2002). While species with small seed size did not show increased emergence in response to seed addition, the cumulative emergence of large-seeded species (*Bromus mollis*, *B. catharticus*, *Eryngium eburneum*, *Hordeum pusillum*, *Lotus glaber*, and *Rumex crispus*) was indeed promoted by seed sowing, and their recruitment did not substantially differ among different combinations of community states and disturbance treatments.

Community invasibility

Our results showed a high inter-patch variation and community specific effects on emergence precocity (Fig. 1a), emergence success (Fig. 1b) plant survival (Fig. 1c) and, consequently, on plant recruitment (Fig. 1d). We found a strong contrast of response variables between the pajonal and the matrix. The uncut pajonal and the richest community state (short-grass matrix) presented the lowest overall recruitment rates (Fig. 1d).

In the presence of an undisturbed canopy, suppression of seed germination is generally indicated as the primary mechanism responsible for low invasion rates and low species richness (Kitajima and Tilman 1996). However, in this experiment, disturbance

Table 5 ANOVA tests on different factors and their interaction on seed emergence precocity (*P*), emergence success (*E*), seedling survival (*S*), and seedling recruitment (*R*) of large and

small-seed size species in different combinations of community patches and disturbance treatments in a tall-tussock grassland of the flooding Pampa of Argentina

Source of variation	Emergence precocity			Emergence success			Seedling survival			Seedling recruitment		
	Df	F	P	Df	F	P	Df	F	P	Df	F	P
Community (C)	1.3	24.46	0.016	1.3	10.35	0.049	1.3	0.05	0.831	1.3	3.69	0.15
Disturbance (D)	1.6	7.94	0.03	1.6	1.60	0.250	1.6	12.36	0.013	1.6	31.07	0.001
Seed size (SS)	1.140	13.32	0.001	1.140	44.31	0.001	1.140	21.24	0.001	1.140	12.63	0.001
C × D	1.6	7.94	0.03	1.6	2.18	0.190	1.6	7.85	0.003	1.6	20.74	0.004
C × SS	1.140	13.32	0.001	1.140	5.77	0.018	1.140	1.18	0.279	1.140	0.12	0.731
D × SS	1.140	0.33	0.611	1.140	0.05	0.828	1.140	4.44	0.037	1.140	5.12	0.025
C × D × SS	1.140	0.33	0.611	1.140	0.15	0.700	1.140	2.01	0.159	1.140	4.02	0.047

P-value-level of significance (*P* < 0.05 printed in bold)

influence and thus microsite limitation were only expressed after seed addition. Therefore, the responses of different recruitment components (emergence success and survival) as well as the emergence rate were associated with disturbance (Fig. 1a–c, Table 5). Whereas, none of the species germinated at the first count date under the uncut matrix, many species germinated better under the thick litter layer of uncut pajonal patches. However, overall species recruitment was higher in cut pajonal. Recruitment components showed different responses in different experimental conditions. Although emergence success was greater in the pajonal regardless of disturbance (Table 5), seedling survival of the species was greater in the cut pajonal (Table 5). Simultaneous seed supply and disturbance occurrence was a necessary condition for any plant recruitment in the pajonal, where high invasibility was primarily reflected by enhanced seedling emergence in response to seed additions, while recruitment success greatly depended on canopy disturbance. Invasion of pajonal communities from seed after severe disturbances (e.g., fire) has been previously described (Laterra 1997; Laterra et al. 1994, 2003; Juan et al. 2000; Ortega and Laterra 2003) and suggested to reflect the relative inability of dominant tussock grass species to rapidly occupy bare-ground gaps after intense disturbances (Laterra et al. 1994, 2003; Ortega and Laterra 2003; Perelman et al. 2003).

Potential mechanisms accounting for the observed differences in recruitment components between the pajonal versus the matrix may include both abiotic and biotic factors. It is well known that soil temperature varies in a wider range under crowded

and undisturbed canopies than under open and disturbed ones; this fluctuation serves as a common signal for the germination of many species (Laterra et al. 1994). Pajonal soils are generally wetter than matrix soils due to low evapotranspiration from undisturbed pajonal patches (Sakalauskas et al. 2001). In contrast, the short-grass matrix composed by a mix of cool- and warm-season species usually shows water deficits, particularly during the summer (Sala et al. 1981). The size of the physical disruption not only dictates the amount of physical space, but also influences greatly the type and amount of resources available for exploitation (McConaughay and Bazzaz 1987). In contrast with the complete or almost complete covering of soil by the pajonal canopy, the basal area of the *Paspalum* spp. tussocks accounts for no more than 40% of the total area (Laterra et al. 1994, 2003). Although the availability of PAR is much more limited in the pajonal than in the matrix, the harvest implies a much greater change for the former than for the latter.

Many studies have shown the strong adverse effect of a litter layer on seedling emergence and recruitment (Carson and Peterson 1990; Isselstein et al. 2002; Emery and Gross 2006), as observed in the pajonal patches (Ortega and Laterra 2003). Litter in uncut pajonal prevents soil surface from being desiccated during dry periods (Pers. Obs.) thus making it a more favorable site for seedling emergence. Despite of the successful emergence under pajonal canopy, the physical interception that impedes seedling from accessing to light before reserves are depleted (Harper 1977; Facelli 1994) renders seedling survival unlikely. The cut pajonal height almost equalled that of the

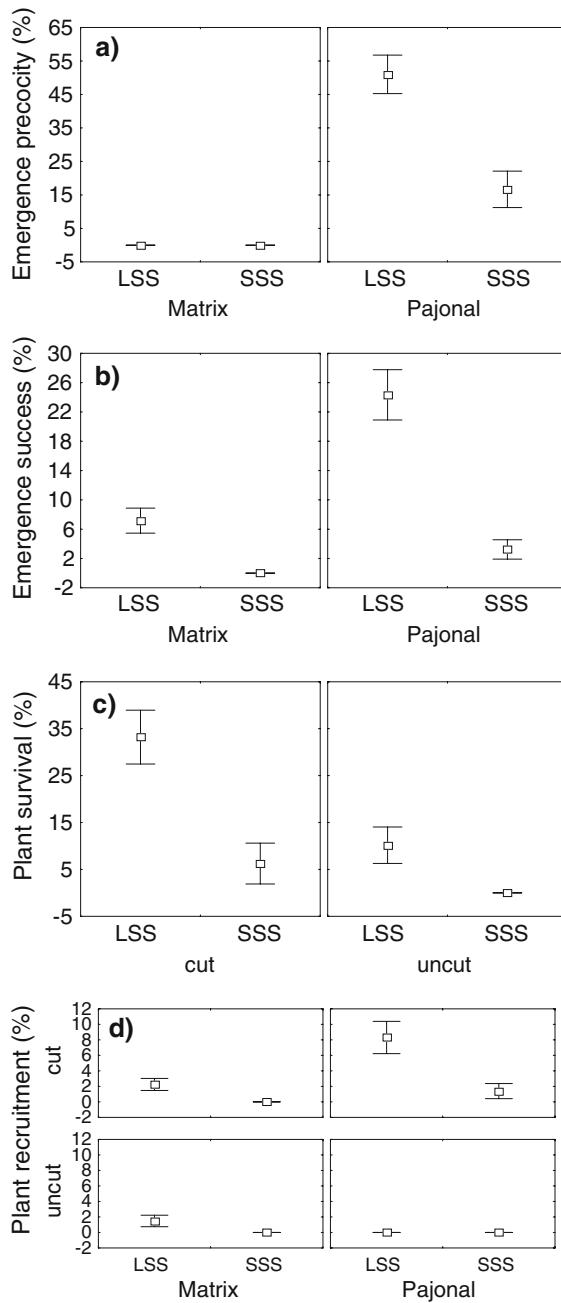


Fig. 2 Cumulative seedling emergence (emergence precocity) (a) and emergence success expressed as percentage of sown seeds (b) for different species, and according to their seed size in the different community states; percentage of emerged seedlings of different size that survive until the last count in the different community states and disturbance treatments (c), and plant recruitment expressed as percentage of sown seeds for species according to their seed size in the different community states and disturbance treatments (d). Cut: cutting treatment, uncut: uncutting treatment, SSS: small seed size, LSS: large seed size. Points represent the mean and bars indicate 1 standard error

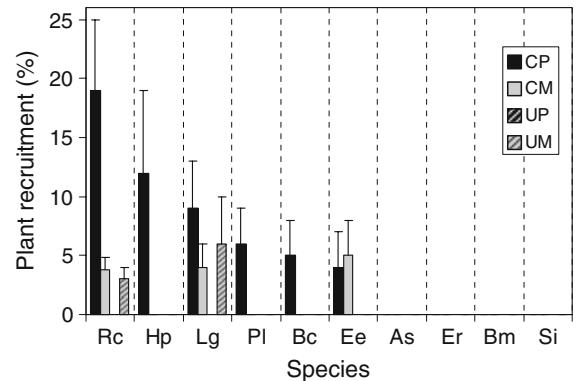


Fig. 3 Plant recruitment expressed as percent of available seeds according to species for different treatments. CP: cut pajonal, CM: cut matrix, UP: uncut pajonal, UM: uncut matrix; Rc: *Rumex crispus*, Hp: *Hordeum pusillum*, Lg: *Lotus glaber*, Pl: *Poa lanigera*, Bc: *Bromus catharticus*, En: *Eryngium eburneum*, As: *Aster squamatus*, Er: *Eragrostis retinens*, Bm: *Bromus mollis*, Si: *Sporobolus indicus*

uncut pajonal at the end of the experiment. Apart from the matrix, there were few but significant differences in vegetation height between cut and uncut treatments. Although the treatments were fenced and plots could not be trampled, cows were able to reach all the sub-sub-plots with their heads and grazed the experimental units. Differential preference by cattle of pajonal and matrix plots determined contrasting rates of canopy recovery, modifying the microsite conditions throughout the 10 months of the experiment. The mentioned conditions at the end of the experiment could be responsible for different emergence precocity, seedling survival, and plant recruitment patterns in both plant communities.

Seed consumption by invertebrates and small vertebrates may be important in temperate grasslands (Pérez et al. 2006), and lower emergence success in the matrix could have been explained by this mechanism. However, results of an experiment conducted at the same study site revealed that rates of seed predation by small vertebrates and invertebrates in the matrix patches are consistently very low (Murillo et al. 2007). Thus, we suggest that microsite availability would be a limiting factor for seedling recruitment in the matrix grassland.

Species invasiveness

Most of the benefits of large seed size species are expected to be manifested during the establishment

phase (Leishman et al. 2000; Westoby et al. 2002). Although seed banks of large-seeded species are generally smaller than small-seeded species (Aarssen and Jordan 2001; Henery and Westoby 2001), these species would be more tolerant to threats taking place during this phase: competition of established vegetation, intensity of shaded vegetation, defoliation, lack of mineral nutrients, droughts, and burial in the ground or under accumulated litter (Leishman et al. 2000; Westoby et al. 2002). In our experiment, this pattern was explained by a higher emergence precocity (Fig. 2a), a higher emergence success (Fig. 2b), a higher plant survival (Fig. 2c), and then plant recruitment (Fig. 2d) for large-seeded than for small-seeded species. The inhibitory effect of ground cover on seedling emergence has been suggested to be inversely related to seed size (Facelli and Pickett 1991; Reader 1993). However, in the present work, uncut treatment did not affect the recruitment of small-seeded species (Fig. 2d), and cut canopy promoted the recruitment of large-seeded species in the same way that the uncut treatment did (Fig. 2d). Thus, results obtained in this work suggest that differences in seed size may help to explain differential species invasiveness across different combinations of resident community structure and disturbance regime. Likewise, the differential invasibility of the studied community states should not be considered without taking into account the seed size of the available species.

Concluding remarks

Our study provides new evidence about the influence of community structure and disturbance on the invasion dynamics of flooding Pampa grasslands by species with different seed size. Invasion processes in the study grassland are not a simple consequence of species invasiveness and seed availability nor of community attributes conferring a generalized level of invasion resistance. We conclude that particular assemblages of grassland colonizer species result from interactions between the invasiveness of available species and the invasibility of available community states and their disturbances stages.

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Feral horses dung piles as potential invasion windows for alien plant species in natural grasslands

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Abstract Small scale disturbances could act as patches that provide sites for the colonization of competitively inferior species, promoting the establishment of non-native species in some cases. We analyzed the vegetation associated with feral horse dung piles in montane pampas grasslands in Mid-East Argentina and described the changes following their abandonment, evaluating whether dung piles act as invasion windows, allowing the entrance of alien plant species. We estimated the portion of the study area directly covered by horse manure and dung height was used to estimate the time elapsed after the abandonment of each pile. Vegetation replacement on dung piles of different ages was assessed and compared with grassland controls using discriminant analysis. We used regression analysis to look for changes in vegetation cover, species richness, species diversity and evenness in response to height (age) of the dung piles, and principal component analyses (PCA) to identify groups

of plants associated with different successional stages. We compared cover of alien plant species on dung piles with grassland controls using one-way ANOVA. On average, 2.5% of the study area was covered by horse dung. Total vegetation cover, species richness, diversity and evenness increased after the piles were abandoned. Characteristic plant groups were associated with initial, middle and last phases of the studied succession. Vegetation on the dung piles significantly differed from that in grassland controls and two species were consistently associated with dung piles: the invasive Red Star Thistle, *Centaurea calcitrapa*, and a native grazing-intolerant grass, *Nassella clarazii*. Non-native species cover was also higher in dung piles than in control plots. Dung piles cover a significant portion of grassland area in our study site, produce significant changes in the vegetation and are associated with some invasive alien plants that could eventually colonize more pristine areas in the vicinity. On the other hand, they might represent refuges for palatable species, since horses seem to avoid them for grazing.

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Introduction

Invasive alien species usually modify the disturbance regime of invaded ecosystems changing patterns of succession and promoting colonization by pioneer or

ruderal species, which results in the modification of natural communities (Vitousek et al. 1996; Mack et al. 2000). The presence of a non-native species quite often favours the introduction of others through a process called “invasion meltdown” (Parker et al. 1999; Grosholz 2005; Facon et al. 2006), as has been cited in several cases when the arrival of plant species was associated with the presence of large alien herbivores (Parker et al. 2006). The introduction of domestic and feral livestock results in degradation of the native flora affecting the whole landscape, decreasing the cover of the herbaceous layer and favouring the dominance of plants that are less susceptible to grazing, such as unpalatable (Huntly 1991; Vázquez 2002; Tood 2006), spiny or rosette species (Fernández-Alès et al. 1993), and of medium to low height species (Lavorel et al. 1999), increasing the richness and diversity of non-native plants in many cases (Chaneton et al. 2002; Vázquez 2002; Parker et al. 2006).

The introduction of herbivores results in higher impacts when introduced animals are functionally different from those already present, or thrive in greater densities in respect to those found naturally in the native community (Hobbs and Huenneke 1992). Large alien ungulates have produced smaller changes in the savannas of Africa and North America, possibly because large, hoofed, congregating grazing mammals already existed in the evolutionary history of these habitats, resulting in the evolution of grasses tolerant to heavy grazing—including both tussock and rhizomatous or turf grasses. The situation is different on the large South American plains where herbivores of this kind were not previously present and so dominant tussock grasses are intolerant to heavy grazing (Mack 1989). Similarly, the grazing intensity by domestic herbivores in southern South America is thought to be about ten times more than it was before their introduction (Oesterheld et al. 1992).

Grazing and trampling are the best known agents of change associated with the presence of introduced herbivores (Trimble and Mendel 1995; Vázquez 2002; Rook et al. 2004), whereas, in comparison, much less is known, about the impact of the accumulation of manure, which is a conspicuous effect of ungulate activity. Feral horses and other ungulates, e.g. vicuñas, guanacos, llamas and Przewalski horses (Bouman 1986; Vilá 1994; Dalmasso et al. 1999; Beever and Brussard 2000; King and Gurnell 2007), behave in a

particular way: herds or family groups deposit their dung at special sites, forming conspicuous mounds, called *dung piles* or *stud piles* (King and Gurnell 2007). It is thought that these accumulations mark the territory occupied by a male and they also serve as a signal for reproduction, letting a male know when females in his territory are on heat (Bouman 1986; Vilá 1994). Manure in dung piles increases the availability of soil nutrients, especially nitrogen and phosphorus (Nielsen 1997; Dai 2000; Aarons et al. 2004) and favours the conservation of humidity. This might contribute to the development of special patches of vegetation growing directly on manure and in its surroundings (Shiyomi et al. 1998; Dai 2000). In many ecosystems, the invasion of non-native plant species is dependent on this type of disturbance that creates appropriate microhabitats for their establishment, especially if the disturbance results in an increase in water and nutrients (Lake and Leishman 2004). These microhabitats can act as “invasion windows” fostering the establishment and potential dispersal of invasive alien species. Moreover, many species are transported in manure, which also contributes to their establishment (Dobbie et al. 1993; Malo and Suárez 1995a, b; Campbell and Gibson 2001; Wells and Lauenroth, 2007). Previous studies are concerned with analysis of the effects of manure of domestic livestock (Shiyomi et al. 1998; Dai 2000; Loucoguaray et al. 2004), but no evaluation of the impact of wild herbivore dung piles has been undertaken, especially feral horses on natural ecosystems.

Horses have been present in the Argentine plains since the beginning of European colonization (Vázquez 2002) and they form feral populations in several natural and semi-natural grasslands and shrublands across the country (INBIAR, Database on Biological Invasions in Argentina 2007). A population of feral horses inhabits the Ernesto Tornquist Provincial Park (ETPP), one of the last few relicts in a relatively good state of conservation in the Pampas Phytogeographic Province (Scorolli 1999). The ETPP has provided a favourable habitat for the growth of this population which started with a nucleus of a stallion and four mares that were introduced in 1942 (Scorolli 1999), reaching a current density of 25 horses per km² (A. Scorolli, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, pers. comm.). The presence and activity of feral horses in the area results in a highly altered matrix of low

grassland from which small areas of taller tussock grasses (*Paspalum quadrifarium*) emerge, in association with low, wet sites. It is known that grazing in the reserve results in a reduction of vegetation cover and height and increases the abundance of shrub species and unpalatable grasses (A.E. de Villalobos and S.M. Zalba pers. comm.), as well as the cover of alien species (Frangi and Bottino 1995; Long and Grassini 1997). However, the impact of dung piles on vegetation communities in the reserve had not been previously analyzed.

In this study, we assess the area directly affected by feral horse manure in the ETPP. We evaluate differences in plant communities growing on dung piles compared with control plots without manure, and describe the successional changes in dung pile vegetation over time and also discuss the possible role of dung piles in the introduction and dispersal of invasive plants in the reserve.

Methods

Study area

The study was carried out in the Ernesto Tornquist Provincial Park (ETPP), which has an area of approximately 6,700 ha in the Ventania mountains, between 38°00'–38°10' S and 61°45'–62°08' W, and includes mountains of around 1,000 m a.s.l. Climate is temperate, with an average annual temperature of 14°C (Burgos 1968). The average precipitation is 800 mm, with occasional snow in the winter.

The study area in the reserve consists of 2,000 ha of piedmont valleys with 5–11% slopes, shallow soils and occasional rocky outcrops. In the absence of intensive grazing the grass canopy is 50–60 cm tall, dominated by *Piptochaetium hakeleii*, *P. napostanense*, *Nassella melanosperma* and *Chascolytrum subaristatum* among others (Frangi and Bottino 1995). Tussock paspalum (*Paspalum quadrifarium*) is dominant on stream edges and in areas where the soil is saturated with water during rainy periods (Frangi and Bottino 1995).

Sampling design and statistical analysis

The sampling was carried out in January and February 2004 over an area where horses were active

(Scorolli 1999). Fifteen linear transects 50-m long were placed at random, in order to determine the percentage of the study area was directly covered by manure.

Fifteen dung piles >1 m in diameter were selected in order to avoid biases due to very small dung piles or isolated dung (i.e. dung not grouped in piles). The area of dung piles was calculated assigning each pile to either a circular or oval shape. The height of each pile was calculated as the average from five measurements taken in the centre of the pile and in the middle of their largest and smallest radii. Mean height was used to estimate how much time had passed since abandonment by the animals (age of the dung pile). Loss of humidity, decomposition of organic material and physical factors such as wind and rain, result in hardening and then disintegration and fragmentation of the manure over time and also in the loss of the boundaries of the lumps (Dai 2000). These changes in turn cause a decrease in the height of the pile and a concomitant increase in its area. Therefore, it can be assumed that dung height shows a negative correlation with time after abandonment of the pile and so it can be used as an indicator of its age. Alternatively, differences in dung height might respond to an original variation in the size of the pile; if dung height reduces with time, we would expect to find a negative correlation between both variables. Actually, the area of dung piles ($2.80 \pm 0.324 \text{ m}^2$, mean \pm SE) and their height were negatively correlated ($R = -0.679$, $P < 0.01$, $n = 15$), supporting the idea of reductions in dung height and increases in size over time.

We determined the percentages of plant species cover and of bare manure by visual estimation for each dung pile. Plant species were collected and identified in the laboratory according to Zuloaga and Morrone (2007).

Plant species richness and Shannon's diversity index and evenness (Begon et al. 1996) were calculated for the vegetation on the dung piles. A linear regression analysis was undertaken between the height of the dung (as an indicator of the time passed since abandonment of the pile) and the total percentage of vegetation cover, species richness, Shannon's diversity index as well as the evenness in the whole dung pile.

A principal components analysis (PCA) was carried out using the correlation matrix between the percentage cover for each species in order to search for patterns in the floristic composition of the dung

piles. Occasional species (i.e. species present on only one dung pile with a percentage cover <1%) were excluded. As the first principal component explained a meaningful part of the total variation according to Kaiser–Guttman criterion (Legendre and Legendre 1998), we correlated it with dung pile height in order to describe plant species replacement with time.

Twenty 1-m² grassland controls placed at least at 10 m from the nearest dung pile were randomly selected. Species composition on these plots was assessed and compared with dung piles using step-wise discriminant analysis. A canonical variate analysis was used to determine which variables were more important in separating dung piles from grassland controls. We also compared non-native species cover per square meter between control plots and dung piles by Kruskal–Wallis non-parametric analysis of variance.

Results

Manure cover at the study site was $254 \pm 52 \text{ m}^2 \text{ ha}^{-1}$, representing 2.5% of the total surface area. About 78 species were found growing directly on the dung piles, among which 35 (45%) were considered occasional. About 61 species were detected in control plots of which nine (15%) were considered occasional. The percentage of uncovered substrate (i.e. non-vegetated substrate covered only by manure) increased with the height of the dung pile, i.e. greater percentages of bare manure were associated with more recent dung piles

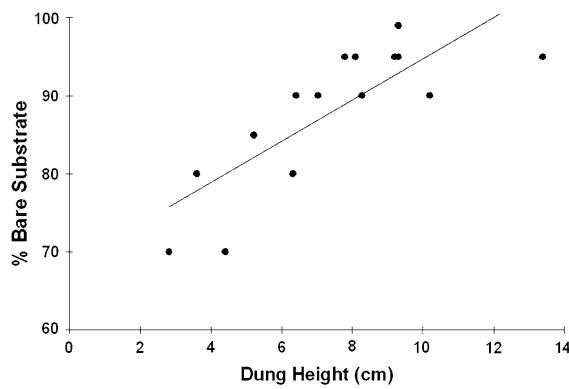


Fig. 1 Linear regression between the percentage of bare substrate and dung height. The height of the dung is used as a proxy inversely related to the time passed since the abandonment of the dung pile. $R^2 = 0.64$, $n = 15$, $P < 0.01$

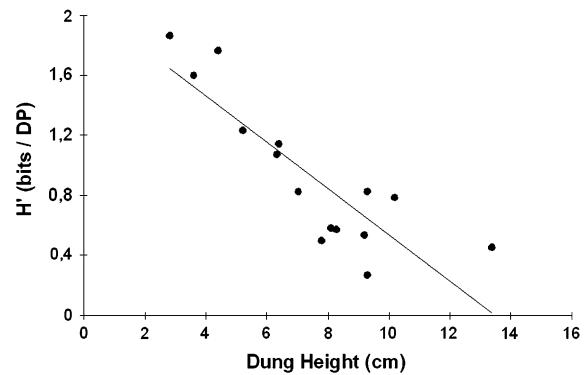


Fig. 2 Linear regression between Shannon's diversity (H') and height of the dung piles. $R^2 = 0.725$, $n = 15$, $P < 0.01$. DP: Dung pile

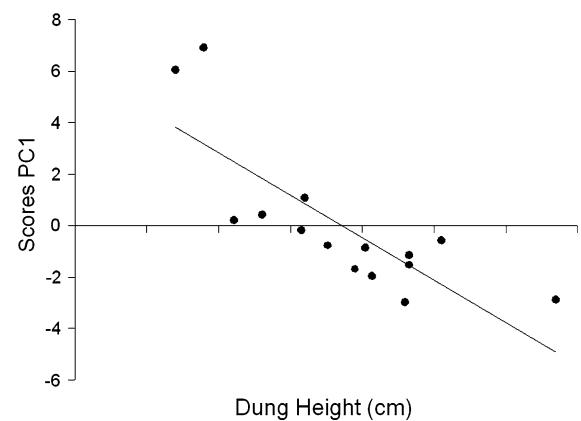


Fig. 3 Linear regression between dung piles scores on the first principal component (PC1) and the height of each pile

($R^2 = 0.640$) (Fig. 1). Shannon's diversity index increased significantly with reductions in height of the dung pile ($R^2 = 0.725$) (Fig. 2). The same occurred with species richness and evenness ($R^2 = 0.461$ and $R^2 = 0.701$, respectively). The first principal component of the PCA obtained from the data of species cover in the dung piles explained 18% of the total variance, while the second component explained 13%. No clearly defined groups of dung piles were detected by their ordination on these first two axes.

The height of the dung pile was negatively correlated with the position of the dung piles on the first principal component (correlation coefficient = -0.798 ; $P < 0.01$) (Fig. 3). The ordering of dung piles on the first principal component reveals changes in the abundance of different species in association with the age of the dung pile, according to species loads in

Table 1 Correlation coefficients (R) between species cover and the coordinates of dung piles on the first principal component (PC1). Only species with correlation coefficients, equal to or greater than half the highest value obtained are shown

Species	R PC1
<i>Nassella clarazii</i>	0.9350
<i>Evolvulus sericeus</i>	0.8444
<i>Chaptalia exscapa</i>	0.7924
<i>Cyperus aggregatus</i>	0.6770
<i>Paronychia</i> spp.	0.6622
<i>Pfaffia gnaphaloides</i>	0.6468
<i>Centaurea calcitrapa</i>	0.5720
<i>Conyza monorchis</i>	0.5642
<i>Piptochaetium medium</i>	0.5275
<i>Acmella decumbens</i>	0.5181
<i>Helenium radiatum</i>	0.4879
<i>Margyricarpus pinnatus</i>	-0.2537
<i>Hedeoma medium</i>	-0.3438
<i>Berroa gnaphalioides</i>	-0.3499
<i>Baccharis rufescens</i>	-0.4058
<i>Chevreulia sarmentosa</i>	-0.4551

the first PCA axis (Table 1). Some species, e.g. *Eragrostis lugens*, *Eryngium nudicaule*, *Margyricarpus pinnatus* and *Pavonia cymbalaria*, were present in most of the dung piles and some of them, e.g. *Aristida*

spegazzinii, *Pfaffia gnaphaloides* and *Piptochaetium stipoides*, showed greater cover in the oldest dung piles. Another group of plants including *Baccharis rufescens*, *Chevreulia sarmentosa*, *Hedeoma medium*, *Piptochaetium montevidense* and *Plantago myosuros* appeared in recent and middle aged dung piles. Other plants associated with dung piles of middle age continued to be present in the oldest ones, e.g. *Centaurea calcitrapa*, *Chaptalia exscapa*, *Cyperus aggregatus*, *Eleusine tristachya*, *Evolvulus sericeus*, *Nassella clarazii*, *Piptochaetium medium* and *Sporobolus indicus* (Table 2).

The best stepwise discriminating model obtained when comparing vegetation on the dung piles and in the control selected thirteen species from a total of 67 (Table 3). *Centaurea calcitrapa* and *Nassella clarazii* only appeared growing on the dung piles, while *Baccharis rufescens*, *Helenium radianum* and *Pfaffia gnaphaloides* showed higher cover on them. Alternatively, *Aristida spegazzinii*, *Baccharis stenophylla*, *Chaptalia exscapa*, *Eragrostis lugens*, *Juncus tenuis*, *Mimosa rocae* and *Piptochaetium stipoides* were consistently associated with the control plots. The discriminant function discriminates significantly between both vegetation groups (Wilks's-Lambda = 0.008, df = 13, $P < 0.001$) with a 100% of the samples correctly allocated.

Table 2 Species cover on dung piles and mean cover in control plots. Cover: 1. 0–1%; 2. 1–5%; 3. 5–25%, 4. 25–50%, 5. 50–75%, 6. 75–100%. Species were selected considering changes in abundance in association with piles of different height

Species	Dung Pile height (cm)														Control Plots	
	13.4	10.2	9.3	9.3	9.2	8.3	8.1	7.8	7.0	6.4	6.3	5.2	4.4	3.6	2.8	
<i>Piptochaetium stipoides</i>	1	0	1	1	1	0	1	1	2	1	1	2	1	1	2	5
<i>Margirucarpus pinnatus</i>	1	1	1	0	1	1	0	1	1	1	0	1	1	0	1	2
<i>Eragrostis lugens</i>	1	1	1	0	1	0	0	0	1	1	1	0	1	1	1	3
<i>Aristida spegazzinii</i>	1	1	1	0	1	0	1	1	0	1	0	0	2	1	0	2
<i>Pavonia cymbalaria</i>	1	1	1	0	1	0	0	0	1	2	0	1	1	1	0	2
<i>Pfaffia gnaphalioides</i>	1	1	1	0	0	0	0	0	1	1	1	0	1	1	2	1
<i>Eryngium nudicaule</i>	1	1	0	0	1	1	0	1	1	0	1	0	1	1	0	1
<i>Baccharis rufescens</i>	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
<i>Hedeoma medium</i>	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1
<i>Chevreulia sarmentosa</i>	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
<i>Piptochaetium haackelii</i>	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Plantago myosurus</i>	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	1
<i>Piptochaetium medium</i>	0	0	0	0	0	1	1	0	0	0	1	2	1	1	2	2
<i>Eleusine tristachya</i>	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	1
<i>Cyperus aggregatus</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1
<i>Sporobolus indicus</i>	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0
<i>Nassella clarazii</i>	0	0	0	0	0	0	0	0	1	0	0	0	2	2	0	0
<i>Evolvulus sericeus</i>	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1
<i>Centaurea calcitrapa</i>	0	0	0	0	0	0	0	0	0	0	2	2	3	0	3	0
<i>Chaptalia exscapa</i>	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1

Table 3 Standardized canonical discriminant function coefficients (*C*) from canonical variate analysis on plant species cover between dung piles and grassland controls

Species	<i>C</i>
<i>Centaurea calcitrapa</i>	-2.021
<i>Nassella clarazii</i>	-1.360
<i>Helenium radianum</i>	-1.244
<i>Juncus tenuis</i>	-1.202
<i>Baccharis crispa</i>	-0.958
<i>Baccharis rufescens</i>	-0.624
<i>Pfaffia gnaphaloides</i>	0.870
<i>Aristida spiegazzinii</i>	1.025
<i>Eragrostis lugens</i>	1.912
<i>Mimosa rocae</i>	1.199
<i>Baccharis stenophylla</i>	2.404
<i>Chaptalia exscapa</i>	3.047
<i>Piptochaetium stipoides</i>	3.435
Functions at group centroids	
Dung piles	-12.273
Grassland controls	9.205

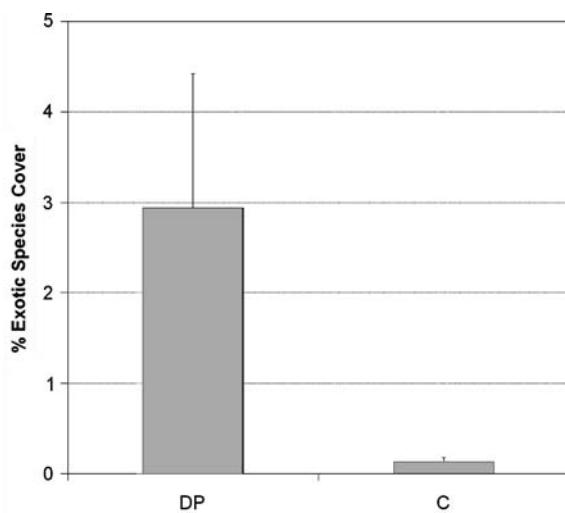


Fig. 4 Alien species cover per square meter in dung piles (DP) and control plots (C). The bars represent standard deviation

Non-native species cover was higher in dung piles than in grassland control plots ($H = 4.69$, $P < 0.05$) (Fig. 4). Only one alien species, *Geranium dissectum*, was found growing in control plots with a mean cover of $<0.15\%$. However, nine alien species were associated with dung piles with *Centaurea calcitrapa* being the most abundant species in some of the piles (Table 4).

Table 4 Alien plant species frequency (Freq.) and mean cover (M.C.) on dung piles and control plots

Species	Dung Pile (n = 15)		Control plots (n = 20)	
	Freq.	M.C. (%)	Freq.	M.C. (%)
<i>Bromus hordeaceus</i>	1	0.03	0	0
<i>Centaurea calcitrapa</i>	4	2.40	0	0
<i>Echium plantagineum</i>	1	0.20	0	0
<i>Geranium dissectum</i>	3	0.10	5	0.13
<i>Petrorhagia nanteulli</i>	2	0.07	0	0
<i>Plantago lanceolata</i>	1	0.03	0	0
<i>Polycarpon tetraphyllum</i>	1	0.03	0	0
<i>Scleranthus annuus</i>	1	0.03	0	0
<i>Taraxacum officinale</i>	1	0.03	0	0

Discussion

Despite the widespread presence of feral ungulates in grassland ecosystems, this is the first effort to assess the effect of feral horse dung piles on plant communities. The area covered by manure in the ETTP (ca. 2.5%) is greater than the results obtained for cattle dung by other authors in other ecosystems of the world. Dai (2000) found around 1% cover of cattle manure in a field on the island of Öland, in the south of Sweden. Omaliko (1981) reports that the percentage of surface covered by cattle manure reached 0.38% in a savanna area in Nigeria. Malo and Suárez (1995c), working in a Mediterranean pasture in Spain, found that the cover of cattle manure reached 0.5% of the area, varying during the year, and locally reaching 2%.

In our study, the percentage of bare substrate decreases with the age of the dung piles whereas species richness, diversity and evenness increase. It is possible that the initial impact of the accumulation of manure restricts the colonization of the site to a reduced group of species capable of tolerating the high concentration of salts that comes from the dung and urine of the horses. With the passage of time, environmental conditions may become less extreme, allowing the establishment of other species. The coexistence of both groups could explain the increase in total vegetation cover, richness, diversity and evenness that follows. This conclusion has to be taken with caution, as there is a risk of confounding

the effect of time with the concomitant increase in area that occurs as dung piles disintegrate. However, plant cover (shown as the complement of bare substrate in Fig. 1) increases more rapidly than dung piles area: since the area of the dung piles augments in a factor of 5 times (ca. 1 m² to ca. 5 m²), the percentage plant cover changes from ca. 1% to ca. 30% in the studied dung piles. On the other hand, the recorded increases in plant species richness and diversity greatly exceed those expected only by an increase in area, considering that minimal sampling area in this grasslands is about 1 m² (Zalba 1994).

A process of successional replacement of the vegetation becomes evident when the variation in the specific composition of dung piles over time is analyzed. The presence of a particular species would depend on its relative capacity to arrive at the site, germinate and become established, as well as to resist competitive replacement by new species. A group of plants (e.g. *Aristida spiegazzinii*, *Piptochaetium stipoides*, *Pfaffia gnaphaliooides*) that commonly appear in the plots sampled in the surrounding grassland matrix was also present on dung piles of different ages, showing greater cover in the older ones. It is possible that at least some of these species were present before the site was covered by dung and were capable of resisting the impact of manure, resprouting from underground structures, such as rhizomes, stolons, root suckers or bulbs, after the abandonment of the dung piles. Another group of species might initially respond in the same way, but then not be able to tolerate the environmental conditions in the dung piles in successive stages, or the competition from opportunistic species that take advantage of this habitat, and eventually disappearing. This group of plants appeared in young dung piles, but was not found in the oldest ones (e.g. *Hedeoma medium*, *Baccharis rufescens*). Finally, a third group of species was only associated with the lowest dung piles, indicating that they appeared after a certain time had passed after the abandonment of the dung pile (e.g. *Centaurea calcitrapa*, *Chaptalia exscapa*, *Nassella clarazii*).

Dung piles represent a sudden and significant disturbance due to the accumulation of a large amount of manure, with the consequent decrease in the cover of the underlying vegetation and the enrichment of nutrients and humidity. According to the Grime's triangular model for representing plant

strategies (CSR theory) (Grime 1977, 2001) plant competition is not important in recently disturbed areas with great availability of resources and rapid colonization. So, the initial stages of plant establishment on a dung pile would be determined by the survival of tolerant species that are already present in the site, plus pioneer species present in the soil seed bank, in seed rain or transported in the dung. Seeds of species of some genera recorded on dung piles in ETTP, e.g. *Carex*, *Conyza*, *Cyperus*, *Daucus*, *Eleusine*, *Erodium*, *Geranium*, *Juncus*, *Oxalis* and *Plantago*, among others, are able to survive the passage through the digestive tract of horses and other ungulates and are effectively dispersed by them (Malo and Suárez 1995b; Campbell and Gibson 2001; Cosyns and Hoffmann 2005; Mouissie et al. 2005). As time passes, the conditions in the dung piles would become more moderate, allowing the arrival of other species. Competition might then become the factor that structures the communities.

Changes in vegetation growing on dung piles respect to grassland controls should be taken with care considering that manure covers an important portion of the area and it is possible that control plots could have been covered by dung in the past. To have a real knowledge of the effect of manure, plots historically free of dung should be used, what is actually not possible due to the extensive use of the area for cattle ranching. Nevertheless, an effort has been made to select controls that were free of dung evidences and we think that the described restriction does not preclude drawing conclusions about the effect of dung piles.

The association of alien species with dung piles is of particular interest. In particular *Centaurea calcitrapa*, the most abundant non-native species found in the dung piles, is relatively uncommon in natural grasslands at the study area, but very frequent on road edges and other disturbed places (Long and Grassini 1997). During this study, *C. calcitrapa* was not recorded in grassland habitats away from the dung piles, but it was very frequent in old dung piles, exceeding percentage covers of 5%. The species was seen flowering on the piles, supporting the idea that it can complete its life cycle before the complete disintegration of the pile and so producing seeds that can disperse from there. Dung piles in which the species is present might therefore function as a source from where the species could colonize grassland

habitats in the future, provided that certain ecological, genetic and/or demographic changes occur, constituting a potential centre of dispersion of propagules over the area.

The case of *Nassella clarazii* is also interesting as it was only found in the oldest dung piles and the specimens found were more than 25-cm tall (A. Loydi, pers. observ.). *N. clarazii* is very palatable and sensitive to grazing (Rúgolo de Agrasar et al. 2005), and so it is not common in this intensively grazed ecosystem and in fact it was not found in the grassland control plots. Abundance of sensitive species to grazing seems to depend on refuge availability in both terrestrial and aquatic ecosystems (Quenga Kerr and Paul 1995; Lavorel et al. 1999; Callaway et al. 2000; Milchunas and Noy-Meir 2002; Oesterheld and Oyarzábal 2004; Callaway et al. 2005; Rebollo et al. 2005; Baraza et al. 2006; Graff et al. 2007). These refuges allow threatened organisms to escape from the effect of herbivores, avoiding local extinction in areas that are heavily grazed (Callaway et al. 2000). The presence of ungrazed specimens of *N. clarazii* associated with dung piles might be because of feral horses avoid grazing there and therefore these areas might act as refuges that favour palatable species. The tendency of domestic horses to avoid grazing in latrine areas has been reported by numerous authors (Nielsen 1997; Shiyomi et al. 1998; Loucogaray et al. 2004), however, Beever and Brussard (2000) and Lamoot et al. (2004) did not record this behaviour when analyzing populations of feral and free-ranging horses. Alternatively, the presence of the species might be related to the particular ecological conditions at dung piles and this could be further tested by experimental manipulation with exclosures.

It can be concluded that dung piles cause local changes in vegetation communities of Pampas grassland that may scale up both their spatial and temporal boundaries. These might still affect plant communities after the disintegration of the manure, the virtual disappearance of the piles and even after the eventual removal of the population of the invasive herbivore, mainly due to their role as facilitators of invasion processes. Dung piles in some cases may act as invasion windows and sources of propagules for invasive alien species, leading to potentially irreversible changes in the grassland. On the other hand, the presence of grazing-sensitive species on the dung piles is indicative of the effect of grazing outside their

boundaries and also of the potential for recuperation of grassland by managing the stocking rate of non-native herbivores.

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Reproductive allocation of *Carex flava* reacts differently to competition and resources in a designed plant mixture of five species

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Abstract In natural plant communities, reproductive allocation can be affected by complex interactions among abiotic resources, species competition and plant size. This topic was addressed using a variety of designed mixed stands of five species (*Carex elata*, *Carex flava*, *Lycopus europaeus*, *Lysimachia vulgaris* and *Mentha aquatica*) under four abiotic conditions to investigate how competition and abiotic resources influence the reproductive allocation of one of the five species, *C. flava*. The plant mixtures varied systematically in both the relative abundance of the five species and the absolute density, and were each established with two levels of water and nutrients. In total, 176 mixtures were maintained for two growing seasons in large pots in an experimental garden. Reproductive allocation of *C. flava* increased from 6.8% to 9.7% under high nutrient application; however, for both nutrient levels, reproductive allocation was independent of shoot mass (size-independent allocation). Under low competition, reproductive allocation of *C. flava* decreased as its shoot mass increased, indicating a relatively high investment in vegetative structures under higher light

availability. However, under strong competition, the allocation pattern changed and a constant reproductive allocation for different plant sizes was observed. Different water levels did not influence the shoot mass, seed mass or reproductive allocation of *C. flava*, indicating that the species was not stressed under dryer conditions. When under competitive pressure, however, the species responded with reduced shoot and seed production under more favourable water conditions. This behaviour indicates a trade-off between the ability to tolerate stress and the competitive and reproductive response of *C. flava*. In conclusion, *C. flava* was adversely affected by competition with some of the species, and competition, mediated by plant size, indirectly affected reproductive allocation. *C. flava* was able to modify its allocation pattern depending on the available resources and retained its reproductive allocation even under unfavourable conditions for varying plant sizes, which is interpreted as an advantageous reaction to greater competition pressure.

Keywords Fen meadow species · Nutrient water supply · Simplex design · Stress tolerance · Trade-off

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Introduction

Plants allocate restricted proportions of resources to either vegetative or reproductive structures.

According to environmental conditions, the species' allocation patterns may be adapted in ways that affect their fitness; therefore, resource allocation is expected to have important implications for plant life history and evolution (Reekie and Bazzaz 2005). Studying a plant species' reproductive allocation should thus highlight its survival strategy (Harper and Ogden 1970).

The mass of a plant's reproduction structures in relation to the total aboveground biomass has been defined as reproductive allocation (RA) (Karlsson and Méndez 2005). Within a species, the most important factor affecting RA is plant size (Weiner 1988; Sugiyama and Bazzaz 1998; Méndez and Karlsson 2004). In an analysis of 41 species, many perennials exhibited a decreasing RA with increasing plant biomass, whereas in the case of annuals, either the reverse was true or RA remained constant (Hara et al. 1988). Studies focussing on single species' RA have confirmed these patterns (Reekie 1998; Susko and Lovett 2000; Sletvold 2002).

Linking RA and plant size can be most effectively achieved by analysing a species' seed mass and shoot mass, which are often positively correlated and increase linearly on a log-log scale (Klinkhamer et al. 1994; Sadras et al. 1997; Sletvold 2002). This correlation becomes $Seed\ mass = \alpha\ shoot\ mass^\beta$ when expressed on a linear scale (Hendriks and Mulder 2008). Different allocation patterns, such as size-dependent or size-independent allocation, can be distinguished according to the relative change of seed mass and shoot mass (Weiner 1988; Sugiyama and Bazzaz 1998).

Intraspecific responses of RA to environmental stress have been thoroughly reviewed (Chiariello and Gulmon 1991; Reekie and Bazzaz 2005). Chiariello and Gulmon (1991) noted that many perennial species reduce RA in response to resource limitation (water, nutrients and light), but annual species often increase RA in response to shortage of water, decrease RA in response to light limitation and exhibit no response to nutrients. As shown by Karlsson and Méndez (2005), these trends are not consistent and vary greatly in response to environmental factors.

So far, the relationship between RA and resources has only been investigated with single species or in pairwise species comparisons (Sugiyama and Bazzaz 1998; Whitfield et al. 1998; Cheplick 2001; Arenas et al. 2002). Conclusions drawn from such

experiments are not easily applied to plant communities, as species interactions in mixtures can be more complex than pairwise. The presence of a strong competitor could reduce the performance of a target species; however, the presence of additional species could considerably mitigate negative competition (Suter et al. 2007).

Interactions among several species can be investigated using experiments with numerous species and varying abiotic conditions. A specific design for the set-up and analysis of multi-component mixtures is the simplex design (Cornell 2002). The simplex design, originally developed for industrial trials, allows quantification of the effects of many species in a mixture on a target variable, including interactions between competition and resources (Ramseier et al. 2005). This design has recently been used to investigate the role of multiple species interactions in plant community development. In an experimental set-up, mixtures of five perennial species were established under two intensities of competition and four environmental conditions by combining two levels of water availability with two levels of nutrient supply (Suter et al. 2007). In the present article, the influence of the various treatments on RA and on shoot and seed production of one of the five species, *Carex flava* L., is analysed in detail.

Investigation of RA in *C. flava* under these semi-complex conditions should allow more accurate conclusions to be drawn on the species' reproductive behaviour in natural communities. *C. flava* is mainly observed in calcareous fens of low nutrient status (*Caricion davallianae*, Ellenberg 1996). In such habitats, nutrient enrichment can drastically influence community composition (Pauli et al. 2002). Because increased nutrient availability also changes the relative allocation to shoots and seeds (Karlsson and Méndez 2005), it is possible that *C. flava* adapts its RA when environmental conditions change. If, for example, *C. flava* is able to resist competition pressure from more productive species that profit from nutrient enrichment, its shoot and seed mass should only be marginally affected by increased density of neighbouring species, and RA should remain constant. In comparison, when strongly suffering from competition, shoot and seed mass should be reduced, but the relative allocation could be adapted so that RA is maintained or even increased (Chiariello and Gulmon 1991). In the latter case, *C.*

flava would act similarly to annual species that produce a high seed output under unfavourable conditions (Grime 2001; Karlsson and Méndez 2005). Specifically, this study examined (1) whether competition of different species and varying abiotic conditions interact in their effect on RA and shoot and seed mass production of *C. flava*, (2) whether species competition affects RA and shoot and seed mass of *C. flava* in the same way as varying nutrient levels and (3) whether a change in RA is an advantageous reaction for *C. flava* to maintain its occurrence under modified abiotic conditions.

Materials and methods

The experimental design is described in detail by Suter et al. (2007). An outline of the most important aspects relating to this study is presented here.

Plant material and experimental design

Plant mixtures consisted of five perennial species common to Swiss fens: *Carex elata* All., *Carex flava* L., *Lycopus europaeus* L. s.str., *Lysimachia vulgaris* L. and *Mentha aquatica* L. (Nomenclature following Aeschimann and Heitz 2005). Twenty-two different mixtures were established in accordance with the simplex design (Cornell 2002); all five species were present in each mixture. Mixtures were either equal stands (20% of each species), dominant stands (60% of one species, 10% of four others) or codominant stands (35% of each of two species, 10% of three others). Equal and dominant stands were planted in two overall densities of 20 (low density) and 50 (high density) seedlings per pot, whereas codominant stands were planted in an overall density of 20 seedlings per pot. Each mixture was established at two water and two nutrient levels and replicated twice.

The seedlings were planted on 7 and 8 May 2001 in pots of 75 l (50 cm diameter, 45 cm tall), containing quartz sand of 1 to 1.7 mm grain size. Within each pot, the interseedling distance was 6 cm for high density and 10 cm for low density mixtures. In total, 176 pots were randomly distributed at an experimental site of the Swiss Federal Institute of Technology, Zurich.

In the high water treatment, water level was maintained at sand surface (42 cm height in the pot), while in the low water treatment, the level was at half-pot height (21 cm), resulting in a dry zone above. Water levels were adjusted every other day with tap water during the growing season.

The pots with high nutrient levels received three times the amount of nutrients as those with low nutrient levels. Nutrients for the high level consisted of 6 g m⁻² nitrogen in the 1st year and 9 g m⁻² nitrogen in the second. The nitrogen/phosphorous (N/P) ratio was set to 4, and N and P were applied with a complete fertiliser that also included micro-nutrients (Wuxal, Maag, Switzerland).

Maintenance and measurements

Initial shoot biomass was estimated by drying and weighing 20 seedlings of each species, randomly selected from those remaining after planting. In the second growing season, when *C. flava* reached reproductive maturity, infructescences were carefully removed (18 and 19 June 2002), leaving the remaining plant biomass until the final harvest of all the species 2 months later. The seeds left within the utricles were cleaned from the bracts, and empty utricles were removed from the samples. Seed mass per pot was determined after drying the samples (75°C) to constant weight. All plants were harvested from 19 to 29 August 2002 by cutting aboveground living shoot mass. Again, biomass samples were dried (75°C) to constant weight, and dry mass per species and pot was determined.

The diaspore mass of *C. flava* was used for the analysis because it was not practical to separate the seed from the surrounding utricle. Nevertheless, the term “seed mass” rather than “diaspore mass” is used for convenience. Since the seed accounts for a large proportion of the diaspore mass, the inclusion of the utricle is unlikely to affect the conclusions drawn from the data.

Data analysis

The definition of mixtures with five species ($i = 1, \dots, 5$) follows Suter et al. (2007). For *C. flava* (*cf*), three response variables were calculated:

$$\text{Shoot mass} = \frac{(\text{Final shoot mass})_{\text{cf}} \text{per pot}}{d_{\text{cf}}},$$

$$\text{Seed mass} = \frac{(\text{Total seed mass})_{\text{cf}} \text{per pot}}{d_{\text{cf}}},$$

$$\text{Reproductive allocation} = \frac{\text{Seed mass}}{\text{Shoot} + \text{seed mass}},$$

with d_{cf} being the number of planted *C. flava* individuals per pot. It follows that these variables refer to a mean value per pot and not to individuals within a pot. The effects of the initial biomass of the five species and of water and nutrients on these response variables were first assessed with

$$\text{Response} = \alpha + \sum_{i=1}^5 \beta_i y_i + \gamma \text{water} + \delta \text{nutrient} + \varepsilon \quad (1)$$

where y_i is the initial biomass of species i in a mixture at the start of the experiment. The effects of increasing species biomass (competition) are given by the β coefficients; γ and δ coefficients indicate the shift from the low to the high water and nutrient levels, respectively, and ε represents the error term. In order to meet the assumptions of linear regression (normal distribution and homogeneity of residual variance), the response variables shoot mass and seed mass were \log_{10} transformed, and RA was arcsine-square root transformed. Quadratic effects of species biomass ($y_i * y_i$) and any two-way interactions between main effects ($y_i * \text{water}$, $y_i * \text{nutrient}$ and $\text{water} * \text{nutrient}$) were also tested (for further details on the analysis, see electronic appendix).

In order to evaluate the confounding effects of plant size on RA, the relationship between seed mass and shoot mass of *C. flava* was analysed in more detail with

$$\log_{10} (\text{seed mass}) = \log_{10} (\alpha) + \beta \log_{10} (\text{shoot mass}) + \gamma \text{water} + \delta \text{nutrient} + \lambda \text{density} + \varepsilon \quad (2)$$

where γ and δ estimates are as stated above. Density was included as a factor with two levels and served as a surrogate variable to assess the role of competition on seed mass and RA (Karlsson and Méndez 2005). Thus, the λ -coefficient indicates an overall competition effect due to the shift from low to high planting density.

Using the relationship $\log_{10} (\text{seed mass}) = \log_{10} (\alpha) + \beta \log_{10} (\text{shoot mass})$, the following considerations enable distinction of different allocation patterns. (1) Provided that β is 1, seed mass and shoot mass will increase proportionally, and RA will remain constant with increasing shoot mass. (2) If $\beta \neq 1$, then seeds will be allocated in a non-linear way and RA will increase ($\beta > 1$) or decrease ($\beta < 1$) with higher shoot mass. (3) An influence of abiotic or biotic factors (nutrients, water and competition) could either change the allocation pattern (change of β) or the α -coefficient. In the latter case, RA will change by a fixed amount, independent of vegetative biomass; thus, such effects are termed size-independent (Weiner 1988). All analyses were performed using the statistical software R (R Development Core Team 2007).

Results

Shoot mass and seed mass

After 2 years, mean shoot dry mass per individual of *C. flava* was 0.710 g and the mean seed mass was

Table 1 Regression coefficients for the effects of initial species biomass, water and nutrient levels on shoot mass, seed mass and reproductive allocation of *C. flava* in experimental plant mixtures with five species after 2 years

Effect	Log_{10} (shoot mass)	Log_{10} (seed mass)	Arcsin-sqrt (reproductive allocation)
Intercept	-0.155***	-1.304***	0.260***
Water	0.012	0.031	0.006
Nutrient	0.211***	0.386***	0.053***
<i>C. elata</i>	-0.837***	-0.351***	0.030
<i>C. flava</i>	-0.235***	-0.234***	-0.004
<i>L. europaeus</i>	-0.139	-0.056	-0.004
<i>L. vulgaris</i>	-0.151	-0.060	0.020
<i>M. aquatica</i>	-0.086	-0.561***	-0.128***
<i>C. elata</i> ²	0.379***	-	-
<i>M. aquatica</i> ²	-	0.257**	0.068**
Water \times <i>L. europaeus</i>	-0.624**	-0.883**	-

Water and nutrient effects express the shift from the low to the high level

** $P \leq 0.01$; *** $P \leq 0.001$; - term has been omitted in the particular model

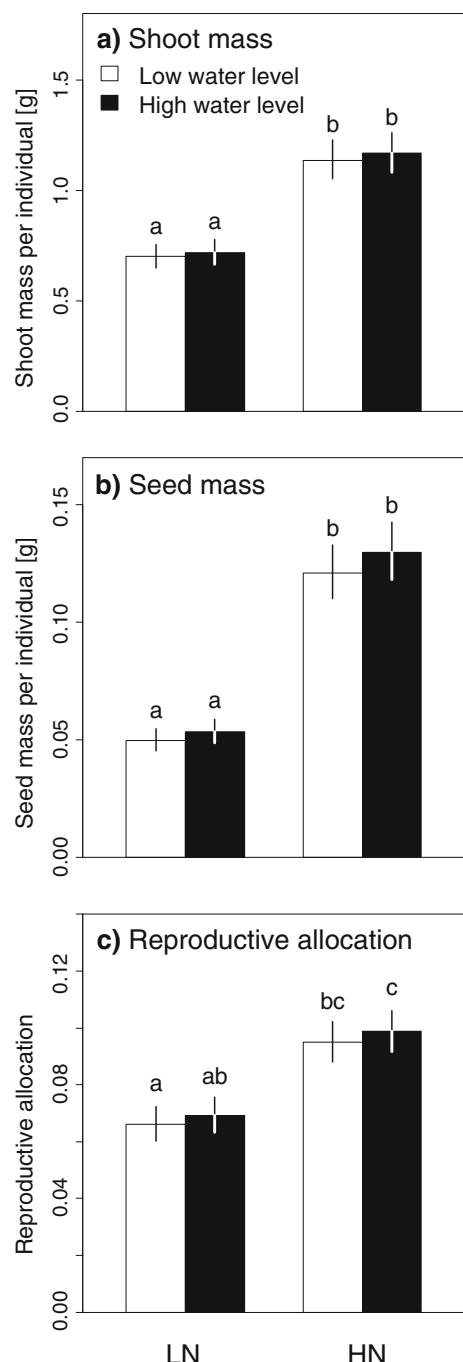


Fig. 1 Shoot and seed production and reproductive allocation of *C. flava* in experimental plant mixtures under four combinations of water and nutrient treatments. Means and standard errors are backtransformed predicted values from regression analysis (Table 1). LN: low nutrients, HN: high nutrients. Different letters indicate a difference at $P \leq 0.05$ (Tukey's multiple comparison test)

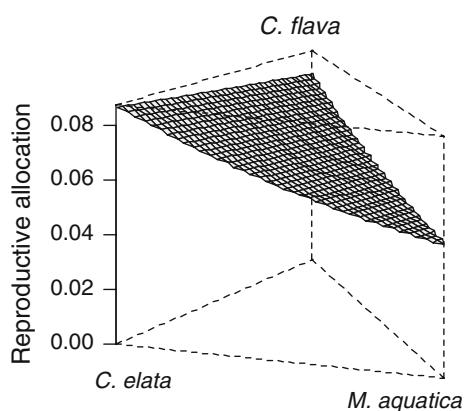


Fig. 2 Reproductive allocation of *C. flava* in experimental plant mixtures with five species after 2 years; the influence of the three most relevant species is displayed. The response surface refers to backtransformed values based on the regression of Table 1 and is for low water–low nutrients conditions. The corners of the surface refer to mixtures with one dominant species (see electronic appendix for further details)

0.052 g under low nutrient and low water conditions (Table 1; Fig. 1a, b). High nutrient application increased shoot mass by 62% and seed mass by 143%, whereas high water level did not affect either parameter (Table 1).

Competition effects depended strongly on the species. Greater initial biomass of *C. elata* (interspecific competition) and *C. flava* (intraspecific competition) caused a decrease in shoot and seed mass, with *C. elata* having a stronger influence than *C. flava* (Table 1). Competition from *M. aquatica* did affect seed mass of *C. flava* but not its shoot mass. *L. europaeus* only had a negative influence on shoot and seed mass with the high water level (water \times *L. europaeus* interactions). The positive quadratic effects of *C. elata* and *M. aquatica* indicate that competition levelled off with a high initial biomass of these species.

Reproductive allocation

Reproductive allocation of *C. flava* was 6.8% under low nutrient conditions. Under high nutrient application, RA increased up to 9.7%, whereas water level had no influence (Table 1; Fig. 1c). Competition from four out of the five species did not change RA of *C. flava*; only *M. aquatica* had a significant negative

Table 2 Regression coefficients for the effects of shoot mass, water and nutrient levels as well as overall density (competition) on seed mass of *C. flava* in experimental plant mixtures after 2 years

Effect	Log_{10} (seed mass)
Intercept	-1.135***
Log_{10} (shoot mass)	0.509***
Water	0.040
Nutrient	0.246***
Density (competition)	-0.050
Density $\times \text{Log}_{10}$ (shoot mass)	0.594***

Water and nutrient effects express the shift from the low to the high level

*** $P \leq 0.001$

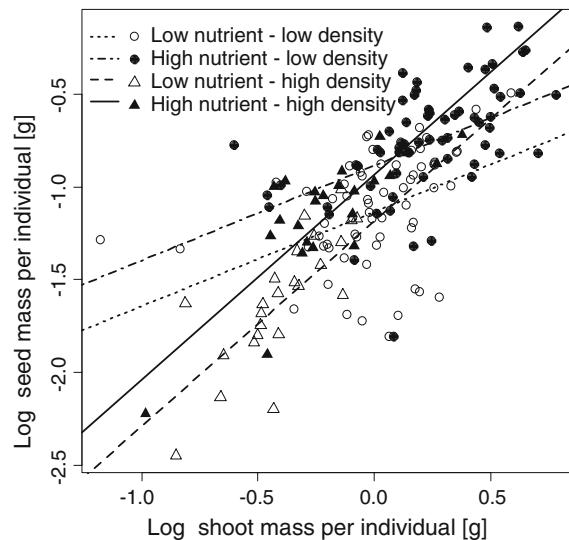


Fig. 3 Seed mass of *C. flava* as a function of its shoot mass, nutrients and density in experimental plant mixtures (log-log graph). Density has been used as a surrogate variable for overall competition. Both lines at low density conditions were significantly shallower than 1 ($P < 0.001$), whereas the lines at high density were not different from 1 ($P = 0.475$) (prediction based on regression of Table 2). Water had no significant effects

influence, which levelled off with an increasing abundance of this species (Table 1; Fig. 2).

Reproductive allocation of *C. flava* was further influenced by its own size, as demonstrated by the relationship between seed and shoot mass (Eqn 2). Seed mass of *C. flava* was positively affected by its shoot mass (Table 2; Fig. 3). The shoot mass coefficient, however, was significantly smaller than 1 at

low density conditions ($t_{170} = 6.159$, $P < 0.001$), indicating that RA decreased with plant size when competition from other species was low. Though higher overall density (high competition) did not further affect seed mass when the shoot mass of *C. flava* was taken into account, high competition did change the allocation pattern (Fig. 3; Table 2, density \times shoot mass interaction). This interaction resulted in a shoot mass coefficient not significantly different from 1 with high density conditions (sum of coefficients: $0.509 + 0.594 = 1.103$, $t_{170} = 0.716$, $P = 0.475$ for difference from 1), indicating that RA became size-independent under increased overall competition. The positive effect of high nutrient application on seed mass represents a size-independent increase of RA (Fig. 3).

Discussion

Effects on shoot and seed mass

C. flava tussocks are perennial, but the species spreads both clonally and with seeds, and seed production of *C. flava* is usually high because of self-compatibility (Schmid 1984). These features could allow *C. flava* to alter its relative investment in shoots and seeds to optimise its survival under changing conditions. The natural habitats of *C. flava* provide high light availability even for smaller plants. Nutrient enrichment in these habitats will considerably impair the smaller plants through increased competition from species with rapid nutrient uptake and biomass production (Pauli et al. 2002; van der Hoek et al. 2004). Therefore, *C. flava*, as a relatively small species (mean size of 30 cm, Lauber and Wagner 2007), should decrease in its relative abundance with high nutrient levels. This is confirmed by the present experiment: though the vegetative growth of *C. flava* benefited from high nutrient levels (Table 1), its biomass proportion relative to the remaining species was significantly reduced (Suter et al. 2007).

C. flava was only partially resistant to adverse conditions and suffered considerably from competition. Under high competitive pressure from *C. elata*, shoot and seed mass of *C. flava* were adversely affected (Table 1). This strong impairment is explained by the large biomass production of *C. elata*, which was more than two times greater than

each of the other four species (Suter et al. 2007). *C. elata* took-up most of the available resources and considerably affected not only *C. flava* but all the species in the plant mixtures. Though the level of RA in *C. flava* was not directly influenced by *C. elata*, this strong competitor reduced the shoot mass of *C. flava* more than its seed mass and changed the allocation pattern (Table 2).

Different water levels did not influence the shoot and seed mass of *C. flava*, indicating that *C. flava* was not stressed under dryer conditions. However, with the higher water level, i.e. more favourable water conditions, shoot and seed mass were reduced by competition with *L. europaeus*. The performance of *L. europaeus* was greatly enhanced by higher water conditions, and primarily affected *C. flava* in this experiment (Suter et al. 2007). The impairment of *C. flava* from competition under an increased water level thus indicates a trade-off between its ability to tolerate stress and its competitive response, a mechanism that is assumed to be of central importance for community dynamics (Campbell and Grime 1992; Suding et al. 2003; Liancourt et al. 2005). This could explain why *C. flava* also occurs under slightly dryer or disturbed conditions at the edges of fen meadows of low productivity (F. Klötzli, personal communication), but is absent in wetlands, where biomass production is high and light becomes a limiting factor for small species (e.g. *Magnocaricion elatae*, Ellenberg 1996).

While the adverse effect of *C. elata* was pervasive over all established resource levels, the competitiveness of *L. europaeus* was only pronounced under the higher water level conditions, and no significant effects could be found for *L. vulgaris* (Table 1). This shows that the interrelations between plants can be very species-specific and can also be modified by abiotic conditions (Ramseier et al. 2005; Emery and Gross 2007). Though the experimental time period may have been too short to detect further interactions between species and resources, the designed plant mixtures allowed quantification of some of these influences.

Effects on reproductive allocation

C. flava, as grown in the experimental plant mixtures, exhibited subtle reactions in RA to the manipulated parameters. The relative allocation of resources to

shoots and seeds was influenced by nutrients and competition, but less so by water (Figs. 1 and 2). Under low planting density (low competition treatment), RA decreased with increasing plant size. Reekie (1998) explained a size-dependent decrease of RA with additional costs in support tissues. In larger individuals, such costs could be higher and, as a consequence, RA would diminish. In the present experiment, the low planting density with an inter-seedling distance of 10 cm allowed *C. flava* to produce larger individuals, and for these individuals, the relative investment in seeds was reduced.

Though vegetative growth of *C. flava* was impaired under the high planting density, competition had little or no direct effects on RA in *C. flava* (Table 1). However, the allocation pattern changed and RA became size-independent (Table 2; Fig. 3). *C. flava* reacts very sensitively to limited light (Edelkraut and Guesewell 2006), and growth of *C. flava* was restricted by the availability of light in the high density treatment. The two strongest competitors, *C. elata* and *L. europaeus*, prevented the growth of larger *C. flava* plants. Thus, the absence of large individuals with proportionally smaller seed mass could explain the relatively constant RA observed under high competition (Fig. 3). The change of the allocation pattern indicates that, under increased competition and shading, *C. flava* can ensure its survival by maintaining its RA and producing seeds even from the smallest individuals (Fig. 3). In fact, *C. flava* is able to produce seeds with less than 20 cm in size (Schmid 1984).

Regarding the change of RA, a similar behaviour has been observed in the annual species *Abutilon theophrasti* (Sugiyama and Bazzaz 1998). Free from competition, *A. theophrasti* has demonstrated decreasing RA with increasing plant size, but under competition and intermediate nutrient conditions, it maintained constant RA for different plant sizes. However, the competition effect on RA was an indirect effect mediated by plant size, rather than a direct effect. In *C. flava*, competition mainly affected shoot mass production, which, in turn, modified the pattern of RA. The present study thus confirms that competition predominantly affects the size of a plant, and that changed plant size affects other features such as RA (Weiner 1988).

With high nutrient levels, the absolute and relative resource allocation to seeds was enhanced (Tables 1

and 2). However, a shortage of nutrients did not have the same effect on RA as competition. With low nutrient application, total produced biomass was strongly reduced, and light availability was less limiting. Under these conditions, *C. flava* also produced individuals with large shoots in relation to seed mass, and the proportions of allocated resources were not affected by plant size.

C. flava was able to modify its allocation pattern and maintain seed output under increased competition. If a permanent nutrient enrichment followed by increased biomass production occurs in the natural habitats of *C. flava*, the species could maintain or increase its vegetative growth and RA for some time. However, in the long term, *C. flava* would be edged out by competition due to light limitation (Edelkraut and Guesewell 2006; Suter et al. 2007) and the species would survive only in the soil seed bank (Schmid 1986).

In summary, specific observation of the model plant communities allowed quantification of the effects of various abiotic conditions on the resource allocation of *C. flava*. *C. flava* modified its allocation pattern and retained its RA even under unfavourable conditions and in varying plant sizes. It is concluded that this modification is an advantageous reaction to higher competition pressure.

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Factors affecting the establishment and growth of annual legumes in semi-arid mediterranean grasslands

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Abstract Legumes are an important component of mediterranean grasslands with a significant ecological and economic role. The aim of this study was to investigate the factors that affect their establishment and growth and how they survive in a highly variable and unpredictable environment. The research was carried out in a grassland characterised by a semi-arid mediterranean climate and located on a calcareous substrate at about 150 m a.s.l., in Macedonia, northern Greece. It was dominated by annual legumes such as *Hippocratea multisiliquosa*, *Medicago disciformis*, *Medicago minima*, *Onobrychis aequidentata*, *Trifolium angustifolium*, *Trifolium campestre* and *Trifolium scabrum*. It was subjected to the following treatments for four consecutive years: prescribed burning, irrigation, digging, cutting, P fertilization and control. Total legume density was measured in late autumn and in the following spring each year, while total legume biomass was measured only in spring. Dominant legume species densities and biomasses were measured only in spring in the last 3 years. Also, monthly precipitation and air temperature were recorded in a nearby weather station. A great

reduction of both legume density and biomass occurred at the third growing season due to adverse weather conditions. Among treatments, P fertilization affected the positively annual legume density and biomass. The other treatments such as burning, irrigation, digging and cutting influenced positively or negatively annual legume density and biomass depending on the climatic characteristics of the particular growing season involved. It is concluded that in semi-arid mediterranean grasslands with cold winters, weather conditions strongly interact with human disturbances in affecting establishment and growth of annual legumes.

Keywords Biomass · Burning · Cutting · Density · Digging · Legumes · P fertilization · Weather changes

Introduction

Grasslands of the mediterranean region cover a relative limited area but constitute an important ecological and economic resource (Papanastasis and Mansat 1996). Their vegetation is dominated by annual species due to the mediterranean climate that is characterised by a wet and cool winter period followed by a hot and dry summer. As a matter of fact, the longer and drier the summer period, the stronger is the seasonability resulting in greater predominance of the annual species (Seligman

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1996). These species are well adapted to the highly variable mediterranean climate because they produce high amount of small seeds and have a long-lived seed bank (Teketay 1996; Pagnotta et al. 1997).

Extreme climatic variables (e.g. drought) may restrict the number of grassland species (Tilman and El Haddi 1992). On the other hand, Koukoura and Papanastasis (1997) and Koukoura et al. (1998) have found that legume abundance is correlated with air temperature and precipitation. Similar results are also reported by Fernàndez Alès et al. (1993) in Spain, who found that legume abundance is particularly influenced by spring precipitation.

Besides climate, several other factors are affecting mediterranean grasslands but their impact on species structure is conflicting. Individual grassland species have evolved specific adaptations to burning that allow them to survive periodic fires (Pausas 1999; Pignatti et al. 2002). Le Houerou (1981) concluded that the structure of mediterranean grasslands after fire is determined by the reaction of every single species as fire not only removes the vegetation but also has a direct effect on germination and seedling survival (Hanley and Fenner 1998). Menke (1989) found low density of grasses after fire while Papavasiliou and Arianoutsou (1993) reported a dominance of leguminous species in recently burnt forest communities.

Grazing in mediterranean grasslands reduces production and affects species composition (Noy-Meir 1998). By increasing grazing intensity, there is a subsequent decrease of grasses and an increase of legume abundance (Naveh and Whittaker 1979). Drought and grazing may interact as defoliation produced by grazing can reduce the plant's ability to sustain drought and vice versa, so drought and grazing are seen as complementary forces (Vesk and Westoby 2001). Grazing also interacts with fire in affecting species composition in mediterranean grasslands (Noy-Meir 1998).

Ploughing is a more serious disturbance than grazing as it totally destroys biomass. Doerr et al. (1984) report that the lighter the soil disturbance, the sooner the grassland species recover. On the other hand, annual legumes are among the first species to colonise abandoned arable fields (Noitsakis et al. 1992).

Fertilization is an effective management practice aiming at quantitative and qualitative improvement of herbage (Menke 1989; Osman et al. 1999).

Phosphorous (P) especially is essential for the legume establishment though it is known that plant response to P fertilization is expected only in wet years (Henkin and Seligman 2000). Generally, P fertilization alters species composition in grasslands by favouring legumes at the expense of grasses (Papanastasis and Koukoulakis 1988; Osman et al. 1999; Henkin et al. 2006).

In arid and semi-arid mediterranean grasslands, species composition is affected more by short-term weather changes than grazing intensities (Tsiouvaras et al. 1998). According to Jackson and Bartolome (2002), variations of species composition and biomass in the annual grasslands of California are associated with weather patterns. In the mediterranean basin, in particular, moisture is the limiting factor affecting primary productivity that fluctuates both within and between years (Le Houérou and Hoste 1977; Naveh 1982; Papanastasis 1982). Irrigation favours annual species but for a very short time, as perennials eventually dominate (Powell et al. 1990).

Annual legumes are an important component of mediterranean grasslands. In semi-arid environments, they may make more than 20% of the total number of species present (Papanastasis 1981). Their importance mainly lies on their ability to fix atmospheric nitrogen, which is the primary functional trait that influences nitrogen accumulation and biomass production (Spehn et al. 2002). In addition, they are superior to grasses in feeding value for animals because they have higher content of protein and minerals resulting in higher voluntary intake and a faster rate of digestion (Van Soest 1994). In this paper, we investigated how fire, soil digging and cutting as well as P fertilization and irrigation affect the establishment and growth of annual legumes in mediterranean grasslands with a semi-arid climate so that their function is better understood and management is properly implemented.

Materials and methods

The research was conducted in a mediterranean grassland of Macedonia, northern Greece ($E\ 23^{\circ}\ 58'49''$, $N\ 41^{\circ}\ 11'30''$) located on a calcareous substrate at about 150 m a.s.l. Long term monthly temperature ranges from $3^{\circ}C$ in January to a high of $25^{\circ}C$ in July.

Mean annual precipitation is 589.4 mm, and the mean minimum temperature of the coldest month (January) is 0.1°C indicating a semi-arid mediterranean climate with cold winters (Le Houerou 1981). The dry period is from July to September, whereas the driest month is September.

The soil of the area is of medium depth, sandy clay with neutral reaction. It is rich in humus but low in concentration of phosphorous while it is adequately rich in cations.

The grassland was dominated by annuals such as the grasses *Avena barbata* Pott ex Link, *A. sterilis* L. and the legumes *Onobrychis aequindentata* (Sibth. and Sm.) D' Urr., *Trifolium angustifolium* L., *Trifolium campestre* Schreber and *T. scabrum* L. *Hippocrepis multisiliquosa* L., *Medicago disciformis* DC., *Medicago minima* (L.) Bartal., but perennial species were also present with most important being the grass *Dichanthium ischaemum* (L.) Roberty.

In late summer of 1993, a homogenous in species composition area of 30 × 30 m was selected and fenced out in order to be protected from sheep and goats, which were grazing in the whole grassland in a communal way. In this fenced area, six treatments were applied in a completely randomized block design with four blocks. Treatments included prescribed burning, irrigation, digging, cutting, P fertilization and control. They were applied in plots 3 × 3 m in size and they were arranged randomly within each block.

Prescribed burning, P fertilization and digging were applied in early autumn of 1993 and repeated in 1994, 1995 and 1996. The first treatment involved burning of aboveground biomass as an imitation of grassland wildfires often set by shepherds at the end of the summer in order to remove the old growth and encourage the establishment of palatable vegetation after the first autumn rainfall (Papanastasis 1981); the second treatment involved the application of 100 P₂O₅ kg/ha, recommended for favouring legumes in grasslands (Papanastasis and Koukoulakis 1988); and the third treatment involved digging of the soil down to 30 cm with a chopping hoe as an imitation of ploughing for sowing winter cereals in grasslands. The cutting treatment was applied in the middle spring of 1994, during the full bloom period of legumes, by removing the aboveground vegetation as an imitation of grazing and repeated in 1995, 1996 and 1997. Irrigation, finally, was applied both in autumn (November) and spring (March) of all the

growing seasons by spreading 30 and 40 mm of faucet water, respectively.

In each treatment, legumes were sampled with quadrats of 0.5 × 0.5 m in size. Within each quadrat, total legume density was measured in late autumn (beginning of December) and the following spring (middle of April) in each of the four growing seasons, by counting the number of individual plants of all legumes present. In spring, the seven dominant species were counted separately. Also, total legume biomass was measured at the end of the growing season (middle of May), by cutting above-ground vegetation within each quadrat and hand separating the legumes from the collected material; the seven dominant species were hand separated only in the last 3 years. In both the measurements five quadrats per plot were taken. The quadrats were taken in different places within each plot every growing season in order to avoid any sampling effect from the previous ones. The non-leguminous species collected in each quadrat are not reported in this study.

In addition, meteorological data were collected from the weather station of the city of Drama, located 10 km away and in the same plain with the study area. They included monthly air temperatures and precipitation.

Density and biomass data were subjected to the analysis of variance both between treatments in a year and between years in a treatment using the software package SPSS. If significant, means were further assessed with the Tukey's test at the 0.05 level of significance (Sokal and Rolf 1981).

Results

Weather conditions

Weather parameters changed widely during the four growing seasons of the experiment. In general, the first two seasons (1993–1994 and 1994–1995) were warmer but drier than the other two (1995–1996 and 1996–1997). More specifically, mean air temperature for the seven months (October–April) of the four growing seasons were 10.2, 12.0, 8.4 and 9.8°C compared with the 20-year value of 9.5°C (1978–1998). For the minimum air temperature, the mean values for the seven months were 5.6, 6.8, 4.1 and 4.9°C, respectively, for the four seasons compared

with the 20-year value of 4.0°C. For the precipitation, finally, the 7-month totals were 345, 277, 466 and 427 mm compared to the 20-year value of 375 mm. These data show that the third growing season (1995–1996) was the coldest but also the wettest. Nevertheless, relatively little rainfall was recorded in the autumn months October (2.6 mm) and November (44 mm) in the third compared with the other three (22 and 65 mm on the average, respectively). At the same two months, mean air temperature was 9.2 and 3.1°C, respectively, compared with the other three (11.7 and 5.9°C on the average, respectively).

Density

Total legume density varied widely among the four growing seasons, between autumn and spring of the same growing season and among the six treatments. More specifically, autumn density was significantly increased from the first to the second growing season in all treatments, but it sharply decreased in the third, to increase again but not significantly in the fourth growing season. This increase, however, did not help reach the density levels of the first two growing seasons. An exception was observed in the P fertilization treatment where legume density was significantly reduced only in the third growing season but remained the same in the other three (Table 1). On the other hand, total legume density was increased from autumn to spring in all four growing seasons, especially in the third (1995–1996). However, spring density was sharply decreased in the third growing season, but it did not recover to the levels of the first two growing seasons except in the P fertilization treatment, as in the autumn season (Table 2).

Table 1 Means of total legume density (plants/m^2) in the autumn in the various treatments for four years

Treatments	1993	1994	1995	1996
Burning	66.5aB	160.0abC	4.2cA	33.2aA
Irrigation	165.3cB	304.0dC	1.0abA	41.2abA
Digging	90.5abB	200.0bcC	1.4abA	32.8aA
Cutting	132.0bcB	242.4cdC	0.2aA	38.4abA
P fertilization	142.5bcB	120.8aB	1.0abA	130.0cB
Control	150.8bcB	289.6dC	2.4bcA	50.0bA

Means of each treatment within the same year followed by the same small letter and between years followed by the same capitals are not significantly different at $p \leq 0.05$

Table 2 Means of total legume density (plants/m^2) in the spring in the various treatments for four years

Treatments	1994	1995	1996	1997
Burning	233.6aB	263.6abcB	26.6bA	55.2aA
Irrigation	238.4aB	270.3bcB	3.4aA	47.2aA
Digging	280.0bcB	334.3cB	6.8aA	70.4aA
Cutting	253.6abC	288.3bcC	2.0aA	60.8aB
P fertilization	292.8cD	152.8abB	6.2aA	223.2bC
Control	220.8aC	192.0aC	7.2aA	96.8aB

Means of each treatment within the same growing season followed by the same small letter and between years followed by the same capitals are not significantly different at $p \leq 0.05$

As far as the individual treatments are concerned, the results were the following. Burning resulted in significantly lower density values than the control in the autumn of the first growing season but it recovered in spring. The same pattern was repeated in the second and fourth growing seasons. In contrast, no significant differences were caused in the autumn of the third season while in the spring the treatment produced significantly higher density than the control (Tables 1 and 2).

Irrigation did not produce significant different legume densities compared with the control in both seasons of the four growing seasons except in the spring of the second year when significantly higher density was recorded compared with the control. Similar were the results obtained in the treatment of cutting as well (Tables 1 and 2).

Digging did not significantly affect legume density in the autumn of the first growing season, but the density obtained in the spring was significantly higher than the control. In the second growing season, however, it significantly reduced density during autumn but in the spring produced significantly higher density than the control. The significant reduction appeared again in the autumn of the forth season without recovery during spring. P fertilization finally produced inconsistent results in the first three growing seasons but significantly higher densities than the control and all the other treatments in both seasons of the fourth year.

Similar results with the total legume density were also found with the density of the individual legume species. More specifically, there was a sharp reduction of their density between the second and third growing season and an impressive recovery in the

forth which, however, did not reach the levels of the first. In *O. aequindentata*, a dominant legume species, its density was reduced by 95% on the average for all treatments between 1995 and 1996 and recovered by 175% between 1996 and 1997. Among the treatments, however, only burning and digging resulted in significantly higher than the control densities in 1996, an effect that was also maintained in 1997 but only for the latter treatment. All the other treatments produced no significant results (Table 3).

Among *Trifolium* spp., *T. scabrum*, the second dominant legume, did not appear at all in 1996 while the density in 1997 was on the average much less than the levels of 1995. Among the treatments, only P fertilization produced significantly higher density than the control in 1997. The other treatments produced no significant results in any of the two years. *T. angustifolium*, a third species in terms of dominance, did not appear at all in 1996, except in the irrigation treatment and the control, while it recovered only in the digging treatment. In *T. campestre*, its density was decreased by 95% on the average between 1995 and 1996 and increased by 92% between 1996 and 1997. Among treatments, only P fertilization produced significant results compared with the control in 1997 (Table 3).

Hippocratea multisiliquosa did not also appear in 1996, except in the irrigation treatment and the control, while it recovered only in the burning, digging and cutting treatments as well as in the control (Table 3).

Among *Medicago* spp., finally, overall density was decreased by 88% between 1995 and 1996 in *M. minima* and almost by 100% in *M. disciformis* while it increased by 93% and almost by 100% between 1996 and 1997 in the two species, respectively. They were the only species where 1997 density levels exceeded those of 1995. The applied treatments though affected only *M. minima*, which was more dominant than *M. disciformis*. Specifically, burning, irrigation and P fertilization reduced significantly its density compared with the control, while the latter treatment seemed to have positively affected it in the fourth season (Table 3).

Biomass

Like density, legume biomass changed both among growing seasons and among treatments. More

Table 3 Mean spring density (plants/m²) of the dominant legume species in the various treatments for three years

Treatments	<i>Onobrychis aequindentata</i>		<i>Trifolium scabrum</i>			<i>Trifolium angustifolium</i>			<i>Trifolium campestre</i>			<i>Hippocratea multisiliquosa</i>			<i>Medicago minima</i>			<i>Medicago disciformis</i>			
	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997
Burning	91.6a	15.0c	10.4b	78.5a	0.0	20.0a	16.0a	0.0a	0.0a	37.5a	0.4a	1.6a	3.2a	0.0a	8.0b	4.4a	0.4a	4.0a	2.4a	0.4a	8.8a
Irrigation	77.6a	1.4a	6.4ab	54.5a	0.0	24.0a	11.3a	0.2a	0.0a	23.5a	1.2a	4.8a	40.0b	0.2a	0.0a	4.2a	0.2a	6.4a	2.2a	0.2a	4.0a
Ploughing	88.0a	5.8b	23.2c	44.1a	0.0	27.2a	7.0a	0.0a	5.6b	10.3a	0.2a	7.2a	44.0b	0.0a	11.2b	11.4b	0.0a	3.2a	7.4b	0.4a	0.8a
Cutting	73.6a	0.6a	1.6a	101.0a	0.0	33.6a	10.5a	0.0a	0.0a	24.8a	0.2a	2.4a	0.0a	0.0a	0.8a	11.0b	0.6a	7.2a	2.6a	0.6a	3.2a
P Fertilization	86.8a	2.6ab	12.8b	53.8a	0.0	92.4b	2.5a	0.0a	0.0a	20.3a	1.0a	49.6b	52.0b	0.0a	0.0a	4.8a	0.6a	56.0b	4.4ab	0.2a	8.8a
Control	78.4a	1.2a	8.8ab	59.8a	0.0	52.8a	17.5a	0.6a	0.0a	17.0a	0.4a	8.0a	12.8ab	0.6a	1.6ab	10.4b	1.6a	9.6a	3.6ab	0.8a	7.2a

Means of each treatment within the same year followed by the same letter are not significantly different at $p \leq 0.05$

Table 4 Means of total legume biomass (g/m^2) in the spring in the various treatments for four years

Treatments	1994	1995	1996	1997
Burning	66.42aB	82.59cdB	7.46bA	18.54abA
Irrigation	106.53abC	39.97abB	0.34aA	7.33aA
Digging	109.61abB	114.62 dB	1.43aA	36.13abA
Cutting	108.50abB	18.96aA	0.36aA	7.57aA
P fertilization	123.82bC	59.99abcB	2.45aA	121.72cC
Control	130.27bC	64.91bcB	4.08aA	47.48bb

Means of each treatment within the same year followed by the same small letter and between years followed by the same capitals are not significantly different at $p \leq 0.05$

specifically, it was significantly reduced in four treatments (except burning and digging) between 1994 and 1995, but in all of them between 1995 and 1996. However, no changes occurred in the four of them between 1996 and 1997, when only P fertilization and the control treatments resulted in significantly higher biomass (Table 4).

As far as the individual treatments are concerned, burning significantly reduced legume biomass compared with the control in the first year but it recovered the second year, while in the third the treatment produced significantly higher biomass than the control to return again to control level during the fourth year. Irrigation did not produce significant results except in the fourth year when the biomass produced was significantly lower than the control. Digging did not produce any significant results at all. Cutting resulted in significantly lower biomass in the second and third years, while P fertilization in significantly higher biomass only in the last year of the experiment. The results suggest, as in the case of density, that the treatments applied had no effect (digging), or temporary effects (burning, irrigation and cutting) or a delayed positive effect (P fertilization) on legume biomass (Table 4).

For the individual species, their biomass reacted the same way as the total biomass; they were greater and persistence differences among years than among treatments. *Onobrychis aequinata*, for example, reduced its biomass on the average from the 1995 to 1996 and it increased it from the 1996 to 1997. Among treatments, only burning resulted in increased biomass compared with the control in 1996; cutting resulted in reduced biomass compared with the control in 1995.

Table 5 Mean biomass (g/m^2) of the dominant legume species in the various treatments for three years

Treatments	<i>Onobrychis aequinata</i>			<i>Trifolium scabrum</i>			<i>Trifolium angustifolium</i>			<i>Trifolium campestre</i>			<i>Hippocratea multifiliolosa</i>			<i>Medicago minima</i>			<i>Medicago disciformis</i>		
	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997
Burning	41.66ab	7.35b	10.87ab	9.14b	0.00	1.95a	3.96a	0.00a	0.00a	2.07a	0.00a	0.05a	0.34a	0.00	1.48a	12.37a	0.07a	0.34a	1.71a	0.04a	0.83a
Irrigation	30.50ab	0.21a	3.97ab	3.45a	0.00	2.11a	1.18a	0.01a	0.01a	0.65a	0.00a	0.22a	2.02a	0.00	0.00a	1.86a	0.04a	0.56a	0.74a	0.02a	0.35a
Ploughing	59.26b	1.19a	24.40c	3.21a	0.00	9.56a	0.60a	0.00a	0.41b	0.30a	0.00a	0.92a	15.03c	0.00	1.39a	4.42a	0.00a	0.42a	6.68b	0.01a	0.06a
Cutting	11.32a	0.32a	0.19a	4.26a	0.00	4.72a	0.22a	0.00a	0.00a	0.63a	0.00a	0.12a	0.00a	0.00	1.18a	1.45a	0.02a	0.77a	0.56a	0.03a	0.62a
P fertilization	42.44ab	0.68a	12.60b	4.54ab	0.00	33.56b	0.11a	0.00a	0.00a	2.01a	0.00a	10.88b	0.26a	0.00	0.00a	1.02a	0.01a	52.06b	0.70a	0.01a	6.82b
Control	50.70b	0.26a	7.58ab	6.24ab	0.00	6.08a	1.40a	0.01a	0.00a	0.94a	0.00a	0.53a	3.55b	0.00	0.18a	1.86a	0.21a	1.46a	0.77a	0.10a	0.88a

Means of each treatment within the same year followed by the same letter are not significantly different at $p \leq 0.05$

All the other treatments produced no significant results (Table 5).

Among *Trifolium* spp., *T. scabrum* did not produce any biomass at all in 1996 like density but it recovered completely in 1997. Only P fertilization produced significant higher biomass than the control in 1997, while irrigation, digging and cutting produced significant lower biomass than the control in 1995. *T. angustifolium* did not produce also biomass in 1996 except in the irrigation treatment while in the other two years the biomass produced was negligible. In this year, digging resulted in increased biomass compared with the control in 1997. *T. campestre* behaved also the same way as *T. scabrum* (Table 5).

Hippocrepis multisiliquosa did not produce also measurable biomass in 1996 and the applied treatments affected it during the second year (1995). More specifically, burning, irrigation, cutting and P fertilization resulted in significantly lower biomass while digging in significantly higher biomass than the control (Table 5).

Among *Medicago* spp., finally, *M. minima* had almost null biomass in 1996 but recovered completely in 1997, especially the treatment with P fertilization which produced significantly higher quantity than the control. Similar behaviour was displayed by *M. disciformis* too, which in addition was favoured by the digging treatment in the second season (1995).

Discussion

The great reduction of legume density in the autumn of the third growing season should be attributed to the adverse weather conditions of the months October and November when legumes mainly germinate and get established. According to George et al. (1984), germination occurs when rainfall is at least 25 mm, which is much more than the 2.6 mm that fell in October of that particular season. In November, although rainfall was sufficient (44 mm), the low temperatures (below normal) apparently prevented the mass germination of legumes. The fact that legume density was increased in the spring suggests that leguminous seeds continued to germinate after November but in limited numbers since this particular season was quite low compared to the other seasons. This late germination (after November)

should explain the increase of the spring density as compared with the autumn one in the other growing seasons, too. The overall decrease though of the spring density in the second growing season compared with the autumn one, on the contrary, should be attributed to the relatively low temperatures of the winter months, especially December and January. This means that moisture and temperature in October and November and temperatures in November and December are the critical factors for legume germination and establishment, while too low temperatures in the winter months may kill the established seedlings. Special research should be carried out to verify all these interactions between weather parameters and legume seed germination.

The recovery of legumes during the fourth growing season, after the great reduction of the third one, should be attributed to the seed reserves in the seed bank. The fact that the seeds of annual legumes are part of the permanent seed bank in the mediterranean environment has been confirmed by several investigators (Rice 1989; Cocks and Osman 1996; Ehrman and Cocks 1996; Cocks 1992a, b; Del Pozo and Aronson 2000; Sulás et al. 2000) and suggests their high adaptation and resilience to mediterranean climate. However, not all the species were equally affected by the adverse weather conditions of the third year or recovered in the fourth growing season. For example, *O. aequindatata* was the least while *T. scabrum* the most affected species, but the latter recovered more impressively than the former. An impressive recovery was also displayed by annual medics, especially *M. minima*, while *T. campestre* recovered much more easily than *T. angustifolium*. These results show that individual annual legumes have different sensitivities to extreme weather conditions and different capabilities to recover after them.

Among the factors studied, P fertilization was the most effective in improving both legume density and biomass and helping most species recover from the shock of the third growing season, especially *T. scabrum*, *T. campestre* and *M. minima*. The reduced density and biomass in the second growing season should be attributed to the limited rainfall that fell during this season (only 74% of the long term average). The results of this factor were expected. Several authors have found that P fertilization favours the establishment and growth of legumes in

mediterranean grasslands at the expense of grasses (Papanastasis and Koukoulakis 1988; Menke 1989; Osman et al. 1991; Henkin et al. 1996; Henkin and Seligman 2000; Papanastasis and Papachristou 2000; Rochon et al. 2004).

Burning is known to favour legumes in two ways: by breaking their seed dormancy and removing the competitive vegetation. Several investigators have found an increased legume density in mediterranean ecosystems, especially in forests after fire (Papavasiliou and Arianoutsou 1993; Kazanis and Arianoutsou 1996; Arianoutsou and Thanos 1996). The negative effects that burning had in this study, especially in the autumn densities, although they did not persist, could be attributed to a possible partial destruction of the surface seeds by fire which produced a great part of the autumn seedlings. Among these, *O. aequindentata* was the most and *M. minima* the least favoured species.

The lack of any appreciable effect of autumn irrigation could be attributed to the fact that it was applied in November and not in October which turned out to be the most critical month for seed germination. As for March irrigation, apparently it was not needed except in the second growing season which had a dry spring.

Although digging was a quite “severe” treatment, legumes are adapted to such a factor, since density could recover by the end of the growing season. This recovery can be attributed to the modification of the permanent seed bank as seeds from the deeper soil layer could come to the surface and germinate. The treatment especially favoured *O. aequindentata*, *T. angustifolium*, *H. multisiliquosa* and *M. disciformis*.

Several researchers claim that cutting favours legumes since it removes competitive vegetation, especially grasses (Naveh and Whittaker 1979; Sternberg et al. 2000). On the other hand, cutting during the bloom stage may limit the capacity of legumes to produce seeds and subsequently persist (Briske 1989; Tsiouvaras et al. 1993). The lack of any appreciable effects of this factor in the present study can be attributed to the large variety of species present which do not bloom at the same time suggesting that the one cut applied in spring was not enough to impose significant results, especially on legume density. Legume biomass, however, was negatively affected in the second growing season, which was relatively dry suggesting a negative

interaction between cutting and drought. The species involved in this interaction were *O. aequindentata*, *T. scabrum* and *H. multisiliquosa*.

Conclusion

It can be concluded that annual legumes are vulnerable both to weather changes and to management interactions. Nevertheless, they display a remarkable capacity to overcome these changes and interventions due to their opportunistic behaviour. This behaviour is dictated by the different strategies of the component species which allows them to survive in a variable and highly unpredictable natural environment. On the other hand, annual legumes constitute an important asset of mediterranean grasslands. The high biomass recorded in this study, although reduced over the four years, indicates their importance both for ecosystem functioning through their capacity to fix atmospheric nitrogen and for feeding grazing animals especially sheep, due to their high nutritional value.

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Are irrigation and grazing effects transferred, accumulated, or counteracted during plant recruitment?

