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Interacting effects of grazing and habitat conditions on seedling recruitment and establishment

Anna Kladivová & Zuzana Münzbergová

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Browsing; Calcareous grassland; Dry grassland; Ellenberg indicator values; Seedling germination; Seedling mortality; Seedling survival; Soil depth; Species composition

Nomenclature
Kubát et al. (2002)

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Kladivová, A. (Corresponding author, anna.kladivova@seznam.cz)[1],
Münzbergová, Z. (zuzmun@natur.cuni.cz)[1,2]

[1]Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-128 01 Prague 2, Czech Republic;
[2]Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ-252 43 Průhonice, Czech Republic

Abstract

Questions: What is the effect of grazing on seedling recruitment and seedling establishment? To what extent is grazing dependent on local habitat conditions?

Location: Český Kras (Czech Karst), central Bohemia, Czech Republic.

Methods: In 2005, grazing was reintroduced on formerly grazed dry grassland. Twelve blocks of grazed and control plots were established. Seedlings of dicotyledonous plants were observed three times a year for 4 yrs, for a total of ten censuses. We tested the effects of grazing on seedling recruitment (entry of new individuals to the seedling stage) as well as seedling establishment (survival of seedlings for 1 yr after initial emergence). In addition, we explored the effect of soil depth – the most important environmental variable at our study site – on seedling response to grazing. The effects of grazing, soil depth and season on seedling number in plots were tested with generalized mixed effect models with a Poisson distribution. The effect of grazing on species composition was tested with canonical correspondence analysis.

Results: Grazing had a significant positive effect on seedling recruitment. Grazing affected the number of seedlings and species composition and facilitated the presence of dry grassland species. Seedlings of xerophilous species were primarily found in grazed plots, while control plots contained species preferring higher, closed sward. Grazing explained only 1.2% of the total variation in composition of recruited seedlings. We presume that the main factor determining species composition could be dispersal limitation. Grazing increased the number of recruited seedlings by 10.55%. The final number of established seedlings was unaffected by grazing. Furthermore, the interaction of grazing with soil depth had a significant positive effect on seedling recruitment, thus showing that the effect of grazing strongly depends on habitat conditions at the specific microsite within our location.

Conclusions: Our results indicate that predicting higher generative reproduction on pastures based on a larger number of seedlings may be misleading if the species are limited by establishment – but not recruitment – as at our study site. Furthermore, our findings show that varying habitat conditions on small spatial scales can modify the impact of management on seedling recruitment and establishment.

Introduction

The seedling stage is probably the most vulnerable part of the plant life cycle (Stebbins 1971; Fenner & Thompson 2005). Seedlings require specific conditions for successful establishment and subsequent growth (Grubb 1977; Panetta 2001), and are more sensitive to environmental conditions and competition than adult plants (Turnbull et al. 2000). As a result, the mortality of seedlings is exceptionally high, sometimes even reaching 100% (e.g., Silvertown & Dickie 1980; Bucharová et al. 2012; Dostálek & Münzbergová 2012; Heinken-Šmídová & Münzbergová 2012). The high mortality of seedlings is also related to the fact that site conditions that are suitable for recruitment

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may be unsuitable for seedling establishment (Schupp 1995; Fenner & Thompson 2005). For example, gaps in the sward may promote recruitment in spring when there is enough moisture (Goldberg 1987); however, during the summer, when the open gaps often dry up faster than the surrounding vegetation, seedlings may die due to drought (Martinkova et al. 2009; Knappová et al. 2013). Thus, monitoring of single seedlings for at least a year is necessary to determine their fate (Fenner 1978; Stampfli & Zeiter 1999).

