

Drought and grazing combined: Contrasting shifts in plant interactions at species pair and community level



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ABSTRACT

The combined effects of drought stress and grazing pressure on shaping plant–plant interactions are still poorly understood, while this combination is common in arid ecosystems. In this study we assessed the relative effect of grazing pressure and slope aspect (drought stress) on vegetation cover and soil functioning in semi-arid Mediterranean grassland–shrublands in southeastern Spain. Moreover, we linked these two stress factors to plant co-occurrence patterns at species-pair and community levels, by performing C-score analyses. Vegetation cover and soil functioning decreased with higher grazing pressure and more south-facing (drier) slopes. At the community level, plants at south-facing slopes were negatively associated at no grazing but positively associated at low grazing pressure and randomly associated at high grazing pressure. At north-facing slopes, grazing did not result in a shift in the direction of the association. In contrast, analysis of pairwise species co-occurrence patterns showed that the dominant species *Stipa tenacissima* and *Anthyllis cytisoides* shifted from excluding each other to co-occurring with increasing grazing pressure at north-facing slopes. Our findings highlight that for improved understanding of plant interactions along stress gradients, interactions between species pairs and interactions at the community level should be assessed, as these may reveal contrasting results.

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1. Introduction

How plant interactions change along environmental gradients has been a central theme in plant ecology over the last few decades. First conceptual models focused on competition, hypothesising it would increase with increasing productivity (Grime, 1979), or that it would be invariant along a productivity gradient (Tilman, 1988). Over the last two decades an increasing number of studies have focused on facilitation, i.e. net positive interactions between plants (for review see: Callaway, 2007; He et al., 2013; Pugnaire et al., 2011). Facilitator plant species (i.e. nurse species) can relieve abiotic stress for other plants, for example by protecting them

against extreme temperatures and high irradiance or by increasing water or nutrient availability (Callaway, 2007). Next to that, facilitators can lower consumer pressure (i.e. grazing) by protecting other species against herbivores, a process known as associational resistance (sensu Hay, 1986).

A popular conceptual model called the Stress Gradient Hypothesis (SGH) predicts that the frequency of positive interactions will increase with increasing abiotic stress or grazing pressure (Bertness and Callaway, 1994; Callaway, 1995; Callaway and Walker, 1997). Many studies have attempted to test the SGH by experimentally investigating pairs of plant species and calculating the facilitation intensity (i.e. the performance of a plant with a protecting neighbour compared to a plant without) at several stress levels (Goldberg et al., 1999). However, studies from semi-arid ecosystems (e.g. Maestre and Cortina, 2004; Maestre et al., 2005) showed contrasting patterns to the original SGH: plant interactions shifted from competition to facilitation and back to competition along a gradient of high to low rainfall. In parallel, other studies in

List of abbreviations: C-score, Checkerboard score; LFA, Landscape Function Analysis; SES, Standardized Effect Size; SGH, Stress Gradient Hypothesis.

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drylands found temporal shifts from facilitation to competition during periods with very low rainfall (Armas and Pugnaire, 2005; Tielbörger and Kadmon, 2000). Also contradicting the predictions from the original SGH, studies in grazed ecosystems have reported an increase in facilitation intensity from low to high grazing pressure but a decrease again with further increases in grazing pressure (Smit et al., 2009). Such collapses in facilitation intensity at high grazing pressure have been observed in both terrestrial (Brooker et al., 2006; Graff et al., 2007; Saiz and Alados, 2012; Smit et al., 2007) and aquatic ecosystems (Bulleri et al., 2011; Le Bagousse-Pinguet et al., 2012; Levenbach, 2009). Plants can protect neighbours by physically sheltering them from herbivore damage, but when grazing pressure becomes very high, facilitative interactions might wane, because the nurse itself gets damaged by grazing or trampling (Michalet et al., 2014), or because consumers might start searching more intensively for resources (Soliveres et al., 2011a). Hence, from the examples above, it is clear that both abiotic conditions (resource and non-resource based, Maestre et al., 2009) and grazing pressure alter plant–plant interactions, but it remains unclear how multiple stressors combined, e.g. drought and grazing, shape plant interactions along combined gradients. Comparing the relative effects of both drought stress and grazing pressure on interaction intensity is important, because both may ultimately result in a decline in facilitative interactions, which may cause rapid degradation of arid ecosystems (Verwijmeren et al., 2013). One of the few available studies to date that tested combined effects of drought stress and herbivory is Soliveres et al. (2011a). This study showed that rabbit herbivory altered plant–plant interactions throughout the year, with positive interactions between *Stipa tenacissima* and *Retama sphaerocarpa* during winter and autumn, but with neutral interactions during summer. Other studies also showed that effects of grazing pressure on plant interactions can depend on water availability (Soliveres et al., 2012; Veblen, 2008), but the interactive effects of drought and grazing on plant interactions is yet surprisingly understudied and therefore unclear. On the one hand, grazing may have a bigger effect on plant interactions under high drought stress, as plants will have lower ability to compensate for herbivory than in lower stressed environments (Gómez-Aparicio et al., 2008). On the other hand, herbivores may play a smaller role in higher drought stressed environments as herbivores density will be less abundant and plants are less palatable due to lower productivity (Smit et al., 2009; Verwijmeren et al., 2013).

Furthermore, the effect of stress on plant interactions is thus far mostly studied by observing one or multiple pairs of species (Soliveres et al., in press). Studies that assess interactions at the entire community level, i.e. considering all species pairs within a community, are still scarce (but see; Saiz and Alados, 2012; Soliveres et al., 2011b, 2012), particularly those that consider multiple (interacting) stress factors. Thus, it still remains unclear how species interact at the community level along an environmental gradient consisting of drought and grazing, and how this relates to changes at species level.