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Abstract How do effects from perturbations such as irrigation and grazing that have an impact at one stage of the recruitment process (e.g., seedling) affect performance at later stages (e.g., adult)? Such effects may be transferred to later stages without any further change (transferred effects), reinforced by a similar effect so that their importance increases (accumulative effect), or counteracted at later stages by an opposite effect (counteractive effect). We analysed the predominance of transferred, accumulative, and counteractive effects depending on (1) organization level (community, functional group, and species levels), (2) life cycle stage (seeds, seedlings, and adults), (3) grass/forb functional group (forbs versus grasses), and (4) seed mass (small-seeded species, medium-seeded species, and large-seeded species). The study was conducted in Alcalá de Henares, central Spain. During one annual cycle, we counted the number of readily germinable seeds (3,909 seeds: 2,156 forbs and 1,753 grasses), emerged seedlings (2,126 seedlings: 1,238 forbs and 888 grasses), and

surviving seedlings up to reproductive adult status (917 adults: 217 forbs and 700 grasses) of all species (74 species) in a factorial field experiment under three different simulated rainfall and three seasonal sheep grazing regimes in a natural Mediterranean annual plant community composed of forbs (dicotyledonous herbs) and grasses (monocotyledonous herbs). Our main results were: (1) the proportion of transferred and counteractive effects was similar between the three studied organization levels (community, functional group, and species levels); (2) we detected many more counteractive effects in the seedling stage than in the adult stage; (3) we did not find more counteractive effects in forbs versus grasses, but transferred effects were more frequent in grasses; and (4) we found more counteractive effects with decreasing seed mass and more transferred effects with increasing seed mass. Our main conclusions were that the prevalence of counteractive effects reduces the sensitivity of the whole recruitment process and enhances the relative importance of the final stage (adults). The prevalence of transferred effects makes the recruitment process more predictable and more dependent on the early stages (seeds and seedlings).

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Keywords Factorial field experiment · Functional groups · Grazing · Mediterranean annual plant community · Rainfall variability · Summer drought · Vole mounds · Water regime

Introduction

A central issue of plant ecology is the understanding of the relative role of different life history stages in successful plant recruitment. The consecutive stages of seed, seedling, and adult are related to each other in a complex way that largely depends on species and the influence of physical and biological factors (Goldberg et al. 2001), for example, irrigation and grazing. As a result of relationships between these stages, the consequences of an ecological factor depend on the way that its effects propagate onto the following stage of the recruitment process. As far as we know, there are no published studies that have addressed this subject.

In this article, we characterize the relationships between the three plant developmental stages. Some effects at a certain stage can be transferred to subsequent stages without any further change, thus inducing transferred effects. Some effects can become more intense through the occurrence of similar effects (accumulative effects) in later stages. In contrast, some effects are counteracted or counterbalanced at a certain stage by opposite effects (counteractive effects) and do not have consequences in subsequent stages. The prevalence of one or another type of effect (transferred, accumulative, or counteractive) is of high ecological interest in order to understand the mechanisms regulating plant recruitment.

The dominance of each type of effect is expected to vary with the level of plant organization (community, functional group, and species levels). At the community level, counteractive effects are expected to be relatively more important, due to differences in sensitivity between species and functional groups (Rebolledo et al. 2003). At this level, the relationships between consecutive stages may differ depending on the community parameter analysed (total density of individuals, species richness, similarity in species composition between consecutive stages of the recruitment process, spatial heterogeneity in species composition and evenness). A stage (e.g., adults) could show huge differences in density of individuals with respect to the previous stage (e.g., seedlings), but small changes in species richness or evenness. Although the importance of each stage may vary strongly at different organization levels, most scientific studies consider only one or a few target species. We found very few field studies addressing the

recruitment of adult plants in entire communities, and all of them focus on changes in total densities of individuals and do not refer to changes in community structure (Moles and Drake 1999; Rebolledo et al. 2001; Goldberg et al. 2001). This field of research has hardly been explored empirically, and we think it may reveal interesting mechanisms for the regulation of individual density and species diversity in plant communities. At the functional group level (which sorts species according to common features), we expect differences depending on growth form (grasses versus forbs) and depending on seed mass (differences between small-seeded, medium-seeded, and large-seeded species). Some authors (Goldberg et al. 2001; Rebolledo et al. 2001) studying annual plant communities have found greater seedling emergence and survival of grasses when compared with forbs. This suggests a stronger influence of transferred effects on grasses, both between seeds and seedlings, and between seedlings and adults. Additionally, seedling emergence and survival are largely determined by seed reserves (Greiling and Kichanan 2002). Transferred effects between seeds, seedlings, and adults are expected to be more important for large-seeded species, because a greater amount of reserves usually improves seedling emergence and survival (Winn 1985). At the species level, the response to environmental factors (e.g. irrigation and grazing) will largely depend on species identity (Silvertown et al. 1993).

The main aim of our study was to determine the relative importance of two life history stages (seeds and seedlings) on recruitment of adult plants in an annual herbaceous plant community subjected to irrigation and grazing. We were interested in studying three environmental variability levels through three water availability regimes simulated by irrigation: (a) stable throughout the year (all-year irrigation), (b) with predictable annual fluctuations (autumn-and-spring irrigation but with summer drought), and (c) with unpredictable annual fluctuations (the usual rainfall regime under a Mediterranean climate). We were also interested in testing three sheep grazing levels: autumn grazing (grazing during seedling emergence), spring grazing (grazing during plant growth and flowering period), and no grazing.

The study was a 1-year factorial field experiment conducted in a 9-year-old Mediterranean plant community under different irrigation regimes (surrogate

for precipitation) and sheep grazing. The successful recruitment of adult plants was analysed on small-scale soil disturbances: mounds of the pine vole (*Microtus duodecimcostatus*, de Sélys-Longchamps, 1839), a common fossorial rodent in the Iberian Peninsula. We addressed the following specific questions at the plant community, functional group, and species levels: (I) what are the effects of irrigation and grazing on the three stages of the plant recruitment process (seeds, seedlings, and adults)? (II) what irrigation and grazing effects are transferred, accumulative, and counteractive effects? This study has an exploratory nature and examines the predominance of transferred, accumulative, and counteractive effects depending on (1) organization level (community, functional group, and species levels), (2) life cycle stage (seeds, seedlings, and adults), (3) grass/forb functional group (forbs versus grasses), and (4) seed mass (small-seeded species, medium-seeded species, and large-seeded species). (III) We discuss the ecological implications of the prevalence of each kind of effect (transferred, accumulative, and counteractive effects) and the importance of counteractive effects in minimizing effects of abiotic (rainfall variability) and biotic factors (grazing) on plant communities. In order to understand the relative importance of seeds and seedlings for the recruitment of adult plants, we discuss the effects of irrigation and grazing on seedling emergence (relates seeds with seedlings) and seedling survival (relates seedlings with adults).

Methods

Site description

The study was conducted at “El Encín” Experimental Farm (IMIDRA) in Alcalá de Henares (central Spain, 40°35' N, 3°25' W) at a mean elevation of 565 m. Climate is semi-arid continental Mediterranean with mean annual temperature and precipitation of 13.1°C and 424 mm, respectively. Precipitation is characterized by a high year-to-year fluctuation in timing and amount and by a pronounced summer drought. The main rainfall periods are autumn and spring. Potential vegetation is an *Ulmus minor* Mill. forest, which has long been converted into arable farmland. The natural plant community is currently dominated by annuals

which germinate mainly during the autumn rainy period (Rebollo et al. 2001). Species overwinter as seedlings or juveniles and reach the reproductive stage (i.e. adults) with the spring rains. Species bear fruit and die at the beginning of the summer drought (Rebollo et al. 2003) (see Appendix for species, growth forms, and seed masses). The size range of the seeds varies from 0.0077 mg of *Fumaria parviflora* to 51.4666 mg of *Vicia sativa*. Mean aboveground net primary production for the 4 years prior to the study was 490 g dry matter/m² year.

Experimental design

The experiment was set up in September 1997 with two blocks (Fig. 1). Each block included nine plots, which represent all combinations (i.e. a 3 × 3 factorial experiment) of three irrigation treatments (non-irrigation, autumn-and-spring irrigation, and all-year irrigation) by three sheep grazing treatments (non-grazed, spring grazed, and autumn grazed). Therefore, one plot per block had no manipulation (i.e. non-irrigation × non-grazed). Experimental plots were 11.5 m × 14 m, and treatments were randomly distributed in each block. This design allows six replicates for testing direct effects

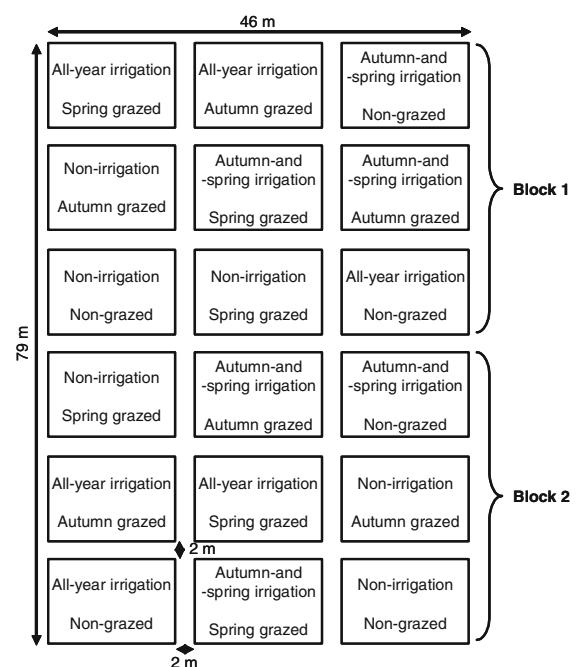


Fig. 1 Experimental design

(irrigation and grazing treatments) and two replicates for analysing interactions.

Irrigation treatments were applied by means of four sprinklers per plot, allowing homogeneous distribution of water and maintaining soil moisture at over 20% volume during irrigation periods. Soil moisture was monitored weekly at two points per plot on undisturbed ground using Time Domain Reflectometry (TDR technique) and a 16-cm-long sensor. The autumn-and-spring irrigation treatment ensured water availability during the critical periods for germination (autumn) and plant growth (spring). The all-year irrigation treatment was applied during these periods and also in summer, eliminating the summer drought. Mean additional water provided by the autumn-and-spring and all-year irrigation treatments in the study year was 82 and 355 mm, respectively. Sheep grazing treatments involved controlled grazing with a stocking rate of 4.3 sheep/ha year. Plots were grazed for five consecutive days at the beginning of the growing season (autumn grazing), which is the main germination and seedling establishment period, or at the end of the growing season (spring grazing), the main period for plant growth and flowering. Mean herb consumption by sheep was 57 g/m² year in autumn and 129 g/m² year in spring.

The Mediterranean pine vole *M. duodecimcostatus* (weight: approximately 25 g) feeds primarily on roots and bulbs in underground tunnels and regularly deposits the excavated soil material on the surface, building mounds of bare soil that cover the burrow entrance (Gómez-García et al. 1995). We found mean (± 1 SE) size and weight of mounds to be ($n = 40$): 34.6 ± 1.31 cm maximum diameter, 25.8 ± 0.96 cm minimum diameter, 6.9 ± 0.32 cm tall, and $2,834 \pm 326.3$ g dry weight of excavated soil. Voles excavate soil up to 1 m in depth (Soriguer and Amat 1980).

We randomly selected eight fresh vole mounds per plot in spring 1999 in order to (1) analyse the readily germinable seed bank in autumn 1999, in four of them, and (2) monitor emerged seedlings in the field, up to reproductive status, in spring 2000, in the four remaining mounds. We also randomly selected a total of 20 fresh vole mounds in the study area (less than 10 h old) in spring 2002, in order to analyse the germinable seed bank at the time of mound formation. The comparison of seeds present in these mounds in spring and autumn allowed us to know

the origin of seeds and estimate seed rain from spring to autumn. All selected mounds were kept 1.5 m from plot fences to avoid border effects.

Seed, seedling, and adult counts

We counted the number of readily germinable seeds (3,909 seeds: 2,156 forbs and 1,753 grasses), emerged seedlings (2,126 seedlings: 1,238 forbs and 888 grasses), and surviving seedlings up to reproductive adult status (917 adults: 217 forbs and 700 grasses) of all species (74 species).

Readily germinable seed bank (estimated in the glasshouse)

Four of the eight mounds selected per plot in spring 1999 were randomly chosen in autumn 1999 (autumn samples). One 10 cm × 10 cm and 4 cm-deep soil sample was collected from the central part of each mound. We also collected a similar soil sample from each of the 20 fresh mounds selected in spring 2002 (spring samples). Both sets of soil samples were placed in 15-cm-deep and 14.5-cm-diameter pots on top of a 8-cm layer of vermiculite, separated by a thin layer of fine gauze. They were then placed in a transparent glasshouse with temperature and light close to outdoor conditions. Soil samples were watered regularly from autumn (autumn samples) and spring (spring samples) onwards. Viable seeds ready to germinate (readily germinable seed bank sensu Thompson and Grime 1979) were estimated by the number of emerged seedlings. Seedlings were identified, counted, and removed throughout 1 year. This period was considered sufficiently long, because most seeds in this type of community germinate during the first year (Levassor et al. 1990). Four pots with sterile soil were placed in the glasshouse to determine potential seed rain, but no seeds from outside the soil samples were detected.

Emerged seedlings and seedlings followed up to reproductive adult status (estimated in the field)

In the four remaining mounds per plot, selected in spring 1999, we set up permanent 10 cm × 10 cm quadrat in the centre of each mound. Quadrats were inspected at 10-day intervals during the first 4 months (mid-August–December 1999), and monthly

thereafter until July 2000 because seedling emergence was less intense. We counted all emerged seedlings and adults. We considered adults those seedlings which survived up to reproductive status and presented flowers or fruits. This was supplemented by mapping plants on photographs (Rebollo et al. 2001).

Parameters for statistical analyses

Five community-level parameters were estimated. (1) Total density, calculated as the number of total individuals per $10\text{ cm} \times 10\text{ cm}$ quadrat. (2) Species richness per plot, calculated as the number of species minus one, divided by the logarithm of the number of individuals (Margalef 1951 quoted by Peet 1974) ($D_{Mg} = (S - 1)/\ln N$, where N is the total number of individuals). This was necessary because species richness is strongly influenced by density of individuals (Goldberg and Estabrook 1998). (3) Similarity in species composition between consecutive stages of the recruitment process: (a) seed bank and seedling pool and (b) seedling and adult pools. Whittaker's (1952) index of community association [Similarity = $1 - (0.5 \times \sum |p_{ia} - p_{ib}|)$], where p_i is the proportion of species i in sample pools "a" and "b", was chosen because it is responsive to the less abundant species. A value of 0 indicates a completely different species composition, and a value of 1 indicates that both stages have the same species in the same proportions. (4) Spatial heterogeneity in species composition: we calculated the mean Whittaker (1952) index of community association between the four $10\text{ cm} \times 10\text{ cm}$ quadrat of each plot. (5) Pielou's (1966) evenness per plot ($J = H'/H'_{max}$) where H' is Shannon and Weaver's (1949) diversity per plot ($H' = -\sum_{i=1}^S p_i \ln p_i$, where p_i is the proportion of species i in the sample pool). In order to investigate differences between the five functional groups, we calculated the density of individuals per $10\text{ cm} \times 10\text{ cm}$ quadrat for (1) grasses, (2) forbs, (3) small-seeded species ($<0.1\text{ mg/seed}$), (4) medium-seeded species ($0.1\text{--}1\text{ mg/seed}$), and (5) large-seeded species ($>1\text{ mg/seed}$) (Rebollo et al. 2001). When allocating seeds into different seed size classes, forbs and grass species were pooled. Density per $10\text{ cm} \times 10\text{ cm}$ quadrat was also estimated for the five most abundant species in the seed bank in autumn (*Stellaria media*, *Galium parisiense*, *Vulpia*

ciliata, *Bromus rubens*, and *Trisetum paniceum*). Values for vegetation parameters estimated per $10\text{ cm} \times 10\text{ cm}$ quadrat were averaged per plot in order to avoid pseudoreplication (Hurlbert 1984).

Statistical procedures

Two-way ANOVAs were performed in order to test the effects of irrigation and grazing on vegetation parameters at the seed, seedling, and adult stages. Irrigation and grazing treatments were used as factors. These analyses allowed us to detect irrigation-sensitive and grazing-sensitive stages. ANOVA analyses were also used to identify the beginning of the effects of treatments (the first stage when a significant effect was detected) and counteractive effects (when the significant effect was not detected at the next stage). ANCOVA analyses were used to identify transferred effects that occurred during the previous stage and accumulative effects that reinforced previous effects. We used the vegetation parameter from the previous stage as a covariate. If the covariate decreased or eliminated the effect of a treatment, this implied that the treatment mechanism acted through the covariate (Cox 1958, p. 65), i.e. the treatment acted at the stage prior to the one tested (transferred effect). If the effect remained after inclusion of the covariate, this implied that the treatment mechanism did not act through the covariate, i.e. the treatment also acted at that stage (accumulative effect). In order to attain normality and homogeneity of variances, vegetation parameters were transformed by the expression "squared root ($x + 1$)". We report means ($\pm 1\text{ SE}$) for data without transformation. We used *t*-tests to compute the significance level for the difference between the proportions of each type of effect between life stages and between organization levels [StatSoft, Inc. (2003). STATISTICA (data analysis software system), version 6. www.statsoft.com].

Results

We found low numbers of seeds (mean: 6.05 ± 0.95 seeds/ 100 cm^2 , ranging from 0 to 14 seeds) on vole mounds at the time of mound formation in spring and before seed rain took place (spring samples). Thereafter, the results refer to seeds

from the autumn samples, collected after the seed rain has occurred.

Effects of irrigation and grazing on seeds, seedlings, and adults

Irrigation affected total seed density (Table 1) in such a way that autumn-and-spring irrigation increased total seed density with respect to the control and all-year irrigation treatments (Fig. 2a). Spring grazing decreased total seed density with respect to the control and autumn grazing (Fig. 2b). These effects of irrigation and grazing on total seed density appeared with marginal significance at the seedling stage (irrigation $P = 0.074$ and grazing $P = 0.089$). A negative effect of grazing on total density of adults was found in irrigated plots. This effect was stronger in the all-year irrigated plots (Fig. 3a). Irrigation decreased species richness of seedlings (Fig. 2c) and decreased species richness and evenness of adults (Fig. 2d and e).

With respect to grasses and forbs, irrigation had positive significant effects on seedlings of grasses in the ungrazed and spring-grazed plots (Table 1 and Fig. 3b). These effects remained significant during the adult stage. Forb seed density increased in the autumn-and-spring irrigated plots (Fig. 2f). The density of adult forbs was greater in non-irrigated and grazed plots (Fig. 3c).

Seed density of small-seeded species increased in the autumn-and-spring irrigated plots (Fig. 2g). Adult density of medium-seeded species increased with irrigation only in ungrazed plots (Fig. 3d). The seedling density of large-seeded species increased with irrigation only in ungrazed plots (Fig. 3e), and this effect remained significant during the adult stage. At the species level (for the five most abundant species: *S. media*, *G. parisiense*, *V. ciliata*, *B. rubens*, and *T. paniceum*), irrigation and grazing effects varied with species identity and were, in general, very different from those acting at the community level. *Trisetum paniceum* was the least sensitive species to the treatments, and *B. rubens* was the most sensitive one.

Transferred, accumulative, and counteractive effects of irrigation and grazing

Transferred effects were those irrigation and grazing effects that occurred at a certain developmental stage

and were transferred to subsequent stages without any further change. We estimated the number of transferred effects from the 20 significant irrigation and grazing effects influencing seedlings and adults (see the 20 effects in Table 1). We used ANCOVA to check (see Table 2) whether these effects derive from irrigation and grazing effects occurred on the previous stage (seed and seedling stages, respectively). Sixty percent of effects that affected seedlings and adults were transferred from a previous stage (12 out of 20). The 12 transferred effects are shown in Table 3. The number of transferred effects was not significantly different between the three organization levels [community (4 out of 5), functional group (6 out of 10), or species level (2 out of 5)].

Accumulative effects were those that reinforced irrigation and grazing effects that had appeared in a previous stage and that become more important in a subsequent stage. We found no evidence of accumulative effects at the community, functional group, and species levels.

Counteractive effects were those that counteracted irrigation and grazing effects that had occurred during earlier stages. We analysed counteractive effects taking into account the 13 significant effects that influenced seeds and seedlings (see the 13 effects in Table 1). We checked whether the 13 effects were still taking place in the following stage (seedling and adult stage, respectively). Sixty-two percent of these effects were counteracted during the following stage (8 out of 13 effects). The eight counteracted effects are shown in Table 3. The three organization levels analysed had similar proportions of counteractive effects (community, 2 out of 3, and functional groups and species, 3 out of 5). More counteractive effects were observed in the seedling stage than in the adult stage (6 out of 6 and 2 out of 7, respectively, $P = 0.024$). All significant effects of irrigation and grazing on seeds were counteracted during the seedling stage.

Discussion

Seed density on mounds largely depends on soil origin, time of formation with respect to the main seed rain period, and the seed trapping potential of the soil (Boeken and Shachak 1994; Rebollo et al. 2001). At the time of mound formation in spring, seed density

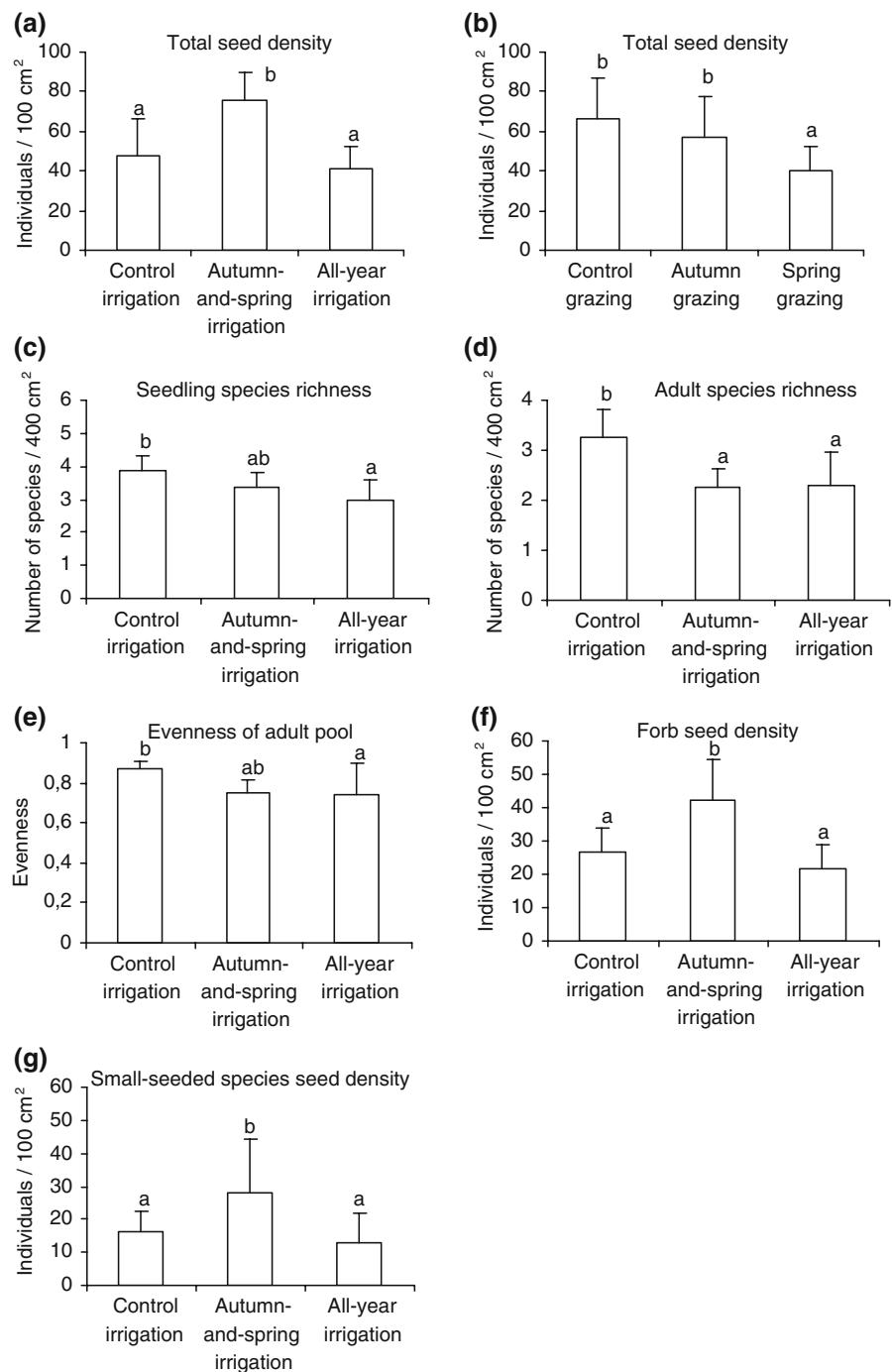
Table 1 Results of the two-way ANOVAs used to test the effects of irrigation and grazing on vegetation parameters at a particular stage (seeds, seedlings, and adults)

(1) Community	Irrigation		Grazing		(2) Functional group		Irrigation		Grazing		(3) Species		Irrigation		Grazing		Irrigation		
	df = 2		df = 2		df = 4		df = 2		df = 2		df = 4		df = 2		df = 2		df = 4		
	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	
Total density																			
Seeds	9.56***	5.46*	0.35	Seeds	3.44#	3.04#	0.35	Seeds	13.63***	0.06	1.30								
Seedlings	3.66#	3.32#	2.07	Seedlings	3.06#	4.77*	6.53*	Seedlings	0.93	0.95	0.58								
Adults	1.60	6.36*	12.29**	Adults	4.16*	4.07#	5.68*	Adults	1.70	0.89	1.28								
Species richness																			
Seed bank	0.69	0.21	0.67	Seeds	8.87***	2.78	1.76	Seeds	0.05	0.38	0.54								
Seedling pool		4.66*	1.23	1.52	Seedlings	1.94	0.74	0.22	Seedlings	0.82	4.83*	2.10							
Adult pool		5.71*	0.27	1.68	Adults	10.30***	0.04	6.49*	Adults	0.37	2.18	1.43							
Evenness																			
Seed bank	1.18	0.31	0.29	Seeds	4.80*	1.83	1.88	Seeds	7.97*	1.70	0.98								
Seedling pool	2.00	0.47	0.61	Seedlings	2.35	0.05	0.13	Seedlings	0.28	0.22	1.80								
Adult pool		4.62*	2.84	3.38#	Adults	0.57	2.92	1.97	Adults	2.34	0.30	3.35							
Spatial heterogeneity																			
Seed bank	0.51	1.04	0.60	Seeds	2.81	1.29	0.59	Seeds	1.68	1.35	2.91								
Seedling pool	1.62	0.66	0.56	Seedlings	0.67	2.06	2.24	Seedlings	10.30***	2.11	9.04**								
Adult pool	1.62	0.04	0.72	Adults	1.70	3.10#	7.27***	Adults	11.63***	1.78	13.72***								
Similarity																			
Seed bank-seedling pool	0.06	0.65	1.35	Seeds	0.93	1.78	1.66	Seeds	0.87	2.25	0.24								
Seedling pool-adult pool	0.18	0.54	0.33	Seedlings	0.68	4.05#	4.19*	Seedlings	1.83	4.06#	1.77								
				Adults	2.04	4.84*	5.14*	Adults	1.25	2.88	2.72#								

Vegetation parameters were ordered according to three organization levels: (1) community level, (2) functional group, and (3) species. We have marked in bold the 20 significant effects observed at the seedling and adult stages, which were used to test for the occurrence of transferred effects (see Table 2). We have marked in italics the 13 significant effects observed at the seed and seedling stages, which were used to analyse the occurrence of counteractive effects

$P < 0.10$; * $P < 0.05$; ** $P < 0.01$

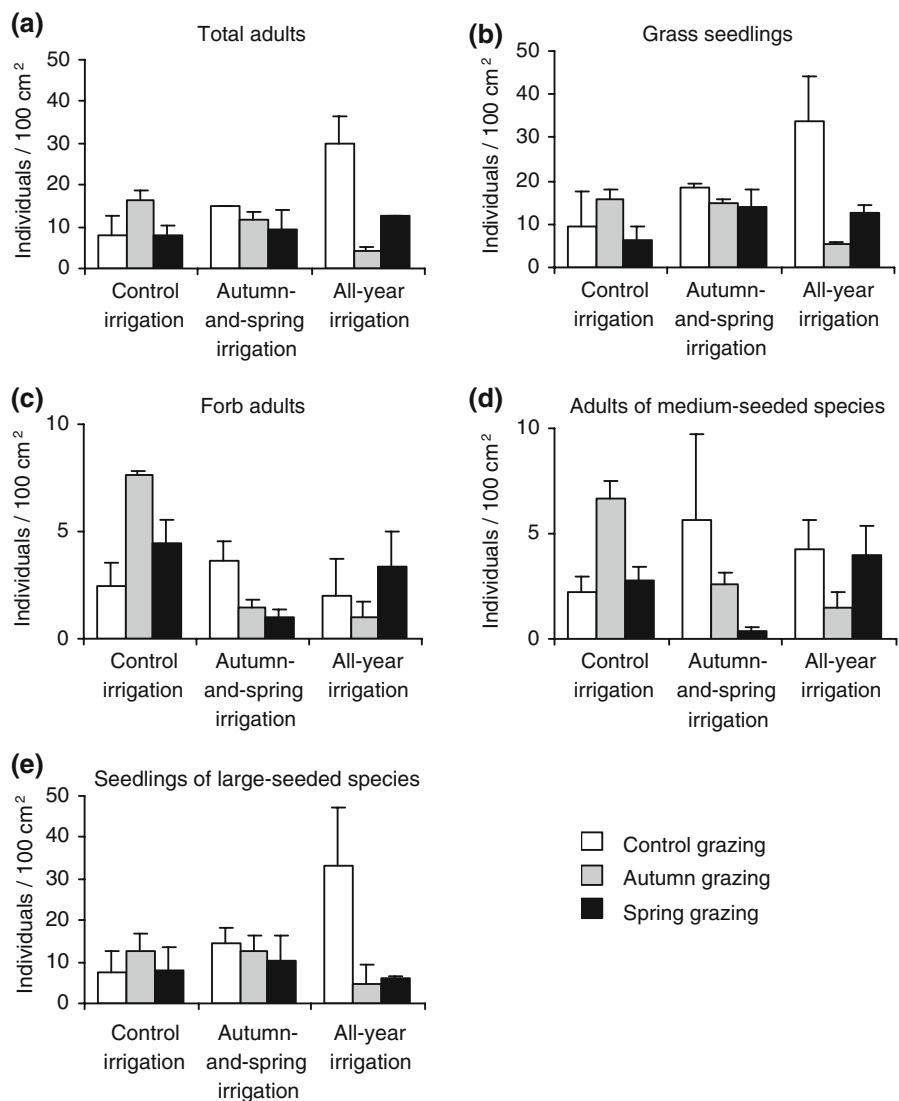
Fig. 2 Significant direct effects of irrigation and grazing on community parameters (**a–e**) and functional groups (**f, g**) (see also Table 1). Means of vegetation parameters for each treatment: **(a)** total seed density in the irrigation treatments, **(b)** total seed density in the grazing treatments, **(c)** seedling species richness in the irrigation treatments, **(d)** adult species richness in the irrigation treatments, **(e)** adult evenness in the irrigation treatments, **(f)** seed density of forbs in the irrigation treatments, **(g)** seed density of small-seeded species in the irrigation treatments. Error bars indicate 1 SE. Bars with the same letter are not statistically different (Tukey test, $P > 0.05$)



on mounds was very low (a mean of 6.05 seeds/100 cm²) compared with the beginning of the germination period in autumn (a mean of 54.7 seeds/100 cm²). Low seed density at the time of mound formation was due to a scarcity of germinable seeds at the depths from which soils were excavated by voles

(up to 1 m deep, Soriguer and Amat 1980). Almost 90% of seeds present during the germination period in autumn depended on the spring and summer seed rain that occurred after mound formation. Hobbs and Mooney (1985) also found low seed densities on gopher mounds in the serpentine grassland

Fig. 3 Significant interactive effects of irrigation and grazing on community parameters (a) and functional groups (b–e) (see also Table 1). Means per 100 cm² quadrat of (a) adult density, (b) seedling density of grasses, (c) adult forb density, (d) adult density of medium-seeded species, (e) seedling density of large-seeded species, for each irrigation and grazing treatment. Error bars indicate 1 SE



community. Our results suggest that seed colonization from seed rain is a crucial process for vole mound revegetation in this Mediterranean annual plant community. By contrast, mounds built by other species (e.g. mounds from scrapes built by European rabbits *Oryctolagus cuniculus*) depend more on the soil seed bank, because these mounds are built from the soil top layer which have many seeds (Ortega 1994).

What are the effects of irrigation and grazing on plant recruitment?

It is well known that irrigation increases seed production in numerous plant species, especially in annual plants under semiarid conditions (Harper 1977). In

agreement with this, we found that seed density in autumn increased with irrigation. However, we found no increase in the all-year irrigated plots, which were also watered during the summer. Several mechanisms may explain this effect of nullifying the summer drought. Irrigation during summer may (a) favour seed mortality by a wider group of soil organisms (e.g., moulds) (Cardina et al. 1996); (b) increase the activity of invertebrates that feed on or harvest seeds (Fenner 2000); and (c) promote a greater abundance of late-summer species (e.g., *Conyza canadensis*), the seeds of which would not be present at the time of sampling because for these species the seed rain occurs later. Additional studies are needed to analyse the relevance and mechanisms of these detrimental effects of

Table 2 Results of the ANCOVA analyses used to determine whether the irrigation and grazing effects detected at a particular stage (see Table 1) occurred in that stage or during a previous stage (transferred effect)

We used the vegetation parameter value from the previous stage as a covariate. If the covariate decreases or eliminates the effect of a treatment, this implies that the treatment acted during the previous stage. Vegetation parameters were ordered according to three organization levels: (1) community level, (2) functional group, and (3) species. We show a summary of the transferred effects in Table 3

$P < 0.10$; * $P < 0.05$;

** $P < 0.01$;

*** $P < 0.001$

	Covariate	Irrigation		Grazing		Irrigation \times grazing df = 4 F		
		Name	df = 1 F	df = 2 F	df = 2 F			
<i>(1) Community</i>								
Total density								
Adults	Seedlings		0.61	0.58	3.23 [#]	6.43*		
Species richness								
Seedling pool	Seed bank		0.37	3.85 [#]	0.92	1.39		
Adult pool	Seedling pool		4.28 [#]	2.33	0.21	0.66		
Evenness								
Adult pool	Seedling pool		10.91*	3.01	2.92	5.74*		
<i>(2) Functional group</i>								
Grasses								
Seedlings	Seeds		0.15	2.24	2.68	5.71*		
Adults	Seedlings		77.92***	3.85 [#]	0.05	1.35		
Forbs								
Adults	Seedlings		0.99	10.59**	0.14	5.90*		
Medium-seeded species								
Adults	Seedlings		5.94*	2.28	2.02	4.39*		
Large-seeded species								
Seedlings	Seeds		0.13	0.45	2.42	3.27 [#]		
Adults	Seedlings		18.39**	1.42	0.42	0.81		
<i>(3) Species</i>								
<i>Galium parisense</i>								
Seedlings	Seeds		5.38*	1.02	5.56*	2.12		
<i>Bromus rubens</i>								
Seedlings	Seeds		1.34	5.85*	0.91	8.14**		
Adults	Seedlings		37.83***	0.72	0.26	1.30		

summer irrigation on seed density under Mediterranean conditions. Irrigation also affected the community structure of seedling and adult pools. Lower species richness (corrected by number of individuals) and evenness were found in irrigated plots. This finding suggests that irrigation favoured the germination and seedling survival of some species but not others, probably by promoting species which are better competitors for light in the more mesic conditions created by irrigation (Padgett et al. 2000). The fact that the decrease in evenness was greater under ungrazed with respect to grazed conditions reinforces this hypothesis, because sheep grazing probably exerts some control on the establishment of these relatively taller and better light-competitor plants.

Grazing decreased seed density, and this depended on the timing of grazing. The greatest effect was

found when grazing occurred during the flowering period (spring grazing treatment). This may be due to inflorescence removal or to decreased plant investment in reproductive output after grazing events (see Obeso 1993 for an extensive review). In contrast with O'Connor (1991) and Bullock et al. (1995), grazing did not affect seedling emergence. These authors found that grazing by ungulates may increase seedling density by removing litter or vegetation covering gaps. In the present study, gaps due to vole mounds presented low litter and adult plant cover during the germination period, regardless of grazing treatment (Rebolledo et al. 2003). This probably limited the effects of grazing on emergence. Grazing decreased adult density, but only in irrigated plots. The presence of taller seedlings in the irrigated treatments may increase overall seedling sensitivity

Table 3 Summary of the transferred and counteractive effects of irrigation and grazing on vegetation parameters at a particular stage (seeds, seedlings, and adults)

(1) Community	Irrigation	Grazing	Irrigation × grazing	(2) Functional group	Irrigation	Grazing	Irrigation × grazing	(3) Species	Irrigation	Grazing	Irrigation × grazing
Total density											
Seeds				Grasses							
Seedlings	Counteractive	Counteractive	Transferred	Seeds							
Adults				Seedlings							
Species richness											
Seed bank				Adults							
Seedling pool	Transferred			Forts							
Adult pool	Transferred			Seeds							
Evenness											
Seed bank				Seedlings							
Seedling pool				Adults							
Adult pool	Transferred			Small-seeded species							
Spatial heterogeneity											
Seed bank				Seeds							
Seedling pool				Seedlings							
Adult pool				Adults							
Similarity											
Seed bank–seedling pool				Medium-seeded species							
Seedling pool–adult pool				Seeds							
Similarity				Seedlings							
Seed bank–seedling pool				Adults							
Seedling pool–adult pool				Large-seeded species							
Similarity				Seeds							
Seed bank–seedling pool				Seedlings							
Seedling pool–adult pool				Adults							
Similarity				Transferred							
Seed bank–seedling pool				Transferred							
Seedling pool–adult pool				Transferred							
Similarity				Transferred							

Vegetation parameters were ordered according to three organization levels: (1) community level, (2) functional group, and (3) species

to sheep grazing. It is known that these herbivores only graze vegetation above a certain height (Hodgson and Illius 1996).

The effects of the irrigation and grazing treatments on the five most abundant species were species dependent, each presenting a different response pattern. This suggests that demographic dynamics at the community level cannot be easily guessed by observing a few particular species and emphasizes the need to address different organization levels to fully understand plant recruitment processes and responses to ecological factors.

What effects of irrigation and grazing are transferred, accumulative, and counteractive effects?

A major aim of this study was to detect which stages promote the transfer of irrigation and grazing effects (transferred effects) from earlier stages such as seeds and seedlings and which effects are reinforced or counteracted (accumulative or counteractive effects, respectively). The balance between transferred, accumulative, and counteractive effects found in this study has revealed four important points.

First, many of the irrigation and grazing effects affecting early stages such as seeds and seedlings were neutralized in later stages (seedlings and adults). Examples of neutralized effects include the irrigation and grazing effects on total density of seeds or the effects of irrigation on the seeds of forbs or small-seeded species (see Tables 1 and 3 for more examples). All these effects on seeds were counteracted in the seedling stage. Sixty-two percent of the irrigation and grazing effects on seeds and seedlings disappeared during subsequent stages. Thus, treatment effects during the seed stage had less influence on final adult density than those affecting seedling survival, after which counteractive effects are not possible. This result shows that the consequences of irrigation and grazing on mound revegetation depended to a great extent on the influence of these factors on seedling survival. This suggests that the more important that counteractive effects are during the recruitment process, the more critical seedling survival is for adult recruitment.

Second, the proportion of transferred and counteractive effects was quite similar between the three studied organization levels, and evidence of

accumulative effects was not found in any organization level. We expected that the different sensitivities of each stage at both functional group and species levels would contribute to plant community stability by favouring neutral balances or compensatory mechanisms (*sensu* Ernest and Brown 2001) at a higher organization level. Maybe certain homogeneity in species characteristics (most are annual opportunistic species) contributed to this result despite the relatively high number of species in the study site (a total of 74 species were detected in 0.5 ha).

Third, many more counteractive effects occurred during seedling emergence than seedling survival. All significant effects of irrigation and grazing on the seed bank were counteracted during seedling emergence. The similarity in species composition between seed bank and seedling pool (45%) was smaller than between seedling and adult pools (72%). Why was seedling emergence a more sensitive phase than seedling survival? We suggest three main reasons. (1) There are changes in the dormant status of viable seeds (readily germinable seeds became dormant seeds and vice versa) in the field mediated by changes in weather conditions during the germination period (Baskin and Baskin 1998). Espigares and Peco (1993) found many differences in species composition of seedling pools, when germination conditions in phytotron-simulated weather patterns were modified in a similar plant community. (2) Composition of the seed pool can change rapidly via seed predation and seed rain (Fenner 2000). In our field experiment, new seed rain from late-summer species after collecting the autumn soil samples might be an important factor. (3) The fact that it is not possible to estimate the germinable seed bank and seedling pool from the same soil sample poses some methodological limitations, when the seed bank is spatially heterogeneous (Thompson 1986; Dessaint et al. 1991).

These findings emphasize the role of germination conditions in this type of community (Espigares and Peco 1993; Peco and Espigares 1994). More changes in species composition took place during seedling emergence than during seedling survival, although the percentage of seeds that did not germinate was lower (36% for the entire community) than seedling mortality (63%). This suggests that, to some extent, qualitative and quantitative changes occurred during different stages of plant recruitment. This finding concurs with those of Bartolome (1979), Rice (1989),

and Rebollo et al. (2001) who found that reproductive plant community composition is largely determined by time of emergence in Mediterranean annual plant communities. Our results indicate that it is necessary to study the changes in both the density of individuals and community structure in order to determine the key stages in the plant recruitment process. As far as we know, studies addressing the recruitment of adult plants in entire communities (Moles and Drake 1999; Rebollo et al. 2001; Goldberg et al. 2001) do not refer to changes in community structure.

Fourth, the irrigation-sensitive and grazing-sensitive stages and the prevalence of transferred effects varied with both seed mass and growth form of grasses and forbs (Table 3). We did not find differences in the amount of counteractive effects in forbs versus grasses (Tables 1 and 3). However, we found more transferred effects in grasses; thus, the early stages (seeds or seedlings) determined the final density of reproductive adults in grasses. Goldberg et al. (2001) observed that graminoids have greater emergence and survival than dicots in seminatural communities of desert annual species. This is consistent with our findings that transferred effects from seeds to seedlings and from seedlings to adults are more frequent in graminoids than forbs. In the case of forbs, the final density of adult forbs depended entirely on the treatment effects on seedling survival. In small-seeded species, the final density of adults of small-seeded species depended entirely on the treatment effects on seedling emergence. In large-seeded species, seedling emergence and seedling survival transferred effects from seeds to adults without any further change. These results agree with Greiling and Kichanan (2002), who found that seed mass (i.e. the amount of reserves in seeds) is a main determinant of seedling emergence and survival, with larger seeded species establishing better than smaller ones. In summary, in our study transferred effects were more frequent for grasses than for forbs and the strength of transferred effects increased with seed mass.

What are the ecological implications of transferred, accumulative, and counteractive effects?

The prevalence of transferred effects makes the recruitment process more predictable and more

dependent on the early stages of the plant life cycle (seeds and seedlings). This simplifies the recruitment process to a series of subsequent stages that propagate the effects of ecological factors up to the final stage, reproductive adults. However, the prevalence of accumulative and counteractive effects increases and reduces, respectively, the sensitivity of the whole recruitment process to environmental factors, and enhances the relative importance of the last stage of recruitment (adults). This increases the complexity of the recruitment process and the uncertainty about the amount of individuals and species reaching the status of reproductive adult.

The existence of transferred effects and the relatively high percentage of counteractive effects found in this study show that the studied annual community presents a complex recruitment process with high levels of uncertainty. Although this community is a species-rich plant community (74 spp. per 0.5 ha) compared with other herbaceous communities, we acknowledge the fact that we have studied a relatively simple system compared, for example, with tropical and temperate systems. Our community is dominated by herbaceous annual plants, with a minor presence of perennials.

In conclusion, this article illustrates the complexity of mechanisms underlying the effects of physical and biological factors on an entire plant community. We found effects at all stages of the recruitment process (seeds, seedlings, and adults). These effects were related to each other in a complex way through transferred and counteractive effects. We employed a useful statistical procedure (ANOVA and ANCOVA analyses) to determine the stage at which effects occur initially and the propagation of these effects through subsequent life stages. This approach is novel and it can be applied to many contexts in ecology, both theoretical and practical, in order to detect key stages during the recruitment process.

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Appendix

Appendix Biological traits of the species used in the statistical analyses

Species	Grass/ forb	Class of seed mass
<i>Adonis aestivalis</i> L.	Forb	Large
<i>Amaranthus blitoides</i> S. Watson	Forb	Large
<i>Anacyclus clavatus</i> (Desf.) Pers.	Forb	Small
<i>Arenaria serpyllifolia</i> L.	Forb	Small
<i>Bellardia trixago</i> (L.) All.	Forb	Small
<i>Bromus maximus</i> Desf.	Grass	Large
<i>Bromus rubens</i> L.	Grass	Large
<i>Bromus squarrosus</i> L.	Grass	Medium
<i>Bromus tectorum</i> L.	Grass	Large
<i>Capsella bursa-pastoris</i> (L.) Medicus	Forb	Small
<i>Cardaria draba</i> (L.) Desv.	Forb	Large
<i>Carduus bourgeanii</i> Boiss. & Reuter	Forb	Large
<i>Carthamus lanatus</i> L.	Forb	Large
<i>Centaurea melitensis</i> L.	Forb	Large
<i>Cerastium glutinosum</i> Fries	Forb	Small
<i>Conyza canadensis</i> (L.) Cronq.	Forb	Small
<i>Convolvulus arvensis</i> L.	Forb	Medium
<i>Crepis capillaris</i> (L.) Wallr.	Forb	Small
<i>Crepis vesicaria</i> L.	Forb	Small
<i>Chenopodium album</i> L.	Forb	Medium
<i>Chenopodium vulvaria</i> L.	Forb	Medium
<i>Chondrilla juncea</i> L.	Forb	Medium
<i>Chrozofora tinctoria</i> (L.) A. Juss.	Forb	Large
<i>Dactylis glomerata</i> L.	Grass	Medium
<i>Diplotaxis erucoides</i> (L.) Dc.	Forb	Medium
<i>Erodium ciconium</i> (L.) L'Hér.	Forb	Large
<i>Euphorbia serrata</i> L.	Forb	Large
<i>Filago pyramidata</i> L.	Forb	Small
<i>Foeniculum vulgare</i> Miller	Forb	Large
<i>Fumaria parviflora</i> Lam.	Forb	Small
<i>Galium parisense</i> L.	Forb	Medium
<i>Geranium molle</i> L.	Forb	Large
<i>Herniaria hirsuta</i> L.	Forb	Small
<i>Hirschfeldia incana</i> (L.) Lagr.-Foss.	Forb	Medium
<i>Hordeum murinum</i> L.	Grass	Large
<i>Hypecoum procumbens</i> L.	Forb	Large
<i>Lactuca serriola</i> L.	Forb	Medium
<i>Lathyrus cicera</i> L.	Forb	Large
<i>Lolium rigidum</i> Gaudin	Grass	Large
<i>Lophochloa cristata</i> (L.) Hyl.	Grass	Small

Appendix continued

Species	Grass/ forb	Class of seed mass
<i>Malva sylvestris</i> L.	Forb	Large
<i>Medicago minima</i> (L.) Bartal.	Forb	Medium
<i>Papaver rhoes</i> L.	Forb	Small
<i>Petrorhagia dubia</i> (Rafin.) G. López & Romo	Forb	Medium
<i>Polygonum aviculare</i> L.	Forb	Large
<i>Scabiosa simplex</i> Desf.	Forb	Large
<i>Scorzonera laciniata</i> L.	Forb	Large
<i>Silene nocturna</i> L.	Forb	Medium
<i>Sonchus asper</i> (L.) Hill	Forb	Medium
<i>Sonchus oleraceous</i> L.	Forb	Medium
<i>Stellaria media</i> (L.) Vill.	Forb	Medium
<i>Tragopogon porrifolius</i> L.	Forb	Large
<i>Trigonella polyceratia</i> L.	Forb	Medium
<i>Trisetum paniceum</i> (Lam.) Pers.	Grass	Small
<i>Veronica arvensis</i> L.	Forb	Small
<i>Veronica polita</i> Fries	Forb	Medium
<i>Vicia cracca</i> L.	Forb	Large
<i>Vicia peregrina</i> L.	Forb	Large
<i>Vulpia ciliata</i> Dumort.	Grass	Medium
<i>Vulpia myurus</i> (L.) C. C. Gmelin	Grass	Medium

Seed mass was classified as small (<0.1 mg), medium (0.1–1 mg), and large (>1 mg). Nomenclature of taxa: Tutin et al. (1964–1981)

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Extent and spatial patterns of grass bald land cover change (1948–2000), Oregon Coast Range, USA

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Abstract Globally, temperate grasslands and meadows have sharply declined in spatial extent. Loss and fragmentation of grasslands and meadows may impact biodiversity, carbon storage, energy balance, and climate change. In the Pacific Northwest region of North America, grasslands and meadows have declined in extent over the past century. Largely undocumented in this regional decline are the grass balds of the Oregon Coast Range, isolated grasslands in a landscape dominated by coniferous forests. This study was conducted to quantify the spatial extent and patterns of grass bald change. Five balds in the Oregon Coast Range were evaluated using historical aerial photographs and recent digital orthophoto quadrangles (DOQ). Over the time period of study (1948/1953 to 1994/2000), bald area declined by 66%, primarily from forest encroachment. The number and average size of bald vegetation patches declined, while edge density increased. Tree encroachment into balds was inversely related to distance from nearest potential parent trees. Spatial patterns of bald loss may result from a forest to bald gradient of unfavorable environmental conditions for tree establishment and/or seed dispersal limitation. Species dependent on balds may be at risk from loss of bald area and increased fragmentation, although metrics of habitat fragmentation may not reflect species-

specific habitat requirements. Tree encroachment patterns and increased bald edge densities suggest increasing rates of bald loss in the future. The remote sensing nature of this study cannot determine the fundamental causes of bald decline, although prior research suggests climate change, cessation of native burning, successional changes in response to prior wildfires, and cessation of livestock grazing all may have potential influence.

Keywords Grassland decline · Land cover change · Forest encroachment · Habitat loss · Fragmentation

Introduction

Declines in the extent of temperate grasslands, montane meadows, and subalpine meadows appear to be a global ecological phenomenon (Mast et al. 1997; Bowman et al. 2001; Didier 2001; League and Veblen 2006; Marie-Pierre et al. 2006; Baker and Moseley 2007; Coop and Givnish 2007). Noting that conversions of temperate grasslands to other land cover types greatly exceeds conservation; Hoeskstra et al. (2005) consider the loss of grasslands as a major component of a global “biome crisis”. Extensive losses of grassland habitat will reduce biodiversity, since habitat modification and fragmentation are believed to be significant threats to biodiversity (Sala et al. 2000;

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Foley et al. 2005). Grasslands and meadows occupy a significant portion of global land surface, and are major contributors to biosphere primary production (Field et al. 1998; Saugier et al. 2001). Large-scale declines of grasslands and meadows may change regional carbon storage (Houghton et al. 1999; Jackson et al. 2002; Knapp et al. 2008), and surface albedo (Bonan et al. 1992), thereby influencing global climate change.

In the Pacific Northwest Region of North America, the loss of lowland, montane, and subalpine grasslands have been largely attributed to tree encroachment (Habeck 1961; Franklin et al. 1971; Woodward et al. 1995; Rochefort and Peterson 1996; Miller and Halpern 1998; Foster and Shaff 2003), although agricultural development has played a major role in the loss of Willamette Valley prairies (Habeck 1961; Johannessen et al. 1971). Largely overlooked within the region have been the grass balds of the Oregon Coast Range, graminoid dominated vegetation which typically occur at the summits of Coast range peaks and contrast strikingly in structure with the taller surrounding coniferous forests (Franklin and Dyrness 1998). Like balds of the Southern Appalachian Mountains of North America, these balds had been relatively stable in extent, while containing rare and endemic plant species, and disjunct populations, suggesting they are relict vegetation assemblages (Merkle 1951; Detling 1953; Wieg and Knowles 1995). However, there is considerable uncertainty regarding the origins, maintenance, and decline of the balds, or the role of humans in bald dynamics. Coast Range balds provide habitat to multiple species with varying levels of Federal and State protected status (USFWS 2001; ONHIC 2007). Their limited spatial extent, unique vegetation, and presence of disjunct and rare species contribute to their ecological importance and value to society.

Limited research within the balds has focused on vegetation classification (Merkle 1951; Aldrich 1972; Snow 1984). Magee and Antos (1992) investigated tree encroachment into one Coast Range bald, but no published documentation exists quantifying current and historical bald extent, or changes in bald area over time. There is no documentation of the types of bald loss (i.e., forest encroachment versus human disturbance), spatial patterns of bald loss, or landscape metrics (i.e., number of bald patches, area of patches, and edge density of patches) of bald vegetation fragmentation over time. Quantifying spatial patterns

of bald change may provide information to make inferences about the underlying proximate processes involved. Landscape metrics of fragmentation are a widely used proxy for habitat degradation. Mapping of bald change, and the extent of bald fragmentation could provide land managers with useful spatially explicit information for establishing reference conditions and prioritizing specific areas for restoration activities.

The objectives of this study were to: (1) quantify the extent of balds in the Oregon Coast Range over the past five decades (1948–2000), (2) determine what types of land cover balds are being converted to (i.e., coniferous forest, roads and building, bare ground, etc.), (3) quantify the spatial patterns of tree encroachment into the balds, and (4) determine if bald vegetation patches have become more fragmented and edgier (i.e., decline in total number of patches and average patch size, while having increased edge densities) over the time period of study.

Methods

Study area

Franklin and Dyrness (1998) listed seven major balds in the Oregon Coast Range, but omitted the extensive bald on Mount Hebo (USFWS 2001). Of these eight balds, only five were used in this study due to the poor quality of historical imagery at three balds (Fig. 1). From north to south, balds studied are: Mount Hebo, Bald Mountain, Marys Peak, Grass Mountain, and Praire Peak (see Table 1 for study area information). Bald elevations range from 968 to 1249 m. All five balds are in the *Tsuga heterophylla* zone, Coast Range Level III Eco-region (Franklin and Dyrness 1998; Thorson et al. 2003). However, higher balds (such as Marys Peak) may lie within the higher elevation, *Abies amabilis* zone (McCain and Diaz 2002). The climate is maritime, with mild wet winters and cool dry summers, but climate varies with proximity to the ocean, latitude, and orographic effects. Over the 1970–2000 time period, climatic data from the nearest weather station (Laurel Mountain Weather Station, National Weather Service Cooperative Network #354776, 44°55' N 123°34' W, 1090 m), reported annual average maximum temperature of 10°C, mean temperature of 6.6°C, annual average minimum temperatures of 3.1°C, annual



Fig. 1 Documented major grass balds within the Oregon Coast Range ecoregion. Grass Balds names are: SM—Saddle Mountain, MH—Mount Hebo, BM—Bald Mountain, MP—Marys Peak, GM—Grass Mountain, PP—Prairie Peak, RN—Roman Nose Mountain, and TY—Tyee Mountain. Grass Balds with solid triangles were studied

precipitation of 307 cm, and total snowfall of 301.5 cm which falls predominantly from November to April. Mount Hebo, Bald Mountain, and Prairie Peak study areas are predominantly underlain by mafic intrusions of Oligocene origin, while Marys Peak and Grass Mountain study areas are underlain by a combination of Oligocene mafic intrusions and middle Eocene sandstones and siltstones (Walker and MacLeod 1991).

Bald vegetation varied between study areas. Common graminoid species include: *Elymus glaucus*, *Festuca rubra*, *Bromus carinatus*, *Poa pratensis* (non-native), *Agrostis diegoensis*, *Carex californica*, and *Carex rossii*. Common forbs and ferns include: *Lomatium martendalei*, *Collinsia parviflora*, *Senecio triangularis*, *Delphinium menziesii*, *Iris tenax*, and *Pteridium aquilinum* (see, Merkle 1951; Aldrich 1972; Schuller and Exeter 2007 for details). Disjunct populations of more typically Eastern Cascades species include *Festuca idahoensis* and *Carex hoodii*, while populations of species more typical of high altitudes include *Phleum alpinum*, *Arctostaphylos uva-ursi*, *Phlox cepitosa*, *Silene douglasii*, *Allium crenulatum*, and *Lupinus lepidus* var. *lobbi* (Merkle 1951; Detling 1953; Oregon Plant Atlas 2007). Multiple species of concern are associated with grass bald vegetation in the Oregon Coast Range, including: the Federally listed Oregon Silverspot Butterfly (*Speyeria zerene hippolyta*), the State listed Coast Range Fawn Lilly (*Erythronium elegans*), and three plants that are candidates for State protection (*Cardamine pattersonii*, *Saxifraga hitchcockiana*, and *Sidalcea hirtipes*) (USFWS 2001; ONHIC 2007). Nomenclature of plant species follows Hitchcock and Cronquist (1976). Multiple conifer species can encroach into the balds. At Mount Hebo *Psuedotsuga menziesii* dominates encroachment (personal observation). *P. menziesii* is also the dominant encroaching species at Bald Mountain and Prairie Peak. *P. menziesii*, *Abies procera*, and to a lesser degree *Tsuga heterophylla* are encroaching at Grass Mountain, while *A. procera* is the dominant encroaching species at Marys Peak (Magee and Antos 1992; Schuller and Exeter 2007).

Aerial photo change detection

Analyses used historical (1948 and 1953) aerial photographs and recent (years 1994 and 2000) digital orthophoto quadrangles (DOQ's) of the five study areas. The historical images were geo-rectified using the DOQ's as reference imagery. Historical photos and DOQ's were filtered to improve classification of imagery into distinct land cover classes. Filtered images were manually classified into four land cover classes (bald, coniferous forest, roads and buildings, and bare ground) within each image. A matrix function was applied to these classified images, resulting in a single image with 16 (4 × 4) potential land cover

Table 1 Grass bald study area maximum elevations, geographic coordinates, and imagery dates

^a Two historical images were mosaicked for Prairie Peak. See Appendix A for details

Study area	Elevation (m)	Latitude	Longitude	Imagery dates (dd/mm/year)
Mount Hebo	968	45°12' N	123°45' W	29/07/1953, 30/05/1994
Bald Mountain	985	44°47' N	123°32' W	29/06/1948, 08/08/2000
Marys Peak	1,249	44°30' N	123°33' W	14/07/1948, 30/08/1994
Grass Mountain	1,098	44°27' N	123°33' W	29/06/1948, 30/05/1994
Prairie Peak	1,043	44°16' N	123°37' W	29/06/1948 ^a , 02/07/1948 ^a , 30/08/1994

change classes for each study area. Many of these 16 change classes were irrelevant for this study (such as coniferous forests converted to roads and buildings), so the numbers of land cover classifications were reduced to aid in visual interpretation of bald change (Fig. 2). The surface area of the remaining relevant cover classes were compiled to determine the amount of historical and current bald area, the extent of bald change, and what types of land cover boulders were being converted to (i.e., coniferous forest, roads and buildings, and bare ground). Unless otherwise mentioned, image processing and analyses took place using ERDAS Imagine 8.7 (2003 Leica Geosystems GIS and Mapping, LLC). For a detailed description of image acquisition and processing methods please refer to Appendix A.

Spatial pattern metrics

Landscape metrics were calculated to quantify bald patch fragmentation. The number of bald patches, average patch size (referred to as patch size), area-weighted average patch size (referred to as weighted patch size), and edge density were calculated for both historical and recent classified images. Weighted patch size was calculated in addition to simple patch size because the distribution of bald patch sizes was skewed to a few large patches and many small patches. In this patch distribution, the simple arithmetic average will not reflect the expected patch size encountered by random placement of points, resulting in a disproportionate effect of small patches (Turner et al. 2001). The weighted patch size was calculated as,

$$S_a = \sum (S_k^2) / \sum (S_k),$$

where S_a is the area-weighted average patch size, and S_k is the size of the k th patch. Edge density was calculated as the sum of the lengths (m) of all edge segments within the bald patches divided by the

landscape area (m^2) of all bald patches, multiplied by 10,000 (to convert to meters of edge per hectare). Higher edge density values are indicative of complex or elongated patches, lower edge density values indicate more compact or simple shaped patches.

The function Distance in the Spatial Analysis of ArcGIS 9.1 (2005 ERSI Inc.) was used to test the relationship between tree encroachment and distance to potential parent trees. The Euclidean distance was calculated between each pixel of bald encroached by trees and the nearest pixel of potential parent tree (i.e., nearest forested pixel in the historical imagery). Euclidean distance was also calculated between each pixel of bald that remained stable (did not change between imagery dates) and the nearest pixel of potential parent tree. The percentage of pixels within both encroached bald and unchanged bald classes were separately rescaled to total 100%, and plotted with respect to distance from nearest potential parent trees. A Wilcoxon signed rank sum test was used to determine whether distribution of pixels with respect to distance from potential parent trees differed between encroached bald and unchanged bald pixels. Distributions were considered different if the Wilcoxon signed rank sum statistic (S value) was significant at $\alpha = 0.05$ level.

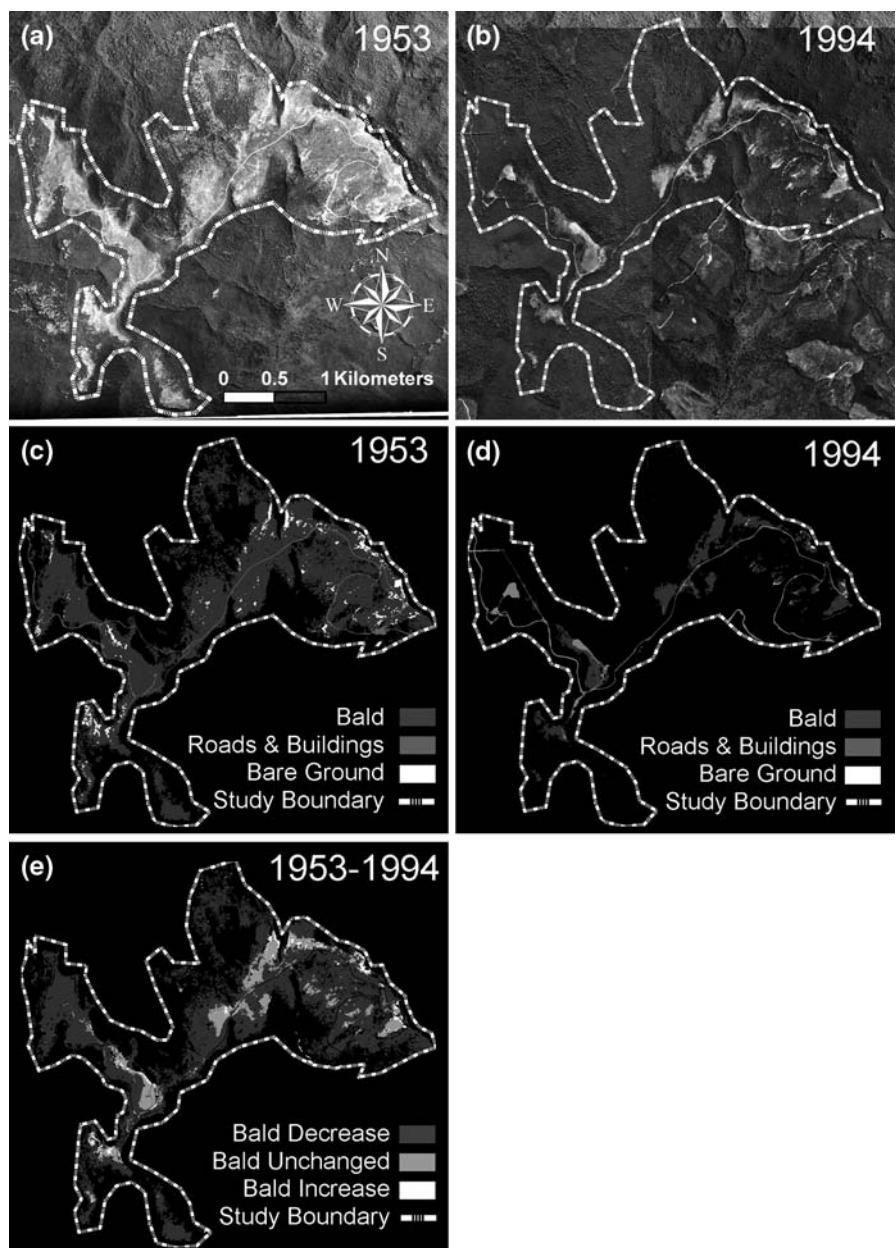
Results

Extent and decline of grass boulders

All five boulders declined in area over the time period of study (Table 2). From 1948/1953 to 1994/2000, boulders declined from 523.6 ha to 179.2 ha (66%). Forest encroachment into boulders was the dominant type of bald decline (348.7 ha, 95%). Conversion of boulders to roads and buildings accounted for 15.4 ha (4%), while conversion to bare ground was minor.

Mount Hebo displayed the greatest bald decline. Bald Mountain was the smallest historical bald, had

Fig. 2 Example (Mount Hebo study area) of aerial photo grass bald change detection process. **a** 1953 historical aerial photo, **b** 1994 DOQ, **c** 1953 classified image, **d** 1994 classified image, and **e** matrix image depicting grass bald change at Mount Hebo from 1953 to 1994



the lowest proportional bald decline, and the greatest proportional amount of bald area decline due to road and building development. Visual interpretation of the Bald Mountain imagery (not shown) suggests the 0.2 ha conversion of bald to bare ground resulted from adjacent logging operations in the southern edge of the study area. Prairie Peak had the second highest percentage of bald decline. Bald decline at Prairie Peak resulted in the near complete elimination of one bald in the eastern part of the study area, while

converting the contiguous main bald into a series of smaller interconnected boulders (not shown).

Spatial patterns of grass bald change

Landscape metrics of bald fragmentation (number of bald patches, patch size, weighted patch size, and edge density) were generally consistent between study areas (Table 3). From 1948/1953 to 1994/2000, the number of bald patches declined at all study

Table 2 Grass bald changes by area and types of land cover change

Study area	Date	Area (ha)	Type change ^a	Δ (ha) ^b	Δ (%) ^c
Mount Hebo	1953	243.4	Bald to forest	202.1	96.09
	1994	39.8	Bald to bare ground	0.2	0.10
	1953–1994	−203.6	Bald to R&B	8.0	3.79
			Conversion to bald	6.6	
Bald Mountain	1948	24.3	Bald to forest	14.8	84.8
	2000	7.3	Bald to bare ground	0.2	1.2
	1948–2000	−17.1	Bald to R&B	2.4	14.0
			Conversion to bald	0.4	
Marys Peak	1948	118.4	Bald to forest	47.0	93.9
	1994	77.2	Bald to bare ground	0.4	0.7
	1948–1994	−41.1	Bald to R&B	2.7	5.5
			Conversion to bald	9.0	
Grass Mountain	1948	43.9	Bald to forest	22.0	98.3
	1994	23.1	Bald to bare ground	0.0	0.0
	1948–1994	−20.8	Bald to R&B	0.4	1.9
			Conversion to bald	1.6	
Prairie Peak	1948	93.6	Bald to forest	62.7	95.4
	1994	31.8	Bald to bare ground	1.1	1.7
	1948–1994	−61.8	Bald to R&B	1.9	2.8
			Conversion to bald	3.9	
All Study Areas	1948/1953	523.6	Bald to forest	348.7	95.3
	1994/2000	179.2	Bald to bare ground	1.9	0.5
	Change	−344.4	Bald to R&B	15.4	4.2
			Conversion to bald	21.6	—

^a Type change refers to what type of land cover bards are converted to. R&B refers to roads and buildings

^b Δ (ha) refers to the area (in hectares) of each type of land cover change

^c Δ (%) refers to the proportion of bald loss due to a specific type of land cover change

Note: Bald area values used to calculate Δ (ha) and Δ (%) are bald loss values corrected for conversions of non-bald land cover classes to bald vegetation

areas (47% total 10–67% for individual study areas). Patch size for four out of the five bards declined (49% average, 20–66% for individual study areas). The fifth study area (Marys Peak) saw a 2% increase in patch size. Weighted patch size declined sharply in all study areas (94% average, 48–97% for individual study areas). Bald edge density increased at four out of the five study areas (18% total, 7–46% for individual study areas). The fifth study area (Marys Peak) saw only a 0.41% increase in edge density.

Forest encroachment into the bards was inversely related to the distance from potential parent trees (Fig. 3). The distribution of encroached bard versus unchanged bard in relation to distance from potential parent trees was significant at Mount Hebo ($S =$

−2184, $P < 0.0001$), Marys Peak ($S = -8333.5$, $P < 0.0001$), Grass Mountain ($S = -1557$, $P < 0.0001$), and Prairie Peak ($S = -908.5$, $P < 0.0001$). Results for Bald Mountain ($S = -876.5$, $P = 0.0836$) suggest a similar relationship, but also displayed longer distance tree encroachment.

Discussion

Grass bard decline and fragmentation

This study is the first to document the widespread decline and fragmentation of grass bards in the Oregon Coast Range. All five study areas displayed

Table 3 Landscape metrics of grass balds by study area and date

Study area	Date	No. of bald patches (m ²)	Patch size (m ²)	Weighted patch size (m ²)	Edge density (m ² /ha)
Bald Mountain	1948	87	2,945	226,910	15,021
	2000	78	1,352	24,893	19,354
Grass Mountain	1948	778	595	66,473	14,957
	1994	515	473	34,398	15,983
Marys Peak	1948	1,651	791	597,727	14,660
	1994	1,066	809	202,988	14,719
Mount Hebo	1953	2,446	1,119	2199,737	13,911
	1994	1,324	380	63,454	16,081
Prairie Peak	1948	2,025	724	788,861	9,563
	1994	674	538	64,133	13,996
See section "Methods" for detailed descriptions of landscape metrics	All study areas	1948/1953	6,987	5,384	3281,981
		1994/2000	3,657	2,744	186,878
					80,134

large losses in bald area due to tree encroachment, with only 35% of 1948/1953 bald area present in 1994/2000. Study areas displayed declining numbers of bald patches, reduced patch sizes and weighted patch sizes, and increased edge densities. However, these metrics of fragmentation varied by study area, suggesting different patterns of bald change. At Bald Mountain there were small declines in the number of patches, large declines of patch size and weighted patch size, and large increases in edge density. This is consistent with the elimination of only a few small bald patches, but larger declines in the area of larger patches, and edgier (i.e., more complex and/or elongated) remnant balds. Mount Hebo and Prairie Peak had the greatest declines in the number of bald patches, patch size, and weighted patch size, but had different changes in edge density. Prairie Peak bald patches had a much greater increase in edge density compared to Mount Hebo, suggesting that while both of these study areas experience declines in both large and small bald patches, the remnant patches are Prairie Peak have more elongated or complex shapes than those at Mount Hebo. In contrast, Marys Peak had a large reduction in the number of bald patches, a slight increase in patch size, a large reduction in weighted patch size, and almost no increase in edge density. These changes are consistent with the predominant disappearance of smaller patches, and reductions of remaining large patches areas while maintaining less complex and/or elongated shapes. Grass Mountain had a small increase in edge density, and when combined with the other patch metrics,

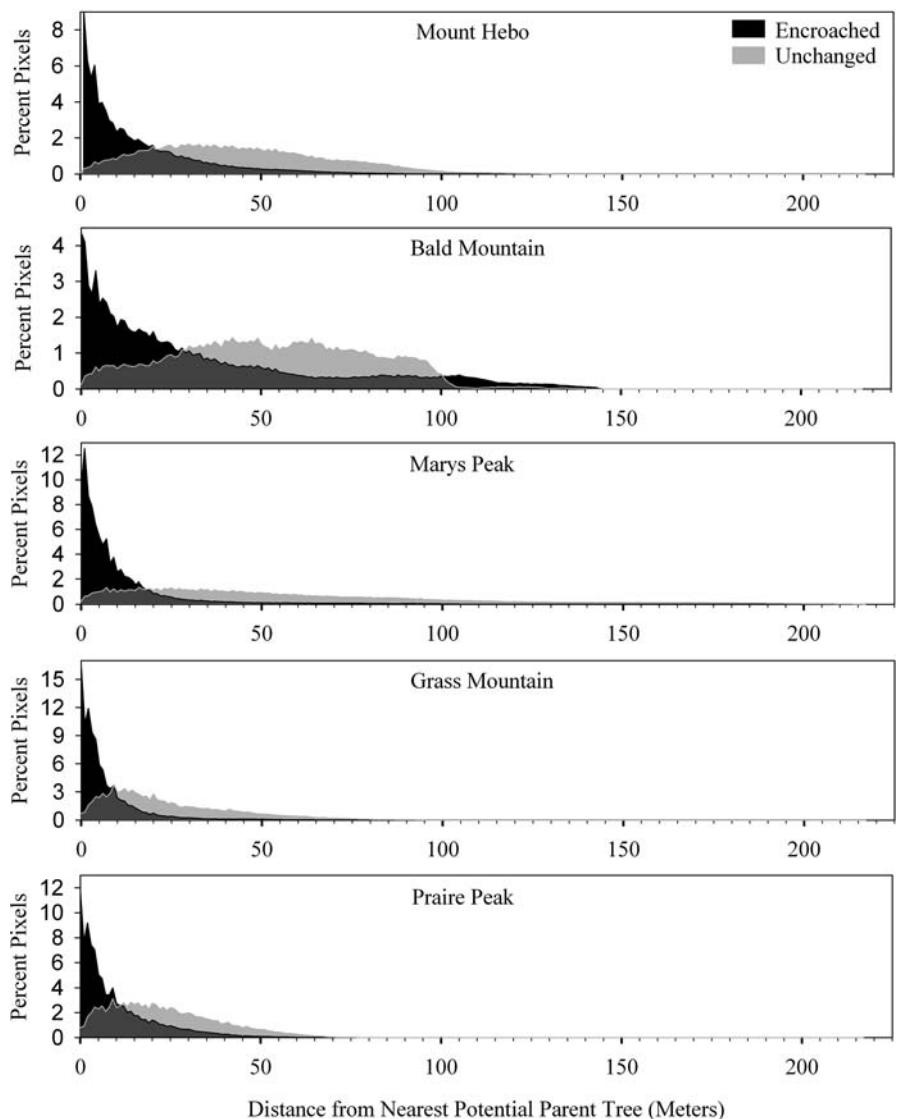
suggests bald decline was largely due to the elimination of small patches, and the shrinking of larger patches without becoming very complex or elongated in shape.

Spatial patterns and proximate causes of tree encroachment

Bald decline was overwhelming due to tree encroachment, which was greatly closer to the forest edge. This infilling pattern of encroachment is consistent with field data from Marys Peak (Magee and Antos 1992), but differs from more clustered patterns of encroachment found in montane and subalpine meadows of the Oregon and Washington Cascades (Franklin et al. 1971; Miller and Halpern 1998).

The spatial patterns of tree encroachment likely result from a combination of unfavorable tree establishment conditions due to high resource competition from graminoids, and dispersal limitation of tree seed with increased distance from the forest edge. The influence of forest edge on non-forest microsite conditions decline with increased distance from the forest edge (Chen et al. 1993). With increased distance from the forest edge there is increased graminoid cover. For many conifer species, both establishment and growth decline with increased graminoid cover (Alan and Lee 1989; Kolb and Robberecht 1996; Dovciak et al. 2008). *A. procera* germinants and seedlings tend to be at a competitive disadvantage against dense bald vegetation (Magee and Antos 1992). *Pseudotsuga menziesii* and *Tsuga heterophylla*

Fig. 3 Distribution of encroached versus unchanged grass bald land cover in relation to distance from potential parent trees



establishment and growth also decline with increases in herbaceous vegetation competition (Rose et al. 1999; Rose and Ketchum 2002). Wind-dispersed seeds can travel quite far (Clark et al. 1999), but seed dispersal displays inverse seed density with increased distance from source trees. The heavy weight of *A. procera* seeds results in a relatively short dispersal distances compared to *P. menziesii* and *T. heterophylla*. However, almost all bald vegetation was within 100 m of pre-existing trees, well within dispersal distances of invading species (Isaac 1930; Franklin and Smith 1974; Carkin et al. 1978).

In the Carpathian Mountains, Dovciak et al. (2008) found invasion of grasslands by *Picea abies* to be

driven by a multivariate gradient from the forest to core grassland of seed dispersal and environmental conditions (overstory cover, moss and grass cover, etc.). Although specific microsite information is lacking for this study, prior studies within the grass balds and in other grasslands suggest both seed dispersal and environmental conditions appear to drive spatial patterns of forest encroachment.

Ecological implications

Multiple species of concern are associated with bald vegetation in the Oregon Coast Range (USFWS 2001; ONHIC 2007). Declines in habitat area and increased

fragmentation are generally associated with the loss of species diversity and reductions in species populations (Saunders et al. 1991; Sala et al. 2000; Krauss et al. 2004; Foley et al. 2005). Although, landscape metrics, such as habitat area and fragmentation can be important, they cannot directly quantify habitat quality or species composition. Reliance on landscape metrics is often necessary in the absence of, but may be less important than, species-specific field-based measures of habitat suitability (Hobbs and Yates 2003; Fischer and Lindenmayer 2007). Additionally, reductions in species populations and species richness may lag temporally behind habitat loss, further complicating the relationship between species and habitat (Tillman et al. 2002; Helm et al. 2006). Despite these uncertainties, large declines in bald area and increased bald fragmentation are likely to negatively impact rare species and disjunct populations.

Patterns of tree encroachment and bald fragmentation suggest a positive feedback of accelerating encroachment in the future. In some meadow types, tree establishment positively alters microsite conditions with respect to future tree regeneration and establishment (Miller and Halpern 1998; Dovčiak et al. 2008). Since the remnant bald patches are closer to forest edge, they are more likely to encounter a higher density of tree seed. Higher edge density of remnant bald patches also suggests the surrounding forest matrix will have increased influence on ecological patterns and processes in the remnant patches (Saunders et al. 1991). In particular, increased shade from the forest edge may reduce graminoid cover that excludes conifer establishment. If conservation and restoration of balds is desired, the potential for additional and accelerating bald loss should be a management concern. Bald decline due to road and building development was minor at most study areas (except the Bald Mountain study area). However, landscape metrics used in this study cannot quantify changes in habitat quality that occur even if the land cover type remains unchanged, while roads can increase the dispersal and establishment of non-native plant species (Parendes and Jones 2000; Trombulak and Frissell 2000; Christen and Matlack 2006).

Fundamental causes of bald decline

The proximate cause of bald decline is tree encroachment, but the primary objectives and data of this

study cannot provide empirical evidence regarding its fundamental causes. However, a review of regional causes of tree encroachment, combined with site specific disturbance and land use history, may provide valuable information. Regional causes of tree encroachment vary by study, but most often include: climate change, fire and fire suppression, grazing history, and succession.

Climate changes (via changes in growing season length, snow free periods, growing season temperatures, etc.) are a likely cause of regional subalpine tree encroachment (Franklin et al. 1971; Woodward et al. 1995; Rochefort and Peterson 1996). Tree establishment in the balds of Marys Peak coincided with favorable climatic conditions (Magee and Antos 1992). However, has previously noted, Marys Peak is at a higher elevation and contains a more subalpine forest type than other studied balds, so conifer establishment-climate relationships at Marys Peak may not be representative of other lower elevation balds. Livestock grazing also can be an important driver of tree encroachment, reducing plant cover and density, while exposing bare soil. Miller and Halpern (1998) concluded that tree encroachment in some upland meadows in the Oregon Cascades occurred following grazing cessation and coincidentally, favorable climatic conditions. Homesteaders grazed livestock on the balds from the 1800s to 1940s (Reynolds 1993), so observed forest encroachment could be partly due to grazing cessation prior to historical imagery

High-severity wildfires are an important disturbance agent in Coast Range forests that has varied considerably over time. About 4600 to 2700 years BP, the Oregon Coast Range used to have a 140 ± 30 year fire intervals, compared to 240 ± 30 year from 2700 years ago to present (Long and Whitlock 2002). Native Americans are believed to have used frequent low-severity fire to manage some of the Coast Range grass balds prior to Euro-American homesteading (personal communication Phyllis Steeves, Archeologist, Siuslaw National Forest). By 1860, disease-driven declines in Native populations likely resulted in dramatic reductions of Native fire ignitions on the grass balds. Elimination of Native American ignitions, combined with active fire suppression, has driven tree encroachment in the prairies of the Puget Sound and Willamette Valley (Johannessen et al. 1971; Foster and Shaff 2003;

Boyd 1999). Evidence of extensive fires in the region could suggest grass balds are early-successional vegetation that has since been invaded after over 50 years of fire suppression. However, there is strong evidence balds have been long term features on the landscape: balds generally lack biological legacies (i.e., large woody debris, stumps, snags, and remnant trees) present even after multiple disturbances, they are dominated by graminoids rather than shrub-dominated early-successional vegetation typical in the Coast Range, and contain disjunct and endemic plant species suggestive of long term vegetation stability (Merkle 1951; Detling 1953; Dyrness 1973; Hemstrom and Logan 1986; Franklin et al. 2002). One potential exception is Mount Hebo, where high intensity fires, active reforestation, commissioning and decommission of the Mount Hebo Air Station, along with mowing and burning to maintain habitat for the Oregon Silverspot Butterfly, create a very complex disturbance history (Munger 1944; Oregon Division of Forestry 2001; USFWS 2001). Climate change and complex disturbance history likely interact to shape spatial and temporal patterns of tree invasion into the grass balds, as found elsewhere (Miller and Halpern 1998). However, addressing these fundamental drivers of tree invasion is beyond the scope of this study. Spatially-explicit tree age and growth chronology data, combined with climate records and detailed grazing history, may allow inference regarding the fundamental causes of tree invasion into these unique landscape features.

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Appendix A. Aerial photograph acquisition and image processing

Aerial photo acquisition

Historical panchromatic 1:12,000 scale aerial photographs were acquired from the University of Oregon

Map Library in Eugene, OR. All historical aerial photographs were taken during the summer of 1948 (except Mount Hebo, taken during the summer of 1953). Historical aerial photographs were scanned on a flatbed scanner at 600 dpi. Scanned historical images had 8-bit radiometric resolution and 0.63–0.89 m postrectification spatial resolution, re-sampled to 1 m pixel size after geo-rectification using nearest neighbor interpolation. Recent images of the study areas are panchromatic digital orthophoto quadrangles (DOQs). DOQs were downloaded from TerraServer-USA (<http://terraserver-usa.com/default.aspx>). The Mount Hebo, Marys Peak, Grass Mountain, and Prairie Peak DOQs were taken in the summer of 1994 (except Bald Mountain, taken during the summer of 2000). DOQs were in Universal Transverse Mercator Projection (UTM, Zone 10N), using the Geodetic Reference System Spheroid of 1980, and the North American Datum of 1983. All DOQs had 8-bit radiometric resolution and 1 m spatial resolution.

Aerial photo geo-rectification

Each scanned historical image was first manually clipped so only grass balds and surrounding forest vegetation were retained. Each clipped image was geo-rectified using the recent DOQ of the same site as the reference image. Control Points (CPs) between historic and recent images were manually selected, focusing on the most temporally stable features (i.e., buildings, roads, rock outcrops, and individual open grown trees). A minimum of 20 CPs between historical and reference imagery was desired, although this was not possible in all cases. Geo-rectification models used polynomial equations, evaluated by the Root Mean Squared Error (RMSE), and rejected if the RMSE exceeded 0.5 pixels. Additionally, rectified images were digitally overlain as partial transparencies over recent DOQs to qualitatively assess rectification accuracy. Rectification and resampling occurred using the nearest neighbor method, conserving raw image brightness values. A mosaic of two rectified images was required for the Prairie Peak study area (Prairie Peak a & b), and was generated using histogram matching, no cutlines, minimum select function, and union of all inputs.

Study area	Date	CPs	RMSE X	RMSE Y	RMSE total	Polynomial order
Mount Hebo	1953	22	0.05	0.03	0.06	1st
Bald Mountain	1948	8	0.03	0.01	0.02	1st
Marys Peak	1948	11	0.02	0.29	0.04	2nd
Grass Mountain	1948	20	0.02	0.01	0.02	2nd
Prairie Peak—a	1948	24	0.06	0.06	0.08	1st
Prairie Peak—b	1948	20	0.04	0.02	0.05	1st

Image filtering, level slicing land cover classification, and matrixing

DOQs and rectified historical images were filtered using a 5×5 pixel focal minimum filter followed by a 5×5 low-pass filter. This filtering combination compensated for highly reflective (high brightness value) pixels within continuous forest canopy that would be misclassified during land cover classification as bald vegetation or bare ground unless their brightness values were reduced. The 5×5 focal minimum filter assigned the lowest brightness value of the neighboring 24 pixels to the central pixel. Focal filtering was followed by a 5×5 low-pass filter to deemphasize high-frequency edges (Lillesand et al. 2004).

Classification of pixels into three landcover classes (grass bald, forest, and bare ground) was accomplished by level slicing of gray-level brightness values. This method has previously been used on panchromatic images to classify pine forests and grasslands in the Colorado Front Range (Mast et al. 1997). Each filtered image was sliced individually, and brightness value ranges for each land cover class varied between each of the 10 images. Attempts to classify conifers and hardwoods separately were unsuccessful; instead a single forest land cover classification included both conifers and hardwoods. Using level slicing to classify the roads and buildings land cover class greatly reduced classification accuracy of the other three classes. Instead, the roads and buildings were manually delineated as polygons in separate shapefiles for each current and rectified historical image in ArcGIS 9.1 (2005 ERSI Inc.). Polygon shapefiles were converted to raster images and incorporated into each three-class classified image using an overlay function, resulting in four-class classified images.

Despite focal minimum and low-pass filtering, visual comparison of classified imagery to the georectified images and DOQs found numerous instances

where small areas of forest were misclassified as grass boulds. Clumping and sieving was used to reclassify these false boulds as forest. An eight pixel window was used to clump contiguous groups of bald pixels into individual bald patches. Bald patches were determined manually by overlaying the clumped bald patches over the associated rectified image or DOQ. Clumped bald patches that were smaller than the smallest visually determined patches within a historical or DOQ image were reclassified as forest, resulting in historical and recent 4-class (forest, meadow, bare ground, and roads and buildings) land cover images for each study area. Change detection of bald land cover 4 between historical and recent classified images used a matrix function, creating a new image showing the coincidence of values between the historical and recent classified images. Each matrix classified image had 16 potential coincident classes (4 classes \times 4 classes). These classes were recoded, and the surface area of each land cover type was calculated from the number of pixels within each class.

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Grass (Poaceae) richness patterns across China's nature reserves

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Abstract Explaining grass richness patterns over broad geographic scales is a central issue of biogeography and macroecology. In this investigation, we document the richness patterns of grasses across China's nature reserves, and discuss their possible explanations at national and regional scales using grass richness data coupled with information from climatic and topographical variables. Our results suggest that the water- and energy-related variables and elevation range (a surrogate of topographical heterogeneity) represent the primary explanations for the variation in grass richness across China's nature reserves. Significant relationships were found between energy and all grasses, water and C₃ grasses, and the combination of water and energy and C₄ grasses at the national scale. Topographical heterogeneity is strongly associated with grass richness across China, whereas climatic constraints to grass diversity vary regionally. In regions of high rainfall, the presence of C₄ grasses is determined by annual potential evapotranspiration and

sunshine hours; the climatic constraint to C₃ grass richness in a large and varied region is sunshine hours; whereas in regions of low soil temperature, the temperature determines the grass richness pattern. Our results also suggest that water variables alone do not represent the primary predictor of grass richness at the regional scale.

Keywords China · Energy · Grass richness · Topographical heterogeneity · Nature reserves · Vegetation region

Introduction

The grasses family (Poaceae) has a worldwide distribution and consists of more than 11,000 species (Watson and Dallwitz 1992). The family spans a wide range of ecological conditions with the majority of Chinese species occurring in arid habitats (Chen et al. 2006). Nearly half of all grass species exhibit C₄ photosynthesis (Sage 2004). A number of studies have shown a relationship between photosynthetic type (C₃ or C₄) and geographical and ecological distribution (e.g., Winter et al. 1976; Teeri and Stowe 1976; Chazdon 1978; Vogel et al. 1978; Tieszen et al. 1979; Rundel 1980; Hattersley 1983; Cavagnaro 1988; Klink and Joly 1989; Paruelo and Lauenroth 1996; Pyankov et al. 2000; Bianchin Belmonte and Rúgolo de Agrasar 2002; Edwards and Still 2008).

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Temperature has been regarded as of prime importance in determining the distribution of C₄ grasses (Ehleringer et al. 1997). Other investigators have concluded that water availability may be the most critical factor influencing the distribution of C₃ and C₄ grasses (Chazdon 1978). However, Rundel (1980) and Pyankov et al. (2000) suggested that no single climatic factor determines the geographic distribution of C₃ and C₄ grasses. The different conclusions may in part reflect broad climatic differences between the different areas investigated. It has also been suggested that some of these studies were undertaken on a limited number of species or in a limited area (Edwards and Still 2008).

China's geographical extent and complex topography confer a broader range of climatic conditions, from tropical to subarctic/alpine, resulting in vegetation types that vary from rainforest to desert. This makes it very suitable for comparative phytogeographical and ecophysiological studies. In this context, the grasses are an ideal study group because they are diverse, including C₃ and C₄ photosynthesis, and widely distributed in various habitats (Watson and Dallwitz 1992; Chen et al. 2006). However, except for a floristic survey on the distribution patterns of a very few C₄ grasses (Yin and Li 1997), little is known about spatial variation in grass species diversity in China in the context of detailed climatic data and topographical variables.

The present study aims to: (1) explore the geographic and richness patterns of grasses in China and their possible explanations using data from China's nature reserves, coupled with corresponding information on climatic and topographical variables and (2) characterize the relationship between C₃ and C₄ grass richness and potential determinants, including climatic and topographical variables at national and regional scales.

Study sites and methods

Data collection

We collected grass genera and species richness data from 115 nature reserves across China, by reviewing recently published floristic surveys (Appendix 1). These nature reserves range from 18°35' to 48°36' N in latitude and from 80°18' to 133°42' E in longitude

(Fig. 1) and cover a total area of 1,312,445 km² (13.6% of the total area of China). The area data of nature reserves compiled from the newest online database to solving the variation in the reserve areas (WCS and IZ 2005). We excluded those nature reserves with an area of <5 and >500,000 km² to avoid spatially overlapping reserves. The data set covers almost the full spectrum of the Chinese variation in abiotic conditions and includes all major biomes and floristic kingdoms of China. Figure 2 shows the size distribution of the 115 nature reserves. Among them, the reserves of intermediate size (i.e., 100–1000 km²) account for 57.4% of the total number of reserves (Xu et al. 2006).

The grass taxon data collected included total numbers of genera and species (including bamboos) and the number of C₃ and C₄ genera and species. We excluded alien species from the data analysis (Appendix 2). The assignment of photosynthetic type was based on data obtained from published works or from closely related species (Brown 1977; Watson and Dallwitz 1992; Hattersley and Watson 1992). Based on this, 20% of Chinese grass species are C₄. A number of genera (e.g., *Panicum*) contain both C₃ and C₄ species, but C₃–C₄ intermediate species have not been identified (Yin and Li 1997). Thus, species are either C₃ or C₄ and are not interconvertible (Liu et al. unpublished data).

Environmental data for each reserve such as reserve area, geographical range, minimum elevation, and maximum elevation were gathered. Elevation range (maximum elevation minus minimum elevation) was used as a measure of topographical heterogeneity. Climatic data, assigned to each reserve based on its polygons (reserve boundary), were used to analyze the relationship between climate and grass richness. These data were compiled from a 1961 to 1999 temperature/precipitation database of China at 0.1° × 0.1° resolution, generated from 480 climatic stations across the country (Fang et al. 2001; CMDSSS 2003). Climatic variables included mean annual temperature, precipitation, wind speed, relative moisture degree, sunshine hours, potential evapotranspiration (PE), and actual evapotranspiration (AE). PE and AE were estimated using Thornthwaite's method (Fang and Yoda 1990). We also calculated water deficit as the difference between PE and AE (Stephenson 1990). Based on the vegetation zonation in the *Vegetation Atlas of China* (CCVVA 2001), we divided the 115 nature reserves

Fig. 1 Location of the 115 nature reserves of China used in the investigation, and the extent of four vegetation regions: subtropical forest region (I, $N = 69$), temperate forest region (II, $N = 24$), temperate steppe and desert region (III, $N = 15$), and Qinghai-Tibet Plateau region (IV, $N = 7$). The background shows the topography of China (South China Sea Islands not included), in an Albers equal-area conic projection

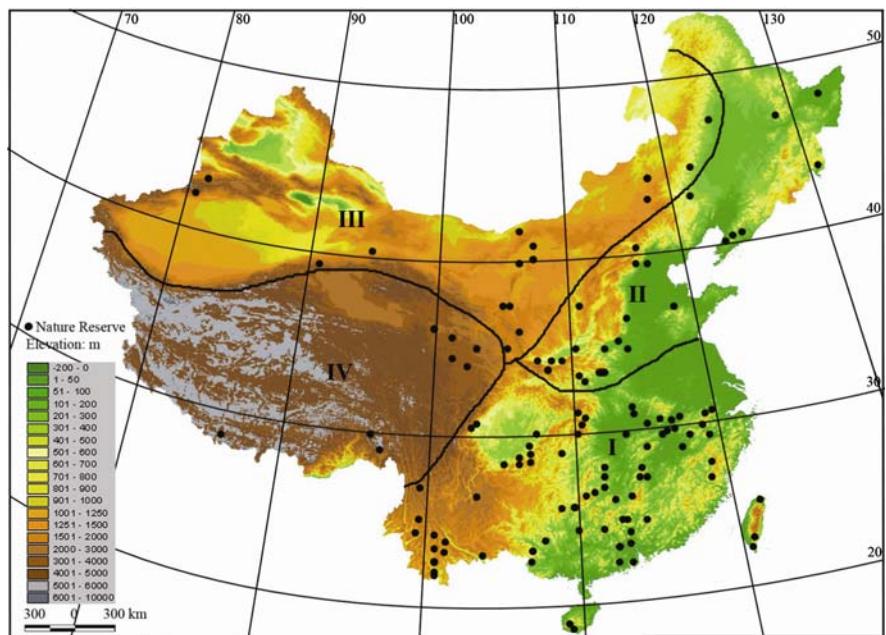
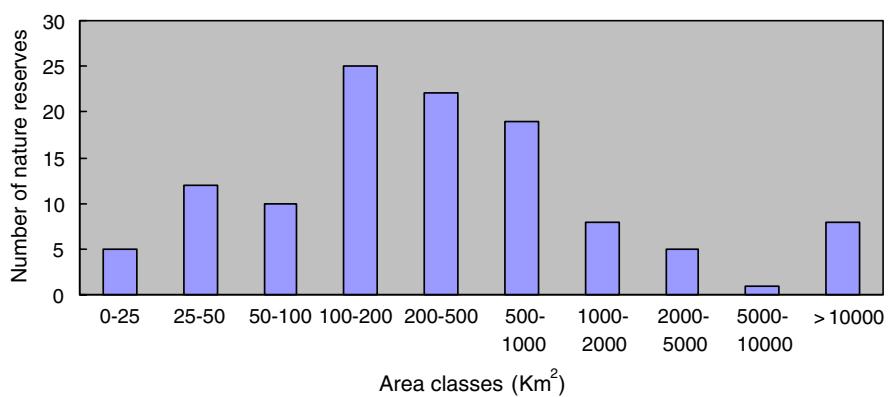


Fig. 2 Area distribution of the 115 nature reserves of China used in this investigation



into four vegetation regions—subtropical forest region, temperate forest, temperate steppe and desert, and Qinghai-Tibet Plateau—to examine grass richness patterns in different regions (Fig. 1). Statistical relationships between richness and latitude or altitude provide no information about causal factors, so latitude and altitude were excluded as variables in this study.