When studying seedlings in grasslands, ecologists often seek to understand the effect of various management regimes on their recruitment (Morgan 2001; Overbeck et al. 2003; Hofmann & Isselstein 2004; Martin & Wilsey 2006; Viard-Crétat et al. 2010). The effect of grazing is one of the most studied factors of grassland ecology (e.g., Watt & Gibson 1988; Crawley 1990; Bullock et al. 1994, 1995; Edwards & Crawley 1999; Wilsey & Polley 2003). The effect of grazing on plant species composition can be exceptionally diverse. Aside from simple reduction of above-ground biomass and subsequent flowering and seed production of grazed species, grazing also affects recruitment and establishment of seedlings. For several plants, gaps in the vegetation are necessary for seedling recruitment (Kotorová & Lepš 1999; Bullock 2000). Grazing animals create gaps in sward by trampling and biomass removal (Nystuen et al. 2014) and by excrement deposition, which leads to an increase in the nutrient content of the stands (Malo & Suarez 1995). Grazing thus increases the number of suitable microsites for seed germination and subsequent seedling recruitment (e.g., Rusch 1988; Watt & Gibson 1988; Silvertown et al. 1992; Bullock et al. 1994, 1995). In addition, grazing animals can also affect vegetation by transferring seeds over both short and long distances (Fisher et al. 1996). Due to the character of our chosen study site (dry grassland on shallow soil over limestone with a history of grazing), grazing was chosen to facilitate gap opening and alter site conditions.

Grazing, a traditional management approach on European grasslands, is currently being reintroduced as a management tool for maintaining species diversity in several species-rich grasslands (Karberg & Beattie 2009). In spite of the high number of studies dealing with seedling recruitment in grasslands (e.g., Crawley 1990; Edwards & Crawley 1996; Morgan 2001; Špačková & Lepš 2004; Viard-Crétat et al. 2010) and especially in pastures (e.g., Bullock et al. 1995; Eriksson & Eriksson 1997), most of these studies have dealt with data from single events; thus, the authors can only report whether the seedling was recruited but not if the seedling survived and established. This fact is unfortunate because the mortality of seedlings is exceptionally high. While ideal information on seedling establishment would be obtained by following the seedling until its generative reproduction, this method was not used in our study. The highest mortality rate of seedlings occurs during the first year after recruitment. Seedlings surviving after the first growing season appeared to have a much better chance of continued survival (Eis 1967; Enright & Lamont 1992; Gilbert et al. 2001). The chances of plant survival also increase with seedling size (van den Driessche 1992; Villar-Salvador et al. 2012). We presume that the survival probability of a seedling over the first year of its life is a good proxy of survival probability until adulthood. Previous studies exploring seedling recruitment and establishment have also demonstrated strong between-year variation in seedling success (e.g., Morgan 2001; Dzwonko & Gawroński 2002; Knappová et al. 2013). Koyama & Tsuyuzaki (2013) demonstrated that the primary factor limiting seedling establishment (drought) might vary between average and extreme years. To obtain reliable information on the effect of a given treatment, data from multiple growing seasons should thus be collected. Previous studies, however, only provide data from one or a maximum of two growing seasons (Kitajima & Tilman 1996 one season; Niederfriniger Schlag & Erschbamer 2000 two seasons; Setterfield 2002 one season; Kleijn 2003 one season; Milbau et al. 2013 two seasons). In this study, we used detailed data on recruitment and establishment of nine different cohorts of seedlings collected over three growing seasons.

Moreover, seedling recruitment has been shown to depend on habitat conditions such as soil moisture (Dodd & Power 2007) or light conditions (Silvertown 1981), and the effect of various treatments on seedling recruitment and establishment may depend on the habitat conditions of the site (Loydi et al. 2013). For example, in the study of Špačková & Lepš (2004), moss and litter removal led to increased seedling emergence under ample moisture conditions of wet grassland. Conversely, on dry grasslands, the presence of mosses can have a positive effect on seedling recruitment (Ryser 1993; Overbeck et al. 2003). Several previous studies have also demonstrated that the effects of grazing can differ between localities with different habitat conditions. For example, grazing on grasslands with low productivity has been shown to have negative effects on species diversity, while herbivores have been shown to have positive effects at high productivity levels when they can limit the intensity of light competition between established species (Olff & Ritchie 1998; Bakker et al. 2006). We are, however, not aware of any study that has examined the effect of habitat conditions on the effects of grazing on a small scale within a single study site. If the habitat conditions vary within a single site, species in different blocks scattered over such a site may be expected to exhibit different responses to grazing.

The aim of this study was to test the effects of grazing on seedling recruitment (entry of new individuals that had germinated and successfully emerged above ground into the seedling stage) and seedling establishment (survival of seedlings for 1 yr after initial emergence). In addition, we explored the effect of soil depth, which is the most important environmental variable at our study site, on seedling response to grazing. Specifically, we aimed to answer the following questions: (1) what is the effect of grazing on seedling recruitment and seedling establishment; and (2) to what extent is the effect of grazing dependent on local habitat conditions?

Methods

Study site

The study was conducted at Pání hora in the Protected Landscape Area Český Kras (Czech Karst), central Bohemia, Czech Republic. The locality is part of the National Nature Reserve Karlštejn. The Czech Karst is the largest calcareous area in the Czech Republic hosting large preserved areas of rock steppes, forest steppes and deciduous forests rich in natural flora and fauna. The mean annual precipitation in the area is 530 mm, and the mean annual temperature is approximately 8–9 °C. Precipitation reaches a maximum in July. In winter, precipitation is minimal and snow cover is low and transient. The experimental site lies in the vicinity of the small town of Bubovice. The site is situated at an altitude of 375 m a.s.l., 49°57′ 43″ N, 14°9′ 53″ E.

The west slope and the top part of Pání hora hill are covered by vegetation with a mosaic of steppe and shrubs. Pání hora was left to spontaneous development in the 1950s, when grazing management was abandoned at the site (Mayerová et al. 2010). Grazing experiments were conducted in 2005 as a means of preservation of xerothermic grassland vegetation with important species such as *Pulsatilla pratensis* (L.) Mill., *Helianthenum grandiflorum* (Scop.) DC., *Veronica prostrata* L., *Teucrium chamaedrys* L. and *Anacamptis pyramidalis* (L.) Rich. The main threats to this vegetation are invasions of shrubs (mainly *Prunus spinosa* L. and *Crataegus* sp.), trees and mesophilous species of grass (*Arrhenatherum elatius* (L.) J. Presl et C. Presl and *Calamagrostis epigejos* (L.) Roth.; Kubát et al. 2002) that outcompete herbaceous plants.

Experimental set-up

The grazing experiment was run from 2005 to 2008. A herd of approximately 30 goats and 60–100 sheep grazed the experimental area. Grazing was restricted by a fence. The fence was moved all over the study site to ensure even grazing on the entire site. The locality was grazed from May to June. In 2008, grazing was also conducted during the autumn to eliminate larger amounts of *A. elatius*. On average, 46% of the above-ground biomass was removed by the grazers each year (Mayerová 2009).

Twelve blocks were established in spring 2005 at the locality. Each block was composed of two square plots (33 × 33 cm) – one grazed plot and one control plot that was not grazed. There were 24 plots in total. Each control plot was covered with a metal cage during the entire experimental period to protect it from grazing (1.2 × 1.2 × 1 m size).

To keep detailed records of seedlings, each plot was divided into a grid of 25 smaller squares with a square size of 6.6 × 6.6 cm. Seedlings in squares were counted, marked with coloured pins and recorded in the chart of the plot with a square grid, thereby creating 'a map of seedlings'. This chart was used for every census so that during subsequent censuses, we were able to identify each individual seedling. Coloured pins were easily found, and such marked seedlings were easily recognized. Only a small number of pins disappeared from the plots. These pins were arranged in a precise line beside the plots, which suggests that there was human interference. Plots were sampled three times a year – in April, July and October–November, starting in the autumn of 2005 and ending in the autumn of 2008 (i.e., ten censuses in total). Only seedlings of dicot plants were recorded due to the difficulties in identifying monocot seedlings to species and distinguishing them from vegetative clones. While we also recorded seedlings of annual species, we did not include annuals in any of our analyses, as annuals can never survive over four censuses and therefore cannot establish according to our definition (see below). Seedlings were determined using the key of Csapody (1968).