Data analysis

We used SPSS version 10.0 for the statistical analyses (Norusis 2000). Descriptive statistics for grass richness and the environmental variables were shown in Table 1. Correlation analyses between grass richness and each independent environmental variable were

carried out to interpret the relationships between them (Appendix 3). Stepwise regression analyses were performed to identify the factors explaining variation in grass taxon (genus/species) richness for China and for different regions. Before performing the multiple regression analyses, all environmental variables were tested for nonlinear relationships with grass genus/species richness using second-order polynomial models, and no significant nonlinearities were found. Therefore, we assumed that the relationships of taxon richness to the environmental variables were linear. We Log_{10} -transformed the total number of grass species, total C_3 species, total C_4 species, reserve area, water deficit, and elevation range to obtain an appropriate distribution for these data so that the skewness

Table 1 Summary statistics of grass genus/species richness, reserve areas, climatic variables, and topographical heterogeneity used in the investigation

Variables	N	Minimum	Maximum	Mean	SD	Skewness	Kurtosis
Species richness							
No. of grass genera	115	8	135	46.8	22.2	0.7	1.2
No. of grass species	115	10	679	88.3	74.1	4.7	35.1
No. of C ₃ genera	115	3	79	25.3	11.9	0.9	2.6
No. of C ₃ species	115	3	518	50.4	52.6	6.4	54.7
No. of C ₄ genera	115	0	58	21.5	12.7	0.3	-0.3
No. of C ₄ species	115	0	168	37.8	29.8	1.5	4.1
Location and area							
Mean latitude (°)	115	18.6	48.6	30.9	7.2	0.6	-0.5
Mean longitude (°)	115	80.3	133.7	110.5	8.9	-0.5	1.3
Reserve area (km ²)	115	5	500,000	11412.5	58,185	6.9	52.4
Climatic variables							
Mean annual temperature (°C)	115	-1.2	24.9	14.4	5.6	-0.5	-0.3
Annual precipitation (mm)	115	26.1	2372.3	1,005	525.9	0.2	-0.6
Mean annual wind speed (m/s)	115	0.5	4.3	2.2	0.9	0.2	-0.9
Mean annual relative moisture degree (%)	115	39	82	70.7	9.9	-1.2	0.9
Mean annual sunshine (h)	115	760.9	3062.1	2024.5	494.6	0	-0.3
Annual PE (mm)	115	414	1,179	845	175.5	-0.5	-0.5
Annual AE (mm)	115	27	1,006	622.8	222.4	-0.4	-0.6
Water deficit (mm)	115	91	886	221.6	126.5	2.3	8.1
Topographical heterogeneity							
Elevation range (m)	115	3	7627	1440.6	1148.9	2.1	7.6

and kurtosis of these variables were greater than 1 and 2.5, respectively (Table 1).

To explore whether vegetation has an effect on grass genus richness, we used a general linear model to perform analysis of covariance by using the vegetation region as a fixed factor; mean annual temperature, mean annual sunshine hours, annual AE, and elevation range as covariates; and grass genus richness as a dependent variable.

Results

Geographical distribution of grasses

The number of grass species per reserve varies from 10 at Inner Mongolia Wulate to 679 at Tibet Hengduan Mt. The means for all nature reserves are 88 total species, 50 C₃ species, and 37 C₄ species per nature

reserve in China, resembling the richness level of Chongqing Jinfoshan Reserve. The C₃ and C₄ grasses are distributed differently in the vegetation regions; C₃ grasses are most abundant at Yunnan Mt. Hengduan in the Qinghai-Tibet Plateau vegetation region and are least represented at Hainan Bawangling in the subtropical forest vegetation region, whereas C₄ grasses are most abundant at Yunnan Honghe in the subtropical forest vegetation region and are absent at Xinjiang Arjinshan in the temperate steppe and desert vegetation region (Appendix 2).

National scale

The multiple regression analyses yielded primary predictors of grass genus/species richness in the nature reserves across China: elevation range (a surrogate of topographical heterogeneity), water (AE or mean annual relative moisture degree), and energy (annual

PE) together accounted for 11.0%, 16.2%, 13.1%, 36.1%, and 30.8% of the variation in the total numbers of genera and species, C₃ genera, and C₄ genera and species, respectively (Table 2). The relationships between grass taxon richness and the three predictors—annual PE, annual AE, and mean annual relative moisture degree—are shown in Figs. 3–5.

A general linear model using the vegetation region as a dummy variable indicated that the region was a significant factor affecting grass genus richness patterns, which, together with annual AE and reserve area, explained 21.4% of the total variation in all grass genus richness (Table 3).

Table 2 Determinants of grass genus/species richness in China's nature reserves at the national level as revealed by multiple stepwise regression analyses

Variables	Coefficient	P	Adjusted R ²
No. of grass genera			
Annual AE	0.781	0.008	0.089
Log ₁₀ elevation range	0.183	0.041	0.110
Log ₁₀ reserve area	0.344	<0.0001	0.186
Log ₁₀ no. of grass species			
Log ₁₀ reserve area	0.376	<0.0001	0.051
Annual AE	0.367	<0.0001	0.162
No. of C ₃ genera			
Mean annual relative moisture degree	0.272	0.004	0.131
Log ₁₀ no. of C ₃ species			
Mean annual wind speed	-0.212	0.026	0.020
No. of C ₄ genera			
Annual PE	0.372	0.013	0.319
Mean annual sunshine hours	0.229	0.017	0.335
Log ₁₀ elevation range	0.215	0.024	0.361
Log ₁₀ no. of C ₄ species			
Annual PE	0.460	<0.0001	0.247
Log ₁₀ elevation range	0.282	0.001	0.288
Mean annual relative moisture degree	0.341	0.007	0.308
%C ₃ species			
Annual PE	-0.687	<0.0001	0.333
Mean annual sunshine hours	-0.199	0.026	0.356
%C ₄ species			
Annual PE	0.511	<0.0001	0.410
Mean annual wind speed	0.251	0.001	0.451
Mean annual relative moisture degree	0.250	0.014	0.476

Regional scale

The species-area relationships for the four different regions indicated that reserve area positively affected the grass species richness in subtropical forests (Fig. 6a) but was not related to species richness in the temperate forests, the temperate steppe and desert, and the Qinghai-Tibet Plateau regions (Fig. 6b–d). This implies that the area variation represents the variations of other environmental factors, which vary inconsistently within the reserves of different area (Martin 1981; Palmer and White 1994).

Simple bivariate relationships between taxon richness and climatic variables were consistent within regions (Fig. 7a–j). Genus and species richness in the four regions covaried with energy-related variables (Table 4). Among the climatic variables we examined, numbers of C₄ genera and species in the subtropical forest region were significantly correlated with mean annual sunshine hours and annual PE (Fig. 7e–h). The number of C₄ genera in the temperate forest region was significantly correlated with mean annual temperature (Fig. 7a), and C₄ species richness in the region was significantly correlated with mean annual temperature and annual PE (Fig. 7b, h). Grass genus richness in the temperate steppe and desert region was only weakly associated with mean annual temperature (Fig. 7i). In the Qinghai-Tibet Plateau region, the total numbers of genera and species as well as the numbers of C₄ genera and species were all significantly associated with mean annual sunshine hours (Fig. 7c–f). Consistent with the results at the national scale, elevation range was positively correlated to C₄ species richness in the subtropical forest region (Fig. 7j).

Stepwise regressions accounted for 17.5% of the variation in C₄ species richness in the subtropical forest, 21.6% of the variation in C₄ species richness in the temperate forest, 17% of the variation in the number of grass genera in the temperate steppe and desert, and 59.8% of the variation in C₄ species richness in the Qinghai-Tibet Plateau regions (Table 4).

Discussion

More than 30 hypotheses exist in the literature to explain the heterogeneous distribution of biodiversity across the Earth (Pianka 1966; Rohde 1992; Heaney 2001; Rahbek and Graves 2001), and these primarily

Fig. 3 Relationships between grass genera (species) richness and annual actual evapotranspiration at the national scale: (a) grass genera, (b) Log₁₀ grass species, (c) C₃ genera, (d) Log₁₀ C₃ species, (e) C₄ genera, and (f) Log₁₀ C₄ species

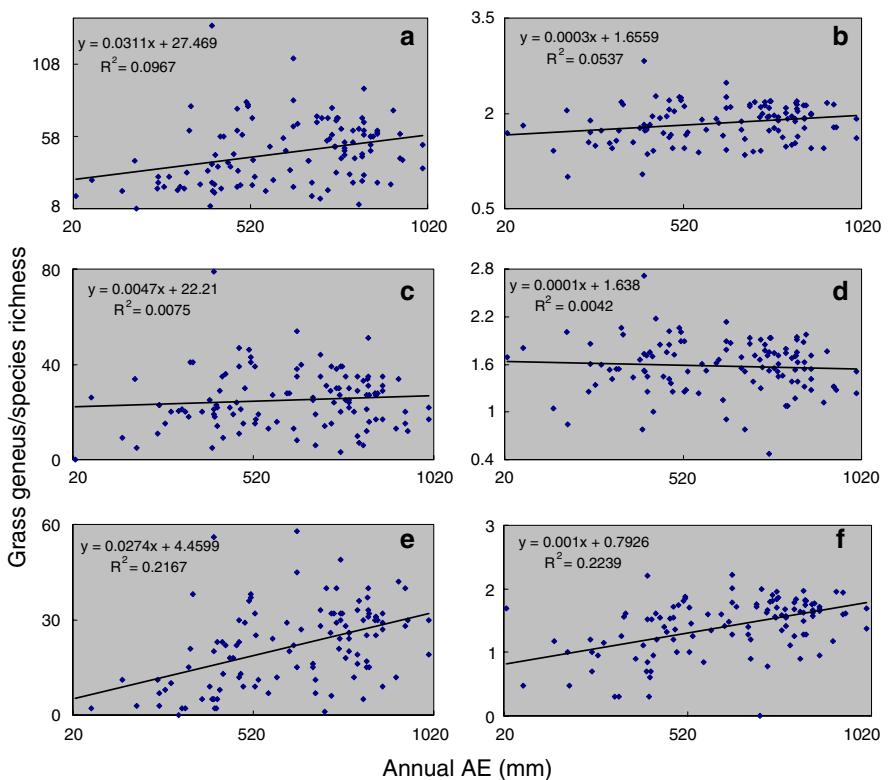


Fig. 4 Relationships between grass genus/species richness and annual PE at the national scale: (a) grass genera, (b) Log₁₀ grass species, (c) C₃ genera, (d) Log₁₀ C₃ species, (e) C₄ genera, and (f) Log₁₀ C₄ species

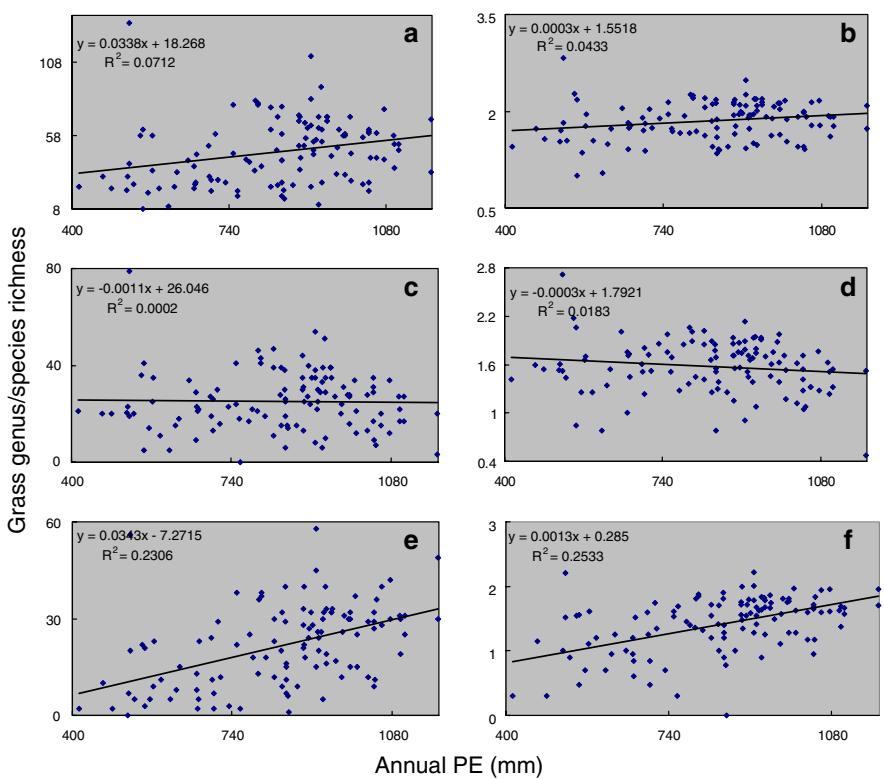


Fig. 5 Grass genus/species richness-AM relationships at the national scale:
(a) grass genera, **(b)** Log₁₀ grass species, **(c)** C₃ genera,
(d) Log₁₀ C₃ species, **(e)** C₄ genera, and
(f) Log₁₀ C₄ species

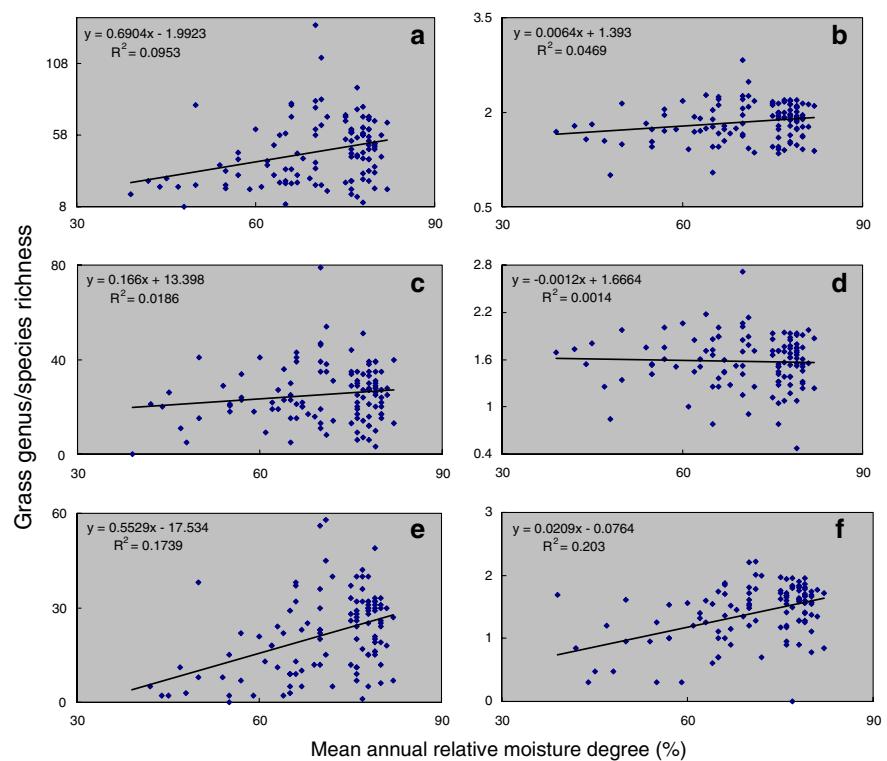


Table 3 Analysis of effect of vegetation region on grass genera richness using a general linear model, with vegetation region added as a dummy variable (see text for details)

Effect	P	F	Type III sums of squares
Annual AE	0.029	4.916	1909.014
Log ₁₀ reserve area	<0.0001	14.234	5527.901
Vegetation region	0.080	2.316	2697.816

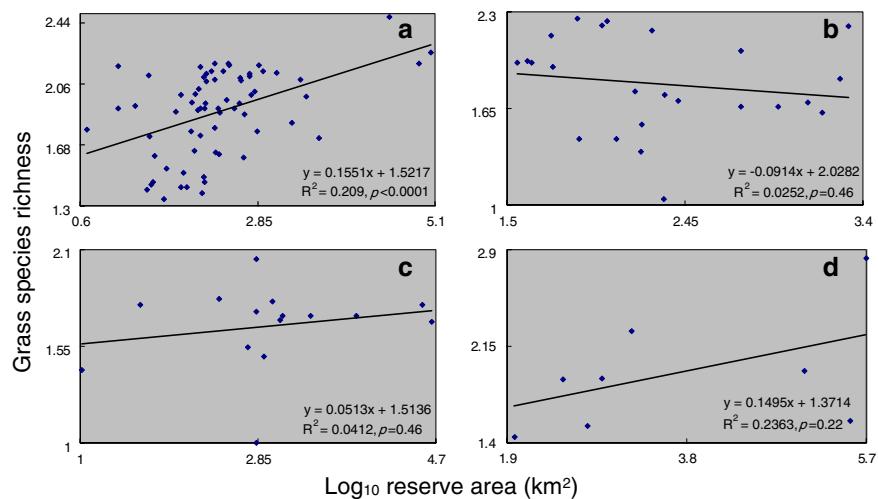
$F_{3,133} = 7.208$, $P < 0.0001$, adjusted $R^2 = 0.214$

break down into three groups. One group of hypotheses proposes that climatic variables, such as seasonal climate, energy, and water availability, are the primary predictors of broad-scale species richness patterns (Klopf 1959; Wright 1983; Francis and Currie 1998; Hawkins et al. 2003). A second group suggests that the variation in species richness is affected by several other factors but not by climatic variables including topographical heterogeneity (O'Brien et al. 2000; Rahbek and Graves 2001) or historical/regional differences based on different speciation or extinction rates, coupled with unique events in the Earth's history (Ricklefs 1987; McGlone 1996; Ricklefs et al. 1999).

A third group of hypotheses suggests that area or spatial constraints can influence diversity patterns (Willig and Bloch 2006; McCain 2007). Our results suggest that spatial variation in grass diversity in China's nature reserves results primarily from the combined influences of climate and topography (Whittaker and Field 2000; Rahbek and Graves 2001).

We discovered that water alone, energy alone or their combination are the measure of climatic factors constraining grass richness, which is consistent with the results of many previous studies (e.g., Hawkins et al. 2003). Hartley (1958a, b) suggested that a globally consistent relationship exists between species richness in the subfamily Panicoideae and wet, hot climates. Our results at the national scale support this prediction. Because all the major ecosystem types found on Earth are presented in China, covering a broad range of climatic variation, from tropical to subarctic/alpine and from rainforest to desert, and across a great altitudinal range from below sea level to the Qinghai-Tibet Plateau, the findings of this study at the national scale are likely to be representative of the global relationship between grass taxon richness and climate. More significantly, our results have extended the consistent relationship between climate and panicoid species richness into that

Fig. 6 Grass species richness-area relationships for four vegetation regions: (a) subtropical forest region, (b) temperate forest region, (c) temperate steppe and desert region, and (d) the Qinghai-Tibet Plateau region



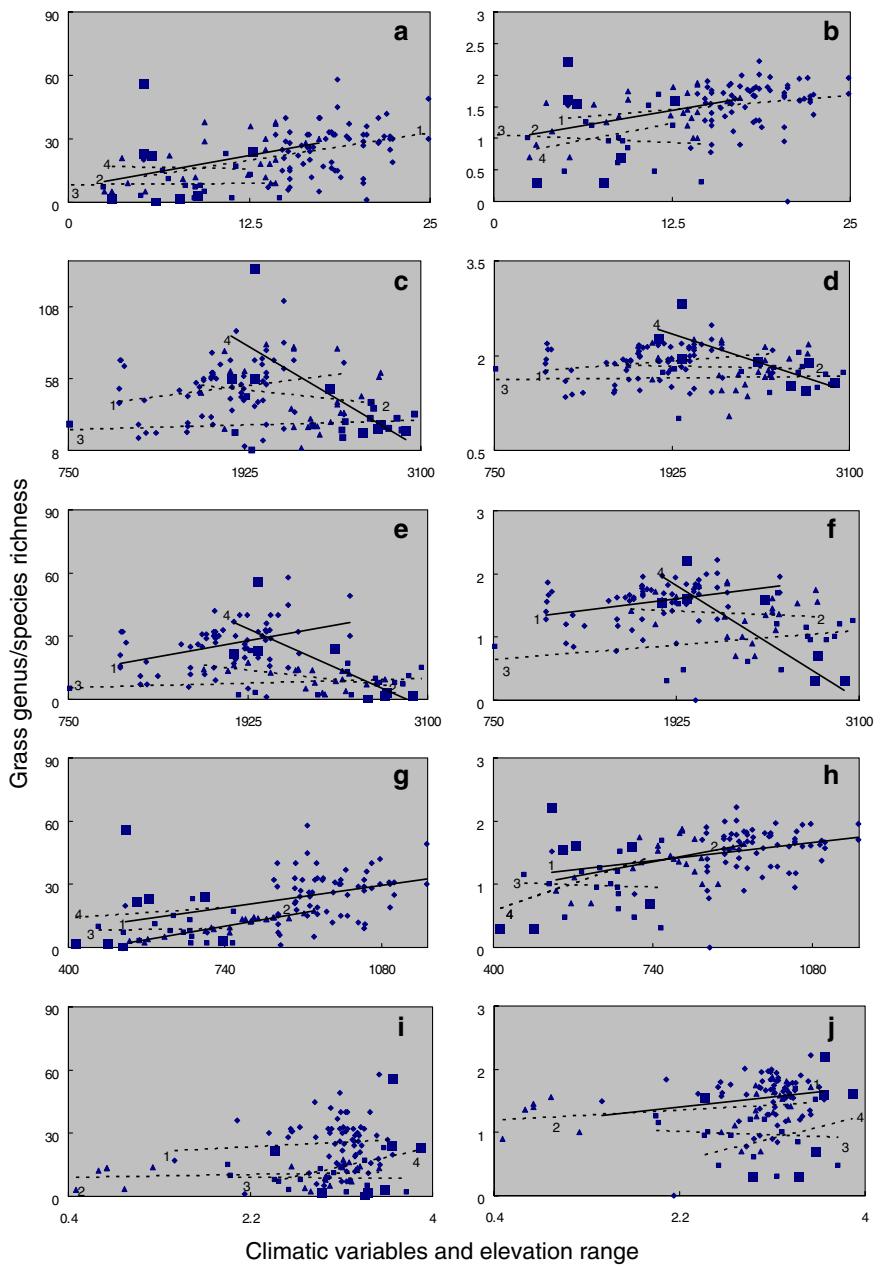
of energy (annual AE) and all grasses (including genera and species), water (annual relative moisture degree) and C₃ grasses, and the combination of water (annual relative moisture degree) and energy (annual PE) and C₄ grasses (Figs. 3–5; Table 2). In contrast, energy variables alone represent the primary predictor of grass richness pattern in different regions (Fig. 7a–h, j; Table 4). In the subtropical forest region, C₄ grasses dominating patches under and away from forest trees are more distinct in high rainfall zones, where the modest enhancement of transpiration in a herbaceous vegetation can lower the stress of water surplus to grasses (Belsky et al. 1993); low dense evergreen canopies enhancing morning and afternoon light levels facilitate the establishment of the unique grassland of C₄ grasses, therefore, annual PE and sunshine hours become the primary predictors of C₄ grass diversity. The Qinghai-Tibet Plateau region spans various vegetation types, including (from east to west) tropical seasonal rainforest, subtropical evergreen broadleaf forest, high-cold shrub, high-cold steppe, and high-cold desert vegetation as a result of the Himalayan uplift event in the Cenozoic era (CCVVA 2001). The total energy budget in this relatively large-scale region is slightly attenuated than the required solar radiation for effective photosynthesis because a large part of the energy is advected to the surrounding environment. Therefore, the climatic constraint to grass richness in this region is sunshine hours (Mordelet and Menaut 1995). In the temperate steppe and desert region and the temperate forest region, grasses have the widest geographical distribution in areas of low soil temperature, which influences

caryopsis germination and seedling establishment in the growing season (Scholes and Archer 1997), and thus temperature also represents the primary limiting factor for grasses. We found that water variables alone never represent the primary predictor of grass diversity in each region, as grasses acquire water primarily from shallow soil layers in grassland ecosystems and can take advantage of smaller precipitation amounts, as was also observed in an analysis of grass richness patterns in the North American tallgrass prairies by McCarron and Knapp (2001).

Elevation range, a surrogate of topographical heterogeneity, was found to represent a strong predictor of diversity for a wide range of taxa, including plants in California (Richerson and Lum 1980), trees in South Africa (O'Brien et al. 2000), birds in South America (Rahbek and Graves 2001), and mammals in North America (Kerr and Packer 1997). Consistent with these patterns, our study suggests that elevation range is strongly associated with grass genus and C₄ genus richness across China, and is associated with genus richness in the subtropical forest region.

Our results show that vegetation region, a proxy for historical/regional processes, was of only marginal importance in predicting the grass richness (the addition of the vegetation region only improved the predictive model R^2 by 5.2%). Because we did not have direct information on historical factors, such as glaciations and dispersal, more refined studies are needed to investigate the relative roles of potential environmental and historical drivers of grass species diversity.

Fig. 7 Relationships between grass genera (species) richness and climatic variables and elevation range in four vegetation region: (1) the subtropical forest region, (2) the temperate forest region, (3) the temperate steppe and desert region, and (4) the Qinghai-Tibet Plateau region. Dashed lines indicate the correlations are not statistically significant. Solid lines are statistically significant: (a) C₄G-T (a2: $r^2 = 0.245$, $P = 0.014$), (b) Log₁₀ C₄S-T (b2: $r^2 = 0.250$, $P = 0.013$), (c) GG-SH (c4: $r^2 = 0.536$, $P = 0.039$), (d) Log₁₀ GS-SH (d4: $r^2 = 0.604$, $P = 0.014$), (e) C₄G-SH (e1: $r^2 = 0.166$, $P = 0.001$; e4: $r^2 = 0.589$, $P = 0.026$), (f) Log₁₀ GS-SH (f1: $r^2 = 0.091$, $P = 0.013$; f4: $r^2 = 0.642$, $P = 0.015$), (g) C₄G-PE (g1: $r^2 = 0.085$, $P = 0.017$; g2: $r^2 = 0.017$, $P = 0.207$), (h) Log₁₀ C₄S-PE (h1: $r^2 = 0.032$, $P = 0.046$; h2: $r^2 = 0.211$, $P = 0.024$), (i) C₄G-Log₁₀ ER (i2: $r^2 = 0.113$, $P = 0.108$), (j) Log₁₀ C₄S - Log₁₀ ER (j1: $r^2 = 0.032$, $P = 0.013$). For abbreviations please see Appendix 3



Because reserve area varied, its relationships to grass richness could be examined. We found that reserve area is a predictor of grass richness at the national scale, but improved or weakened only a little (about 7–11%) the variation explained by climate and topographical heterogeneity. Undoubtedly, area and other environmental variables affect the grass richness patterns. The strength of the contribution depends on the range of their variability. For example, reserve area

represents a strong predictor of species richness in the subtropical forest region, yet had no effect in the other three regions. This finding indicates that the nature reserves of optimal area (region I), rather than a few very large ones (regions III and IV) or many very small ones (region II) should be established in order to reflect the area effects in separate regions (Zhou and Wang 2002), otherwise the climates and topography across regions are sufficiently broad to mask the area

Table 4 Determinants of grass genus/species richness in the different vegetation regions as revealed by stepwise multiple regressions

Richness	Variables	Coefficient	P	Adjusted R^2
Subtropical forest vegetation region				
No. of grass genera	Log ₁₀ elevation range	0.205	0.045	0.064
Log ₁₀ C ₃ species	Mean annual temperature	-0.0241	0.049	0.044
No. of C ₄ genera	Mean annual sunshine hours	0.398	0.001	0.153
	Annual PE	0.289	0.014	0.195
Log ₁₀ C ₄ species	Mean annual sunshine hours	0.303	0.01	0.077
	Log ₁₀ elevation range	0.298	0.013	0.114
	Annual PE	0.280	0.019	0.175
Temperate forest vegetation region				
No. of C ₄ genera	Mean annual temperature	0.495	0.014	0.211
Log ₁₀ C ₄ species	Mean annual temperature	0.500	0.013	0.216
Temperate steppe and desert vegetation region				
No. of grass genera	Mean annual temperature	0.479	0.071	0.170
Qinghai-Tibet Plateau vegetation region				
No. of grass genera	Mean annual sunshine hours	-0.732	0.039	0.459
Log ₁₀ grass species	Mean annual sunshine hours	-0.777	0.023	0.538
No. of C ₄ genera	Mean annual sunshine hours	-0.768	0.026	0.522
Log ₁₀ C ₄ species	Mean annual sunshine hours	-0.810	0.015	0.598

effect in a homogenous environment (McCain 2007; Zhao et al. 2006). Therefore, area alone cannot be the principal driving force of grass diversity at the national level, but it is a regional factor that needs to be considered in future studies.

Bioclimatic models do not represent forecasts of future distributions, but rather provide an indication of the potential magnitude of the impact of global warming on the species distributions (Hill et al. 1999). Literature on responses of grass distribution to global warming shows that total grass richness is significantly linked to temperature at the time of reproduction (Cavagnaro 1988; Collatz et al. 1998). Continued rising temperatures correlate with rising water and soil temperature at large spatial scale. Increased water and soil temperatures have the potentiality to both increase water loss through increased leaf growth and to reduce water uptake through increased evaporation. Therefore, it is entirely possible that more drought-tolerant grasses will flourish as the less-tolerant grasses currently present decline. C₄ grasses have greater potential for ecological success relative to C₃ grasses because they have greater water-use efficiency (Zhang et al. 2003), thus C₃ grasses will lose their competitive advantage in arid regions as water becomes more limiting (Collatz et al. 1998). Our

analyses offer a guideline for C₃/C₄ plant distributions under a global change scenario.

In conclusion, our results revealed that water- and energy-related variables and elevation range represent the primary explanations for the variation in grass richness in China's nature reserves. Significant relationships were found between energy and all grasses (C₃ and C₄), water and C₃ grasses, and the combination of water and energy and C₄ grasses at the national scale. Climatic constraints to grass diversity varied by vegetation region. We found that energy variables alone represent the primary predictor of grass richness pattern at the regional level. Topographical heterogeneity, measured by elevation range, is strongly associated with grass richness in the subtropical forest region, but has no effect in the other regions. The results reported herein have important implications in predicting vegetation response to future climate change, i.e., global warming could cause C₄ grass expansion in China's nature reserves.

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Appendix 1: Literature list used to document species richness for Poaceae and other information of reserves in China

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Appendix 2

Appendix 2 Original information on grass species richness and other basic characteristics for the 115 nature reserves in this investigation

Reserve name	No. of total grasses	No. of C ₃ species	No. of C ₄ species	LAT (°N)	LONG (°E)	Area (km ²)
Subtropical forest vegetation region						
Anhui Huangshan	87	52	35	30.1	118.1	154
Anhui Jixiqingliangfeng	54	28	26	30.1	118.8	30
Anhui Jinzhaitianma	91	56	35	30.5	115.4	289
Anhui Yaoluoping	79	32	47	30.2	116.8	123
Fujian Longxishan	120	56	64	26.6	117.3	156.9
Fujian Wuyishan	133	76	57	27.7	117.7	565.3
Chongqing Jinfoshan	87	51	36	29.1	107.2	418.5
Chongqing Wulingshan	127	81	46	27	111	146.3
Three Gorges reservoir area	154	86	74	30.5	107.5	79,000
Guangdong Babaoshan	28	19	9	24.7	113	33
Guangdong Chebaling	98	35	53	24.5	114.2	75.5
Guangdong Dawuling	41	17	24	22.3	111.2	35.3
Guangdong Ehuangzhang	81	32	49	21.6	111.2	150
Guangdong Dinghushan	148	58	90	23.2	112.5	12
Guangdong Xiangtoushan	61	21	40	23.3	114.4	202.8
Guangdong Neilingding Island	60	19	41	22.4	113.8	4.8
Guangdong Nanling	98	51	47	24.8	112.8	584
Guangdong Shimantai	138	21	87	24.2	113.2	822.6
Guangdong Northern <i>Panthera tigris amoyensis</i>	96	26	45	24.3	113.5	2,905
Guangxi Nonghua	81	35	46	22.9	105.9	134
Guangxi Longhushan	84	42	42	22.4	107.3	20
Guangxi Dayaoshan	81	41	40	24	110.1	360
Guangxi Huaping	28	13	15	25.6	109.9	151.3
Guangxi Mulun	26	11	15	25.2	108	89.7
Guangxi Nonggang	58	21	37	22.4	106.9	100.8
Guizhou Chishui <i>Alsophila</i>	55	36	19	28.4	106	133
Guizhou Fodingshan	24	17	7	27.3	108.1	140.3
Guizhou Xishui Forest	74	49	25	28.3	106.2	486.7
Guizhou Zhujiashan	26	20	6	27	107.6	76.4
Guizhou Leigongshan	40	32	8	26.4	108.2	473

Appendix 2 continued

Reserve name	No. of total grasses	No. of C ₃ species	No. of C ₄ species	LAT (°N)	LONG (°E)	Area (km ²)
Guizhou Liupanshuiyeyezhong	22	6	16	26.2	104.8	46
Hainan Bawangling	53	3	50	19.2	108.8	4,287
Hainan Jianfengling	123	33	90	18.6	108.9	201.7
Henan Jigongshan	130	72	58	31.8	114.1	29.3
Henan Liankangshan	138	85	53	31.6	114.8	185
Henan Taihangshan	128	77	51	35.3	112.4	566
Hubei JIugongshan	154	86	68	29.3	114.5	201.1
Hubei Houhe	88	46	42	30.1	110.6	103.4
Hubei Shennongjia	58	36	22	31.5	110.3	704.7
Hunan Badagongshan	42	29	13	29.7	109.9	232
Hunan Mangshan	77	40	37	25	112.9	65.4
Hunan Bamianshan	34	15	19	25.7	113.5	49.1
Hunan Taoyuandong	76	34	42	26.4	114	237.9
Jiangxi Jinggangshan	43	24	19	26.6	114.2	207
Jiangxi Dagangshan	107	42	65	27.3	114.4	126
Jiangxi Jiulianshan	146	52	94	24.6	114.5	134.1
Jiangxi Poyang lake	81	50	31	29.2	116	224
Jiangxi Wuyishan	133	94	59	27.9	117.8	160.1
Jiangxi Lushan	154	87	67	29.5	116.4	304.9
Sichuan Longxi-Hongkou	126	74	52	30.7	103.6	427.2
Taiwan Chaishan	81	12	69	22.4	120.2	12
Taiwan Hehuanshan	25	24	1	23.6	120.6	27.9
Taiwan Yangmingshan	99	37	62	25.2	121.6	114.6
Yunnan Daweishan	121	61	60	23	103.6	439.9
Yunnan Wuliangshan	151	51	100	24.3	100.4	309.4
Yunnan Nujiang	66	33	33	27.8	98.3	1,947
Yunnan Gaoligongshan	135	60	64	25.5	98.7	1245
Yunnan Amushan	30	12	18	23.1	102.2	147.6
Yunnan Honghe region	303	135	168	23.4	102.9	32,931
Yunnan Honghe Cycas	27	8	19	23.8	103.3	31.9
Yunnan Tongbiguan	151	70	81	24	97.4	732.2
Yunnan Dazhongshan	44	14	30	24.5	100.5	112.2
Yunnan Xishuangbanna	123	63	60	21.7	101.1	2,474
Yunnan Luchun Huanglianshan	103	55	48	22.5	102.1	650.6
Yunnan Upper Reaches Region of Hongshuihe	180	78	102	24.9	105.9	110,000
Zhejiang Gutianshan	32	24	8	29.2	118.1	81.1
Zhejiang Baishanzu	137	82	55	27.7	119.1	260.5
Zhejiang Qingliangfeng	91	48	43	30.2	119	108
Zhejiang Tianmushan	93	55	38	30.4	119.4	42.8
Temperate forest vegetation region						
Beijing Songshan	91	57	34	39.6	115.3	166
Beijing Baihuashan	139	95	41	39.9	115.6	217.4
Hebei Hengshuihu	85	70	25	37.3	115.4	187.9
Henan Funiushan	179	115	64	33.4	111.8	560
Henan Yellow River Wetland	28	18	10	34.8	112.6	1,900

Appendix 2 continued

Reserve name	No. of total grasses	No. of C ₃ species	No. of C ₄ species	LAT (°N)	LONG (°E)	Area (km ²)
Henan Baotianman	161	86	75	33.3	111.5	54.1
Henan Xiaoqinling	173	102	71	34.4	110.5	40.8
Henan Old Yellow River	28	18	10	35.2	114.3	259.8
Heilongjiang Maoershan	58	45	13	45.3	127.4	76.6
Heilongjiang Liangshui	23	18	5	47.1	128.5	121.3
Heilongjiang Honghe	35	27	8	47.8	133.7	218.4
Heilongjiang Zhalong	150	114	36	47.2	124.2	2,100
Jilin Huichun	11	6	5	43	130.2	889.1
Liaoning Baishilazi	55	33	22	40.9	124.8	74.7
Liaoning Laotudingzi	50	32	18	41.3	124.9	152.2
Liaoning Xianrendong	109	53	56	40	123	35.8
Shandong Nansihu	46	19	28	35	116.7	1,275
Shandong Yellow River Delta	46	23	23	37.9	118.9	1,530
Shanxi Manghe	49	28	21	35.3	112.4	55.7
Shaanxi Niubeiliang	42	26	16	33.8	108.9	164.2
Shaanxi Qingmuchaun	71	45	26	32.8	106.2	102
Shaanxi Taibaishan	159	106	53	34	107.6	563.3
Shaanxi Zhouzhi	115	70	35	33.8	108	563.9
Tianjin Baxianshan	26	10	16	40.1	117.3	10.5
Temperate steppe and desert vegetation region						
Gansu Gahai-Zecha	53	51	2	32	102.3	2,474
Gansu Xiaolongshan	61	28	33	34.1	106.5	42
Gansu Dunhuang	61	54	7	40.1	95.3	36,000
Hebei Saihanwula	66	57	9	42.2	116.9	276.3
Inner Mongolia Dalai Lake	53	39	14	48.6	117.5	7,400
Inner Mongolia Dalinuoer	50	40	10	43.3	116.7	1,194
Inner Mongolia Keerqing	53	35	18	45.1	122	1,270
Inner Mongolia Wulate	10	7	3	42.1	106.6	680
Inner Mongolia West Erdos	35	19	16	39.7	107.2	555.8
Ningxia Helan Mountain	112	102	10	38.6	105.4	677.1
Ningxia Liupanshan	56	52	4	35.7	106.3	678
Ningxia Baijitan	31	22	9	38.1	106.6	818
Xinjiang Arjinshan	49	49	0	39.1	90.3	45,000
Xinjiang Tuomuerfeng	64	61	3	41.9	80.3	1,000
Xinjiang Gongliuxueleng Spruce	78	73	5	43.2	83	312.2
Qinghai-Tibet Plateau vegetation region						
Tibet Big Bend Gorge of Yalu Tsangpo	91	50	41	29.6	95.5	111,600
Qinghai Beichuanhe	34	34	0	37.2	100.5	559
Qinghai Mengda	28	26	2	35.8	102.7	95.4
Sichuan Xiaman	185	150	35	33.7	102.7	1,666
Tibet Hengduan Mountains	679	518	161	28.7	99.5	500,000
Tibet Ali	37	35	2	29.4	86	340,000
Yunnan Cangshanerhai	80	40	40	25.7	95.6	797

Abbreviations: LAT, latitude; LONG, longitude; Area, reserve area

Appendix 3

Appendix 3 Correlation matrix of species richness for Poaceae, reserve area, climatic variables, and elevation range in the study

Appendix 3 continued

	GG	\log_{10} GS	C_3G	$\log_{10} C_3S$	C_4G	$\log_{10} C_4S$	LAT	LONG area	\log_{10} AT	AP	\log_{10} AW	AM	SH	PE	AE	\log_{10} WD	\log_{10} ER		
AP	PC	0.252	0.187	0.057	-0.056	0.385	0.407	-0.705	0.220	-0.344	0.712	1	-0.189	0.779	-0.499	0.742	0.953	-0.714	0.150
	P	0.00	0.04	0.54	0.55	0.00	0.00	0.01	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115
\log_{10} AW	PC	-0.095	-0.117	-0.166	-0.170	-0.018	-0.027	0.278	0.342	-0.006	-0.224	-0.189	1	-0.264	0.513	-0.182	-0.194	0.122	-0.291
	P	0.31	0.21	0.07	0.06	0.85	0.77	0.00	0.00	0.95	0.01	0.04	0.00	0.00	0.00	0.05	0.03	0.19	0.00
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115
AM	PC	0.309	0.217	0.137	-0.038	0.417	0.451	-0.693	0.246	-0.354	0.678	0.779	-0.264	1	-0.631	0.719	0.831	-0.493	0.132
	P	0.00	0.02	0.14	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115
SH	PC	-0.155	-0.089	-0.102	-0.013	-0.163	-0.227	0.531	0.059	0.134	-0.511	-0.499	0.513	-0.631	1	-0.527	-0.562	0.297	-0.317
	P	0.09	0.34	0.27	0.89	0.08	0.01	0.00	0.53	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115
PE	PC	0.267	0.208	-0.016	-0.135	0.480	0.503	-0.784	0.093	-0.357	0.958	0.742	-0.182	0.719	-0.527	1	0.823	-0.095	0.143
	P	0.00	0.02	0.86	0.15	0.00	0.00	0.00	0.32	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.12
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115
AE	PC	0.311	0.232	0.086	-0.065	0.465	0.473	-0.763	0.241	-0.359	0.791	0.953	-0.194	0.831	-0.562	0.823	1	-0.613	0.136
	P	0.00	0.01	0.35	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.14
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115
\log_{10} WD	PC	-0.146	-0.101	-0.136	-0.065	0.111	-0.130	0.314	-0.225	0.122	-0.102	0.714	0.122	-0.493	0.297	-0.095	-0.613	1	-0.097
	P	0.11	0.28	0.14	0.49	0.24	0.16	0.00	0.01	0.19	0.27	0.00	0.19	0.00	0.00	0.31	0.00	0.00	0.30
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115
\log_{10} ER	PC	0.211	0.197	0.191	0.145	0.194	0.140	-0.339	-0.505	0.101	0.175	0.150	-0.291	0.132	-0.317	0.143	0.136	-0.097	1
	P	0.02	0.03	0.04	0.12	0.04	0.13	0.00	0.00	0.28	0.06	0.10	0.00	0.16	0.00	0.12	0.14	0.30	0.00
N	115	115	115	115	112	115	115	115	115	115	115	115	115	115	115	115	115	115	115

Abbreviations: PC, Pearson correlation coefficient; P, significance level; GG, grass genera; GS, grass species; C_3G , no. of C_3 genera; C_4G , no. of C_4 genera; C_4S , no. of C_4 species; $\%C_3$, $\%C_4$; $\%GS$; LAT, longitude; LONG, latitude; Area, reserve area; AT, mean annual temperature; AP, annual precipitation; AW, mean annual wind speed; AM, mean annual relative moisture degree; SH, mean annual sunshine hours; PE, annual potential evapotranspiration; AE, annual actual evapotranspiration; WD, water deficit; ER, elevation range

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Interacting effects of grass height and herbivores on the establishment of an encroaching savanna shrub

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Abstract Shrub encroachment is a widely observed problem in Southern African savannas. Although the effects of herbivory and grass height on woody species recruitment have been studied individually, little information exists about how these factors interact. In this study seeds and seedlings of the encroaching shrub *Dichrostachys cinerea* were planted in clipped and unclipped grass plots, with and without large herbivores present. Seed germination, seedling survival and seedling predation were monitored for 8 months. Germination started earlier in plots where herbivores were excluded. Overall, the earlier the seeds germinated, the longer the seedlings survived.

Clipping positively affected the number of germinated seeds, seedling growth and survival but effects varied among herbivore exclusion treatments and sites. Invertebrates caused the majority of the seedling damage. We conclude the recruitment of *D. cinerea* is influenced by the interplay of grass height and herbivory. In this study, the presence of large herbivores early in the wet season, and the absence of simulated grazing later on, affected the regeneration of *D. cinerea* negatively. However, differences in effects among sites suggest that the mechanisms found here may work differently in other habitats.

Keywords Bush encroachment · *Dichrostachys cinerea* · Herbivory · Precipitation · Seed germination · Seedling survival

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Nomenclature *Dichrostachys cinerea* (L.) Wight and Arn. · *Digitaria longiflora* (Retz.) Pers. · *Eragrostis curvula* (Schrad.) Nees · *Eragrostis superba* Peyr. · *Panicum maximum* Jacq. · *Sporobolus africanus* (Poir.) A. Robyns and Tournay · *Sporobolus nitens* Stent · *Themeda triandra* Forsk. · *Urochloa mosambicensis* (Hack.) Dandy

Introduction

Bush and tree encroachment poses significant problems to farmers and wildlife managers in many parts

of the world and has been well documented in North America (Hobbs and Mooney 1986; Archer et al. 1988; Archer 1995), South America (Adamoli et al. 1990), Australia (Burrows et al. 1990) and Africa (Van Vegten 1983; Watson and MacDonald 1983; Skarpe 1990; Prins and van der Jeugd 1993; Moleele et al. 2002). Particularly in formerly open grasslands an increase in woody plant biomass can alter the water balance (Pressland 1973), fire intensity (van Langevelde et al. 2003), nutrient cycling (Belsky 1992), and primary productivity of the grass layer (Stuart-Hill and Tainton 1989). As livestock and large herbivores are highly dependent on the quality and quantity of grass (Coe 1983; Demment and van Soest 1985; Belovsky 1997), they are also likely to be negatively affected by bush encroachment. In South Africa it has been estimated that 13 million ha of savanna have been subject to recent bush encroachment (Trollope et al. 1989) due to changed grazing and fire regimes.

Herbivores and fire are the two major interacting drivers of tree–grass dynamics in African savannas (Pellew 1983; Dublin 1991; McNaughton 1992). Grazers can indirectly and positively affect tree seedling establishment by reducing the surrounding grass cover (Roques et al. 2001; Goheen et al. 2004). This decreases competition with grasses for resources such as water, nutrients and light (Belsky and Blumenthal 1997) and prevents seedlings from burning through reduced fuel loads (Walker et al. 1981; van Vegten 1983; Archer 1995; Milton and Dean 1995). Fire may additionally promote the regeneration of woody plant species by promoting the release and germination (Brown and van Staden 1997; Keeley and Fortheringham 2000; Brown et al. 2003) of seeds. Consumption of seed pods followed by defecation of intact seeds by browsers can also promote shrub recruitment (Brown and Archer 1987; Reyes et al. 1994) as scarification through ingestion is known to break seed dormancy (van Staden et al. 1994b). On the other hand, large herbivores can inhibit tree establishment directly through browsing, uprooting or trampling of seedlings (Dublin et al. 1990; Mwalyosi 1990; Sinclair 1995). High browsing pressure by selective herbivores (such as impala) can prevent the establishment and recruitment of woody seedlings (Prins and van der Jeugd 1993) and make them more exposed to fire (Mills 1983). Furthermore intense grazing can lead to a drier microclimate and lower soil moisture which increases the risk of

seedling desiccation, especially in the dry season. In addition to large herbivores, smaller consumers (e.g. rodents and insects) have been reported to negatively influence recruitment of woody species via seed- and seedling predation (Sullivan 1979; Andersen and Lonsdale 1990; Ostfeld and Canham 1993; Miller 1994; Auld 1995; Crawley and Long 1995; Harju and Tahvanainen 1997; Weltzin et al. 1997; Manson et al. 2001), especially during population peaks (Pusenius et al. 2000). Although the effects of herbivory and grass height on the recruitment of woody species in savannas have been studied individually (Walker et al. 1981; Trollope 1984; Prins and van der Jeugd 1993; O'Connor 1995), the importance of interactions among these factors is still poorly understood, and experimental studies are rare.

The leguminous shrub *Dichrostachys cinerea* is an important bush encroacher in South African savannas (van Vegten 1983; O'Connor 1995; Roques et al. 2001; Moleele et al. 2002). In the present study we investigate the direct and indirect effects of mammalian herbivores on *D. cinerea* seed germination and seedling survival in a South African savanna. In field experiments, we manipulated grazing intensity by excluding large herbivores and grass height by clipping at four study sites (two in mesic and two in arid savanna). We assumed the time of year during which germination takes place can be crucial for the survival of the seedling. Seeds that germinate early in the wet season may have better chances to survive, which consequently leads to a higher risk for severe bush encroachment. We hypothesised the survival of established seedlings may then be affected by the height of the surrounding vegetation. In tall vegetation seedlings should be protected from browsing herbivores but in short vegetation they would experience less competition with grasses for water, nutrients and light. On the other hand, in short vegetation grazing herbivores may graze them along with grasses and also make them more visible for browsers. We expected that the presence of large herbivores would decrease seedling establishment through predation particularly in clipped grass where seedlings are more visible. Previous studies have shown that the exclusion of large herbivores leads to increased small mammal densities (Hagenah et al. submitted) which in turn may have a negative impact on tree seedling survival. Therefore, both the effects of grazing intensity and grass height on time of germination and survival time, as well as the

proportions of germinated seeds and surviving seedlings at the end of the wet and dry season, were investigated.

Materials and methods

Study site

This study was conducted between March and December 2004 at the Hluhluwe-iMfolozi Park (HiP) in KwaZulu-Natal, South Africa ($28^{\circ}13' S/32^{\circ}00' E$). HiP is a 90,000 ha protected fenced area and consists of the Hluhluwe Game Reserve in the North and the iMfolozi Game Reserve in the South. The altitude in the park ranges from 60 to 750 m (Conway et al. 2001). The climate is coastal and seasonal, with temperatures from $\pm 13^{\circ}\text{C}$ to $\pm 35^{\circ}\text{C}$. Precipitation varies greatly across the year and generally falls between October and March. The mean annual rainfall (measured from 1980–2004) for Hluhluwe is 985 mm (min: 68 mm, max: 4060 mm), whereas in iMfolozi it is 650 mm (min: 2 mm, max: 3200 mm), measured at five weather stations each in Hluhluwe (Gontshi, Gunjaneni, Memorial Gate, Nqumeni and Research Centre) and iMfolozi (Makhamisa, Masinda, Mbuzane, Mpila and Tobothi). The park is characterised by savanna vegetation ranging from open grasslands to closed *Acacia* and broad-leaved woodlands (Brooks and MacDonald 1983). Skowno et al. (1999) documented that the woody plant biomass in HiP has rapidly increased over the last 40 years. *Dichrostachys cinerea* (legume of the Mimosoidae), a deciduous multi-stemmed shrub/small tree (Pooley 1993) is an important bush encroacher in HiP, invading large areas of previously open grasslands (Skowno et al. 1999). The shrub produces highly nutritious leaves and pods and the infructescences have a strong rich aroma that attracts browsers (van Staden et al. 1994a). The seeds of *D. cinerea* are impermeable to water and dormant at release (van Staden et al. 1994b; Witkowski and Garner 2000). Dormancy is mainly broken through diurnal temperature fluctuations, by scarification through ingestion and by bruchid exit holes (van Staden et al. 1994b). A fire management regime is simulating natural fires in the park, in which different areas are burnt with varying frequencies. HiP harbours a high diversity and biomass of indigenous large herbivores including elephant (*Loxodonta africana*),

white rhinoceros (*Ceratotherium simum*), black rhinoceros (*Diceros bicornis* L.), African buffalo (*Syncerus caffer*), giraffe (*Giraffa camelopardalis*), wildebeest (*Connocheates taurinus*), zebra (*Equus burchelli*), waterbuck (*Kobus ellipsiprymnus*), kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angansi*), impala (*Aepyceros melampus*) and warthog (*Phacochoerus aethiopicus*).

Experimental design

The present study utilised herbivore exclosures established at four study sites in HiP within the context of a different study (Bond and Olff, unpublished). Fences 2.50 m tall excluded all mammalian herbivores larger than hares from 40×40 m plots of vegetation since early 2000. To quantify small mammal densities, permanent trapping grids inside and outside the exclosure treatment were established ($n = 8$). Overall, the absence of large herbivores leads to a significant increase in small mammal numbers (Hagenah et al. submitted). Furthermore the exclusion of large herbivores resulted in increased grass height at all sites. The grass species that were most frequently dominant in the mesic savanna of Hluhluwe included *Sporobolus africanus* and *Digitaria longiflora*. Other frequently dominant grasses were *Panicum maximum* and *Themeda triandra* (Hagenah et al. submitted). For the arid savanna of iMfolozi the dominant grass species were *Urochloa mosambicensis* and *P. maximum*, but also *T. triandra* and *Sporobolus nitens* were found frequently (all mentioned grass species are C4 species). The amount of rainfall was measured at weather stations situated at our study sites and varied seasonally (Fig. 1). In 2004 the amount of rainfall was similar to that of previous years since 1980, described in the *study site* section. The soils at all study sites were lithosols with ca. 17% clay content (FAO). All study sites are burned every second year; in 2004 they were burnt approximately 4 months after seeds and seedlings were planted. *D. cinerea* can survive bushfires and resprouts easily after burning (Bond, pers. comm.).

Seed-germination and seedling survival experiments

Fresh seeds of *D. cinerea* were collected during July to September 2003 from more than 50 trees well

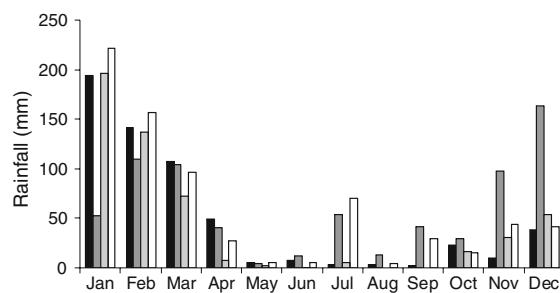


Fig. 1 Mean monthly rainfall (mm) at the four study sites in the Hluhluwe-iMfolozi Park from January to December 2004. Bars present data of different study sites; black: Nombali, dark grey: Maqanda, light grey: Mona, white: Tobothi. *Dichrostachys cinerea* seeds were sown mid of March 2004

spread over the HiP. Seeds were stored in a sealed box in a fridge for several days, scarified between sandpaper to break dormancy and imbibed in water for 12 h. A pre-germination test of *D. cinerea* seeds under controlled conditions revealed a germination rate of approximately 20% after 30 days.

The best time to conduct this study would have been to sow the seeds at the beginning of the wet season. However, flooding due to heavy rains forced us to sow the seeds later in the rainy season. Similarly, understanding when and under which conditions seedlings die may suggest factors influencing patterns of seedling establishment. A survival analysis makes it possible for us to monitor these patterns and also at what time of the year possible interacting effects take place.

Experiment 1: germination and survival of seedlings established from sown seeds

To examine the effects of herbivory and grass height on tree seedling germination and survival, two study sites were established in each mesic savanna in Hluhluwe (Nombali and Maqanda) and arid savanna in iMfolozi (Mona and Tobothi). Each study site had two large herbivore treatments: exclusion of large herbivores vs. unfenced control. Within the herbivore treatments four 1 × 1 m plots with tall grass and four 1 × 1 m plots with short grass were created by clipping the grass in half of the plots to approximately 8 cm grass height. A total of 64 plots were thus established. Plots inside and outside the herbivore enclosure were established with at least 10 m spacing to each other. The distance of the plots to the fences inside the enclosure was no less than 5 m.

fences inside the enclosure was no less than 5 m. In mid-March, 25 imbibed seeds were sown in each plot (resulting in 1600 sown seeds in total) approximately 1 cm deep in the soil and watered. Seed germination, seedling survival and herbivory were monitored and vegetation height recorded in the plots on a monthly basis throughout the experiment (8 months). Seedlings were considered to be damaged by mammalian herbivores when their stems, leaves, or branches had been cut off and by invertebrates when leaves were partly eaten. If a seedling had lost one or more leaflets it was considered to be caused by partial wilting or disease. Wilted seedlings were also recorded.

Experiment 2: survival of transplanted seedlings

For this experiment one study site in mesic savanna (Nombali) and one study site in arid savanna (Mona) were used. Each study site had two herbivore treatments: exclusion of large herbivores vs. unfenced control. Within the herbivore treatments three 1 × 0.5 m plots with tall grass and three 1 × 0.5 m plots with short grass were created by clipping the vegetation to approximately 8 cm grass height. A total of 24 plots were thus established. Plots inside and outside the herbivore enclosure were established with at least 10 m spacing to each other. The distance of the plots to the fences inside the enclosure was no less than 5 m. Imbibed seeds were sown separately approximately 1 cm deep in plastic trays with soil from the park and stored in a nursery. Seeds were left to germinate and watered daily. After 8 weeks seedlings were approximately 2 cm in height. Nine seedlings were then transplanted in each 0.5 × 1 m plot into the field (resulting in 216 seedlings in total) and watered once a week during the first month. Because of the low number of seedlings transplanted our plot size requirements for this experiment was smaller than for experiment 1. Seedling survival, herbivory and vegetation height were monitored as described for experiment 1.

Data analysis

The effects of the treatments on time until germination and survival time of *Dichrostachys Cinerea* was analysed with Cox regression hazard model survival analyses. The model compared the number of seeds

that germinated with the number of seeds that did not germinate at a given time of the experiment (after Kleinbaum 1996). Furthermore, the number of seedlings that were alive at a given time was compared with the number of seedlings that had died. In those analyses germination time or survival time was the time factor, the status ‘germinated’ or ‘alive’ the event and herbivore exclusion (fenced vs. unfenced control), clipping treatment (clipped vs. unclipped control) and site the predictors. In the analysis of treatment effects on survival time, germination time was included as a covariate factor. The output of the model gives the coefficient estimates and associated estimated standard errors, chi-square statistics and *p*-values of Wald test. The higher the value of the Wald test the stronger the effect of the predictor(s) on the event. The survival analysis is designed to allow analyses before all events have been observed and to accommodate for the fact that not all seeds germinate at the same time. Because of these features, survival analysis was considered to be the most suitable method in this case, even if it was not possible to take the split-plot design of this experiment into account.

The effects of herbivore exclusion (fenced vs. unfenced control), clipping (clipped vs. unclipped control) and the interactions of site × clipping (herbivore exclusion) and site × herbivore exclusion on the proportion of seeds that germinated were analysed as a split-plot design. Clipping, clipping × enclosure, clipping × site, and clipping × enclosure × site were treated as effects within the herbivore exclusion treatment (at the sub-plot level), and herbivore

exclusion, site and exclusion × site were treated as effects between the herbivore exclusion treatment (at the whole-plot level). Similar analyses were conducted for the proportion of seedlings that was still alive at the end of the wet season in April and at the end of the dry season in September, for both seedlings that established from sown seeds and transplanted seedlings. Too few seedlings remained at the end of the experiment (December) to be analysed statistically. The proportions of germinated seeds and seedlings were arcsine square-root transformed to normalise residuals. Seedling growth was tested using a factorial ANOVA with herbivore exclusion, clipping treatment and site as predictors and seedling height as dependent variable. Furthermore a Pearson’s Chi-square test was used to analyse the overall differences of seedling damage in fenced/unfenced and clipped/unclipped plots.

Results

Experiment 1 – seed germination

The time of germination was not affected by the clipping treatment (Table 1). The effect of large herbivore exclusion on the time of seed germination varied among sites (significant site × herbivore exclusion interaction, Table 1). Germination started earlier in fenced plots compared to unfenced plots at all sites, but the germination rate levelled out in fenced plots at three sites (Maqanda, Mona and

over the course of the experiment (8 months), analysed with a Cox regression hazard model

Table 1 Effects of site, herbivore exclusion (fenced vs. unfenced control) and clipping treatment (clipped vs. unclipped control) on time of germination and survival time of *D. cinerea*

Source of variation	df	Germination		Survival of seedlings established from seeds		Survival of transplanted seedlings	
		Wald	<i>P</i>	Wald	<i>P</i>	Wald	<i>P</i>
Site	1	6.953	<i>0.008</i>	0.05	0.823	3.674	0.055
Herbivore exclusion	1	3.631	0.057	10.74	<i>0.001</i>	1.351	0.245
Clipping	1	0.387	0.534	4.60	0.032	0.275	0.600
Site × Herbivore exclusion	1	4.295	<i>0.038</i>	14.94	<i>0.000</i>	3.522	0.610
Site × Clipping	1	2.658	0.103	0.52	0.472	3.621	0.570
Herbivore exclusion × Clipping	1	0.009	0.926	4.97	0.026	2.778	0.096
Site × Herbivore exclusion × Clipping	1	0.001	0.970	8.206	<i>0.004</i>	0.513	0.474
Germination time	1	–	–	36.91	<i>0.000</i>	–	–

P-values in italics are significant

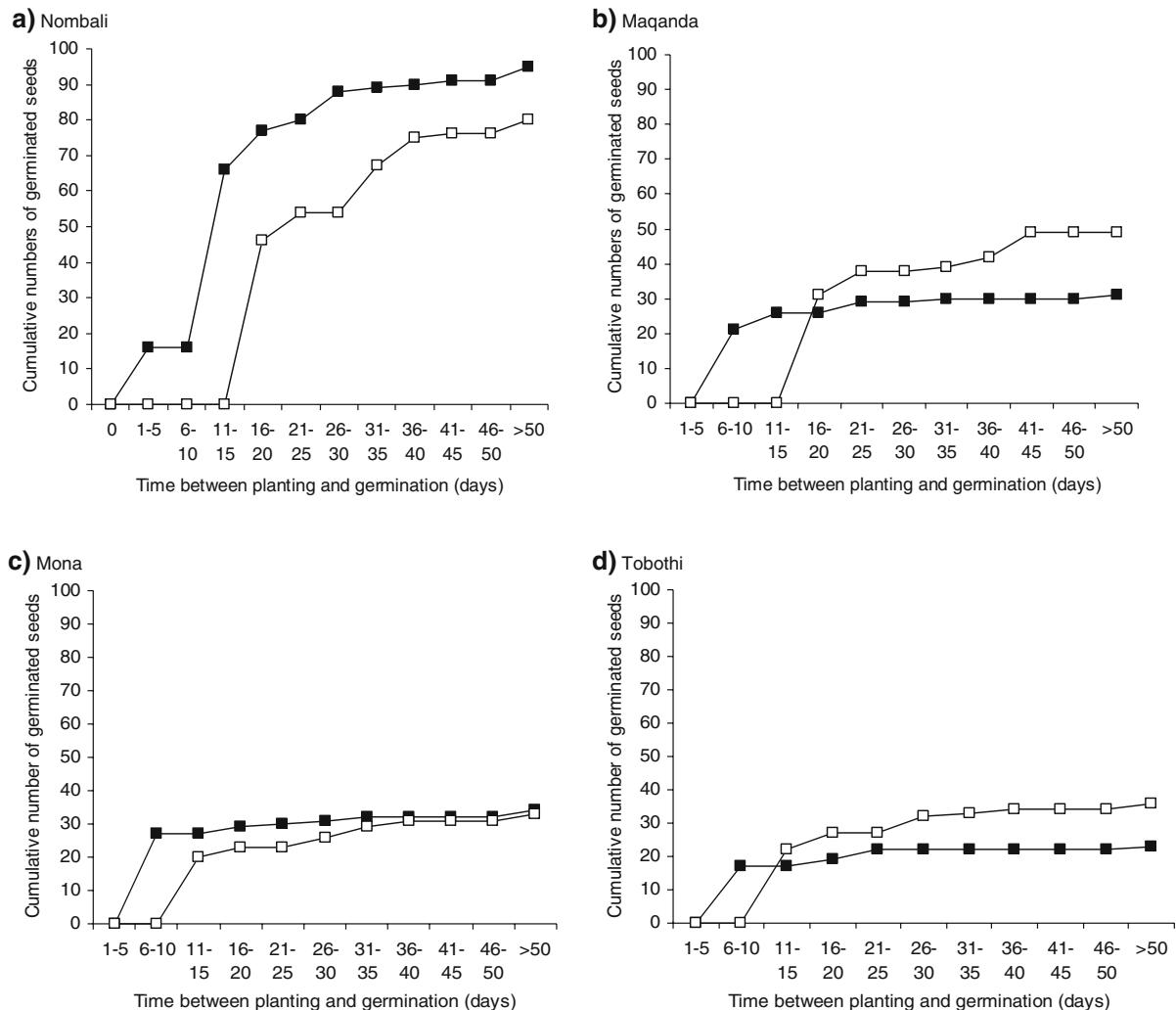


Fig. 2 Cumulative number of seeds that germinated in plots inside the large herbivore exclusion (solid squares) and unfenced control (open squares) within a given period after

Tobothi) after about a week. At Nombali the germination rate in fenced plots continued to be as high as in unfenced plots over the course of the experiment (Fig. 2a-d).

The proportion of seeds that germinated was positively affected by clipping and varied among sites (Table 2 and Fig. 3). At Nombali the proportion of germinated seeds was approximately 20% to 32% higher than at the three other sites, for unfenced and fenced plots, respectively (Fig. 3). For the first 15 days after seed planting, both the precipitation and the germination rate of seeds in fenced/unfenced plots were similar at all sites (Figs. 1, 2a-d). However, 16 to 25 days after seed planting, precipitation

planting at the two mesic sites Nombali and Maqanda (a and b) and two arid sites Mona and Tobothi (c and d; $N = 380$)

and seed germination rate was higher at the mesic sites than at the arid sites.

Experiment 1 – survival of seedlings established from sown seeds

Effect of germination time

Germination time affected time of survival of seedlings significantly (Table 1). Seedlings that established from seeds that germinated in the wet season had higher survival time than seedlings of germinated seeds from the dry season. Overall, the earlier the seeds germinated, the longer the seedlings survived.

Table 2 Experiment 1 (Seedlings established from sown seeds): Effects of site, herbivore exclusion (fenced vs. unfenced control) and clipping treatment (clipped vs. unclipped control) within the factor “herbivore exclusion”, and of the between-

subject factors “herbivore exclusion” and “site” on the total proportion of seeds that germinated and seedlings survived of *D. cinerea* at the end of the wet season (April) and at the end of the dry season (September), analysed with split-plot ANOVAs

Source of variation	df	Germination		Survival of seedlings established from seeds, April		Survival of seedlings established from seeds, September	
		F	P	F	P	F	P
Site	3	11.53	<i>0.000</i>	8.15	<i>0.001</i>	5.51	<i>0.005</i>
Herbivore exclusion	1	1.18	0.288	0.59	0.449	0.107	0.747
Clipping	1	5.32	<i>0.030</i>	9.11	<i>0.006</i>	19.30	<i>0.000</i>
Site × Clipping	3	0.185	0.906	2.44	0.089	4.34	<i>0.014</i>
Herbivore exclusion × Clipping	1	0.094	0.762	4.08	0.055	0.27	0.608
Site × Herbivore exclusion × Clipping	3	0.347	0.792	0.21	0.887	1.80	0.173
Herbivore exclusion × Site	3	0.69	0.565	1.67	0.201	3.01	<i>0.050</i>

P-values in italics are significant

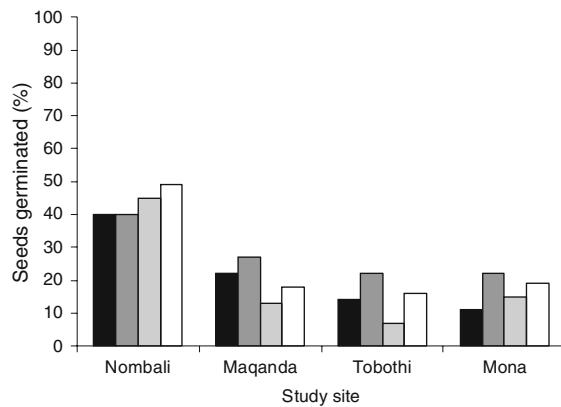


Fig. 3 Effects of the clipping treatment (clipped vs. unclipped) and exclusion of large herbivores (fence vs. unfenced) on the total percentage of *D. cinerea* seeds that germinated at two mesic sites (Nombali and Maqanda) and two arid sites (Tobothi and Mona). Bars present data of different combinations of treatments; black: unfenced, unclipped; dark grey: unfenced, clipped; light grey: fenced, unclipped; white: fenced, clipped

Interacting effects of clipping, herbivores and site

Seedling survival time was affected by large herbivore exclusion and clipping (Table 1). The combined effects of the grass clipping treatment and herbivore exclusion on the survival time of *D. cinerea* seedlings varied among sites (significant site × clipping treatment × herbivore exclusion interaction; Table 1). At the mesic site Nombali clipping had a positive effect on survival time where large herbivores were excluded, while at two other sites a positive clipping effect was found when herbivores were present (Fig. 4a–d).

The proportion of surviving seedlings was significantly higher in clipped than in unclipped grass and varied among sites, both at the end of the wet season and at the end of the dry season. There were also significant interactions between site and clipping treatment and between site and herbivore exclusion treatment at the end of the dry season (Table 2). In total only 21 seedlings that established from sown seeds were still alive at the end of the experiment in December, which was too few to analyse statistically. 13 of these seedlings were found at Nombali.

Cause of damage to seedlings

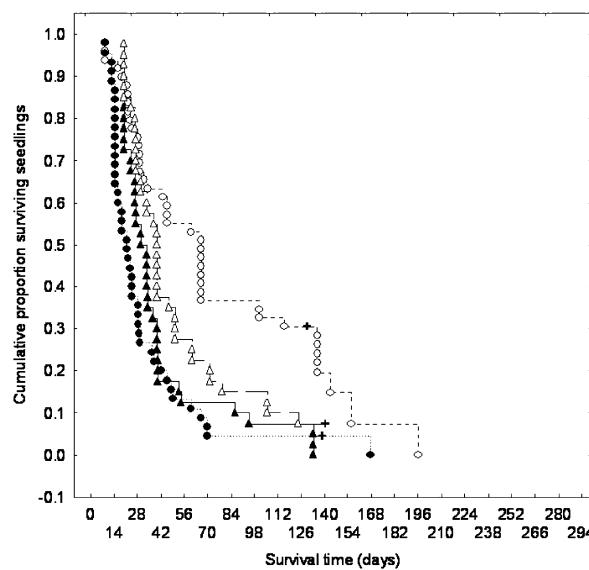
Overall, 32% of the seedlings were damaged. We observed significant differences in the damage types that seedlings experienced in the different treatments ($X^2_9 = 808.957$, $P < 0.000$, Fig. 5). For all treatments the most frequent damage to seedlings was leaflet loss because of wilting or disease. Most of the herbivory was caused by invertebrates but also mammals (most likely rodents) were found to be consumers of seedlings. Furthermore, both invertebrates and rodents damaged more seedlings in clipped than in unclipped grass, whereas the presence or absence of larger herbivores had no effect.

Experiment 2: survival of transplanted seedlings

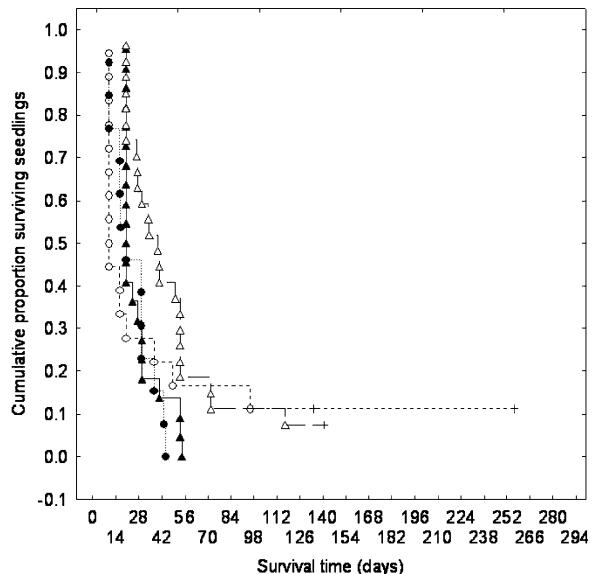
Interacting effects of grass height, herbivores and site

There were no significant effects of treatments on survival time (Table 1; Fig. 6a, b). The effect of

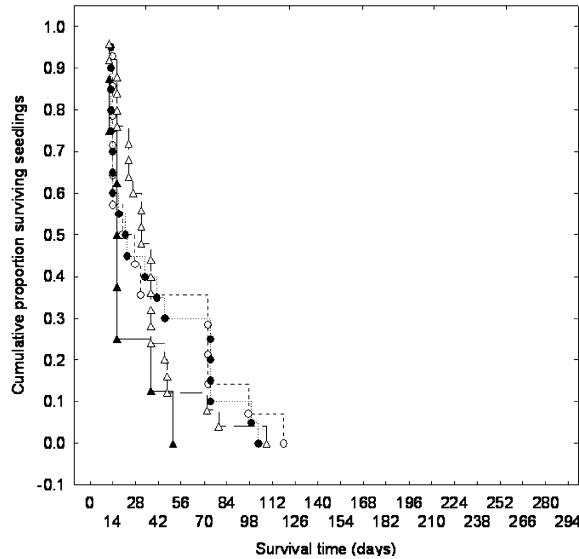
a) Nombali



b) Maqanda



c) Mona



d) Tobothi

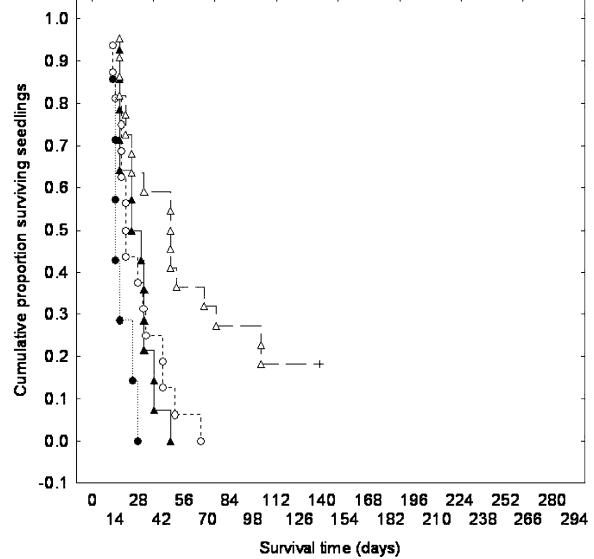


Fig. 4 Interacting effects of the clipping treatment (clipped vs. unclipped) and large herbivores (fence vs. unfenced) on the survival time of *D. cinerea* seedlings at the two mesic sites Nombali and Maqanda (**a** and **b**) and two arid sites Mona and Tobothi (**c** and **d**; Kaplan Meier). + = censored, other symbols = complete observations. Symbols present data of

different combinations of treatments; solid circles: unclipped, fenced; open circles: clipped, fenced; solid triangles: unclipped, unfenced; open triangles: clipped, unfenced. The combined effects of the clipping treatment and large herbivore exclusion varied significantly among sites ($N = 380$, Wald = 8.206, $P = 0.004$)

clipping on the proportion of surviving seedlings in April varied among sites. In September, the proportion of surviving seedlings was not significantly

affected by either treatments or interactions between them (Table 3). Only six transplanted seedlings were still alive at the end of the experiment; all of them

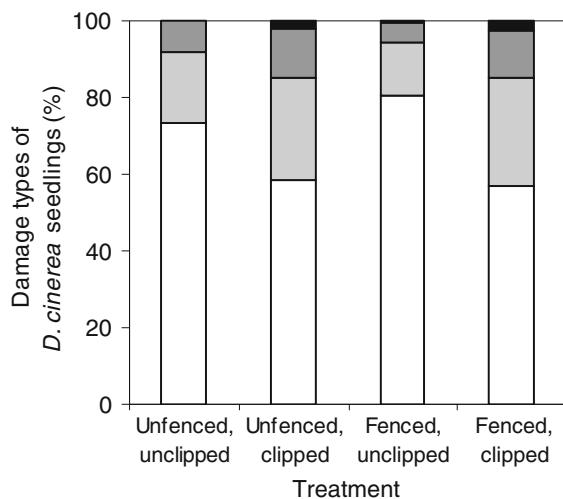


Fig. 5 Percentage of different damage types of *D. cinerea* seedlings for the different treatments at all sites for March to December 2004. Black boxes: rodent herbivory, dark grey boxes: invertebrate herbivory, light grey boxes: other damage, white boxes: no damage. Seedling damage was significantly different between treatments ($N = 890$, $X^2_9 = 808.957$, $P < 0.000$)

were recorded in clipped grass at the mesic site Nombali (Fig. 6a). This was too few to conduct further statistical analyses.

Seedling growth

Overall, seedling growth (as measured by height) was affected by both clipping treatment and site. Seedlings in clipped grass grew more than in unclipped grass plots ($F_{1,181} = 26.29$, $P < 0.001$) and seedling growth was higher at the mesic savanna site Nombali than at the arid savanna site Mona ($F_{1,181} = 10.06$, $P = 0.002$). We found no evidence that seedling growth was influenced by large herbivores.

Cause of damage to seedlings

Overall, 20% of the seedlings were damaged. Significant differences in the types of damage to seedlings in the different treatments were observed ($X^2_9 = 96.056$, $P < 0.000$, Fig. 7). Except for leaflet loss because of wilting or disease, most of the damage was caused by invertebrates. Rodent-type damage to seedlings was rarely observed. Furthermore, seedlings were more often damaged in unclipped than in clipped grass, and more seedlings were damaged in the absence of large herbivore than in their presence.

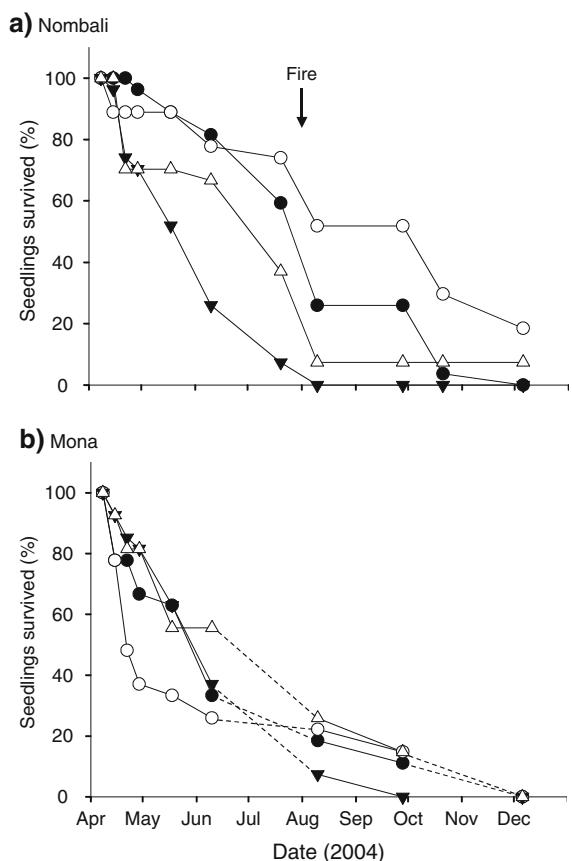


Fig. 6 Interacting effects of large herbivores (fence vs. unfenced) and clipping treatment (clipped vs. unclipped) on the survival of transplanted *D. cinerea* seedlings at the mesic site Nombali (a) and arid site Mona (b) for April to December 2004. Symbols present data of different combinations of treatments; solid circles: unclipped, fenced; open circles: clipped, fenced; solid triangles: unclipped, unfenced; open triangles: clipped, unfenced. The seedling survival at the two sites was not significantly influenced by the combined effects of the clipping treatment and large herbivore exclusion ($N = 3$ plots per treatment, Wald = 0.513, $P = 0.474$)

Discussion

Seed germination and seedling survival

It is often suggested that competitive inhibition by the grass layer may be a crucial filter for tree recruitment in savannas (Sankaran et al. 2005) but also herbivores have been considered important (Pellew 1983; Dublin 1991; McNaughton 1992; van Langevelde et al. 2003). In the present study, seed germination and seedling survival of *D. cinerea* was affected by the interplay of competition between trees and grasses for resources

Table 3 Experiment 2 (Transplanted seedlings): Effects of site, herbivore exclusion (fenced vs. unfenced control) and clipping treatment (clipped vs. unclipped control) within the factor “herbivore exclusion”, and of the between-subject

factors “herbivore exclusion” and “site” on total proportion of seedlings survived of *D. cinerea* at the end of the wet season (April) and at the end of the dry season (September), analysed with split-plot ANOVAs

Source of variation	df	Survival of transplanted seedlings, April		Survival of transplanted seedlings, September	
		F	P	F	P
Site	1	1.45	0.263	0.91	0.368
Herbivore exclusion	1	0.02	0.893	3.22	0.110
Clipping	1	1.14	0.318	3.24	0.110
Site × Clipping	1	52.12	<i>0.000</i>	0.16	0.704
Herbivore exclusion × Clipping	1	1.21	0.303	0.001	0.971
Site × Herbivore exclusion × Clipping	1	0.02	0.880	1.87	0.209
Herbivore exclusion × Site	1	1.26	0.295	0.000	0.984

P-values in italics are significant

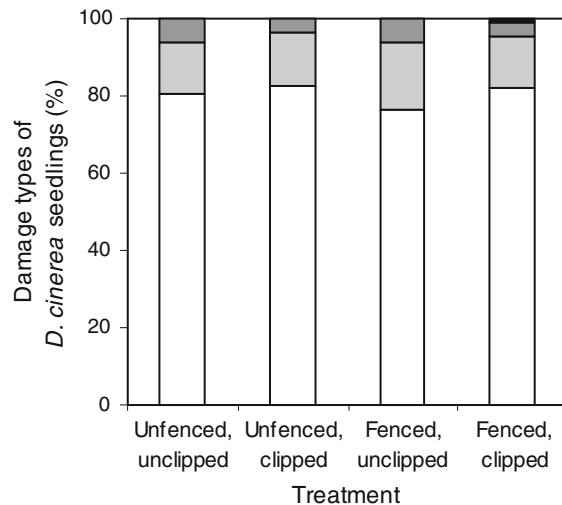


Fig. 7 Percentage of different damage types of *D. cinerea* seedlings for the different treatments at both sites for April to December 2004. Black boxes: rodent herbivory, dark grey boxes: invertebrate herbivory, light grey boxes: other damage, white boxes: no damage. Seedling damage was significantly different between treatments ($N = 366$, $X^2_9 = 96.056$, $P < 0.000$)

and habitat modifications caused by grazing large herbivores. For instance, by suppressing the growth of graminoids through intense grazing, herbivores may indirectly facilitate seed germination and seedling survival through improved light conditions. It has been shown that high light levels can improve seed germination and seedling survival (Denslow 1987; Brokaw 1985; Fischer et al. 1991; Belsky and Blumenthal 1997), which is demonstrated in our study. Furthermore, when the grass sward is damaged, e.g. by

overgrazing, above- and below-ground biomass is reduced (Holland and Detling 1990) and more water becomes available for woody plants (Caldwell et al. 1987; Walker and Noy-Meir 1982; Bland 1985) that otherwise could have been utilised by the grasses (Walker and Noy-Meir 1982; Bland 1985). Water relations have been indicated to be particularly important during seed germination (Hoffmann 1996) and also for seedling survival. Therefore intense grazing by large herbivores may indirectly facilitate seed germination and seedling survival by suppressing the growth of competing grasses and thus accelerate tree recruitment through improved light and water availability.

On the other hand, grazing also can have negative effects on seedling survival. For instance, overgrazing often results in a significant decrease in vegetation cover and the lack of protective cover in turn may increase the seedlings' risk of being directly killed through trampling of large grazing herbivores. Additionally large grazers could graze them along with grasses and make them more visible to browsers. Furthermore, reduced shade levels in overgrazed areas may lead to higher desiccation which could promote a rise in seedling mortality when compared with a shady environment (Gerhardt 1996). In our study, seedling survival increased in clipped plots, but seedlings responded both negatively and positively to herbivore exclusion, indicating that the positive effects of herbivore presence on *D. cinerea* seed germination and seedling survival under some circumstances outweigh its negative effects but in other cases they did not.

In the first week of the study, seeds only germinated in the generally higher ungrazed grass inside the herbivore exclusion. The undisturbed grass sward might have kept the moisture and created a favourable microclimate for germination. After 2–3 weeks, coinciding with decreasing precipitation, germination rate in fenced plots slowed down in three of the four sites. The lighter rains at the beginning of dry season may not have reached the soil surface where the seeds were if the grass was dense, high and not disturbed by trampling. Because clipping had no significant effect on germination time, it appears that in this case trampling and thinning of the grass sward were more important factors than grass height. At Nombali, the germination rate was not affected by herbivore exclusion. This might be partly explained by the sometimes higher precipitation at this site. The total proportion of seeds that germinated was significantly affected by clipping but not by herbivore exclusion. The positive effect of early germination on survival time suggests that the total proportion of germinated seeds may be less important than a high germination rate at the beginning of the rainy season.

Seedling survival time of *D. cinerea* was affected by the interplay of competition between trees and grasses for resources and habitat modifications caused by grazing large herbivores. Overall, survival time was longer in clipped plots where large herbivores had access, as the combination of clipping and large herbivores resulted in short trampled grass that allowed rain and light to reach down to the soil surface. However, at Nombali survival time was longer in clipped plots in the absence of large herbivores. The positive effects of trampling seemed to be less important than the positive effects of clipping. These results are consistent with the expectations that less competition for light and water promotes seed germination and seedling survival. However, the high seedling mortality in this study may also partly be attributed to the late planting of the seeds.

Browsing mammals have been suggested to have a relatively low impact on tree seedling recruitment, while invertebrates may account for most of the herbivory (Miller 1994; Mucunguzi 1995; Meiners et al. 2000; Shaw et al. 2002). Roques et al. (2001) found that large browsers only had a minor impact on *D. cinerea* dynamics in Swaziland. In accordance with these studies we observed that invertebrates were responsible for most of the herbivory on *D. cinerea*,

whereas large browsers were only of minor importance. Small mammals, most likely rodents, were found to be seedling predators. In African savannas the reported role of rodents in influencing tree recruitment ranges from potentially important (e.g. Miller 1994) to negligible (e.g. Barnes 2001). Shaw et al. (2002) found that rodents in East African savannas have only relatively little effects on *Acacia drepanolobium* seedling survival, whereas insects were considered to be important agents of seedling mortality.

Implications of the study

There is considerable debate on how rainfall (Fensham and Holman 1999; Fernandez-Gimenez and Allen-Diaz 1999; Fynn and O'Connor 2000) and grazing (Hulme et al. 1999; Bokdam and Gleichman 2000; Sternberg et al. 2000) should be taken into account in terms of management of shrub encroachment. The results of this study indicate that reductions in *D. cinerea* regeneration may occur when drought acts in concert with light grazing. Drought might inhibit germination of seeds and seedling survival. Light grazing may result in a closed grass sward that has the potential to reduce or prevent shrub encroachment (Roques et al. 2001) by out-competing seeds and seedlings for resources such as light. Furthermore, when the grass sward has sufficient above- and below-ground biomass the grasses would be able to utilise the water in the upper soil that otherwise would have been used by the woody species. Additionally, increased plant growth could lead to both increased fire frequencies (Roques et al. 2001) and intensities (Norton-Griffiths 1979) and fire would thus be another important tool to suppress bush encroachment.

In this study the grazing and browsing pressure during the early wet season, in addition to the absence of simulated grazing later on, affected the regeneration of *D. cinerea* negatively. The differences in effects among sites suggest that the mechanisms detected in this study may work differently in habitats with other conditions regarding grazing intensity, precipitation and invertebrate- and rodent herbivory. This is important to consider when planning land management to fight bush encroachment. Based on the results of our study it can be suggested that by modifying the vegetation structure large herbivores have an impact on the recruitment of *D. cinerea*. Managers can influence the grazing regime, but they

have little control over drought in order to prevent or reverse bush encroachment.

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Effects of competition on root–shoot allocation in *Plantago lanceolata* L.: adaptive plasticity or ontogenetic drift?

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Abstract We investigated how shoot and root allocation in plants responds to increasing levels of competitive stress at different levels of soil fertility. In addition, we analyzed whether different responses were due to adaptive plasticity or should be attributed to ontogenetic drift. *Plantago lanceolata* plants were grown during 18 weeks at five plant densities and four nutrient supply levels in pots in the greenhouse. Thereafter root and shoot biomass was measured. There were clear negative effects of increasing plant densities on plant weights revealing strong intraspecific competition. At the lower N-treatments, the proportional allocation to root mass increased with increasing competitive stress, indicating the important role of belowground competition. At the higher N-supply rate, the relationship between competitive stress and shoot to root ratio was neutral. These responses could not be attributed to ontogenetic drift, but could only be explained by assuming adaptive plasticity. It was concluded that at lower N-supplies belowground competition dominates and leads to increased allocation to roots, while at the higher N-supply competition for soil resources and light had balanced impacts on shoot and root allocation. An alternative hypothesis explaining the observed pattern

is that light competition has far less pronounced impacts on root–shoot allocation than nutrient deprival.

Keywords Root–shoot allocation · Competition · Ontogenetic drift · Adaptive plasticity · *Plantago lanceolata* L.

Introduction

In grassland communities, plant species composition and plant species diversity vary significantly with productivity and soil fertility (Whittaker 1975; Grime 2001). Competition between plant populations is often assumed to have decisive effects on these patterns (Grime 1979; Berendse 1983; Tilman 1988). A classical, but still unresolved issue is whether the relative importance of belowground and aboveground competition shifts with increasing soil fertility and whether this has consequences for competition intensity and the rate of species replacement (Grime 1979, 2001; Tilman 1988; Wilson and Tilman 1991; Goldberg and Novoplansky 1997; Peltzer et al. 1998; Keddy et al. 2000; Wardle 2002). Answering this question would enable us to predict what plant features increase plant fitness at different levels of soil fertility.

Competing plants deprive each other of resources such as light, soil nutrients, and water. Brouwer (1962a, b) and many others showed unambiguously

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that changing levels of resource supply lead to significant changes in the partitioning of growth between the below- and the aboveground parts of the plant. Shading increases the allocation to leaves and stems, while reduced supplies of nitrogen or water increase the allocation to root growth. Recently, these general patterns were reconfirmed by a meta-analysis of literature data (Poorter and Nagel 2000). Brouwer (1962a,b) explained this adaptive response by his Functional Equilibrium Hypothesis, where he assumed that below- and aboveground parts are competing for the resources that the plant has acquired. The consequence is that we should expect that root competition would lead to increased allocation to belowground parts, while aboveground competition would lead to increased allocation to shoots. These opposite responses would enable a sharp distinction between the effects of stress due to above- or belowground competition. Such distinction can possibly help to answer questions about the relative importance of above- and belowground competition from the point of view of the plant.

The single use of shoot:root ratios to test whether plants show an adaptive response in allocation pattern to changes in resource supply has been criticized (Weiner 1990; Jasinski and Bazzaz 1999). It has been argued that biomass allocation patterns may change due to allometric relationships that are maintained during plant development (Evans 1972; Gedroc et al. 1996). Such ontogenetic relations might lead to changes in biomass partitioning only as a consequence of an increase in plant size. Using an allometric analysis, several authors observed that the effects of nutrient supply (Müller et al. 2000) or root competition (Cahill 2003; Litton et al. 2003) on shoot:root ratios could be explained by their effects on individual plant mass.

In this article, we investigate the effects of nutrient supply and competitive stress on allocation to above- and belowground parts. We attempted to distinguish between changes in biomass partitioning due to adaptive plasticity or fixed allometric relationships during development. We tried to answer the following questions: (1) What are the effects of different levels of competitive stress on the proportional allocation to shoot and root mass? (2) Is the response of shoot:root ratios to increasing levels of competitive stress different at low and high nutrient supplies? (3) Are the effects of nutrient supply and competitive

stress on the shoot:root ratio a consequence of adaptive plasticity or should they be attributed to ontogenetic drift?

We attempted to answer these questions by performing an experiment where we compared the allocation to shoot and root mass at a large range of competitive stress and soil fertility levels. It is often impossible to harvest the complete root mass of the target species as roots of the competing species are strongly intermingled. We resolved this problem by creating different levels of competitive stress by growing *Plantago lanceolata* L. plants at different monospecific densities, so that we were able to harvest all roots in all treatments. *Plantago lanceolata* L. is a perennial herb that grows in hay meadows at a wide range of soil fertilities.

Materials and methods

Design of the experiment

The experiment was designed to investigate the effects of different levels of N supply on the stress that plant individuals experience by intra-specific competition. The plants were grown in the greenhouse in pots with a diameter of 20 cm and a depth of 20 cm. In order to avoid edge effects that would reduce the differences in light environment between densities and nutrient supply levels, the plants were grown in pots surrounded by an outer pot in which plants were grown at the same density. The target plants were grown in the inner pot with a ground area of 314 cm² and a pot volume of approximately 6 l. These pots were placed in larger pots that had dimensions such that the outer part also had a ground area of 314 cm² and a soil volume of 6 l. The diameter of the pot including the buffer part was 28.3 cm. These buffer zones were planted with the same numbers of plants as the inner pots, so that not only plant densities, but also ground area and soil volume per individual were equal in the inner and outer pots.

We compared five plant densities (1, 2, 4, 8, and 16 plants per 314 cm²) and 4 N-fertilization levels (N0, N5, N10, and N20) in a complete factorial combination. Each combination of plant density and N-treatment was repeated in five replicated blocks. In each of these blocks the treatments were located randomly.