In our study, we distinguish between seedling recruitment – entry of a new individual that had germinated and successfully emerged above ground to the seedling stage (Ribbens et al. 1994) – and seedling establishment – survival of the seedling for 1 yr after initial emergence (Bullock 2000) so that the seedling can be considered to be a permanent part of the vegetation. For seedling recruitment, recruited seedlings were identified as those that still possessed their primary leaves or were smaller than 4 cm (depending on species and cover of surrounding vegetation) and were not recorded in a previous census. Individual seedlings were then monitored for four subsequent censuses (1 yr). Seedlings that survived longer than 1 yr after recruitment (e.g., seedling that was recruited in spring 2006 and was still alive in summer 2007) were classified as successfully established survivors (Bullock 2000; Laborde & Thompson 2013).

To characterize the habitat conditions of each experimental plot, we measured soil depth in all plots, all the

Doi: 10.1111/jvs.12395

way down to the bedrock. Soil depth was measured in four corners of each plot, and mean soil depth was calculated for each plot. We used metal sticks that were driven into the soil down to the bedrock. We also dug small holes near each plot as a control for the accuracy of our measurements. Because the maximum soil depth at the study site was 21.5 cm, our method of measurement was sufficient. The soil at the locality is generally fairly shallow, and soil depth was thus expected to be a robust proxy of moisture and temperature of the plots. To demonstrate the effect of soil depth on habitat characteristics, we calculated Ellenberg indicator values of light, temperature, moisture, soil reaction and nutrients for each of the plots based on the species composition in each of the plots. The Ellenberg indicator values were previously used in a wide range of studies and were shown to robustly predict habitat conditions in a wide range of habitats (e.g., Diekmann & Dupré 1997; Dostálek & Münzbergová 2010; Dupré et al. 2010, Münzbergová & Skuhrovec 2013). For the purpose of Ellenberg indicator calculations, we recorded a phytosociological relevé for each plot using the Braun-Blanquet cover–abundance scale in 2005 before the start of the experiment. Plant species names were unified according to Kubát et al. (2002). Each species in the phytosociological relevé was assigned an Ellenberg indicator value that expressed its requirements for five environmental variables (light, temperature, moisture, soil reaction and nutrients; Ellenberg et al. 1992) and the mean of these values weighted by abundance was calculated. Ellenberg indicator values of light, temperature, moisture, soil reaction and nutrients calculated for individual plots were correlated with soil depth as inferred from Pearson's correlation coefficient calculated in S-plus 2000 Professional (MathSoft Inc., Cambridge, MA, US).

Data analysis

Number of seedlings

To assess the effect of grazing, soil depth and season and their interaction on the number of seedlings in plots, we used generalized mixed effect models as implemented in the LME4 package in R (R Foundation for Statistical Computing, Vienna, AT) assuming a Poisson distribution of the dependent variable. The independent variables tested as fixed effects were grazing (grazed/control), soil depth, season (spring, summer, autumn) and their interactions. Census was used as a random effect in all of the models. In addition, we used block as a random effect in all predictors recorded at the plot level (i.e., grazing, soil depth and their interaction) and plot as a random effect in the predictors recorded at the level of each recording (i.e., season and its interactions).

Two analyses were performed. In the first analysis, the dependent variable was seedling recruitment, i.e., number of recruited seedlings in the given census. The dependent variable of the second analysis was the number of seedlings in the given period that successfully established, i.e., all seedlings marked as survivors in the given census. This second analysis included only data from censuses four to ten. The effects on the number of established seedlings were tested using two approaches, with and without the number of seedlings in a previous period as a covariate (Appendix S1). Because the two tests gave very similar results for all of the main tested factors and their interactions, we report only the test without the covariate in the subsequent text. All of the univariate tests were conducted using R (v 3.0.2, R Foundation for Statistical Computing, Vienna, Austria).