Experimental procedures

Plants were grown in the greenhouse at day and night (12/12 h) temperatures of 20 and 18°C, respectively. Air humidity was maintained at 70%. A nutrient-poor soil substrate was prepared by mixing five parts sand without organic matter and one part black top soil removed from an arable field on a sandy subsoil. The organic matter content of the mixture was 1.3%, and pH(H₂O) and pH(KCl) were 6.4 and 6.0, respectively. Total N and P contents were 345 and 163 mg kg⁻¹, respectively. We supplied both the inner and outer pot weekly with 50 ml of nutrient solution with 37 mg N, 5 mg P, and 12.5 mg K at the highest fertilization level. The total amount applied during the course of the experiment were 0, 4.4, 8.8, and 17.7 g N m⁻² (and corresponding amount on P and K) in the N0, N5, N10, and N20 treatments, respectively. Pots were supplied daily with water to prevent water limitation. The soil water content was maintained at field capacity (14.4%, w/w) by weighing the pots twice a week and adding sufficient amount of water to maintain constant pot weights. On the five days each week when this was not done, the amount of water to be added were determined from estimates based on the weights of a random sample of pots from each N treatment.

On 26 April 2001 seeds of *Plantago lanceolata*, collected from natural populations in a hay meadow near Wageningen, were planted in trays containing fine sand. On 17 May 2001 the seedlings were planted in the pots. Plants were harvested on 3 September 2001. All shoots in the inner pots were cut off at ground surface (including the caudex). Roots in the inner pots were washed out of the soil and thereafter cleaned by hand using tweezers to remove humus particles. All plant parts were dried at 70°C during 48 h and subsequently weighed.

Statistical analysis

The homogeneity of variances of log-transformed individual plant weights and shoot:root ratios were investigated using a Gamma GLM (GENSTAT 1993). The assumptions with respect to the homogeneity of the variances were met by our data. Subsequently, a GLM was applied with plant density and nutrient treatment as factors and the log-transformed values of individual plant weights and

shoot to root ratios as dependent variables. Blocks were included as random factor. In a second analysis of the shoot to root ratios (S_w/R_w) we included plant density (D) and N-treatment (N) as fixed factors with plant weights (P_w) as a covariate. We used the model

$$\log(S_w/R_w) = a + b \cdot \log P_w + N + D + N \cdot D + N \cdot \log P_w + D \cdot \log P_w \quad (1)$$

We also analyzed the allometric relation between log-transformed shoot and root weights. Shoot weight (S_w) was set as a dependent variable, root weight (R_w) was set as a covariate, and density and N-treatment were set as factors. Here, we applied the following model

$$\log S_w = a + b \cdot \log R_w + N + D + N \cdot D + N \cdot \log R_w + D \cdot \log R_w \quad (2)$$

Three way interactions, which did not have significant effects, were excluded since they would lead to an undesired loss of degrees of freedom given the number of data. Significant effects of the factors N and D are assumed to reveal differences between the intercepts of the relationships across fertilization treatments and plant densities, respectively. Significant interactions between N or D and the covariates P_w or R_w are supposed to reveal significant differences among the slopes of the allometric relation across different levels of nutrient supply or intra-specific competition, respectively (Shipley and Meziane 2002). Pair-wise differences between regression coefficients were tested using variances and covariances of the estimates.

Results

Individual plant weights

At all N-supply rates, individual plant weights declined strongly with increasing plant density, indicating the great stress that the plants experienced due to intra-specific competition (Fig. 1). Plant density and N-treatment each had significant effects ($P < 0.001$). The interaction between these two factors was not significant ($P = 0.11$), but distinction between the linear, quadratic, and cubic terms of these effects shows that the interaction between the linear effects was highly significant ($P < 0.001$),

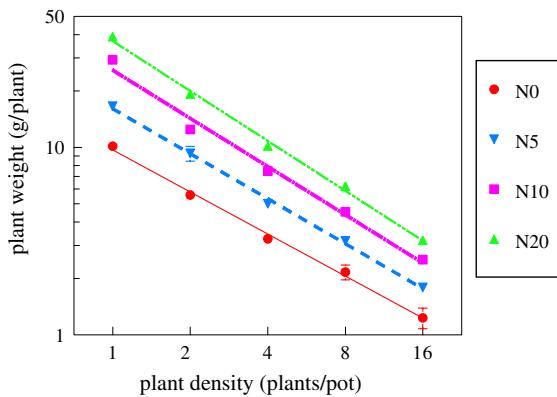


Fig. 1 Effects of increasing plant densities on individual plant weights at four levels of nutrient supply (dots, N0; reverse triangles, N5; squares, N10; triangles, N20). Bars indicate standard errors of the mean. Both the vertical and the horizontal axis have a logarithmic scale

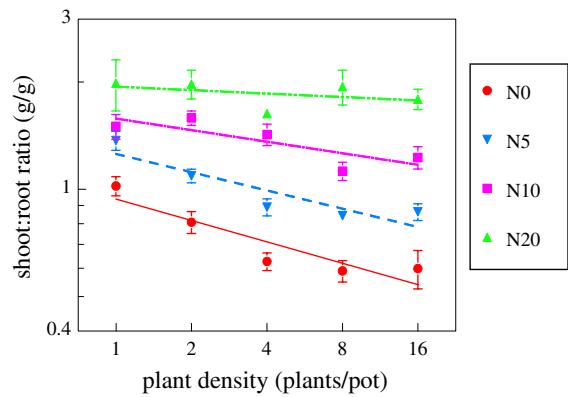


Fig. 2 Effects of increasing plant densities on shoot to root ratios at four levels of nutrient supply (dots, N0; reverse triangles, N5; squares, N10; triangles, N20). Bars indicate standard errors of the mean. Both the vertical and the horizontal axis have a logarithmic scale

while the other interactions were not. The slope of the effects of plant density on plant weight became steeper with increasing N-supply (-0.52 , -0.55 , -0.59 , -0.61 in the N0, N5, N10, and N20 treatments, respectively). The slope for treatment N20 was significantly steeper than for the treatments N0 ($P = 0.001$) and N5 ($P = 0.024$) and that for treatment N10 differed from that for treatment N0 ($P = 0.014$).

Shoot to root ratios

Shoot to root ratios declined with increasing plant density ($P < 0.001$) and decreasing N-supply ($P < 0.001$; Fig. 2). The interaction between plant density and N-treatment ($P = 0.023$) shows that the effects of plant density on shoot to root ratios varied across different N-levels. Distinction between linear and higher order terms revealed a highly significant interaction between the linear terms ($P < 0.001$), while the interactions which included higher order terms were not significant. The slope for the N20 treatment was less steep than for the N0 ($P < 0.001$) and N5 treatment ($P = 0.004$). The difference between the slopes in the N10 and the N0 treatment was marginally significant ($P = 0.059$).

Including individual plant weight as a covariate showed that within each nutrient treatment this variable explained a large part of the variation in shoot to root ratio (Fig. 3). Shoot to root ratios declined with decreasing plant weight. Since there

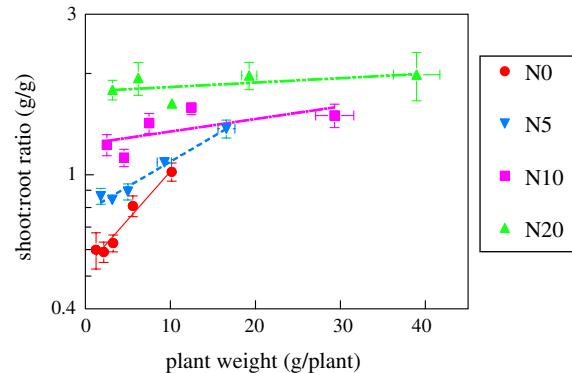


Fig. 3 The relation between shoot to root ratios and individual plant weight at four levels of nutrient supply (dots, N0; reverse triangles, N5; squares, N10; triangles, N20). Vertical and horizontal bars indicate standard errors of the means. The vertical axis has a logarithmic scale

was a highly significant interaction between the effects of N-supply and individual plant weight ($P < 0.001$), we compared the slopes of the regression lines (Fig. 3). These slopes decreased with increasing N supply. In the N0, N5, N10, and N20 treatments the slopes were 0.25, 0.21, 0.11, and 0.01, respectively. The slope for the N0 treatment differed significantly from the slope in the N10 and N20 treatments ($P = 0.041$ and $P = 0.001$, respectively) and the slope of the N5 treatment differed from that in the N20 treatment ($P = 0.003$). The interaction between plant weight and plant density was not significant.

Allometric relation between shoot and root mass

An analysis of the relationships between shoot and root weights produced similar results (Table 1). The interaction between the effects of root weight and N-treatments was highly significant, while the interaction with plant density was not. The relationships between shoot and root mass had declining slopes with increasing N-supply: 1.24, 1.21, 1.09, and 0.96 in the N0, N5, N10, and N20 treatments, respectively. The slope for the N20 treatment was significantly smaller than that for the N0 and N5 treatments ($P = 0.003$ and $P = 0.001$, respectively). In the N0, N5, and N10 treatments the slopes were significantly ($P < 0.05$) greater than 1, meaning that the shoot to root ratios declined with decreasing plant size, while in the N20 treatment the slope did not deviate from unity. The single factors nutrient treatment and plant density had significant effects indicating different intercepts both among nutrient treatments and plant densities.

Discussion

We found that at the lower nutrient supplies the proportional allocation to root mass increased with increasing competitive stress, while in the higher nutrient treatment the relationship between competitive stress and shoot to root ratio was neutral. The relation observed in the nutrient-poor treatments has earlier been found in field studies (Cahill 2003; Litton et al. 2003). These authors attributed the effects of competitive stress on the shoot to root ratio entirely to

Table 1 Effects of plant density (D), nutrient supply (N), and root mass (R_w) on shoot mass

	df	F	P
Intercept	1	82.73	<0.001
Density	4	20.24	<0.001
Nutrients	3	24.72	<0.001
Density * nutrients	12	1.59	0.114
Log(root weight)	1	1.59	0.114
Density * log(root weight)	4	0.49	0.773
Nutrients * log(root weight)	3	4.28	0.008

A General Linear Model was applied with plant density and nutrient supply as fixed factors and root mass as covariate (cf. Eq. 2). Blocks did not have significant effects

the ontogenetic relation between shoot and root mass (sensu Gedroc et al. 1996) rather than to adaptive phenotypic plasticity. In most experiments it is not possible to distinguish between the effects of plant size and the effects of competitive stress, since these two variables are strongly correlated. However, in our experiment, in the high N-supply treatment the variation in plant sizes was not only much greater, but also overlapped the variation in the three other treatments (cf. Fig. 3). Nevertheless, in the higher N treatment there was no relationship between individual plant mass and shoot to root ratio, while there was a strong relationship in the low N supply treatments. Also the allometric analysis of the relations between shoot mass and root mass (cf. Table 1) supports the conclusion that at the different nutrient supply levels there were different effects of competitive stress on the allocation to shoots and roots. The relationships between root and shoot mass were different both across the four nutrient treatments (intercepts and slopes) and five levels of competitive stress (intercepts; cf. Shipley and Meziane 2002). Apparently, the negative relation between plant density and shoot to root ratio in this experiment cannot be attributed to ontogenetic drift. However, the observed patterns can be explained very well by adaptive plasticity in accordance with the functional equilibrium hypothesis (Brouwer 1962a, b).

The intensity of competition was measured as the slope of the decline of plant weight with increasing density. There were small, but significant differences between these slopes among fertilization levels. At the highest nutrient supply level the slope of the relationship between plant density and plant weight was 17% steeper than in the treatment where no nutrients were applied. The higher intensity of competition at the higher nutrient supplies was probably due to the more rapid growth of the seedlings, so that the plants started sooner to interfere with each other. Although intra-specific competition in the high N treatment appeared to be even more intense than at the lower N-supplies, there was no response of allocation pattern to increased plant densities. The aboveground biomass that was present at the higher N supplies seemed to be sufficient for light competition to develop. At the higher plant densities, the harvested aboveground biomass was 236, 424, 706, and 1,044 g m⁻² in the inner pots with the N0, N5, N10, and N20 treatments, respectively,

so that the aboveground biomass in the highest N-supply treatment was clearly well beyond the level ($400\text{--}500 \text{ g/m}^2$) at which Grime (1979, 2001) predicted light competition would become the dominant process.

Twolan-Strutt and Keddy (1996) measured the effects of full and belowground competition in wetlands varying in standing crop and soil fertility. The belowground competition intensity did not differ between sites with low and high standing crops, but the estimated aboveground competition intensity was clearly higher at the high productive site. Peltzer et al. (1998) found no change in root competition intensity along a soil fertility gradient, but other studies found a decrease in root competition after fertilization (Cahill 1999). Many of these field studies suffer from great methodological difficulties. Root competition is often excluded by the insertion of tubes in the soil that not only exclude roots, but also change the volume of soil available for the target plants. In the treatments without tubes it is often extremely difficult to harvest all roots, since they are intermingled with the roots from the surrounding vegetation. In other studies, the intensity of aboveground competition is estimated on the basis of the difference between the effects of full and belowground competition, ignoring possible interactions.

In our experiment these pitfalls were avoided, but it should be emphasized that we measured the response of the whole population of plants in the pot. It is possible that there is correlated variation in plant size and allocation pattern among plant individuals within the pot with smaller plants showing a stronger response (cf. Fig. 3), but larger individuals contributing more to the response that we measured. Since large plants had probably experienced less competitive stress than smaller plants, we might have underestimated the change in allocation in response to increased competition. So, taking this possible pitfall into account, we can still only conclude that there are strong effects of competitive stress on the allocation of biomass to below- and aboveground organs.

The relationships between shoot to root ratios and plant weights show that at high levels of competitive stress these ratios vary markedly among the four N-supply levels (Fig. 3). At the low levels of competitive stress this variation is much smaller. It even appears that the four lines converge to a constant shoot to root ratio at lower plant densities, i.e., under

conditions where both nitrogen and light limitation are low.

We did not find a complete shift from a negative to a positive effect of plant density on the shoot to root ratio with increasing nutrient supply as we expected, but rather a shift from a negative to a neutral relationship. We suggest that there are two possible hypotheses that explain this pattern. The first explanation is that belowground competition is relevant both on nutrient-poor and nutrient-rich substrates, while the relevance of aboveground competition increases with increasing aboveground biomass. In the low N treatment only belowground competition plays its role, while in the high N treatment the effects of above- and belowground competition would more or less balance each other. This hypothesis would be in agreement with the finding of Twolan-Strutt and Keddy (1996), that total and aboveground competition were greater in wetlands with higher standing crop, while belowground competition intensity did not differ between sites. The second hypothesis is that the effects of nutrient deprival on root-shoot allocation are far more important than the effects of light competition, while we would expect such nutrient depletion effects to be stronger on poor substrates. Casper et al. (1998) did not find any effect of increased plant densities on shoot to root ratios, when they excluded root competition, while the plants grown at high densities exhibited typical aboveground responses to neighbors, such as higher ratios of stem to leaf biomass and greater specific leaf areas. Poorter and Nagel (2000) attempted to evaluate the adaptive value of changes in shoot to root ratio in response to changes in light and nutrient supply using a growth analysis, taking into account the effects of various parameters on the relative growth rate. They concluded that changes in allocation to roots had strong adaptive effects on plant growth, while the consequences of changes in allocation to aboveground biomass were relatively small as compared to the effects of changes in other parameters such as specific leaf area and rate of photosynthesis per unit leaf area. It is important that future experiments attempt to test these two alternative hypotheses so that we can conclude at last what plant features contribute to the competitive ability of plant individuals on fertile and less fertile substrates.

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Rock-colonizing plants: abundance of the endemic cactus *Mammillaria fraileana* related to rock type in the southern Sonoran Desert

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Abstract Establishment, colonization, and permanence of plants affect biogenic and physical processes leading to development of soil. Rockiness, temperature, and humidity are accepted explanations to the influence and the presence of rock-dwelling plants, but the relationship between mineral and chemical composition of rocks with plant abundance is unknown in some regions. This study documents plant species growing on rocks, their capacity as rock colonizers measured by the Importance Index, and the relationships between the chemical composition of rocks and the abundance of the dominant plant. The community is composed of eight species and is dominated by the small cactus *Mammillaria fraileana*. Sites with low abundance of this species contain volcanic breccias, high amounts Ca, Fe, Mg, Ti, Al,

and Mn as part of moderately weatherable minerals, such as plagioclase and pyroxene. Sites with higher abundance contain rhyodacite, rhyolite, and andesite rocks rich in more weatherable minerals, such as volcanic glass and minerals containing Si, K, and Na. K and Na were present in equal proportions only at the site with more plants. Since Na is toxic for most plants, an experiment was carried out to assess its effect on the survival of *M. fraileana* seedlings. Decreased survival occurred as the concentration of Na increased. Even in the treatment without Na, survival decreased slightly. In summary, presence and abundance of plants is related to the type of bedrock, their weathering characteristics, and proportion of elements. The interactions among elements, rather than the isolated effect of specific elements, could be the most reliable explanation for local variations in the abundance and dominance of *Mammillaria fraileana* in rocky habitat in the southern Sonoran Desert.

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Rock weathering · Saxicolous desert plants

Introduction

Rocky environments constitute a suitable location for study of ecological phenomena, such as colonization (Ryti 1984), plant succession (Martinez 1999), and rock weathering (Adams et al. 1992; Puente et al. 2004a). Colonization by plants can occur on primary

sites (newly created) or secondary sites (previously vegetated) and participate in rock weathering through the establishment and persistence of species (Glenn-Lewin et al. 1992). Rock and mineral weathering by physical and biochemical processes eventually lead to formation of soils (Barber 1995). Biological weathering is the result of metabolic activity of bacteria, cyanobacteria, fungi, lichens, and vascular plants releasing organic acids and chelating compounds that completely or selectively extract mineral components from rocks and exploit them for their own use (Hinsinger 1998; Belnap and Lange 2003; Schulze et al. 2005). Plant roots enhance the rate of soil formation as a result of changes in the physico-chemical environment of the rhizosphere, for example, by increasing substrate porosity (Gibbs and Reid 1988) and rhizosphere acidity, the latter by production of root exudates and protons that modify the nutrient concentration and availability of nutritional elements for plants and microorganisms (Hinsinger 1998; Jones 1998; Carrillo et al. 2002; Akter and Akagi 2005).

Saxicolous plants (plants inhabiting rocks) occur in many ecosystems, temperate (Franklin and Dyrness 1973), semi-arid (Zwieniecki and Newton 1995), and very arid (Bashan et al. 2002) environments. Saxicolous plant communities share similar limiting environmental conditions, such as shallow soil or bare rocks, low soil moisture and nutrients, and exposure on slopes (Martinez 1999; Nobel and Zutta 2007).

In hot, dry deserts, colonization of rocks by plants is an additional challenge that requires adaptations to this harsh environment. Even so, perennials, succulents, cacti, and shrubs are common on rocky substrates in the Sonoran Desert of northwestern Mexico and southwestern USA (Turner et al. 1995; Nobel and Loik 1999; Anderson 2001; Chadwick and Steinmetz 2006; Nobel and Zutta 2007). Rock-colonizing plants become established on bare rock, that is, without the benefit of soil (Bashan et al. 2002, 2006). Apparently, the key to their outstanding performance in harsh environments lies in their association with microorganisms (Puente et al. 2004a, b; Bashan et al. 2007). However, the relationship between environmental factors, such as specific plants colonizing rocks and the composition of the rocks are almost unknown.

This study assessed the capabilities of the small cylindrical cactus *Mammillaria fraileana* (Britt. &

Rose) Boedecker, which is endemic in the State of Baja California Sur, Mexico, to colonize rocky areas within the desert. This cactus is common in rocky habitats and many individuals grow in fissures or directly on the rock surface (Wiggins 1980; Bashan et al. 2002). The hypotheses of this study were that rock-dwelling cacti, even small species, are major colonizers of rocks in the desert and that rocks are a suitable habitat for pioneering desert plants. This study attempted to (1) determine the importance of the population of *M. fraileana* compared to other plant species growing on representative rocky habitats and (2) evaluate whether abundance of *M. fraileana* can be explained by the composition and weatherability of the rocks in its habitat. This was done by combining quantitative field surveys, chemical and mineralogical analyses, a plant growth experiment, and comprehensive statistical analyses of the data from these sources. This is the first study specifically intended to understand patterns of abundance of cacti and environmental factors related to rock composition.

Materials and methods

Plant species

Mammillaria fraileana (Britt. & Rose 1923) Boedecker (common local name ‘viejito’ or ‘small old man’) is an endemic cactus from Baja California Sur, Mexico. Its main habitat is rocky hillsides and desert islands, where it is found in large populations that dominate the habitat, despite the small size of individual plants. Its range is about 250 km along the east coast of the Baja California Peninsula from Isla Catalina (25°36' N, 110°48' W) southward to the city of La Paz (Wiggins 1980). *M. fraileana* grows in clusters, 10–15 cm high, 3 cm average diameter with narrow cylindrical stems, pink flowers, red fruit, and small black seeds (Anderson 2001). The biology of this species has not been studied (JL Leon de la Luz, pers. comm.). Field observations suggest similarities to other *Mammillaria* that it strongly resembles phenologically, that is, the flowering periods are associated with the oncoming rainy season and fruit maturation often occurs inward toward the stem, between the tubercles (Bravo-Hollis and Sanchez-Mejorada 1991; Zavala-Hurtado and Valverde 2003).

Study area

We studied three sites about 2 km north of the city of La Paz, Baja California Sur, Mexico ($24^{\circ}11'15''$ N, $110^{\circ}17'50''$ W) (Fig. 1). Sampling sites A, B, and C were located on adjacent hills landward of the first ridge of hills bordering the coastal plain. The vegetation of the study area is sarcocaulous or desert scrub vegetation. The overstory is dominated by several shrubs, *Jatropha cuneata* Wigg. & Rollis, *Fouquieria burragei* Rose, *Bursera microphylla* A. Gray, *Bursera epinnata* (Rose) Engler, *Agave sobria* Brandegee, *Aeschynomene vigil* Brandegee, and the treelike cactus *Pachycereus pringlei* (S. Wats.) Britt. & Rose. The lower story is composed primarily of the small cacti, *Mammillaria fraileana* (Britt. & Rose) Boedeker, *Echinocereus brandegeei* (J.M. Coulter) K. Schumann. Among herbaceous plants, only *Euphorbia leucophylla* Benth. is common (JL Leon de la Luz, pers. obs.).

The climate is subtropical, mostly hot and dry. The multi-year average rainfall is 180 mm ranging from 35 mm in dry years to 424 mm in the mountainous areas. The rainfall is usually associated with Eastern Pacific hurricanes and tropical storms that occur from

August through October. Winter rain is likely, but uncommon, and is usually about 10% of annual precipitation. Data from the CIBNOR meteorological station near La Paz indicates that average monthly temperature ranges from 14.8°C in January to 32.4°C in the three summer months, with several hours each day reaching $38\text{--}42^{\circ}\text{C}$. The 6-year average insolation during the hottest period of the day may reach $2,300\text{--}2,500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for several hours.

The lithology is interbedded sandstones and conglomerates derived from rhyolite-based ash-flow and tuff and andesite-based lahars and lava flows. The soils are immature, brown to gray in color, composed of fragmental debris (Hausback 1984).

Field methods

This area was chosen because the hillsides display perceptible differences in rock substrate, different plant densities occur on the rocks, and the vegetation seems undisturbed.

To avoid effects of coastal salt spray, prevailing high winds, and variable humidity in an east-west direction, only south-facing slopes were chosen. The sample plots at each site covered about $1,000 \text{ m}^2$ at

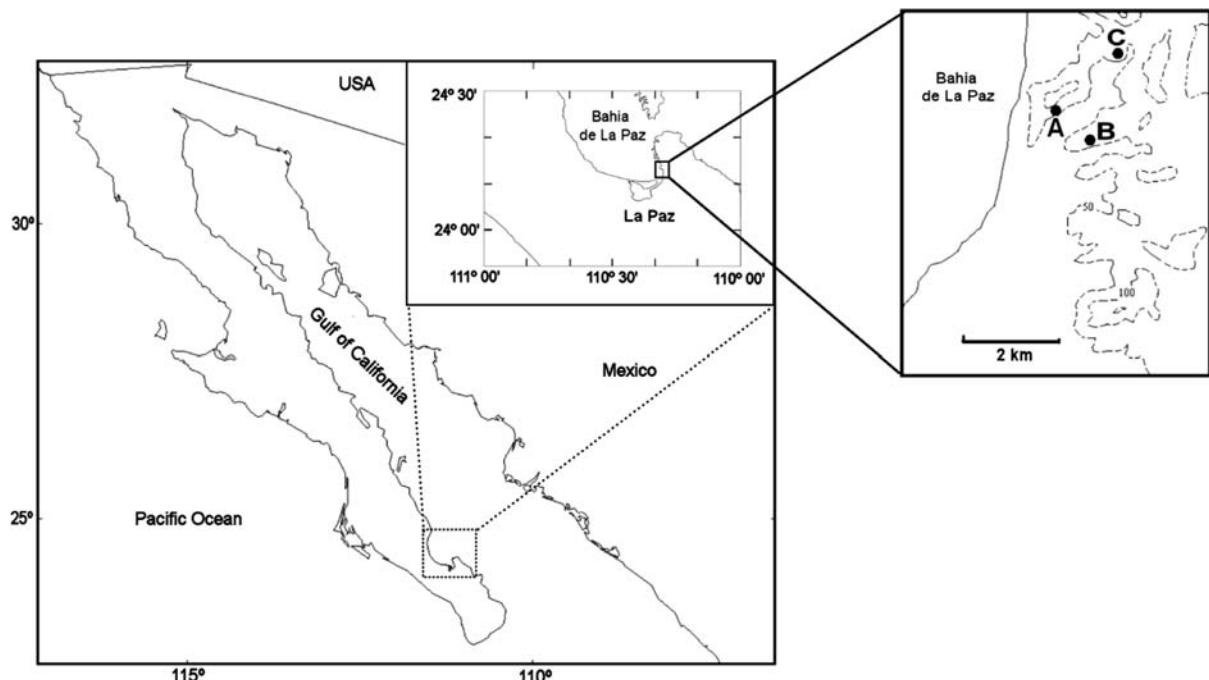


Fig. 1 Location of the study area and sampling Sites A, B, and C on three hillsides. Contours represent 50 and 100 m

an elevation between 50 and 100 m. At Sites A and B, the surface of the sample plots were boulder-sized rocks. At Site C, the surface of the sample plots was massive sedimentary rock consisting of angular or sub-angular breccias larger than 2 mm. Each site consists of 10 sample plots ranging from 0.1 to 19 m².

Measurement of plant abundance and volume

To measure plant species abundance in each sample unit, the volume of individual plants and the frequency of occurrence were calculated. Volume is a measurement calculated from the plant cover and its height. Plant species, their height, number of stems, and stem diameter were recorded for each individual growing on the sampled rocks. Most desert plants located in the sampled area did not have leaves at the time of sampling. Volume is usually used to reflect the size of the plant and is an essential growth parameter for cacti growth related in general to survival in the wild (Gibson and Nobel 1986; Bashan et al. 1999). For woody and herbaceous species that were measured, the volume denotes stems and branches without leaves; for cacti, this attribute indicates stem volume; and for agaves, this indicates the volume of the fleshy leaves. The volume of cacti stems were calculated from the volume equation for a cylinder; the volume of each agave leaf was calculated by estimating a pyramid using the height from the base to the terminal spine, the width at the base, and the thickness of the leaf, expressed as cm³ of plant per m² of rock.

Identification of rock types

One composite sample was collected from each rock; some fragments were taken from the vicinity of the plants but without any fragments smaller than cobble size. Representative samples were identified by J. Hirales at the Department of Geology of the Universidad Autonoma de Baja California Sur, La Paz, B.C.S., Mexico.

Mineralogical analysis

Because most of the rock fragments available for this analysis was gravel, the relative composition of the minerals were analyzed with a microscopic thin section method used in soils and sediment analyses (Murphy 1986). Analysis was performed at the

GeoAnalytical Laboratory at Washington State University, Pullman, WA. In this case, the gravels were glued with a special epoxy resin (Epon 815C, Hexion Specialty Chemicals, Columbus, OH) and ten parts of triethylenetetramine (Sigma-Aldrich, #13,209-8) on a round, 5-cm diameter aluminum tray and dried for two days at room temperature. The flat-bottom side of the solidified samples was polished into a thin section by standard methods. The minerals were identified and counted under a petrography microscope.

Analysis of elements in rock minerals

The samples were analyzed at the GeoAnalytical Laboratory of the Washington State University for common and trace element abundances with X-ray fluorescence spectrometer (XRF; ThermoARL Advant'XP+ sequential X-ray, Thermo Fisher Scientific, Lausanne, Switzerland), following the procedure of Johnson et al. (1999). This low-dilution fusion method is reliable and robust with high analytical precision (Johnson et al. 1999). During XRF analyses, elements in the unknown samples are measured by comparing the X-ray intensity of each element with the intensity of United States Geological Survey standard samples and pure vein quartz as blanks for all elements except Si. The elemental concentrations are expressed as wt%, volatile-free, with iron expressed as FeO.

Effect of NaCl on survival of *Mammillaria fraileana* seedlings

Since sodium was abundant at all the sites, and it is frequently referred as toxic for plants, we evaluated the effect of Na on the survival of *M. fraileana*. Using concentrations of Na similar to those found at the study sites, batches of about 100 seeds were rinsed with de-ionized water and then placed in plastic cups (5 cm diameter × 3 cm height) containing 6 g perlite (Supreme Perlite, Portland, OR) and 4 g of 0.2-mm diameter white quartz sand. The medium was kept at saturation by adding 9 ml de-ionized water until 80% germination occurred (~5 days). Four salinities were tested (0, 1.5%, 2.5%, 3% wt/v of analytical grade NaCl) in nine replicates, where each replicate consisted of 50 seedlings (450 seedlings per treatment). Young, 20-day-old seedlings were watered with 10 ml of the corresponding salt treatment and later

watered daily with 4 ml of de-ionized water to keep saturation level constant. The experiment was conducted in an incubator (model 815, Precision Scientific, Chicago, IL) under the conditions considered optimal in previous experiments: $30 \pm 1^\circ\text{C}$ and $31 \pm 2 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ for an 8-h light period during a 24-h cycle. Survival of seedlings was recorded weekly for 1 month.

Statistical analysis

Statistical analyses were related to the type of experiment and the measured parameters.

(1) *Importance of rock-colonizing plants:* The importance of rock-colonizing plants was evaluated with the Importance Value Index (IVI). This index indicates the contribution of a species within the community (Barbour et al. 1999); in this case, to the plant community growing only on the rocks. The IVI is calculated by adding the separate values of relative density, relative frequency, and relative dominance of each species reaching maximum values of 300 or 200 (expressed without units) (Mueller-Dombois and Ellenberg 1974), depending on the components used for its calculation. In this study, we calculated the IVI as the sum of the relative volume (as the parameter of relative dominance) plus relative frequency, so that the maximum possible value is 200. Relative frequency was the frequency of one species as a percentage of total number of plants at a site (Barbour et al. 1999). Plant density was estimated, rather than measured, because of the difficulty of precisely counting individual, caespitose cacti (arranged in clusters). Therefore, plant density is used as a complementary value and not for statistical comparisons.

(2) *Spatial variations in abundance of *M. fraileana*:* The volume of *M. fraileana* plants was not normally distributed. The differences between sites were analyzed first by Kruskal-Wallis ANOVA by rank and then by Dunn's test to determine which sites have significant differences. Based on the number of groups and their size, Dunn's test compared the sum of ranks between two groups with the expected average difference (Zar 1999).

- (3) *Spatial variation of elements at the study sites:* Principal Component Analysis (PCA) was used to explore spatial variability based on the correlation matrix of concentration of the major elements determined by X-ray fluorescence. The PCA multivariate analyses were performed with Multivariate Statistical Package (MVSP 3.1; Kovach 1998). Standardization and centering of the matrix seemed appropriate to perform the PCA eigenanalysis to reduce extreme variation among variables (Kovach 1998). The measure of the relative importance (load) for each element on the extracted PCA axes was given by the PCA loadings. To detect differences among sites, elements with higher PCA loadings were compared with one-way ANOVA and Tukey's HSD test at $P < 0.05$ with statistical software (JMP v. 5.1.2; SAS Institute 1989).
- (4) *Correlation of abundance of plants and chemical composition:* The chemical elements that contributed to differences among sites were tested to seek statistical correlation between the concentrations of chemical elements and plant volume. The correlation matrix was constructed with standardized data of the concentration of major and trace elements and \ln of plant volume.
- (5) *Effect of NaCl on survival of *M. fraileana* seedlings:* Survival was calculated as the proportion of the starting number of cacti surviving to the next week (Barbour et al. 1999). Survival of seedlings under different concentrations of NaCl was tested by one-way ANOVA and Tukey's HSD at $P \leq 0.05$ with statistical software (JMP v. 5.1.2; SAS Institute 1989).

Results

Relative importance of rock-colonizing plants

Species richness of saxicolous plants at the three sites was low. Of eight species identified, five were succulents (four cacti, *Mammillaria fraileana*, *M. brandegeei*, *Echinocereus brandegeei*, *Pachycereus pringlei*, and one agave *Agave* sp.); two were small herbaceous plants *Euphorbia leucophylla* and *Coleosanthus* sp., and one was a woody species (*Fouquieria burragei*). The eight species are common in the surrounding area. The relative importance of the

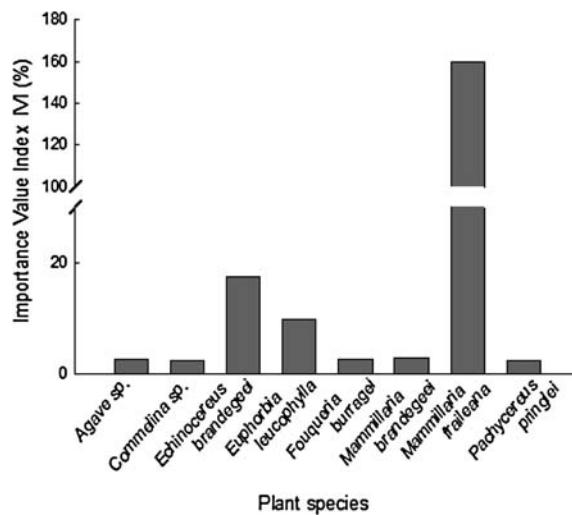


Fig. 2 Importance Value Index (IVI) for the plants growing among 30 rocks. IVI is an additive value calculated as the sum of ‘relative dominance’ plus ‘relative frequency,’ so that the maximum possible value is 200

species at each site was measured by the IVI index as the sum of two parameters: relative volume of plants and relative frequency. Each parameter can reach a maximum of 100% (Fig. 2). Therefore, the maximum value for the IVI of one species would be 200 (value without units) in strongly dominated communities.

The IVI for *M. fraileana* was 159.70; the second species was the cactus *Echinocactus brandegeei* with 17.46, which was present only at site A. About 63% of the eight species were found only once growing on rocks (*Fouquieria burragei* IVI = 2.64, *Agave* sp. IVI = 2.57, and *Pachycereus pringlei* IVI = 2.48), even though they were abundant in the surrounding vegetation (Fig. 3a). Only *M. fraileana* grew on 100% of the sampled rocks; therefore, this species was considered dominant (Fig. 3a, b). In general, the population of *M. fraileana* looked healthy, ranging in size from seedlings of 5 mm in height to adults up to 15 cm high and 5 cm across. Plants usually grow in cracks or fissures that are deeply penetrated by the root system (Fig. 3c). Seedlings (<5 mm high) were more frequently found in deep cracks, protected from direct solar radiation and reflected light.

Spatial variations in abundance of *M. fraileana*

The plant volume of this species varied among sites. The maximum volume occurred at Site B ($744.3 \text{ cm}^3 \text{ m}^{-2}$) and the minimum ($5.6 \text{ cm}^3 \text{ m}^{-2}$)

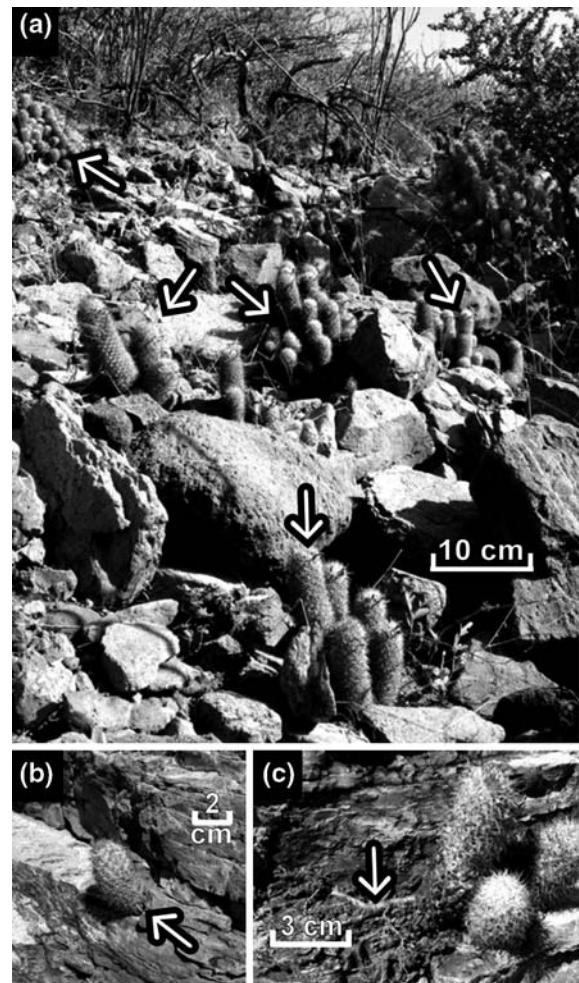


Fig. 3 **a** Plant community at Site A in the study area (arrows indicate clusters of *Mammillaria fraileana*), **b** *M. fraileana* growing on andesite, **c** *M. fraileana* with roots exposed after removing rock fragments surrounding the plant

at Site C. Even though volume was not normally distributed, for a better understanding, average of plant volume and its corresponding plant density were calculated for each sampled plot. In ascending degree, Site C had $19.0 \text{ cm}^3 \text{ m}^{-2}$ ($\sim 0.38 \text{ plants m}^{-2}$), Site A had $110.7 \text{ cm}^3 \text{ m}^{-2}$ ($\sim 4.17 \text{ plants m}^{-2}$), and Site B had $350.3 \text{ cm}^3 \text{ m}^{-2}$ ($\sim 9.91 \text{ plants m}^{-2}$). Kruskal-Wallis ANOVA by ranks, showed significant differences in the volume of *M. fraileana* between sites ($H = 18.56$, $P \leq 0.0001$). Dunn’s Test showed significant differences between sites A and C ($Q = 2.936$; $P \leq 0.05$), and C and B ($Q = 4.165$; $P \leq 0.05$), but no differences were found between sites A and B ($Q = 1.397$;

Table 1 Dunn's nonparametric test for multiple comparisons of volume of *Mammillaria fraileana* at the three sites at $P \leq 0.05$

Site comparisons	Ranks difference	Standard error	Q	$Q_{0.05,3}$	Conclusion concerning hypothesis ^a
B vs. A	5.45	3.902	1.397	2.394	Accept
B vs. C	16.25	3.902	4.165	2.394	Reject
C vs. A	10.8	3.679	2.936	2.394	Reject
Sites grouping					
A	a				
B	a				
C	b				

$Q_{0.05,3}$ = one-tailed hypotheses, 3 df

^a Hypothesis that volume of *Mammillaria fraileana* is the same at Sites B and A

$P \leq 0.05$). Therefore, the A–B group had the highest volume and the C group had the lowest plant volume per m² (Table 1).

Lithology and mineralogy

The rocks from the three sites were part of the Comondu Formation (Hausback 1984). Sites A and B (Fig. 4a, b) are volcanic rocks called Providencia rhyodacites (gray flow-laminated lava), ranging in composition from rhyodacite to rhyolite and andesite containing horizontal fissures and vertical fractures. Site C is composed of rhyolite breccias that are more massive-like and with much fewer crevices and fractures (Fig. 4c). In our field observations, cracks, which are numerous at Sites A and B, provide a firm anchor for the roots that grow in intimate association with the parallel surfaces of fissures or penetrate deeply into rock fractures of the solidified andesite breccia at Site C. Data on fracture density is not available.

Microscopically, the ratio of plagioclase, pyroxene, and volcanic glass were identified and characterized. The mineralogy at Sites A and B was significantly different from Site C. Sites A and B had less plagioclase (general formula (Na, Ca)[Al₂Si₂O₈]), pyroxene (general formula (Ca, Mg, Fe, Al, Na)₂[Al, Si]₂O₆) and a high percentage of volcanic glass (combination of several minerals). Site C had the least volcanic glass and the highest percentage of plagioclase and pyroxene (Table 2). Rocks at the three sites contained felsic minerals, that is, high amounts of silicon, oxygen, aluminum, sodium, and potassium. Site C had a distinctly more mafic composition, that is, enriched with magnesium and

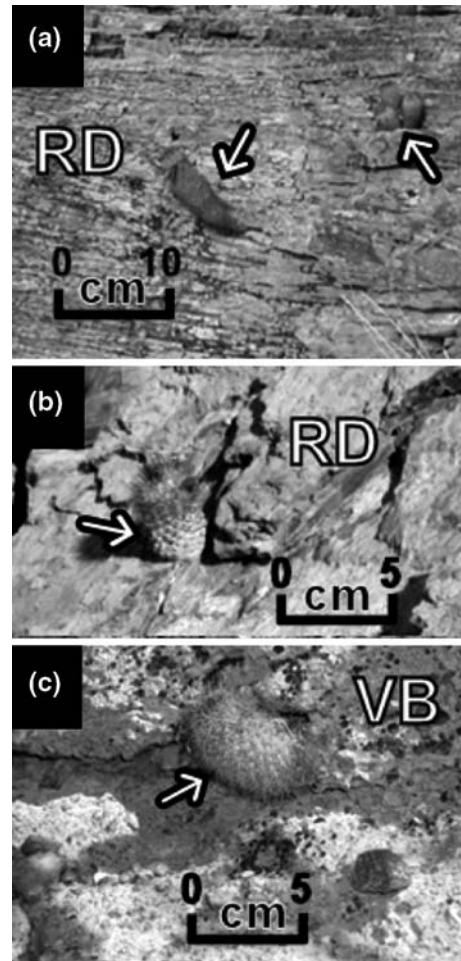


Fig. 4 Typical volcanic rocks in the study sites. Arrows indicate *Mammillaria fraileana*. **a** Site A composed of Providencia rhyodacite. **b** Site B composed of Providencia rhyodacite. **c** Site C composed largely of andesitic breccia. RD = Providencia rhyodacite, VB = Volcanic breccias

Table 2 Mineral composition of rocks at the three study sites

Minerals	Site			
		A	B	C
Volcanic glass ^a		~96%	~90%	~60%
Plagioclase	1–2%	5–8%	25–40%	
Pyroxene	1–2%	2–5%	~5%	
Other	Hornblende	<1% magnetite, trace of calcite, weathered hornblende	~2% magnetite, 2% weathered olivine	

Source: GeoAnalytical Laboratory, Washington State University, Pullman, WA, USA

^a Combination of several minerals, mostly related to rhyolite, dacite, and possibly andesite

iron and less silicon, yet still somewhat felsic. In general, the study area had very low phosphorus, while sulfur was close to the limit of detection and chlorine was insignificant (Table 3).

Spatial variation of elements at the study sites

PCA analysis of elements showed that along the first variation axis PCA 1 (70.66% of total variance), there is a clear separation of conditions at Site C (Number 1 on the left side in Fig. 5a) from conditions at Site A and Site B (on the right side in Fig. 5a). Although the second variation axis PCA 2 (10.35% of total variance) seems to separate Site A and Site B, its explained variance is much lower than the variance explained by PCA 1, such that the distance is not comparable to the distance from Site C to Site A–Site B. Consequently, Site A and Site B were considered a single group. Site C is predominantly related to the abundance of the major elements Ca, Fe, Mg, Ti, Al, and Mn (PCA loadings –0.209, –0.209, –0.208, –0.207, –0.206, and –0.202, respectively); Site C was also partly explained by the trace elements Sc, V, Sr, Cr, and Ni (PCA loadings –0.208, –0.204, –0.201, –0.189, and –0.174, respectively). Site A and Site B were primarily associated with high concentrations of three major elements Si, K, and Na (PCA loadings 0.209, 0.208, and 0.187, respectively), and the trace elements Zr, La, Pb, Rb, Ce, Nd, U, Nb, and Th (PCA loadings 0.197, 0.193, 0.190, 0.188, 0.187, 0.181, 0.171, 0.165, and 0.160, respectively).

Table 3 Elemental content of rocks at the three study sites based on X-ray fluorescence analyses

Element	Site			
		A	B	C
Major elements (mg element g rock ^{–1} ± SE)				
Al	137.6 ± 0.7	135.1 ± 3.1	180.8 ± 1.9	
Ca	9.1 ± 0.2	10.7 ± 1.0	69.8 ± 1.4	
Fe	25.3 ± 0.2	23.8 ± 0.8	70.4 ± 1.1	
K	45.5 ± 0.2	48.2 ± 1.0	14.3 ± 0.5	
Mg	1.9 ± 0.1	2.7 ± 0.3	30.5 ± 0.9	
Mn	0.5 ± 0.0	0.5 ± 0.0	1.3 ± 0.0	
Na	37.6 ± 0.5	36.9 ± 0.7	29.4 ± 0.5	
P	0.9 ± 0.1	1.6 ± 0.3	2.3 ± 0.2	
S	0.1 ± 0.0	0.3 ± 0.2	0.4 ± 0.2	
Si	704.6 ± 1.2	707.4 ± 5.7	566.1 ± 4.1	
Ti	4.5 ± 0.0	3.8 ± 0.3	9.0 ± 0.2	
Trace elements (mg kg-rock ^{–1})				
As	16.0 ± 1.0	16.2 ± 3.4	3.0 ± 1.0	
Ba	1700.7 ± 67.6	1330.4 ± 158.1	946.6 ± 138.8	
Ce	94.8 ± 3.0	126.2 ± 9.3	44.9 ± 1.7	
Cr	2.6 ± 0.3	4.0 ± 0.6	17.7 ± 1.8	
Cs	4.3 ± 0.4	10.1 ± 1.1	5.4 ± 0.6	
Cu	5.8 ± 0.3	8.8 ± 1.2	12.1 ± 0.8	
Ga	21.2 ± 0.2	22.8 ± 1.0	26.0 ± 0.4	
La	45.9 ± 1.3	59.7 ± 3.3	20.9 ± 0.9	
Nb	23.5 ± 0.3	34.6 ± 5.5	7.6 ± 0.3	
Nd	44.1 ± 1.5	53.9 ± 4.5	23.1 ± 1.2	
Ni	2.5 ± 0.4	4.4 ± 0.4	9.1 ± 0.7	
Pb	24.4 ± 1.6	21.7 ± 0.8	8.2 ± 0.5	
Rb	176.8 ± 0.8	238.2 ± 18.6	59.2 ± 3.5	
Sc	12.4 ± 0.2	9.4 ± 0.8	31.1 ± 2.8	
Sr	181.7 ± 3.4	210.6 ± 23.9	472.7 ± 9.8	
Th	17.5 ± 0.3	31.2 ± 4.2	4.3 ± 0.3	
U	5.4 ± 0.3	7.6 ± 1.0	1.2 ± 0.4	
V	28.5 ± 1.2	49.7 ± 5.3	234.4 ± 9.5	
Y	47.0 ± 1.8	53.8 ± 5.9	35.0 ± 1.3	
Zn	72.0 ± 1.8	76.4 ± 8.4	108.9 ± 1.5	
Zr	493.6 ± 2.9	534.9 ± 44.9	172.1 ± 3.2	

Data were calculated by converting wt% to wt/wt units

Source: GeoAnalytical Laboratory, Washington State University, Pullman, WA, USA

From PCA, all elements associated with the described pattern were analyzed first by ANOVA and then by Tukey's HSD test to find the most important variables related to the ordination pattern

and to confirm differences between Site C and Sites A-B. Significant differences ($P < 0.001$, 2/26 df) between sites were found for all the elements related to the ordination pattern. Tukey's HSD test at $P < 0.05$ showed similarities between Sites A and B for seven of the major elements: Si, Al, Fe, Mn, Ca, Mg, and Na (Figs. 6a–f, 7a).

Essential nutrients for plant growth, such as K and P, were significantly different among sites but there was no consistent grouping of sites (Fig. 7b, c). Nevertheless, when the cumulative amount of K and P was calculated, significant differences between sites and groupings were displayed ($F = 684.86$, $P < 0.0001$, 2/26 df), which indicates higher cumulative K + P at Sites A and B (Fig. 7e). In general, significant differences ($F = 1475.22$, $P < 0.0001$, 2/26 df) occurred when the combination of Fe + Mn + Ca + Mg was higher at Site C and lower at Sites A and B (Fig. 7f). Titanium showed significant differences between sites and was higher at Site C, which had fewer plants.

Regarding noxious trace elements (Taiz and Zeiger 2006), Zn was lower at Sites A and B ($F = 18.65$, $P < 0.0001$, 2/26 df) and higher at Site C ($F = 14.58$, $P < 0.0001$, 2/26 df), but Cu and Ni did not show a pattern ($F = 14.67$, $P < 0.0001$, 2/26 df, and $F = 40.47$, $P < 0.0001$, 2/26 df, respectively). When the combination of Al + Zn + Cu + Ni was compared between sites, significant differences occurred ($F = 182.24$, $P < 0.0001$, 2/26 df). Sites A and B had less of these elements (Fig. 7g). Other significant findings involved the ratio Na:K, which was lower at Sites A and B (Na:K = 1:1), meaning that Na and K were present in equal amounts at these two sites. Site C was depleted in K and the ratio of Na:K was about 2:1.

Correlations between abundance of *M. fraileana* and chemical composition of rocks

About 70% of the major elements were strongly correlated to the abundance of *M. fraileana* and 40% of the trace elements showed strong and mostly positive correlations. Positive significant correlations ($P < 0.001$) were found only for three major elements: K, Si, Na, and seven trace elements, Nd, La, Ce, Zr, Nb, Y, and Rb. Negative correlations were found for Al, Ca, Fe, Mn, Mg, and Ti (Table 4).

Table 4 Correlation coefficient between abundance of *Mammillaria fraileana* and concentration of elements in rocks at the three sites

Major elements		Trace elements			
Al	-0.74	As	0.42	Pb	0.56
Ca	-0.67	Ba	0.52	Rb	0.60
Fe	-0.65	Ce	0.75	Sc	-0.69
K	0.68	Cr	0.15	Sr	-0.45
Mg	-0.60	Cs	0.25	Th	0.49
Mn	-0.63	Cu	-0.02	U	0.53
Na	0.62	Ga	-0.31	V	-0.63
P	-0.25	La	0.76	Y	0.61
S	-0.16	Nb	0.64	Zn	-0.32
Si	0.66	Nd	0.78	Zr	0.72
Ti	-0.59	Ni	0.14		

Bold letters indicate significant correlations ($P < 0.001$)

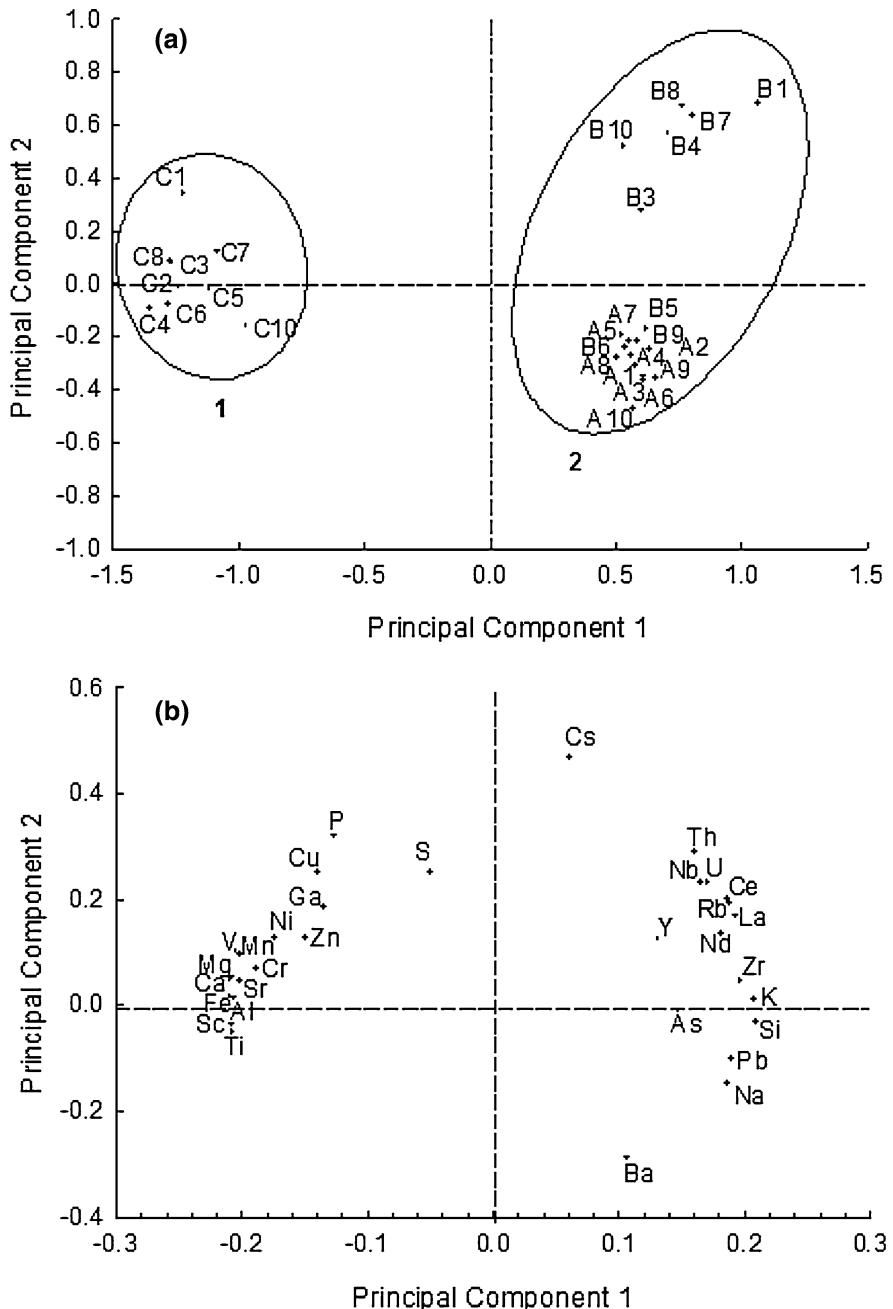
Effect of NaCl on survival of *M. fraileana* seedlings

With time, survival rate of seedlings decreased in all treatments; control seedlings, watered only with deionized water, showed some ‘natural’ mortality. Survival rates declined with increasing NaCl concentrations (Fig. 8a). Statistical differences among treatments ($P < 0.0001$, $F = 140.01$) and Tukey's HSD comparison ($P < 0.05$) indicated that the effect on survival at the end of the experiment is similar at a 1.5% and 2.5% NaCl solution (survival = $19.77\% \pm 2.32$ and $19.3\% \pm 2.73$), but very different from seedling growing at 0% NaCl solution (survival $79.5\% \pm 4.11$) and 3.0% NaCl solution $5.55\% \pm 1.19$) (Fig. 8b).

Discussion

Plants colonizing barren desert rocks have a significant ecological advantage over species incapable of handling extreme substrate conditions (Bashan et al. 2002, 2006). In this study, the saxicolous community contains only eight plant species, mostly cacti *M. fraileana*, *E. brandegeei*, *M. brandegeei*, and *P. pringlei*. This result agrees with other studies describing cacti often growing on rocky substrates in Mexico (Valverde et al. 2004; Bashan et al. 2002). Particularly in our study, these cacti were observed growing in nearby vegetation in soil patches among

Fig. 5 Principal Components plots. **a** Ordination of sites. Large ovals represent Groups 1 and 2, A1 to A10, B1 to B10, or C1 to C10 indicate sampling units (●) at Sites A, B, or C, respectively. **b** Ordination of elements based on loading values



the rocks, but despite their abundance in rocky soil, colonization of barren rocks was almost exclusively *M. fraileana*. Taken together, a restricted number of species and high dominance of one species represent a plant community of low diversity (Crawley 1986). This usually occurs in stressful environments; in this case, the rocky habitat represents a limiting habitat characterized by extremely high temperatures, high

insolation, low availability of water, and a highly impervious substrate for roots, limited soil volume and scarce nutrients (Nagy and Proctor 1997; Bashan et al. 2002, 2006).

For desert plants inhabiting rocky soils, plant development is related to physical factors, such as reflected light, rock hardness, rock and sediment porosity, water-holding capacity of the substratum

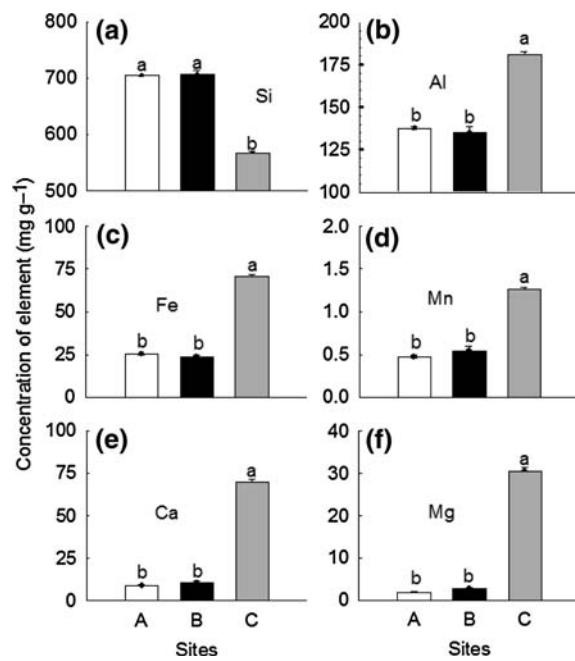


Fig. 6 Tukey's HSD test for concentration of elements at Sites A, B, and C with comparisons for: **a** Silicon, **b** aluminum, **c** iron, **d** manganese, **e** calcium, and **f** magnesium. Columns denoted with a different lower case letter differ significantly at $P < 0.05$. Whisker bars indicate standard error (SE). Absence of a bar indicates negligible SE

(Martre et al. 2002; Nobel and Zutta 2007). However, very little is known about physical factors controlling the abundance of rock colonizers. In our study, *M. fraileana*, like other saxicolous plants, exploit cracks and crevices as do other cacti and arid-zone plants (Zwieniecki and Newton 1995; Bashan et al. 2002). In fact, the numerous fissures and crevices in rhyodacites (Hausback 1984) were related to the abundance of *M. fraileana* at Site A and Site B, although it was not statistically demonstrated. Fissure density, fracture depth, and other microenvironmental conditions will be important to evaluate in further studies. We observed that cracks provide microsites that are safe against herbivores and protected from extreme environmental conditions, such as direct radiation.

In addition to physical factors, it is known that the presence and concentration of specific minerals (Nobel and Zutta 2007), the rate of weathering of rock minerals to clay minerals and salts (Nagy and Proctor 1997), and availability of plant nutrients may be crucial factors affecting rock colonizers. We hypothesized that

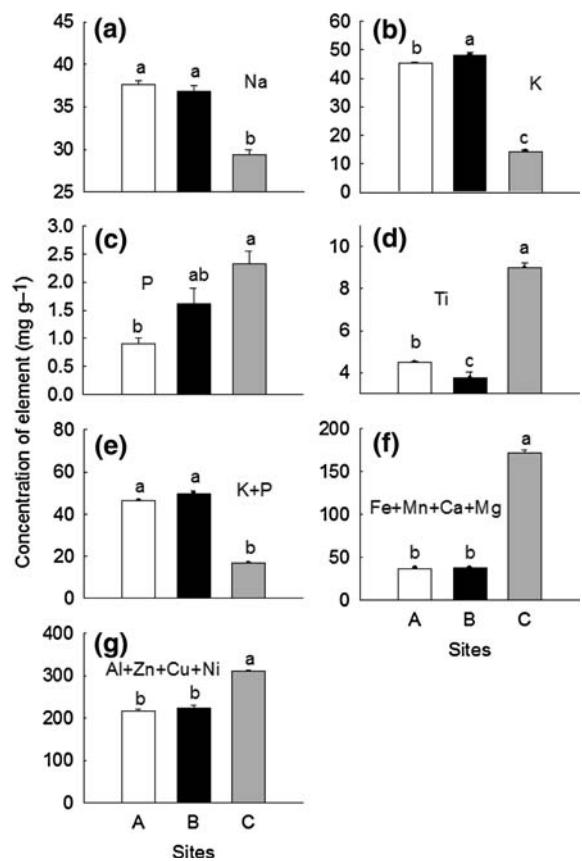


Fig. 7 Tukey's HSD test for concentration of elements at Sites A, B, and C with comparisons for **a** Sodium, **b** Potassium, **c** Phosphorous, **d** Titanium, **e** cumulative amount of macronutrients potassium + phosphorous, **f** cumulative amount of iron + manganese + calcium + magnesium, and **g** cumulative amount of toxic elements: aluminum + zinc + copper + nickel. Columns denoted with a different lower case letter differ significantly at $P < 0.05$. Whisker bars indicate standard error (SE). Absence of a bar indicates negligible SE

such factors, specifically mineralogy and the chemical composition of the rocks, contribute to the ecological success of *M. fraileana* and possibly control its local distribution.

The mineral analysis of the rocks in our study area was similar to earlier reports of these rock types (Hausback 1984), generally, andesite and rhyolite outcrops and breccias. We found higher abundance and volume of *M. fraileana* at Sites A and B associated with larger amounts of volcanic glass. This mineral is more susceptible to weathering according to the following scale; volcanic glass > plagioclase > pyroxene (Colman 1982). Site C, with the smallest

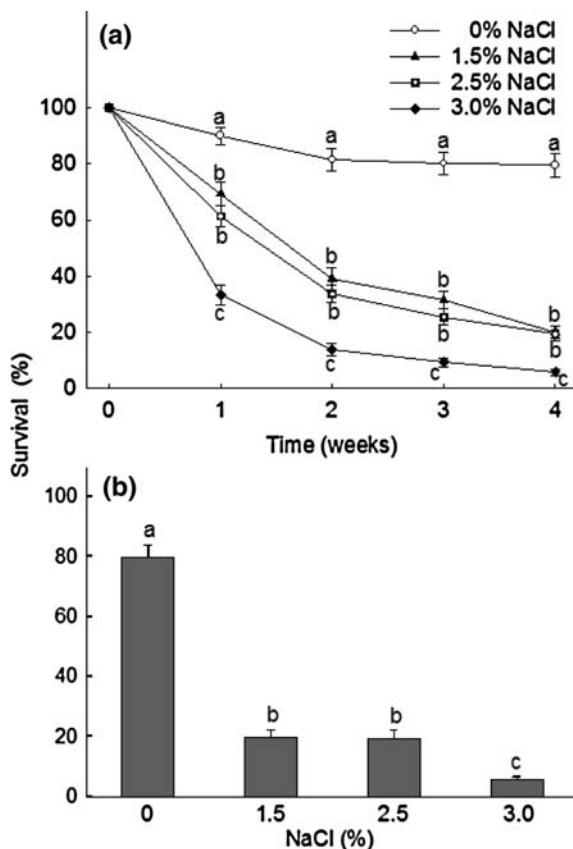


Fig. 8 Effect of NaCl on survival of *Mammillaria fraileana*. **a** Survival curves, **b** Tukey's HSD test comparison of survival at end of experiment. Values of survival at each incubation time (a) denoted with a different lower case letter differ significantly by one-way ANOVA and Tukey's HSD post hoc analysis at $P < 0.05$. Columns denoted with a different lower case letter differ significantly by one-way ANOVA and Tukey's HSD post hoc analysis at $P < 0.05$. Whisker bars indicate standard error

population of *M. fraileana*, has more slowly weathering minerals (plagioclase and pyroxene).

Since *M. fraileana* colonizes barren rocks, we analyzed the elements present in the rock and their availability as the rocks weather to determine if this has relevance in accounting for the abundance of plants. We found that sites with low abundance of this species contain high amounts Ca, Fe, Mg, Ti, Al, and Mn, whereas sites with higher abundance were rich in Si, K, and Na. While weathering of rocks was not directly determined, it is known that weathering of andesite, characteristic of our study sites, initially releases Ca, Mg, K, and Na, but metals (i.e., Al and Fe) are less mobile (Mulyantno et al. 1999); however,

certain plants can enhance dissolution of Si, Mn, Al, and Fe (Akter and Akagi 2005). Given the negative correlation with Ca, Mg, Fe, and Mn, it is likely that these cations are available, perhaps in excessive amounts, and therefore limiting the abundance of *M. fraileana* at Site C.

Contrary to expectations, positive correlations between Na and Si and abundance of *M. fraileana* at Site A and Site B hinted at a possible advantage for *M. fraileana*, although both elements are not considered essential for plants (Raven 2003). Na is even toxic to most plant species at moderate concentrations and Si forms cellular deposits that help plants keep their cell structure or it can lessen the toxicity of metals, including Al and Mn (Taiz and Zeiger 2006). According to the weathering sequence of rocks described above, Na at sites rich in *M. fraileana* is likely to affect plants; however, in the field, Na would be easily dissolved and washed away during rainfall. We tested the hypothesis that resistance to NaCl gives an environmental advantage to *M. fraileana* over other plants at these sites that are colonizers of rocks. In general, the negative effect of NaCl could be associated with the sensitivity of terrestrial plants to moderate concentrations of Na and Cl. The higher concentrations of NaCl in the growth medium (256–513 mM) was related to lower survival, except that *M. fraileana* has higher resistance to NaCl than other cacti. In this study, *M. fraileana* seedlings tolerated higher concentrations of NaCl than seedlings of other cacti, *Ferocactus acanthodes*, *Trichocereus chilensis*, and *Carnegiea gigantea*, which can survive NaCl to 130 mM (Nobel 1983). Despite significant mortality of *M. fraileana* seedlings on high saline treatments, the decrease in survival with the nonsaline treatment suggests that *M. fraileana* may require low levels of Na⁺ for growth, perhaps lower than the levels tested in this study. This is a likely possibility because Na is considered essential in some C₄ plants and plants with crassulacean acid metabolism (CAM; Winter and Ziegler 1992; Winter and Holtum 2005). Indeed the response to Na in plants with CAM varies, depending on the age of plants; it may also be influenced by drought conditions (Winter and Ziegler 1992; Winter and Holtum 2005).

Another explanation for the advantage of *M. fraileana* in colonizing bare rocks and rock crevices might be related to the amount of K and its relations with other elements. Although relative

low levels of K were found in the rocks, high populations of *M. fraileana* were positively correlated with higher K levels. When the effect of K + P is measured, the sites with greater abundance have larger amounts of these two elements than sites with lower abundance. Potassium may play an important role because, under saline conditions, K can counteract toxic levels of Na and the K:Na ratio could represent an indicator of balanced ions (Poole 1971; Cramer et al. 1987). Analysis showed a ratio of about 1:1 K:Na at Sites A and B, which had an abundance of *M. fraileana*. Site C, with a small population of *M. fraileana* contained about twice as much Na as K. It is likely that an imbalance in the K:Na ratio in the rocks and the weathered products in the crevices influenced the lesser abundance of *M. fraileana* at Site C. Regarding other major plant nutrients, the source of nitrogen that supported colonization of rocks by *M. fraileana*, was not studied. Past studies have shown that rock-dwelling cacti have large populations of nitrogen-fixing bacteria on their roots (Puente et al. 2004a, unpubl. data); hence, we accepted that nitrogen would be generally available.

In summary, our results highlighted the importance of *M. fraileana* in rocky habitats and showed that local variations in its abundance are based on the interaction of several elements, rather than the effect of single elements. We have provided evidence of relationships between chemical composition of rocks and the abundance of *M. fraileana*. However, it is necessary to explore physical and biological factors to elucidate their relative contribution and the relations among them.

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Ecophysiological responses of nine floodplain meadow species to changing hydrological conditions

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Abstract Here, we investigated how species from different floodplain meadow plant communities differ in their ecophysiological responses to an abrupt change in hydrological conditions. We simulated two contrasting hydrological scenarios for 5 weeks under controlled conditions: the waterlogging of a mesophilic species mixture (flooding scenario) and the drying of a hygrophilic species mixture (drying scenario). The mesophilic mixture was composed of three characteristic species of dry habitats (dry species) and three indifferent species with regard to the hydrological conditions; the hygrophilic mixture was composed of three characteristic species of wet habitats (wet species) and the same three indifferent species. The flooding scenario induced a significant decrease in photochemical efficiency (F_v/F_m) of all species for at least one day, and four of these species did not readjust their F_v/F_m at the end of the

experiment. The F_v/F_m of four species was significantly reduced by the drying scenario, but was readjusted within the time of the experiment. Significant leaf plastic responses were mainly detected under the drying scenario, through increasing specific leaf area (SLA) and decreasing leaf dry matter content (LDMC) for two indifferent species, and through decreasing SLA and increasing LDMC for two wet species. Finally, the flooding scenario significantly decreased the aboveground biomass of one dry species, but increased the aboveground biomass of the three indifferent species, suggesting an acclimation response to waterlogging through increasing shoot/root ratio. Since the dry species did not show ecophysiological acclimation to changing hydrological conditions (in contrast to the wet species and the indifferent species), this study demonstrated that mesophilic meadow communities should be particularly affected by a change from dry to wet conditions.

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Keywords Drying · Leaf plasticity · Photochemical efficiency · Waterlogging

Introduction

The hydrological regime is the most important physical factor influencing the floristic composition of floodplain meadows (Grevilliot et al. 1998; Muller et al. 1998; Casanova and Brock 2000; van Eck et al. 2004). Plant communities are differentiated along a flooding

gradient, which is defined by an increase of flooding duration and frequency with decreasing elevation (Gowing and Spoor 1998). Many human activities may strongly alter the hydrology of floodplains (Postel et al. 1996; Poff et al. 1997). For example, dam construction along rivers increases flooding in upstream areas and decreases flooding in downstream areas (Nilsson and Berggren 2000). Understanding the responses of plant communities to such hydrological changes is a major challenge for conservation and/or restoration of floodplain ecosystems. Modification of species diversity after a change in hydrology is well documented (e.g. van der Valk et al. 1994; Elmore et al. 2003; Toogood et al. 2008); however, few studies have investigated how the impact of hydrological changes on plant communities may be related to the ecophysiological responses of individual species (Parolin 2001; Insausti et al. 2001). The species' responses to a change in hydrology should depend on (1) the intensity of stress (*sensu* Grime 1979) induced by the change and (2) the acclimation abilities of the species to the new hydrological regime.

The relative importance of abiotic and biotic factors controlling species' distribution along the flooding gradient varies between lower and upper limits (Austin 1980; Pennings and Callaway 1992). The lower distribution limits are abiotically constrained by flooding stress (Vervuren et al. 2003; van Eck et al. 2004), which strongly affects plant performances by reducing gas exchanges between plant and atmosphere (Crawford and Brändle 1996; Dat et al. 2004). In contrast, competition is recognised as the most important factor at the upper distribution limits (Grace 1990; Keddy 1990, Shipley et al. 1991; Budelsky and Galatowitsch 2000), although studies suggested that drought might contribute to restrict flood-tolerant species to low elevations (Silvertown et al. 1999; Lenssen and de Kroon 2005). Consequently, as flooding gradients would correspond to a shift from abiotic to biotic constraints, the intensity of stress induced by a change in hydrology should differ according to the direction of change. More precisely, plant communities should be physiologically more affected by a change from dry to wet conditions than the opposite.

Species' abilities to cope with contrasting hydrological conditions depend on the plasticity of ecophysiological traits, particularly leaf plasticity (Voesenek et al. 2006). The leaf response of species may hence contribute to reduce the severity of

changing hydrology on plant communities (Bazzaz 1996). Thus, previous studies demonstrated that a decrease in specific leaf area (SLA) and an increase in leaf dry matter content (LDMC) might reduce the water loss in plant tissues under drought stress (Kalapos et al. 1996; Peñuelas et al. 2004); a similar acclimation response has been emphasised under soil waterlogging (Dale and Causton 1992, Vartapetian and Jackson 1997), which is experienced as a 'physiological drought' because oxygen deficiency in the root environment prevents water uptake by plants (Forbes and Watson 1992).

This study examines the short-term ecophysiological response of floodplain meadow communities to two contrasting hydrological scenarios: (1) a 'flooding scenario', consisting of waterlogging a mixture of species from high elevations and (2) a 'drying scenario', consisting of drying a mixture of species from low elevations. The scenarios were simulated under controlled conditions, and species mixtures were composed from field vegetation data. Species' responses were evaluated through measurements of photochemical efficiency, leaf traits and aboveground biomass. As plants from high elevations should be more hydrologically constrained than those from low elevations, we hypothesised that species photochemical efficiency should be more affected by the flooding scenario. Moreover, we expected that species from low elevations should be better able to adjust their leaf traits to varying hydrological conditions.

Materials and methods

Field study and species selection

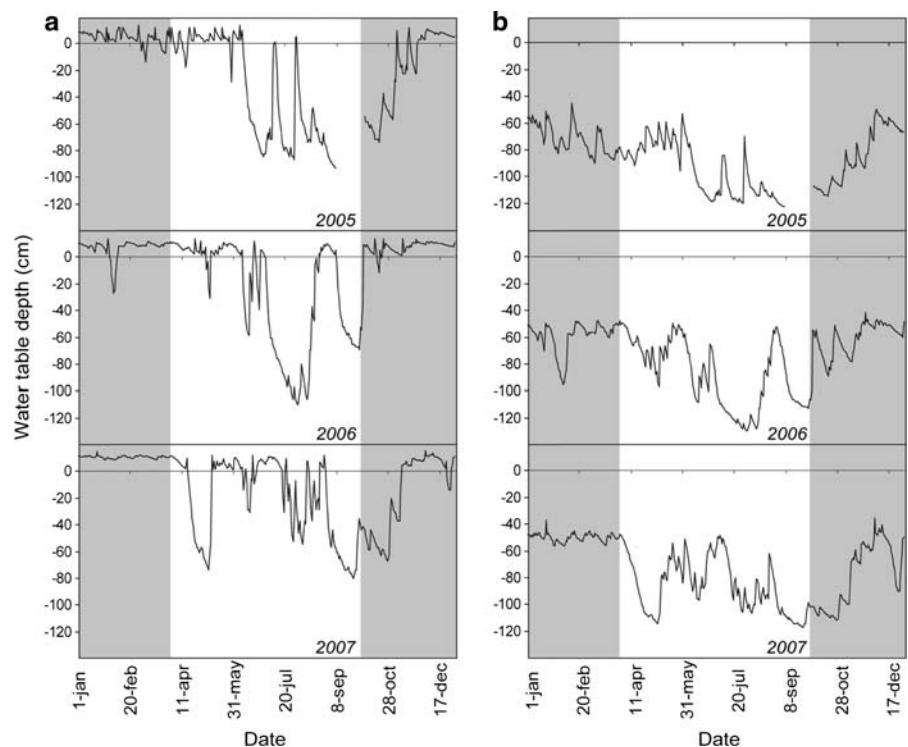
A field study was conducted in order to analyse the plant species composition at both ends of a natural flooding gradient. The study area consists of hay meadows located in the Alzette floodplain (Grand-Duchy of Luxemburg, 49°31' N 6°06' E). The climate is of humid-temperate type; the mean annual temperature is 9°C and the mean annual rainfall is 850 mm, with the highest value during winter and the lowest value during summer (Pfister et al. 2000). This rainfall regime directly controls the temporal fluctuations of the water-table level (Fig. 1). The flooding gradient is spatially defined by the topographic variations, which influences the soil water-table

depth. The lowest elevations of the floodplain support a flooding duration higher than 6 months per year (Fig. 1a), whereas the highest elevations are never flooded (Fig. 1b). Thirty-three plant relevés were recorded at both elevation ranges, permitting recognition of two different plant communities (see supplementary material): a hygrophilic community, belonging to the phytosociological order *Eleocharite-talia palustris* (de Foucault 1984), at low elevations and a mesophilic community, belonging to the phytosociological order *Arrhenatheretalia elatioris* (de Foucault 1989), at high elevations. Species were classified as ‘wet species’ (i.e. species recorded in the hygrophilic community only), ‘dry species’ (i.e. species recorded in the mesophilic community only) and ‘indifferent species’ (i.e. species present in both communities). Nine species were selected for the experiment, comprising three of the most abundant species within each group: *Phalaris arundinacea* L., *Agrostis stolonifera* L. and *Carex disticha* Huds. were selected as wet species; *Dactylis glomerata* L., *Anthoxanthum odoratum* L. and *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl et C. Presl as dry species; *Festuca arundinacea* Schreb., *Holcus lanatus* L. and *Alopecurus pratensis* L. as indifferent species.

Fig. 1 Variations in the water-table level experienced by meadow communities at the lowest elevations (a) and the highest elevations (b) in the study area. White area indicates the growing season of meadow species. Data comes from two piezometers located in representative mesophilic and hygrophilic communities of the Alzette floodplain (relevés 6 and 30, see supplementary material)

Experimental design

Individuals of the selected species were combined in two six-species plant mixtures: (1) a ‘wet mixture’, from the three wet species (*P. arundinacea*, *A. stolonifera* and *C. disticha*) and the three indifferent species (*F. arundinacea*, *H. lanatus* and *A. pratensis*) and (2) a ‘dry mixture’, from the three dry species (*D. glomerata*, *A. odoratum* and *A. elatius*) and the three indifferent species. Plants were grown from seeds collected in the study area and cold stratified at 5°C in the dark for 20 weeks to break seed dormancy (Milberg and Andersson 1998). Before sowing, the seeds of *C. disticha* were placed under wet conditions at 30°C in the light, in order to enhance germination (Schütz and Rave 1999). Seeds of all species were germinated for 2 weeks in greenhouse trays filled with a 1:4 mixture of sand and commercial garden soil at 25(day)/15(night)°C. In April 2007, the plant mixtures were made by transplanting seedlings in plastic trays (35 × 35 × 13 cm) filled with the same substrate, as used for germinations. Nine seedlings per species were uniformly distributed in each tray, resulting in a total density of 441 plants m⁻². The trays were randomly placed in six outdoor ponds (150 × 100 × 32 cm)



assigned to two hydrological conditions: (1) waterlogged conditions, with a water level maintained at 1 cm above the soil surface and (2) dry conditions, with trays only watered by rain. The wet and the dry mixtures were established for 5 weeks in the waterlogged and the dry conditions, respectively. Waterlogged conditions were chosen rather than complete submergence, because field observations revealed that shoots of most meadow species, and especially tall species like grasses, remain above the water level during flooding events (pers. obs.).

The experiment started at the end of May 2007 and consisted of a combination of the two plant mixtures (wet/dry) by two water treatments (control/modified), with seven replicates for each factor combination. The water treatments were applied during 5 weeks, by permuting a random selection of half the trays of each plant mixture. During the period of the experiment, the hydrological conditions experienced by the control mixtures were consistent with those that may be encountered by the natural plant communities under field conditions (especially in 2007 for the wet conditions, Fig. 1).

Measurements

The effect of the water treatments on the species' photochemical efficiency was assessed by the means of chlorophyll fluorescence measurements. Chlorophyll fluorescence is widely used to evaluate the effect of environmental stresses on the maximum efficiency of photosystem II (Maxwell and Johnson 2000) estimated by the ratio of variable fluorescence to maximum fluorescence (F_v/F_m). The F_v/F_m ratio was measured seven times during the experiment, at days 2, 4, 6, 10, 17, 24 and 31. Measurements were made in the control and in the modified treatments (waterlogged or dried) on two plants per species per tray, resulting in 14 replicates per species per treatment. Plants were selected in the centre of trays to avoid border effects and were marked with coloured clips in order to repeat measurements on the same individual. Before measurements, leaves were dark adapted for at least 30 min using leaf clips (Georgieva and Lichtenthaler 1999). Since F_v/F_m shows a marked diurnal course, all individuals of each species were measured within a time which did not exceed 1 h (comprising dark adaptation), by alternating measurements in the control and in the

modified treatment. Measurements were made with a portable fluorometer Handy PEA (Hansatech Instruments Ltd, Norfolk, UK).

Leaf trait responses induced by the hydrological changes were assessed through measurements of the SLA and the LDMC. Leaf traits were measured from days 32 to 34 on two individuals per species per tray. For each individual, the youngest fully expanded leaves was collected and then stored following a standardized protocol (Garnier et al. 2001). After weighing of saturated fresh mass, the leaves were scanned using a flatbed scanner at a resolution of 500 dpi (Agfa Snapscan 1212u). Their projected area was determined using PHOTOSHOP (ver. 8, Adobe Systems Inc., San Jose, CA). Leaves were weighed after drying at 70°C for 48 h. SLA ($m^2 kg^{-1}$) was calculated as the ratio between leaf area and leaf dry mass, and LDMC ($g kg^{-1}$) as the ratio between leaf dry mass and leaf saturated fresh mass.

At day 35, the aboveground biomass was harvested in each tray, sorted by species, oven dried at 60°C for 48 h and weighed.

Data analysis

For each species and each measurement day, the differences in F_v/F_m between control and modified mixtures (dried or waterlogged) were tested by Mann–Whitney U-tests, as data were not normally distributed. Significant differences in SLA, LDMC, species biomass per trays and total biomass per trays were tested by Student *t*-tests, after verifying normality (Shapiro–Wilk test, $P < 0.05$) and homoscedasticity (Levene test, $P < 0.05$) assumptions. Statistical analyses were carried out using the program STATISTICA (ver. 7.0, Statsoft Inc., USA).

Results

Photochemical efficiency

In the wet mixture, the three wet species showed a significant decrease of their photochemical efficiency under drying (Fig. 2), arising from the 4th day for *A. stolonifera* and *P. arundinacea*, and from the 10th day for *C. disticha*. The highest difference between the control and the dried mixtures observed during the experiment for the wet species was between 4.8%

and 11.8% (Table 1). Among the indifferent species, only *F. arundinacea* showed a significant decrease of F_v/F_m on the 4th day (3.3% lower than the control). However, none of the significant differences between the control and the dried treatment remained at the end of the experiment.

Waterlogging significantly decreased the photochemical efficiency of all species in the dry mixture for at least 1 day (Fig. 3). The highest difference between the control and the waterlogged mixtures observed during the experiment was between 3.4% and 5.1% for the indifferent species, and between 2.8% and 48.5% for the dry species, *A. elatius* being the most affected (Table 1). Species differed in their latent period before significant differences between the control and the waterlogged treatment occurred. For two indifferent species (*H. lanatus* and *A. pratensis*) and two dry species (*A. elatius* and *D. glomerata*), a significant difference between the control and the waterlogged treatment was still observed on the 31st day.

Leaf traits

Four species of the wet mixture (*F. arundinacea*, *H. lanatus*, *A. stolonifera* and *P. arundinacea*) showed significant variations in LDMC and/or SLA under drying scenario (Fig. 4a). The responses of the

Fig. 2 Changes in F_v/F_m under the drying scenario (dashed lines) compared to changes in the F_v/F_m in the control mixture (solid lines) for each species during the experiment. Values are medians and error bars indicate first and third quartiles ($n = 14$). Statistical results from Mann–Whitney U-tests comparing F_v/F_m between species in the control mixture and species in the dried mixture:
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. not significant

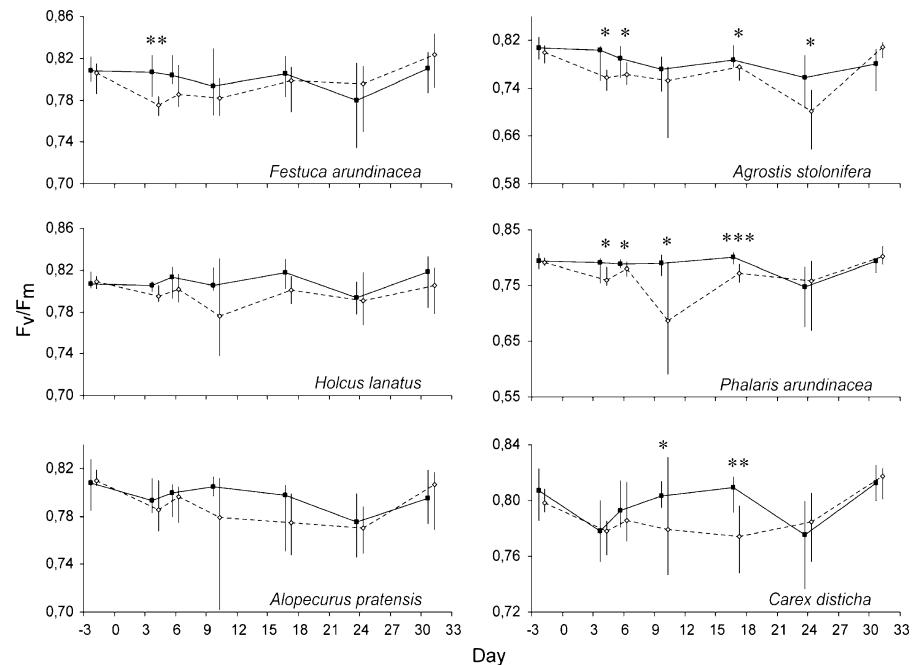


Table 1 Maximum variation in F_v/F_m (F_v/F_m , in %) under each hydrological scenario compared to the F_v/F_m in the control mixture (=100%) for each species during the experiment

	Drying scenario			Flooding scenario		
	$\Delta F_v/F_m$	U	P	$\Delta F_v/F_m$	U	P
<i>Agrostis stolonifera</i>	-7.2	43	*			
<i>Phalaris arundinacea</i>	-11.8	56	*			
<i>Carex disticha</i>	-4.8	39	**			
<i>Festuca arundinacea</i>	-3.3	44	**	-3.7	61	*
<i>Holcus lanatus</i>	-2.3	85	n.s.	-5.1	29	***
<i>Alopecurus pratensis</i>	-4.4	67	n.s.	-3.4	58	*
<i>Arrhenatherum elatius</i>				-48.5	22	***
<i>Anthoxanthum odoratum</i>				-2.8	33	**
<i>Dactylis glomerata</i>				-5.0	37	**

Statistical results from Mann–Whitney U-tests

n.s. not significant

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

indifferent species (increase of SLA by 10–20% and decrease of LDMC by 7% between control and dried mixtures) were in contrast to those of the wet species (decrease of SLA by 9–10% and increase of LDMC by 13% between control and dried mixtures). In the

dry mixture (Fig. 4b), a significant waterlogging-induced response was found only for *A. elatius*, which showed a strong decrease of LDMC (22%), as well as a strong increase of SLA (44%).

Aboveground biomass

No significant effects of the hydrological changes were observed on the total aboveground biomass within trays (Fig. 5). However, significant differences were found in individual species aboveground biomass. Thus, in the dry mixture (Fig. 5a), waterlogging significantly decreased the aboveground biomass of *A. elatius* by 96% when compared with the control. In contrast, waterlogging significantly increased the aboveground biomass of the indifferent species, by 67% for *F. arundinacea*, by 63% for *H. lanatus* and by 175% for *A. pratensis*. The effect of drying on species comprising the wet mixture was less important, as *A. pratensis* was the only one showing a significant decrease of biomass by 18% (Fig. 5b).

Discussion

As predicted by our hypotheses, the flooding scenario had a stronger impact on species photochemical

efficiency than the drying scenario. Thus, the flooding scenario affected all species from the dry mixture (both dry and indifferent species), whereas in the wet mixture the drying scenario mainly affected the wet species; however, the F_v/F_m of the wet species reached the same level as the control mixture at the end of the experiment. These results demonstrated that an abrupt change from waterlogged to dry conditions did not represent an important stress for the wet mixture, because (1) the photochemical efficiency of the indifferent species were mostly not affected and (2) the wet species were able to readjust their photochemical efficiency. More generally, these results confirm the idea that species from dry locations along flooding gradients are hydrologically more constrained than species from wet locations (Vervuren et al. 2003; van Eck et al. 2004).

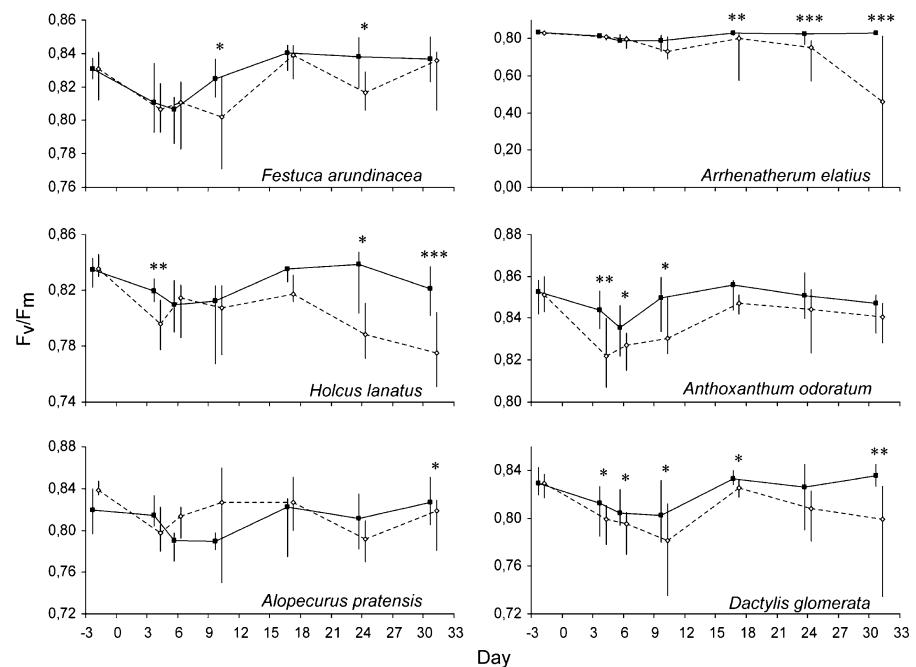
Each scenario also differed in its effect on induced leaf plasticity. Four species showed a leaf response under the drying scenario, whereas only *A. elatius* showed significant variations in SLA and LDMC under the flooding scenario. However, such variations have to be interpreted with caution, since almost all *A. elatius* material from the waterlogged dry mixture was dead when leaf traits were measured, as illustrated by the chlorophyll fluorescence and the growth data. Thus, although we found some

Fig. 3 Changes in F_v/F_m under the flooding scenario (dashed lines) compared to the changes in F_v/F_m in the control mixture (solid lines) for each species during the experiment. Values are medians and error bars indicate first and third quartiles ($n = 14$).

Statistical results from Mann–Whitney U-tests comparing F_v/F_m between species in the control mixture and species in the dried mixture:

*** $P < 0.001$, ** $P < 0.01$,

* $P < 0.05$, n.s. not significant



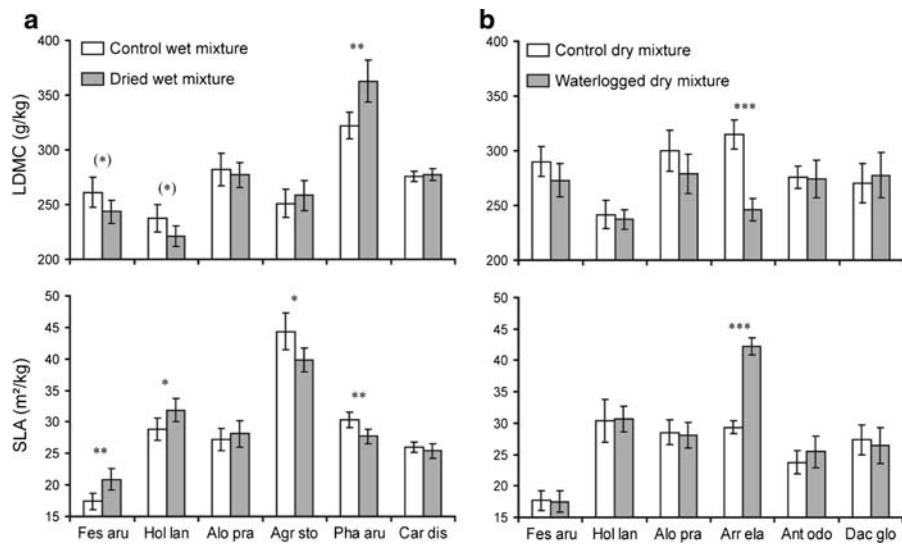


Fig. 4 Variations in leaf dry matter content (LDMC) and specific leaf area (SLA) under the drying scenario (a) and the flooding scenario (b) for *Festuca arundinacea* (Fes aru), *Holcus lanatus* (Hol lan), *Alopecurus pratensis* (Alo pra), *Agrostis stolonifera* (Agr sto), *Phalaris arundinacea* (Pha aru), *Carex disticha* (Car dis), *Arrhenatherum elatius* (Arr ela)

Anthoxanthum odoratum (Ant odo) and *Dactylis glomerata* (Dac glo). Values are means and error bars represent standard deviations ($n = 14$). Asterisks indicate significant differences between the control and the dried mixtures (Student t -tests): *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, (*) $P < 0.1$

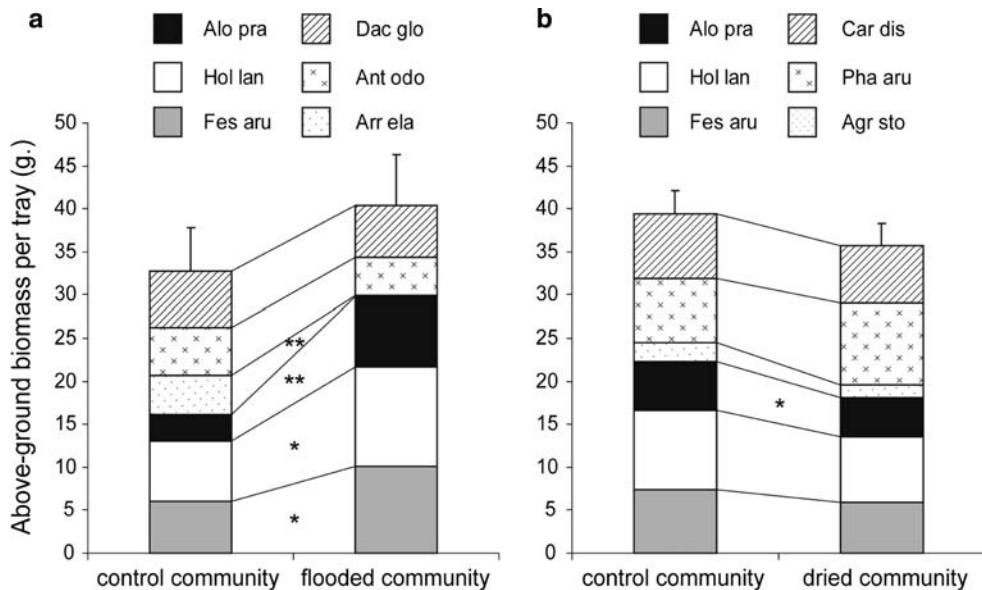


Fig. 5 Effects of each hydrological scenario (a flooding scenario, b drying scenario) on the aboveground biomass of *Festuca arundinacea* (Fes aru), *Holcus lanatus* (Hol lan), *Alopecurus pratensis* (Alo pra), *A. stolonifera* (Agr sto), *Phalaris arundinacea* (Pha aru), *Carex disticha* (Car dis), *Arrhenatherum elatius* (Arr ela), *Anthoxanthum odoratum* (Ant

odo) and *Dactylis glomerata* (Dac glo). Error bars indicate the standard deviation of the total aboveground biomass, where no significant differences were found between control and modified mixtures. Asterisks indicate significant differences in species' aboveground biomass between control and modified mixtures (Student t -tests): ** $P < 0.01$, * $P < 0.05$

remaining green leaves to make the measurements, the observed differences in SLA and LDMC should be regarded as a leaf necrosis symptom, rather than an as a induced plastic response. In the dried wet mixture, the leaf responses of the wet species (decrease of SLA; increase of LDMC) were in contrast to those of the indifferent species. Previous studies demonstrated that leaf plasticity along flooding gradients might be regarded as variations in stomatal conductance (Givnish 2002). Particularly, a decrease in SLA may be related to stomatal closure, which is relevant under drought and waterlogging stresses (Kalapos et al. 1996, Dale and Causton 1992; Vartapetian and Jackson 1997) because it reduces water loss by transpiration (Jones 1998). Although stomatal conductance was not measured in our experiment, the leaf responses of the wet species might be related to stomatal closure due to the drying scenario. On the contrary, the leaf responses displayed by the indifferent species might be related to stomatal opening due to the arrival of more favourable conditions.