Species composition

To test the effect of grazing and time (census) on species composition, canonical correspondence analysis was performed using Canoco for Windows (v 4.5; Biometris, Wageningen, NL). The dependent variables were the number of recruited seedlings of each species. Only species with a minimum of ten recruited seedlings were included in these analyses. The effect of grazing and the interactions between grazing and census, and grazing and soil depth were tested. To take into account the initial differences between blocks, the effect of soil depth was used as a covariate in all tests. In testing the interactions between grazing and census, the main effects of grazing and census were also used as covariates. Grazing was also used as a covariate in testing the interaction of grazing and soil depth. A Monte Carlo test (499 permutations) was used to test the effects.

Only seedling recruitment was tested. The species composition of established seedlings was not tested because out of all of the 36 established species, only three species had at least ten established seedlings.

Results

Soil depth was significantly negatively correlated with the Ellenberg indicator value of temperature ($R = -0.632$, $P = 0.035$) and of light ($R = -0.618$, $P = 0.029$). Indicator values of moisture, soil reaction and nutrients were independent of soil depth ($P > 0.1$ in all cases).

Recruitment

Seedling recruitment was significantly affected by soil depth, grazing and most of their interactions (Table 1). There were 2627 seedlings recorded in total. Out of these,

Table 1. Effects of grazing, soil depth and season and their interaction on numbers of seedlings in plots, tested using a generalized mixed effect model with a Poisson distribution. Dependent variables were seedling recruitment and establishment. Independent variables were soil depth, season (spring, summer, autumn), grazing (grazed/control) and all of their interactions.

Dependent Variable	Seedling Recruitment			Seedling Establishment		
Independent Variable	*df*	*P*	Chi	*df*	*P*	Chi
Soil Depth	1	**<0.001**	59.162	1	0.049	3.86
Season	2	0.203	1.624	2	0.086	4.27
Grazing	1	**<0.001**	93.268	1	0.052	3.789
Soil Depth × Season	2	**<0.001**	12.487	2	0.998	0.001
Soil Depth × Grazing	1	**<0.001**	22.764	11	0.974	0.001
Grazing × Season	2	**0.005**	7.819	2	0.397	1.848
Grazing × Soil Depth × Season	9	0.145	2.121	5	0.198	3.237

Variables with statistically significant effects are emphasized by bold numbers.

373 seedlings belonged to annual species and were omitted from the analyses. Of the remaining 2254 seedlings of perennial species, there were more recruited seedlings in grazed plots (1246 seedlings, 55.28%) than in control plots (1008 seedlings, 44.72%). Grazing had different effects at different soil depths but not during different seasons (Table 1).

Seedling recruitment was also significantly affected by the interaction of grazing and soil depth (Table 1). The numbers of recruited seedlings increased with increasing soil depth in grazed plots and decreased with increasing soil depth in control plots (Fig. 1).

The composition of recruited seedlings was significantly affected by grazing ($P = 0.002$, $F = 2.44$). Grazing explained 1.2% of the total variation in composition of recruited seedlings. Recruited seedlings of xerophilous species of steppes and pastures were primarily observed in grazed plots. These species included *Dianthus carthusianorum* L., *P. pratensis*, *H. grandiflorum*, *Verbascum lychnitis* L., *T. chamaedrys* and *Pseudolysimachion spicatum* (L.) Opiz. These species at the study site preferred spots with short, open sward. In contrast, grazing had a negative effect on the recruitment of species that grew mostly on spots with higher, closed sward. These species included *Securigera varia* (L.) Lassen, *Fragaria viridis* (Duchesne) Weston, *Hypericum perforatum* L. and *Daucus carota* L. (Fig. 2). The interaction of census and grazing did not have any significant effect on the species composition of seedlings ($P = 0.322$, $F = 1.01$). Additionally, the interaction of soil depth and grazing did not have any significant effect on the species composition of seedlings ($P = 0.127$, $F = 1.82$).