Two reasons may be advanced to explain why leaf responses to a change in hydrology were mainly found in the wet mixture. The first reason would be that within natural floodplains, plant communities from low elevations experience more contrasting hydrological conditions than those from high elevations. Indeed low elevations are subjected to more important water table fluctuations than high elevations (Fig. 1). Moreover, as the fluctuations at low elevations impact upon the root zone, they affect plants more than at high elevations (Crawford 1996). A high degree of leaf plasticity would therefore be required at low elevations, where species have to cope with hydrological conditions varying from flooding to drought during the same growing season. The second reason refers to the assumption that species from low elevations are mainly excluded from drier habitats by competition rather than hydrology (Grace 1990; Keddy 1990, Shipley et al. 1991; Budelsky and Galatowitsch 2000). This assumption would mean that low-elevation species should be more plastic than high-elevation species, since they tolerate a wider range of hydrological conditions. The above-mentioned reasons are in line with the idea that morphological plasticity is correlated with the heterogeneity of the environment, at both spatial and temporal scales (Alpert and Simms

2002; Garbey et al. 2004). Our results therefore corroborated the hypothesis that heterogeneous environments favour species with high plasticity (Schmid 1992) and, thus, improve the capacity of plant communities to acclimate to a new environment (Ghalambor et al. 2007).

Festuca arundinacea and *Holcus lanatus* showed a significant leaf response only under the drying scenario. This demonstrated a better ability of these species to adjust their leaf traits to a change from waterlogged to dry conditions than the opposite. Although the ecological range of *F. arundinacea* and *H. lanatus* encompassed the entire flooding gradient, their higher abundance in the mesophilic community (see supplementary material) indicates that the dry conditions used in our experiment should be closer to their physiological optimum than the waterlogged conditions. It may be argued that these species produced stress-acclimated leaves during their seedling stage in the wet experimental mixture, in order to cope with waterlogging (Voesenek et al. 2006); on the contrary, no stress-acclimated leaves were produced in the dry mixture, where species established under more favourable conditions. Thus, the drying scenario corresponded to a shift from stressful to favourable abiotic conditions. This could explain why the particular ‘stress leaf strategy’ maintained by *F. arundinacea* and *H. lanatus* during their establishment in the wet mixture was easily reversed by the drying scenario.

Significant differences in species aboveground biomass were mainly found under the flooding scenario. *A. elatius* was the only dry species that showed a significant decrease in the aboveground biomass. No decreases were found for *D. glomerata* and *A. odoratum*. These results demonstrated that despite the decline in their photochemical efficiency and the lack of leaf plastic responses, these species are able to resist waterlogging during several weeks. The indifferent species showed an increase in their aboveground biomass under the flooding scenario. This is probably attributable to an increase in the shoot/root ratio, which is a well-known response of flood-tolerant species (Loreti and Oesterheld 1996; Miller and Zedler 2003). Thus, the indifferent species should have compensated for the absence of photochemical and leaf adjustments to waterlogging by increasing the total photosynthetic area.

Conclusion

Maintaining the natural flooding regime has been advocated as a prerequisite for the conservation of species-rich floodplain meadows (Joyce and Wade 1998). However, it is spatial heterogeneity, and especially microtopographic variations that promote species richness (Silvertown et al. 1999) by generating a multitude of hydrological conditions, from long-flooded to not-flooded. An important consequence of human-induced changes in floodplain hydrology is the loss of spatial heterogeneity (Brooks et al. 2002). The impact of dams on river and groundwater levels is so strong that both low- and high-floodplain elevations are flooded in upstream areas and drained in downstream areas. The present study provides an experimental basis for predicting the impact of such a modification on plant species richness. We demonstrated that floodplain meadow communities differ in their short-term susceptibility to hydrological changes. The dry species were not able to acclimate to the flooding scenario. Mesophilic communities submitted to waterlogging should therefore be affected by a decrease in species richness through the decline of the dry species. Mesophilic alluvial meadows communities are designated under Natura 2000 (Muller 2000), and many threatened species occupying high floodplain elevations (such as *Filipendula vulgaris*, *Scabiosa pratensis* or *Colchicum autumnale*) should be particularly sensitive to a change from dry to wet conditions. Under the drying scenario, both wet species and indifferent species showed acclimation responses, suggesting a relative ability of hygrophilic communities to resist dry conditions. However, in a longer term than the duration of our experiment, it is likely that the wet species would be indirectly affected through changes in species-competitive interactions. Multi-annual surveys are needed to study the long-term outcome of changing water regime on floodplain communities and, particularly, to assess how the hydrologically induced responses of species may affect their competitive abilities.

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Tolerance of a perennial herb, *Pimpinella saxifraga*, to simulated flower herbivory and grazing: immediate repair of injury or postponed reproduction?

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Abstract Perennial, polycarpic herbs can respond to herbivory either by (1) regrowth in the same season in order to compensate for lost reproductive structures or by (2) postponing reproduction until the following growing season. We tested these response patterns with the perennial umbellifer *Pimpinella saxifraga* by simulating flower herbivory and shoot grazing both in the field and in a common garden experiment. In the field, both simulated flower herbivory and grazing effectively suppressed current reproduction, whereas no statistically significant effects of previous-year treatments on growth or reproduction were found in the following year. In the common garden, in the first year the species fully compensated for simulated flower herbivory in vegetative parameters but seed set was reduced by 26%. After 2 years of flower removal, the plants overcompensated in shoot and root biomass by 47 and 46%, respectively, and compensated fully in reproductive performance. Simulated grazing resulted in 21%

lower shoot biomass in the first season, but the root biomass was not affected. In the second season the root biomass increased by 43% as compared to the control plants. However, regrowth following simulated grazing resulted in a significant delay in flowering with the consequence that the seed yield of fertile plants was reduced by 55% as compared to the control plants. These results suggest that in resource-rich garden conditions *P. saxifraga* may immediately repair injuries caused by flower herbivory, but repairs more extensive shoot injury less successfully. Delayed phenology decreases the benefits of immediate repair. In resource-poor conditions, the benefits of regrowth can be negligible. Accordingly, in our field population, the plants postponed their reproduction until the following year in response to simulated grazing and frequently in response to flower removal. When the plants gain very little from regrowth, the costs of reproduction would select for postponed reproduction in response to injury.

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Keywords Burnet saxifrage · Cost of herbivory · Cost of reproduction · Fitness · Plant life-history · Tolerance

Introduction

When strict biennials and other monocarpic species are grazed during the flowering stage, they have only

one choice. They must immediately repair the injury to compensate for the lost biomass and damaged reproductive structures. If conditions are favourable, the immediate repair may result in equal and even overcompensation compared to the reproductive success of ungrazed plants (Paige and Whitham 1987; Whitham et al. 1991; Lennartsson et al. 1998; Huhta et al. 2000a). However, compensatory regrowth is often associated with delayed flowering and seed maturation (Benner 1988; Bergelson and Crawley 1992) which may, in unfavourable conditions, result in a complete reproductive failure or at best only a partial compensation for the lost reproductive potential (Lennartsson et al. 1998). Consequently, monocarpic plants are in a “bet-hedging” situation, not only in relation to herbivory (Vail 1992; Nilsson et al. 1996), but also in relation to resource and weather conditions during regrowth and seed maturation (Lennartsson et al. 1998; Huhta et al. 2000b; Levine and Paige 2004).

In response to grazing (or mowing), polycarpic species have more options as compared to monocarpic species (Fig. 1). A perennial plant may postpone its reproductive attempt into the following season

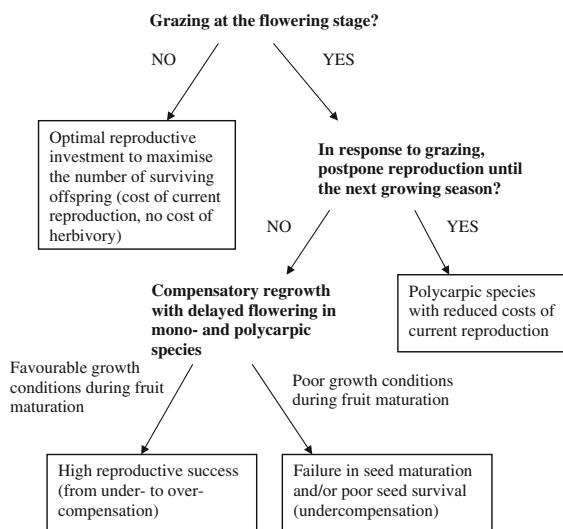


Fig. 1 Strategy tree of mono- and polycarpic species in relation to grazing and immediate repair versus postponing reproduction until the next growing season. Undercompensation = the current reproductive success of grazed plants is lower compared to ungrazed plants, equal compensation = grazed and ungrazed plants have an equal seed production, overcompensation = the current reproductive success of grazed plants is higher than that of ungrazed plants

rather than to retry flowering and seed setting within the same season. Besides grazing and mowing, insect herbivory may cause considerable losses for flowering plants (Louda 1984; Hendrix and Trapp 1989; Cox and McEvoy 1983; Karban and Strauss 1993). One could expect that it would be easier to immediately repair flower herbivory than extensive injury by grazing and browsing. On the other hand, the plant may postpone reproductive investment also in this case. In fact, clipping of flower stalks and removal of developing flowers are treatments sometimes used to manipulate the current reproductive investment in order to test how the reduced reproductive effort affects future reproductive success in perennial plants (e.g. Tolvanen and Laine 1997; Hemborg 1998; Houle 2001; Obeso 2002; Knight 2003). If the plants do postpone their reproductive investment in response to flower removal, their improved future reproductive success would indicate the costs of reproduction.

An important implication of these manipulative studies is that herbivory during the flowering stage of a perennial plant has in fact three consequences: (A) the immediate cost of herbivory referring to the immediately lost reproductive capacity of injured plants in relation to intact plants, (B) the delayed cost of herbivory in terms of reduced future reproductive capacity of previously injured plants and (C) altered reproductive investment of injured plants influencing the potential costs of reproduction. According to Veneczel and Aarssen (1998), if current reproduction is prevented by herbivory or clipping, resources that would have been otherwise invested in reproduction may be stored below-ground and invested in reproduction in the following year. In such a case, the greater fruit and seed yield of injured plants in the subsequent year compared to uninjured plants would indicate the costs of reproduction (i.e. $C > B \geq 0$). On the other hand, when the injured plants immediately regrow and flower in order to mitigate the costs of herbivory damage on current reproduction (A), this investment may reduce their future fecundity as a consequence of the costs of compensatory reproduction. This implies that it would not be useful to postpone reproduction in conditions where the injured plant gains more in reducing the costs of injury than it loses in terms of the costs of compensatory reproduction. If there are no costs associated with reproduction, it would be

always useful to restart reproduction in order to reduce the adverse effects of herbivory. However, if the reproductive costs are high and the compensatory reproduction is likely to fail, it would be useful to postpone reproduction until the following growing season.

We studied the responses of the perennial umbellifer, Burnet saxifrage (*Pimpinella saxifraga*), after simulated flower herbivory and ungulate grazing or browsing in two consecutive years in garden conditions (1998–1999), and in a field population (1999–2000). *P. saxifraga* occurs mainly in human-influenced habitats, such as dry to mesic meadows and pastures, fields, road verges and ridges (Wells et al. 1976; Hämet-Ahti 1980; Grime et al. 1988). It is a mid-seral species and occurs in moderately stressful but undisturbed habitats (Grime et al. 1988). Although it contains several chemical defence compounds (Cornu et al. 2001), it is a food source for Swallowtail butterfly larvae (*Papilio machaon*) (Marttila et al. 1990) and also ungulate grazing may cause serious injuries to *P. saxifraga*. Because it does not occur very abundantly in intensively grazed grasslands (Jantunen and Saarinen 2003), we hypothesized that the species may be well able to compensate for the loss of reproductive organs caused by insect herbivores but less so for more comprehensive biomass losses caused by mowing or ungulate grazing. We monitored the effects of two levels of damage both in vegetative and reproductive

performance parameters in order to test for the costs of injury and the costs of reproduction.

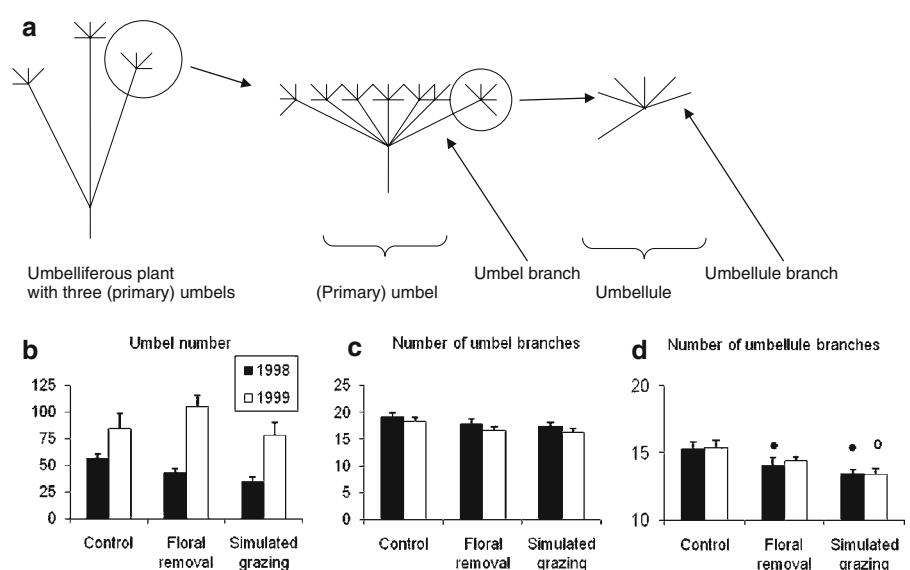
Material and methods

Study species

Pimpinella saxifraga (L.) is a perennial plant that has large and deep penetrating rootstock. Most of the above-ground parts die at the end of the growing season. Rosette leaves overwinter and support new growth in the following spring. In field conditions the plants grow about 50 cm tall (Kalela and Väänänen 1960). White to pale reddish flowers are commonly hermaphrodite and the inflorescence is typically umbelliferous with main umbels and umbellules (Fig. 2a). Each hermaphroditic flower can produce a fruit consisting of two-one-seeded mericarps.

The worldwide distribution of *P. saxifraga* includes the whole of Europe, Asia ranging to the Baikal area and north-eastern parts of North America (Hämet-Ahti 1980). According to Kalela and Väänänen (1960), *P. saxifraga* is native in Finland as a seashore species. In contrast, according to Hämet-Ahti (1980), it occurs only in human-influenced habitats as an archaeophyte. In Finland *P. saxifraga* is common extending to northern parts of Central Finland (Kalela and Väänänen 1960). In northern Finland, it occurs in only a few locations, such as

Fig. 2 Schematic presentation a of an umbelliferous plant and flower structure along with the treatment effects (b–d) on reproductive characteristics (umbel structure) of *Pimpinella saxifraga* in the garden during the years 1998 and 1999 (mean \pm SE). The bars denoted by a dot differ statistically from the unclipped control plants of the corresponding treatment group (filled = 1998, open = 1999, $P < 0.05$)



Kiiminki (the origin of one of the populations studied here), the Kuusamo district and Kemi on the coast of the Gulf of Bothnia.

Experimental design

The garden experiment was carried out during 1997–1999 in the Botanical Gardens of the University of Oulu, Finland (65° N, 25.5° E). The target plants were grown from seeds collected from two separate populations located in Joensuu (62.5° N, 30° E) and in Kiiminki ($65^{\circ}10'$ N, $25^{\circ}50'$ E). The seedlings were planted (1997) at regular distances (50 cm) into planters in five adjacent rows. At the time of sprouting (1998) the plants ($N = 23\text{--}33$ per treatment) were randomly assigned into three different treatment groups: (1) control, (2) removal of all developing flower buds (referred to hereafter as simulated flower herbivory) and (3) 75% clipping of shoot biomass measured as height of an each individual, which represents realistic amount of biomass loss during heavy grazing pressure by domestic animals (Huhta, pers. obs.), and is hereafter referred to as simulated grazing. In the first growing period (1998), after measuring the initial height and number of stems, the treatments were carried out just before flowering (at the bud stage), on 29 June 1998. The phenological state of each plant was surveyed on 21 July, 20 August and 5 October using four classes: (I) the plant with flowers in the bud stage, (II) flowering, (III) unripe (green) fruits and (IV) ripe (brown) fruits. At the end of the season (19–20 October) the plants from rows two and four ($N = 27, 26$ and 25 per treatment) were harvested in order to evaluate the impacts of the treatments after the first season, while the plants in the three remaining rows ($N = 23, 33$ and 33 per treatment) were allowed to grow for another year. During the second season (1999), the treatments (1, 2 and 3 above) were repeated at a phenologically corresponding time on 24–25 June 1999. The phenology of plants was monitored on 15 July and 18 August. In addition to the phenological stages of flowering plants I–IV, sterile and dead plants were recorded. The surviving plants were harvested for analysis on 28–29 October 1999.

The harvested plants (the above-ground structures were cut away first, whereafter the underground structures were dug out from the soil) were dried at

room temperature for several weeks and the following parameters were recorded: stem height, stem number, shoot and root weight, root:shoot ratio, the number of umbels, and seed weight. Further, five to six plants from each treatment were randomly chosen and six of their primary umbels were chosen for closer examination. This included counting the number of umbellules per umbel and the number of seed bearing branches per umbellule (umbellule branches, see Fig. 2a). All this together allowed us to calculate an estimate for total seed production per plant: seed number = number of umbels \times number of umbellules per umbel \times number of umbellule branches \times 2. The multiplier 2 is used because of the two-one-seeded mericarp is carried by each umbellule branch.

In 1999, sterile plants were included in the vegetative parameters and dead and sterile plants were included when calculating reproductive success in order to find out the true effects of clippings in time, i.e. if the treatments were to be realized in exhaustion of resources in the following year leading into resource deficiency causing “resting period” or even death. In vegetative parameters we did this by recording sterile plants as zeros in case that particular parameter was not available (e.g. number of stems). In the seed parameters only fertile plants were included as these data were not measured from every plant (e.g. umbellule branches). In case of reproductive success we estimated the final performance by multiplying the number of seeds per fertile plant by survival probability (% of sterile and fertile plants in 1999) and by flowering probability (% of fertile plants in 1999) as well as by seed weight.

The treatments (control, flower removal and 75% clipping) were repeated in the Kiiminki field population on 6 July 1999. The soil in Kiiminki habitat is nutrient poor compared to the garden soil, nitrogen concentration being only about 1/4 of that in the garden (0.41% vs. 1.83%) and while the organic layer in Kiiminki is on average less than 5 cm, the soil in garden is mainly organic material (OM% 67.3). Similar sized plants growing on two small hillocks, an area of 0.1 ha, were randomly allotted to treatments. The plant performance was followed until the 2000 season during which no clippings of the target plants were performed in order to see if the effects of the treatments in 1999 were still affecting plant performance. Their phenology was estimated on 20

July, seeds were collected on 21 September and plants were harvested on 6 October 2000. This experiment also included the removal of vegetation around the immediate surroundings of the target plants by mowing the neighbouring plants (referred to below as no competition). The original number of replicates per specific treatment combination was 15.

Data analyses

Because we tested a single hypothesis with multiple variables we performed a protected ANOVA on the data. This approach combines MANOVA and ANOVA (i.e. univariate analyses are performed if the multivariate analysis yields a significant result) and is less conservative than, for example, Bonferroni correction used to correct the P -values of multiple tests of a single hypothesis (Scheiner 1993). Because in the garden experiment the MANOVA yielded significant results (Table 1), we also conducted ANOVA for the data. In the garden experiment in both MANOVA (Pillai's trace) and the subsequent ANOVA, we applied a two-way factorial design with treatment (control, simulated flower herbivory and simulated grazing) and the number of treatments (clippings during 1 or 2 years) as grouping factors. Further, because we collected seeds from two populations (Joensuu and Kiiminki) and since seeds from the Joensuu population seemed to produce plants that were both longer (Joensuu 50.4 ± 1.2 cm and

Kiiminki 47.2 ± 1.1 cm; $F_{1,191} = 3.74$, $P = 0.054$) and tended to have more stems (9.5 ± 0.4 and 8.5 ± 0.4 , $F_{1,191} = 3.17$, $P = 0.077$) (measured before the treatments were carried out in 1998), we used the origin as a blocking factor (here considered as a fixed factor) in further analyses. Our intention is not, however, to study closer the possible differences between the two populations, and hence, we did not include origin in the interactions terms in the models (cf. Newman et al. 1997). MANOVA was performed separately for those variables measured in all studied plants (Table 1a: height, stem number, umbel number, above-ground weight and root weight) and for those parameters measured only in a subsample of studied plants (Table 1b: umbellule branch number, umbel branch number, number of seeds per umbel, seeds per fertile plant and seed weight). We present here the MANOVA results, but instead of all the individual ANOVA results we present planned contrasts (control versus other treatments performed separately for plants treated during 1 or 2 years) performed under the above ANOVA model.

For the data from the field, we performed the MANOVA using two-way factorial design with cutting treatment (control, simulated flower herbivory and simulated grazing) and competition (neighbouring plants mown or left intact) as grouping factors. The test was performed separately for those variables measured in all studied plants (Table 3a: height, stem number and above-ground weight) and those measured only in plants that produced flowers (Table 3b: number of umbels, number of umbellules per umbel, number of umbellule branches and number of seeds per umbel as well as number of seeds per plant). In the field data no significant cutting effect was found (Table 3), and hence we did not continue to ANOVA and planned cutting treatment comparisons. Seed production and initial height, number of leaves and stem diameter of the plants that re-flowered in 1999 were compared to those that postponed flowering to 2000 by means of one-way ANOVA.

The results of garden- and field experiments are presented in Table 2 where we tabulated vegetative and reproductive traits case-wise and calculated ratios expressing relative performance of plants under different treatments in the following formula: $100 \times [(treatment - control)/control]$. Thus, positive values indicate overcompensation and negative values, undercompensation (sensu Belsky 1986), respectively.

Table 1 MANOVA results (Pillai's trace) for parameters: (a) height, stem number, umbel number, above-ground weight and root weight, (b) umbellule branch number, umbel branch number, number of seeds per umbel, seeds per fertile plant and seed weight

Effect	Pillai's trace	df H/E	F	P
(a)				
Origin	0.081	5/159	2.79	0.019
Year	0.512	5/159	33.42	<0.001
Injury	0.227	10/320	4.09	<0.001
Injury × Year	0.050	10/320	0.814	0.62
(b)				
Origin	0.109	5/61	1.49	0.208
Year	0.454	5/61	10.16	<0.001
Injury	0.408	10/124	3.17	0.001
Injury × Year	0.117	10/124	0.77	0.654

Table 2 Comparison of the simulated flower herbivory and simulated grazing experiments in the garden and the field experiment (%-change compared to control (i.e. intact) plants within that particular year)

Year:	Garden experiment (1998–1999)				Field experiment (1999–2000)			
	Flower removal		Simulated grazing		Flower removal		Simulated grazing	
	1998	1999	1998	1999	2000	2000	2000	2000
Vegetative traits								
Stem height	4	18	-6	3	1	8	3	-1
Stem number	12	57*	-3	32	11	-29	11	-15
Above-ground biomass	1	47*	-21	16	-4	-25	3	-25
Below-ground biomass	16	46*	7	43*	-	-	-	-
Root:shoot ratio	12	-47*	48	-22	-	-	-	-
Reproductive traits								
Umbel number	-24	24	-39	-7	14	-4	25	-2
Number of umbel branches	-7	-10	-9	-12	-3	8	14	-10
Number of umbellule branches	-8*	-6	-12*	-13*	7	12	17	-2
Seeds per umbel	-14	-11	-20*	-23*	3	20	20	3
Seeds per fertile plant	-26	-23	-36	-55*	-1	18	9	5
Seed weight	-4	1	-13*	-11	-	-	-	-

Note that in the garden the treatment effects in 1999 indicate plant responses to repeated simulated flower herbivory and grazing performed both in 1998 and 1999. Asterisk (*) denotes a statistically significant difference ($P < 0.05$, measured from the basic data) between the control and the particular treatment

The impact of injury level and origin on plant phenology during the two seasons in the garden experiment was tested using a four-way contingency table, where the grouping factors were phenological stage (I–IV, see above) of the plant, treatment, population and time of observation, whereas the dependent variable was the number of the plants in each class. The effects of grouping factors (and their interactions) were examined using a loglinear model. Because the phenology in 1998 was monitored three times and in 1999 only twice, the examination described above was performed separately for each year. We performed the analysis using SPSS software's "Loglinear model selection" option suitable for hierarchical loglinear models to multidimensional cross tabulations using an iterative proportional-fitting algorithm. Iterations start from a saturated model of which factors (and their interactions) are gradually dropped out until the best-fit model has been reached. The best-fit model only has factors (or factor combinations) that explain the observed frequencies. Since we are testing (with log-likelihood coefficient G^2) the compatibility between observed and expected frequencies, a good model takes a low

G^2 -value (and high P -value). As the data included zero frequencies, a constant (0.5) was added to each frequency before the analysis (cf. Caswell 1989). Statistical differences between the number of dead, sterile and fertile plants in different treatments in 1999 was tested with the G^2 -test.

Results

Growth and reproductive success in the garden

In the garden experiment (1998–1999), the treatments significantly affected plant growth and shoot structure (Table 1a) and reproductive parameters (Table 1b). In the first year (1998), there were only minor treatment effects on stem height and number (Table 2). Neither flower removal nor simulated grazing had any marked effects on above- or below-ground biomass (Table 2). In the second year, flower removal increased above- and below-ground biomass by 47 and 46%, respectively, and simulated grazing by 16% (NS) and 43% compared to control plants.

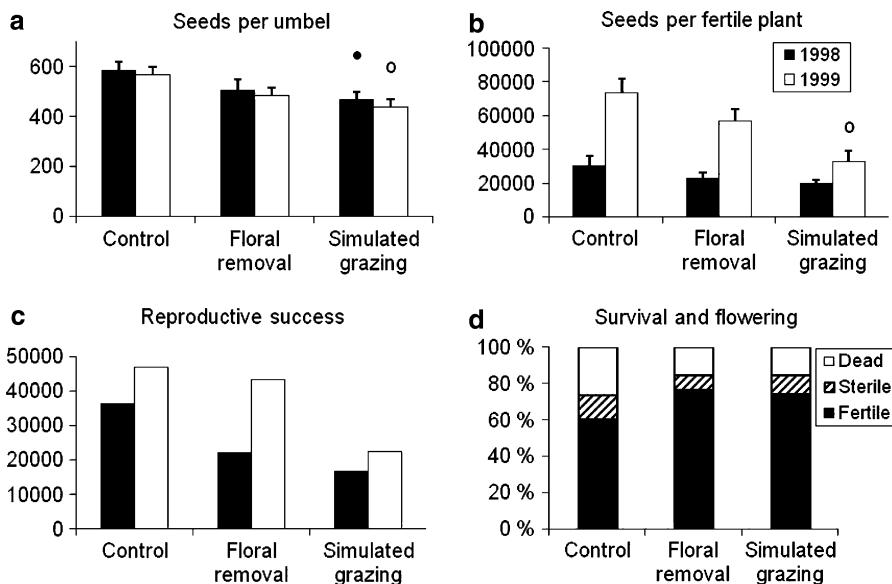


Fig. 3 Treatment effects on reproductive characteristics and fitness of *Pimpinella saxifraga* in the garden during the years 1998 and 1999 (mean \pm SE): **a** mean seed number per umbel, **b** mean seed number of fertile plants, **c** reproductive success taking account probability of survival and flowering and mean seed number of fertile plants, **d** proportion of dead, sterile, and fertile plants in 1999. In **a** and **b** the bars denoted by a dot

differ statistically from the unclipped control plants of the corresponding treatment group (filled = 1998, open = 1999, $P < 0.05$). Number of replicates for each treatment group (control, floral removal and simulated grazing) are: 1998: 11, 11 and 10 and for 1999: 10, 11 and 12, respectively. For arrays used in calculation of reproductive success and survival and flowering, see Material and methods

Reproductive parameters showed a different pattern as most of the treatment effects were negative (Table 2). The adverse effects of simulated grazing were slightly more pronounced than the effects of flower removal, throughout the hierarchical structure of the inflorescences (Fig. 2a-d). The pattern was the same in the seed number per umbel (Fig. 3a), the seed production of fertile plants (Fig. 3b), and seed weight (Table 2). When the proportion of dead and sterile plants was taken into account for evaluating reproductive success (total seed production per plant), this did not qualitatively change the result (Fig. 3c), because all plants survived to flowering in 1998 and sterility and mortality did not significantly differ between the treatment groups in 1999 (Fig. 3d; $G^2 = 2.4$, $df = 4$, $P = 0.662$).

Phenological effects in the garden

The injured plants flowered and set seed considerably later than the uninjured controls and this delay increased with injury intensity. In the first year, the peak of flowering was around 21 July among control

plants, while the injured plants were still in the bud-stage (stage I). On 20 August, 37% of control plants had passed flowering but the fruits were still immature (stage III) while injured plants had just started to flower (stage II). On 5 October, 90, 50, and 30% of the control, flower removed, and plants subjected to simulated grazing, respectively, had fully matured seeds (stage IV). In the second year, the same pattern was repeated. The injured plants had their flowering peak approximately 4 weeks later than the controls. On 20 August, control and flower-removed plants had produced immature fruits (stage III), but a half of the plants subjected to simulated grazing were still flowering (stage II). The observed frequencies could be best explained by the log linear model of the three factor interaction “phenological stage \times injury level \times observation time” in the both years (1998: $G^2 = 11.17$, $df = 45$, $P = 1.00$; 1999: $G^2 = 6.19$, $df = 30$, $P = 1.00$). Hence, the effect of clipping on the phenological stage was dependent on the time of observation, but the origin of populations did not significantly explain plant phenology (i.e. this factor could be dropped from the model).

Table 3 MANOVA results (Pillai's trace) for field parameters: (a) height, stem number and above-ground weight, (b) number of umbels, number of umbellules per umbel, number of umbellule branches and number of seeds per umbel as well as number of seeds per plant

Effect	Pillai's trace	df H/E	F	P
(a)				
Injury	0.038	6/152	0.494	0.812
Competition	0.138	3/75	3.99	0.011
Injury × Competition	0.047	6/152	0.606	0.726
(b)				
Injury	0.137	10/134	0.983	0.462
Competition	0.113	5/66	1.686	0.15
Injury × Competition	0.083	10/134	0.578	0.829

Compensation ability in the field

In the field experiment (1999–2000), *P. saxifraga* could hardly compensate at all by repairing injuries in the first summer. Although some injured plants re-flowered in most treatment groups (40% in flower removal + no competition, 47% in flower removal + competition, 0% in simulated grazing + no competition and 7% in simulated grazing + competition), only two flower-removed plants managed to produce seeds. When the plants were left untouched during summer 2000, no statistically significant effects of previous flower or shoot damage were detected in vegetative (Table 3a) or reproductive variables (Table 3b). In the presence of competition, there was a slight decreasing trend in the number of stems, above-ground biomass and number of umbels in previously injured plants (Table 2).

Competition as such reduced plant growth (Tables 2 and 3a), but had no effect on reproductive performance (Table 3b). There was no indication of the cost of reproduction among plants recovering from previous flower removal since the seed production in 2000 of the plants that re-flowered in 1999 was the same (1378 ± 293 , mean \pm SE) as those that postponed flowering until the following season (1373 ± 261 , one-way ANOVA: $F_{1,28} < 0.001$, $P = 0.991$). At the time of injury in 1999, these two groups of plants did not differ from each other in relation to initial height (flowered 51.1 ± 1.8 cm vs. postponed 54.1 ± 2.0 cm; $F_{1,28} = 1.217$, $P = 0.279$), number of leaves (flowered 10.2 ± 1.2 vs. postponed 10.2 ± 1.9 ; $F_{1,28} < 0.001$, $P = 0.998$)

or stem diameter (flowered 3.2 ± 0.1 mm vs. postponed 3.2 ± 0.2 mm; $F_{1,28} = 0.069$, $P = 0.794$).

Discussion

Immediate repair or postponed reproduction?

In perennial plants, the fitness consequences of herbivory are rather complicated since the costs of injury on current and/or future reproductive success are confounded by the costs of reproduction. If the plant escapes grazing altogether (Fig. 1), the optimal reproductive tactics include its investment in current reproduction and its expected future reproductive success. In conditions where reproduction is costly, the plant has to trade current reproduction for expected future reproduction (e.g. Obeso 2002). A possible strategy is that the plant invests resources in current reproduction and, as a consequence, suffers costs in the survival and future fertility. Alternatively, the plant may totally refrain from current reproduction and thus it will avoid the potential costs of reproduction. The injured plants may also postpone their reproduction until the following growing season, and therefore they will suffer from the costs of herbivory but not from the costs of reproduction. Thus the overall effect of herbivore injury on reproductive success in the second season could be positive if, as suggested by Veneczel and Aarssen (1998), by postponing reproduction, the injured plant can avoid the costs of reproduction and can accumulate more resource reserves for the following growing season. This notion fits rather well with the responses of *P. saxifraga* in our field experiment. The control plants escaped the costs of grazing, but suffered from the potential costs of reproduction. The plants which were subjected to simulated grazing postponed their reproduction until the following growing season (2000). They thus suffered the cost of grazing in terms of reproductive failure in 1999. However, 1 year later, the previously grazed and control plants performed equally which does not support the presumption that there is a cost of reproduction. In the case of flower removal, the situation was similar except that 40–47% of the injured plants re-flowered in the same year that they were damaged (1999), but could not produce seeds. The failure in setting seeds may result from missing peak of pollinator availability (e.g. Juenger and

Bergelson 1997) or insufficient response time before the end of the growing season (Trumble et al. 1993; Venecz and Aarsen 1998). Anyhow, it is likely that the reproductive failure is related to a shift in the phenology of the injured plants that started to re-flower after injury, as we observed in the garden conditions.

The fact that compensatory reproduction is likely to fail in the field suggests that the costs of reproduction need not be high in order to select for postponed reproduction in *P. saxifraga*. We found no evidence supporting the cost of compensatory reproduction, since the plants which re-flowered in the first season (1999) in the field produced seeds in the following summer as equally well as the plants that postponed flowering until the following summer. There was no indication that, e.g. differences in plant size could have confounded this comparison.

Tolerance to repeated grazing

The field experiment indicates that both simulated flower herbivory and grazing have direct costs on the reproductive success of *P. saxifraga* in the same growing season. There are no delayed costs of grazing in the following growing season, or they are outweighed by the costs of reproduction when comparing ungrazed flowering plants and grazed plants which fail to flower due to grazing. From these results, one could expect that repeated grazing in subsequent years will more or less completely suppress flowering of *P. saxifraga*. However, the results of our garden experiment were not in line with this expectation. In the garden, the plants tolerated both flower removal and simulated grazing well. The treatments did not suppress the current reproduction as dramatically as in the field. In the garden, vegetative traits showed mainly positive response to the injury, whereas seed production declined and this decline was more pronounced in plants subjected to repeated grazing. As a result of the greater current reproductive investment one could expect that control plants would have accordingly suffered a greater cost of previous reproduction in the following season (Venecz and Aarsen 1998). Indeed, in the second season, the control plants had 47 and 16% lower above-ground biomass and 47 and 43% lower below-ground biomass compared to flower removal and simulated grazing. They also produced 24% fewer umbels than plants under flower removal, but 7% more umbels than plants subjected to simulated

grazing. The costs of previous reproduction may also be realized as lower survival rates of the control plants compared to the flower removal treatment, or to a greater probability of remaining sterile in the second season. The figures supported this idea (mortality: 26% vs. 16%; sterility: 13% vs. 8% in control and flower removal, respectively), but the differences were not statistically significant.

Resource allocation and availability

Both flower removal and simulated grazing promote the increase of below-ground biomass. This in turn made extensive compensation of above-ground structures possible. This observation strongly supports the notion that the ability to shunt carbon reserves from root to shoot is one of the most central mechanisms of herbivore tolerance in perennial plants (e.g. McNaughton 1983; Rosenthal and Kotanen 1994; McNaughton et al. 1998; Strauss and Agrawal 1999; Wise et al. 2008). In our case, it is possible that the reserves are related to the costs of reproduction which are, at least partially, avoided in injured plants compared to the uninjured flowering plants. Alternatively, the resources are gained as a result of improved shoot growth (i.e. increased photosynthetic biomass) in the year of injury. The increased vegetative growth enhances the ability to assimilate carbon and acquire mineral nutrients (through increased sink demand) compared to uninjured controls (Sadras 1996). The acquired resources are stored in the root and in the following season are invested in above-ground growth and reproduction (Venecz and Aarsen 1998). In the garden experiment, flower removal and simulated grazing had no effect on shoot or root growth in the first year, but in the second year flower removal improved both shoot and root biomass and simulated grazing root biomass compared to the control plants.

It is interesting to note that the plants grown in the garden attempted to repair the injuries rather than postpone their reproduction until the following season. This is consistent with the fact that tolerance via compensatory growth seems to be more beneficial in the resource-rich garden conditions than in the field (cf. Maschinski and Whitham 1989; Huhta et al. 2000a). If the costs of reproduction were greater among the uninjured plants in the garden than in the field conditions, this would accordingly favour compensatory growth in the garden compared to the field conditions.

We can neither confirm nor reject this possibility in the case of *P. saxifraga*. In our experiment, the compensation responses are linked to soil fertility. In the garden, during the second year the plants compensated seemingly well despite that they were injured for the second time in their lifetime. Also control plants maintained their high productivity, although their combined sterility and mortality was over 10% units higher compared to both clipping treatments. The control plants in the garden (in 1999) were about 14 times heavier (46.2 g vs. 3.34 g) and produced nearly 50 times more seeds (69,808 vs. 1460) than plants in the field in a non-competitive environment. Comparison of the results from the field experiment to the results from the garden experiment suggests that the compensation capacity is resource limited: after herbivory damage in a resource-poor environment reproduction is postponed until the following growing season, but not necessarily in resource-rich environments. In the field, there was also a slight decreasing trend in the number of stems, above-ground biomass and number of umbels in previously injured plants in the presence of competition. There was not, however, any statistically significant injury × competition interaction on plant performance.

In all, our results suggest that polycarpic plants may more readily allocate resources to immediate compensation in the resource-rich environment (garden) and postpone reproduction until the next growing season in the resource limited field conditions. Consequently, injured polycarpic perennials may either postpone their reproduction until the following summer or try to compensate immediately after the injury during the first summer (Fig. 1). Our results showed that the reproductive tactics that the plants use depends on resource availability. In the field, grazed plants postponed their reproduction until the following growing season and in the second season the previously injured plants performed equally well as the control plants. The plants that re-flowered during the first season could not produce seeds which suggest a time-limitation for seed maturation. In the resource-rich garden conditions, on the other hand, plants compensated fully for simulated flower herbivory in vegetative parameters but seed set was reduced. Hence, it seems that after herbivory damage in a resource-poor environment reproduction is postponed until the following growing season, but in resource-rich environments plants seem to be ready to take the chance to compensate already in the first season.

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Differential herbivory tolerance of dominant and subordinate plant species along gradients of nutrient availability and competition

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Abstract We tested whether differences in the herbivory tolerance of plant species is related to their abundance in grassland communities and how herbivory and nutrient availability affect competitive balances among plant species through changes in their tolerance. The experimental approach involved a simulated grazing treatment (clipping) of two competitive grass species (*Arrhenatherum elatius* and *Holcus lanatus*) and two subordinate forb species (*Prunella vulgaris* and *Lotus corniculatus*) along a gradient of nutrient availability and under conditions of competition. Total standing, aboveground, root,

and regrowth biomass were evaluated at the end of the experiment as an estimate of the capacity to compensate for twice removing aboveground biomass at different nutrient levels (NPK). Although clipping had a more pronounced negative effect on dominant plant species (*Arrhenatherum* and *Holcus*) than on subordinate species, the negative effects on dominant species were offset by the application of fertilizer. The combined effect of fertilizer and competition had more negative effects on the performance of *Lotus* and *Prunella* than on the dominant species. In terms of competition, the regrowth ability of *Arrhenatherum* and *Holcus* increased with the application of fertilizer, while the opposite pattern was observed for *Lotus* and *Prunella*. The addition of fertilizer has a positive effect on both grass species in terms of growth in clipped pots and competition, while subordinate species did not respond to the addition of fertilizer to the clipped pots and were negatively affected by competition with both grass species. The results suggest (1) that species replacement towards subordinate species as a function of herbivory is partially dependent on the herbivory tolerance of that species, (2) competitive relations between competitive grass species and subordinate forb species change under different environmental conditions, and (3) although grazing disturbance significantly influences competitive relations in favor of less competitive species, increasing nutrient levels counteract the negative effect of grazing on dominant competitive plant species.

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Introduction

Herbivory is one of the major forces shaping the composition and structure of plant communities throughout the world (Briske 1996; de Mazancourt et al. 1999; Van der Wal et al. 2000; Olff et al. 2002). Nevertheless, there is still a lack of knowledge on the underlying mechanisms by which herbivory induces species replacement due to the fact that both direct and indirect feedbacks with soil conditions and antagonistic species contribute to the complex mechanism of this force (Anderson and Briske 1995; Van der Wal et al. 2000; Hendon and Briske 2002).

Grazing may affect plant communities directly by the selective and differential removal of plant tissues or species, and indirectly when the grazing of dominant species reduces their vigor and persistence. The latter favors the establishment of less competitive species (subordinate plant species; after Briske 1996) that may be able to cope with grazing by the partial or full compensation for the loss of vegetative biomass or by avoidance strategies (Paige and Whitham 1987; Whitham et al. 1991). It has been suggested that tolerance to herbivory plays an important role in explaining the relative abundance patterns of plant species in grassland where grazing animals are present (Crawley 1990; del-Val and Crawley 2004, 2005). However, the relative importance of herbivory tolerance and competitive ability in the interactions between dominant and subordinate species is still unknown. A potentially important mechanism by which competitive interactions among plant species may be altered in response to grazing is the differential ability of species to regrow after defoliation (Prins et al. 1989; Crawley 1997). This ability implies a capacity to compensate for tissue loss due to a high relative growth rate, reallocation of resources from roots to shoots, the availability of rapidly resprouting meristems, or an increase in nutrient uptake (Belsky et al. 1993; Briske 1996). Species with these compensatory attributes gain a competitive advantage over associated species that grow more slowly after defoliation (Grime 2001).

It is also widely acknowledged that a plant's tolerance towards herbivore-induced damage is affected by abiotic and biotic factors, such as water availability or soil nutrients and competition (Maschinski and Whitham 1989; Rosenthal and Kotanen 1994; Wise and Abrahamson 2005). In particular, soil nutrient availability can be expected to play an important role in compensatory responses because nutrient levels determine biomass production and shoot growth patterns as well as foliage quality (Huisman et al. 1999; Van der Wal et al. 2000). The 'compensatory continuum hypothesis' (CCH) (Maschinski and Whitham 1989) proposes that the ability of plants to compensate for herbivory can be situated along a continuum, depending on the levels of nutrients and competition. The CCH additionally predicts that the probability of compensation for herbivory (i.e., tolerance) increases with increasing resource levels and decreasing competition. Competitive species generally have the ability to rapidly allocate resources to aboveground parts, which enables them to reach high aboveground biomass more rapidly than less competitive species. However, this allocation capability depends on the amount of nutrients available in the soil (Tilman 1982; Huisman et al. 1999; Kuijper et al. 2005). Consequently, under conditions of higher nutrient availability levels in undisturbed situations, low-competitive plant species may be rapidly excluded. Grazing disturbance can be expected to significantly influence this competitive relation, since the relative profit of producing large quantities of aboveground biomass is undermined by the biomass removal by herbivores. However, increasing nutrient levels counteracts the effect of grazing and may once again favor dominant competitive plant species. Most tests of herbivory on native plants have evaluated the direct effects of consumption on plant growth or fitness. However, the joint effect of herbivory and plant competitive interaction along a gradient of productivity has been neglected.

We tested the following hypotheses empirically under standardized conditions: (1) subordinate plant species have a great tolerance to grazing than dominant plant species in terms of regrowth ability after grazing; (2) the tolerance ability of plant species is reduced under competition and increases with nutrient availability; (3) despite the fact that grazing may induce a shift towards a less competitive species, increased nutrient availability may counterbalance

this effect (i.e., competitive relations between competitive dominant species and subordinate species change with different levels of nutrient availability).

The experimental approach involved a simulated grazing treatment (clipping), competition and a gradient of nutrient availability on two plant species known as competitive dominant species (*Arrhenatherum elatius* and *Holcus lanatus*) as well as on two subordinate plant species (*Prunella vulgaris* and *Lotus corniculatus*). The former species are known to be partially replaced by the latter in the presence of large herbivores in coastal dune grasslands (Tahmasebi Kohyani et al. 2008a).

Materials and methods

Four typical grassland species, *Arrhenatherum elatius*, *Holcus lanatus*, *Prunella vulgaris*, *Lotus corniculatus*, were used as model species to investigate tolerance ability to herbivory in relation to competition and nutrient availability in a greenhouse experiment. All four species were grown from seeds purchased from Herbiseed (Ghent, Belgium). All seeds were germinated in trays following which three individuals were transplanted—one species per pot—into 1.5-l treatment pots containing 80% sand and 20% peat. For each plant species, we used a factorial design with five environmental treatments: two levels of clipping (unclipped, clipped twice) and three levels of fertilization (control, addition of 2 and 5 g/kg fertilizer, respectively; N:P:K, 1:1:1). In the experiments aimed at investigating competitive relations among the competitive grass species and the subordinate forb species under the above-mentioned conditions, each experimental pot was planted with three individuals of one of the grass species and three individuals of one of the subordinate species. Every treatment was repeated three times. All treatments were randomly assigned, and grazing was simulated by clipping twice 80% of the aboveground biomass with a scissors with a time interval of 20 days between clippings (compare Bel-sky et al. 1993). At the end of the experiment, the aboveground biomass was separated from the roots and the roots washed thoroughly to eliminate soil particles. Above- and belowground biomass were dried in an oven at 75°C for 48 h and then weighed. The experiment started in early April and was terminated 5 months later, in early August 2007.

The data were analyzed by General Linear Models (GLM) using total biomass, aboveground biomass, root biomass, and root:shoot ratio as response variables for each species separately. In order to compare the tolerance ability of each plant species, first plant regrowth was calculated for each species as the final biomass after two clippings for a given treatment divided by that for the control. A GLM was then run with plant regrowth as a response variable and competition, nutrient, and species as fixed factors. A backward procedure was used until the model contained only main effects and significant interaction terms. Differences between treatments within each nutrient level and competition were tested using the Tukey multiple comparison test. All statistical analyses were conducted in SPSS ver. 13 (SPSS, Chicago, IL).

Results

Total aboveground and root biomass of all four species were negatively affected by clipping (Table 1). Clipping increased the root:shoot ratio of *H. lanatus* and *A. elatius* ($df = 1$, $F = 37$, $P = 0.000$ and $df = 1$, $F = 5$, $P = 0.03$, respectively) but no effect was observed for *L. corniculatus* ($df = 1$, $F = 0.7$, $P = 0.4$) and *P. vulgaris* ($df = 1$, $F = 0.06$, $P = 0.8$). The responses of *Holcus* and *Arrhenatherum* to fertilizer were different from those of *Prunella* and *Lotus*, with a less pronounced increase in above-ground biomass for the latter (Fig. 1). Root biomass of *Arrhenatherum* and *Prunella* increased with fertilizer application, while no changes were observed for *Holcus* and *Lotus* (Table 1).

All measured variables (total standing, above-ground and root biomass) for *Lotus* and *Prunella* were negatively affected by competition with *Holcus* as well as with *Arrhenatherum*, with a more pronounced effect for *Holcus* (Table 1, Fig. 1). In contrast, both *Arrhenatherum* and *Holcus* increased in biomass when plants were grown in competition with *Lotus* and *Prunella* (Table 1, Fig. 1). We also found significant interactions between treatments for all species (Table 1). For *Arrhenatherum*, the significant interaction between grazing and fertilization was due to a larger positive effect of fertilization on total aboveground and root biomass for unclipped compared to clipped individuals (Fig. 1). The same

Table 1 Results (F statistics and P value) of the General Linear Model testing for the effect of grazing, fertilization, competition, and their interaction on different response

variables: total, aboveground, and root biomass and root: shoot ratio for two dominant and two subordinate plant species

Experimental parameters	Grazing (G)			Fertilization (F)			Competition (C)			G × F			G × C			F × C			G × F × C		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
<i>Arrhenatherum elatius</i>																					
Total standing biomass	1	353.0	0.00	2	16.0	0.00	2	2.0	0.20	2	15.0	0.00	2	3.2	0.06	4	1.0	0.30	4	1.0	0.30
Aboveground biomass	1	248.0	0.00	2	15.0	0.00	2	4.5	0.02	2	14.0	0.00	2	5.2	0.01	4	1.6	0.20	4	1.6	0.18
Root biomass	1	239.0	0.00	2	5.0	0.01	2	0.8	0.40	2	4.5	0.01	2	0.2	0.80	4	0.1	1.00	4	0.2	1.00
Root/shoot ratio	1	5.0	0.03	2	5.0	0.01	2	18.0	0.00	2	0.7	0.50	2	9.2	0.00	4	3.0	0.02	4	2.0	0.05
<i>Holcus lanatus</i>																					
Total standing biomass	1	525.0	0.00	2	5.0	0.01	2	7.0	0.00	2	5.4	0.00	2	8.2	0.00	4	1.8	0.13	4	1.9	0.13
Aboveground biomass	1	586.0	0.00	2	13.0	0.00	2	10.0	0.00	2	13.0	0.00	2	11.0	0.00	4	1.2	0.30	4	1.3	0.30
Root biomass	1	231.0	0.00	2	1.5	0.25	2	1.8	0.17	2	1.2	0.30	2	2.5	0.10	4	2.2	0.08	4	2.3	0.08
Root/shoot ratio	1	37.0	0.00	2	18.0	0.00	2	5.0	0.01	2	0.7	0.50	2	1.5	0.23	4	6.0	0.00	4	6.0	0.00
<i>Lotus corniculatus</i>																					
Total standing biomass	1	52.0	0.00	2	3.0	0.05	2	54.0	0.00	2	3.0	0.06	2	35.0	0.00	4	0.8	0.54	4	1.2	0.34
Aboveground biomass	1	59.0	0.00	2	4.0	0.02	2	55.0	0.00	2	4.5	0.05	2	39.0	0.00	4	1.5	0.21	4	1.7	0.15
Root biomass	1	34.0	0.00	2	1.2	0.30	2	42.0	0.00	2	1.2	0.30	2	24.0	0.00	4	0.0	1.00	4	0.1	0.80
Root/shoot ratio	1	0.7	0.40	2	8.0	0.00	2	10.0	0.00	2	10.0	0.00	2	7.0	0.00	4	5.5	0.00	4	7.0	0.00
<i>Prunella vulgaris</i>																					
Total standing biomass	1	326.0	0.00	2	4.2	0.02	2	325.0	0.00	2	1.0	0.37	2	273.0	0.00	4	5.0	0.00	4	7.0	0.00
Aboveground biomass	1	506.0	0.00	2	2.8	0.07	2	502.0	0.00	2	1.0	0.30	2	422.0	0.00	4	13.5	0.00	4	15.0	0.00
Root biomass	1	121.0	0.00	2	5.0	0.01	2	122.0	0.00	2	1.2	0.30	2	102.0	0.00	4	0.8	0.50	4	1.5	0.22
Root/shoot ratio	1	0.0	0.80	2	3.5	0.04	2	22.0	0.00	2	3.3	0.06	2	3.0	0.06	4	10.0	0.00	4	10.0	0.00

pattern was observed for *Holcus*, except for root biomass, which showed an equal increase after the addition of the fertilizer for both clipped and unclipped plants. In contrast, this interaction was not significant for *Lotus* and *Prunella* for any of the response variables (Fig. 1). The interaction between clipping and competition was also significant for total and aboveground biomass of both *Arrhenatherum* and *Holcus* in a way that the aboveground biomass increased in the presence of *Lotus* when they were unclipped but remained unchanged when the plants were clipped (Fig. 1). Clipping also interacted with the competition for all response variables in the case of *Lotus* and *Prunella*: plants were more negatively

affected by clipping in the presence of competition with the two other species (*Arrhenatherum* and *Holcus*) than when they were grown without competition (Table 1, Fig. 1).

A comparison of the tolerance ability of four plant species (defined as plant regrowth after two clippings divided by control) revealed that the two subordinate plant species (*Lotus* and *Prunella*) had a higher amount of regrowth than the dominant species (*Arrhenatherum* and *Holcus*) (Table 2, Fig. 2). The negative effect of clipping on regrowth of the dominant species was offset by the addition of fertilizer (indicated by the interaction between fertilization and species; Table 2, Fig. 2). The combined

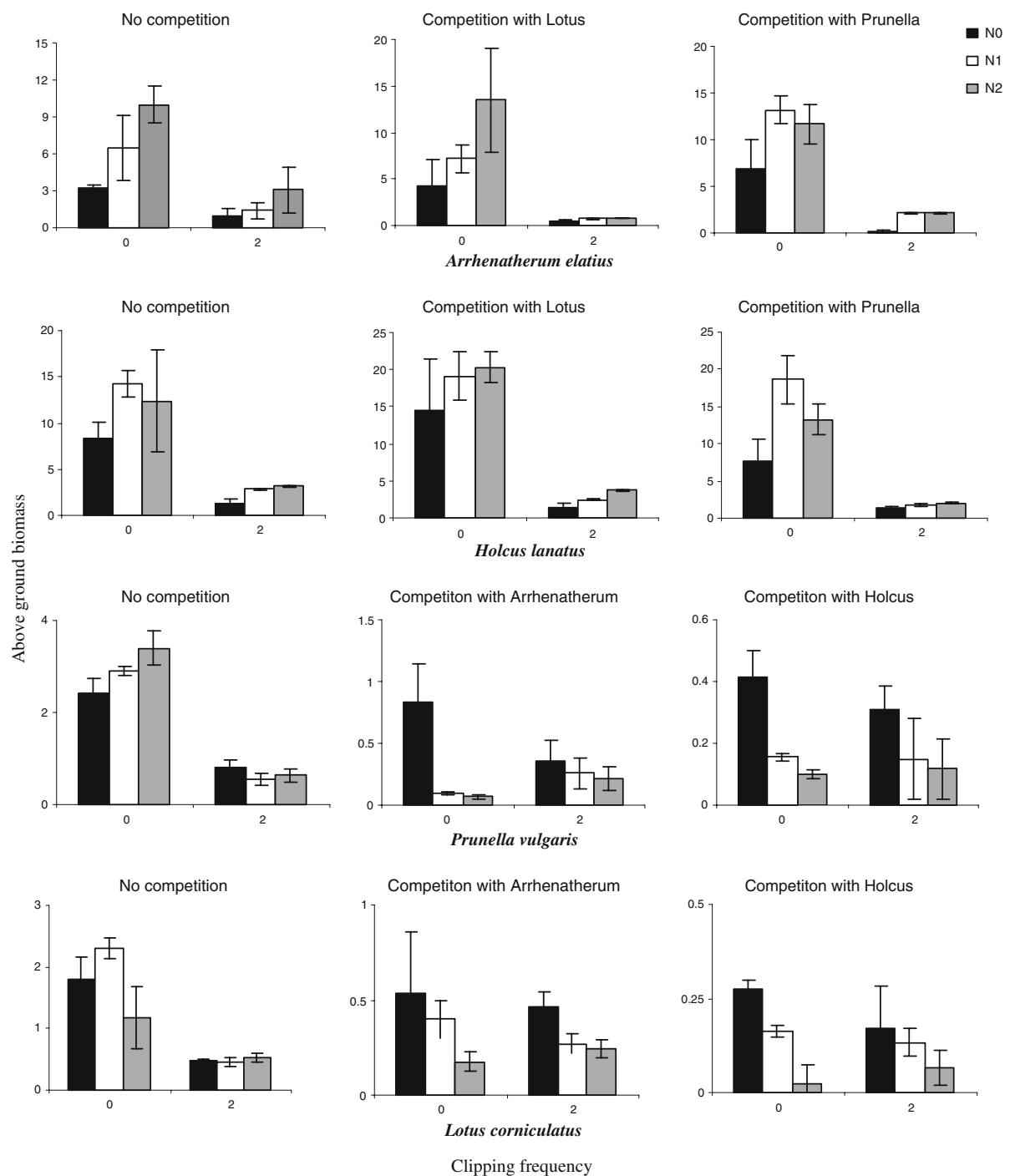


Fig. 1 The combined effect of grazing, fertilization and competition on aboveground biomass (means \pm 1 SD) of two dominant species (*Arrhenatherum elatius* and *Holcus lanatus*) and two subordinate species (*Prunella vulgaris* and *Lotus corniculatus*). N0, N1, N2 No fertilization and addition of 2 and 5 g/kg fertilizer (N:P:K, 1:1:1), respectively. Note the different scales of the x-axis

effect of adding fertilizer and competition had more negative effects on the regrowth of *Lotus* and *Prunella* than on the dominant species (indicated by

Table 2 Results (*F* statistics and *P* value) of the General Linear Model testing the comparative tolerance ability (in terms of plant regrowth) of plant species after two clippings along the gradients of nutrient availability and competition

	Plant regrowth		
	df	<i>F</i>	<i>P</i>
Species	3	39.93	0.00
Fertilization	2	0.27	0.75
Competition	4	30.20	0.00
Fertilization × species	6	3.39	0.00
Competition × species	4	6.09	0.00
Fertilization × competition	8	1.24	0.28
Fertilization × competition × species	8	2.99	0.00

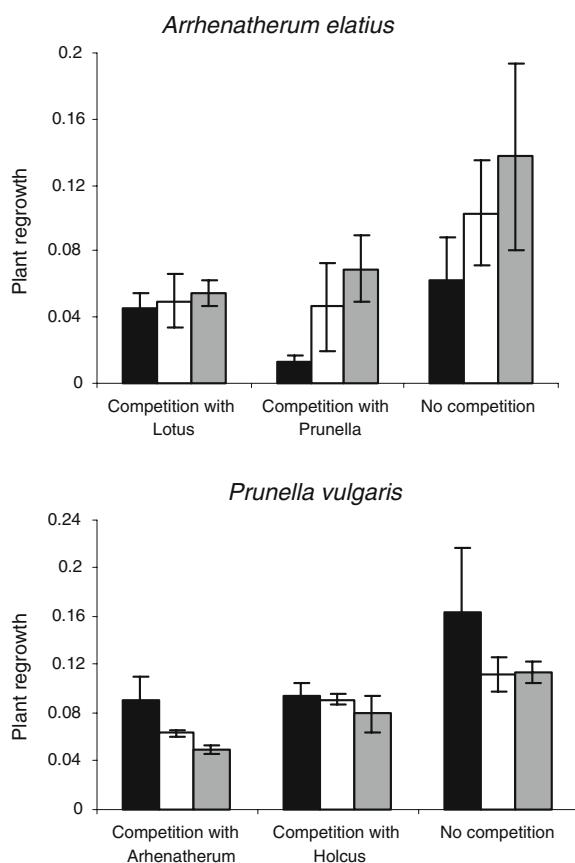


Fig. 2 Comparison of the tolerance ability (defined as plant regrowth after two clippings divided by control; means \pm 1 SD) of two dominant species (*A. elatius* and *H. lanatus*) and two subordinate species (*P. vulgaris* and *L.*

corniculatus) after two clippings along the gradient of fertilization and competition. *N0*, *N1*, *N2* No fertilization and addition of 2 and 5 g/kg fertilizer (N:P:K, 1:1:1), respectively. Note the different scales of the *x*-axis

Discussion

The aim of this study was to compare the tolerance of four plant species to grazing along gradients of nutrient availability and competition. As expected, the species-specific response in terms of biomass compensation was different for competitive and subordinate species. The competitive species (*H. lanatus* and *A. elatius*) had a higher biomass than the subordinate species at the end of the experiment

(Fig. 1). However, when compensated biomass was expressed as the ratio of total aboveground biomass that was produced by the plant (i.e., plant regrowth), subordinate species were able to compensate better than both competitive grass species (Fig. 2). The limited ability of regrowth of both *Arrhenatherum* and *Holcus* relative to *Lotus* and *Prunella* under repeated defoliation correlates with the observation that the former are significantly reduced when large herbivores are present, as recorded by Tahmasebi Kohyani et al. (2008a, b) in dry coastal dune grassland. This is in contrast with the suggestions made in some studies that subordinate species have a lower or similar tolerance to defoliation as dominant species (Pacala and Crawley 1992; Belsky et al. 1993; Hendon and Briske 2002). However, this species-specific difference in compensation may not necessarily result in species replacement since the amount of biomass produced by subordinate species was not high enough to override any of the competitive species. This result is consistent with the theoretical model suggested by Ritchie and Olff (1999) that if in a system herbivores consume different plant species (simulated here by clipping both dominant and subordinate species) that compete for the same limiting resource, the direct negative effect of grazing on each species is balanced by the indirect positive effect due to decreased competition. As a consequence, no effect on species replacement would be observed. Tolerance mechanisms are, however, not the only ones that contribute to shifting species composition. Most experimental studies have shown that preferential grazing on dominant species suppressed their greater competitive ability, rather than their differential tolerance ability. Based on these observations, many scientists have argued that the effect of herbivory should be related to its differential impact on competing species and may in fact be independent of the absolute amount of damage inflicted (Anderson and Briske 1995; Augustine and McNaughton 1998). This is likely to be the primordial process behind herbivore-induced species shifts (Fowler and Rausher 1985; Ellison 1987; Ash and McIvor 1998).

Several studies have noted that a higher compensation might occur on soils with a higher amount of nutrient availability than when environmental constraints limit plant responses and compensatory growth (CCH hypothesis; Maschinski and Whitham 1989; Whitham et al. 1991; Huhta et al. 2000).

Consumer effects can often be observed along gradients: productivity gradients, elevational gradients, and local gradients (Lauda et al. 1990). Plants with higher nutrient requirements or growth rate are most likely vulnerable to foliage losses (Lauda et al. 1990; Augustine and McNaughton 1998). The quantitative impact of herbivory is then related to factors that determine the individual plant's ability to compensate for herbivore-caused foliage losses, such as its nutritional status or specific growing conditions (Lauda et al. 1990; Huhta et al. 2000; Van der Wal et al. 2000; Wise and Abrahamson 2005). Thus, generally, for plants with high resource requirements, herbivory should influence resource-mediated interactions when the limiting resource is scarce. As expected, in our study, the increase in the biomass of the dominant species with increasing nutrient levels was relatively higher than that of the subordinate species, indicating that the growth of the former is predominantly nutrient-limited, while it is less so for subordinate species. Interestingly, the species used as models for both groups responded differently to the addition of fertilizer when either clipped or growing together (Figs. 1, 2). In clipped pots, the dominant species responded positively to the application of fertilizer—both with and without competition. In contrast, the subordinate species showed no response towards increased fertilizer levels in clipped pots and were even negatively affected (in terms of both total and root biomass) when competition with both grasses was induced (Figs. 1, 2). This suggests that although a given level of herbivory may induce species replacement, increased nutrient availability is likely to change the competitive balance again towards competitive species (Kuijper et al. 2005).

An additional external factor related to plant performance is competition (Lee and Bazzaz 1980; Newingham et al. 2005). As expected, the total aboveground and root biomass of both *Lotus* and *Prunella* decreased in the absence of clipping and in competition with both *Holcus* or *Arrhenatherum* (Fig. 1). In contrast, the biomass of *Holcus* and *Arrhenatherum* was not reduced under conditions of competition with subordinate species and even tended to increase when in competition with *Lotus* (Fig. 1), suggesting some kind of facilitation which may be the result of nitrogen fixation by *Lotus*. This result clearly indicates that competition enforced by tall grass species may lead to the exclusion of subordinate

species in the absence of grazing (Rodriguez and Brown 1998). In such a situation, perennial competitive grasses tended to expand and suppress the growth of smaller neighbors through the expanding zones of depletion, the most conspicuous of which are for light (Grime 2001). In this type of vegetation, high rates of mortality and low rates of reproduction are characteristic of those plants that are outstripped by their neighbors. This may eventually result in vegetations approaching a state of monoculture by tall graminoid species, such as *A. elatius*, *H. lanatus* or *Calamagrostis epigejos*. This was documented by a field study of dune grasslands in which animals were excluded (Tahmasebi Kohyani et al. 2008a).

A long-lasting debate among experts in the field of vegetation ecology concerns the interactive influence of competition and herbivory along the gradient of productivity (Tilman 1988; Grime 2001). It is clear from this study that both a gradient of nutrient availability and clipping have a direct impact on species performance, and it is equally evident that interspecific competition also influences species composition. Although the effect of herbivory was significant for all species, the amount of regrowth by subordinate species was more than that of dominant grass species, suggesting that tolerance to herbivory may also be an important strategy adopted by subordinate species to persist under grazing. Both theoretical considerations (Pacala and Crawley 1992) and empirical studies (Van Der Meijden et al. 1988; Mauricio et al. 1997) suggest that tolerant and defensive plants should succeed equally well in grazed grasslands. This is important for plant growth, because a plant cannot always avoid damage (in terms of escape in space, time, and possession of secondary compounds), even if it has developed defense mechanisms against herbivores. Thus, natural selection may simultaneously favor both greater defense and tolerance (Mauricio et al. 1997). Our results also confirm that although grazing disturbance significantly influences this competitive relation, increasing nutrient levels contract the negative effects of grazing on dominant competitive plant species, suggesting that competitive relations between competitive grass species and subordinate forb species change under different environmental conditions. As such, an improved understanding of these species-specific differences is necessary to enable better insight in species replacement in grasslands.

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Responses of clonal architecture to experimental defoliation: a comparative study between ten grassland species

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Abstract Clonal architecture may enable plants to effectively respond to environmental constraints but its role in plant tolerance to defoliation remains poorly documented. In several non-clonal species, modifications of plant architecture have been reported as a mechanism of plant tolerance to defoliation, yet this has been little studied in clonal plants. In a glasshouse experiment, five rhizomatous and five stoloniferous species of grazed pastures were subjected to three frequencies of defoliation in order to test two hypotheses. (1) We expected plant clonal response to defoliation to be either a more compact architecture (low clonal propagation, but high

branching), or a more dispersed one (long-distance propagation and low branching). Such plastic adjustments of clonal architecture were assumed to be involved in tolerance to defoliation i.e. to promote genet performance in terms of biomass and number of ramets. (2) The response of clonal architecture to defoliation was expected to be dependent on the species and to be more plastic in stoloniferous than in rhizomatous species. Most genets of each species were tolerant to defoliation as they survived and developed in every treatment. Architectural modifications in response to defoliation did not match our predictions. Clonal growth was either maintained or reduced under defoliation. Relative growth rate (RGR) decreased in eight species, whereas defoliated genets of seven species produced as many ramets as control genets. Biomass allocation to ramet shoots remained stable for all but one species. In defoliated genets, the number and mean length of connections, and mean inter-ramet distance were equal to or lower than those in control genets. Four groups of species were distinguished according to their architectural response to defoliation and did not depend on the type of connections. We hypothesised that dense clonal architectures with low plasticity may be the most advantageous response in defoliated conditions such as in grazed pastures.

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Introduction

Vegetative multiplication is widely spread in plants, particularly in Angiosperms. Many species are able to reproduce both by seeds (sexual reproduction) and by clonal growth (asexual reproduction), while some others are even exclusively clonal (Price and Marshall 1999). A genet consists of the vegetative production of genetically identical offsprings (*ramets*) that are potentially independent units as they develop their own shoots and roots (Hutchings and Bradbury 1986; Klimeš et al. 1997). This propagation allows the genet to persist and spread both in space and time (Gardner and Mangel 1997; Oborny and Kun 2002). Vegetative growth modes are variable and major attention has been paid to clonal plants forming aboveground or belowground horizontal stems (stolons and rhizomes respectively, hereafter often referred as *connections*) carrying ramets (Klimeš et al. 1997). In the following, the term *clonality* will refer to these particular growth modes.

Clonal architecture provides singular plant characteristics relying mainly on the integration between ramets, which presents a potential adaptive role (Hutchings and Wijesinghe 1997; Suzuki and Stuefer 1999). Clonal plants are likely to effectively respond to environmental constraints that may explain their abundance in a variety of environments (Hutchings 1999; Price and Marshall 1999). Clonal plants are particularly able to show plastic adjustments of clonal architecture, a strategy involved in selective foraging for light quality (Stuefer and Huber 1998), light intensity (Dong and Pierdominici 1995), nutrient availability (Liao et al. 2003) and even soil temperature (MacDonald and Lieffers 1993) and competition (MacDonald and Lieffers 1993; Kleijn and van Groenendael 1999; Macek and Lepš 2003).

For plants submitted to defoliation, the ability of growth and reproduction after damage is defined as tolerance, while the term compensation is often used to characterise the degree of this tolerance (Strauss and Agrawal 1999). Tolerance can be considered as a plastic trait and ranged along a gradient (Maschinski and Whitham 1989, Stowe et al. 2000). Incomplete tolerance occurs when defoliated plants survive and develop but their performance is lower than for undefoliated plants (undercompensation sensu Strauss and Agrawal 1999). Compensating and even

overcompensating tolerance respectively refer to maintained and increased performance for damaged plants compared to undamaged ones (Stowe et al. 2000). Amongst a variety of mechanisms, modifications of aboveground plant architecture (i.e. branching pattern) had often been mentioned as a frequent response to clipping in non-clonal plants (Owen 1980; Paige and Whitham 1987; Lennartsson et al. 1998).

The aim of this study was to investigate whether active adjustments of clonal architecture are involved in genet tolerance to defoliation. Few studies have been carried out on the responses of clonal architecture to disturbance such as clipping or grazing (see however Moen et al. 1999; Piqueras 1999; Li et al. 2004; Wang et al. 2004). We investigated such questions by considering the comparative response to experimental clipping for 10 species, embracing both stoloniferous and rhizomatous species.

We first tested whether clonal architecture-related traits are involved in species compensating tolerance of defoliation, enabling the maintenance of genet performance (in terms of biomass and ramet production). Their response is expected to vary between traits, and either a more compact architecture (low clonal propagation, but high branching) or a more dispersed one (long-distance propagation and low branching) is expected to occur in response to defoliation (Table 1). The second hypothesis was that the response of clonal architecture to defoliation might vary according to species and in particular

Table 1 Variations of clonal traits predicted by the hypothesis of compensating tolerance to defoliation

Survival and development	→
Clonal performance	
Relative growth rate	→
Number of ramets	→
Biomass allocation to ramets	→
Clonal architecture-related traits	
Number of connections	↘
Mean length of connections	↗
Mean inter-ramet distance	↗
Clonal growth form	Dispersed Compact

Arrows indicate the direction (decrease, maintenance or increase) of trait variation between undefoliated and defoliated plants

among stoloniferous and rhizomatous species. As stolons are more often involved in spatial propagation and show higher morphological plasticity than rhizomes (Dong and de Kroon 1994; Dong and Pierdominici 1995), clonal architecture is expected to be more responsive to defoliation in stoloniferous than in rhizomatous species.

Methods

The response of 10 clonal species to three frequencies of defoliation was recorded in terms of genet performance and architecture-related traits.

The plant species for study were selected out of the 23 clonal herbaceous perennials from grazed pastures in the Marais poitevin, Western France ($46^{\circ}28' N$ and $1^{\circ}30' W$). These species are the most abundant clonal species in these plant communities, where the biomass consumption by grazing ranges from 55% to 87% of the available biomass (Ros-signal et al. unpublished). They belong to several families and show different modes of clonal growth. All species can produce long connections, either aboveground (stolons) or belowground (rhizomes), or both. Some of them can also form tussocks through very short connections (caespitose growth form) (Table 2).

Experimental set-up

Ramets were collected in February 2006, from a grazed area of 1 ha. Thirty-three ramets per species were chosen and randomly assigned to one of the three treatments of defoliation. The experimental design thus consisted of 10 species \times 3 defoliation treatments \times 11 replicates with a total of 330 experimental units.

Each ramet was cleaned, weighed (fresh mass, FM) and rooted in the centre of a pot (20-cm-diameter and 16-cm-height) containing fine garden soil. Approximately 6–10 cm³ of substrate from the collection site were added close to the roots of the ramet in order to provide symbiotic microorganisms. Ramets were first allowed to grow freely for a five-week acclimation period during which dead ramets were replaced. The ratio FM/DM (fresh and dry mass respectively) was determined for 10 additional non-planted ramets per species, and the mean per species used to estimate initial dry biomass of each planted ramet.

The experiment was conducted in a non-heated glasshouse at the campus of Beaulieu (University of Rennes 1, France) from 29th March up to 17th May 2006. In the glasshouse, temperature was maintained below 25°C. Pots were watered daily with tap water, and weeds were regularly removed.

Table 2 Studied species and their clonal growth type (adapted from Klimeš et al. 1997)

Species	Abbreviation	Class	Family	Clonal growth type
<i>Agrostis stolonifera</i>	Asto	Monocotyledons	Poaceae	Stoloniferous Caespitose
<i>Carex divisa</i>	Cdiv	Monocotyledons	Cyperaceae	Rhizomatous
<i>Eleocharis palustris</i>	Epal	Monocotyledons	Cyperaceae	Rhizomatous
<i>Elytrigia repens</i>	Erep	Monocotyledons	Poaceae	Caespitose Rhizomatous
<i>Glyceria fluitans</i>	Gflu	Monocotyledons	Poaceae	Caespitose Stoloniferous
<i>Juncus articulatus</i>	Jart	Monocotyledons	Juncaceae	Rhizomatous Stoloniferous
<i>Juncus gerardii</i>	Jger	Monocotyledons	Juncaceae	Rhizomatous
<i>Ranunculus repens</i>	Rrep	Dicotyledons	Ranunculaceae	Stoloniferous
<i>Trifolium fragiferum</i>	Tfra	Dicotyledons	Fabaceae	Stoloniferous
<i>Trifolium repens</i>	Trep	Dicotyledons	Fabaceae	Stoloniferous

For species having the ability to develop two types of connections, the major type developed during the experiment is mentioned first. Caespitose growth type corresponds to the production of short rhizomes (tussock forming species)

Experimental treatments

The three frequencies of defoliation tested were: no defoliation (control treatment), defoliation every 30 days (mid-frequency defoliation treatment) and defoliation every 15 days (high-frequency defoliation treatment). To make a realistic simulation of defoliation caused by cattle, all aboveground parts of Monocotyledons were cut to 7 cm height (Loucugay et al. 2004). As Dicotyledons were generally lower than 7 cm, defoliation consisted in the removal of 50% of developed leaves by severing the petiole at its base (both petiole and lamina were removed). Genets were harvested 8 weeks after the first clipping. Genets under mid-frequency treatment were thus cut twice and those under high-frequency treatment were cut four times.

Trait measurements

At harvest the number of ramets per genet was counted and architectural traits were measured. Then, each genet was divided into vegetative shoots, flowers, connections, and roots, dried to constant mass at 60°C and the dry mass of each of these parts was weighed. As the study focused on clonal architecture, biomass allocation to roots was not taken into account. As only a few genets had produced flowers during the experiment, only traits related to vegetative development were taken into account.

Traits related to clonal performance

The investment in clonal reproduction was estimated as the final number of ramets. The total growth of each genet was measured as the relative growth rate calculated as follows:

RGR

$$= \frac{\ln(\text{final biomass} + \text{clippings}) - \ln(\text{initial biomass})}{\text{number of days}},$$

where final biomass is the dry mass of the whole genet (including roots) at the end of the experiment, clippings, the dry mass of clipped tissues, and initial biomass, the dry mass of the planted ramet.

Finally, the biomass of ramets corresponded to the sum of the final dry mass of shoots of all ramets produced by the genet.

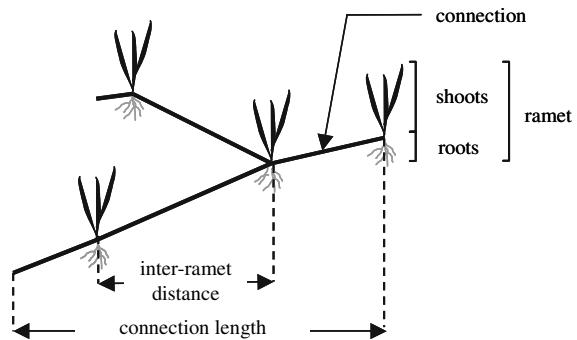


Fig. 1 Schematic representation of a genet, and definition of architectural traits

Architecture-related traits

Measured traits were: the total number of connections (stolons or rhizomes) per genet, the mean length of connections (with a precision of 1 mm) produced per genet, and the mean inter-ramet distance (with a precision of 1 mm) per genet. This latter corresponded to the mean distance between two consecutive ramets. It could be calculated only for genets that had produced connections carrying ramets (Fig. 1).

Four out of 10 species can develop two types of connections (Table 2). However, *Juncus articulatus* produced very few stolons and only data related to rhizomes were taken into account. For *Agrostis stolonifera*, *Elytrigia repens* and *Glyceria fluitans*, the calculation of mean inter-ramet distance did not include caespitose connections.

A species was considered as tolerant when genets had survived and developed even under the high-frequency defoliation treatment. The response of clonal performance to defoliation (from a decrease to an increase) were used to characterise the degree of tolerance (from incomplete to compensating tolerance).

Statistical analysis

For all species, one-way ANOVAs showed no differences in ramet initial biomass between the three treatments, confirming the assumption of ramet randomisation between treatments at the beginning of the experiment. The percentage of biomass removed by defoliation was assessed through ANOVA with species and treatment as main effects. The correlation between the percentage of biomass removed and the

values of architecture-related traits was tested. We used the non-parametric Spearman correlation coefficient (ρ) as traits did not follow a normal distribution.

Within-species effects of defoliation treatments on final number of ramets, RGR, mean length of connections and mean inter-ramet distance were assessed through one-way ANOVAs, after checking for normality and homogeneity of variances (Kolmogorov–Smirnov and Levene tests respectively), and log-transformation of data when necessary. Post-hoc comparisons between treatments were tested by the Tukey–Kramer test. In the particular case of final number of connections, for which assumptions of normality and homogeneity of variances were not met, non-parametric Kruskal–Wallis tests were used and post-hoc comparisons were made by Mann and Whitney U -tests with Bonferroni correction. The effect of defoliation on biomass allocation to ramets was analysed by ANCOVAs using final biomass as a covariate. The aim was to increase the power of the F -statistic by adjusting for the influence of the covariate, and to avoid the use of biomass ratios, which may be misleading to study allocation patterns (Jiaseński and Bazzaz 1999). Interactions between treatment and covariate were first introduced into the model and removed when non-significant.

The comparison of architectural responses to defoliation between the 10 species was done by multivariate analyses, taking into account the three architecture-related traits. After a Principal Components Analysis (PCA) on correlation matrix, traits were centred independently per species (within-species PCA) and compared between treatments by a between-class PCA (bc-PCA), each treatment considered as one class (Dolédec and Chessel 1991). Such analysis enabled to eliminate scale effects linked to differences of average trait values between species. Following this analysis, hierarchical ascendant classification (HAC using Ward method of clustering, Legendre and Legendre 1998) was used to group species according to their multivariate trajectory of response. The coordinates of each treatment along the two first axes of bc-PCA constituted the six variables.

ADE-4 software (Thioulouse et al. 1997) was used for bc-PCA, and JMP software (SAS procedure) for other statistical calculations. In all the cases, we rejected null hypothesis at the $P = 0.05$ level.

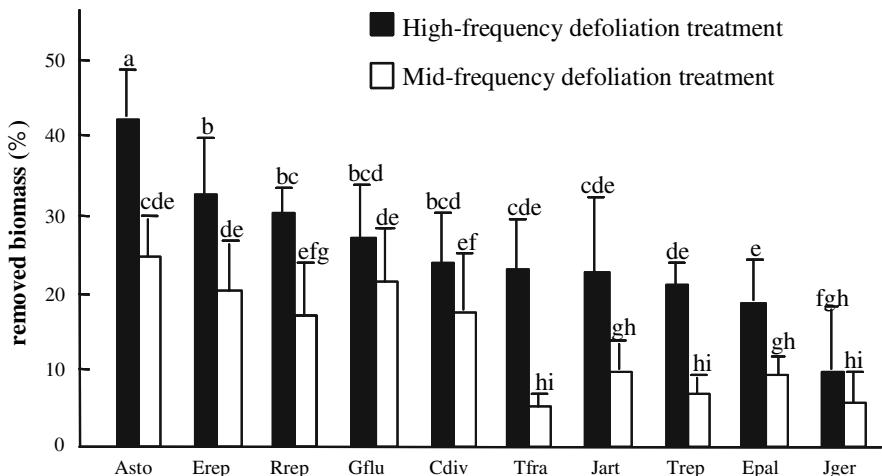
Results

Effects of defoliation treatments on clonal performance and architecture-related traits

The amount of biomass removed by defoliation differed significantly between species and treatments (species \times treatment: $F_{284}^{18} = 12.05$, $P < 0.0001$, species: $F_{284}^9 = 36.78$, $P < 0.0001$, treatment: $F_{284}^2 = 750.04$, $P < 0.0001$). For the high-frequency defoliation treatment, it ranged from 42.5% (*Agrostis stolonifera*) to 10% (*Juncus gerardii*). The three grasses were the most severely impacted (between 27% and 42.5% for high-frequency defoliation, and between 20% and 24% for mid-frequency treatment), whereas *J. gerardii* and the two *Trifolium* species were less impacted, especially by mid-frequency defoliation treatment (only from 5% to 7% biomass removed) (Fig. 2).

At the end of the experiment, 311 out of the 330 genets had survived and developed, all species and treatments taken together. The impact of treatments on trait values depended on species and traits. Significant differences of trait values occurred either between the control and both defoliation treatments, or between the control and the high-frequency defoliation treatment only. Therefore, hereafter the term defoliation will most frequently be used without distinction between the two levels of the defoliation treatment. *J. articulatus*, *J. gerardii* and *Ranunculus repens* were the three species for which almost no trait was significantly impacted by defoliation. By contrast, the final number of ramets of defoliated genets was 60% to 30% compared to the one of control genets for *Eleocharis palustris*, *G. fluitans* and *Trifolium fragiferum*. It did not significantly change for the other ones (Table 3, see also Table S1-A in Supplementary material). Defoliation generated a significant reduction of RGR for eight species (Table 3, Table S1-B), and there was a significant covariation between the final biomass and the biomass of ramet shoots for all species except *T. fragiferum*. This last trait was significantly impacted by clipping treatment only for *Trifolium repens*, indicating that, for the nine other species, biomass allocation to shoots (i.e. the part of the genet final biomass allocated to the ramet shoots) was not changed by defoliation (Table 3, Table S1-C). For *Carex divisa* only, all architecture-related traits

Fig. 2 Means and standard deviations of percentage of biomass removed [biomass removed/(final biomass + biomass removed)] for each species. Letters indicate significant differences between treatments and species (post-hoc Tukey tests)



showed a significant drop after defoliation (Table 3). For *E. palustris*, *E. repens* and *T. fragiferum*, the number of connections decreased, up to 80% for *E. palustris* (Table 3, Table S2-A). Mean length of connections decreased by 75% for *A. stolonifera* and 50% for *C. divisa* and *G. fluitans* (Table 3, Table S2-B). Finally, mean inter-ramet distance decreased in *A. stolonifera*, *C. divisa* and *T. repens* (Table 3, Table S2-C).

Multivariate responses of clonal architecture to defoliation

Over all 10 species, there was no significant correlation between the percentage of biomass removed and the three architectural traits ($\rho = -0.08, P = 0.15$ for

the number of connections; $\rho = 0.02, P = 0.68$ for the mean length of connections, and $\rho = 0.02, P = 0.68$ for the mean inter-ramet distance). But their response to defoliation varied between traits and species. The two first axes of bc-PCA represented 96% of total variance (69% and 27% respectively, Fig. 3). The F1 axis carried out mean length of connections and mean inter-ramet distance. The F2 axis was negatively correlated with the number of connections (Fig. 3a). The amplitude of variation between the extremes of the trajectories along the first axis was weak for all species but *A. stolonifera*. The trajectory between control and high-frequency defoliation treatment along the second axis varied in the direction of a decreased number of connections for nine species. This variation was the most important

Table 3 Effects of defoliation on clonal traits for all the 10 species

	First hypothesis	Asto	Cdiv	Epal	Erep	Gflu	Jart	Jger	Rrep	Tfra	Trep
Survival and development	→	→	→	→	→	→	→	→	→	→	→
Clonal performance											
RGR	→	↘	↘	↘	↘	↘	→	→	↘	↘	↘
Number of ramets	→	→	→	→	→	→	→	→	→	↘	→
Biomass allocation to ramets ^a	→	→	→	→	→	→	→	→	→	→	→
Clonal architecture											
Number of connections	↗	↘	→	↘	↘	↘	→	→	→	↘	→
Mean length of connections	↘	↗	↘	↘	→	→	→	→	→	→	→
Mean inter-ramet distance	↘	↗	↘	↘	→	NA	NA	→	→	→	↘

Arrows indicate the variations of trait values between control and both defoliation treatments taken altogether: ↗ significant increase, ↘ significant decrease, → no significant difference, NA not available. Abbreviations of species follow Table 2. Results of statistical tests are presented in Supplementary material (Table S1 for traits indicative of performance, Table S2 for architectural traits)

^a Biomass allocation to ramets corresponds to the effect of treatment (main factor) on the biomass of all ramets of a genet, tested by the ANCOVA (final genet biomass as a covariate)

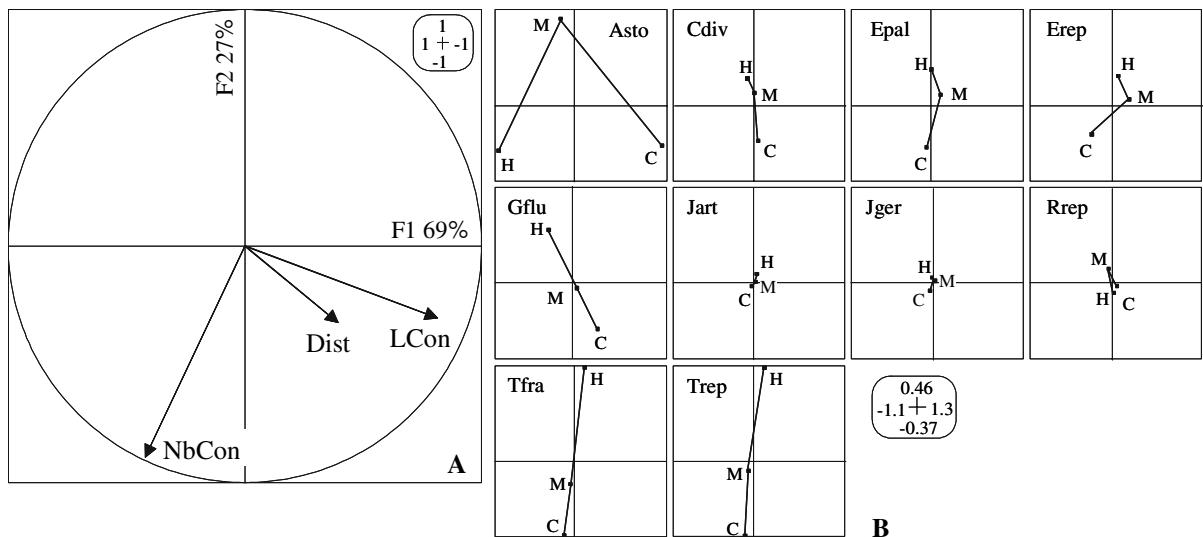


Fig. 3 Multivariate responses of clonal architecture to defoliation for each species. Traits values have been centred per species (within-species PCA) and compared between treatments (between-treatment PCA). The factorial plan is thus the same for all species. **a** Correlation circle of architectural traits in the factorial plan 1–2 of between-treatment PCA. **b** Trajectories of multivariate responses to the three treatments

for each species in the factorial plan 1–2. Each point represents the barycentre of all replicates of a defoliation treatment. Points *C*: Control treatment, *M*: mid-frequency defoliation treatment, *H*: high-frequency defoliation treatment. *Dist*: mean inter-ramet distance, *LCon*: mean length of connections, *NbCon*: number of connections. See Table 2 for the meaning of species abbreviations

for *Trifolium* species, weaker for *C. divisa*, *E. palustris*, *E. repens* and *G. fluitans*, and was close to zero for Juncaceae and *R. repens*. The trajectory of *A. stolonifera* along this axis had a singular shape, with a great increase between control and mid-frequency defoliation and a decrease between mid-frequency and high-frequency defoliation (Fig. 3b).

The HAC based on architectural responses to defoliation resulted in four groups of species. The first group consisted of both *Trifolium* species. *J. articulatus*, *J. gerardii* and *R. repens* formed the second group, while *C. divisa*, *E. palustris*, *E. repens* and *G. fluitans* constituted the third group. Finally, the last group corresponded to *A. stolonifera*, due to the singular shape of its trajectory of response (Fig. 4).

Discussion

Consequences of defoliation on clonal performance

All species showed tolerance to defoliation as 311 out of 330 genets survived and developed even when severely clipped.

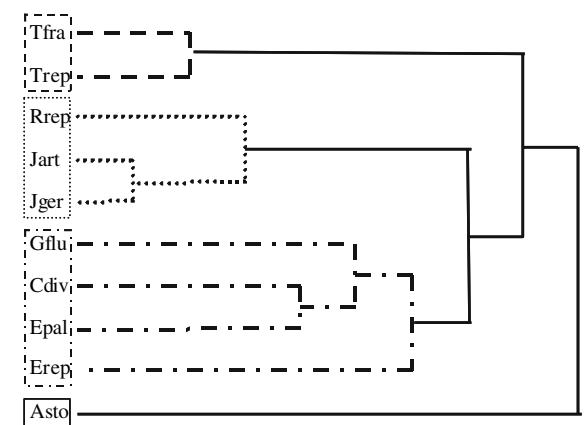


Fig. 4 Dendrogram of species resulting from HAC according to the multivariate responses of clonal architecture to defoliation. Species were clustered into four groups on the basis of Euclidean distance (Ward's method). See Table 2 for the meaning of species abbreviations

Biomass allocation to ramets was not affected by the treatment, except for one species. This indicates that genets were able to sustain damaged ramets and even to compensate for aboveground biomass removal caused by defoliation. While the design did not allow to identify the mechanisms involved,

Brown and Allen (1989) reported that clipping treatment may cause the translocation of resources from belowground organs to support the regrowth of aboveground tissues.

We recorded, however, a great discrepancy on the degree of tolerance depending on traits and species considered. The RGR decreased with clipping, which is typical of undercompensation (Strauss and Agrawal 1999) and incomplete tolerance (Stowe et al. 2000), while together, seven out of 10 species maintained a similar number of ramets in all three treatments, arguing for compensating tolerance to clipping. Previous studies had already reported a variety of responses to clipping by clonal plants, among species and among environments for a given species. For example, ramet number has been reported to decrease with leaf removal for three clonal perennials, (Hicks and Turkington 2000), to be unchanged for *Leymus chinensis* (Wang et al. 2004), either to be unchanged or to increase according to nutrient availability for *Cyperus esculentus* (Li et al. 2004) and even to increase for five caespitose grasses (Richards et al. 1988). Biomass responses to defoliation were also shown to vary from undercompensation (Li et al. 2004) to overcompensation (Hicks and Turkington 2000), probably due to both the differing species studied and the defoliation treatment applied. Interspecific differences in compensatory ability were notably found both in clonal and non-clonal species (McNaughton and Chapin 1985; Belsky 1986; Del-Val and Crawley 2005). Response to defoliation has also been shown to vary within the same species depending on environmental conditions (Maschinski and Whitham 1989). The lack of generality in clonal plant responses to clipping may also originate from the variety of defoliation treatments used in the different studies. Their impact on plant growth may indeed differ whether they are applied at a given date (e.g. Hicks and Turkington 2000; Wang et al. 2004), at a given development stage (e.g. Richards et al. 1988) or several times (e.g. Li et al. 2004).

Responses of clonal architecture-related traits to defoliation

Clonal architecture-related traits did not match the predicted responses. Species followed four types of architectural response to defoliation, going from nil

to high variation. Trait values never increased with clipping and defoliation thus led to fewer connections and/or shorter connections and mean inter-ramet distances. Similar results in response to clipping or grazing have already been observed in other herbaceous clonal plants such as *Trifolium repens* (Hay and Newton 1996), *Acaena magellanica* (Moen et al. 1999), *Trientalis europaea* (Piquerias 1999) or *Lymus chinensis* (Wang et al. 2004).

Furthermore, the results obtained here show that there is no relationship between the multivariate pattern of architectural response and the degree of tolerance to defoliation. For instance, both the first and the third response groups included together species for which defoliation induced no change in the number of ramets (*C. divisa*, *E. repens* and *T. repens*), and species for which defoliation induced a decreased number of ramets (*E. palustris*, *G. fluitans* and *T. fragiferum*). Compensating tolerance can be related to various responses of architectural traits. For *E. repens*, the production of ramets was not affected by defoliation despite a decrease of the number of connections, as only a few ramets were produced by these connections, the majority being caespitose (tussock forming). An alternative strategy was shown by *C. divisa*, *T. repens* and *A. stolonifera*. They maintained the number of ramets unchanged with clipping by way of the decreased mean inter-ramet distance, whatever the variation of the other traits.

Interspecific comparison of the responses of clonal architecture to defoliation

Previous studies have shown that higher nutrient and/or light supply increased branching intensities of both stolons and rhizomes, whereas the morphology of stolons (mean connection length and mean internode length) was more plastic than the morphology of rhizomes (Dong and de Kroon 1994; Dong and Pierdominici 1995). According to our study, only the stolons of *A. stolonifera* showed a high degree of variability, with a high amplitude of variation of all architecture-related traits. The responses of other stoloniferous species were not clearly distinct from those of rhizomatous species. The second and the third response groups contained both stoloniferous (*G. fluitans* and *R. repens*) and rhizomatous species (*C. divisa*, *E. palustris*, *E. repens*, *J. articulatus* and *J. gerardii*). Thus, contrary to the predictions that clonal

architecture should be more responsive to defoliation in stoloniferous than in rhizomatous species, the response of clonal architecture to defoliation was not constrained by the type of connections. Other developmental constraints may be implied in architectural responses to defoliation. For instance, branching pattern is related to the number and activity of axillary meristems (Huber and During 2001), which may play a key role in architectural response to defoliation (Briske 1996). In monopodial species (e.g. *T. fragiferum* and *T. repens*) the number of connections is constrained by the limited number of meristems available per ramet (Huber and During 2001). As a result they are likely to be more sensitive to defoliation (Huber et al. 1999). Indeed, the trajectories of response of the two studied *Trifolium* species did show a great decrease of the number of connections, compared to the other eight species (sympodial species, Klimeš and Klimešová 1999).

However, the species constitutive of the second (*J. articulatus*, *J. gerardii* and *R. repens*) and the third (*C. divisa*, *E. palustris*, *E. repens* and *G. fluitans*) response groups can be linked neither by their phylogenetic nor by their developmental features. Our results thus demonstrate that architectural response to defoliation can neither be predicted on the sole basis of the type of clonal connection (stolons or rhizomes), nor by the phylogenetic and developmental features.

Conclusion

Species responses to defoliation were either the stability of clonal architecture or the decreased investment in the production of connections and a lower clonal propagation. Gross et al. (2007) showed that low lateral spread was a constitutive trait of species tolerant to clipping. Such growth forms can be interpreted as the expression of the ‘consolidation strategy’ (as defined by de Kroon and Schieving 1990) characterised by short and little plastic connections. In grazed pastures, where the risk of defoliation is high, plants with short propagation (either constitutive or induced by defoliation) and capable of producing a dense population of ramets when defoliated are very likely to be more competitive than plants that allocate energy in the production of long connections. However, small variations of

architectural traits may have great consequences on spatial distribution of ramets within a genet, and consequently on genet performance, resource acquisition and competition (Huber et al. 1999). Such parameters are likely to be modified by recurrent defoliation that occurs in grazed pastures. The four architectural patterns of response to defoliation distinguished during the present short-term experiment are very likely to constrain competitive ability, and hence the relative species abundances along the grazing gradient.

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Diffuse knapweed (*Centaurea diffusa* Lam.) seedling emergence and establishment in a Colorado grassland

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Abstract Knapweeds (*Centaurea* spp.) are damaging invaders of grasslands and other North American rangelands. A field study was conducted to determine conditions that promote diffuse knapweed (*C. diffusa*) emergence and establishment in a native Colorado grassland (North America). Knapweed was planted in native grassland under treatments with different opening sizes, levels of competition, knapweed seed burial and season of seeding. There was no effect of opening size where competing natives were alive, but knapweed emergence in 5- and 15-cm openings was higher than 0-cm openings where natives were killed. Reducing competition reduced fall diffuse knapweed emergence, but did not affect spring emergence. Seed burial increased knapweed emergence, but the effect varied by season. Although diffuse knapweed emergence reached 35%, only four plants survived from 3,600 seeds. This native grassland did not prevent knapweed emergence or establishment, but both were so low that rapid knapweed invasion is unlikely.

Keywords Emergence · Exotic plants · Invasive plants · Invasibility · Noxious weeds · Soil disturbance · Openings

Introduction

Diffuse knapweed (*Centaurea diffusa* Lam.) is one of the most economically and ecologically damaging invasive plants on North American rangelands (Lacey et al. 1990; Sheley et al. 1998) including grasslands. Presently, diffuse knapweed is widely distributed across North America (Sheley et al. 1998; USDA NRCS 2002). Much is known regarding the biology and life history of knapweeds (Watson and Renney 1974; Schirman 1981; Roze et al. 1984; Jacobs and Sheley 1998; Sheley et al. 1998; Jacobs and Sheley 1999; Marler et al. 1999a; Marler et al. 1999b), but it remains unknown whether or not knapweeds have the ability to establish and subsequently invade late-seral native plant communities in the absence of human-caused disturbances. Manipulative field experiments that identify whether or not late-seral native communities resist invasion, and the conditions under which they are susceptible to invasion are needed to improve the present understanding of knapweed invasions. Several authors have identified this as a critical precursor to improved management and prevention of noxious weed invasions (Bergelson et al. 1993; Levine and D'Antonio 1999; Davis et al.

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2000; Jesson et al. 2000; Prieur-Richard and Lavorel 2000).

Field studies of spotted (*C. stoebe* L.) and diffuse knapweed establishment have generally been conducted in disturbed areas or adjacent to existing infestations (Wolfson et al. 2005; Watson and Renney 1974; Roze et al. 1984; Myers and Berube 1983; Tyser and Key 1988; Larson and McInnis 1989; Schirman 1981) and have not addressed the question of whether or not these knapweeds can establish and subsequently invade native communities at greater distances from existing infestations. Very little work has focused on diffuse knapweed, and questions about this plant's ability to establish in native communities located greater distances from existing infestations are particularly important, given its potential for long distance seed dispersal (Watson and Renney 1974). Seed heads of diffuse knapweed have small (3–5 mm) distal openings that release achenes slowly as mature plants break off at ground level and become "tumbleweeds" blown by the wind (Watson and Renney 1974). Given this potential for long distance seed dispersal, information about diffuse knapweed emergence and establishment in native communities may be particularly important. Greenhouse studies using field collected soil indicated that diffuse knapweed emergence in native rangeland soil did not differ from emergence in soil from the core and perimeter of a diffuse knapweed infestation, but diffuse knapweed biomass was slightly higher in native rangeland soil (Meiman et al. 2006). Even though diffuse knapweed seedlings emerged and grew well in native rangeland soil, this plant does not appear to be an aggressive invader of undisturbed grasslands in Colorado (personal observation) or in Washington (Kulmatiski 2006). Identifying the controls on diffuse knapweed seedling growth in a late-seral native rangeland is important for understanding community resistance or susceptibility to invasion by exotic plants. Information can be found regarding the emergence and growth of native seedlings among established native plants (Aguilera and Lauenroth 1993), but no information is available for diffuse knapweed.

The overall goal of our study was to evaluate emergence and establishment of diffuse knapweed seedlings in a late-seral, foothills grassland in northwestern Colorado (USA). We were specifically interested in testing whether or not existing interspaces between

the above ground portions of established plants (openings) represent safe sites for diffuse knapweed emergence and establishment and if disturbances that incorporate knapweed seed into the soil or reduce competition from established native plants are required for diffuse knapweed seedling emergence and establishment. We hypothesized that the native grassland at our study site would prevent emergence of diffuse knapweed seedlings but that emergence and establishment would increase with seed burial, reduction of competition from existing native plants, and increasing opening size. The selected treatment combinations inform predictions regarding a variety of disturbances that might weaken established native plants and result in seed burial. These would include, but not be limited to the activities of burrowing animals and large ungulates (wild or domestic), recreational use, and some forms of vegetation management.

Materials and methods

Study area

This study was conducted in a grassland near Fort Collins, Colorado USA at an elevation of 1,540 m (UTM 13 487150E 4492800 N). Annual precipitation averages 365 mm, more than half of which falls between April and August. Total above ground current year plant production (oven dry basis) in early July, 2002 was $97 \pm 5 \text{ g m}^{-2}$. Twenty native species accounted for approximately 97% of the above ground plant biomass on the site. Two exotic species were encountered, but only in trace amounts and diffuse knapweed was not present. The site was dominated by native perennial plants including needle and thread (*Hesperostipa comata* (Trin. & Rupr.) Barkworth), New Mexico feathergrass (*Hesperostipa neomexicana* [Thurb. ex Coul.] Barkworth), green needlegrass (*Nasella viridula* (Trin.) Barkworth), little bluestem (*Schizachrium scoparium* (Michx.) Nash), big bluestem (*Andropogon gerardii* Vitman), prairie flax (*Linum lewisii* Pursh), white sagebrush (*Artemisia ludoviciana* Nutt.), and yucca (*Yucca glauca* Nutt.). Soils are sandy clay loams (54% sand, 16% silt, and 30% clay) and are mapped as the Minnequa-LaPorte complex (3–15% slopes) (USDA 1980).

In the summer of 2001, we randomly located three 30-m × 60-m blocks leaving 10- to 30-m buffers between blocks. We constructed a fence around the study site to minimize impacts by humans and large animals. Exactly 240 diffuse knapweed introduction plots were located at 2-m intervals along 20 transects spaced 2 m apart in each block (720 introduction plots total). We used 10-cm diameter by 5-mm thick by 5-mm tall polyvinyl chloride rings to mark each introduction plot. Each introduction plot was randomly assigned to one of 12 treatment groups and one of two runs of the experiment (fall 2001 or spring 2002).

Treatments

Treatments consisted of a full factorial arrangement of three opening sizes (0-, 5-, and 15-cm diameter), two levels of competition (existing native plants alive or killed) and two levels of seed burial (seed buried and seed not buried). The entire experiment was conducted twice (fall 2001 and spring 2002) using each introduction plot only once.

Openings were defined as existing interspaces among the bases of established native plants using an approach similar to Aguilera and Lauenroth (1993). Opening treatments were randomly assigned to introduction plot locations. At each 2-m mark along the transects, the introduction plot was located in the nearest existing opening of the appropriate size. A 0-cm opening was defined as the center of an existing perennial bunchgrass crown. Five- and 15-cm diameter openings were located where plastic discs of 5- or 15-cm diameter fit among the bases of established plants (Aguilera and Lauenroth 1993).

Knapweed seed was sown in two different ways. Seed was dropped into the introduction plot and left where it fell without ensuring seed-to-soil contact to simulate seed falling from a mature parent plant (seed not buried). The other method involved removal of litter or any other objects that would prevent seed contact with the soil, pressing the seed firmly into the soil and covering with approximately 5 mm of soil (seed buried). Litter moved prior to placement of the seed was returned to its original location.

We reduced competition from established native plants by treating a 50-cm diameter circular area centered at the introduction plot with a 3% glyphosate solution ('killed' native plants treatment). All

plots receiving this treatment (fall and spring runs) were sprayed on 10 September 2001. The glyphosate treatment was effective at killing the existing established plants in the treated plots.

Seed collection, preparation, planting and site cleanup

We collected diffuse knapweed seed on 31 August 2001 from plants growing 2 km from the study site. Seed was kept dry and stored at room temperature for 30 days before being cleaned with a seed blower to separate chaff and unfilled seed from filled seed. The filled (heavy) seed remaining after cleaning was used for this study.

Two hundred seeds were randomly selected after cleaning for 3-week germination trials in the greenhouse prior to each run of the experiment (four germinators, 50 seeds/germinator). The cleaned diffuse knapweed seed was fairly large (750 seeds g^{-1}) and germination was $93 \pm 2\%$ and $93 \pm 3\%$ at the time of the fall seeding and spring seeding, respectively (mean \pm SE, $n = 4$). We planted diffuse knapweed seed in the field by sowing five seeds in each introduction plot. Introduction plots used for the fall run were seeded on 17 September 2001 and those used in the spring run were seeded on 2 April 2002. For the first 30 days after seeding, we watered all introduction plots for a given run daily if soil was dry to the touch and no natural precipitation was received. Each watering consisted of adding 60 ml of water to each 10-cm diameter introduction plot (~8 mm). Introduction plots received a total of 80–90 mm of moisture from precipitation and supplemental watering in the 30-day periods following seeding, which is between the 30-year normal, and high monthly precipitation for that period.

At the end of the study, all diffuse knapweed plants were hand pulled to ensure extraction of the taproot. One year later, all introduction plots were treated to ensure that no live diffuse knapweed plants remained.

Data collection

We quantified seedling emergence as the percentage of diffuse knapweed seeds that developed into seedlings at any time within 30 days of sowing. Introduction plots were checked at 2–3 day intervals

for diffuse knapweed seedlings and thorough seedling inventories were conducted 15 and 30 days after sowing. Emergence for each treatment combination was based on 50 seeds (5 seeds/introduction plot \times 10 introduction plots) per treatment combination per block in each season.

Diffuse knapweed establishment was determined in October, 2002 for both fall and spring runs, by recording whether or not each introduction plot supported a live diffuse knapweed plant and by counting the total number of leaves (live and dead). Thompson and Stout (1991) determined that over half of diffuse knapweed plants with 13 or more leaves can be expected to bolt after cold vernalization so we used the 13-leaf stage as our establishment criteria.

Experimental design and statistical analyses

We used Analysis of Variance to test for effects of opening size, seed burial, presence or absence of competition from established native plants, season of seeding, and all possible interactions on diffuse knapweed seedling emergence. Blocks were treated as a random effect and all other factors were fixed. Plots of residuals versus predicted values indicated that data transformation was unnecessary. Tukey's Studentized Range Tests were used to compare treatment means.

Results

Diffuse knapweed emergence ranged from 10 to 35% and was explained by four, two-way interactions of treatment factors. None of the higher order interactions were significant. Burial of diffuse knapweed seed increased emergence in both fall and spring runs of the experiment, but the effect was much greater in the fall-seeded run ($P < 0.0001$) (Fig. 1). In the fall, emergence of buried seed was approximately three times that of unburied seed, but in the spring, emergence of buried seed was about twice that of unburied seed. The effects of killing vegetation in and around introduction plots varied by season ($P = 0.013$) (Fig. 2). In the fall run, live native vegetation facilitated diffuse knapweed emergence (26 vs. 20%). However, emergence was not different in the spring run between plots with live or killed neighboring vegetation. There was no effect of

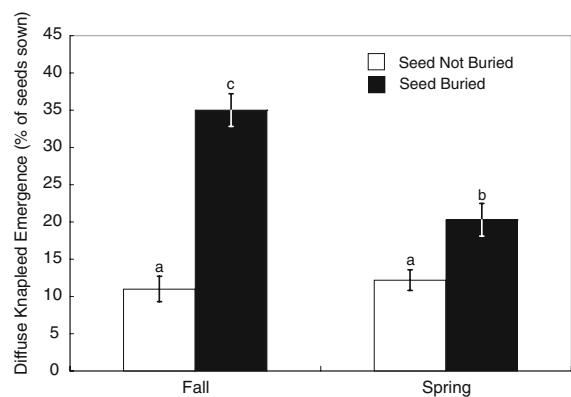


Fig. 1 The effects of diffuse knapweed (*Centaurea diffusa* Lam.) seed burial and season of seeding on the mean (\pm SE, $n = 18$) percentage of seeds that emerged in each treatment combination in a native grassland west of Fort Collins, Colorado, USA. Means with the same letter are not different, Tukey's Studentized Range Tests, $\alpha = 0.05$

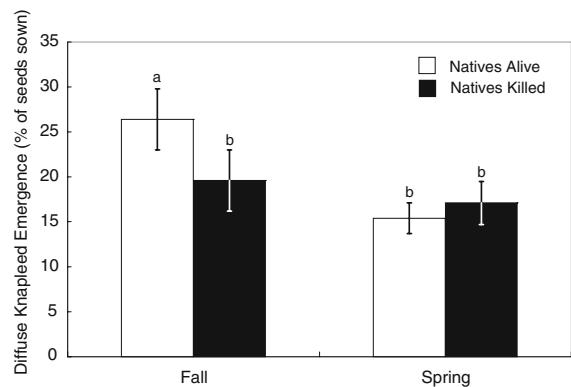


Fig. 2 The effects of competition and season of seeding on the mean (\pm SE, $n = 18$) percentage of diffuse knapweed (*Centaurea diffusa* Lam.) seeds that emerged in each treatment combination in a native grassland west of Fort Collins, Colorado, USA. Means with the same letter are not different, Tukey's Studentized Range Tests, $\alpha = 0.05$

opening size where natives were left alive, but emergence in the 5- and 15-cm diameter openings was slightly higher than in the 0-cm opening where established native plants had been killed ($P = 0.0355$) (Fig. 3). Diffuse knapweed emergence was higher for buried seed than unburied seed in all opening sizes and increased with increasing opening size only for buried seed ($P = 0.0808$) (Fig. 4). Emergence of buried seed was highest in the 15-cm diameter opening and lowest in the 0-cm opening, while emergence of unburied seed was 10–13% regardless of opening size.

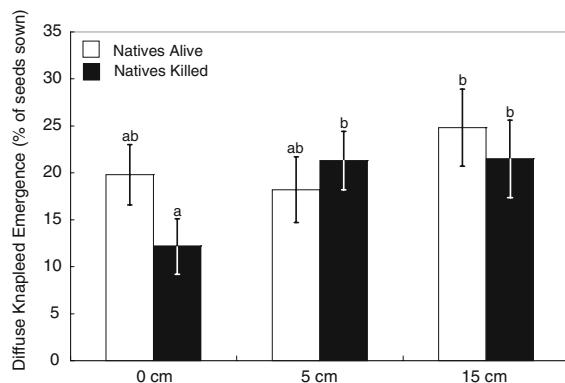


Fig. 3 The effects of competition and opening size on the mean (\pm SE, $n = 12$) percentage of diffuse knapweed (*Centaurea diffusa* Lam.) seeds that emerged in each treatment combination in a native grassland west of Fort Collins, Colorado, USA. Means with the same letter are not different, Tukey's Studentized Range Tests, $\alpha = 0.05$

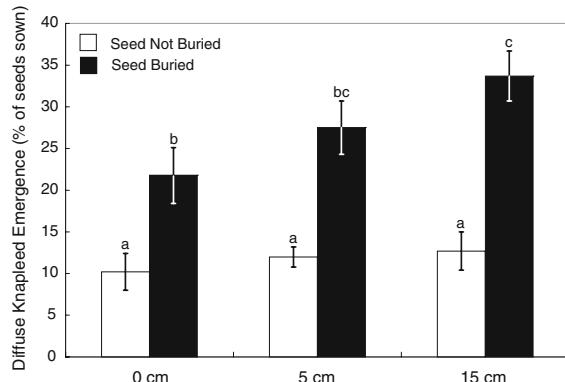


Fig. 4 The effects of opening size and seed burial on the mean (\pm SE, $n = 12$) percentage of diffuse knapweed *Centaurea diffusa* Lam. seeds that emerged in each treatment combination in a native grassland west of Fort Collins, Colorado, USA. Means with the same letter are not different, Tukey's Studentized Range Tests, $\alpha = 0.05$

Despite emergence values as high as 35%, very few diffuse knapweed seedlings survived. Out of 360 introduction plots in the fall-seeded run, 339 contained no live diffuse knapweed seedlings in early June of 2002. Similarly, we found no live diffuse knapweed seedlings in 327 of 360 introduction plots in the spring-seeded run as of 1 July 2002. By the end of the study only four diffuse knapweed plants remained alive. Three of these occurred in openings (5- and 15-cm diameter) and grew from buried seed where surrounding native plants had been killed. The other surviving diffuse knapweed plant was found in

a plot with a 0-cm opening and grew from unburied seed where surrounding native plants were alive. Only one of the four plants alive on 11 October 2002 met the establishment criteria so establishment analyses were not possible.

Discussion

We quantified diffuse knapweed emergence and the establishment in a native late-seral grassland, and detected little difference between existing interspaces and areas occupied by the crowns of established native bunchgrasses. Our results suggest that the undisturbed native grassland where this study was conducted did not prevent emergence and the establishment of diffuse knapweed, but it did exhibit a high level of resistance to invasion. Kulmatiski et al. (2006) observed similar resistance of native communities to exotic plant growth (mix of four exotic species including diffuse knapweed), but the specific responses of diffuse knapweed were not reported. As hypothesized, emergence increased with seed burial but the effects of opening size and reduced competition were complex and likely influenced by microclimate conditions.

Existing interspaces among the bases of established native plants at our study site did appear to be safe sites for diffuse knapweed emergence and establishment. However, interspaces did not appear to be the only safe sites for emergence given that there was little evidence to suggest that emergence was higher in these small (≤ 15 cm) openings compared to the crowns of established native bunchgrasses. Where native plants were alive, and when averaged across seed burial treatments, emergence did not vary by opening size. The only indication of higher emergence in openings was for buried seeds. Interestingly, although no analysis of establishment was possible, three of the four diffuse knapweed rosettes that survived until the end of the study, including the one that met our establishment criteria, grew in 5- and 15-cm diameter openings.

Although the native grassland where this study was conducted did not prevent emergence or establishment of diffuse knapweed, there was evidence of resistance to invasion which is consistent with other recent studies of spotted and diffuse knapweed in intact or reconstructed grasslands (Pokorny et al.

2005; Seastedt and Suding 2007). Emergence observed in this field study (10–35%) was not limited by the viability of diffuse knapweed seed (greenhouse germination of 93%). Survival of diffuse knapweed observed in this study was much lower than suggested by Watson and Renney (1974) and toward the low end of what has been reported by others working in disturbed areas or close to existing infestations (Myers and Berube 1983; Roze et al. 1984). Still, diffuse knapweed seedlings did emerge and establish in this study. Even when seed was not buried, 10–13% emergence was documented and diffuse knapweed emergence was as high or higher where established native plants were left alive compared to plots where native plants had been killed to reduce competition.

The resistance of our study area to invasion may be linked to a variety of factors including interactions with soil biota (Klironomos 2002; Meiman et al. 2006), functional group diversity (Pokorny et al. 2005), or the combined effects of spatial and temporal dynamics of disturbance and resource availability (Belote et al. 2008). This resistance to invasion should be viewed with caution. We observed one established diffuse knapweed plant as a result of sowing 3,600 seeds. However, a single diffuse knapweed plant may produce 2,800–12,000 seeds m^{-2} year $^{-1}$ (assuming that one plant occupies 0.25 m^2 and seed production estimates of Schirman 1981) which is 0.8–3.3 times the total number of seeds used in our study. Watson and Renney (1974) estimated the annual reproductive capacity of a single diffuse knapweed plant to be 740 plants. Therefore, one surviving diffuse knapweed plant could eventually lead to rates of spread associated with common definitions of invasion (Richardson et al. 2000).

The higher emergence of diffuse knapweed from buried versus unburied seed is consistent with the findings of others suggesting that diffuse knapweed emergence is highest from seed that has good contact with mineral soil and is near the soil surface (Watson and Renney 1974). Berube and Myers (1982) observed two to four times more diffuse knapweed seedlings and rosettes in plots that had been physically disturbed compared to undisturbed plots.

The interaction between seed burial and opening size (Fig. 4) suggests that seed contact with the soil is more limiting to diffuse knapweed emergence than

opening size. Low emergence of nonburied seed may have been driven by unfavorable conditions for emergence or by seed predation. Rodent utilization of knapweed seed has been observed (Watson and Renney 1974), and other granivores likely use knapweed seed as well. Seed loss to predation may be high for seed lying on the soil surface.

The effects of microclimate in plots where competing native plants were killed were likely more important for knapweed emergence than competition from surrounding plants. Close proximity to live, transpiring plant material may provide more favorable growing conditions than would be expected adjacent to dead plant material. Light is generally not regarded as a limiting factor in this sparsely vegetated system. Larson and McInnis (1989) found that growing conditions for early seedling growth of diffuse knapweed were more favorable close to crowns of seeded grasses than in interspaces between grass plants. Killing plants surrounding the introduction plots had no effect on diffuse knapweed emergence in the spring-seeded run of our study and this may have been driven by the drought of 2002. Precipitation for the 6-month period immediately prior to seeding of the spring run (83 mm) was 74% of average, but precipitation for the 30-day period corresponding to the emergence monitoring for the spring-seeded run (7 mm) was 15% of average. The plants surrounding all introduction plots in the spring-seeded run were dry and yellow regardless of whether or not plots had been sprayed. Therefore, micro climatic conditions in plots that were dry and yellow because of a dry spring were likely similar to plots that were dry and yellow because they had been sprayed the previous fall. In contrast, unsprayed plots in the fall-seeded run supported live, green plants throughout the 30-day period following the fall seeding and only the sprayed plots were yellow and dry. Precipitation for the 6-month period immediately prior to the fall seeding (237 mm), and during the 30-day period when emergence was monitored for the fall-seeded run (22 mm), was 87% and 68% of average, respectively. The greater effect of seed burial on diffuse knapweed emergence in the fall versus spring (Fig. 1) was likely also driven by precipitation differences.

The effect of killing plants around introduction plots also varied depending on opening size (Fig. 3). Where surrounding plants were left alive, diffuse

knapweed emergence was about 20% regardless of opening size. Where surrounding plants had been killed, diffuse knapweed emergence in the 0-cm opening was half that observed in 5- and 15-cm openings. These emergence patterns are consistent with the earlier discussion of microclimate effects, and it makes sense that such effects would be observed where diffuse knapweed seedlings were closest to killed plants (0-cm opening).

Propagule pressure is an important final consideration. Colautti et al. (2006) reported that although propagule pressure was considered in only 29% of the invasion ecology studies they reviewed, it was an important predictor of both community invasibility and species invasiveness 85% of the time. Levine (2000) also stressed the importance of propagule supply and suggested that this may be an important factor affecting the relationship between diversity and invasibility. The total number of seeds sown in our experiment (3,600) was intentionally low to simulate initial stages of invasion, and we observed only one established plant. As more plants become established, or for areas close to large infestations, propagule pressure could be much higher and the rate of invasion may be more rapid than observed in our study. Other studies have used high seed numbers (Pokorny et al. 2005; Seastedt and Suding 2007) that likely represent the propagule pressure associated with established knapweed infestations. Prolific seed production is highlighted as a major factor contributing to the aggressiveness of knapweeds (Watson and Renney 1974), but recent findings by Story et al. (2008) suggest that seed head-attacking biological control agents for spotted knapweed have led to significant reductions in the number of seeds per flower head and per unit area over a 30-year period. Those authors also propose that knapweed plant density would not decrease until seed numbers fall below a threshold level. Given the low seed numbers we used, our results may have important management implications related to such thresholds. However, studies like ours, which use low seed numbers, are rare and additional manipulative experiments designed to determine the effects of propagule pressure on emergence and establishment of diffuse knapweed invasion are needed.

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Biodiversity and tallgrass prairie decomposition: the relative importance of species identity, evenness, richness, and micro-topography

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Abstract Biodiversity has been declining in many areas, and there is great interest in determining whether this decline affects ecosystem functioning. Most biodiversity—ecosystem functioning studies have focused on the effects of species richness on net primary productivity. However, biodiversity encompasses both species richness and evenness, ecosystem functioning includes other important processes such as decomposition, and the effects of richness on ecosystem functioning may change at different levels of evenness. Here, we present two experiments on the effects of litter species evenness and richness on litter decomposition. In the first experiment, we varied the species evenness (three levels), identity of the dominant species (three species), and micro-topographic position (low points [gilgais] or high points between gilgais) of litter in three-species mixtures in a prairie in Texas, USA. In a second experiment, we varied the species evenness (three levels), richness (one, two, or four species per bag), and composition (random draws) of litter in a prairie in Iowa, USA. Greater species evenness significantly increased decomposition, but this effect was dependent on the environmental context. Higher

evenness increased decomposition rates only under conditions of higher water availability (in gilgais in the first experiment) or during the earliest stages of decomposition (second experiment). Species richness had no significant effect on decomposition, nor did it interact with evenness. Micro-topographic position and species identity and composition had larger effects on decomposition than species evenness. These results suggest that the effects of litter species diversity on decomposition are more likely to be manifested through the evenness component of diversity than the richness component, and that diversity effects are likely to be environmentally context dependent.

Keywords Ecosystem functioning · Litter bag · Litter quality · Grassland · Moisture · Time

Introduction

Biodiversity has been declining in many areas due to habitat loss (Rosenzweig 1995), eutrophication (Tilman 1987), and human management practices (Engle et al. 1993). The effect of declining species diversity on ecosystem process rates (ecosystem functioning, EF) is of both theoretical and applied interest (Loreau et al. 2002). Most studies of biodiversity—EF have focused on primary productivity because of its obvious importance to ecosystems (Loreau et al. 2002). However, both decomposition and primary

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productivity control nutrient and energy flow. The rate of return of nutrients from decomposing plant tissue can limit primary productivity in the long run, and litter forms the basis for the detritivore food web, which contains more energy than the herbivore food web in most ecosystems (Swift et al. 1979). Furthermore, a full understanding of decomposition processes is important in predicting carbon sequestration rates and feedbacks that impact global warming (Swift 2001). Thus, it is important to develop a better understanding of how decomposition is affected by changes in litter diversity.

Biodiversity encompasses both species richness and evenness (Magurran 1988). Different components of biodiversity such as beta diversity can also encompass the change in species composition over space. However, in this study we focus on alpha diversity by varying litter diversity at individual points. Early studies of biodiversity and EF emphasized the species richness component of biodiversity (Loreau et al. 2002), but more recent studies have examined the effects of both species richness and evenness on EF (Nijs and Roy 2000; Mulder et al. 2004; Wilsey and Polley 2004; Emery and Gross 2006; Boyero et al. 2007; Kirwan et al. 2007; McKie et al. 2008; Swan et al. accepted). In studies focusing on the effects of species richness on EF, all species were generally added at the same relative proportions (i.e., at maximum evenness), whereas natural systems typically have low evenness (Preston 1962; Tokeshi 1993; Schwartz et al. 2000; Wilsey et al. 2005). A literature survey found that plant species evenness accounted for 53% of variation in plant species diversity (Shannon's H'), while plant species richness accounted for only 6% of the variation across community types (with covariation between evenness and richness accounting for the remaining variation; Stirling and Wilsey 2001). Thus, studies which do not take into account species evenness do not examine the full effects of changes in diversity because they do not encompass both components of biodiversity.

The applicability of species richness—EF studies has also been questioned by researchers, who suggested that the apparent effects of high species richness were actually due to the greater likelihood of including particular species that strongly affect EF (the “sampling effect”; Aarssen 1997; Huston 1997; Tilman et al. 1997). The sampling effect could

underlie some of the effects of litter species richness in studies of decomposition (Hector et al. 2000; Knops et al. 2001), although it is important to emphasize that Tilman et al. (2001) found transgressive overyielding effects of species richness that go beyond the sampling effect. In contrast, the effects of species evenness cannot be attributed to the sampling effect, since the identity of species does not change at different evenness levels, only the relative abundance of species (Wilsey and Potvin 2000; Loreau et al. 2001; King et al. 2002). Thus, by varying species diversity through changes in evenness, we are able to test for diversity effects in the absence of the sampling effect (Wilsey and Potvin 2000; Loreau et al. 2001; King et al. 2002).

Species evenness could commonly interact with richness to affect EF (Doak et al. 1998; Schwartz et al. 2000; Dangles and Malmqvist 2004; Swan et al. accepted). In sites with low evenness, one or more dominant species could have strong effects on EF and the remaining rare species could have a negligible effect on EF (e.g., Smith and Knapp 2003). However, in sites with high evenness, each species has a similar abundance and the addition or removal of a species may have appreciable effects on EF. If the effect of species richness depends on evenness, then this interaction would need to be fully understood before making inferences from richness-EF studies to field situations (Schwartz et al. 2000; Srivastava and Vellend 2005).

The two previous studies on the effects of litter species evenness on decomposition found mixed results. King et al. (2002) found no effects of litter evenness on litter decomposition in laboratory microcosms. Swan et al. (accepted) found that litter evenness and richness interacted to significantly affect leaf litter decomposition in a stream. The highest decomposition rates were found when evenness was closer to field values.

Plant species diversity could affect litter decomposition through mechanisms involved with litter mixing. The chemical environment and decomposer community changes when litter is mixed as long as species differ in resource quality or structure (Blair et al. 1990; Smith and Bradford 2003; Gartner and Cardon 2004). By mixing species at different litter evenness levels, the ratios of different types of phenolics, textures, and nutrients in the mixture are altered. Evenness could affect decomposition rates

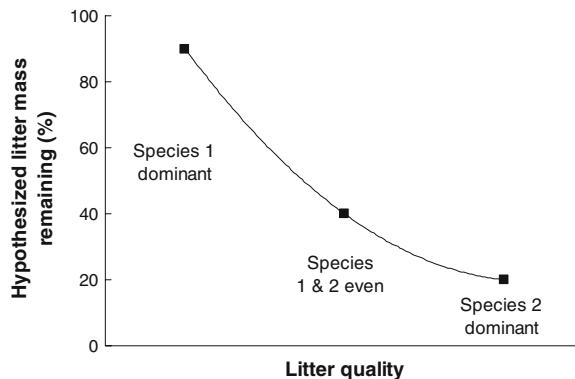


Fig. 1 Hypothesized relationship between decomposition rate and litter mixes at different levels of litter species evenness. If the litter decomposition rate is non-linearly related to litter quality then mixing litter at high evenness could increase mean

due to non-linear responses of decomposition to changes in stoichiometry and litter quality (Fig. 1; Melillo et al. 1982).

Decomposition is strongly affected by factors other than biodiversity, and therefore, the effects of biodiversity must be studied in the context of abiotic and other biotic variables. Rates of litter decomposition can vary with soil moisture (Zhang and Zak 1995; Walser et al. 1998), temperature (Hobbie 1996), and microbial community composition (Chapela and Boddy 1988; Zhang and Zak 1995). Abiotic and biotic variables not related to biodiversity can sometimes have a greater direct effect on decomposition than biodiversity (Wardle et al. 1997b), and could interact with biodiversity.

We tested the general hypothesis that decreases in litter species diversity will cause declines in litter decomposition. In two separate experiments, we manipulated species evenness and species identity or composition in litter bags. We also manipulated species richness in one experiment and micro-topographic location (i.e., in a soil depression [gilgai] or not) in the other experiment. We used these experiments to test the following predictions: (1) higher litter species evenness will cause an increase in litter decomposition; (2) evenness will interact with micro-topographic factors such that the effect of evenness on litter decomposition will be larger at lower topography, where soil moisture is higher; and (3) effects of species richness on litter decomposition will be smaller in the presence of realistically low levels of litter evenness.

decomposition rates. In this example, low evenness bags would have a hypothetical mean remaining biomass of $(90\% + 20\%)/2 = 55\%$, whereas high evenness bags would have a mean of 40%

Methods

Clymer Prairie experiment

The Clymer Meadow Prairie Preserve is located in the blackland prairie region of northeast Texas, USA near the town of Greenville. The preserve encompasses several hundred hectares and is owned by The Nature Conservancy. Soils are vertisols, and the dominant vegetation at the site is represented by tallgrass prairie species (Polley et al. 2005). Mean annual precipitation is 111 cm, and average monthly precipitation shows a bimodal distribution with peaks in May (14 cm) and October (12 cm) and a minimum in August (6 cm). Daily average temperatures range from a low of 0°C in January to a high of 34°C in July and August (Greenville weather station).

In August 2000, leaves of three common species (the forb *Helianthus maximiliani*, and the C₄ grasses *Schizachyrium scoparium*, and *Sporobolus compositus*) were collected and placed in litter bags (all Texas taxonomy follows Diggs et al. 1999). Litter bags were made from 12 × 30 cm polypropylene fabric with openings of 0.25 × 0.5 mm, which is the same material used in a multiple-site study across Canada (Moore et al. 1999). A litter species richness of three was used for all litter bags in this experiment because that is typical of the number of species that would be found at the very small scale of the litter bag. All bags contained 6 g of leaf litter. Litter evenness, dominant species identity, and placement of bags varied in a 3 × 3 × 2 factorial treatment arrangement, with three

species evenness treatments, three dominant species identity treatments, and two micro-topographic location treatments. Litter species evenness treatments were applied by varying the proportion of the dominant species, such that high evenness had 3 g of the dominant species and 1.5 g of the other two species, medium evenness had 4 g of the dominant species and 1 g of the other two, and low evenness had 5 g of the dominant species and 0.5 of the other two. The identity of the dominant species was either *H. maximiliani*, *S. scoparium*, or *S. compositus*. These proportions of species correspond to Simpson's evenness levels of 0.89, 0.67, and 0.47 from high to low evenness, which is somewhat higher than the Simpson's evenness calculated from the plant biomass of moderately sized plots (0.71×0.71 m) from nearby Texas prairie remnants and restorations (range of evenness 0.08–0.30; Polley et al. 2005). The micro-topographic treatment was either in a gilgai or between gilgaies (a gilgai is a seasonally wet depression created by soil expansion and contraction due to soil cracking in dry weather; Edelman and Brinkman 1962; Russell and Moore 1972). In our system, gilgaies created large differences in moisture availability and elevation, and these differences greatly affected plant species composition, with more mesic species within gilgaies and more xeric species between gilgaies (B. Wilsey, personal observations).

Litter bags were placed on the soil surface in September 2000. All bags were placed in random locations so that all treatments (litter evenness, dominant species, micro-topography, and time) were fully interspersed. There were five collection periods (3 weeks; 6 weeks; 12 weeks; 24 weeks; and 48 weeks after September 2000), and each of the treatments was replicated twice within each collection period, leading to 180 litter bags in total (3 litter evenness levels \times 3 dominant species \times 2 micro-topographic treatments \times 5 collection periods \times 2 replicates). Thus, there was low replication for the higher order interactions, but high replication of the main effects ($n = 60$ for each litter evenness and species identity level, and $n = 90$ for each micro-topographic treatment) and one-way interactions (e.g., $n = 20$ for each litter evenness \times gilgai combination) due to hidden replication inherent in factorial designs (Steel and Torrie 1980).

We estimated the carbon (C) to nitrogen (N) mass ratio from litter shortly after it was collected (time zero). We analyzed three replicates of each of the

three species used. Litter C and N was estimated by grinding samples through a Wiley mill and then running samples through a CE Elantech flash EA 1112 combustion C:N analyzer.

Doolittle Prairie experiment

The Doolittle Prairie is a tallgrass prairie in the state preserve system located near Ames, Iowa, USA in the central portion of the state. Soils are mollisols, and vegetation is a highly diverse mix of grasses and forbs (described by Wetzel et al. 1999). Mean annual precipitation is 87 cm, and average monthly precipitation shows a unimodal distribution with a peak in June (13 cm) and a minimum in January (2 cm). Daily average temperatures range from a low of -13°C in January to a high of 29°C in July (Ames weather station).

In late October 2001, leaves of eleven common species were collected from Doolittle and other local prairies (C_4 grasses *Andropogon gerardii*, *Sorghastrum nutans*, *Panicum virgatum*, *Schizachyrium scoparium* and *Sporobolus compositus*, the C_3 grass *Elymus canadensis*, and the forbs *Ratibida pinnata*, *Eryngium yuccifolium*, *Silphium laciniatum*, *Solidago canadensis*, and *Solidago rigida*; all Iowa taxonomy except *S. compositus* follows Eilers and Roosa 1994). Litter bags were made from the same material as the Clymer Prairie experiment: 12×30 cm polypropylene fabric with openings of 0.25×0.5 mm (Moore et al. 1999), and again, all bags contained 6 g of leaf litter. Mixture bags were established with 2×3 factorial treatment arrangement that varied litter species richness (two or four species), and litter species evenness (high, medium, and low denoted by $k = 0$; $k = 2$; $k = 4$, respectively). The chosen values of litter species evenness encompassed the values calculated from the plant biomass of moderately sized plots (0.4×1.0 m) from nearby Iowa prairie remnants and restorations (range of k 1.4 to 2.6; Losure et al. 2007). Richness levels were at the lower end of the number of plant species rooted within 12.5×12.5 cm plots at Doolittle Prairie (range 2–12; Yurkonis Unpubl.). Richness levels were established by randomly drawing either two or four species from the 11 species pool, with four random draws per richness level. Evenness was varied by changing the slope of rank-abundance relationships as outlined by Wilsey and

Polley (2004). Litter mass of each species was varied so that species rank-abundance curves become more negative as k increases in a geometric series model (Tokeshi 1993). For example, a four-species mixture including *S. nutans*, *S. laciniatum*, *E. canadensis*, and *S. rigida* with $k = 4$ contained 4.52 g *S. nutans*, 1.13 g *S. laciniatum* (i.e., 4.52 g/4), 0.28 g *E. canadensis* (1.13 g/4), and 0.07 g *S. rigida* (0.28 g/4), whereas a four species mixture with $k = 0$ contained 1.5 g for all four species. Given a particular value of k , the slopes of rank-abundance curves stay the same across litter richness treatments, thereby allowing a test of the richness \times evenness interaction (Wilsey and Polley 2004). Each of the 11 species used was also placed in single-species monoculture bags.

Litter bags were placed on the soil surface in March 2002 within two areas (blocks). Blocks were established by aligning litter bags along a compass line. All bags were placed in random locations within blocks so that all treatments (litter evenness, litter richness, and time) were fully interspersed. There were three collection periods (4 weeks, 8 weeks, and 32 weeks), and each of the treatments was applied to two blocks within each collection period, leading to 144 mixture bags in total (4 random species draws \times 2 litter richness levels \times 3 litter evenness levels \times 3 collection periods \times 2 blocks), and 66 monoculture bags in total (11 species \times 3 collection periods \times 2 blocks).

Statistical methods

All analyses were completed with SAS 8.0 using type III sum of squares to account for unequal sample sizes (a few litter bags were lost in the field). Proc GLM was used for the Clymer Prairie analysis, with all variables entered as fixed effects in a fully factorial analysis. Proc Mixed was used for the Doolittle Prairie analysis, with species draw as a random factor nested within litter richness levels (two and four species). All other factors were fixed. We included time as a fixed factorial factor because we specifically chose the timing of each collection period and because litter bag location was random across collection periods (i.e., time was not a nested treatment). When interactions with litter evenness occurred, we performed linear and quadratic contrast statements in SAS to test whether evenness affected litter decomposition in a linear or quadratic manner at different levels of the interacting variable (ex. gilgai

at Clymer and time at Doolittle; Littell et al. 2002). The x -axis values for the contrasts were Simpson's evenness levels in the Clymer experiment (0.89, 0.67, 0.47 from high to low evenness), and rank-abundance slopes in the Doolittle experiment (0, 2, 4 from high to low evenness; Wilsey and Potvin 2000; Wilsey and Polley 2004). C:N ratios in the Clymer experiment were analyzed with a non-parametric Kruskal-Wallis analysis due to heterogeneity of variances.

We could not use monocultures in a factorial analysis including evenness because the evenness of monocultures cannot be varied. To test the effects of richness in the Doolittle Prairie while including monocultures, we performed the same analysis as above, except we included monocultures and did not test for evenness effects. This analysis separated effects of species composition from effects of litter species richness by including the effects of species draws at all richness levels.

Results

Clymer Prairie experiment

Litter mass decreased 46.7% on average over the 48 weeks of the experiment. Decomposition over time varied between dominant species and gilgai treatments, with effects of dominant species identity and gilgai becoming more pronounced over time (significant time \times species identity \times gilgai interaction; Table 1; Fig. 2). Litter mass decreased the fastest when *Helianthus maximiliani* was dominant within gilgais, decreased moderately when *Sporobolus compositus* was dominant, and decreased the slowest when *Schizachyrium scoparium* was dominant outside gilgais (Fig. 2). Greater decomposition was found in species with lower initial C:N ratios since *H. maximiliani* had the lowest initial C:N ratio (mean 40.9, standard error 0.3) followed by *S. compositus* (mean 51.8, standard error 1.3) and *S. scoparium* (mean 76.9, standard error 6.5; the C:N ratios were significantly different; χ^2 with 2 d. f. = 7.2, $P = 0.027$).

Although species identity and gilgai had the largest effects on litter decomposition, we also found effects of species evenness. The effects of evenness were variable depending on whether litter bags were placed within gilgai or between gilgai (significant evenness \times gilgai interaction; Table 1; Fig. 3). Litter

Table 1 ANOVA results for observed remaining litter mass at Clymer Prairie, Texas

Predictor variable	d. f.	Sum of Squares	F-values	P-values
Evenness	2	0.52	4.21	0.019
Dominant species identity	2	6.08	48.77	<0.001
Gilgai	1	0.59	9.41	0.003
Evenness × dominant species ID	4	0.24	0.97	0.431
Evenness × gilgai	2	0.67	5.36	0.007
Dominant species ID × gilgai	2	0.52	4.20	0.019
Evenness × dominant species ID × gilgai	4	0.29	1.17	0.329
Time	4	111.09	445.80	<0.001
Time × evenness	8	0.42	0.84	0.574
Time × dominant species ID	8	1.32	2.65	0.013
Time × gilgai	4	1.31	5.27	<0.001
Time × evenness × dominant species ID	16	1.41	1.41	0.158
Time × evenness × gilgai	8	0.45	0.89	0.526
Time × dominant species ID × gilgai	8	1.06	2.13	0.043
Time × evenness × dominant species ID × gilgai	16	0.91	0.91	0.563
Residual	77	4.80		

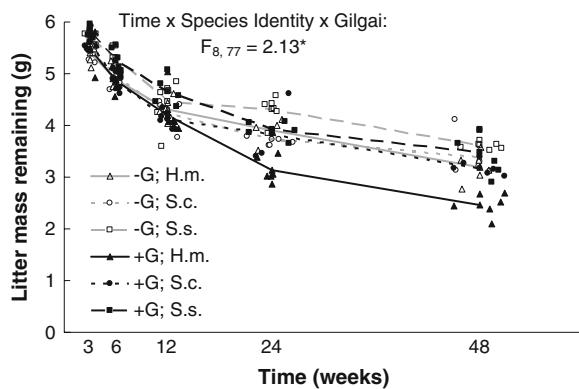


Fig. 2 Amount of litter mass remaining in different treatments at Clymer Prairie, Texas (* $P < 0.05$; G = gilgai; H.m. = *Helianthus maximiliani*; S.c. = *Sporobolus compositus*; S.s. = *Schizachyrium scoparium*; data points are jittered on the x-axis)

evenness had no significant effect on decomposition outside gilgais (linear and quadratic contrasts $P > 0.1$), but higher evenness caused a 5.7% saturating decline in litter mass inside gilgais (linear contrast $F_{1,77} = 15.5$, $P < 0.001$; quadratic contrast $F_{1,76} = 3.21$, $P = 0.077$).

Doolittle Prairie experiment

Litter mass decreased 26.4% on average over the 32 weeks of the experiment. Similar to the Clymer

Prairie study, we found large effects of species composition (random species draws) at all time periods (Table 2).

Litter evenness affected decomposition primarily during the early part of the experiment (significant time × evenness interaction; Table 2; Fig. 4). There were opposite quadratic relationships between evenness and decomposition after 4 weeks (5.5% decrease in litter mass from moderate to high evenness; $F_{1,105} = 6.60$, $P = 0.012$) and 32 weeks (4.6% increase in litter mass from moderate to high evenness; $F_{1,105} = 4.99$, $P = 0.028$), and no other significant linear or quadratic relationships ($P > 0.1$). An extreme data point (3.47 g litter mass remaining) at 32 weeks moderate litter evenness had a large impact on the quadratic relationship at this time ($F_{1,104} = 2.97$, $P = 0.088$ when data point was removed), but had little impact on the overall time × evenness interaction ($F_{4,103} = 3.08$, $P = 0.019$ when data point was removed). Importantly, evenness did not interact with richness ($P > 0.1$) and there were no significant differences in decomposition between litter bags containing two and four species ($P > 0.1$). Richness also had no significant effects on decomposition when including monocultures in the analysis (i.e., between litter bags containing one, two, and four species; $P > 0.1$).

Fig. 3 The effects of litter species evenness on average litter mass across time periods at Clymer Prairie, Texas both outside and inside gilgais (** $P < 0.01$). All error bars are ± 1 standard error

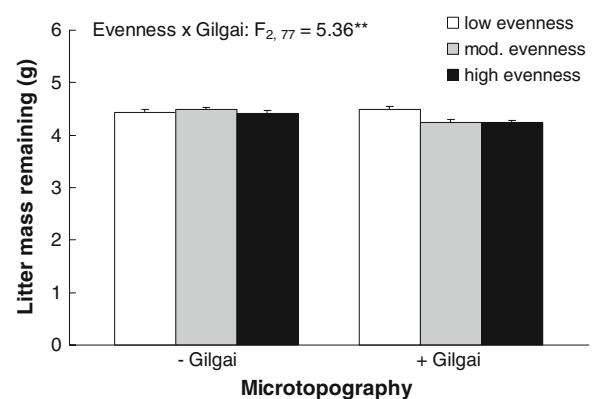


Table 2 ANOVA results for observed remaining litter mass at Doolittle Prairie, Iowa

Predictor ^a variable	d. f.	Sum of squares	F-values	P-values
Block	1	0.08	1.02	0.315
Richness ^b	1	1.48	1.97	0.210
Species draw(richness) ^c	6	4.53	17.05	<0.001
Evenness	2	0.20	1.24	0.294
Richness x evenness	2	0.23	1.44	0.241
Time ^c	2	19.73	222.37	<0.001
Time x richness ^c	2	0.01	0.10	0.905
Time x species draw	12	0.53	0.55	0.878
Time x evenness	4	1.11	3.43	0.011
Time x richness x evenness	4	0.22	0.69	0.600
Residual	104	8.38		

^a F-values are determined by dividing the mean square value for each variable by the residual mean square value unless otherwise noted

^b F-value is determined by dividing the mean square value of this variable by the mean square value of species draw

^c F-value is determined by dividing the mean square value of this variable by the mean square value of time x species draw

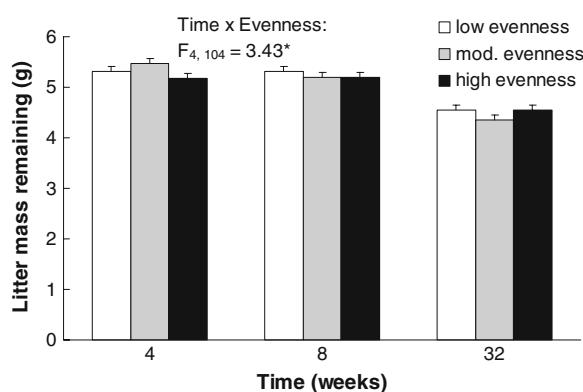


Fig. 4 The effects of litter species evenness on litter mass at Doolittle Prairie, Iowa after 4, 8, and 32 weeks (* $P < 0.05$). Error bars are ± 1 standard error

Discussion

Our experiments tested the general hypothesis that both components of litter species diversity, species evenness and richness, would affect litter decomposition individually and interactively, respectively. Our first prediction was that higher species evenness would cause an increase in litter decomposition. This prediction received some support, although evenness effects were dependent on the environmental context. In support of our second prediction, higher litter evenness caused the greatest decomposition, when litter bags were placed inside gilgais (soil depressions) in the Clymer experiment. Our third prediction was that the effects of litter species richness on decomposition would be smaller at realistically low levels of

litter species evenness. This prediction was not supported. Similar to Knops et al. (2001) and Wardle et al. (1997a), we found no significant effects of litter richness on decomposition, and richness did not interact with litter evenness to affect decomposition. The absence of a richness effect was not likely due to problems with statistical power. Our experimental design was able to detect relatively small differences (<10%) in litter mass, which suggests that we had sufficient statistical power with our design. Finally, the effects of biodiversity on ecosystem functioning (EF) appear to be more strongly dependent on environmental conditions than species diversity.

Other studies have also found that biodiversity is not the primary determinant of EF. Hooper and Vitousek (1997) first noted that plant species richness effects on productivity are smaller than species identity effects. Wardle et al. (1997b) found that fire frequency and species identity had larger effects than plant richness on single-species litter decomposition, standing-crop biomass, and nitrogen mineralization across islands. Walse et al. (1998) found that increased moisture availability led to higher rates of decomposition, which is consistent with our finding of higher decomposition within gilgais. It is interesting to note, however, that even though topography strongly affected decomposition on its own, it strongly interacted with evenness as well. Therefore, the effects of species evenness on EF must be considered within the context of abiotic effects.

We are aware of only two other studies that deal with the effect of plant species evenness on litter decomposition. Swan et al. (accepted) found an effect of litter species evenness on decomposition, but, unlike our study, this effect varied at different litter richness levels. In contrast to our experiments, King et al. (2002) found no effects of woody species leaf litter evenness on litter decomposition, microbial respiration, dissolved organic carbon, or inorganic nitrogen release. However, the King et al. (2002) study was completed in a laboratory with little environmental variability. Higher evenness may lead to greater decomposition in mixtures in variable environments but may have little effect when the environment is stable. This notion is supported by litter evenness effects appearing in the more variable wet-dry environment found within gilgais but not in the more consistent environment outside gilgais. Gilgais contained standing water during January

(mean 67 mm, range 42–97) and March (mean 53 mm, range 27–77) of the growing season but did not contain standing water from April until the following winter. Between gilgais it was more consistently dry.

The interaction between evenness and time in the Doolittle experiment may have been due to the different litter fractions (labile and recalcitrant) that exist over time or the differing importance of bacterial versus fungal decomposition through time. For example, phenolic compounds make up a greater percentage of remaining litter weight over time (Minderman 1968), and evenness may affect litter decomposition differently across time if decomposition is limited by nutrients in the early stages and phenolics in the later stages (Berg and Staaf 1980). It has also been shown that bacteria and fungi are more effective at decomposing different fractions of litter material (Benner et al. 1984), which creates a situation where bacteria and fungi differ in their relative rates of decomposition at different times (Aumen 1980; Ingham et al. 1985). Although not studied here, we hypothesize that the differing effects of litter evenness over time in the Doolittle experiment could potentially be explained by evenness affecting the decomposition of different fractions of litter material differently, by evenness affecting bacterial and fungal communities differently, or both. Future studies should examine the potential mechanisms by which the effects of evenness may change over time.

Several authors have hypothesized that the relationship between species richness and EF would change at different levels of evenness (Doak et al. 1998; Schwartz et al. 2000). A reduced richness effect at realistically low levels of evenness could reduce the applicability of many species richness—EF studies since these studies are generally designed for maximal evenness, whereas most natural communities have much lower evenness (Preston 1962; Loreau et al. 2002; although Hooper and Dukes 2004 showed maximal evenness can sometimes decline to more natural levels over time). Dangles and Malmqvist (2004) found that the effects of detritivore species richness can change across different levels of detritivore evenness, and Swan et al. (accepted) found that litter richness and evenness interacted to affect decomposition of leaves in a stream. Our study is the first to test whether an interaction exists between litter

species richness and evenness on terrestrial plant decomposition, and because we found no interaction, the effects of litter species richness on decomposition in past terrestrial studies (Loreau et al. 2002) may not change qualitatively at different evenness levels, even though further tests are needed.

Our results suggest that the effects of litter species diversity on decomposition are more likely to be manifested through the evenness component of diversity than the richness component, and that diversity effects are likely to be environmentally context dependent. Past studies of the effects of plant litter richness on decomposition have found little to no effect of richness on decomposition (Wardle et al. 1997a; Hector et al. 2000; Knops et al. 2001). Our study and another study (Swan et al. accepted) suggest that changes in litter evenness can affect litter decomposition. Therefore, the evenness component of biodiversity is important in understanding the effects of biodiversity on decomposition. Most studies have shown that increased plant species richness can increase net primary productivity (Loreau et al. 2002). Large standing litter pools could develop if increased richness causes an increase in primary productivity without an accompanying increase in decomposition. These litter pools could eventually have negative feedback effects on productivity in environments without disturbance (Knops et al. 2001). In contrast, the effects of plant species evenness on productivity were not consistent between studies (reviewed by Hillebrand et al. 2008). However, higher evenness may increase productivity and nutrient availability over time by increasing decomposition rates and decreasing standing litter pools, at least in some environments.

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Herbivory and local genetic differentiation in natural populations of *Arabidopsis thaliana* (Brassicaceae)

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Abstract To explore genetic variation in defence against the natural herbivores of *Arabidopsis thaliana*, we transplanted genotypes between a dune habitat and inland habitat in both of which *A. thaliana* occurred naturally. In previous years we had observed that the specialist weevils *Ceutorhynchus atomus* and *C. contractus* (Curculionidae) fed conspicuously on flowers and fruits of *A. thaliana* in the dunes, while these weevils were always rare in inland habitats. Taking all plants together, total fruit damage was indeed much higher in our experimental plots in the dune habitat (59.7%) relative to the inland garden habitat (18.9%). Within a habitat, additional differences existed between plants of different origins, pointing to genetic differences in ecologically relevant characters; plants of inland origin flowered a week earlier, grew better and produced more fruits than plants of dune origin. However, plants of inland origin experienced more total fruit damage by the specialist weevils (75.4%) than plants of dune origin (44.0%) when the two types grew side by side in the dune habitat. Escape from herbivory gives dune

genotypes an advantage in their native habitat, whereas stronger growth and higher survival gives inland genotypes an edge under garden conditions.

Keywords Adaptation · Plant defence · Reciprocal transplantation · *Ceutorhynchus*

Introduction

Adaptive genetic variation in plants has been convincingly demonstrated in relation to abiotic factors, like elevation (Clausen et al. 1940, 1948; Gurevitch et al. 1986), latitude (Mooney and Billings 1961), heavy metals (McNeilly and Antonovics 1968), water availability (Farris 1987), soil type (Snaydon 1970) and salinity (Antlfinger 1981). Biotic factors have received less attention. Turkington and Harper (1979) and Schoen et al. (1986) studied genetic variation in relation to competition, while other studies examined plant pathogen interactions (Kaltz et al. 1999; Thrall et al. 2002). Whether herbivores can cause local adaptation in plants is relatively unknown (but see Prins 1989; Linhart and Grant 1996; Valverde et al. 2003; Nunez-Farfán et al. 2007). Herbivory can have a strong effect on plant fitness and if herbivore pressures differ consistently between habitats in the field, this could well select for plant characters that avoid or reduce herbivory. Whether this occurs in

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nature, or that abiotic factors are more important for adaptation, remains to be seen.

Arabidopsis thaliana (L) Heyn. (Cruciferae) is a small annual plant of Eurasian origin, which is now widely distributed in many parts of the northern-temperate zones of the world (Ratcliffe 1961). *Arabidopsis thaliana* shows a wide climatic amplitude, which makes it suitable for analysing variation in adaptive traits (Koornneef et al. 2004). While homozygosity within populations is high in this predominantly selfing species (Bergelson et al. 1998), genetic differences among Eurasian populations are apparent (Nordborg et al. 2005, see also Jorgensen and Mauricio 2004, for a comparison with Northern America). Several studies have used among-population variation to study fitness consequences of herbivore resistance (Mauricio 1998; Weinig et al. 2003; Murren et al. 2005) and other traits (Griffing and Scholl 1991; Pigliucci 1998; Nordborg and Bergelson 1999; Mitchell-Olds 2001; Pigliucci and Marlow 2001; Kover and Schaal 2002; Koornneef et al. 2004). However, most of these studies typically used a large set of accessions, randomly selected from the species' range. It is still unknown how much genetic variation exists when comparing *A. thaliana* populations at a small scale between habitats that are ecologically different. It is also unclear how well *A. thaliana*, or any other species, can adapt against specialist herbivores that are under continuous strong selection to break its defence (van der Meijden 1996).

While common garden and greenhouse experiments have shown significant variation in the resistance traits in *A. thaliana* (Weinig et al. 2003; Griffith et al. 2004), they do not fully address the potential adaptive nature of genetic variation (Rice and Mack 1991). In several such studies, different genotypes were raised in a single common garden (for instance, Weinig et al. 2003; Griffith et al. 2004) or herbivores were used that probably have little impact on *A. thaliana* in the field (Harvey et al. 2007). However, common garden studies may miss relevant ecological factors that vary at a small scale. For instance, naturally occurring herbivores, specialists in particular, are likely to be present in low numbers when a new garden population is established and their populations may build up over time. As a result effects of specialist herbivores are likely to be underestimated in such studies. Reciprocal transplants in established field populations are more

realistic for addressing the question of local adaptation (Antonovics and Primack 1982).

This study is based on the observation that in a dune habitat in the Netherlands the specialist weevils *Ceutorhynchus atomus* and *C. contractus* (Freude et al. 1983) feed frequently on *A. thaliana*, while in other habitats the weevil is rarely observed. By comparing plants covered with a net with uncovered control plants, we found that weevils reduced seed production by 40% in the dunes (Mosleh Arany et al. 2005). This situation provides a unique opportunity to study differences in plant defence, especially with respect to defence against a specialist herbivore.

In this study we swap *A. thaliana* genotypes between one dune site (high herbivory) and one inland site (low herbivory). We address the following questions: (1) Is variation in growth and herbivory in the field due to genetic differences between populations or environment?, (2) Do home genotypes outperform foreign genotypes? and (3) Do herbivory levels differ between plants from dune and inland origin?

Materials and methods

Habitat description

Arabidopsis thaliana can be found in the coastal regions of the Netherlands, where it grows in two habitat types. This species occurs locally on the calcareous new dunes that were formed partly on top of the old soil profile c. 800 years ago (called dune hereafter). It is also locally common on disturbed roadsides in the west of Holland (called inland hereafter). The former is a natural habitat for *A. thaliana* in the sense that it was not recently created by large-scale anthropogenic disturbance and presumably plants have been evolving under similar conditions over many hundreds of generations.

Our study area in the dunes was located in Meijendel, north of The Hague (the Netherlands), where we sampled seeds from three local populations. Population 1 and 2 were only 5 m apart and the third one was separated from the other two by 500 m. Humus content of the top 10 cm of the soil is low (between 0.45 and 0.96% in the top 10 cm) and consequently the dune sand is infertile with a low water holding capacity. The sandy surface at the dunes sites was covered with moss, grasses and small herbs

with about 10% bare soil. Accompanying species included, among others, *Erophila verna*, *Cardamine hirsuta*, *Rubus caesius* and *Calamagrostis epigejos*. All populations in the dunes were found within 20 m of woody vegetation with small *Hippophae rhamnoides* shrubs and trees such as *Populus nigra*, *P. alba*, *Betula pubescens* and *Crataegus monogyna*.

For inland habitats seeds were collected along a roadside at the edge of Leiden (population 1) and next to a small canal in Noordwijk (population 2). Humus content was high compared to the dune populations (see above): inland population 1 had 1.18% and population 2 had 1.62% humus in the top 10 cm of the soil. Both sites were covered with *Lolium* species, leaving small open patches for the herbs. Accompanying species included, amongst others, *Erophila verna*, *Cardamine hirsuta* and *Plantago lanceolata*. The distance between the two inland populations is about 8 km and the minimal distance between the dune and the inland habitat is about 6 km (Mosleh Arany 2006).

Herbivores

We observed *Ceutorhynchus* beetles conspicuously feeding on *A. thaliana* flowers, especially on the carpels, and ovipositing in the developing fruit. Many of the fruits contained beetle larvae after opening. We collected adult beetles from the flowers and all were identified by Dr. Th. Heijerman (Biosystematics Group of Wageningen University) as either *Ceutorhynchus atomus* or *C. contractus*. Based on observations made three times per week during the flowering period, we concluded that these beetles caused most of the observed damage to flowers and fruits. However, some herbivory by some rare additional herbivore species cannot be excluded. Voucher specimens of the beetles have been deposited at the National Museum of Natural History Naturalis in Leiden (the Netherlands). The RMNH.INS registration numbers are 050 001 and 050 002 for *C. contractus* and 050 003 and 050 004 for *C. atomus* (contact person at Naturalis A. van Assen).

The beetles cause two types of damage that follow each other in time. First, direct herbivory on the flowers by the adult beetles causes some flowers not to develop fruit. Second, beetles also oviposit in developing fruit and the developing larvae then feed on the fruit contents, after which they leave the fruit,

fall to the ground and survive as pupae until the next spring. The damage caused by larvae is evident after opening of the fruits. We find that adults feed mostly on flowers and oviposit in developing fruits in which the larvae develop. This behaviour is different from the description of Freude et al. (1983), who emphasized that the weevils induce galls in *A. thaliana* (*C. atomus*) or mine leaves (*C. contractus*). When we took beetles from the field and placed them on flowering *Arabidopsis* plants in the greenhouse, the beetles climbed the plants and began feeding on the flowers where they stayed most of the time. Later on beetle larvae emerged from the fruits. When adult beetles were placed on vegetative plants, they did not feed on the leaves.

The weevils, *Ceutorhynchus atomus* and *C. contractus* (Curculionidae), are common in disturbed, sandy habitats in the Netherlands. While *C. atomus* is found and reported throughout the Netherlands, *C. contractus* occurs mainly in the coastal areas of the Netherlands (T. Heijerman, personal communication, 2006). As far as we know, these common European species have not been reported in the US.

Transplant experiment

In three dune and two inland populations we randomly selected ten plants per population. In July 2002 seeds were collected from each plant. To reduce maternal effects, plants were grown for one generation under controlled conditions in a growth chamber (potting soil, 20°C, 18 h light, 70% humidity and 2 months in a cold room at 4°C at the rosette stage). Seeds resulting from self-pollination were kept at room temperature until October 2003 when they were germinated. Rosettes (diameter 2 cm) were then transplanted into an enclosure of 16 m² at the dunes close to dune population 3 and a second enclosure in a common garden at Leiden, close to inland population 1. Enclosures had a large mesh size (1.5 cm) and kept people and grazing cattle out, while allowing small herbivores free access to all plants. We were not allowed to set up an enclosure at the original inland sites but consider the chosen garden site in Leiden to be similar to the two inland sites: the garden site had a humus content of 1.0% and *A. thaliana* grew there naturally. For each of the 5 populations, seeds from 10 plants were germinated and for each parent plant 8 small rosettes were

transplanted into each of the 2 enclosures. Hence, a total of 800 plants were transplanted. Within each enclosure, rosettes were positioned at 10-cm intervals. The rosettes were transplanted with potting medium adhering to the roots and with minimal disturbance to the surrounding vegetation. Survival (from rosette stage to seed production), morphological characteristics such as rosette size, stem number and plant height were measured before harvesting the plants in May 2004.

To estimate seed production of the damaged plants we used the following method. When flowering ended we harvested the plants and measured the number of flowers without a fruit (A), with an infested fruit (B) and with an undamaged fruit (C). We interpret the fraction of all flowers that did not produce fruit ($A/(A + B + C)$) as the flower damage caused by adult beetles. This is consistent with the observation in the previous year (2003) that, when beetles were kept away from plants, all flowers produced fruit (Mosleh Arany et al. 2005). The fraction of damage caused by beetle larvae is $B/(A + B + C)$. The total fraction of herbivore damage is the sum of the fraction of flowers eaten by the adult beetles fruits plus the fraction of flowers with fruits containing a larvae ($(A + B)/(A + B + C)$). To estimate the number of seeds produced, rather than the number of fruits, we assumed that the fraction of seeds eaten by larvae in an infested fruit is 80% (Mosleh Arany et al. 2005) and is the same for plants of all origins. Fitness was then estimated as plant survival until reproduction multiplied by units of intact fruits produced after herbivory had taken place [fitness = survival \times (intact fruit + 0.2 \times fruit damaged by larvae); each fruit damaged by larvae counted as 0.2 units of intact fruit]. For comparing the genotypes we assumed that the genotypes have similar numbers of seeds per intact fruit. Because we planted seedlings of different origin, our fitness measure does not include possible differences in seedling germination and survival of seedlings.

All data were analyzed with SPSS 15 (SPSS Inc., Chicago, USA). We first performed a statistical analysis on the data of individual plants. This analysis was complex because residuals were overdispersed. To improve normality, we decided to simplify the analysis by taking the average of all surviving (8 maximum) seedlings from the same mother plant. We then used these averages as dependent variable in the Generalized Linear Model. Main effects include

the site where plants were grown (dunes or garden) and the origin of the plant (dune/inland). The specific population from which the plants were derived was nested with origin. After log-transformation all residuals fitted well to a normal distribution.

Results

Site effects

A significant effect of the site where plants are grown existed for all traits examined (see Generalized Linear Model in Table 1). This simply reflects that all plants grew much better in the garden; the number of stems, stem height and the number of fruits (before and after herbivory) were highest when plants were grown in the inland garden (Fig. 1). In addition, the percentage of damage by adult weevils and their larvae was typically lower when plants grew in the inland garden (Fig. 1e, f). For plants of dune origin, total % fruits with herbivory was 44.0% in the dunes and 17.3% in the inland. For plants of inland origin, total % fruits with herbivory was 75.4% in the dunes and 20.6% in the inland.

Origin effects

A significant effect of origin of the plants existed for all measured traits (Table 1), indicating genetic differences between plants originating from dune and inland. The effect of population within origin is only significant for percentage damage caused by weevil larvae; for all other measured traits the population effect is not significant. This shows that within the dune or inland origin the populations are quite similar.

Plants from inland origin typically produced more stems (Fig. 1a) and more fruits in total (Fig. 1c) but were more heavily damaged by weevil adults (Fig. 1e) and larvae (Fig. 1f). Plants from dune origin survived better than those from inland origin, but the effect was only significant at the dune site (Chi-square test $P = 0.05$, Fig. 1g).

Interaction between site and origin

The Generalized Linear Model showed an origin \times site interaction for the number of intact fruits

Table 1 Generalized Linear Model analysing differences in various morphological and life history traits of plants from two different origins (dune/inland), grown in two sites (dune/garden) (SPSS 15, Generalized Linear Models, type III Sums of Squares, all data were log-transformed)

Trait	Source	df	Wald statistic	P
Number of stems	Site	1	120.88	$P < 0.001$
	Origin	1	13.41	$P < 0.001$
	Pop(origin)	3	6.27	$P = 0.10$
	Origin × site	1	0.53	$P = 0.47$
Stem height	Site	1	589.70	$P < 0.001$
	Origin	1	6.83	$P = 0.009$
	Pop(origin)	3	1.34	$P = 0.72$
	Origin × site	1	2.73	$P = 0.10$
Fruit number	Site	1	490.36	$P < 0.001$
	Origin	1	6.43	$P = 0.011$
	Pop(origin)	3	6.64	$P = 0.08$
	Origin × site	1	0.07	$P = 0.78$
Intact fruits	Site	1	463.98	$P < 0.001$
	Origin	1	5.19	$P = 0.018$
	Pop(origin)	3	3.69	$P = 0.32$
	Origin × site	1	11.77	$P < 0.001$
% Damage by adult weevils	Site	1	244.54	$P < 0.001$
	Origin	1	13.41	$P < 0.001$
	Pop(origin)	3	5.86	$P = 0.12$
	Origin ×	1	11.92	$P = 0.001$
% Damage by weevil larvae	Site	1	66.27	$P < 0.001$
	Origin	1	23.86	$P < 0.001$
	Pop(origin)	3	11.27	$P = 0.010$
	Origin × site	1	0.83	$P = 0.36$
Fitness	Site	1	541.72	$P < 0.001$
	Origin	1	7.33	$P = 0.007$
	Pop(origin)	3	5.36	$P = 0.147$
	Origin × site	1	17.38	$P < 0.001$

Different populations are nested with origin. Error degrees of freedom = 86

after herbivory and for the percentage damage by adult weevils (Table 1). These significant interactions reflect norms of reaction or plasticity of genotypes (in this case inbred mother lines). Plants of dune origin are more constant and the inland plants diverge, suggesting that the latter are more plastic in response to environmental change.

Fitness

There is a main effect of origin on fitness, which means that overall dune plants do slightly better. In addition, the interaction between origin and site is highly significant (Table 1). When grown in the dunes, dune plants perform better than inland plants. When grown in the garden the fitness difference

between dune and inland plants is only marginal and not significant (Table 2). Differences between populations within the dune origin or within the inland origin are not significant (Table 2). The data indicate local adaptation (Kawecki and Ebert 2004) in the dune habitat (Table 2). However, fitness differences are only marginal and insignificant in the garden.

Discussion

We found many differences between *A. thaliana* plants originating from ecologically different habitats (dunes versus ruderal inland populations), which are only several kilometres apart. Since these differences persisted when plants were grown together in the

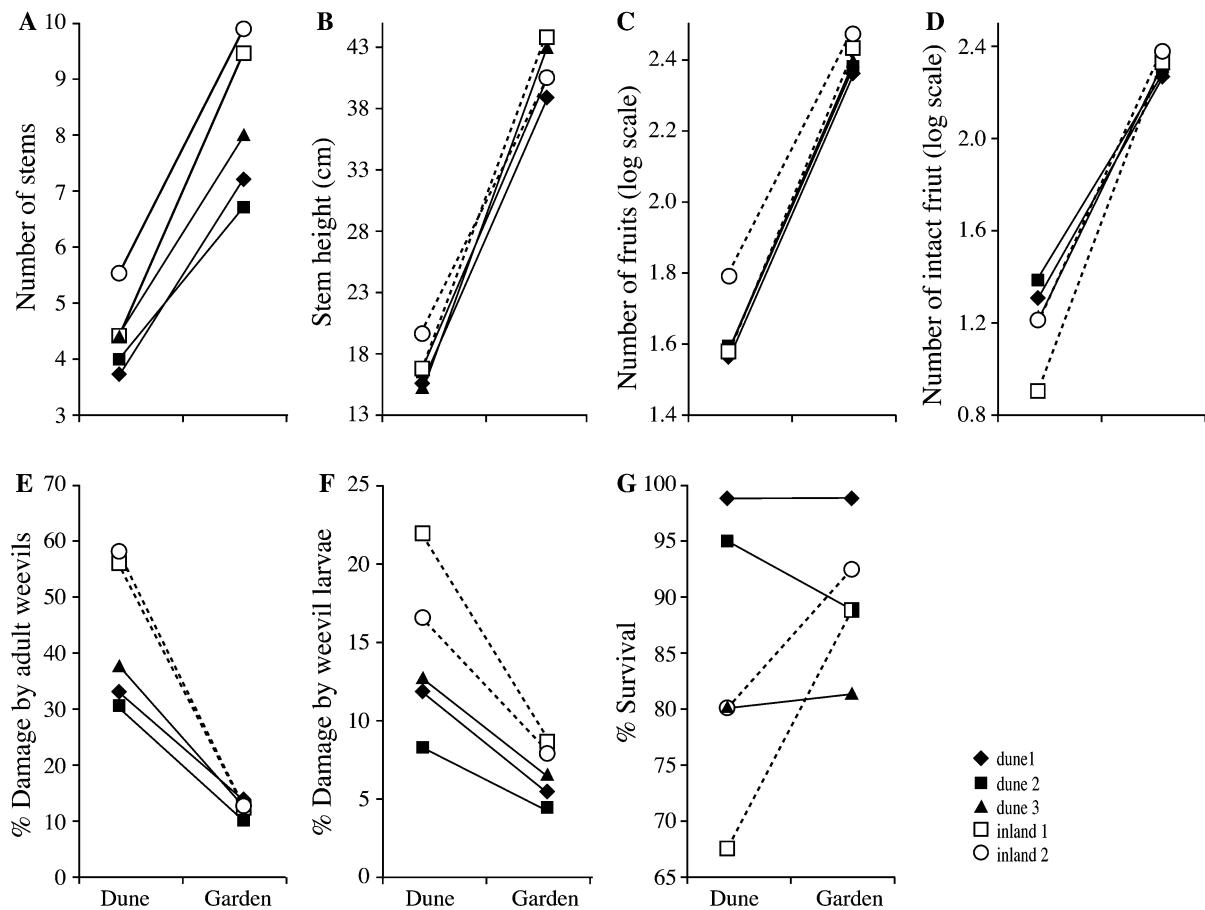


Fig. 1 Mean values for **a** number of stems, **b** stem height (cm), **c** total number of fruits, **d** number of intact fruits, **e** % flowers damaged by adult weevils, **f** % damaged by weevil larvae, and **g** % survival, for plants originating from 5

populations of *Arabidopsis thaliana* (3 in dunes, 2 in inland) when plants were grown in two habitats (dune and an inland garden)

same habitat, the differences have at least partly a genetic background. It is an interesting finding that plants of dune origin are more plastic than plants of inland origin. This might suggest adaptation to the

dune habitat, which is, over the long run, perhaps more stable than the inland habitats with strong and unpredictable human disturbance.

Dune and inland plants showed consistent chemical differences. After growing plants in the lab for one generation we analyzed their chemical content by NMR spectroscopy and multivariate data analysis. These methods showed a clear separation between dune and inland genotypes based on metabolites (especially glucosinolates) in both seeds and leaves (Mosleh Arany et al. 2008). Glucosinolate concentrations were also typically higher in plants of dune origin.

The dune type was better defended against beetle herbivory. The mechanism behind the defence is, however, not yet clear. In crucifers glucosinolates are

Table 2 Log-transformed differences in fitness (see text for explanation) between plants derived from different populations nested with dune or inland origin when transplanted into a dune or garden site (95%-CL, $n = 10$)

Origin ↓	Dune site	Garden site
Dune 1	1.27(0.12)	2.22(0.11)
Dune 2	1.34(0.14)	2.23(0.13)
Dune 3	1.12(0.15)	2.23(0.12)
Inland 1	0.76(0.24)	2.28(0.11)
Inland 2	1.01(0.27)	2.32(0.11)

known to be involved in defence against herbivores. In a subsequent study (Mosleh Arany et al. 2008) we found differences in herbivory when plants of dune and inland plants were offered to larvae of generalist herbivore *Spodoptera exigua* under standard lab conditions. The larvae ate less from the dune plants, which may be due to their higher glucosinolate level. However, the feeding of *Ceutorhynchus* in the field appeared uncorrelated to glucosinolate content (Mosleh Arany 2006), suggesting that other factors play a role in plant defence against this specialist.

It is of interest to compare our results with those collected on other species in the *Brassicaceae*. Over small distances (<15 km) *Brassica oleracea* populations growing along the Atlantic coastlines of Great Britain and France, differed in glucosinolate content and composition, which correlated negatively with herbivore performance (Gols et al. 2008). In an earlier study, Mithen et al. (1995) reported up to a tenfold difference in glucosinolate composition between *B. oleracea* plants in different habitats. They ascribed this difference to selection for high glucosinolates in habitats with generalist herbivores and to selection for low glucosinolates in habitats with specialist herbivores. Moyes and Raybould (2001) found that the seed-eating beetle *Ceutorhynchus assimilis* did not oviposit on *Brassica nigra* but did on *B. oleracea*, regardless of the glucosinolate profiles of the individual plants.

We did not record date of first flowering for individual *A. thaliana* plants, but noticed that plants of dune origin flowered about a week later than plants from inland origin. Since the weevils were feeding most conspicuously on the plants towards the end of the experiment when few flowers were left, we think that late flowering of dune plants does not explain why they have less herbivory. It is rather the opposite, if dune plants would have flowered at the same time as inland plants, they probably would have even less herbivory and the effect of origin on herbivory would have been even higher. Furthermore, we did not collect data on the number of remaining seeds per fruit for plants of different origin, but instead used for all plants an average of 20% seed survival that was established in a previous year in the dune population (Mosleh Arany et al. 2005). It is conceivable that beetle larvae leave fewer seeds uneaten before they depart from a fruit on an inland plant. Such a difference would increase the difference in herbivory

levels between plants of dune and inland origin. Clearly more detailed observations are needed here to understand the mechanism behind plant defence.

Herbivory was consistently higher in the dunes, and plants of dune origin were less eaten than plants of inland origin. We suggest this is due to a history of selection exerted by the beetles. However, conditions in dunes and inland differ in many respects and other selective factors may well have played a role. To test the hypothesis that the beetles are indeed the main selective factor, one would have to monitor seed herbivory in a large number of European *A. thaliana* populations and relate these measurements to plant characters. If high levels of field herbivory correlate positively with plant defence under standard conditions, this would strengthen our tentative suggestion that the specialist beetles indeed drive selection for plant defence in *A. thaliana*. Similar questions have been addressed for *Brassica* species and their herbivores. The model species *A. thaliana* has the advantage that it flowers early and is not affected by the many cabbage butterflies that attack the later flowering *Brassica* species. This would make the *Arabidopsis* system simpler and perhaps suited for studying the controversial role of specialist herbivores on evolution of plant defence.

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The effect of nutrient supply and light intensity on tannins and mycorrhizal colonisation in Dutch heathland ecosystems

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Abstract (1) Increased atmospheric nitrogen deposition has shifted plant dominance from ericaceous plants to grass species. To elucidate the reduced competitiveness of heather, we tested the hypothesis that additions of nitrogen reduce the concentrations of phenolics and condensed tannins in ericaceous leaves and retard mycorrhizal colonisation in ericaceous plants. We also tested the negative effects of reduced light intensity on carbon-based secondary compounds and mycorrhizal colonisation in ericaceous plants. (2) We performed a field inventory at three heathland sites in the Netherlands varying in nutrient supply and light intensity. Leaves of ericaceous plants and grasses were collected and analysed for concentrations of tannins, phenolics and nutrients. Similarly, we took root samples to record mycorrhizal colonisation and soil samples to measure the soil mineralisation. In addition, we conducted two-factorial experiments with *Calluna vulgaris* plants, in which we varied fertiliser and shade levels under greenhouse and field conditions. (3) The field inventory revealed that nitrogen addition and shading both negatively affected the concentration of total phenolics. The total phenolics and condensed tannin concentrations were positively correlated ($P < 0.001$), but in the field experiment, the condensed

tannins were not significantly affected by the treatments. Our results provide the first evidence that the carbon nutrient balance can be used to predict the amount of total phenolics in the dwarf shrub *C. vulgaris*. (4) In the field experiments, shading of plants resulted in significantly less mycorrhizal colonisation. Only in the greenhouse experiment did addition of nitrogen negatively affect mycorrhizal colonisation. (5) Our results imply that increased atmospheric nitrogen deposition can depress the tannin concentrations in ericaceous plants and the mycorrhizal colonisation in roots, thereby reducing the plants' competitiveness with respect to grasses. Additionally, if ericaceous plants are shaded by grasses that have become dominant due to increased nitrogen supply, these effects will be intensified and competitive replacement will be accelerated.

Keywords *Calluna vulgaris* · Carbon-nutrient balance · Dominant grasses · Ericaceae · Ericoid mycorrhiza · Nitrogen deposition

Introduction

In The Netherlands, increased atmospheric nitrogen deposition has shifted plant dominance in heathlands from ericaceous plants towards grass species (Berendse and Aerts 1984; Aerts 1993; Berendse et al. 1994). It is estimated that around 35% of the

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heathland area has become grass-dominated (Bobbink et al. 1998). The increased dominance of grasses has been attributed to their higher growth rate potential than that of dwarf shrubs (Berendse and Elberse 1990). Furthermore, outbreaks of heather beetle in nitrogen-fertilised vegetation have also strongly accelerated the expansion of grasses (Heil and Diemont 1983; Bobbink and Heil 1993; Power et al. 1998). Other factors (e.g. drought- and frost resistance of dwarf shrubs) can also be affected by changes in nitrogen deposition (Power et al. 1998).

An alternative hypothesis, proposed by Northup et al. (1995, 1998) suggested that the dominance of plant species in nutrient-poor ecosystems is the outcome of the production of high levels of carbon-based secondary compounds, e.g. tannins. Mineralisation has traditionally been considered to be the critical factor in nitrogen cycling, the decisive factor in nutrient-poor ecosystems (Chapin 1995; Northup et al. 1995). However, evidence is increasing that the production of large amounts of tannins and the concomitant utilisation of organic nitrogen enables plants in nutrient-poor ecosystems to short-cut the nitrogen cycle, so reducing their dependence on soil nitrogen mineralisation (Bradley et al. 2000; Fierer et al. 2001; Schimel et al. 1996, 1998). So, within these ecosystems plant species, which are able to use organic nitrogen, are expected to have a competitive advantage over plants which are not able to use these nitrogen sources.

Tannins can be subdivided into the hydrolysable tannins (sugar molecules esterified by a number of gallic acid moieties) and the condensed tannins, also referred to as proanthocyanidins (polymers of flavan-3-ols) (Harborne 1997). Both types of tannins can affect nutrient availability in the soil. They interact with proteins during the decomposition of litter material (Schimel et al. 1996; Fierer et al. 2001; Haslam 1998). Tannin–protein complexes are difficult to mineralise, and subsequently, they determine the proportions of nitrogen released in dissolved organic and inorganic forms (NH_4^+ , NO_3^-) (Handley 1961; Northup et al. 1995).

Ericaceous plants can use organic nitrogen more efficiently due to symbiosis with ericoid mycorrhizal fungi (Bending and Read 1997). Via the exudative enzymes, these fungi are able to solubilise tannin–protein complexes (Bending and Read 1997). Sokolovski et al. (2002) showed that *Calluna vulgaris* (L.)

Hull roots are able to use more organic nitrogen when colonised with these fungal symbionts. Therefore, at low mineralisation rates, ericaceous plants are thought to outcompete plant species that form arbuscular mycorrhiza, or non-mycorrhizal plants unable or less able to absorb organic nitrogen (Northup et al. 1998; Read et al. 2004).

Additions of nitrogen can have negative effects on the carbon-based secondary compounds in ericaceous leaves (Bryant et al. 1983). According to the carbon nutrient balance (CNB) hypothesis (Bryant et al. 1983), an increase in nitrogen concentration—as a result of increased nitrogen deposition—would reduce the production of carbon-based secondary compounds. In this case, the available carbon is invested in growth rather than in defence. It is well established that nitrogen addition increases nitrogen concentrations in heather, *C. vulgaris* (Berdowski and Siepel 1988; Iason et al. 1993; Duncan et al. 1994; Hartley et al. 1995; Carrol et al. 1999; Gordon et al. 1999) and other ericaceous plants (Mallik 1996; Prescott et al. 1993; Nordin et al. 1998). However, the expected decrease in total phenolics or condensed tannin levels due to solely nitrogen additions has not yet been empirically shown either in the greenhouse or in the field (Iason and Hester 1993; Iason et al. 1993; Hartley et al. 1995; Kerslake et al. 1998; Bradley et al. 2000; Alonso et al. 2001; Hansen et al. 2006). In contrast, Hansen et al. (2006) recently showed that a combination of nitrogen addition with increased air temperature seems to induce a positive response of condensed tannins in one year old leaves of *Vaccinium vitis-ideae* (L.) and *Cassiope tetragona* (L.) D. Don.

Increased levels of atmospheric nitrogen can also lead to decreases in ericoid mycorrhizal colonisation of ericaceous roots. With increased inorganic nitrogen supply, one would expect that the plant invests less carbon to symbiosis with root-associated fungi, and so ericaceous dwarf shrubs would hardly be able to compete with grasses. Two greenhouse experiments have shown that ammonium addition can negatively affect the extent of ericoid mycorrhizal colonisation in *C. vulgaris* roots (Mickel et al. 1991; Yesmin et al. 1996). However, there is no indication from field experiments that adding nitrogen reduces mycorrhizal colonisation (Lee et al. 1992; Caporn et al. 1995; Johansson 2000).

Light intensity can also influence the amounts of carbon-based secondary compounds and mycorrhizal

colonisation in ericaceous plants. In the context of shrub-grass dynamics, this can be important as when ericaceous plants become shaded by grasses that have become dominant due to increased nitrogen supplies competition replacement can be accelerated through these effects. Conflicting results on the effects of shading on the amounts of carbon-based secondary compounds have been published. Iason and Hester (1993) showed in a field experiment that shading reduced the concentration of total phenolics in *C. vulgaris*. In contrast, Hartley et al. (1995) found no effects of shading on the concentration of total phenolics and condensed tannins in *C. vulgaris* plants. Whereas, Hansen et al. (2006) even found a positive response of shading on the concentration of condensed tannins in *V. vitis-idaea* and *C. tetragona*. In non-ericaceous plants, shading usually reduces the levels of carbon-based secondary chemicals (Hartley et al. 1997; Henriksson et al. 2003, Ruohomäki et al. 1996; Iason et al. 1996). In plants with ectomycorrhizal fungal symbionts, shade effects on the amount of colonisation or amounts of structures are ranging from no effect (Bearly et al. 2007; Dehlin et al. 2004) to decreased colonisation rates (Becker 1983; Ingleby et al. 1998) or even increased colonisation rates (Prajadinata and Santoso 1993; Be'reau et al. 2000). As these ectomycorrhizal fungi produce a Hartig net around the roots, we assume that they show a different response to shading compared to ericoid mycorrhizal fungi, which only proliferate within the epidermal cells of roots. A field study in a subarctic birch forest understorey with *Empetrum*, *Vaccinium* and *Andromeda* species suggests that there is a positive correlation between photosynthetic activity and ergosterol concentration in ericoid roots (Olsrud et al. 2004). However, the same authors also showed that ergosterol content in ericoid mycorrhizal is not correlated with ericoid mycorrhizal colonisation, but is only indicative for the presence of dark septate endophytes (Olsrud et al. 2007). So far, the effect of shading on mycorrhizal colonisation in ericaceous plants is still unclear. We, therefore, decided to test the hypothesis that shaded plants will invest less in mycorrhizal symbiosis and mycorrhizal colonisation will be reduced.

The relation between phenolic levels in plant material and mycorrhizal colonisation has been hypothesized, but it was never thoroughly examined

(Hättenschwiler and Vitousek 2000; Northup et al. 1998). In this research, we asked which abiotic factors (nitrogen and light) would significantly influence the levels of both phenolic compounds and of mycorrhizal colonisation. To circumvent the shortcoming of one particular research method, we combined field observations with greenhouse and field experiments. Initially, we carried out a field inventory on several heathland plants, to study the natural variation of phenolics, condensed tannins and mycorrhizal colonisation in the field. We selected two heathland sites and two *Quercus–Vaccinium* forest sites in the centre and north of The Netherlands. At the heathland sites, we selected two areas, which differed in soil nitrogen supply (soil subjected to recent sod removal vs. soil with a thick organic layer) and at the *Quercus* sites, we selected plots, which varied in natural light intensity (shaded vs. non-shaded communities). Additionally, at one of the sites we choose an area where we collected four ericaceous plant species and two dominant grass species, to analyse the interspecific variation in phenolic and condensed tannin levels. Subsequently, we conducted fertiliser experiments, with *C. vulgaris* in the greenhouse and under field conditions. In these experiments, we also applied shading to test the negative feedback of reduced light intensity on phenolic compounds and mycorrhizal colonisation.

Methods

Field inventory

Field sites and selected species

The field locations were selected at three sites in The Netherlands: in the north, Dwingelderveld National Park (A, 52° 47'N, 6° 25'E); in the centre, De Hoge Veluwe National Park (B, 52° 4'N, 5° 50'E) and Hoog Buurlo (C, 52° 10'N, 5° 54'E). Table 1 shows the collected plant species and relevant soil data for each site. To compare variation in plant chemistry as affected by soil nutrient supply, we selected two dwarf shrub vegetation sites (A and B) with *C. vulgaris* and *Deschampsia flexuosa* (L.) Trin. Plots in recently turf-stripped sites were chosen adjacent to older heathland sites. At the stripped plots the turf layer had been removed up to four years prior to

Table 1 Field inventory overview of the investigated site pairs contrasting with respect to soil nutrients (A) or light intensity (B), showing growth conditions, the plant species collected at each site, and related soil nutrient factors (mean \pm SE, d.f. = 4)

A: Soil nutrients					Sod removed	Site	Interaction
Site	A	A	B	B			
Growth conditions	Sod removal (N-)	Thick organic layer (N+)	Sod removal (N-)	Thick organic layer (N+)			
Plant species	Cv	Cv	Cv	Cv			
N mineralisation (g N/m ² /year)	2.5 (2.1)	4.6 (3.1)	0.8 (0.2)	12.0 (4.2)	***	*	**
C:N soil	34.5 (1.9)	28.7 (0.6)	24.6 (0.4)	25.9 (0.6)	NS	***	**
pH-KCl	3.1(0.1)	2.9 (0.0)	3.4 (0.1)	2.9 (0.0)	***	NS	NS
Org. matter (%)	0.10 (0.0)	0.13 (0.02)	0.06 (0.0)	0.12 (0.01)	*	NS	NS
B: Light intensity					Shade	Site	Interaction
Site	A	A	C	C			
Growth conditions	No shade (S-)	Shade (S+)	No shade (S-)	Shade (S+)			
Plant species	Vvi	Vvi	Vm	Vm			
N mineralisation (g N/m ² /year)	5.2 (7.2)	3.4 (4.6)	10.7 (6.3)	15.9 (6.0)	NS	**	NS
C:N soil	25.9 (1.5)	24.3 (0.7)	25.6 (0.3)	20.1 (0.3)	***	*	*
pH-KCl	2.8 (0.2)	2.9 (0.1)	2.8 (0.0)	2.9 (0.0)	NS	NS	NS
Org. matter (%)	0.17 (0.02)	0.11 (0.04)	0.16 (0.01)	0.08 (0.01)	***	NS	NS

Sites: A = Dwingelderveld National Park; B = De Hoge Veluwe National Park; C = Hoog Buurlo. Plant species: Cv = *Calluna vulgaris*; Vvi = *Vaccinium vitis-idaea*; Vm = *V. myrtillus*. Significance levels for the main effects and the interaction between them are given

*P < 0.05; ** P < 0.01; *** P < 0.001

NS = non-significant

plant collection. The groundwater levels of the sites were similar. The effects of light intensity on *Vaccinium* and *Deschampsia* were investigated by comparing the natural occurring variation in shaded (50% incident light) and non-shaded plots in *Quercus robur* L. woodlands on sites at Dwingelderveld National Park and Hoog Buurlo. The *Quercus robur* L. woodlands consisted of a herbaceous layer dominated by the ericaceous dwarf shrubs *Vaccinium vitis-idaea* L. (A) or *Vaccinium myrtillus* L. (C) and the grass *D. flexuosa*. We preferred to select the *Quercus* woodlands as these are one of the few available places in The Netherlands where we could find shaded ericaceous plants. The additional site at Hoog Buurlo was a mixed heathland with *C. vulgaris*, *Erica tetralix* L., *V. myrtillus*, *V. vitis-idaea* L., *Molinia caerulea* (L.) Moench and *D. flexuosa*. The field sites were roughly 25 × 50 m. Within each site, five plots of 1 m² were chosen 10–25 m apart.