Establishment

Seedling establishment was not significantly affected by any of the independent variables (Table 1). The total number of established seedlings (seedlings that survived more than three censuses after recruitment) was 146 (out of 2373 seedlings recruited in the corresponding censuses). In contrast to seedling recruitment, grazing did not have any significant effect on seedling establishment (Table 1).

Seedling establishment was not significantly affected by interactions of grazing with soil depth (Table 1). The species composition of established seedlings was not analysed due to the exceedingly small number of established seedlings. Among the 36 species with established seedlings, only three species passed the criterion of ten established seedlings: *Alyssum montanum* L. 16 seedlings, *Galium album* Mill. 14 seedlings and *Medicago lupulina* L. 13 seedlings. Among woody species (*Carpinus betulus* L., *Crataegus* sp., *Sorbus torminalis* (L.) Crantz, *Acer campestre* L. and *Cornus mas* L.), only *C. betulus* exceeded the criterion of ten recruited seedlings, with 38 seedlings in grazed and 32 seedlings in control plots. No seedlings of woody species had established.

Discussion

Seedling number

In our study, grazing had a positive effect on seedling recruitment; more seedlings recruited on grazed plots than on control plots. This finding is consistent with previous studies that reported grazing creates open gaps in sward that are suitable for seedling recruitment (Rusch 1988; Watt & Gibson 1988; Silvertown et al. 1992; Bullock et al. 1994, 1995). This effect probably stems from the increase in light availability (Silvertown 1980, 1981) and reduction in competition with the surrounding vegetation (Goldberg & Werner 1983). From the observed rate of seedling mortality, grazing was inferred to increase seedling recruitment but not seedling establishment. While significantly more seedlings recruited on grazed plots, these seedlings also died after being recruited. As a result, grazed plots had a higher level of seedling turnover than control plots. This high turnover means that research based only on data on seedling recruitment from a site would lead to false conclusions. Only a combination of recruitment and establishment provided us with a complete picture. This recommendation should be considered, especially in the

Journal of Vegetation Science
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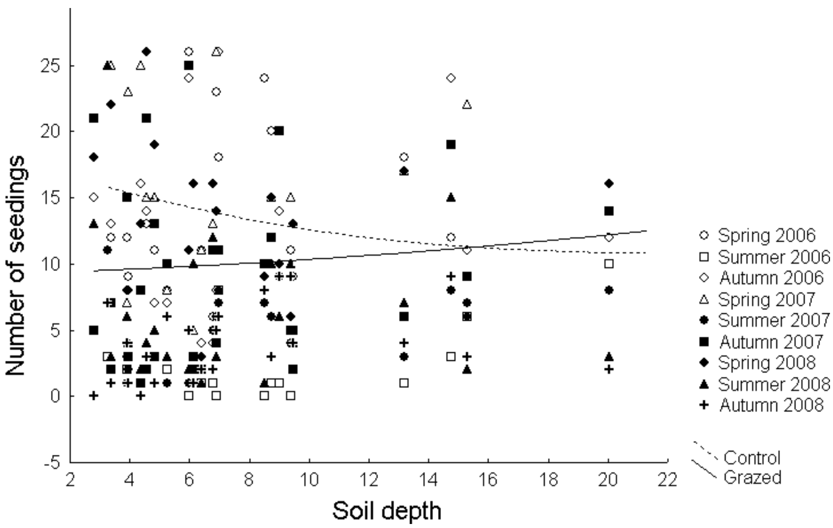

Fig. 1. Effect of the interaction of soil depth with grazing on the numbers of seedlings recruited in plots. Seedling numbers in different censuses are distinguished as points. Trends for grazed and control plots are shown according to GLM with a Poisson distribution. $P < 0.001$.