Leaf measurements

Plant material was collected on 5th and 6th June and 7th and 8th September 2000. Only the first year growth of green leaves was sampled. For proper handling of the leaf material prior to analyses, we followed the guidelines described in Waterman and Mole (1994). The leaf material was kept cool and brought to the laboratory where it was frozen immediately (-18°C) until handled for analyses. Leaves were dried for two days at 38°C. The leaves were separated from stems and flowers by sieving (2 mm) before grinding. For extraction 20 ml of 50% (v/v) methanol was added to 0.19 g dry leaf material. The mixture was covered and placed in a water bath (75°C) for 1 h. The sample was then filtered through a glass filter and the extract adjusted to 50 ml with 50% (v/v) methanol. Total phenolics were determined following the Folin-Ciocalteu method (Waterman and Mole 1994). Condensed tannins were

analysed following the butanol–HCl method of Porter et al. (1986). All analyses were performed in duplicate. Given the problems and complexities of applying an appropriate standard for the proanthocyanidin method (Waterman and Mole 1994), the data are presented as final absorbance at 550 nm. Another portion of dried leaves (70°C) was pulverised and C and N concentrations were measured using an elemental analyser (Fisons Instruments, EA 1108).

Soil measurements

Soil cores (10 cm deep, 5 cm diameter) were taken on 5th and 6th June 2000 and stored at 4°C overnight. After removing any coarse roots and stones, the extractable NH₄–N and NO₃–N were determined in 10 g fresh soil extracted in 25 ml 1 M KCl. The extracts were filtered through filter paper (Schleicher and Schüll no. 589³). Concentrations of the extractable ions in the soil were calculated from the concentrations in the extract using the soil water content. The soil pH was also measured in the same soil extract. To estimate the net mineralisation rate, a subsample of 10 g soil was incubated for 6 weeks at 20°C and then the extractable NH₄–N and NO₃–N were measured. Net mineralisation rates were calculated from the difference between the amount of NH₄⁺ and NO₃[–] before and after incubation. To measure the soil water content a subsample of 5 g soil was dried (105°C) overnight. Organic matter content was determined after combustion at 550°C. The C and N concentrations were measured using an elemental analyser (Fisons Instruments, EA 1108).

Collection of plant roots and mycorrhizal analyses

For each species, roots of one individual plant were collected in each plot on 7th and 8th September 2000 with a soil auger of 20 cm (10 cm diameter). The roots were kept moist in a plastic bag (25 × 10 cm). The soil was removed by washing the roots over a 2 mm sieve. In the laboratory, roots were further cleaned from organic material with forceps, and stored in 50% ethanol. They were then stained in 0.2% solution of trypan blue in lactic acid:glycerol:water (3.25:3:4 by vol.) and transferred to a storage solution of lactic acid:glycerol:water (1:2:1 by vol.). From each root system of an individual plant, 30 healthy root tips were then randomly

selected, mounted on a microscopic slide and, using a light microscope, were examined at 40× magnification for the presence of ericoid mycorrhizal structures in ericaceous roots and for arbuscular mycorrhizal structures in grass roots. Ericoid mycorrhizal structures are most abundantly present in root tips and can be recognized by hyphal coils in epidermal root cells (Read 1996). AMF colonisation was recognized by the presence of vesicles and arbuscules connected to broad, aseptate hyphae. Colonisation of epidermal cells in each root tip was estimated as percentage of colonized cells in the superficial cell layers in 1 cm root.

Greenhouse experiment

From May 2001 to March 2002, we conducted a two-factorial experiment with shading and fertilisation in a greenhouse with controlled climatic conditions (light/dark: 14/10 h., light intensity 50 W m^{–2}, temperature day/night: 20°C/15°C, 70% R.H.). The treatments were: no shade or fertiliser (S–F–), fertiliser (S–F+), shade (S+F–), shade and fertiliser (S+F+). Each treatment was replicated five times. Twenty plastic pots (14 cm diam) were filled with 2.5 kg sand (sand mixed with organic-rich soil, 5:1, v/v). We added one part organic-rich soil to provide the control treatments with a basic level of nutrients. *C. vulgaris* seedlings (~2.5 cm tall) were collected from the De Hoge Veluwe National Park and placed three in each pot. Given the results of the field study, we assumed that all roots from the collected *C. vulgaris* seedlings were colonised by mycorrhizal fungi. The shade treatment involved excluding 50% of incident light using shade netting around each individual pot. The pots were spaced widely to avoid the shade netting constructions shading other plants. Fertilised plants received amounts equivalent to 75 kg N, 25 kg P and 50 kg K ha^{–1} year^{–1}. The fertiliser was applied in June and August. In this experiment, we chose a compound fertiliser, to prevent phosphate limitations. The pots were placed randomly. The plants were watered regularly to keep the soil moisture at 60% of water saturation; for this purpose, the pots were weighed twice per week. After 12 months the above-ground plant parts were clipped off at soil level and dried at 38°C, to measure the dry weight. The roots were gently removed from the soil, then washed to remove any adhering soil and also

dried at 38°C. Total phenolics, condensed tannins, nitrogen and carbon content and mycorrhizal colonisation were measured as described in the previous section. The amounts of inorganic nitrogen and pH KCl of the soil were determined.

Field experiment

At a site in De Hoge Veluwe National Park a field experiment was carried out from September 2001 to March 2003. The field site (50×60 m) was a heathland dominated by *C. vulgaris*. Other plant species were *E. tetralix*, *M. caerulea* and *D. flexuosa*. The field treatments were similar to the treatments in the greenhouse experiment. Each treatment was replicated at five sites in this area, following a randomised block design. Within a site, individual plots of 1 m² were chosen 5–10 m apart and the sites were spaced at 40–50 m. Each plot was fenced with fine-meshed wire to exclude large herbivores. To reduce the light by 50% in the shade treatments, shade nets were put around and above the enclosures (1 m height). The fertilised plots received 50 kg N ha⁻¹ year⁻¹ (NH₄NO₃) in a single treatment. Initial amounts of soil nitrogen were measured before the enclosures were erected and again at harvest. Inorganic nitrogen was measured as described earlier and the total dissolved nitrogen (DON + inorganic N) was determined conductimetrically after persulfate oxidation of the extract (Yu et al. 1993). DON was calculated by subtracting inorganic nitrogen from the total dissolved nitrogen. At the end of the experimental period, the first year's growth of green leaves is harvested. Per plot, we collected one root sample. Shoots and roots were analysed as described for the field inventory.

Statistical analyses

Field inventory

The effects of sod removal or light intensity on soil chemistry parameters was analysed using ANOVA with sod removal and site, or light intensity and site as the respective fixed factors ($P < 0.05$). The effects of sod removal on plant characteristics were analysed using ANOVA with sod removal and site as the fixed factors ($P < 0.05$). The effects of light intensity on plant characteristics was analysed by ANOVA with

shade and plant species as the fixed factors ($P < 0.05$). If the assumption of heterogeneity of variance was violated, the data were log-transformed. To compare total phenolic levels between plants under different treatments at one site, one-way ANOVA was carried out followed by the Tukey post hoc test ($P < 0.05$).

Field and greenhouse experiment

The results of the greenhouse and field experiments were analysed using two-way ANOVA with shade and fertiliser as fixed factors ($P < 0.05$).

Results

Field inventory

On sites where the turf layer had recently been stripped, the net mineralisation of the soil was lower than on older heathland sites with thicker organic layers (Table 1). No differences in net mineralisation were found between the shaded and non-shaded sites. The sites with *V. myrtillus* showed higher mineralisation rates compared to the sites with *V. vitis-idaea* (Table 1). The concentration of total phenolics in the plant ranged from 50–437 mg tannic acid equivalents/g dry weight (Fig. 1a–c). Differences between the amounts of phenolics were related to plant species, growth conditions and site (Table 2). Ericaceous leaves contained larger amounts of total phenolics than the grasses (Fig. 1c, $P < 0.001$). There was also considerable variation among the ericaceous species. At the species-rich site at Hoog Buurlo, *V. myrtillus* leaves contained the highest amounts of total phenolics, *V. vitis-idaea* and *C. vulgaris* were intermediate, while levels were lowest in *E. tetralix* (Fig. 1c, $P < 0.05$). Soil nutrient supply significantly decreased the concentration of total phenolics and condensed tannins. *C. vulgaris* showed higher levels of total phenolics and condensed tannins in leaves when grown on humus-poor soils compared to humus-rich soils (Fig. 1a; Table 2). Shade also decreased the levels of phenolics and tannins (Table 2). Plants growing under shaded conditions produced remarkably less total phenolics and condensed tannins, not only in the ericaceous plants, but also in the grass *D. flexuosa* (Fig. 1b, e; Table 2).

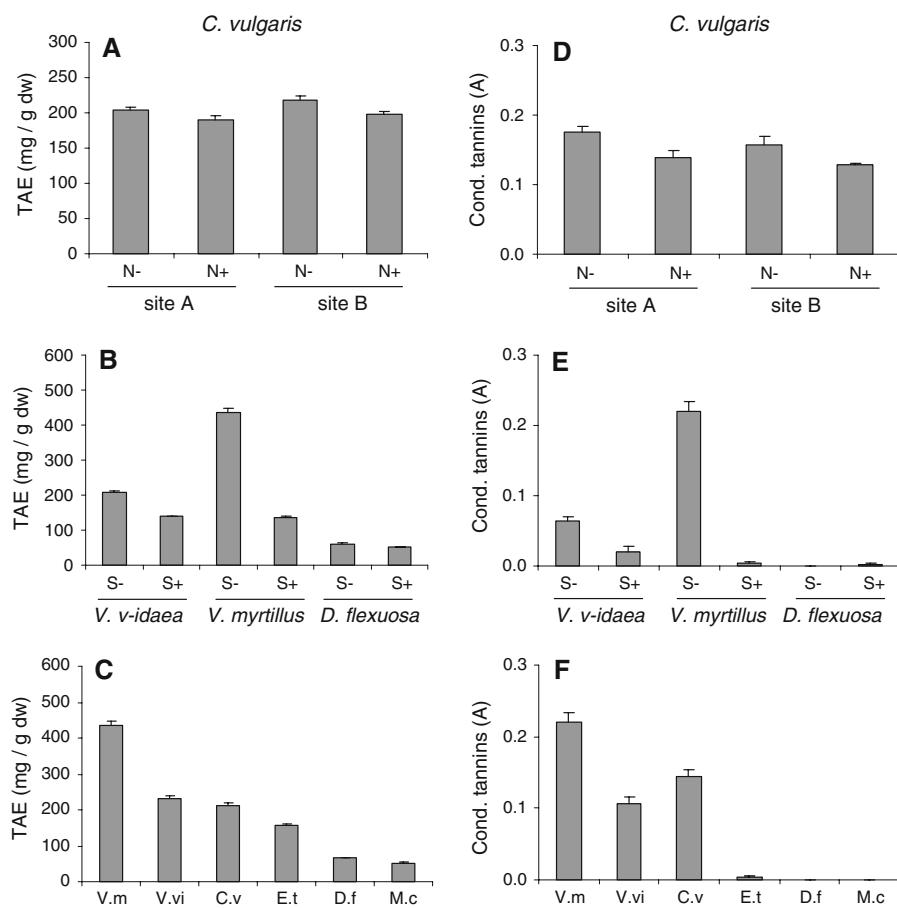


Fig. 1 Results of the field inventory. Mean values of total phenolics (TAE = tannic acid eq. mg/g dw) (**a**)–(**c**) and condensed tannins (absorption value) (**d**)–(**f**) in ericaceous plants and grasses at contrasting growth conditions and sites. **a** and **d** Nutrient contrasts: N– = sod removed, N + = thick organic layer. Site A = Dwingelderveld National Park; site B = De Hoge Veluwe National Park. **b** and **e** Light intensity

contrasts within *Vaccinium vitis-idaea* (site A), *V. myrtillus* (site C) and *D. flexuosa* (site C); S+ = shaded, S– = non-shaded. **c** and **f** Non-shaded site at Hoog Buurlo (site C) with four ericaceous plants and two dominant grasses: C.v, *Calluna vulgaris*; E.t, *Erica tetralix*; D.f, *Deschampsia flexuosa*; M.c, *Molinia caerulea*; V.vi, *Vaccinium vitis-idaea*; V.m, *V. myrtillus*. Data are \pm SE ($n = 5$)

There was a strong positive relationship between the amount of total phenolics and the amount of condensed tannins (Table 2, $r^2 = 0.72$, $P < 0.001$), especially in the ericaceous species. The nitrogen concentration and C:N ratio in plant leaves depended on plant species, growth condition and site (Table 2). The level of mycorrhizal colonisation depended on growth conditions and plant species (Fig. 2; Table 2). We did not find a significant effect of sod removal on the level of mycorrhizal colonisation at the *C. vulgaris* sites. In contrast, shading had a clear negative effect on mycorrhizal colonisation in roots of *Vaccinium* plants. Shading also reduced mycorrhizal

colonisation in grass roots, but to a lesser extent compared to the ericaceous plants.

Greenhouse experiment

Total biomass of the *C. vulgaris* plants was affected by both shade and fertiliser treatments (Table 3). With nitrogen fertilisation, the plants produced more shoots and roots. Shading induced a reduction of the root biomass: the related shoot:root ratios were increased by more than 100%. Flower production was increased by fertilisation and decreased by shade. Surprisingly, the amount of total phenolics increased

Table 2 Analysis of variation in plant chemistry: amount of total polyphenols, condensed tannins, mycorrhizal colonisation, concentration of nitrogen, concentration of carbon, and leaf C:N ratio

	Total phenolics	Condensed tannins	Mycorrhizal colonisation	C (%)	N (%)	C:N ratio
<i>Soil nutrients</i>						
Sod removal	14.0**	16.6***	0.0	0.2	230.8***	314.2***
Site	7.2*	2.8	1.9	16.6***	31.9***	23.2***
Interaction	0.6	0.2	12.9**	0.5	2.5	0.0
<i>Light intensity</i>						
Shading	980.3***	217.5***	16.1***	3.8	211.7***	225.8***
Plant species	1016.1***	123.5***	8.6**	80.8***	153.9***	300.2***
Interaction	461.1***	129.5***	2.1	6.5**	9.8***	0.1

F values and significance levels for the main effects of the factors sod removal, site, shading and plant species, and the interaction between them are given

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

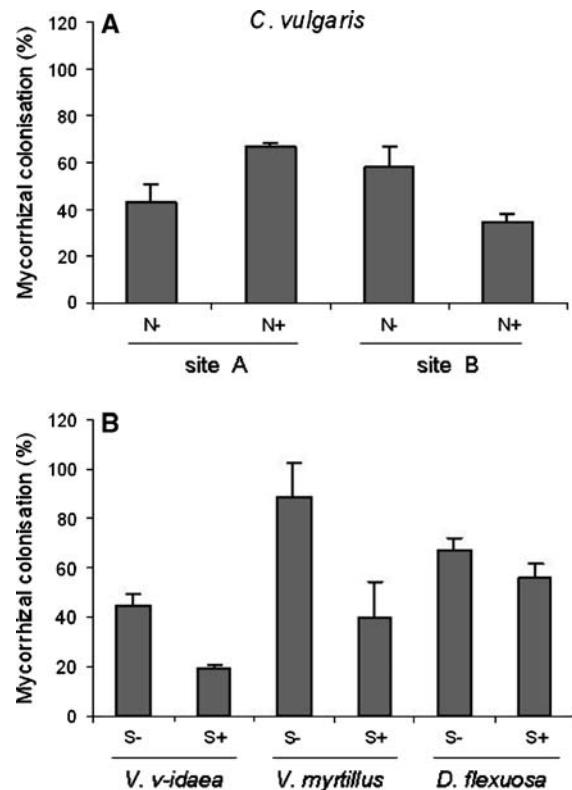


Fig. 2 Mycorrhizal colonisation (%) in roots of *Calluna vulgaris*, *Vaccinium vitis-idaea*, *V. myrtillus* and *Deschampsia flexuosa* under contrasting growth conditions. **a** Nutrient contrasts: N- = sod removed, N+ = thick organic layer. Site A = Dwingelderveld National Park; site B = De Hoge Veluwe National Park. **b** Light intensity contrasts within *Vaccinium vitis-idaea* (site A), *V. myrtillus* (site C) and *D. flexuosa* (site D): S+ = shaded, S- = non-shaded. Data are means \pm SE ($n = 5$)

significantly when plants were shaded, whereas shading plus fertilisation resulted in the lowest amounts of total phenolics. As expected, the C:N ratios in the leaves decreased due to fertilisation and shading. In this experiment, fertilisation had a significant negative effect on the amount of mycorrhizal colonisation in the *C. vulgaris* roots. The mycorrhizal colonisation was positively related to the amount of inorganic nitrogen (linear regression, $r^2 = 0.34$, $P = 0.007$), but negatively related to total biomass (linear regression, $r^2 = 0.23$, $P = 0.03$) and root biomass (linear regression, $r^2 = 0.29$, $P = 0.02$).

Field experiment

In the field experiment, the amount of total phenolics and C:N ratio in ericaceous plants were negatively affected by both shading and fertilisation (Table 4). In contrast, the amount of condensed tannins was not affected. As expected, the fertilised plants had higher nitrogen concentrations than unfertilised plants. Mycorrhizal colonisation was only affected by shading. The shade treatment increased the amount of inorganic nitrogen and DON in the soil, but reduced the ratio DON:inorganic nitrogen. Mycorrhizal colonisation was not related to any of the measured plant or soil parameters. The amount of inorganic nitrogen was negatively correlated with the amount of total phenolics (linear regression, $r^2 = 0.29$, $P = 0.02$) and condensed tannins in the plants (linear regression, $r^2 = 0.20$, $P = 0.06$).

Table 3 Results of the greenhouse experiment with *Calluna vulgaris* seedlings

	S-F-	S-F+	S+F-	S+F+	S	F	S × F
<i>Plant</i>							
Total biomass (g dw)	2.24(0.07)	5.68 (0.40)	2.05 (0.31)	3.82 (0.72)	*	***	NS
Shoots (g dw)	1.48 (0.10)	3.54 (0.25)	1.56 (0.28)	2.51 (0.61)	NS	***	NS
Roots (g dw)	0.76 (0.18)	1.71 (0.30)	0.33 (0.08)	0.42 (0.03)	***	*	*
Shoot:root ratio	2.31 (0.47)	2.47 (0.61)	5.06 (0.44)	5.99 (1.30)	***	NS	NS
Flowers (g dw)	0.25 (0.04)	0.99 (0.11)	0.21 (0.04)	0.34 (0.05)	***	***	***
Total phenolics (mg tae/g dw)	158 (4)	180 (5)	165 (8)	131 (6)	**	NS	***
C (%)	47.6 (0.4)	48.4 (0.2)	49.1 (1.2)	48.1 (0.4)	NS	NS	NS
N (%)	1.2 (0.1)	1.3 (0.0)	1.6 (0.3)	1.8 (0.1)	**	NS	NS
C: N ratio	41.0 (1.9)	37.3 (1.1)	33.6 (3.6)	27.4 (0.9)	***	*	NS
Total N shoots (mg)	26.0 (1.4)	74.0 (5.2)	31.0 (4.3)	66.0 (1.1)	NS	***	NS
Mycorrhizal colonisation (%)	27.0 (4.4)	14.4 (1.2)	23.3 (2.5)	22.6 (2.6)	NS	*	NS
<i>Soil</i>							
Inorganic N (mg N/kg dw)	3.66 (0.16)	0.52 (0.09)	2.72 (0.48)	1.61 (0.27)	NS	***	**
pH KCl	7.1 (0.0)	7.2 (0.0)	7.2 (0.1)	7.2 (0.0)	NS	**	NS

Average values (\pm SE) are shown for the plant and soil characteristics. Significance levels for the main effects and the interaction between them are given

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

NS = non-significant

S+ = with shade

S- = no shade

F+ = with fertiliser

F- = no fertiliser

Discussion

Responses of mycorrhizal colonisation to N supply and light intensity

Our study shows that nitrogen supply negatively affects mycorrhizal colonisation of *C. vulgaris* roots under greenhouse conditions. This finding is in line with the results reported by Yesmin et al. (1996) and Mickel et al. (1991). It suggests that effects of nitrogen deposition are detrimental for the mycorrhizal colonisation of the dwarf shrub roots—and related to this—for the capacity of these plants to use the soluble organic nitrogen. However, in the field experiment, we found no detrimental effects of nutrient addition on colonisation rates. This is in agreement with the results of Lee et al. (1992), Caporn et al. (1995) and Johannsson (2000), who also found no effects of nutrient addition on mycorrhizal colonisation in field experiments. Apparently, the soil litter layer in the field containing large amounts of

organic acids stimulate more microorganisms than the sand medium used in the greenhouse experiment with alkaline pH (Tables 1 and 3). It seems that additional $\text{NH}_4^+ \text{-N}$ inputs become immobilised in the heath more layer (Adams 1986, Whitehead et al. 1997, Kristensen and Hendriksen 1998). Furthermore, the difference between the response to nutrient supply in terms of the amount of mycorrhizal colonisation of heather roots in the field and greenhouse experiments might be explained by the fact that only in the greenhouse was it possible to establish strong nitrogen-limited conditions.

In the Dutch heathlands, nitrogen deposition has increased soil nutrient supply significantly and heather plants are now less nitrogen-limited than several decades ago (Bobbink et al. 1998). The nitrogen concentrations in the plant can be used as estimators of the atmospheric nitrogen deposition (Hicks et al. 2000). In the control plots of our field experiment, the nitrogen concentration in the leaves of *C. vulgaris* was, on average 1.61%, compared with

Table 4 Results of the field experiment at De Hoge Veluwe National Park with *Calluna vulgaris* plants

	S – F–	S – F+	S + F–	S + F+	S	F
<i>Plant</i>						
Total phenolics (mg tae/g dw)	707 (86)	462 (56)	443 (51)	383 (52)	*	*
Cond. tannins ($A_{550}/0.19$ g dw)	0.042 (0.006)	0.021 (0.007)	0.021 (0.008)	0.016 (0.008)	NS	NS
% C	52.0 (0.5)	52.6 (0.3)	52.0 (0.2)	51.9 (1.0)	NS	NS
% N	1.6 (0.1)	1.8 (0.1)	1.8 (0.1)	2.0 (0.1)	NS	*
C: N ratio	32.5 (1.1)	28.9 (1.1)	29.0 (0.9)	26.3 (1.4)	*	*
Mycorrhizal colonisation (%)	20.92 (0.67)	20.12 (0.45)	11.36 (0.36)	10.70 (0.04)	*	NS
<i>Soil</i>						
Inorganic N (mg N/kg dw)	5.78 (1.10)	8.25 (1.23)	13.46 (1.18)	14.35 (1.61)	***	NS
DON (mg N/kg dw)	19.34 (1.09)	23.39 (2.04)	27.21 (1.61)	27.69 (2.21)	**	NS
DON: inorg. N ratio	3.94 (0.90)	3.03 (0.34)	2.04 (0.08)	1.97 (0.09)	**	NS
pH KCl	2.8 (0.0)	2.8 (0.0)	2.8 (0.0)	2.8 (0.0)	NS	NS
pH H ₂ O	3.8 (0.0)	3.8 (0.1)	3.8 (0.1)	3.9 (0.1)	NS	NS

Average values (± 1 SE) are shown for the plant and soil characteristics. Significance levels for the main effects between them are given

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

NS = non-significant

There were no significant interactions between the effects of the two treatments

S+ = with shade

S– = no shade

F+ = with fertiliser

F– = no fertiliser

1.17% in the greenhouse (Tables 3 and 4). Therefore, it is not surprising that the long-term effects of nitrogen supply on mycorrhizal colonisation under our field conditions were less pronounced than the effects observed in the greenhouse. We conclude that the negative effects of increased atmospheric nitrogen on mycorrhizal colonisation of ericoid heathland plants still remain hypothetical. More experiments under field conditions are needed to investigate the effects of increased atmospheric nitrogen on the reduction of mycorrhizal colonisation and the possible consequences for organic nitrogen uptake.

In the field inventory, we found in general a positive correlation between the amount of mycorrhizal colonisation in ericaceous plants and the concentration of total phenolics in the leaves. In contrast, neither of the experiments with *C. vulgaris* showed such a relationship. Although Sokolovski et al. (2002) also showed an increased organic nitrogen uptake by *C. vulgaris* root cells colonised by a mycorrhizal symbiont, the actual importance of the role of mycorrhiza for amino acid uptake in the

field is still being debated (Persson and Näsholm 2001).

Our finding that shading reduced the amount of mycorrhizal colonisation in ericaceous plants under field conditions is consistent with predictions of the CNB hypothesis and the protein competition model (Bryant et al. 1983, Jones and Hartley 1999). Simard et al. (2002) report that mycorrhizal fungi may receive carbon (e.g. sugars) amounting to 15–30% of the net photosynthate of their host plants. Also other studies (Bryant et al. 1983, Smith and Read 1997, Jones and Hartley 1999) have reported that shading reduced the amount of photosynthetic assimilates and therefore limited the amount of carbon translocated to the mycorrhizal symbiosis.

Responses of tannins to N supply and light intensity

Our results show that the CNB hypothesis can be very useful when predicting the amount of total phenolics in *C. vulgaris* under field conditions. The results from

the *Vaccinium* plants in the field inventory and the results from the field experiment confirmed our hypothesis that shade negatively affects the foliar concentration of total phenolics—a result previously found by Iason and Hester (1993). The deviating results in the greenhouse experiment where we found an increase of phenolic concentrations in shaded plants can be caused by the lower intensity of the overall shade reduction compared to the field conditions by which the shaded plants showed less contrast with the control plants. This is also shown by the small differences between the total biomasses of the treatments. However, the direction of the responses in the shade, fertiliser and combined treatments is comparable between the field and the greenhouse experiment.

Our experiment is the first report that the addition of fertiliser results in lower concentrations of total phenolics in *C. vulgaris* under field conditions. The reason for the failure in previous field experiments with *C. vulgaris* to detect a significant fertiliser effect on the foliar content of total phenolics (Iason and Hester 1993, Iason et al. 1993, Hartley et al. 1995, Alonso et al. 2001) is probably that these experiments were too short (most lasted less than 1 year).

We found that the amount of condensed tannins was not significantly affected by the addition of fertiliser. This is in accordance with the results of Iason and Hester (1993) and Bradley et al. (2000). Shading also had no detectable effect on the tannin levels. Concentrations of tannins in *C. vulgaris* plants do not seem to vary strongly. The contrasting results of Hansen et al. (2006) in which concentrations of condensed tannins increased with 8–13% in ericaceous shrubs after shading and a combined treatment with fertiliser can be due to the cold subarctic conditions, which could cause different translocation processes of carbon-based secondary compounds in the plant and in the studied 1-year old leaves.

We conclude from our field study that there is much natural variation in total phenolic and condensed tannin contents within and among ericaceous plants and that this depends strongly on site characteristics (light, soil nutrients) and plant species. The deciduous species, *V. myrtillus* seemed to have more plastic leaf characteristics than the evergreen species. The variation within and between the two grass species was remarkably smaller. The dominant grass species, which have higher growth rates than the

dwarf shrubs, showed lower concentrations of secondary plant compounds. So, it is not surprising that there is a strong negative correlation between the foliar concentration of nitrogen and the concentrations of total phenolics and condensed tannins among plant species. The protein competition model (Jones and Hartley 1999) also suggests that the regulation of protein and phenolic synthesis are tightly linked due to the use of the same precursor phenylalanine. Therefore, plant cells do not appear to be capable of simultaneously synthesising proteins and phenolics at the same rate (Haukioja et al. 1998).

Consequences for the competition between dwarf shrubs and grasses

Berendse and Elberse (1990) hypothesized that ericaceous plants growing in nutrient-poor ecosystems have a competitive advantage over grass species because they are rich in carbon-based secondary compounds that prolong their life span and reduce nitrogen losses. We formulate the additional hypothesis that the symbiosis of ericaceous plants with their ericoid mycorrhizal fungi that degrade protein–phenolic complexes in the soil enables them to use organic nitrogen sources not available to other plants like grasses with their arbuscular mycorrhizal fungi (Berendse and Elberse 1990; Northup et al. 1995; Hättenschwiler and Vitousek 2000; Hodge et al. 2001; Aerts 2002).

Increased nitrogen deposition can seriously hamper the competitive advantage of ericaceous plants, not only by increasing the nitrogen availability in the soil, but also as this study and others have shown, by enhancing the nitrogen concentration in ericaceous litter (e.g. Hartley et al. 1995). This leads to the reduction of the carbon:nitrogen ratio in the litter, which accelerates its decomposition, and finally accelerates the mineralisation of the soil nitrogen (Berendse et al. 1994, Bret-Harte et al. 2004). When nutrient-poor soils become enriched with nitrogen, the grasses have a competitive advantage over ericaceous plants as they are able to benefit faster from the increased nitrogen supplies (Berendse et al. 1994). For example, the competition experiment of Berendse and Aerts (1984) showed that the dwarf shrub *Erica tetralix* was only able to outcompete *Molinia cearulea* at the non-fertilised, nutrient-poor sites, while *Molinia* replaced *Erica* after nutrient addition.

Secondly, our study revealed that the levels of total phenolics in the ericaceous plants can decrease in response to nitrogen additions, thereby enhancing the degradation of the litter and accelerating N mineralisation (Schofield et al. 1998). Due to the reduced concentrations of phenolic compounds in the litter, the inorganic forms of soil nitrogen can increase relative to the organic forms (Northup et al. 1995, 1998). The field experiment with *C. vulgaris* showed that organic nitrogen was the most important labile nitrogen form, exceeding the amount of inorganic nitrogen by approximately 2–4 times (Table 4).

Under nitrogen-poor condition, the symbiosis of ericaceous plants with their mycorrhizal fungi, which are able to use complex organic nitrogen sources, supposedly gives them an advantage. So, when the organic nitrogen sources become relatively less important due to increased atmospheric nitrogen, the grasses—which can benefit more from the inorganic nitrogen sources than ericaceous plants—will outcompete the heathland shrubs (Berendse and Aerts 1984). Notwithstanding the fact that the grass, *Deschampsia* have been shown to be also able to use organic nitrogen (Näsholm et al. 1998; Falkengren-Grerup et al. 2000) and even can be colonized by ericoid endophytic fungi (Zijlstra et al. 2005), the highest N uptake rates are realised on ammonium and nitrate sources (Persson et al. 2003). Although the responses in our experiments were different, our data from the greenhouse experiment show that increased nitrogen addition can reduce mycorrhizal colonisation, which can result in less organic nitrogen being available to the ericaceous species (Sokolovski et al. 2002).

Finally, our data strengthen the idea of a positive feedback in the competition between ericaceous plants and grasses as a result of reduced light intensities, when nitrogen inputs increase. Shaded heathland plants seem to not only produce less phenolics and tannins and higher nitrogen concentrations in the leaves, but also tend to show reduced levels of mycorrhizal colonisation. In this way, shading reduces the competitive ability of heathland plants by directly reducing their nitrogen uptake capacity so that the expansion of the grasses at the cost of the dwarf shrubs will be strongly accelerated.

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Spatial and temporal dynamics of floating and drift-line seeds at a tidal freshwater marsh on the Potomac River, USA

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Abstract The dispersal of seeds through hydrochory can be an important driver of community dynamics and play an integral role in the colonization of restored wetlands. We assessed sources of seeds on the shoreline and in adjacent waters of the Potomac River to Dyke Marsh in Virginia. Drift-line samples were taken at 40 random points four times during 2005–2006, and water surface net trawling took place on the Potomac River from 2003–2005 using six, 200-m transects around the perimeter of the marsh. Seed supply through hydrochory and species richness was low at Dyke Marsh when compared to other regional tidal freshwater marshes. We discovered distinct temporal patterns, where high species richness and seed density were found in the fall for the water trawl samples but in the spring for the drift-line

samples. High fall dispersal in the water trawls may exist owing to peak plant senescence and seed release, while high seed germination in the spring along shorelines may arise due to sufficient cold stratification of marsh seeds.

Keywords Hydrochory · Seed dispersal · Seedling emergence · Water surface net trawl

Introduction

Wetland species can be distributed by various mechanisms including wind and animals, but water dispersal, or “hydrochory,” is the primary method of seed dispersal in riparian and tidal wetlands (Bakker et al. 1985; Middleton 1995). Hydrochory can be an important driver of community structure in wetland ecosystems (Schneider and Sharitz 1988) because it can result in the introduction of new species (Mitsch et al. 1998) and the introduction of exotic species (Thomas et al. 2005; Tabacchi et al. 2005) leading to more diverse wetland plant communities (Leyer 2006).

Seed dispersal via water has been found to play an integral role in the regeneration of restored wetland systems. Gurnell et al. (2006) investigated the vegetation colonization of a newly created channel in the United Kingdom. Hydrochory was the dominant source of seeds deposited along river banks in the winter. Plants that were recruited from these

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deposited seeds then acted as a significant local seed source the second year, which created a feedback loop trapping additional propagules and sediment, thereby propelling river bank succession. Huiskes et al. (1995), Wolters et al. (2005), and Gurnell et al. (2006) all found the seasonality of seed dispersal through waterways to strongly influence colonization of newly created wetland areas. For example, during the reestablishment of a salt marsh in Germany, Wolters et al. (2005) found peak dispersal of salt marsh species in the fall. Furthermore, hydrochory played a more critical role than dredge material in the revegetation of a tidal freshwater marsh restoration in Washington, D.C. (Syphax and Hammerschlag 1995; Baldwin and DeRico 2000; Baldwin 2004; Neff and Baldwin 2005).

The species pool hypothesis (Taylor et al. 1990) states that the most important determinant of plant species richness is the number of species available for local colonization (Partel and Zobel 1999), and hydrochory has been found to increase the pool of colonizing species (Jansson et al. 2005; Gurnell et al. 2006). When species richness is increased at a restored site through hydrochory, many niches may be filled, thereby decreasing the opportunity for invasive species to dominate (Jansson et al. 2005; Boers et al. 2007). Our main objective was to estimate the number of species in the species pool that is available to recolonize Dyke Marsh, a fragmented urban tidal freshwater marsh, using two different methods: the number of species that have seeds in the drift-lines on its shores, and the number of species that are floating in the Potomac River adjacent to the marsh. While it was not possible to directly link the composition and abundance of floating and drift-line seeds, we sampled seed pools as an indicator of potential propagule dispersal to future restored wetlands at the site.

Methods

Study area

Dyke Marsh is a tidal freshwater marsh on the Potomac River, just south of Alexandria, Virginia (38.77° N, 77.05° W; Fig. 1). The marsh is an ideal location for our study because it is (1) situated on a large river in a highly urbanized area, (2) near other

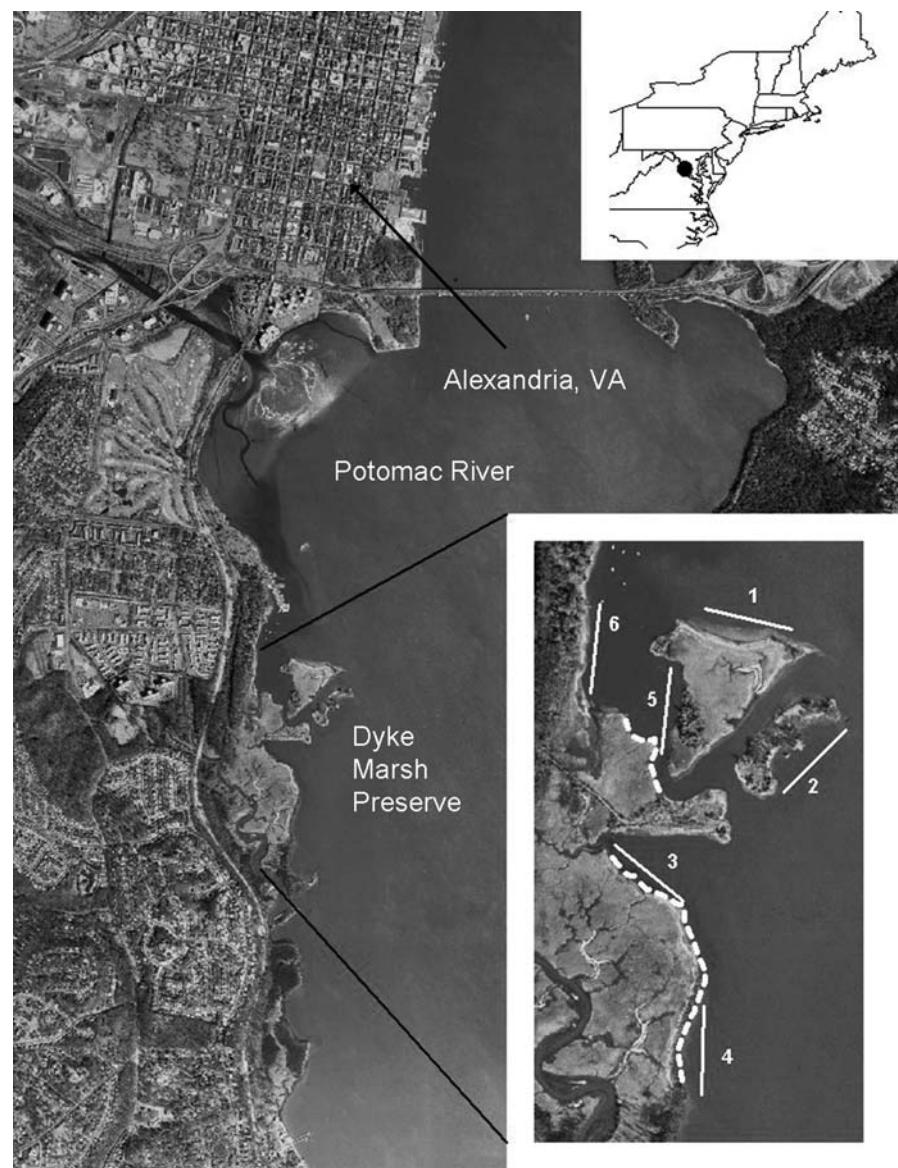
studied Washington, D.C. marshes, and (3) being considered for restoration by the National Park Service (the marsh is part of the George Washington Memorial Parkway, a National Park Service property). Throughout the 1940s–1970s, ~110 hectares of marshland along the outer fringes of Dyke Marsh was dredged and removed for underlying sand and gravel. The dredging operation left holes up to 9.14 m deep in the river surrounding the marsh, created new channels through outer marsh edges, and reduced the total marsh size by half (NPS 1977). The federal government was given control of the existing 196 hectares of forested (72 ha) and non-forested marshland (124 ha) in 1976 (Xu 1991). Currently, the National Park Service is studying possible restoration scenarios to replace some or all of the dredged area at Dyke Marsh, but restoration has not yet been completed. At the study site, mixed tides (two high and low tides per day of differing heights) occur daily with a mean tidal range of 0.5–0.9 m (Carter et al. 1994).

The vegetation composition of Dyke Marsh is typical of tidal freshwater marshes on the east coast (Whigham and Simpson 1977; Leck and Simpson 1995). Dominant annual species in Dyke Marsh include *Impatiens capensis* Meerb., *Amaranthus cannabinus* (L.) Sauer, *Bidens laevis* (L.) B.S.P., and *Polygonum arifolium* L. Dominant perennial species in the marsh include *Typha angustifolia* L., *Peltandra virginica* (L.) Schott, *Nuphar luteum* (L.) Sm., *Acorus calamus* L., and *Schoenoplectus fluviatilis* (Torr.) M.T. Strong. All species names follow the nomenclature of USDA NRCS (2009).

Field sampling

In fall 2003, six 200-m transects were established around the perimeter of the non-forested marsh (Fig. 1). Water surface trawling took place in fall 2003, and spring, summer, and fall of 2004 and 2005 on the Potomac River near the Dyke Marsh shoreline for a total of seven sampling events. During each sampling event, three trawls were made along each transect using a modified plankton net (40.9 cm width by 25.6 cm height) with a seed trap that was towed by a motor boat (Neff and Baldwin 2005) to sample the floating seed rain. The plankton net and the seed trap had buoyant floats attached to ensure the surface of the water was sampled by keeping the top

Fig. 1 Location of Dyke Marsh on the Potomac River. Inset includes transect (solid) and drift-line (dashed) sampling locations



of the net above the water surface (see photo in Neff and Baldwin 2005). The net was dragged approximately 0.6 m from the side of the boat, ahead of the wake, at a speed of 2.7–2.9 km/hr. The sampling area of each transect was ~82 m². After each of the three trawl passes on each transect, the seed trap was scraped and rinsed into an individual plastic bag ($n = 18$ subsamples per sampling event).

The abundance and species richness of seeds was quantified along 920 m of non-forested marsh drift-line along the Potomac River. The drift-line area is located along the upper tidal limits of the shore line

where coarse organic debris settles (Bakker et al. 2002). Drift-line samples were 100 cm² by 2 cm deep and were taken at 40 random points in spring, summer, and fall of 2005 and spring 2006 (Fig. 1). Drift-line material was collected within a 10 × 10 cm metal sampling frame at randomly spaced sample points along the 920-m-long marsh edge; distances between sample points ranged from 5 to 75 m. Each drift-line sample was placed into a plastic bag and returned to the greenhouse for seedling emergence assay. For both the water surface trawl and drift-line sampling the subsamples were placed in

a cooler at the field site for transportation to a 4°C room at the laboratory. Subsamples were processed the next day using the following treatment procedure.

Sample treatment

The seedling emergence technique (Poiani and Johnson 1988; Gross 1990; Baldwin et al. 2001; Neff et al. 2009) was used to determine germinable seed density and species composition of the trawl and drift-line samples. Subsamples were spread over vermiculite in individual black-plastic bedding trays ($W \times L \times H = 27 \times 53 \times 5$ cm) and placed in a greenhouse misting room for germination. Coarse material including leaves, sticks, and trash were removed from the samples, rinsed with water over the tray to remove any seeds, and was then discarded. In late autumn, all trays were stored at $\sim 4^\circ\text{C}$ over winter to cold-stratify any remaining seeds and so they would not freeze solid, and then placed back into the greenhouse to continue germination in the spring. Seedlings emerging from each subsample were identified as young as possible, and removed from the tray when identified. Seedlings of unknown species were transplanted and allowed to mature for identification.

Data Analyses

Data were analyzed using SAS (SAS Institute, Cary, NC) with significance defined at $\alpha = 0.05$.

Variables we calculated included seedling density (seedlings/m²), species density (Number of species/sample), and species richness (Number of species/sampling event). Mean, maximum, and standard error of seedling and species density of subsample greenhouse trays were calculated for each transect at each sampling event. We tested for the effect of sampling event on seedling and species density of trawl and drift-line samples to determine if seasonality had an effect using repeated measures analysis of variance (RMANOVA) with Tukey comparisons of least square means (using the Mixed procedure of SAS). The fixed effect was sampling event, the response variable was seedling density, and the repeated measure was transect. Drift-line species density was log + 1 transformed to conform to normality assumptions. Drift-line seedling density,

seed trawl seedling density, and seed trawl species richness were square-root transformed to conform to normality assumptions.

Due to the difference in number of individuals collected between the two sampling methods, and to be able to compare our results to a previously published study (Neff and Baldwin 2005), we calculated sample-based rarefaction curves and species richness estimations using EstimateS 5.0.1 (Colwell 1997). Rarefaction curves are a type of species accumulation curve formed by repeatedly determining richness for random samples of individuals from the data. The rarefaction curves remove sampling effects and allow for comparisons of species richness when samples contain different numbers of individuals (Colwell and Coddington 1994, Gotelli and Colwell 2001). We used rarefaction curves to compare the species richness of floating and drift-line seed pools in addition to species density data, because the latter are strongly dependent on densities of individuals observed and therefore level of sampling effort (Gotelli and Colwell 2001). Using EstimateS, we estimated the total number of species in the sampled floating and drift-line seed pools using nonparametric asymptotic species richness estimators to determine if our sampling effort was adequate for both the water trawl and drift-line sampling techniques. Chao 1 (Chao 1984) is an estimator of the total number of species in an assemblage based on the number of rare species in the sample (Magurran 2004). Chao 2 (Chao 1987) is a modified approach of Chao 1 that uses presence/absence data based on the distribution of species among the samples (Magurran 2004). Chao 1 was the first estimator to reach an asymptote for both sampling techniques; therefore we will only discuss the Chao 1 data (Colwell and Coddington 1994).

Results

A total of 42 species were identified using both the drift-line and seed trawl methods. The number of emerged seedlings was much greater in the drift-line (1,669 seedlings; 2005–2006) than in the seed trawl samples (292 seedlings; 2003–2005). Species with a high number of emerging seedlings found in both the drift-line and seed trawl samples were *A. cannabinus* and *Pilea pumila* (Table 1).

Table 1 Number of seedlings emerging, all seasons sampled collectively, for each species, from the water surface trawling and drift-line sampling techniques

Species	Number of seedlings emerging	
	Water surface trawling	Drift-line sampling
<i>Amaranthus cannabinus</i> (L.) Sauer	35 ^{a, b, c}	74 ^{a, c}
<i>Ammania latifolia</i> L.	0	1 ^b
<i>Bidens frondosa</i> L.	2 ^c	6 ^b
<i>Bidens laevis</i> (L.) B.S.P.	32 ^{a, b, c}	4 ^a
<i>Carex grayi</i> Carey	0	3 ^a
<i>Cuscuta gronovii</i> Willd. ex J.A. Schultes	2 ^c	17 ^{a, c}
<i>Echinochloa muricata</i> (Beauv.) Fern.	0	1 ^b
<i>Eclipta prostrata</i> (L.) L.	4 ^c	0
<i>Eleocharis engelmannii</i> (Steud.)	0	2 ^a
<i>Helenium autumnale</i> L.	0	2 ^a
<i>Hibiscus moscheutos</i> L.	2 ^c	0
<i>Hydrilla verticillata</i> (L.f.) Royle	1 ^c	5 ^c
<i>Impatiens capensis</i> Meerb.	67 ^{a, c}	0
<i>Iris sibirica</i> L.	1 ^b	0
<i>Juncus effusus</i> L.	0	4 ^{b, c}
<i>Leersia oryzoides</i> L. (Sw.)	11 ^{a, c}	10 ^{a, c}
<i>Ludwigia palustris</i> L. (Ell.)	2 ^c	134 ^{a, b, c}
<i>Lycopus amplectens</i> Raf.	7 ^{a, b}	0
<i>Microstegium vimineum</i> (Trin.) A. Camus	0	2 ^a
<i>Mikania scandens</i> L. Willd.	0	2 ^a
<i>Najas minor</i> All.	1 ^c	54 ^{a, b, c}
<i>Nuphar lutea</i> (L.) Sm.	1 ^c	1 ^a
<i>Peltandra virginica</i> (L.) Schott	15 ^c	0
<i>Pilea pumila</i> (L.) Gray	62 ^{a, b, c}	39 ^{a, b}
<i>Polygonum pensylvanicum</i> L.	0	1 ^a
<i>Polygonum persicaria</i> L.	1 ^c	4 ^{a, c}
<i>Polygonum punctatum</i> Ell.	2 ^c	1 ^c
<i>Ranunculus cymbalaria</i> Pursh	4 ^{a, c}	0
<i>Rorippa palustris</i> (L.) Bess.	0	2 ^a
<i>Rotala ramosior</i> (L.) Koehne	0	2 ^b
<i>Rumex crispus</i> L.	1 ^b	0
<i>Sagittaria latifolia</i> Willd.	0	3 ^{a, b, c}
<i>Schoenoplectus fluviatilis</i> (Torr.) M.T. Strong	5 ^{b, c}	17 ^{a, b}
<i>Solanum dulcamara</i> L.	2 ^c	0
<i>Typha angustifolia</i> L.	16 ^c	1276 ^{a, b, c}
<i>Zizania aquatica</i> L.	1 ^c	2 ^a
Unknown species	15 ^{a, c}	0

Superscripts represent which season the species was found in: ^aspring, ^bsummer, ^cfall

Water surface trawling

The seed trawls contained an average of about 0.03 seedlings/m² (280 seedlings/ha; Table 2) with the lowest sampling event of the study being zero seedlings/m² in summer 2005, and the highest being

0.09 ± 0.01 seedlings/m² (± SE) in fall 2004. Seed density did not vary among trawl replicates (data not shown), indicating that the first trawl pass for any transect did not impact subsequent trawl passes on the same transect. Rarefaction analysis indicated that species accumulate rapidly with sampling effort

Table 2 Density and species richness metrics measured and estimated at Dyke Marsh and the near-by Anacostia River

	Water surface trawl		Drift-line	
	Dyke Marsh	Anacostia River	Dyke Marsh	Anacostia River
Average density \pm SE (seedlings/m ²)	0.03 \pm 0.01	0.59 \pm 0.10	1,043 \pm 424	1,500 \pm 485
Average species density \pm SE (species/sample) ^a	1.1 \pm 0.36	27 \pm 2.08	1.8 \pm 0.38	16 \pm 2.22
Observed species richness	30	82	27	21
Maximum estimated species richness ^b \pm SE	34 \pm 0.56	104 \pm 5.5	29 \pm 0.19	Not available
Species estimated at 250 individuals sampled	28	25	12	37

^a Sample size for the water surface trawl at both locations was 81.8 m²; sample size for drift-line at Dyke Marsh was 0.01 m², and at the Anacostia River was 0.05 m²

^b Chao 2 was used for the Anacostia River study and Chao 1 was used for the Dyke Marsh study

Source: Neff and Baldwin (2005)

(Fig. 2a). For comparison purposes, the rarefaction estimated 28 species when sampling 250 individuals (Table 2). The species richness estimator Chao 1 estimated a total of 34 species (Table 2). Collectively from all sampling events, the seed trawls produced 30 species, indicating that the trawl sampling was effective in capturing almost all species present in the hydrochorous species pool (30 out of 34 species).

Four annual species dominated the floating seed pool: *I. capensis*, *A. cannabinus*, *B. laevis*, and *P. pumila* (Table 1). Fall sampling events contained 22 species, 14 of which were only found in the fall, when *I. capensis* and *B. laevis* were dominant. Eight species were found during spring sampling events, none of which were unique to spring samples, and *P. pumila* dominated the spring species pool. Seven species were sampled during summer sampling events, two of which were only found in summer samples, and *Lycopus amplectens* dominated the species pool in the summer.

The highest number of seedlings and species were found during the fall sampling events, versus spring and summer (RMANOVA overall model for seedling density: $F = 15.54$, $df = 2, 39$, $P < 0.01$, and species density: $F = 17.84$, $df = 2, 39$, $P < 0.01$; Fig. 3). Fall sampling produced significantly higher seedling density (0.05 ± 0.009) than spring (0.02 ± 0.006 ; $p = 0.01$) and summer (0.004 ± 0.002 ; $P < 0.01$) sampling, however spring and summer seedling density did not differ ($P = 0.13$). Similarly, fall sampling events had higher species density (0.02 ± 0.004) than spring (0.007 ± 0.002 ; $P < 0.01$) and summer (0.003 ± 0.001 ; $P < 0.001$), however spring and summer species density did not differ ($P = 0.27$).

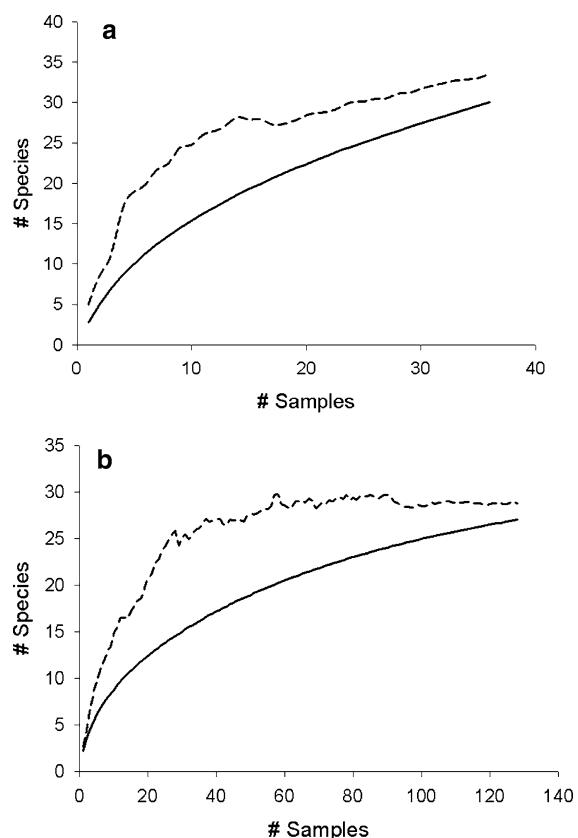


Fig. 2 Sample-based (solid line) and individual-based (dashed line) rarefaction curves for **a** water surface trawls and **b** drift-line sampling at Dyke Marsh

Interestingly, the summer 2005 sampling event did not have any seedlings emerge at all.

The seed trawl transects did not differ in seedling density or species density with all sampling events

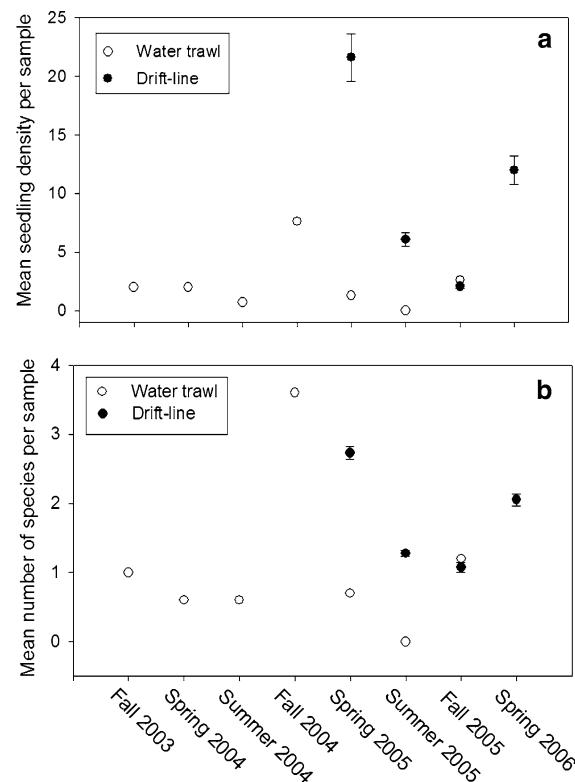


Fig. 3 **a** Average number of seedlings per sample (± 1 SE) and **b** average number of species per sample (± 1 SE) from water trawl samples (white dots; all $SE < 0.01$) and drift-line samples (black dots) for each sampling date. Water trawl sample = 81.8 m^2 , and drift-line sample = 0.01 m^2

combined (ANOVA for seedling density: $F = 1.82$, $P = 0.11$). However, transects containing the greatest seedling density differed among seasons (ANOVA overall model: $F = 6.90$, $P < 0.01$). In spring sampling, transect 1 (see Fig. 1) had the highest seedling density (0.07 ± 0.03 seedlings/ m^2), followed by transects 2 (0.03 ± 0.02) and 3 (0.02 ± 0.008). Transects 2 (0.06 ± 0.02), 3 (0.08 ± 0.03), and 6 (0.06 ± 0.03) had the highest seedling density in fall; however no differences existed among the transects in fall (ANOVA $F = 0.98$, $P = 0.44$) or summer (ANOVA $F = 1.13$, $P = 0.36$).

Drift-line

An average of about 1,000 seedlings/ m^2 (10 million seedlings/ha) was sampled from the drift-line (Table 2), with the lowest being 208 ± 56 seedlings/ m^2 in fall 2005, and the highest being

$2,160 \pm 645$ seedlings/ m^2 in summer 2005. The drift-line sampling resulted in 27 species from all sampling events. Fifteen of the species found in the drift-line samples were also found in the seed trawl samples. Rarefaction analysis indicated that species accumulated with sampling effort (i.e., number of samples) more slowly with drift-line sampling than for trawl samples (Fig. 2b). For comparison with trawl samples, rarefaction estimated only 12 species at 250 individuals, and the species richness estimator Chao 1 estimated a maximum richness of 29 species (Table 2), indicating that our sampling effort collected almost all species in the drift-line pool (27 out of 29 species).

Species with a high number of emerging seedlings found in the drift-line included *T. angustifolia*, *Ludwigia palustris*, *A. cannabinus*, *Najas minor*, and *P. pumila* (Table 1). Spring sampling events captured 20 species, and 10 of those were found only in the spring. Eleven species were found in summer samples, four of which were unique to summer. The fall drift-line samples contained 11 species, two of which were unique to fall samples. *Typha angustifolia* and *L. palustris* dominated samples from all seasons, and *A. cannabinus* was most prevalent in spring, while *Najas minor* was found most in fall samples.

In contrast to the seed trawl samples, the highest number of species and seedlings for the drift-line samples were found in spring (RMANOVA overall model for seedling density: $F = 9.48$, $P < 0.01$; and species density: $F = 10.20$, $P < 0.01$; Fig. 3). Seedling density was significantly higher in the spring 2005 than summer and fall 2005. However, summer and fall seed density did not differ. Similarly, species density was also higher in spring 2005 than in summer ($P < 0.01$) and fall ($P < 0.01$) 2005, but summer and fall did not differ in species density.

Discussion

We observed about 30 species each in both the floating and drift-line seed pools. The two pools shared about 15 species and contained a total of about 40 species. In addition, we found that relatively few individual seedlings were required to adequately sample the floating seed pool through our water trawl sampling compared to the drift-line sampling, suggesting that the spatial distribution of drift-line

seeds is patchier. For both the water trawl and drift-line sampling, the low standard error of the Chao 1 estimates and the fact that the rarefaction curves leveled off near the Chao 1 estimates demonstrate that both sampling efforts were successful in capturing most species within present. However, we must note that while the Chao 1 maximum richness values are similar for the floating seed (34) and drift-line (29) communities, the estimates were derived separately from different populations of seeds. The two species pools are comprised of different species (Table 1), thereby indicating a more species-rich ecosystem than suggested by Chao 1 values for each sampling method alone. Estimating the number of species at a common number of individuals is a useful way to compare seed collection methods and studies. We found 82% of total species at a site should be sampled when collecting 250 individuals using the water surface trawling technique, and only 41% of total species are sampled at 250 individuals for the drift-line technique, again suggesting a patchier distribution of drift-line seeds.

Spatial and temporal variation

After examining the locations of the water trawl transects on a digital aerial orthophoto of Dyke Marsh and a map of marsh elevations created in 2006 (Elmore 2007), we discovered that transects with the highest density of seedlings were located downstream of major tidal creek outlets, for example transect 3 (Fig. 1). This demonstrates the importance of transect location selection for assessing seed sources.

Both the seed trawl and drift-line sampling resulted in seasonal variation of species richness. Higher species richness was found in the fall water trawls versus spring and summer. Wolters et al. (2005) found a similar pattern in diaspore dispersal in an England tidal salt marsh where the main dispersal of diaspores took place between October and December. High dispersal may occur in the fall owing to the end of peak biomass and plant senescence. During this time, seeds are being released everywhere—into the air, the water, and the substrate below the parent plant. Many of the seeds find their way to the water, either directly or indirectly through tidal flushing (Bakker et al. 1985; Huiskes et al. 1995). Finally, they enter the river in the fall.

Contrary to peak richness in the water trawls, we found the highest drift-line species richness in the spring and summer. High richness and density in the spring is consistent with the above pattern of mass-seed dispersal in the fall. In autumn, seeds are being dispersed, being flushed towards the channels, and floating in the water. In winter, seeds begin to collect and concentrate in the drift-line. Once in place in the drift-line, seeds cold-stratify, which is a requirement for germination for many tidal freshwater marsh species (Leck and Simpson 1993). However, seeds floating in the water over winter and during spring may not be properly cold-stratified and therefore do not germinate.

Regional seed sources

Our results indicate that Dyke Marsh has low floating and drift-line seedling density, species density, and species richness compared to other regional tidal freshwater marshes (Table 2). Neff and Baldwin (2005) studied the floating seed pool in fall, spring, and summer around two restored tidal freshwater marshes on the nearby Anacostia River in Washington, D.C. using the same surface water trawl techniques that we used. Kenilworth and Kingman Marshes on the Anacostia River are located less than 25 km from Dyke Marsh and the Anacostia River flows directly into the Potomac River about 10 km north of Dyke Marsh. Using the species richness estimator Chao 2, Neff and Baldwin (2005) estimated many more total species in their seed trawls versus Dyke Marsh, but when sampling 250 individuals, rarefaction indicates that about the same number of species occurred for the Anacostia River and Dyke Marsh (Table 2). However, richness metrics estimated many more species when sampling 250 individuals for the drift-line at the Anacostia River versus the Dyke Marsh drift-line (Table 2). This indicates that species accumulated at a similar rate initially but leveled off at a lower total number of species at Dyke Marsh than at the Anacostia River. A major difference between the two locations is river discharge; the Potomac River is much larger than the Anacostia River. In addition, the Anacostia River has more marsh areas in close proximity than Dyke Marsh (Neff and Baldwin 2005). Thus, the area of wetlands relative to watershed size and the proximity of wetlands that can serve as seed sources are

important factors controlling the density and richness of hydrochorous seeds.

Studies documenting the floating seed pool and drift-line communities are rare in the literature. However, similar to the lower floating seed species richness found at Dyke Marsh (30) versus the nearby Anacostia River (>80), we found total species richness around Dyke Marsh to be dramatically lower than the Upper Eider River in Germany (76 species; Vogt et al. 2004). We could not find any studies on drift-line seed pools in tidal freshwater marshes; however our results (27 species) were comparable to studies in salt marshes and rivers: Wolters and Bakker (2002) examined a 100-year-old salt marsh drift-line in the Netherlands and found a total of 19 species. In addition, two drift-line river studies found a total of 23 and 37 total species (Cellot et al. 1998; Vogt et al. 2007). We could not compare drift-line seed abundance among studies because each study used different sampling and processing methods.

Through our study we have learned more about floating seed pools in and around a tidal marsh in a large, urban river, as well as the importance of using more than one sampling method. We found both the surface water trawl and drift-line techniques successful in analyzing the seed pool. However, seed density and species richness surrounding Dyke Marsh were substantially lower than at near-by Anacostia River marshes, suggesting that seed dispersal may be a more local phenomenon than was previously thought for tidal freshwater marshes. Managers of tidal freshwater marshes should examine if regional or watershed influences affect the marsh seed pool before initiating restoration projects. We found many of the dominant aboveground marsh species in the floating seed pool and drift-line samples, suggesting that a diversity of marsh propagules may be available to promote a heterogeneous restored wetland. However, not all dominant species were found, so initial planting of some species may be advantageous to prevent colonization of non-natives and to ensure a complete representation of native marsh species.

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Estimating plant competition coefficients and predicting community dynamics from non-destructive pin-point data: a case study with *Calluna vulgaris* and *Deschampsia flexuosa*

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Abstract A method is proposed for estimating plant competition coefficients and predicting the dynamics of herb and grassland plant communities from non-destructive pin-point measurements. The method is applied to inter-specific competition in a natural heathland community with relatively few interacting species. The study shows that the dynamics of the heathland plant community may be thought of as essentially a two-species system of *Calluna vulgaris* and *Deschampsia flexuosa*. There were significant competitive interactions between *C. vulgaris* and *D. flexuosa*. *D. flexuosa* affected both the cover and compactness of *C. vulgaris* individuals as a function of the compactness the previous year, whereas *C. vulgaris* significantly affected only the compactness of *D. flexuosa*. There was a significant negative effect of drought on the compactness of both *C. vulgaris* and *D. flexuosa* individuals, whereas night warming had no significant effects on either species. The predicted long-term outcome of the competitive interaction between *C. vulgaris* and *D. flexuosa* was

that of unstable equilibrium, where the more dominant of the two will outcompete the other. However, when both species are found at relatively high plant covers the two species are predicted to co-exist for a long time period relatively to the time scale of the ageing process of *C. vulgaris*. Direct analyses of the inter-specific competitive interactions in natural plant communities with non-destructive measurements can provide important new insight into the processes that determine the composition of plant communities.

Keywords Heathland · Plant competition model · Point-intercept · Succession

Introduction

One of the processes that is known to influence the composition of natural plant communities is inter-specific competition (e.g. Gotelli and McCabe 2002; Silvertown et al. 1999; Weiher et al. 1998), although the relative importance of inter-specific competition as a regulating factor in natural plant community dynamics has been a point of discussion (Hubbell 2001; Shmida and Ellner 1984). The role of inter-specific competitive interactions in natural plant communities has mainly been investigated by indirect methods comparing different plant communities at different points in times, the so-called “space for time substitution” (Bakker et al. 1996; Barclay-Estrup 1970; Barclay-Estrup and Gimingham 1969), or by

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testing whether the species composition deviates from a specified null-model where species are assumed to be independent of each other (Conner and Simberloff 1979; Gotelli and McCabe 2002; Hubbell 2001; Wilson et al. 1996).

In order to investigate the role of competitive interactions between different plant species in natural plant communities more directly, we need to investigate relevant plant ecological data using plant competition models that are tailored to the relevant ecological data. Historically, plant competition models have been developed for annual plants, e.g. crop or weed plants, by expressing yield, biomass or fecundity of individual plants as a function of plant density (Bleasdale and Nelder 1960; Damgaard 1998, 2004; Firbank and Watkinson 1985; Law and Watkinson 1987; Pacala and Silander 1990; Rees et al. 1996). Such competition models are less relevant for natural plant communities dominated by perennial plants, where it is often difficult to distinguish the individuals and, consequently, to obtain individual-level data. When individual plants can be counted, they almost always vary markedly in size, so the number of individuals is of limited value for describing the amount of competition. However, the use of non-manipulative techniques in order to estimate competitive effects directly in natural plant communities is a topic of rising interest (Freckleton and Watkinson 2001), and several methods have been suggested for different types of natural herbal plant communities and ecological measures (e.g. Law et al. 1997; Rees et al. 1996; Roxburgh and Wilson 2000a, b; Turnbull et al. 2004). For example, Rees et al. (1996) used the counts of annual plants in thousands of small quadrates and Turnbull et al. (2004) made a neighbourhood analysis of individual plants of the same dune population of annual species, whereas Law et al. (1997) in a pioneering study estimated competition coefficients from spatial turnover data of four perennial grass species. The present study aims to describe the competition where ramet or individual-level data is not available or is too laborious to obtain.

The ecological measures used in this study come from a non-destructive pin-point analysis (Kent and Coker 1992; Levy and Madden 1933), where a frame with a fixed grid pattern is placed above the vegetation. A pin that is inserted vertically through one of the grid points into the vegetation will

typically touch a number of plants, and the number of times the pin touches different plant species is recorded. This procedure is repeated at each grid point. A pin-point analysis provides the estimates of two important plant ecological variables: plant cover and 3D-space occupancy of the plants. The cover of a specific plant species is defined as the relative number of pins in the grid that touch the species; thus plant cover measures the cover of the plant species when it is projected onto the two-dimensional ground surface. Rather than measuring the size of individual plants, the 3D-space occupancy of the species within the frame is measured as the average number of hits per pin, which has been shown to be correlated to plant biomass (Jonasson 1983, 1988). In order to simplify the writing, the average number of hits per pin will be referred to as “compactness” instead of using the term “3D-space occupancy.”

In this paper, we propose a method for modelling competition and predicting plant community dynamics, which is applicable to many herb and grassland communities, by expressing the compactness as a function of plant cover, where both the compactness and plant cover are measured by a single non-destructive pin-point analysis. Most importantly, and critically for the application of the suggested method, is the fact that, due to the growth form of most plant species, the compactness will increase relatively faster than the plant cover during the growing season. This change in the measures of compactness and plant cover during the growing season is analogous to the change in cumulative biomass relative to the density of annual plants, which also increases during the growing season. The underlying assumption of the method used here is that the species-specific measure of compactness at the end of the growing season relative to plant cover may be used as a measure of growth or the ecological success of the species over the growing season. This measure of ecological success is expected to depend on the abiotic and biotic environment and the cover of other species, which compete for resources such as light, water and nutrients. Furthermore, it is assumed, everything else being equal, that a plant species that grow to a relatively high compactness has a relatively high plant cover the following year, i.e. plants allocate resources into occupying resource space the following year. We apply the method to a

well-studied case of inter-specific competition in a heathland plant community with relatively few interacting species.

Materials and methods

Site description

The study was started in 1998 in a heathland at Mols Bjerge ($56^{\circ}23'N$; $10^{\circ}57'W$) 5 km from the east coast of Jutland, Denmark, in a hilly glacial sandy moraine from the late phase of the Würm glaciation. The climate is relatively dry with an average annual precipitation of 550 mm (1960–1990). The average annual air temperature and precipitation during this study were $8.7^{\circ}C$ and 806 mm, respectively. The soil type is a sandy podzol with a 1–4 cm organic mor layer on top.

The site was cultivated until the 1950s and was grazed by sheep and cattle until 1992. Since 1992, the area has been a nature reserve with no management except selective removal of pine trees and bushes. The vegetation was dominated by the evergreen dwarf shrub *Calluna vulgaris* (L.) Hull until the 1960s, thereafter an increasing amount of grass, mainly *Deschampsia flexuosa* (L.) Trin, has been observed. When the experiment was initiated, the aboveground biomass was c. $1,000\text{ g m}^{-2}$. The vegetation was dominated by a mixture of *Calluna* and grasses, mainly *D. flexuosa* comprising 45% and 46% of the biomass, respectively (for more details, see Beier et al. 2004a; Beier et al. 2004b; Schmidt et al. 2004).

Climate manipulations

The original aim of the study was to investigate the effect of climatic changes on plant communities. In the first year, 1998, there were no climate manipulations (control year), but in the beginning of 1 March 1999 warming at night and extended summer drought were simulated on parts of the study area in $4 \times 5\text{ m}$ plots. Three blocks with three $4 \times 5\text{ m}$ plots were laid out at the heath. In each block, plots were assigned to night warming (W), drought (D) or control (C) treatments. The treatments were non-intrusive and consisted of a scaffolding of galvanised steel tubes carrying a reflective aluminium curtain

(W) or plastic curtain (D). The study plots were open at the sides. The movement of the curtains was automatically controlled by incoming light and rain. At sunset, the aluminium curtains were drawn over the vegetation in the W plots to reduce the IR reflection, thereby conserving energy and leading to an increased temperature, although during rain events at nights the curtains were activated by a rain sensor and automatically removed. At sunrise, the curtains were automatically removed to keep the plots open during the day. Extended summer drought (D) was simulated with plastic curtain that automatically covered the plots during rain events. The drought curtains were activated from 18 May to 29 July, 1999.

The warming treatment increased the monthly average midnight air temperatures in the W plots by 0.6 – $1.9^{\circ}C$ (average yearly increase $1.1^{\circ}C$). The drought treatment reduced the annual water input from 806 mm in the control plots to 536 mm in the plots subjected to summer drought. This is equivalent to a 33% reduction of the annual precipitation (for more details, see Beier et al. 2004a). In the summer period when the drought treatment was applied, the curtains removed 94% of the precipitation.

Vegetation analyses

Four permanently marked $1 \times 0.5\text{ m}$ subplots for vegetation analyses were placed within each of the $5 \times 4\text{ m}$ plots, giving a total of 36 vegetation subplots. For the vegetation analysis, a $1 \times 0.5\text{ m}$ frame with a $10 \times 10\text{ cm}$ fixed grid pattern was constructed. A 2 mm diameter pin was lowered vertically into the vegetation at the 50 points in the grid. Each hit on plant parts by the pin was registered by species until the tip of the pin was no longer visible. Further, it was recorded if the pin hit the newly dead or alive plant parts. In a few cases, the pin did not hit any plants, which was recorded as bare soil. The field work was carried out 10–14 August 1998 and 21–28 July 1999, which is approximately the time of maximum biomass for *C. vulgaris*. For *C. vulgaris*, the pin-point method was modified. Each long shoot with its short shoots was considered as one sausage-shaped entity. Only the first hit on a shoot was registered as long as the tip did not leave the sausage shape and did not hit short shoots longer than 1 cm.

For *D. flexuosa* (and other herb and grass species), hits on both green leaves and red to yellow coloured newly dead leaves were registered as separate variables. The analyses were carried out after flowering of *D. flexuosa*, which causes a temporary decrease of the green leaf biomass due to death of leaves on the flowering shoots. Addition of the current year standing dead leaves is an estimate of the maximum cover of grasses. *D. flexuosa* experiences peak biomass in June, whereas peak biomass in *C. vulgaris* is in August. By including newly dead leaves from the grasses, we were able to compare maximum biomass for the two species in one analysis.

Only data from 1998 to 1999 have been used in the competition model. In 1999, *C. vulgaris* suffered from a major heather beetle (*Lochmaea suturalis*) attack. However, the analyses were made just at the time when the attack was being apparent and before the plants suffered heavily. The shoot structure was unaffected, and thinning of the shoots was generally not conspicuous. Thus, most of the production in spring and early summer 1999 was measured. Later, the plants suffered heavily and most died during the winter.

Competition model

In a given year, the compactness of species i , Y_i , is measured as the average number of pin-point hits per pin within a pin-point frame, and the plant cover of species i , X_i , is measured by a binomial process as the number of grid points where species i is hit by the pin out of n grid points (Damgaard 2008).

It is assumed that the compactness of species i in year t is an increasing function of the plant cover of species i in year t and a decreasing function of the plant cover of other species j, k, \dots in year t due to competition for limiting resources.

$$y_i(t) = a_i x_i(t)^{b_i} \left(1 - c_{ji} x_j(t)^{d_j}\right) \left(1 - c_{ki} x_k(t)^{d_k}\right)$$

$$a > 0, b > 0, c \geq 0, d > 0, \quad (1)$$

where c are competition coefficients and a, b and d are shape parameters.

Since we expect that perennial species with a relative large compactness has a relatively larger plant cover the following year, it is assumed that the plant cover of species i in year $t + 1$ is an increasing

function of the compactness of species i in year t and a decreasing function of the compactness of other species j, k, \dots in year t , again due to competition for limiting resources.

$$x_i(t+1) = \frac{1}{1 + e_i(y_i(t) + f_{ji}y_j(t) + f_{ki}y_k(t) + \dots)^g}$$

$$e > 0, f \geq 0, g > 0, \quad (2)$$

where f are competition coefficients and e and g are shape parameters. Relationships (1) and (2) have been chosen because that (i) the stochastic variable plant cover is distributed between zero and one (ii) the stochastic variable compactness is non-negative. We tried different relationships to obtain a good fit with the data without making the models too complicated.

To illustrate the ecological significance of the estimated competitive interactions among species, it is helpful to predict the long-term ecological equilibrium under the assumption that the estimated competitive interactions will persist unaltered in the following years. Thus, it is possible to construct a recursive equation $h(\cdot)$, which describes the change in the plant cover of species i over years by inserting model (1) in model (2).

$$x_i(t+1) = h(x_i(t), x_j(t), x_k(t)). \quad (3)$$

Estimation and statistical inference

The plant cover and compactness data were fitted to the regression models (1) and (2) under the assumptions that the compactness measure was normally distributed after both the data and the model had been log transformed, and that the plant cover measure was binomially distributed. The maximum likelihood estimates were obtained using the FindMaximum procedure in Mathematica (Wolfram 2003). The fit of the models was checked by visual inspection of various standard plots of model fitting, i.e. a graph of expected values versus observed values, histogram of residuals and fractile diagram of the residuals.

The effects of drought and heat on all the above-mentioned parameters were investigated using simple linear generalisations of the models (1) and (2). For example, the effect of drought on the parameter a of *C. vulgaris* was tested by a likelihood ratio test of the two hypotheses; $H_0: a_i = a_{i,C}$ and $H_1:$

$a_i = a_{i,C} + a_{i,D} D$ with one degree of freedom and where D is a dummy variable from an effect matrix. Due to the complexity of the full model, we adopted a forward testing procedure: if a treatment was found to have a significant effect on a parameter (on the 5% level), the more complicated model (H_1) was chosen for this parameter in the successive tests, otherwise the simple model was chosen (H_0).

Results

The studied heathland plant community was co-dominated by *C. vulgaris* and *D. flexuosa* (Table 1). Associated species, which on average occurred with very low cover, included grasses such as *Holcus mollis* and herbs characteristic to acid grassland as *Achillea millefolium* and *Hieracium umbellatum*. The most prominent mosses were *Pleurozium schreberi* and *Hypnum cupressiforme*.

The observed plant cover of both *C. vulgaris* and *D. flexuosa* varied considerably, and within plots the plant cover of the two species were significantly negatively correlated (Fig. 1). The different species classes were aggregated into a total of three species classes: *C. vulgaris*, *D. flexuosa* and a third class comprising the rest of the higher plant species (Table 2). Based on the visual inspection of the model fitting plots, it was concluded that the aggregated data fitted the regression models (1) and (2) for both *C. vulgaris* and *D. flexuosa*. The two models generally explained a high degree of the observed variation; the r^2 was above 95% for each of the fitted models.

There were no significant effects of warming on either *C. vulgaris* or *D. flexuosa*, but there was a significant effect of drought on both *C. vulgaris* and *D. flexuosa* (Fig. 2). The competitive interactions were not affected by warming or drought, although drought tended to augment the competitive effect of *D. flexuosa* on *C. vulgaris* ($P = 0.06$).

The competitive interactions were further investigated in a sub-sample of the data, in which the control and the warming treatment, which had no significant effects, were pooled. There were no significant competitive effects of the aggregated class of the other higher plant species on either *C. vulgaris* or *D. flexuosa*, neither on the compactness of the two species as a function of the plant cover of the

different species ($P > 0.9$ for both species), nor on the plant cover of the two species as a function of the compactness of the different species the previous year ($P > 0.9$ for both species). This result shows that the plant–plant interactions of the studied plant community may be thought of as essentially a two-species system of *C. vulgaris* and *D. flexuosa*. On the other hand, there were significant competitive interactions between *C. vulgaris* and *D. flexuosa* (Table 3), and in Fig. 3 it is illustrated how cover-affected compactness and the way compactness influenced the plant cover the following year. In summary, *D. flexuosa* affected both the compactness of *C. vulgaris* and the plant cover as a function of the compactness the previous year, whereas *C. vulgaris* significantly affected only the compactness of *D. flexuosa*.

In order to illustrate the ecological significance of the estimated competitive interactions between species (Table 3), the predicted ecological equilibrium of *C. vulgaris* and *D. flexuosa* was investigated, under the assumption that the estimated competitive interactions will persist unaltered in the following years, using the maximum likelihood estimates of the parameters in the recursive equations (3). It was not possible to solve the recursive equations analytically, but the predicted ecological state after 50 years and at equilibrium for different starting conditions was found through numeric simulations (Fig. 4). The stability conditions were checked by numeric calculations of the Lyapunov exponents (Kulenovic and Merino 2002).

The predicted outcome of the competitive interaction between *C. vulgaris* and *D. flexuosa* is that of an unstable equilibrium, where one of the species will outcompete the other depending on the initial plant covers, i.e. the initially more dominant species will outcompete the rare species (Fig. 4). However, when both species are found at relatively high plant covers the two species are predicted to co-exist for a long period of time relatively to the ageing life cycle of *C. vulgaris*. Consequently, if both species are present at relatively high plant covers the competitive interactions will not determine the ecological success of the two species. Rather, the rejuvenating process of *C. vulgaris* (Watt 1947) will determine which species will prevail in the habitat, which again depends on a number of natural disturbances and conservation/management initiatives (Bruggink 1993; Diemont and Linthorst Homan 1989).

Table 1 Average plant cover (pct.) at the Climoor site, Mols, Denmark, in 1998 and 1999

Group	Cover	1998	1999
Dwarfshrubs	<i>Calluna vulgaris</i> (L.) Hull	62.78	60.39
	-incl. dead	63.17	60.72
Grasses	<i>Deschampsia flexuosa</i> (L.) Trin.	65.33	62.28
	-incl. dead	72.56	69.28
Other graminoids	<i>Holcus mollis</i> L.	3.06	3.28
	<i>Festuca rubra</i> L.	0.89	0.89
Herbs	<i>Agrostis capillaris</i> L.	0.50	0.61
	<i>Agrostis stricta</i> J.F.Gmel.	0.22	0.44
Ferns	<i>Helictotrichon pratense</i> (L.) Bess.	0.06	0.06
	<i>Elytrigia repens</i> (L.) Nevski		
Mosses and liverworths	<i>Poa pratensis</i> L.		
	<i>Carex arenaria</i> L.	0.22	
Lichens	<i>Luzula campestris</i> (L.) Dc.	0.06	0.11
	<i>Achillea millefolium</i> L.	1.33	1.39
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Hieracium umbellatum</i> L.	1.78	0.72
	<i>Rumex acetosella</i> L.	0.11	1.06
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Rumex acetosa</i> L.	1.06	0.06
	<i>Stellaria graminea</i> L.	0.33	0.22
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Galium verum</i> L.	0.33	0.17
	<i>Veronica officinalis</i> L.	0.33	0.11
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Galium saxatile</i> L.	0.33	0.06
	<i>Knautia arvensis</i> (L.) Coult.	0.28	0.11
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Pimpinella saxifraga</i> L.	0.11	0.17
	<i>Rumex thyrsiflorus</i> Fingerh.		0.06
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Hieracium pilosella</i> L.		
	<i>Solidago virgaurea</i> L.		
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Campanula rotundifolia</i> L.		
	<i>Teesdalia nudicaulis</i> (L.) R.Br.		
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Polypodium vulgare</i> L.		
	<i>Pleurozium schreberi</i> (Brid.) Mitt.	6.17	4.11
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Hypnum cupressiforme</i> Hedw.	7.22	2.11
	<i>Rhytidiodelphus squarrosus</i> (Hedw.) Warnst.		0.33
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Dicranum scoparium</i> Hedw.	0.17	0.06
	<i>Dicranum polysetum</i> Sw.	0.17	
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Scleropodium purum</i> (Hedw.) Limpr.		0.06
	<i>Brachythecium rutabulum</i> (Hedw.) B. S. G.		
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Lophocolea bidentata</i> (L.) Dum.		
	<i>Cephaloziella</i> sp.		
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	Mosses, not determined		0.17
	<i>Cladonia</i> sp. (<i>chlorophaeae</i> agg., <i>glauea</i> Flörke)	0.78	0.39
Species without cover values are present in the area. Cover determined by pin-point with a 2 mm diameter pin	<i>Hypogymnia physodes</i> (L.) Nyl.		

If both species are rare, the model predicts that *C. vulgaris* will outcompete *D. flexuosa*, but this prediction is rather uncertain due to the limited domain of the data in this parameter space (compare

with Fig. 1). Based on the ecological experiences with this rather simple heathland ecosystem, the obtained predictions seem plausible: *C. vulgaris* is expected to outcompete *D. flexuosa* when no other species occupy

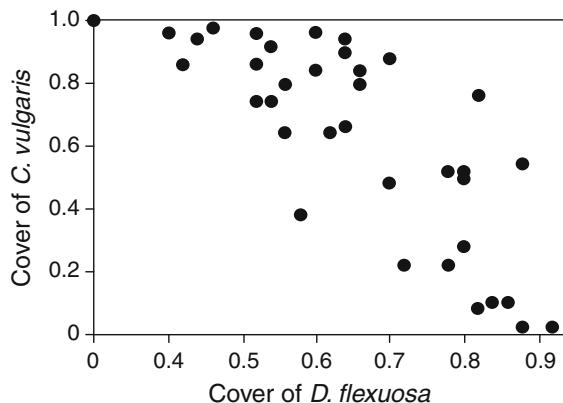


Fig. 1 Observed plant covers of *C. vulgaris* and *D. flexuosa* in 1998 ($r = -0.73$, $P < 0.0001$)

Table 2 Average plant cover (pct.) and compactness in the studied pin-point frames as observed in 1998 and 1999

	Cover		Compactness	
	1998	1999	1998	1999
<i>C. vulgaris</i>	62.8	60.4	3.00	2.92
<i>D. flexuosa</i>	65.3	62.3	1.93	1.93
Other vascular plants	10.1	8.6	1.49	1.30

Pin-point touches on newly dead plant parts are included for the class “other vascular plants”

the space, and the vegetation is dominated by young, competitive *C. vulgaris* plants. However, if the bare ground is the result from ageing *C. vulgaris* plants and *D. flexuosa* is present with high cover, germination and establishment of *C. vulgaris* will be inhibited by the thick litter layer and *D. flexuosa* is expected to be the superior competitor.

Discussion

The method presented allows non-destructive *in situ* measurements of the effects of inter-specific competition in natural plant communities, and since both cover and compactness were measured it is possible to distinguish between different competitive effects, i.e. competitive effects on the processes that control the growth in biomass (compactness) within a growing season, and processes that control the “translation” of biomass into cover the following year. For example, the results presented in Fig. 3 and Table 3 suggest that the competitive effect of *C. vulgaris* on *D. flexuosa*

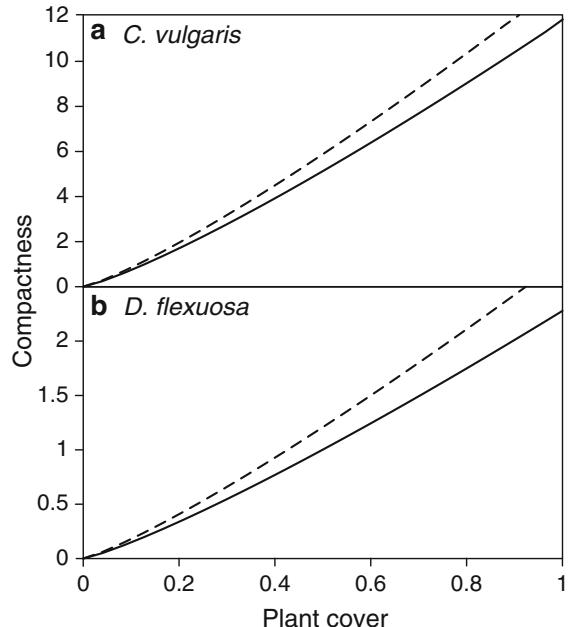


Fig. 2 The effect of drought on the compactness of *C. vulgaris* (a) and *D. flexuosa* (b) as a function of plant cover (significantly affected parameters: $a_{C. vulgaris,D} \neq 0$, $P = 0.008$; $a_{D. flexuosa,D} \neq 0$, $P = 0.0003$). The lines are calculated by inserting the maximum likelihood estimates of the different hypotheses into the regression model (1) under the assumption of no inter-specific competitive effects, i.e. H_0 (dotted line): $y_i = \hat{a}_{i,C}x_i^{\hat{b}_{i,C}}$, and H_1 (full line): $y_i = (\hat{a}_{i,C} + \hat{a}_{i,D})x_i^{(\hat{b}_{i,C} + \hat{b}_{i,D})}$. For both species, the effect of drought is seen as a predicted reduced compactness at a given plant cover

may be fully understood by interference of the growth processes within a growing season, whereas the competitive effect of *D. flexuosa* on *C. vulgaris* also may depend on processes that take place between growing seasons. Furthermore, this approach will allow quantitative ecological predictions for non-senescent herbal plant communities, e.g. grasslands, from observations made on a relatively short time scale.

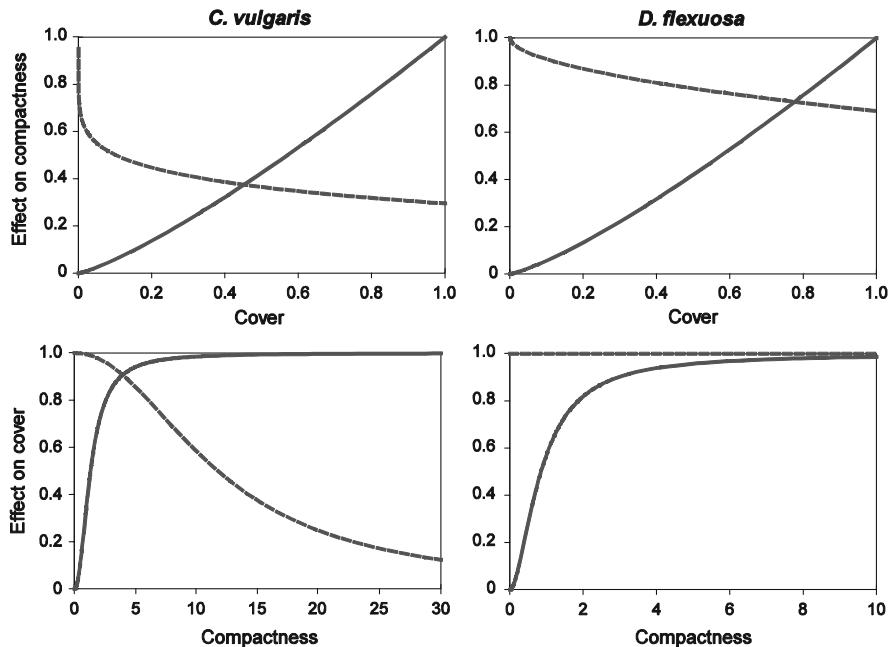
It is often assumed that *C. vulgaris* and *D. flexuosa* affect each other negatively in heathland ecosystems (e.g. Heil and Bobbink 1993; Terry et al. 2004; van Vuuren et al. 1992; van Vuuren and van der Eerden 1992), but the hypothesis has never been tested directly before. Indeed, this study confirms the hypothesis that *C. vulgaris* and *D. flexuosa* affect each other negatively by pin-point measurements of the plants in a heathland ecosystem. Furthermore, there was a significant negative effect of drought on the compactness of both *C. vulgaris* and *D. flexuosa*

Table 3 Maximum likelihood estimates of the competitive effects of species j on species i

j, i	\hat{c}_{ji}	\hat{d}_i	$P(\neq \hat{c}_{ji} = 0, \hat{d}_i = 1)$	\hat{f}_{ji}	$P(\neq \hat{f}_{ji} = 0)$
<i>D. flexuosa, C. vulgaris</i>	0.70	0.15	0.007	0.11	<0.0001
<i>C. vulgaris, D. flexuosa</i>	0.31	0.54	0.0001	0	>0.9

The P -values are from likelihood ratio tests against the hypotheses of no competitive effects

Fig. 3 The effect of cover on compactness in the same year and the effect of compactness on cover the following year. The increasing full lines illustrate the intra-specific effects, e.g. the expected increase in compactness with increasing cover, and the decreasing stippled lines illustrate the inter-specific competitive effects, e.g. the reduction in compactness of species A with increasing cover of species B. The y-axis in the upper panels “Effect of compactness” is normalised to one



individuals, whereas night warming had no significant effects on either species.

Due to the asynchrony in maximum biomass of the two species, both living and the recently dead grass leaves were recorded in the pin-point measurements, but including the dead leaves of *D. flexuosa* in the analysis did not qualitatively change the results.

The long-term ecological success of *C. vulgaris* and *D. flexuosa* (Fig. 4) was predicted on the basis of only 2 years of observation. This is unrealistic because of variation in abiotic and biotic factors and senescence of *C. vulgaris*, and generally it is expected that unmeasured environmental factors may covary with plant cover. Furthermore, since the analysis was conducted in mature vegetation, there are no observations of regeneration with low cover of both species. Due to these limitations, we have not attempted to quantify the level of uncertainty due to the variation in the available data, and the calculated predictions are simply the most likely ecological scenarios given the data we have. Nevertheless, the

predicted long-term ecological scenarios (Fig. 4) were judged to be biologically plausible when compared to the ecological experience that has been accumulated by empirical observations of these well studied and relatively simple ecosystems over the years.

The suggested method assumes that the measure of compactness will increase relatively faster than the measure of plant cover during the growing season and that the species-specific measure of compactness at the end of the growing season relative to plant cover can be used as a measure of the ecological success of the species in the present growing season. It is also assumed that, everything else being equal, a plant species that grows in size has a higher plant cover the following year, i.e. plants allocate resources into occupying space the following year. The long-term ecological success of a species is consequently assumed to be a function of the measured plant cover, and in this respect the suggested methodology is an empirically based analogue to “zone-of-influence”

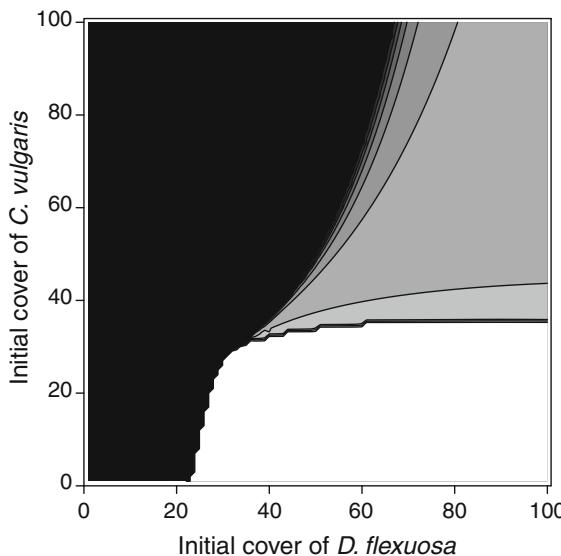


Fig. 4 Predicted ecological equilibrium of the estimated competitive interactions between *C. vulgaris* and *D. flexuosa* for different initial plant covers was calculated using the maximum likelihood estimates of the parameters in the recursive equations (3). Black: *C. vulgaris* will outcompete *D. flexuosa*. Grey: Both species will coexist during relatively long time (more than 50 years), but eventually *C. vulgaris* will outcompete *D. flexuosa*. White: *D. flexuosa* will outcompete *C. vulgaris*

competition model (Gates and Westcott 1978; Hara and Wyszomirski 1994; Weiner and Damgaard 2006; Weiner et al. 2001; Wyszomirski 1983). The assumption seems to be upheld for both species studied here with respect to the above-ground competition. Both *C. vulgaris* and *D. flexuosa* have an upright phenology and are of approximately the same height, and it is assumed that the two species compete for light at this rather nutrient rich heathland. Below ground competition for nutrient and water is expected to dominate in the most nutrient poor heathland (Riis-Nielsen 1997). Competition for resources below ground is more difficult to study, but in heathland similar to the present study site it has been observed that below *D. flexuosa* only grass roots were present, whereas below *C. vulgaris* both shrub roots and grass roots were present (Louise Andresen, pers comm). These observations suggest that the below ground competitive ability of *D. flexuosa*, and consequently its ecological success, may be somewhat underestimated by leaf cover measurements even in rather nutrient rich heathland. Furthermore, if plants allocate a flexible portion of their resources depending on environmental conditions or plant age, then the estimated change in

above-ground biomass may not fully capture the plants competitive ability.