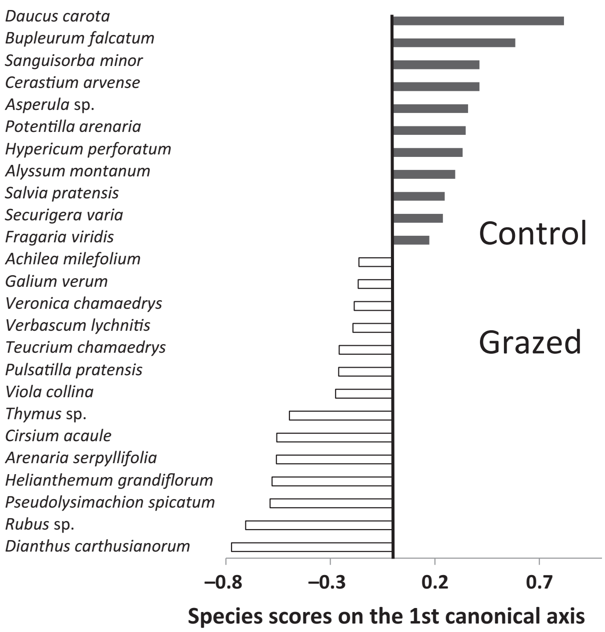

Fig. 2. The effect of grazing on the species composition of seedling recruitment. The graph includes 25 species with the strongest response to grazing, where positive values denote species prevailing in control plots (black columns) and negative values denote species prevailing in grazed plots (white columns). Grazing explains 1.2% of the overall variation in the species composition of seedlings.

context of the conservation of particular plant species. While high turnover of seedlings on a small scale has already been documented in grasslands in a number of studies (e.g., Rusch & van der Maarel 1992; Herben et al. 1993; Otsus & Zobel 2002), we are not aware of any study that has compared the recruitment and establishment of seedlings between plots with different treatments on grassland. In the study of Laskurain et al. (2013), small-seeded species of trees, shrubs and vines were promoted through sheep grazing in birch–beech secondary forest, but no significant effect on establishment was observed. In contrast, sheep did not affect the recruitment of large-seeded species but reduced their establishment.

Seedling survival and establishment is considered to be an important factor that can determine the composition of the vegetation (Grubb 1977) and its response to grazing and other disturbances (Willems 1983; Watt & Gibson 1988). In previous studies, grazing has been reported to promote seedling establishment in grasslands (Rusch 1988; Oesterheld & Sala 1990; Bullock et al. 1994). The absence of such a significant effect in our study is likely attributed to the high mortality of seedlings on the site (93.85%). This number is consistent with the results of previous studies that have reported high values of seedling mortality on dry grasslands, sometimes even up to 100% (Silvertown & Dickie 1980; Ryser 1993; Stampfli & Zeiter 1999; Bucharová et al. 2012; Dostálek & Münzbergová 2012; Heinken-Šmídová & Münzbergová 2012).

An important factor affecting seedling survival on dry grasslands is drought, particularly during the summer (Ryser 1993). Although we do not have any direct data on water availability at our locality, the direct southern orientation of the location with a mean soil depth of 7.67 cm and a slope of 14.04° suggests that drought, together with high temperatures, may be an important factor underlying the high mortality of seedlings.

Species composition

In our study, grazed plots held more seedlings of dicot species than control plots. Seedlings of xeric species from steppes and pastures such as *D. carthusianorum, P. pratensis, H. grandiflorum, V. lychnitis, T. chamaedrys* and *P. spicatum* recruited primarily on grazed plots. In contrast to grazed plots, more seedlings belonging to mesophilous meadow species such as *S. varia, F. viridis, H. perforatum* and *D. carota* occurred in the control plots. The species composition of the established seedlings could not be studied due to the small number of established seedlings (only three species established at least ten seedlings). We are not aware of any study comparing the species composition of seedlings on plots with and without grazing. Bullock et al. (1994) studied the effect of artificial gaps created in grazed enclosures on grassland. The species composition of seedling recruitment in these gaps was positively correlated with the species composition of the vegetation in enclosures. Unfortunately, Bullock et al. (1994) only recorded seedlings of dicot species inside grazed enclosures, but no data on recruitment outside of enclosures (in plots without grazing) were collected.

Only 1.2% of the total variation in species composition of recruited seedlings was explained by grazing. We believe that the primary factor determining species distributions in our location is dispersal limitation (Münzbergová & Herben 2005). The species composition of seedlings thus reflects species mosaics of adult plants in the vicinity of our study plots. In calcareous grassland, Rusch & Fernández-Palacios (1995) found a strong association between seedlings and conspecific adults for certain species. Verkaar et al. (1983) observed seed dispersal distances of only 0.3–3.5 m in chalk grassland. Stampfli & Zeiter (1999) showed that there was dispersal limitation in grasslands, concluding that several grassland species are unable to disperse to unoccupied sites that otherwise would be suitable for new populations. Although the proportion of variance explained by grazing in our study is low, this proportion could increase as the duration of grazing management increases. Our study followed grazing management for three growing seasons. Further research on the long-term effects of grazing would be beneficial.

Our results show that grazing can shift the composition of recruiting seedlings from mesophilous species to xeric species. Relatively few grassland species have been shown to form persistent seed banks (Davies & Waite 1998; Handlová & Münzbergová 2006). When a site is disturbed, the seed bank may be instrumental in changing the vegetation (Williams 1984). Grazing can also affect seed bank density (both positively and negatively; Aboling et al. 2008) and support the recruitment of dicotyledonous plants (Bullock et al. 1994). Another possibility is that higher seedling turnover of xeric species (recruitment without subsequent establishment and seed production) may further limit their input into the seed bank and deplete their seed reserves in the soil. In such cases, grazing would have a negative effect on the composition of the soil seed bank. Further research is needed to confirm or reject this expectation.

Effect of habitat conditions

Soil depth clearly modified the effect of grazing on seedling recruitment. Seedling recruitment decreased with increasing soil depth on control plots, while the trend on grazed plots was the opposite. Soil depth was also negatively correlated with Ellenberg indicator values of light and temperature. This pattern means that on control plots with closed vegetation, seedlings prefer stands with shallow soil that has abundant light and higher temperature. This phenomenon is already well known: calcareous grassland seedlings prefer more open stands with abundant light for recruitment (Silvertown 1980, 1981; Goldberg 1987). In contrast, on grazed plots where part of the vegetation cover was removed by grazing animals, formerly suitable stands with shallow soil and high levels of light and temperature were almost completely bare of vegetation and consequently were the least suitable for the seedlings. More seedlings grew on formerly less suitable stands with at least some shading and shelter provided by neighbouring plants. This effect has been described in a number of habitats (Valiente-Banuet et al. 1991; Fibich et al. 2013). Ryser (1993) described this pattern for the establishment of seedlings on limestone grassland, using some of the same plant species included in our study.

Overall, grazing on our study site facilitated seedling recruitment in plots with increased soil depth and therefore lower light and temperature values. Grazing's impact on stands with opposite conditions was negative. These results suggest that the effect of grazing differs for various habitat conditions at more than just large scales (e.g., wet meadow vs dry meadow; Bakker et al. 2006; Gibson 2009). Even at small scales within a single location with short abiotic gradients, the effect of grazing was different in microsites with different conditions. This finding has implications for conservation, particularly when grazing is used to conserve localities with a distinct pattern of different sward stands.

Conclusions

In our study, grazing had a positive effect on seedling recruitment. Grazing increased the numbers of seedlings and the composition of species by supporting species typical of dry grasslands. The final number of established seedlings was, however, unaffected by grazing. Seedlings in grazed plots had a higher turnover than seedlings in control plots. This difference in turnover clearly indicates that studies concluding that grazing enhances generative reproduction based on the results of only seedling recruitment may be misleading, especially if the species are limited by establishment, as was the case at our study site. In addition, our findings show that even within a single site, the effect of grazing strongly depends on the habitat conditions at the specific microsite.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Table of Effects of grazing, soil depth and season and their interaction on the numbers of seedlings in plots, tested by a Generalized mixed effect model with a Poisson distribution. Dependent variable was seedling establishment. The number of seedlings in a previous period was used as a covariate. Independent variables were soil depth, season (spring, summer, autumn), grazing (grazed/control) and all of their interactions.