The results of the relative few studies that have investigated the role of inter-specific competition are still too sparse to allow much generalisation across different plant communities or even among years (Turnbull et al. 2004). The suggested method needs to be tested in several plant communities before its generality and usefulness may be assessed, and this work is currently underway. In the process of applying the method to other species in different plant communities, it may be necessary to modify the relationships (1) and (2) to fit the general model. Furthermore, the experimental design of permanent pin-point frames allows the detailed study of the importance of competition and demography at the spatial level of individual plants, and we are currently exploring these possibilities with a longer time series.

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Disturbance by mowing affects clonal diversity: the genetic structure of *Ranunculus ficaria* (Ranunculaceae) in meadows and forests

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Abstract To study the impact of disturbance by mowing on clonal variation, we compared the genetic structure of *Ranunculus ficaria* (Ranunculaceae) in meadows and forests located in southeast Germany. We applied random amplified polymorphic DNA (RAPD) analysis to investigate the clonal and genetic diversity and analysed a total of 117 samples from three study plots in each habitat type. Polymerase chain reaction with six primers resulted in 57 fragments. Clonal diversity differed clearly between the two analysed habitat types and was significantly higher in the study plots from meadows than in those from forests. The mean percentage of distinguishable genotypes (PD) was 0.80 in meadow plots and 0.36 in forest plots, and the detected genets were smaller in meadow plots than in forest plots. Mean genetic diversity measured as percentage of polymorphic bands, Shannon's information index and Nei's gene diversity was also higher in meadows (44.4, 0.22 and 0.14) than in forests (25.1, 0.09 and 0.05). The higher level of clonal diversity in meadow plots is most likely due to the effects of disturbance by mowing, which increases the dispersal of bulbils and promotes the establishment of new plants in meadows compared to forests.

Keywords Genetic and genotypic variation · RAPD · Molecular marker · Mowing · Spatial genetic structure · Dispersal

Introduction

The central European landscape has been affected by human activities since the Neolithic age. As a result the landscape consists of a large number of different, man-made habitat types, which strongly vary in respect of disturbance by land use. Land use practices, such as mowing or grazing, have a large impact on the plant species within their habitats, especially on the traits related to their reproduction. Population growth, proportion of flowering shoots, flower and seed production, flowering time, seedling recruitment, individual size and the extent of vegetative reproduction all are factors being influenced by mowing and grazing (Brys et al. 2004; Böhler and Schmid 2001; Ehrlén et al. 2005; Hegland et al. 2001; Lennartsson and Oostermeijer 2001; Piqueras 1999). Reproduction in turn strongly affects the genetic and genotypic variation of populations (Hsiao and Lee 1999; Källiker et al. 1998). Different patterns of molecular variation can, therefore, be expected when plants occur in habitats with different levels of disturbance by land use. Previous studies already revealed that fertilization and defoliation can

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affect the genetic structure of populations (Kölliker et al. 1998). Both the influence of grazing (Bühler and Schmid 2001; Kotanen and Bergelson 2000; Lennartsson et al. 1997) and mowing (Billeter et al. 2002; Kölliker et al. 1998) have been studied in previous studies. However, much more can be learned about the impact of mowing than of grazing.

Many plant species combine both clonal and sexual reproduction to increase their survivability within specific ecosystems, and the proportion of both strategies in the reproduction of a certain species strongly determines their genetic structure. Plant species which exclusively reproduce clonal exhibit very low levels of genotypic diversity. However, since many plants combine both strategies, clonal species generally exhibit a level of genotypic variability which is comparable to non-clonal species (Ellstrand and Roose 1987; Hamrick and Godt 1996; Widén et al. 1994). Different forms of clonal growth and the combination of sexual and vegetative reproduction lead to a complex spatial genetic structure on both micro and macro scale (Reisch et al. 2007). The diversity of clonal species has been analysed in numerous studies (Gabrielsen and Brochmann 1998; Garnier et al. 2002). There are, however, only few studies dealing with the impact of disturbance by land use on the clonal diversity of plant populations (Kleijn and Steinger 2002; Piqueras 1999). The most detailed study addressed the contrasting effects of grazing and hay cutting on the genetic structure of the clonal species *Veratrum album* and revealed lower levels of genotypic diversity in grazed pastures compared to hay meadows (Kleijn and Steinger 2002).

In the study presented here, we compared the genetic structure of *R. ficaria* in disturbed and non-disturbed plots located in mown meadows and unmown forests to analyse the impact of disturbance by mowing on clonal diversity and discussed how the observed differences can most likely be explained. We compared the clonal and genetic diversity of *R. ficaria* in meadows and forests using random amplified polymorphic DNA (RAPD) analysis. This technique was successfully applied in previous studies to analyse the genetic structure of clonal plant species (Hangelbroek et al. 2002; Pluess and Stöcklin 2004; Reisch and Poschlod 2004; Reisch et al. 2007), since it allows the identification of samples belonging to the same genet by the detected banding patterns.

Materials and methods

Species description and sample sites

Ranunculus ficaria L. is a glabrous, ascending, perennial herb (Tutin et al. 1964). It reaches a size of up to 25 cm and has ovate basal leaves with long petioles. The species flowers from March to May and exhibits yellow flowers which are 10–30 mm in diameter. *R. ficaria* can be found in damp meadows, woods or hedge banks, which strongly differ in respect of land use. Generally, two subspecies are recognized (Stace and Thompson 1995). *R. ficaria* ssp. *ficaria* produces no bulbils but many seeds. The flowers are pollinated by flies, bees and beetles, and the seeds are distributed by ants (Sebald et al. 1998). In contrast, *R. ficaria* ssp. *bulbifer* forms bulbils but produces only few viable seeds. This is due to the degeneration of the embryo-sac and the nucellus (Metcalf 1939). In Germany only *R. ficaria* ssp. *bulbifer* can be found (Oberdorfer 2001). Therefore, all individuals analysed in this study belonged to ssp. *bulbifer*.

To analyse the impact of disturbance by mowing on the clonal diversity of *R. ficaria* three meadow (M 1–3) and forest (F 1–3) study plots were established at two localities (Penk, Oberisling) about 15 km from each other near Regensburg ($48^{\circ}58'14''$ N/ $12^{\circ}07'32''$ E) in southeast Germany (Fig. 1). The distance between the study plots at the localities was about 500 m. The meadow plots were located on damp ground along the river Naab. The meadows were highly intensively used by fertilization and mowing two times a year. The forest plots were located at the margin of an adjacent larger woodland and mainly consisted of alder. The herb layer of these forests was not subjected to mowing.

In each of the selected plots, 18–20 flowers and/or leaf buds were sampled for molecular analyses. Following a previous study, two axes of 400 cm length crossing in a right angle were laid out in a patch of *R. ficaria* (Stehlik and Holderegger 2000). Twenty sampling points were established at fixed distances from the point of intersection of the axes on each of the four rays, starting at 5 and advancing to 25, 50, 100 and 200 cm (Fig. 2). At each sampling point, one leaf was taken randomly within a circle of 3 cm around the point. At sampling points, where no plant material could be found within this circle, samples were classified as missing.

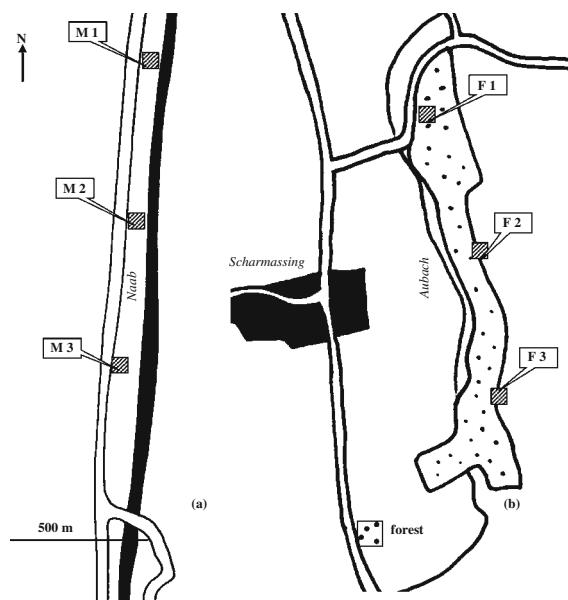


Fig. 1 Geographic position of the meadow and forest study plots (M 1–3 and F 1–3) at the two selected locations (a: Penk, b: Oberisling) in southeast Germany near Regensburg

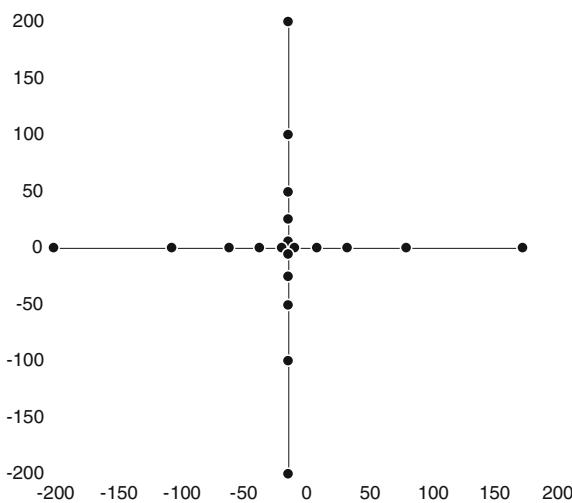


Fig. 2 Sampling design of *R. ficaria*. In each of the six plots, samples were taken along two axes, which cross in a right angle. Twenty samples were collected at fixed distances from the point of intersection on each of the four rays, starting at 5 and advancing to 25, 50, 100 and 200 cm

Molecular analyses

DNA isolation

The fresh leaf material was placed into plastic bags and stored in a cryogenic container in the field for

transportation and at -80°C in the laboratory. The DNA was isolated from the frozen plant material of individual plants using the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich 1994) adapted as follows: approximately 10–30 mg leaf material was ground in liquid nitrogen in a 1.5 ml Eppendorf tube followed by addition of 700 μl extraction buffer (100 mM Tris–HCl, pH 9.5; 20 mM EDTA, pH 8.0; 1.4 M NaCl; 1% PEG; 2% CTAB; 2.5 $\mu\text{l}/\text{mL}$ β -mercapto ethanol). Samples were incubated at 74°C for 30 min being shaken every 5–10 min. Subsequently, the mixture was extracted twice with an equal volume of chloroform/isoamylalcohol (24:1) and centrifuged at 15,000g at 4°C for 10 min. Adding an equal volume of isopropanol (4°C), the DNA was precipitated and pelleted by centrifugation at 20,000g at 4°C for 15 min. The DNA was washed with 70% cold ethanol (4°C) for 5 min and air-dried for 15–30 min. The DNA was resuspended in 200 μl TE buffer (10 mM Tris–HCl, pH 8.0; 1 mM EDTA, pH 8.0). The concentration was estimated spectrophotometrically (Genesys 6, ThermoSpectronic, Germany) at 260 nm, and the purity measured by the ratio of the absorbance at 260 and 280 nm. For PCR only template DNA was used with a purity of 1.8–2.1 in a dilution of 15 ng/ μl .

RAPD amplification

The clonal and genetic diversity of *R. ficaria* within the study plots were investigated using RAPD (Williams et al. 1990) amplification. For DNA amplification, the polymerase chain reaction (PCR) was used with arbitrary 10-mer oligonucleotide primers (Roth, Karlsruhe, Germany) for RAPD amplification. In a final volume of 15 μl , the amplification mixtures contained 10 mM Tris–HCl (pH 8.7), 3 mM MgCl₂, 10 mM KCl, 200 μM dNTP, 1 μM primer, 0.45 U Taq polymerase (Qiagen, Hilden, Germany) and 30 ng of genomic DNA. The PCR was run in a thermal cycler (Cyclone Gradient, PeqLab, Nürnberg, Germany). The thermal cycling program started with denaturation for 120 s at 94°C , followed by 35 cycles of 12 s denaturation at 94°C , 48 s annealing at 36°C and 90 s extension at 72°C . A final extension at 72°C for 10 min concluded the DNA amplification. From each population, four samples were selected and subjected to RAPD

analysis with 100 primers for screening. Six primers were selected giving rise to clear reproducible and distinct banding patterns (Table 1).

PCR products were kept at 4°C until they were loaded into the gel. The amplified products were separated on 1.5% agarose gels in 1× Tris–borate–EDTA (TBE) buffer at 180 V for 2 h, using a 100-bp ladder as a fragment size marker (PeqGold Ladder Mix, PeqLab, Nürnberg, Germany) and visualized by ethidium bromide staining. At the beginning of the study, reproducibility was tested by extracting the same leaf material for several amplifications, which revealed identical banding patterns. Additionally, each sample was repeated at least once in a separate amplification reaction.

For data scoring, the banding patterns were recorded using a trans-illuminating gel documentation system (GeneGenius, Syngene, Cambridge, UK). The image profiles and molecular weight of each band were determined by the program BIONUMERICS 3.0 (Applied Maths, Kortrijk, Belgium). Pictures were examined for strong, clearly defined bands which were scored across all individuals as either present or absent.

RAPD-statistics

In the data matrix, the absence of the band was coded as 0 to create a binary (0/1) matrix, representing the scored RAPD markers. Since RAPD markers are dominant, it was assumed that each band represented the genotype at a single bi-allelic locus (Williams et al. 1990). Samples with identical banding patterns were considered as belonging to the same genet. Based on the binary matrix, the clonal diversity within the plots was determined. The proportion of distinguishable multilocus genotypes (PD) was calculated as G/N (Ellstrand and Roose 1987), where G

is the number of distinct genotypes and N is the number of individuals sampled. Furthermore, the matrix was used to analyse the genetic diversity within populations. We calculated the percentage of polymorphic bands ($P = B_p/B_t$), where B_p is the number of polymorphic bands and B_t is the total number of bands as well as Nei's gene diversity ($H_e = 1 - \sum p_i^2$) and Shannon's information index ($I = \sum p_i \ln p_i$), where p_i is the frequency of the bands at the locus i , for each population, using the program POPGENE V 1.32 (Yeh et al. 1997). Clonal spread within plots was visualized by the mapping of genotypes along the sampling axes (Fig. 3). Being aware of the fact that genets can continue outside the study plot, the size of the genets within the plots was estimated by determining the maximum distance between samples from the same genotype along the axes. Mann–Whitney U -tests were used to test for significant differences between meadow and forest plots concerning genotypic and genetic diversity and the size of the genets.

Results

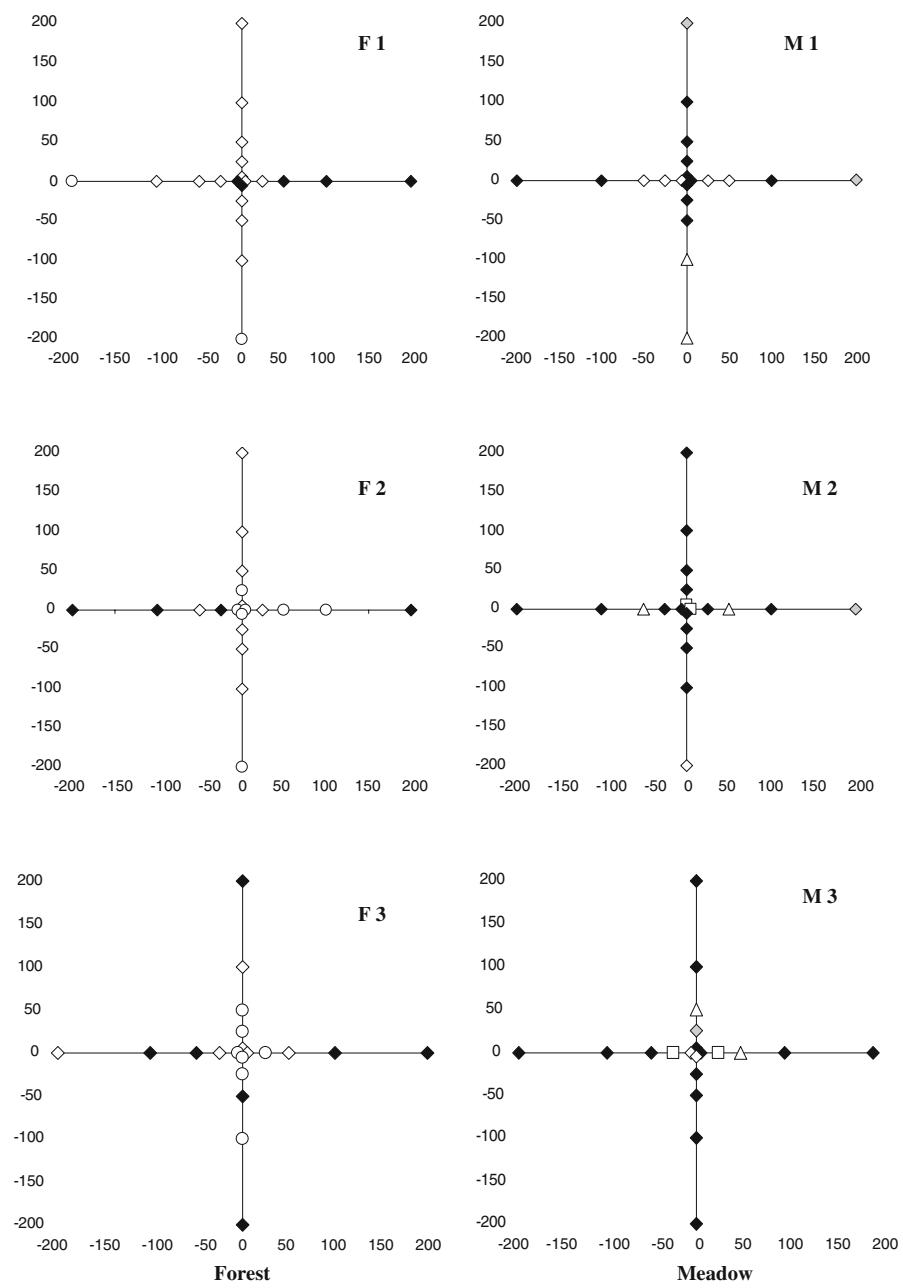
In the RAPD analysis of 117 individuals from six populations of *R. ficaria* with six primers, 57 reproducible fragments were amplified with a varying number per primer (Table 1). For example, primer AB03 produced only four scorable bands, while primer D20 amplified 12 fragments (Table 1). The size of the amplified fragments ranged from 280 to 2,060 bp, and the percentage of polymorphic bands per primer ranged from 50% (AB03) to 100% (A09) with an average of 76%. The 117 analysed individuals produced 65 multilocus RAPD genotypes.

Clonal diversity was substantially higher in plots from meadows than from forests (Table 2). The

Table 1 Primers employed with the number of RAPD markers obtained, their sequence, the size of the fragments for each primer, the number of analysed fragments and the percentage of polymorphic bands (PB) per primer

Primer	Sequence (5' → 3')	Size (bp)	Polymorphic	Monomorphic	Total	PB (%)
A07	-GAA ACG GGTG-	340–1,580	8	2	10	80
A08	-GTG ACG TAG G-	450–1,240	9	0	9	100
A19	-CAA ACG TCG G-	320–780	7	2	9	78
C08	-TGG ACC GGT G-	540–1,270	7	4	11	64
D20	-ACC CGG TCA C-	640–2,060	12	2	14	86
AB03	-TCG CGC ACA C-	280–1,270	2	2	4	50
Total		280–2,060	45	12	57	76

Fig. 3 Clonal structure of *R. ficaria* within the analysed plots (distances in centimetre). Black symbols display multilocus genotypes, which were represented only once in the study. White symbols represent the detected genets consisting of more than one sample. Samples with the same white symbol (circle, rhomb, triangle, square) belong to the same genet. Grey symbols mark positions, where no samples were taken due to the lack of plants at this position



proportion of distinguishable genotypes (PD) ranged from 0.72 to 0.85 (mean 0.80) in meadow plots and from 0.30 to 0.45 (mean 0.36) in forest plots. Measures of genetic diversity were also higher in meadows than forests. The percentage of polymorphic bands was 38.60–54.39 (mean 44.4) in meadow plots and 24.56–26.32 (mean 25.1) in forest plots. Shannon's information index ranged from 0.18 to 0.28 (mean 0.22) and from 0.08 to 0.10 (mean 0.09) in

meadows and forests. Finally, Nei's gene diversity varied between 0.12 and 0.18 (mean 0.14) in meadow plots and between 0.05 and 0.06 in forest plots. For all measurements, differences between habitat types were significant (Mann–Whitney *U*-tests, $P < 0.05$, respectively). One genotype was shared between the forest plots F 1 and F 2.

The observed genets consisting of more than one sample were larger within plots from forests than

Table 2 Genotypic and genetic variation within the analysed plots of *Ranunculus ficaria*

Population	<i>N_{PL}</i>	<i>N_{GT}</i>	PD	PB (%)	SI	GD	Max. <i>D_G</i> (cm)
Meadow							
M 1	18	13	0.72	38.60	0.18	0.12	100
M 2	19	16	0.85	40.35	0.21	0.14	100
M 3	19	16	0.85	54.39	0.28	0.18	50
Mean	18.6	15	0.80	44.4	0.22	0.14	83.3
Forest							
F 1	20	7	0.35	24.56	0.10	0.06	300
F 2	20	6	0.30	24.56	0.08	0.05	200
F 3	20	9	0.45	26.32	0.09	0.05	150
Mean	20	7.3	0.36	25.1	0.09	0.05	216.7

Given are the number of analysed samples (*N_{PL}*), the number of detected genotypes (*N_{GT}*), the proportion of distinguishable genotypes (PD), percentage of polymorphic bands (PB), Shannon's information index (SI) and Nei's gene diversity (GD). Additionally, for each plot the maximum distance between samples belonging to the same genet along the axes is given (Max. *D_G*) as an indicator for genet size. For each habitat, mean values are given. Levels of diversity were significantly lower (*U*-tests, $P < 0.05$) in forest than in meadow plots

meadows (Table 2). Keeping in mind that the genets can continue outside the plots, mean maximum distance between samples belonging to the same genet along the axes was 83.3 cm in grassland plots and 216.7 cm in forest plots. Mapping of the genets along the sampling axes visualized the strong differences in clonal diversity between forest and meadows plots (Fig. 3). The number of single genets was substantially higher in meadows (mean PD = 0.80) than in forests (mean PD = 0.36). The genets stretched nearly across the whole sampled area in forests, while being more restricted in size in the meadows.

Discussion

In the study presented here, we inferred the level of clonal diversity of *R. ficaria* using the proportion of distinguishable genotypes (PD), which was 0.36 in the forest and 0.80 in the meadow plots. In general, PD ranges from 0 for a set of completely identical samples to 1 for a set of totally different samples. According to previous studies, the diversity of

clonal species varies from PD = 0.02 to PD = 1.0 (Ellstrand and Roose 1987). Based on an extensive literature survey, a mean of PD = 0.27 was calculated for clonal plants (Widén et al. 1994). In previous RAPD studies, clonal diversity was 0.13 for *Carex curvula* (Steinger et al. 1996), 0.23 for *Vaccinium idis-idea* (Persson and Gustavsson 2001), 0.68 for *Vaccinium stamineum* (Kreher et al. 2000) or 0.93 for *Viola riviniana* (PD = 0.93) (Auge et al. 2001). Considering these very different levels of variation, it can be stated that the species-specific proportion of clonal and non-clonal reproduction strongly affects the level of clonal diversity in different plant species. This proportion can even vary from habitat to habitat, as demonstrated for *R. ficaria* here.

Clonal and genetic diversity of *R. ficaria* were significantly higher within meadow than within forest plots. Furthermore, in meadow plots more and smaller genets could be found. These differences seem most likely to be due to the different levels of disturbance by mowing in the analysed habitat types. The impact of contrasting land use practices on the genetic diversity of plant populations has already been demonstrated in previous studies (Bockelmann et al. 2003; Reisch and Poschlod 2003; Zopfi 1997, 1998), although these studies mainly deal with the development of ecotypic variants due to differences in management. Fewer analyses revealed significant impacts of land use practices on the diversity within populations (Kleijn and Steinger 2002; Kölliker et al. 1998), such as the study about *Festuca pratensis* which revealed lower levels of genetic diversity in mown populations (Kölliker et al. 1998). Concerning land use practices and clonality nearly no data are available. However, a study of the clonal species *V. album* revealed differences in clonal diversity between populations from grazed pastures and mown hay meadows (Kleijn and Steinger 2002). In the case of *V. album*, clonal diversity (PD) was almost twice as high in mown (0.97) than in grazed (0.53) habitats. Clonal reproduction was, therefore, more common in unmown than in mown habitats. The results of our study support this observation, since the clonal diversity of *R. ficaria* was significantly larger in meadows than in forests. The pattern of clonal diversity, we observed in this study, seems to be caused by the process of mowing, which represents a disturbance regime enhancing the transport of bulbils

and the establishment of young plants from these bulbils. This effects an increased commingling of genotypes and enhances clonal diversity in meadow plots.

Furthermore, previous studies revealed increased dispersal of diaspores by mowing (Bonn and Poschlod 1998). In former investigations it has already been demonstrated that populations in strongly disturbed habitats tend to have higher genotypic diversity compared to populations in stable habitats (Piquot et al. 1998; Xie et al. 2001). This is often due to a better establishment of seedlings under conditions of disturbance (Poschlod and Biewer 2005), and simulation experiments have shown that even a rare establishment from seeds is sufficient to maintain genetic diversity within populations of clonal species (Watkinson and Powell 1993). Higher genotypic variation in mown habitats could, as observed in our study, therefore be also due to a stronger generative reproduction of *R. ficaria* in the highly disturbed environment of meadows compared to the mainly undisturbed conditions in the forest. A comparable pattern has, for example, been observed for the clonal species *Passiflora incarnata*, which exhibited little evidence of clonal reproduction in recently disturbed sites, but a shift from sexual to vegetative reproduction is supposed as the plant community ages (Tague and Foré 2005). Concerning this shift towards clonal reproduction in non-disturbed habitats, the selection of genotypes is an important aspect. The result that genets in forest plots are larger than genets in meadow plots may at least partly result from selection, although it must always kept in mind that we did not analyse selection in this study since we applied neutral genetic markers. However, in the case that after establishment, genotypes with vigorous clonal growth may be favoured, as clonality is an efficient strategy to spread within a habitat or to persist in a stable habitat. Hence, in more stable habitats few genotypes should dominate theoretically due to competitive exclusion (Eriksson 1993; Gray 1987). This process should then lead to a decrease in genotypic diversity and an increase in the size of genets.

Conclusions

The results presented here clearly support the assumption that the level of disturbance by mowing affects the

clonal genetic structure of plant populations. However, further studies comprising a larger number of plots and other species would be suggestive to confirm our results and to place them in a broader context.

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Soil amendment effects on the exotic annual grass *Bromus tectorum* L. and facilitation of its growth by the native perennial grass *Hilaria jamesii* (Torr.) Benth

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Abstract Greenhouse experiments were undertaken to identify soil factors that curtail growth of the exotic annual grass *Bromus tectorum* L. (cheatgrass) without significantly inhibiting growth of native perennial grasses (here represented by *Hilaria jamesii* [Torr.] Benth). We grew *B. tectorum* and *H. jamesii* alone (monoculture pots) and together (combination pots) in soil treatments that manipulated levels of soil phosphorus, potassium, and sodium. *Hilaria jamesii* showed no decline when its aboveground biomass in any of the applied treatments was compared to the control in either the monoculture or combination pots. Monoculture pots of *B. tectorum* showed a decline in aboveground biomass with the addition of Na₂HPO₄ and K₂HPO₄. Interestingly, in pots where *H. jamesii* was present, the negative effect of these

treatments was ameliorated. Whereas the presence of *B. tectorum* generally decreased the aboveground biomass of *H. jamesii* (comparing aboveground biomass in monoculture versus combination pots), the presence of *H. jamesii* resulted in an enhancement of *B. tectorum* aboveground biomass by up to 900%. We hypothesize that *B. tectorum* was able to obtain resources from *H. jamesii*, an action that benefited *B. tectorum* while generally harming *H. jamesii*. Possible ways resources may be gained by *B. tectorum* from native perennial grasses include (1) *B. tectorum* is protected from salt stress by native plants or associated soil biota; (2) when *B. tectorum* is grown with *H. jamesii*, the native soil biota is altered in a way that favors *B. tectorum* growth, including *B. tectorum* tapping into the mycorrhizal network of native plants and obtaining resources from them; (3) *B. tectorum* can take advantage of root exudates from native plants, including water and nutrients released by natives via hydraulic redistribution; and (4) *B. tectorum* is able to utilize some combination of the above mechanisms. In summary, land managers may find adding soil treatments can temporarily suppress *B. tectorum* and enhance the establishment of native plants. However, the extirpation of *B. tectorum* is unlikely, as many native grasses are likely to facilitate its growth.

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Salt tolerance

Introduction

Bromus tectorum L., also known as cheatgrass or downy brome, is an exotic grass species whose invasion has had monumental consequences in western US ecosystems (Mack 1981; Upadhyaya et al. 1986). *Bromus tectorum* has replaced native plant communities throughout the West, resulting in changes in the type and timing of food and cover that have reduced native plant and animal diversity (Vail 1994). Greater wildfire frequency in *B. tectorum* habitat has further reduced native biodiversity and altered native vegetation structure (Whisenant 1990). *Bromus tectorum* can alter soil food webs, biogeochemistry, and nutrient relations in ecosystems, and these effects often reinforce its continued presence (Woodward et al. 1984; Harper et al. 1996; Belnap and Phillips 2001; Belnap et al. 2006; Sperry et al. 2006). There are also large economic consequences of *B. tectorum* invasions, given the deterioration of farm and rangeland habitat and the high cost of fire suppression (Mack 1981; Upadhyaya et al. 1986).

The Colorado Plateau is a 340,000-km² area that covers northeastern New Mexico, northern Arizona, western Colorado, and southern Utah. Grasslands in this region occur on areas with deeper soils and are generally dominated by a patchwork of two plant assemblages. The first assemblage contains a combination of the C₃ *Stipa hymenoides* R. & S., the C₃ *S. comata* Trin. & Rupr., the C₄ *Sporobolus* spp. (often *S. airoides* [Torr.] Torr., *S. flexuosus* [Thurber] Rydb.), and/or the C₄ *Bouteloua gracilis* (H.B.K. Lag. ex Steudel; all taxonomy is according to Welsh et al. 1993). The second assemblage is dominated by the C₄ grass *Hilaria jamesii* (Torr.) Benth. The *H. jamesii* assemblage contains a greater diversity of native annuals than the *Stipa/Sporobolus/Bouteloua* assemblage (Kleiner and Harper 1972, 1977). Grasslands in this region have experienced substantial invasion by *B. tectorum*. However, this invasion has been mostly limited to the patches dominated by *H. jamesii*, while patches dominated by the *Stipa/Sporobolus/Bouteloua* assemblage (which may be less than 1 m away) remain mostly uninvaded (Kleiner and Harper 1972, 1977; Miller 2000; Belnap and Phillips 2001). Patches of *H. jamesii* in other biogeographic regions, such as the salt deserts of the southern Great Basin, are also frequently invaded by *B. tectorum* (e.g., Freeman and Emlen 1995). A few other native plant communities have been

found to be resistant to invasion by *B. tectorum* (Bookman and Mack 1982; Kotanen et al. 1998; Booth et al. 2003; Humphrey and Schupp 2004). It is not known to what degree these invasion patterns are driven by the characteristics of the dominant native plants present, by the soil biota, by the differences found in soil characteristics in these two grassland patch types, or by some combination of the above factors.

Studies in both the lab and field show that soil chemistry and texture likely have a large influence on invasion patterns. Soils where *H. jamesii* and *B. tectorum* co-occur have a higher content of silt and potassium (K) and a higher ratio of K to magnesium (Mg) when compared to soils where *B. tectorum* cover is very low or non-existent, such as patches dominated by *Stipa hymenoides/S. comata* (Kleiner and Harper 1972, 1977; Miller 2000; Belnap and Phillips 2001). This supports other studies showing that soils with high K/Mg ratios favor annual plants over perennial plants (Crooke and Knight 1962; Scott and Billings 1964; Woodward et al. 1984) and that K additions to soils can stimulate growth of *B. tectorum* (Howell 1998; Morrison 1999). Miller et al. (2006) showed *B. tectorum* emergence was inhibited when magnesium oxide (MgO) was added to sandy, calcareous soils. The addition of MgO may have increased soil pH or the acid neutralizing potential (ANP, a measure of the pH buffering capacity of the soil, attributable to carbonates and reactive oxides), thus inhibiting the ability of the seedlings to access calcium (Ca)-bound phosphorus (P) or other micro-nutrients (Tyler 1992, 1994). Miller et al. (2006) also found that *B. tectorum* growth in winter was positively associated with bio-available P and negatively associated with soil ANP. Together, these studies suggest that *B. tectorum* performance in sandy, calcareous soils may be co-limited by K and P and that the life-history stage of the plant may influence when a given resource is limiting (Eckert and Evans 1963).

Restoring native plants to *B. tectorum*-infested ecosystems is a high priority for many land managers. However, seeding natives into established *B. tectorum* stands often fails, and this failure has been attributed to competition from *B. tectorum*. However, it could also be due to interactions between *B. tectorum* and the resident native plants or to the effects *B. tectorum* has on soil biota and thus nutrient

availability. In this study, we examined soil amendments that have the potential to suppress growth of *B. tectorum*, but have minimal effect on the growth of adjacent native grasses. We also examined the effect of those amendments when *B. tectorum* was grown with a native plant to help predict the likely field outcome when native plants were restored into *B. tectorum*-dominated areas. For these experiments, we chose to use the native grass *H. jamesii* because it is an important forage plant throughout the southwestern US, and it is often dominant or subdominant in grasslands and shrublands that are invaded by *B. tectorum*. Therefore, this species has a high likelihood to be involved in restoration efforts throughout this region. Based on the previous studies cited above, our treatments focused on altering soil levels of K and P. Our predictions were that 1) decreasing K availability would inhibit *B. tectorum* growth, whereas adding K would increase growth; and 2) *B. tectorum* would respond positively to P additions and negatively to the addition of compounds that reduce P bio-availability due to geochemical reactions. We expected the treatments to have only a limited effect on the native perennial grass *H. jamesii*, given that desert plants are adapted to growing in environments with generally low, but highly fluctuating, soil nutrient concentrations.

Methods

Studied species

Bromus tectorum is a C₃ exotic invasive annual grass that occurs throughout the cooler deserts of the western US. It is found in many habitat types, ranging from low-elevation grasslands and shrublands to higher-elevation sagebrush and pinyon-juniper communities (Monsen 1994). *Bromus tectorum* is facultatively mycorrhizal and is known to pick up mycorrhizae quickly in desert soils (Allen 1984), including in our study soils (Belnap unpublished data). About 75% of *B. tectorum* roots are concentrated in the top 30–40 cm of soil, depending on rainfall (Peek et al. 2005).

Hilaria jamesii is a C₄ native perennial grass. It is widespread throughout the western US, occurring from southern Wyoming and western Kansas to Utah, Nevada, Arizona, and New Mexico to southeastern California. It occurs in many habitats in these states,

ranging from low-elevation grasslands and shrublands (e.g., salt desert shrubs) to mid-elevation sagebrush and pinyon-juniper woodlands. It can be dominant in the communities where it occurs and produces an abundance of nutritious forage for wildlife and livestock throughout the western US. It is an obligate mycorrhizal plant that is strongly rhizomatous; thus, most roots are near the soil surface. Unlike the caespitose bunchgrasses (e.g., *Stipa*), *H. jamesii* forms a low-growing and more continuous mat of plant material.

Soils and root cation exchange capacity

We collected calcareous, sandy loam Begay soils from Canyonlands National Park (CNP), a cold semiarid ecosystem in southeastern Utah (~1500 m above sea level, average annual precipitation and temperature, 214 mm and 11.6°C, respectively [Miller 2000]), two weeks before the study began. Begay soils support *B. tectorum* and *H. jamesii*. A subset of the soils was sieved to 2 mm and sent to the Brigham Young University (BYU) Soil and Plant Analysis Lab for analysis. Phosphorus and available K were extracted with NaHCO₃ (Olsen et al. 1954; Schoenau and Karamonos 1993; respectively). Exchangeable cations were extracted with NH₄C₂H₃O₂ buffered to pH 8.5 to match the soil pH (Thomas 1982). Micronutrients (copper, iron [Fe], manganese, and zinc) were extracted using diethylene triamine pentaacetic acid (DTPA; Lindsay and Norvell 1978). Organic matter (OM) was determined with the Walkley-Black (1934) procedure and electrical conductivity (EC) and pH with a saturated soil paste (Rhoades 1982). Texture was determined by the hydrometer method, cation exchange capacity (CEC) by sodium saturation (Chapman 1965), total nitrogen (N) by Kjeldahl analysis (Bremner 1996), and the buffering capacity of the soil (acid-neutralizing potential [ANP]) by HCl neutralization (Allison and Moodie 1965). Exchangeable ammonium and nitrate were assessed using the steam distillation method (Bremner and Keeney 1965). The texture and chemistry for the soils used for planting, before amendments were added, are presented in Table 1. Roots from ten plants each of *B. tectorum* and *H. jamesii* were also collected in the field and analyzed for CEC. Roots were acid washed and placed in cooled KCl (Drake et al. 1951). Samples were titrated to a pH of 7.0 over a 5-min period with KOH,

Table 1 Characteristics of soils from the Canyonlands National Park site used for the pot trials before addition of soil amendments

<i>Bromus tectorum</i> soils	
P	17
Available K	198
Exchangeable Ca	2568
Exchangeable K	295
Exchangeable Mg	129
Exchangeable K/Mg	2.2
Exchangeable Na	40
Organic matter (%)	0.4
Electrical conductivity (dS/M)	1.1
pH	7.8
Sand (%)	72
Silt (%)	14
Clay (%)	14
Total N	74
NH ₄	7.4
NO ₃ ⁻	20
ANP (%)	3.2
Cu	0.6
Fe	3.7
Mn	4.6
Zn	0.3

ANP = acid neutralizing potential. Units are $\mu\text{g g}^{-1}$ unless otherwise noted

and then washed and dried. CEC is calculated as $\text{cmol}_{(+)} \text{kg}^{-1}$ dry root.

Soil amendments

In February 2000, each of 270 fiberglass pots (4×16.8 cm) was filled with 161 g of the CNP soil. The different soil amendments were added at equivalent osmolar rates (Table 2) except for zeolite, a solid (see below). Because some treatments could have unforeseen side effects or not fulfill the intended goal, we used multiple ways of altering plant-available soil nutrients. To increase plant-available P, we added Na₂HPO₄ and oxalic acid. Oxalic acid is an organic acid produced by plant roots, mycorrhizae, and other organisms (Allen et al. 1996) that can solubilize or compete for exchange sites with soil Ca²⁺, Fe²⁺, K⁺, Mg²⁺, and PO₄³⁻, keeping these elements and compounds available to local biota (Staunton and LePrince 1996). To increase K, we used KCl and

K₂HPO₄. To decrease K, we used zeolite and MgCl₂. Zeolite is a high-CEC ($220 \text{ cmol}_{(+)} \text{kg}^{-1}$), crystalline, hydrated aluminosilicate of volcanic origin that can preferentially bind K⁺ by electrochemical adsorption on exchange sites (Ming and Mumpton 1989). We hypothesized that MgCl₂ may reduce plant uptake of K through competitive displacement of K⁺ from root exchange sites by Mg²⁺ (Crooke and Knight 1962; Scott and Billings 1964; Woodward et al. 1984). When MgCl₂ is added to calcareous soils, it can also form MgCO₃, which may diminish P bio-availability due to surface sorption of HPO₄²⁻ on carbonate particles (Lajtha and Harrison 1995). NaCl was added both as an osmotic control and as a treatment increasing Na. Treatments, except zeolite, were added as an aqueous solution to the pots. Clinoptilolite (a form of zeolite, GSA Resources, Inc., Tucson, AZ) was charged with Na by equilibration with 2 M NaCl for 5.5 days; the solution was changed out every 24 h. Once charged, enough zeolite to constitute 10% of the soil by volume was then mixed with the soil in a larger container. No amendments were added to the control pots.

Seeding and harvesting of plants

Bromus tectorum seeds were collected from CNP and were fully after-ripened. *H. jamesii* seeds were purchased from Southwest Seed Co. in nearby Cortez, CO because it is field-collectible only after exceptionally wet years. The purchased seeds were from field-grown plants germinated from wild plant seed that had been collected within a 100-km² radius of our site. Because *H. jamesii* has a wide distribution in the southwestern US, is wind pollinated, and wild plants are found adjacent to the fields where the seeds are grown, we felt confident that the obtained seeds were comparable to those found at our site. In early March 2000, 30 pots were planted for each treatment. Ten *H. jamesii* seeds were planted in each of ten pots, ten *B. tectorum* seeds were planted in each of ten pots ("monoculture" pots), and in the remaining ten pots, five *H. jamesii* and five *B. tectorum* seeds were planted together ("combination" pots). Pots were placed in the greenhouse at Denver University. To avoid competition among the plants and to more closely mimic field densities and conditions, the seedlings were thinned to two *B. tectorum* individuals and, because they are smaller and slower growing, five *H. jamesii* individuals

Table 2 Soil amendments added to the pots, their intended effects, the amount of each amendment, and their actual effect

Additive	Intended effect	Amount added (mg g ⁻¹ soil)	Actual effect
No additions	Control	0	None
Na ₂ HPO ₄	+P	1.58	+P
Oxalic acid	+P	1.00	No effect on P
KCl	+K	1.24	+K
K ₂ HPO ₄	+P and K	1.93	+P and K
MgCl ₂	-K	1.06	No effect on K
Zeolite	-K	(10% by volume)	None
NaCl	+Na, osmotic control	0.97	+Na, osmotic control

in the monoculture pots. The combination pots had two *B. tectorum* and five *H. jamesii* plants per pot. Pots were monitored daily in the greenhouse and received deionized water when the surface soil was dry. Temperature minima and maxima averaged 17 and 27°C, respectively, during the growth trial. To avoid competition for soil nutrients, plants were harvested after 8 weeks so that total root biomass was still small relative to the soil volume. At harvest time, the aboveground biomass of both species was collected separately, dried, and weighed. In a subset of the monoculture pots, roots were gently separated from the soil, dried, and weighed for both *H. jamesii* and *B. tectorum*.

Soil nutrient availability

Resin capsules (Unibest PST-1, Bozeman, MT) were placed in each pot (thus, $N = 10$ per treatment) and left in the pots for the duration of the 8-week experiment. Resin capsules were used as they more accurately assess the availability of soil nutrients to plants than traditional soil analyses. When pots were harvested, the resin capsules were removed from the soil and rinsed with distilled water. The capsules were then extracted in 2 N HCl, and the extract was analyzed using Inductively Coupled Plasma mass spectrometry at the BYU Soil and Plant Analysis Lab.

Statistical analyses

Normality of the biomass and resin data was tested using the Kolmogorov-Smirnov test; only the *H. jamesii* in combination pots required transformation to meet normality assumptions, for which we took 4th-root of *H. jamesii* aboveground biomass. All

data were analyzed using ANOVA to distinguish differences among treatment effects. Post hoc differences between treatments were assessed using Tukey's B test if variances were equal and Dunnett's T3 if unequal. *T*-tests were used to analyze biomass between monoculture and combination pots within the same treatment. All statistics were analyzed using SPSS (Version 15, SPSS Inc., Chicago).

Results

Resin capsules showed that direct increases in the target nutrients were achieved in each treatment type for each plant combination although the desired indirect effect was not always achieved (Table 3). Values for K were higher in the KCl and K₂HPO₄ treatments than in the other treatments or the controls. However, K values were not lower in the zeolite treatment than the control as was intended. Values for P were significantly higher in the K₂HPO₄ and Na₂HPO₄ treatments than the control or other treatments. However, they were not higher in the oxalic acid treatment as intended. Values for Mg were higher in the MgCl₂ treatment, but this treatment did not reduce K or P availability as was intended. Values for Na were higher than the control in the zeolite, Na₂HPO₄, and NaCl treatments; among these three, the zeolite treatment added significantly lower Na.

In the *H. jamesii* monoculture pots, there was no treatment where aboveground biomass/pot differed significantly from the control (Fig. 1A). In the combination pots (*H. jamesii* grown with *B. tectorum*), only the Na₂HPO₄ treatment differed from the control. However, the *H. jamesii* aboveground biomass/pot was much greater in the monoculture pots than when it was grown with *B. tectorum* in the combination pots,

Table 3 Mean values (\pm standard error) of P, Mg, K, and Na from resin capsules buried in pot soils throughout the experiment across a row

		Control	Na_2HPO_4	Oxalic acid	KCl	K_2HPO_4	MgCl_2	Zelite	NaCl
P	<i>Hilaria jamesii</i>	1 a	112.5 \pm 24.2 b	5.0 \pm 1.0 a	0.9 \pm 0.3 a	150.6 \pm 25.1 b	2.2 \pm 0.7 a	25.2 \pm 1.2 a	11.8 \pm 2.1 a
	<i>Bromus tectorum</i>	1 a	182.3 \pm 33.5 b	1.8 \pm 0.3 a	0.9 \pm 0.3 a	235.5 \pm 37.3 b	2.0 \pm 0.5 a	2.5 \pm 0.9 a	2.7 \pm 0.3 a
	Combination pots	1 a	32.6 \pm 4.3 c	0.3 \pm 0.05 a	1.1 \pm 0.4 a	13.2 \pm 1.1 b	0.1 \pm 0.01 a	4.6 \pm 0.5 a	4.5 \pm 0.7 a
Mg	<i>Hilaria jamesii</i>	1 a	2.1 \pm 0.6 a	2.0 \pm 0.3 a	1.9 \pm 0.1 a	2.1 \pm 0.4 a	185 \pm 2.6 b	4.7 \pm 0.2 a	5.3 \pm 0.4 a
	<i>Bromus tectorum</i>	1 a	3.1 \pm 0.6 ab	2.3 \pm 0.3 ab	3.7 \pm 0.2 ab	3.8 \pm 0.6 ab	36.2 \pm 2.9 c	1.3 \pm 0.4 a	6.8 \pm 0.6 b
	Combination pots	1 ab	2.5 \pm 0.3 c	0.4 \pm 0.04 a	1.7 \pm 0.2 bc	1.7 \pm 0.1 bc	4.1 \pm 0.6 d	1.8 \pm 0.2 bc	2.3 \pm 0.1 c
K	<i>Hilaria jamesii</i>	1 a	1.6 \pm 0.3 a	2.1 \pm 0.3 a	13.6 \pm 1.5 b	39.0 \pm 4.1 c	2.7 \pm 0.4 a	2.5 \pm 0.1 a	3.7 \pm 0.2 a
	<i>Bromus tectorum</i>	1 a	4.9 \pm 0.6 a	2.8 \pm 0.4 a	31.8 \pm 3.6 b	96.8 \pm 11.4 c	6.2 \pm 0.4 a	0.9 \pm 0.3 a	5.5 \pm 0.4 a
	Combination pots	1 a	1.2 \pm 0.2 a	0.4 \pm 0.04 a	12.2 \pm 1.3 b	15.5 \pm 0.7 c	0.7 \pm 0.1 a	1.1 \pm 0.1 a	2.0 \pm 0.1 a
Na	<i>Hilaria jamesii</i>	1 a	33.2 \pm 5.3 b	1.2 \pm 0.1 a	1.0 \pm 0.1 a	1.4 \pm 0.1 a	1.3 \pm 0.1 a	47.1 \pm 0.9 c	44.8 \pm 1.6 c
	<i>Bromus tectorum</i>	1 a	34.2 \pm 4.4 b	1.1 \pm 0.1 a	0.8 \pm 0.02 a	1.5 \pm 0.1 a	1.4 \pm 0.1 a	6.6 \pm 1.3 a	32.5 \pm 2.6 b
	Combination pots	1 a	62.5 \pm 2.7 c	1.0 \pm 0.03 a	1.0 \pm 0.04 a	1.2 \pm 0.04 a	1.1 \pm 0.04 a	57.2 \pm 4.4 bc	51.7 \pm 4.1 b

Different letters indicate statistically distinct values ($P < 0.05$) among treatments within a species. For ease of comparison, the values presented are standardized to the control, while the statistical tests were run on actual values ($\mu\text{g}/\text{capsule}$)

except in the two treatments that added P (Na_2HPO_4 , K_2HPO_4).

The aboveground biomass/pot of *B. tectorum* monoculture pots declined in the two treatments that added P (Na_2HPO_4 , K_2HPO_4 ; Fig. 1B). In the combination pots, only Na_2HPO_4 showed a negative effect on *B. tectorum* aboveground biomass/pot. Most interestingly, when *B. tectorum* was grown with *H. jamesii*, the aboveground biomass per pot of *B. tectorum* was 1.6–9.1 times greater than when *B. tectorum* was grown in the monoculture pots.

Root biomass was also measured in a subset of the monoculture pots (Fig. 2A). Zeolite was the only treatment that had an effect, and it increased the root biomass/pot of both *H. jamesii* and *B. tectorum* relative to the control (Fig. 2A). Although there was no significant increase in aboveground tissue with this treatment (Fig. 1), zeolite significantly increased total biomass in both species relative to the control (Fig. 2B).

Analysis of the roots of field-grown *B. tectorum* showed they had an average CEC of $11.5 \text{ cmol}_{(+)} \text{ kg}^{-1}$ root (SE = 1.9). As would be expected when comparing the roots of an annual plant with roots from a perennial plant, we found the root CEC of the native perennial *H. jamesii* was much lower, averaging $5.1 \text{ cmol}_{(+)} \text{ kg}^{-1}$ root (SE = 0.5; $P < 0.01$).

Discussion

Abiotic controls on plant response: effects of soil amendments

As expected, our added treatments had little effect on *H. jamesii*. Desert perennial plants are adapted to low soil nutrients and relatively high salinity, and many studies have shown they often have little response to short-term fertilization treatments (e.g., Newingham and Belnap 2006). However, *H. jamesii* biomass/pot did respond to two treatments: zeolite increased the belowground and total (above + belowground) biomass/pot in the monoculture pots (Fig. 2B) and Na_2HPO_4 increased *H. jamesii* aboveground biomass in the combination pots (Fig. 1A). Both treatments substantially increased the availability of soil Na, while the Na_2HPO_4 treatment also increased P (Table 3). These results suggest that *H. jamesii* can not only tolerate high soil Na but may also belong to a group of C₄ grasses that can manifest supplementary

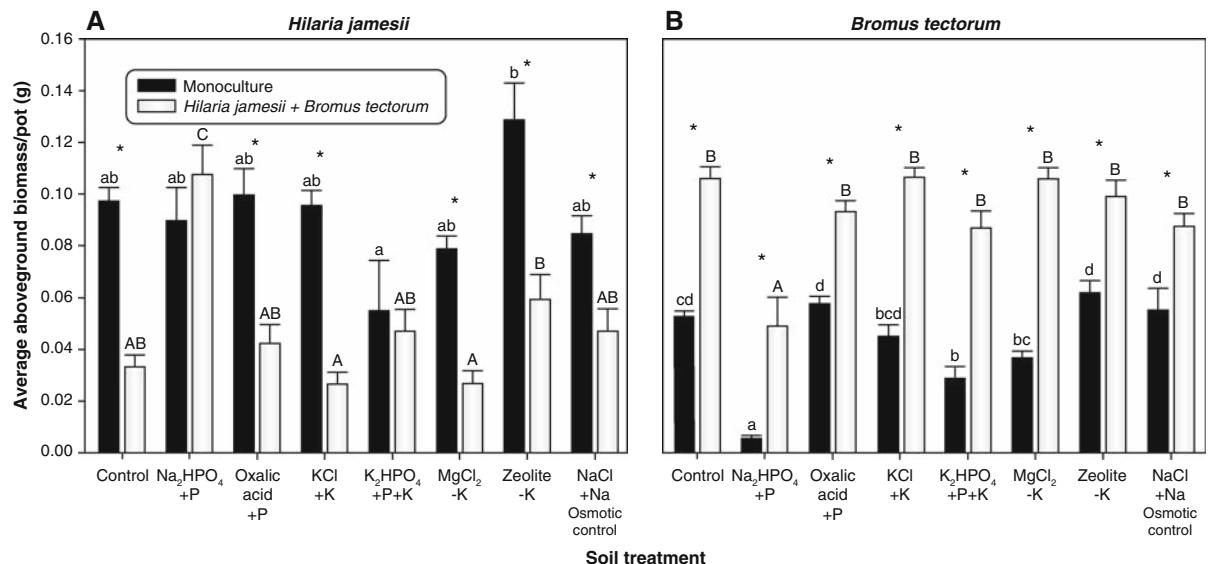


Fig. 1 Aboveground biomass per pot of *Hilaria jamesii* (A) and *Bromus tectorum* (B) in the monoculture and combination pots. Different lowercase letters indicate significant differences ($P < 0.05$) among treatments in the monoculture pots; different

uppercase letters indicate significant differences among treatments in the combination pots. Comparisons between the monoculture and combination pots within a plant type that were significantly different are marked with an *

growth in response to additional Na (Marschner 1995). However, it should be noted that there was no response in aboveground biomass/pot to the NaCl treatment, despite this also increasing soil solution Na (Table 3). Thus, the combined effects of this treatment and other soil factors (e.g., the combined effect of Na and P; the effect of these amendments on soil biota), or other soil factors alone, likely influenced the observed increase in *H. jamesii*.

In contrast to *H. jamesii*, the aboveground biomass/pot of *B. tectorum* declined in the Na_2HPO_4 treatment in both the monoculture and combination pots. Because all treatments were added at an equivalent osmolar concentration (except zeolite, which is a solid), the observed negative response cannot be attributed to osmotic effects. Whereas the addition of this treatment increased both Na and P concentrations in the soil, it also increased soil pH from 8.5 to 9.9, rendering multiple elements less or not available to plants (Troeh and Thompson 1993). In C_3 species such as *B. tectorum*, Na is a non-essential element (Troeh and Thompson 1993; Marschner 1995). Thus, additional Na may have inhibited growth, as found in previous studies of *B. tectorum* and other brome species (Rasmussen and Anderson 2002; Kolb and Alpert 2003; Shen et al. 2003). However, the zeolite and NaCl treatments also elevated soil Na concentrations, and neither treatment

showed a negative response relative to the control. Thus, it would appear that factors other than Na were responsible for the observed effect.

The extremely negative effect of the Na_2HPO_4 addition on *B. tectorum* in the monoculture pots was ameliorated when *B. tectorum* was grown in combination with *H. jamesii*. This treatment also had a similar effect on *H. jamesii* in the combination pots, as it prevented a reduction in *H. jamesii* aboveground biomass. It is possible that Na uptake by *H. jamesii* reduced Na stress on *B. tectorum*, while benefiting *H. jamesii*. This may be a major issue for restoration efforts: whereas Na_2HPO_4 could be used to suppress *B. tectorum* when it is growing in a monoculture, the addition of native plants may override much of the negative effect of the soil amendment, compromising the long-term success of the restoration effort.

Biotic controls on plant response: effects of plant–plant interactions

We observed a reduction in the aboveground biomass of *H. jamesii* when grown in combination with *B. tectorum* in most of our treatments. This was not unexpected, as similar results have been found in previous laboratory and field studies examining the impact of *B. tectorum* on the growth or aboveground

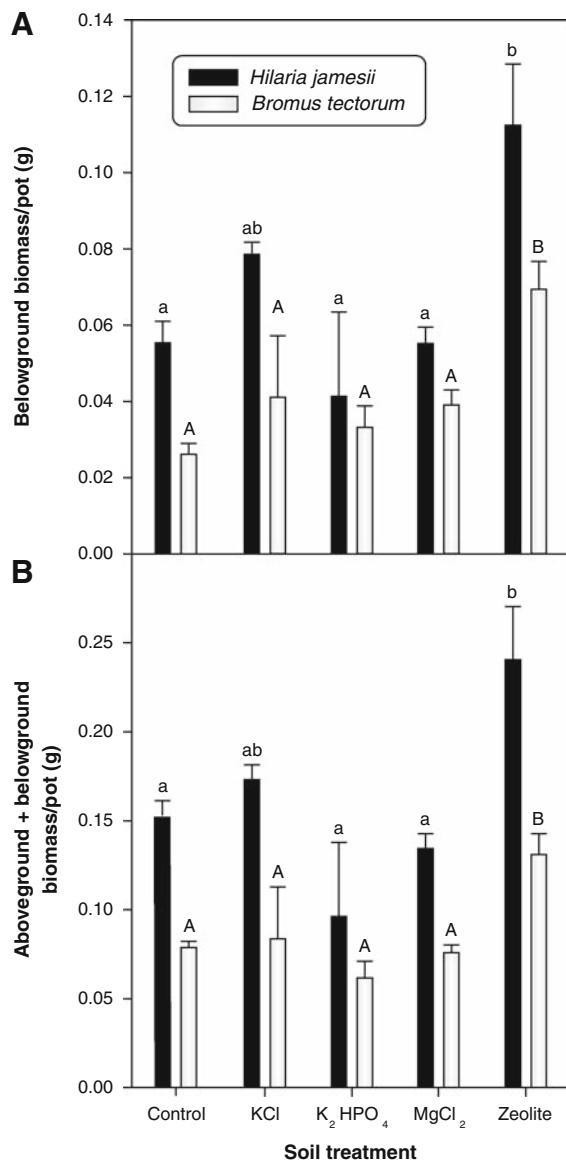


Fig. 2 Belowground biomass (**A**) and total biomass (aboveground + belowground; **B**) for *Hilaria jamesii* and *Bromus tectorum* growing in monoculture pots. Different letters indicate significant differences among treatments within each species

biomass of native grasses (e.g., *Agropyron spicatum*, *Festuca idahoensis*, *F. microstachys*, *F. octoflora*, *Bromus carinatus* [Mack 1981], *Stipa speciosa* [Tausch et al. 1994]) and shrubs (*Artemisia tridentata*; Booth et al. 2003).

We also observed an increase in *B. tectorum* biomass when grown with *H. jamesii*. While this was possibly due to reduced intra-specific competition

(monoculture pots had two *B. tectorum* plants and combination pots had two *B. tectorum* and five *H. jamesii* plants), the very low root biomass/soil volume and constant watering in this study makes this explanation unlikely. It is more likely that the presence of *H. jamesii* facilitated *B. tectorum* growth. Although most studies focus on negative interactions, positive and bidirectional interactions among plants exist in habitats as diverse as salt marshes, oak woodlands, drylands, or sand dunes (e.g., Aguiar and Sala 1994; Greenlee and Callaway 1996; Holzapfel and Mahall 1999; Callaway et al. 2004), and such facilitative relationships between exotic and native plants may explain the success of many invaders (e.g., Callaway et al. 2004; Callaway and Pugnaire 2007).

Facilitation of *B. tectorum* growth by native grasses has been previously reported for *Agropyron desertorum* (Evans 1961), *H. jamesii* (Freeman and Emlen 1995), and *Bouteloua gracilis* (Lowe and Laurenroth 2003), and there are many reports of *B. tectorum* growing larger and denser under shrubs compared to the plant interspace (e.g., Kelrick 1991; Freeman and Emlen 1995). The biomass of the non-native *Bromus diandrus* has greater biomass when grown with the native *B. carinatus* (Kolb and Alpert 2003). *Centaurea maculosa* biomass increases when grown with native grasses (e.g., Herron et al. 2001; Zabinski et al. 2002; Callaway and Pugnaire 2007). Facilitation can be species specific: *C. melitensis* biomass increases when grown with the native grass *Nassella pulchra*, but decreases when grown with the grass *Avena barbata* (Callaway et al. 2003). Biomass of *C. maculosa* increases when grown with the grasses *Festuca idahoensis* and *Koeleria cristata*, but decreases, or is not affected, when grown with other plants (Callaway et al. 2004). Yoder and Nowak (2000) showed *Bromus madritensis* acquired more P when next to *Larrea tridentata* and *Lycium andersonii* than when next to *Ambrosia dumosa*.

Possible mechanisms for facilitation of *Bromus tectorum* by native plants

There are multiple scenarios that could explain the facilitation of *B. tectorum* growth by native plants such as *H. jamesii*:

Scenario 1: *B. tectorum* is protected from salt stress by native plants or associated soil biota. As discussed above, previous studies have shown that many C₃

plants are intolerant to high soil salt levels, whereas many C₄ plants can benefit from these levels. In the combination pots, any salt uptake by the C₄ *H. jamesii* would reduce the salt exposure of the C₃ *B. tectorum*. Mycorrhizal fungi may have aided in reducing salt uptake by *B. tectorum* tissue, as a previous study showed the mycorrhizal fungi *Glomus intraradices* reduced salt stress in *Lotus glaber* (Sannazzaro et al. 2006).

Scenario 2: *B. tectorum* can tap into the mycorrhizal network of *H. jamesii* and obtain its resources, or *H. jamesii* alters other soil biota in a way favorable to *B. tectorum*. Both *H. jamesii* and *B. tectorum* are mycorrhizal (Trappe 1981; Allen 1984; Hawkes et al. 2006). There is ample evidence exotic plants can increase their biomass by tapping into the mycorrhizal networks of native plants, obtaining their resources (e.g., Fischer-Walter et al. 1996; Callaway et al. 2001, 2003; Carey et al. 2004) and increasing the biomass of the exotic plant. *Centaurea maculosa* biomass is greater when grown with the native grasses *Festuca idahoensis* or *Koelaria cristata*, while the biomass of *C. melitensis* increases when grown with the native grass *Nassella pulchra* (Callaway et al. 2001, 2003). Carbon, N, and P can be directly transferred (Grime et al. 1987; Zabinski et al. 2002; Carey et al. 2004; Moyer-Henry et al. 2006). Such transfers are likely more common in grasses, as their fibrous fine roots support more mycorrhizae than the more woody roots of shrubs. Uptake of soil N and P can also be stimulated by the presence of mycorrhizae associated with native plants (Herron et al. 2001).

Recent studies have shown that various components of the soil food web can have a strong influence on vascular plant community structure and composition by influencing competitive outcomes via pathogens or soil nutrient availability (e.g., Bever 1994; Klironomos 2002; Wardle and van der Putten 2002). Thus, the presence of *H. jamesii* may also influence soil food webs in an unknown way that favors *B. tectorum*. However, decomposition and N mineralization rates did not differ when soils dominated by *H. jamesii*, *H. jamesii* plus *B. tectorum*, or *B. tectorum* were compared (Belnap et al. 2005; Belnap et al. 2006).

Scenario 3: *B. tectorum* can take advantage of root exudates from native plants. Plants exude C, N, water, and other compounds through their roots (Biondini et al. 1988; Caldwell 1990). These exudates can increase both the total and available nutrient pools in

soils (Cannon et al. 1995; Marschner 1995). Species growing in calcareous soils exude more compounds than plants growing in less alkaline soils (Ström et al. 1994; Gries and Runge 1995; Tyler and Ström 1995), and exudates can be a common response to nutrient stress (e.g., Zhang et al. 1991, 1997; Awad et al. 1994; Cakmak et al. 1996; Deubel et al. 2000). Water and dissolved nutrients released during hydraulic redistribution can be scavenged by neighboring plants (Caldwell 1990; Dawson 1993; Caldwell et al. 1998). Thus, exudates from *H. jamesii* may increase soil nutrient availability and because *B. tectorum* has a much higher root CEC than *H. jamesii*, *B. tectorum* could outcompete *H. jamesii* for these nutrients. The intermingling of roots, both in our study and in the field, makes such an interaction highly likely.

Scenario 4: *H. jamesii* facilitates *B. tectorum* growth through some combination of the above scenarios. This may be a direct effect, with *H. jamesii* increasing the availability of soil resources to *B. tectorum* via root exudates and mycorrhizal fungal connections between the two plants. There may also be cascading effects of *H. jamesii* presence that increase resource availability to *B. tectorum*. For example, an increase in C exudation could stimulate soil fungi, many of which secrete phosphatases, which then increase P bio-availability. Similarly, stimulation of rhizosphere bacteria by exuded C can accelerate the transformation of N into bio-available forms. It is also possible that the response of *B. tectorum* in this study was a combination of both positive and negative effects created by the presence of *H. jamesii*, with positive effects outweighing negative effects (Callaway and Pugnaire 2007). For example, added salts may have negatively affected *B. tectorum*, but the presence of *H. jamesii* had sufficient positive effects to outweigh the negative effects and create the observed results.

Management implications and future directions

Given our current state of knowledge, eliminating *B. tectorum* from western rangelands is probably an unrealistic goal for land managers. However, suppression of *B. tectorum* during active restoration of natives is desirable and likely achievable. This study found two soil amendments that negatively affected *B. tectorum* without affecting *H. jamesii*. However, the success of these treatments may be overshadowed by the ability of *B. tectorum* to increase growth in the

presence of native grasses. Therefore, we need further studies to better understand the nature of the relationship between *B. tectorum* and native species to determine what and how native species facilitate *B. tectorum* growth and if there are ways to lessen this interaction.

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Effects of fire on the vegetation of a lowland heathland in North-western Italy

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Abstract This study focuses on the effect of fire on lowland heathlands at the extreme southern edge of their European distribution (Vauda Nature Reserve, NW Italy). Forty-nine plots (50 m radius) were surveyed between 1999 and 2006. Each year, fire occurrences were recorded and per cent cover of four vegetation types (grassland, heath, low shrubland, and tall shrubland) was estimated in each plot. Vascular plant species richness was also recorded in 255, 1 m² quadrats. After a fire, grassland vegetation expanded, but then declined rapidly as heath and shrubland recovered: 7 years after a fire, tall shrubland encroached on to more than 40% of the plots, and grassland declined from 50% to 20% cover. Between 1999 and 2006, *Betula pendula* shrubland greatly expanded, while grassland decreased over most of the Reserve, even where fire frequency was high. Tall shrubland had low plant diversity and was dominated by widespread species of lower conservation value. By contrast, early successional vegetation (grassland and low shrubland) had higher richness and more narrowly distributed species, indication that the development of tall shrubland causes significant species loss in the heathland. Italian lowland heathlands are characterized by high rates of shrubland

encroachment that threatens both habitat and species diversity. Burning frequencies of once in 3–6 years seem appropriate in this habitat, but burning alone might not suffice without actions to increase herbivore grazing.

Keywords *Betula pendula* · Birch · *Calluna vulgaris* · Conservation · Fire · Heather · Shrubland

Introduction

Lowland dry heathlands are mosaics of dwarf ericaceous shrubs (among which *Calluna vulgaris* (L.) Hull is the commonest species), intermixed with patches of grassland and pioneer trees. Although they occur on acidic, nutrient-poor soils that check the growth of woody vegetation, lowland heathlands are almost never a true climax, but rather an anthropogenic formation maintained by man through cutting and burning the original woods (Ellenberg 1988). Thus, heathland maintenance depends on a mix of human activities, among which grazing by domestic herbivores, burning, and mowing are the most commonly practiced (Webb 1986, 1998). These activities prevent the development of woody vegetation by physically damaging plants and restricting the accumulation of nutrients in the soil. In this way, the heathland remains in an early successional stage dominated by dwarf shrubs and grasses.

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Heathlands characterized the landscape of central and western Europe at least since the late Neolithic, or c. 5,000 years ago (Webb 1986; Ellenberg 1988). However, the abandonment of traditional management practices and developments in agricultural techniques that allowed the expansion of crops on infertile soils caused a spectacular decrease of heathlands across the continent. Conservative estimates of loss in western Europe range between 60% and 95%, and the remaining heathlands are severely fragmented and threatened by a variety of factors (Rebane and Wynde 1997). The conservation of lowland dry heathlands is one of the key targets of the environmental policy of the European Union, as stated in the European Habitat Directive (92/43/CEE).

One of the conservation issues in European heathlands is woodland encroachment, which causes the disappearance of the majority of the species of conservation concern (Gimingham 1992; Price 2002). When burning and grazing are discontinued, the expansion of woody species is an inevitable natural process that, in Italy, is initiated by pioneer trees such as *Betula pendula* Roth. and *Populus tremula* L. In time, pioneers facilitate the invasion of late successional species such as *Quercus* spp., and *Robinia pseudoacacia* L., an invasive tree introduced from North America (Sindaco et al. 2003).

Heathland encroachment was studied intensively in northern Europe (e.g. Gimingham 1992; Bullock and Webb 1995; Mitchell et al. 1997; Snow and Marrs 1997; Marrs et al. 2000; Pakeman et al. 2002; Manning et al. 2004, 2005), but little research was done in southern Europe, where heathlands reach the edge of their distribution. Lack of research on southern European heathlands is particularly problematic, since they are floristically different from similar habitats in northern Europe, and contain rich floras as well as unique habitat types (Devillers et al. 1991; Webb 1998). Available information suggests that encroachment is faster in southern Europe than in the northern part of the continent (Bartolome et al. 2005). Thus, the last heathland remnants in southern Europe are particularly threatened and in need of management, which, however, currently cannot be based on a sound base of scientific information as is possible in northern Europe.

This paper has two aims. First, data collected between 1999 and 2006 are used to illustrate patterns of fire recurrence and their effects on vegetation in a

poorly known habitat, the lowland heathland of North-western Italy. Second, plant species richness is compared across habitat types to assess the impact of periodic burning (or lack of burning).

Material and methods

Study area

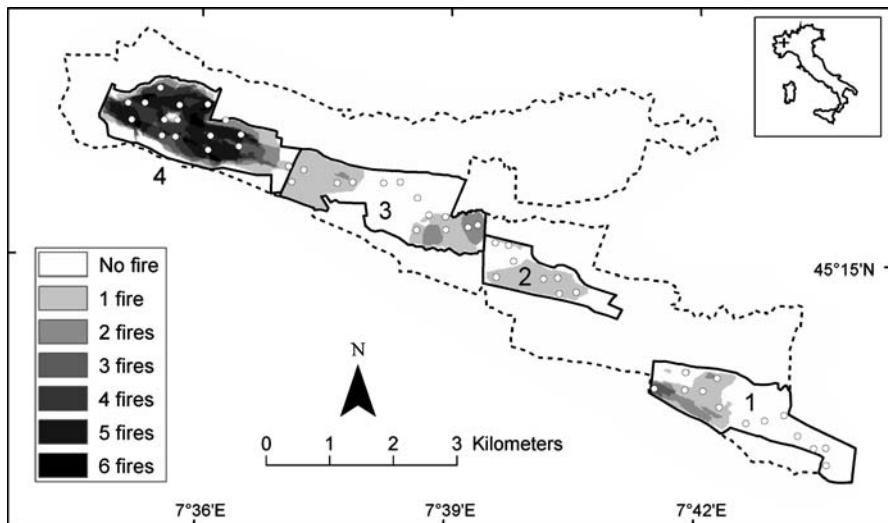
The Vauda Nature Reserve (45°15'N 7°39'E) was created in 1993 to preserve a fragment of the "Po basin heathland", a habitat unique to northern Italy, located at the extreme southern edge of the range of European heathlands (CORINE code 31.229, (Devillers et al. 1991)). The total heathland area in the Reserve is 910 ha, divided into four sectors (Fig. 1; sector 1: 234 ha; 2: 116 ha; 3: 276 ha; 4: 284 ha).

The Reserve is located on a fluvio-glacial terrace datable to the Mindel glaciation (c. 400,000 years ago). Soils are ancient and leached, with fairly low pH (4.8 ± 0.1 , $n = 14$, L. Borgesio, unpublished data) and high clay content. Elevation ranges from 270 to 440 m. The climate is Prealpine, with marked yearly temperature excursions (>20°C difference between the hottest and the coldest month), and a relatively well-spread rainfall, with no arid months (Sestini 1957). Rainfall averages 1100 mm/year, with maxima in May and October and minima in January and July. Average yearly temperature is 12°C, with minima (1.3°C) in January, and maxima (22°C) in July (Biancotti and Bovo 1998).

The Vauda is a fragment of a much larger heathland that occupied the area until the beginning of the 20th century. Since then, most of the heathland has been converted to agriculture and settlements. The Nature Reserve was spared because it has been used as military training area since the early 19th century. Military activities continue to this day.

The study area has a rich flora, with 750 species of vascular plants reported in the early 20th century (Ferrari 1913). However, many species have not been observed for many years, and the flora of the Reserve is impoverished compared with one century ago (L. Borgesio, unpublished). Evidence for widespread biodiversity erosion was also reported for birds, butterflies, and dung beetles (Borgesio 2004) and was linked to a variety of causes, including conversion of the heathland to agriculture, military

Fig. 1 Map of the study area, showing the borders of the Vauda Nature Reserve (hatched line), the extent of the heathland habitat within the Reserve (bold lines), and the areas burnt between 1996 and 2006. Darker shades correspond to higher burning frequency. The small circles mark the 49 plots; numbers (1–4) refer to sectors described in the text



activities, change in traditional management practices, and encroachment of woody plants and allochthonous herbs.

The Vauda has been used for centuries as a pasture for cattle and sheep, and grazing is still practiced. However, grazing intensity has decreased in recent decades. In particular, sector 2 of the Vauda has not been grazed since 2003. Three herds of cattle (totaling c. 200 animals) graze sectors 1, 3, and 4 from late March to early July. One herd of c. 500 sheep also grazes sectors 3 and 4 in May. Fires occur usually in winter or early spring (December to April), and are probably lit by the shepherds who graze their animals in the Vauda, but no clear information is available on this, since burning natural vegetation is illegal, and those who lit the fires do so anonymously.

Field methods

Burnt areas were mapped each year between 1999 and 2006. Data were collected in late May, at a time of the year when no fire occurs and burnt vegetation is easily identifiable. Between 1999 and 2001, burnt areas were mapped manually on 1:10,000 maps, with an estimated resolution of c. 50 m. Beginning in 2002, a GPS was used to draw the boundaries of burnt areas with 10 m horizontal resolution. Surfaces were calculated with ArcInfo 8.3 with the Spatial Analyst extension (©ESRI 2003).

Vegetation development was studied in a network of 49 circular plots (50 m radius) located on randomly

selected nodes of a 250 m-sized grid superposed to the study area. Positioning error due to GPS selective availability (Adrados et al. 2002) resulted in some slight irregularities in the placement of the plots (Fig. 1). The plots were visited each year between 15 May and 15 June and percent cover of the following four vegetation types was visually estimated:

- Grassland: areas with prevailingly grassy vegetation. Common species include *Molinia arundinacea* Schrank, *Brachypodium pinnatum* (L.) Beauv., *Danthonia decumbens* (L.) D.C., and *Festuca tenuifolia* Sibth. (Poaceae); *Genista tinctoria* L. and *Genista germanica* L. (Fabaceae); *Carex panicea* L. and *C. pilulifera* L. (Cyperaceae); and *Potentilla erecta* (L.) Rauschel (Rosaceae). These species are also found in the following vegetation types, which differ from open grassland due to the high cover of particular species
- Heath: areas with 40% or more cover of *Calluna vulgaris*
- Low shrubland: with at least 40% cover of shrubs and low trees (<1.5 m high), such as *Betula pendula*, *Populus tremula*, and *Frangula alnus* Miller
- Tall shrubland: same species as low shrubland, but average height >1.5 m and per cent cover of woody plants 70% or more

Accuracy of visual estimates was checked with aerial (1999 and 2000) and satellite photos (2004) of the study area. When necessary, plots were revisited to improve the accuracy of the estimates.

Vascular plant richness within each of the four vegetation types was estimated by surveying 1 m² quadrats between 21 June and 9 July 2002. Two quadrats were located 10 and 50 m from the center of each of the 49 main plots along three compass directions (north, southeast, and southwest). Thus, six quadrats were located in each of the 50-m radius plots; 39 quadrats were discarded as they fell on ponds, roads, mown meadows, or *Robinia pseudacacia* woods growing on former cultivated land. The remaining 255 quadrats were unequivocally assigned to one of the four vegetation types and categorized as burnt or not burnt during the current year. There were no quadrats in the burnt-heath category, because fire strongly reduces *Calluna*, thus changing the vegetation to the burnt-grassland type. All the species of vascular plants whose aerial parts fell inside the quadrat were recorded to estimate species richness at the quadrat level. In total, 122 species of native vascular plants were observed; eight allochthonous species (73 records, 2.2% of total) were excluded from the analyses. The range size of each species was estimated by tallying their presence in the 55 administrative provinces of the Alps according to Aeschimann et al. (2004). Use of this reference dataset is justified as the Vauda lies at the foot of the Alps, within the area covered by Aeschimann et al. (2004). As range size and population abundance are positively correlated (Gaston et al. 2000), species with smaller ranges are assumed to have a higher conservation value.

Statistical analyses

The data allowed the fire history of the 49 circular plots to be followed between 1999 and 2006. Additional information allowed the detection of fires that occurred in 1997 and 1998 in 12 of the 49 plots. The resulting database contained 353 yearly estimates of vegetation cover in plots whose fire history was known. Plots were grouped according to the number of years elapsed from the last fire (i.e., group 0 contains plots that burned on the current year, group 1 those that burned in the previous year, etc). Plots that had not burned for seven or more years were lumped in a single group. Due to repeated burning, some plots went through the same development stage multiple times (i.e. a plot that burned three times on alternate years went through stages 0 and 1 three

times). To avoid overrepresentation of some plots in particular development stages, multiple estimates of the same plot in the same stage were averaged, resulting in a final dataset comprising 238 estimates. Sample size varied between 17 (age classes 5 and 6) and 44 plots (age class 2).

General linear models (GLM) were used to study changes of per cent vegetation cover across the time elapsed since the last fire. Data were arcsin square-root transformed to achieve normality. Spatial autocorrelation was controlled by regressing the vegetation cover estimates on Easting coordinate and using the residuals of this regression in the analyses. Moran's I values showed that residuals of this regression did not have any significant residual spatial autocorrelation. Simple (YEAR) and quadratic terms (YEAR²) were entered as continuous covariates in the GLM to account for non-linear effects of time. Plot identity was modeled as a random factor to account for the non-independence of repeated estimates done in the same plot on multiple years.

To better describe changes from one year to the following, per cent variation of each vegetation type in each plot between pairs of subsequent years was estimated, and Spearman's rank order correlations were calculated between all pairs of vegetation types. This analysis highlights spatial relationships between vegetation types, i.e., which vegetation type expands when another decreases due to fire or succession.

To show how vegetation changed in the four sectors of the study area over the entire period, two-way repeated measures ANOVA (sector X year) was used to compare per cent cover (arcsin square-root transformed) of the four vegetation types in the 49 plots from 1999 to 2006. When two-way ANOVA was significant, each sector and vegetation type was analyzed separately with one-way repeated measures ANOVA to highlight significant variations more precisely.

One-way ANOVA was used to compare average species richness and range sizes per 1 m² quadrat across the four vegetation types and between burnt and unburnt quadrats. Preliminary analyses showed a spatial trend of species richness in the study area: quadrats in the westernmost sector are more species-rich than those in the eastern sectors. Slopes of regression lines did not differ among the four vegetation types (ANCOVA, interaction species richness X coordinate, $F_{7,247} = 0.2, P = 0.86$). Thus,

$\log(\text{Easting coordinate})$ was entered as a covariate in the analyses to control this spatial trend. Untransformed variables conformed to normality and homogeneity of variances.

Throughout the paper, data are presented as mean \pm SE and the threshold for statistical significance was set at $P \leq 0.05$. Statistical calculations were done with Statistica 6.1 (©Statsoft 2003). Taxonomic nomenclature follows Pignatti (1982).

Results

Patterns of fire recurrence

During the period considered, 25 fires occurred in the study area. The average extent of a fire was 47 ± 12 ha. The four largest ones (>100 ha each) made up 57% of the total area burnt between 1999 and 2006. Fires whose date were known occurred between 15 December and 16 April ($N = 7$). Fires usually started on days with strong eastward wind, and burned the heathland but stopped at the edges of dense *Betula* or *Quercus* woodland.

During the eight-year period, 53% of the study area burned one or more times. However, fires were unequally distributed over the four sectors (Figs. 1 and 2). More than 80% of sector 4 burned one to six times, while 71% of sector 1 never burned.

Effects of fire on vegetation development

All vegetation types were significantly affected by fire. Heath cover increased after a fire (GLM, $F_{50,187} = 15.1$,

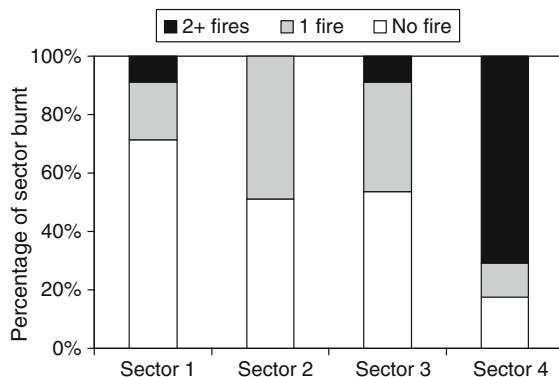


Fig. 2 Percentage of area burnt in the four sectors (totals of 1999–2006)

$P < 0.0001$). Both linear and quadratic trends were highly significant (YEAR, $F_{1,48} = 68.9$, $P < 0.0001$; YEAR 2 , $F_{1,48} = 68.9$, $P < 0.0001$), suggesting that the expansion of *Calluna* slows about 3–5 years after a fire (Fig. 3a). Grassland declined sharply and linearly after fires (GLM, $F_{50,187} = 21.8$, $P < 0.0001$; YEAR, $F_{1,48} = 46.9$, $P < 0.0001$; YEAR 2 , $F_{1,48} = 0.1$, $P = 0.85$; Fig. 3b). Low shrubland initially increased and then decreased, as shown by the significant effect of YEAR 2 (GLM, $F_{50,187} = 9.3$, $P < 0.0001$; YEAR, $F_{1,48} = 16.2$, $P < 0.0001$; YEAR 2 , $F_{1,48} = 26.3$, $P < 0.0001$; Fig. 3c). Tall shrubland increased over time (GLM, $F_{50,187} = 23.7$, $P < 0.0001$). Only quadratic terms in the model were highly significant (YEAR, $F_{1,48} = 2.5$, $P < 0.11$; YEAR 2 , $F_{1,48} = 58.6$, $P < 0.0001$), suggesting an accelerating spread of tall shrubland in late succession years (Fig. 3d).

Considering change in percent cover between pairs of consecutive years (Fig. 4), grassland had a large, negative correlation with heath and low shrubland, suggesting that the expansion of these two vegetation types occurs at the expense of grassland. Low shrubland was negatively correlated with tall shrubland. Heath had a marginally significant positive correlation with low shrubland, suggesting that these two vegetation types follow parallel trends of development after a fire, and that shrubland has little ability to encroach *Calluna*-dominated areas.

Between 1999 and 2006 there were marked changes in the study area, but these differed in amplitude and direction among the four sectors. Heath cover changed significantly, but the four sectors followed contrasting trajectories (Two-way repeated-measures ANOVA, Year, $F_{1,45} = 19.0$, $P < 0.0001$; Sector, $F_{3,45} = 13.0$, $P < 0.0001$; interaction $F_{3,45} = 16.8$, $P < 0.0001$). Heath increased in sectors 2 and 3, which burned in 1999, decreased in sector 1, which burned rarely (Fig. 2), and remained on low levels in sector 4, which burned frequently (Table 1).

Grassland cover decreased significantly in sectors 1, 2, and 3 between years (Table 1), although the magnitude of change differed (Year, $F_{1,45} = 99.5$, $P < 0.0001$; Sector, $F_{3,45} = 5.0$, $P = 0.004$; interaction $F_{3,45} = 10.5$, $P < 0.0001$).

At the scale of the entire study area, low shrubland cover did not change across years, but this concealed contrasting trends in the sectors (Year, $F_{1,45} = 0.3$, $P = 0.57$; Sector, $F_{3,45} = 18.7$, $P < 0.0001$; interaction $F_{3,45} = 4.0$, $P = 0.01$). Low shrub increased in

Fig. 3 Changes in per cent vegetation cover with increasing time after a fire.
a: heath; **b:** grassland; **c:** low shrubland; **d:** tall shrubland

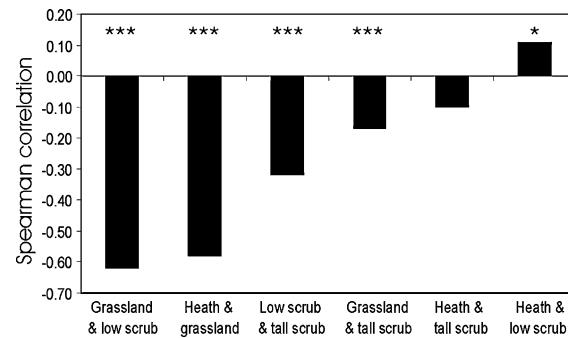
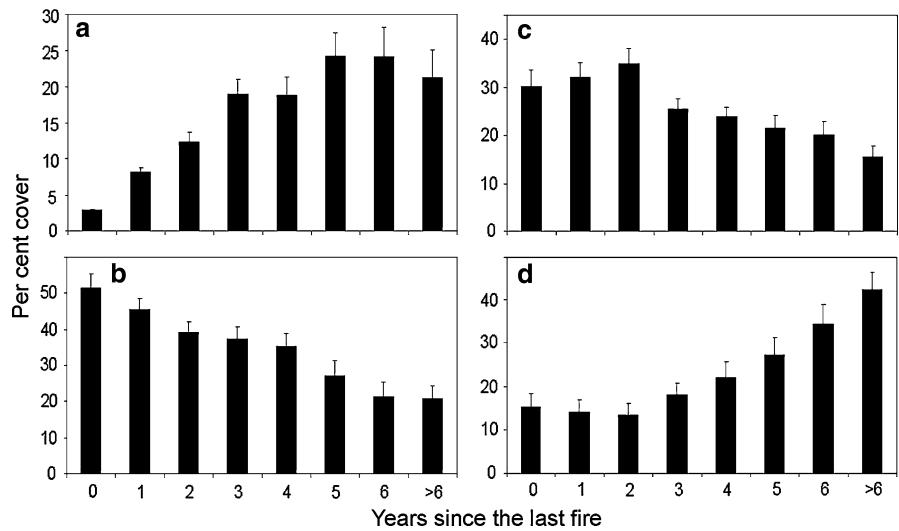


Fig. 4 Spearman rank correlation coefficients between differences of per cent cover of the four vegetation types in plots assessed on two consecutive years ($N = 249$ estimates). *** $P < 0.01$; * $P = 0.1$

sector 1, had a nearly significant negative trend in sector 3, and did not change in sectors 2 and 4 (Table 1).

Tall shrubland expanded significantly in the entire area (Year, $F_{1,45} = 36.8$, $P < 0.0001$; Sector, $F_{3,45} = 1.5$, $P = 0.23$; interaction $F_{3,45} = 1.0$, $P = 0.40$), although the increment was only marginally significant in sector 2 (Table 1).

Effects of fire and vegetation type on vascular plant richness

Burnt and unburnt quadrats did not differ either in species richness (ANOVA, $F_{1,207} = 0.1$, $P = 0.71$), or in the average range size of the plants ($F_{1,207} = 0.04$, $P = 0.83$).

Species richness differed between vegetation types (ANOVA, $F_{3,250} = 9.3$, $P < 0.0001$), which were ranked as low shrubland > grassland > tall shrubland > heath (13.1 ± 0.4 , 11.0 ± 0.3 , 9.8 ± 0.4 , 9.7 ± 0.4 species/quadrat, respectively). Multiple comparisons showed significant differences between low shrubland and the other vegetation types (Tukey test, all $P < 0.001$) and a marginally significant difference between grassland and tall shrubland (Tukey test, $P = 0.1$). Range size differed between vegetation types (ANOVA, $F_{3,250} = 10.7$, $P > 0.0001$): species observed in grassland (49.9 ± 0.2 provinces/species) and low shrubland (49.7 ± 0.3) had smaller ranges than those recorded in tall shrubland (51.6 ± 0.3) (Tukey test, all $P < 0.001$).

Discussion

This work is one of the first analyses of the effects of fire in an Italian lowland heathland. In this study, vegetation followed a predictable succession after a fire. Grassland increased in recently burnt areas while heath decreased, but, as time progressed, *Calluna*-dominated heath grew and reinvaded the grassland. Low shrubland initially expanded in areas occupied by grassland immediately after a fire (Fig. 4), but, after 3 years, it started to decrease, as woody plants grew above 1.5 m and entered the tall shrubland stage. This pattern has been found in other studies (e.g., Clement and Touffet 1981; Mallik and

Table 1 Per cent vegetation cover in the four sectors in 1999 and 2006.

Vegetation type	Sector	Year		F	d.f.	P
		1999	2006			
Heath	1	19.8 ± 5.3	14.8 ± 3.6	0.8	1,11	0.38
	2	5.1 ± 2.4	33.4 ± 4.7	34.1	1,7	>0.001
	3	8.4 ± 2.6	13.6 ± 3.4	4.7	1,12	0.05
	4	1.3 ± 0.2	1.5 ± 0.5	0.5	1,15	0.49
Grassland	1	59.6 ± 5.4	39.9 ± 5.3	7.2	1,11	0.02
	2	76.0 ± 3.9	20.4 ± 4.8	65.1	1,7	>0.001
	3	45.8 ± 5.4	16.8 ± 4.5	80.2	1,12	>0.001
	4	37.2 ± 3.0	30.6 ± 4.8	3.5	1,15	0.08
Low shrubland	1	8.2 ± 2.0	17.2 ± 4.2	5.1	1,11	0.05
	2	14.9 ± 3.4	15.3 ± 1.9	0.3	1,7	0.61
	3	31.9 ± 4.4	19.9 ± 3.4	4.5	1,12	0.06
	4	51.2 ± 4.4	44.4 ± 4.6	1.5	1,15	0.24
Tall shrubland	1	12.3 ± 4.4	28.1 ± 6.3	10.0	1,11	0.01
	2	3.9 ± 1.5	30.9 ± 3.8	5.3	1,7	0.06
	3	13.9 ± 3.9	49.7 ± 5.5	29.4	1,12	>0.001
	4	10.4 ± 5.4	23.6 ± 6.9	9.0	1,15	0.01

Table gives means ± SE and the results of one-way repeated-measures ANOVA

Gimingham 1983; Hobbs and Gimingham 1984; Forgeard 1990), but a major difference with more northern latitudes is that, in the Vauda, shrubland expands more rapidly after a fire. Bullock and Webb (1995) reported an increase of *Betula pendula* shrubland from about 8% to 15% 8 years after a severe fire in southern England, while in this study tall shrubland expanded to more than 40% cover in seven years (Fig. 3). In the UK, rates of shrubland encroachment similar to those found in Italy are usually observed only after 35–40 years (Marrs et al. 1986; Rose et al. 2000). Plant diversity was low in tall shrubland, and vascular plants observed in this vegetation were more widespread in the Alps than those found in grassland. This suggests that rapid expansion of woody species reduces both habitat and species diversity, and is probably the most important direct threat to the heathlands of Southern Europe.

Variation of *Calluna vulgaris* cover between consecutive years was strongly and inversely correlated with that of grassland, but much less so with tall and low shrubland, suggesting that the increase of heather after a fire occurs mainly at the expense of grassland. The leveling out of *Calluna* cover after the third year suggests a competitive equilibrium between shrubland and *Calluna*. Mature *Calluna* strongly suppresses other plants, as is confirmed by the low species richness of *Calluna*-dominated quadrats. In

northern Europe, *Calluna* suppresses the establishment of competitors for 20 or more years (Watt 1955; Gimingham 1972). As this study lasted only 8 years, it is not clear if that would be the case also in the Italian heathlands, or if the encroachment of *Calluna* stands by shrubland will occur in shorter times. In any case, this study showed that in the Italian heathlands the dominance of *Calluna* is less marked than in northern Europe, where this species often blankets large expanses of land. On the contrary, Italian heathlands are mosaics where grassland and shrubland occupy 70% or more of the area (Table 1). This makes Italian heathlands constitutionally more prone to encroachment by woody species such as *Betula pendula* or *Populus tremula*.

Between 1999 and 2006 there were marked changes in the amount and distribution of vegetation types. The most consistent change was the increase of tall shrubland in all sectors, in spite of strong differences in fire frequency. If the increase of shrubland in unburnt areas is easily explained, that observed in frequently burnt sectors might be due to two factors. First, fire stimulates seed germination and seedling establishment by providing patches of bare soil where competition is low. As a result, *Betula pendula* and other woody species often increase in burnt areas (Atkinson 1992; Bullock and Webb 1995). Second, due to reduced fuel abundance, fire intensity decreases in

frequently burnt areas, and low-intensity fires spare many woody plants, especially when they have developed a protective layer of bark around their foot (Mallik and Gimingham 1983). The rapid growth of woody plants observed in this study enhances their chances of reaching a stage where they are safe from low-intensity fires. Thus, in the Vauda, both the absence of fires and frequent, low-intensity fires appear to stimulate shrubland expansion.

How could Italian heathlands persist over time? Ancient maps show that the Vauda has been a heathland since the beginning of the 19th century, and, in all likelihood, heathland existed there since late Middle Age. This is remarkable, because Italian heathlands rest on a fragile equilibrium between fires and the rapid encroachment by shrubland and woodland. Most European heathlands are created and preserved by human action, but the rapid vegetation succession observed in this study suggests that Italian heathlands will require a particularly intense and frequent management. Frequent fires have probably been important to maintain Italian heathlands over the centuries, but this study also showed that shrubland expands even in frequently burnt areas. Indeed, other research in the area confirms that the short-term effects of fire in controlling woody plants are unclear (Ascoli et al. 2006). This suggests the importance of other factors, especially of herbivore grazing, to complement the action of fire in checking the growth of woody plants.

Conclusion: the conservation of lowland heathland in Italy

This study suggests that fire frequency is a critical variable in the management of Italian heathlands, since both too high and too low frequencies will stimulate shrubland encroachment. Burning once in 3–6 years appears to be the most appropriate frequency, as this will maximize the amount of heath, and at the same maintain species-rich grassland, while controlling the expansion of tall shrubland dominated by *Betula pendula*. This frequency is markedly shorter than the 15–20 years usually recommended in the British Isles (Price 2002). Thus, attempts should be taken to reduce fire frequency in the western part of the Vauda, while the opposite target should be pursued in the eastern sectors. At the

same time, this study suggests that burning alone might not be sufficient to stop shrubland expansion. Increased and more prolonged grazing might also be an essential management action.

Considering the large size of the Vauda, fire management will likely pose a huge logistic and financial challenge. Intervals of 3–6 years between fires will require burning a total of 150–300 ha in each year, which, considering the low amount of resources available for environmental management in Italy, is probably well beyond the current possibilities of the Reserve administrators. The likely outcome of this lack of resources will be a foreseeable large decrease, if not the total disappearance, of the last remnants of lowland heathland in Italy.

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