The aim of this study is to investigate how plant interactions shift along a combined gradient of grazing pressure and drought stress. We expected grazing to increase the amount of positive associations, because of increased importance of associational resistance (Saiz and Alados, 2012; Verwijmeren et al., 2013). We performed an observational study in semi-arid grassland–shrubland in southeastern Spain, using aspect (north- vs south-facing slopes, reflecting a drought contrast) and grazing pressure (distance from stable). By performing transects we measured the grazing pressure, the vegetation cover and degradation level and also quantified species co-occurrence from species presence-absence data in quadrats along the same transects. We describe

the effect of grazing pressure and aspect on vegetation cover and soil functioning, and indicate how these stressors interact in affecting aggregation and segregation patterns at plant community and at species-pair level.

2. Methods

2.1. Study site

We performed our study at goat grazed semi-arid fields in southeastern Spain (Murcia region 37°57'28.37"N – 1°0'16.14"W). Average annual rainfall here is 301 mm, with on average 38 rainy days with more than 1 mm of precipitation annually. Highest rainfall occurs during spring and autumn and there is an extensive dry period in summer; average rainfall in August and July is only 10 and 5 mm, respectively. Mean monthly temperature ranges from 10.1 °C in January up till 26.7 °C in August (Agencia Estatal de Meteorología, AEMET).

Altitudes in our field site range from 175 to 302 m above sea level. Soil type consists of loamy sand. We performed our observations on slopes where vegetation mainly consists of a mixture of woody shrubs, grasses and chamaephytes (dwarf shrubs). Most occurring species are (in order of abundance): *Teucrium polium* (chamaephyte), *Rosmarinus officinalis* (unpalatable shrub), *Anthyllis cytisoides* (palatable shrub), *Fumana ericoides* (chamaephyte), *S. tenacissima* (unpalatable tussock grass) and *Brachypodium retusum* (short grass).

The study area has been grazed since April 2009 by a herd of approximately 200 goats of the Murciano Granadina breed. We selected three large zones at increasing distance from the herd shelter; the furthest zone (800–1400 m from shelter) was ungrazed by goats for decades, the middle zone (650–800 m from shelter) was grazed at low pressure since 2009, and the nearest zone (100–400 m from shelter) was grazed at high pressure since 2009. The nearest zone (high grazing pressure) was visited daily by the herd, while the middle zone (low grazing pressure) was visited weekly. The two grazed zones were grazed year-round except for the driest months July and August. We verified the assigned grazing pressure levels by counting droppings (described below). Besides grazing by goats, other – natural – herbivores in our site that may have a significant influence on the vegetation are rabbits (*Oryctolagus cuniculus*).

2.1.1. Study design and data collection

To assess the impacts of grazing pressure and drought stress on degradation and plant–plant interactions, we established a study design with three levels of goat grazing along a range of slopes with aspect varying from north to south, thus differing in drought stress. This is because drought is more intense on sunnier and dryer south-facing slopes, and this is linked to decreased plant performance in semi-arid regions in previous studies (e.g. Gómez-Aparicio et al., 2004).

Within every grazing treatment we measured the perennial vegetation cover, soil functional status, and the species co-occurrence on replicated line transects. We selected 10–12 (1.5 × 30 m) transects within every grazing treatment, with transects following the maximum slope angle and being equally distributed over north-facing and south-facing slopes. The selected slopes varied in aspect and inclination. Aspect (expressed as degrees deviation from north) ranged from 0 (pure north) to 180 (pure south) and did not significantly differ between the three grazing levels (Kruskal–Wallis $\chi^2 = 0.252$; $p = 0.882$). Slope inclination ranged from 20 to 74%, with an average of 42% and did not significantly differ among the three grazing levels (Kruskal–Wallis

$\chi^2 = 0.515$; $p = 0.773$). The minimum distance between selected slopes was 50 m.

2.2. Herbivory

For every grazing level goat and rabbit droppings were counted. For this, we subdivided each transect in 20 quadrats of 1.5 by 1.5 m in which we recorded pellet presence. From this we calculated pellet density per transect, as a percentage of quadrats with droppings present. For rabbits we followed a likewise routine, but instead of presence of individual droppings we counted presence of latrines along transects. At no grazing, we did not observe any goat droppings. A generalized linear model (normal errors, identity link function) with grazing as a factorial variable and aspect deviations from the north as a continuous covariable showed that goat droppings were more abundant at sites with high grazing pressure (Wald $\chi^2 = 124.148$, $df = 2$, $p < 0.001$), but aspect also had a significant effect with overall lower dropping counts at south-facing slopes (Wald $\chi^2 = 18.871$, $df = 1$, $p < 0.001$). Also, we found a significant interaction between grazing pressure and aspect (Wald $\chi^2 = 44.936$, $df = 2$, $p < 0.001$), with higher dropping count on highly grazed slopes that were more oriented to the north. Pairwise contrasts (at $p < 0.001$) showed a significant higher dropping count at slopes with low grazing pressure compared to slopes with no grazing and also a significantly higher dropping count for slopes with high grazing pressure compared to slopes with low grazing pressure. A similar generalized linear model (normal errors, identity link function) showed that the number of rabbit latrines was not significantly different between the three goat grazing pressure levels (Wald $\chi^2 = 0.2626$, $df = 2$, $p = 0.269$), but we found significantly more rabbit droppings at north slopes than at south-facing slopes (Wald $\chi^2 = 37.444$, $df = 1$, $p < 0.001$). We found no significant interaction between goat grazing pressure and aspect (Wald $\chi^2 = 0.918$, $df = 2$, $p < 0.632$) for rabbit latrine number.

2.3. Vegetation cover and soil functioning (LFA)

Along all transects we recorded vegetation cover and soil functioning. In order to measure these two variables, we followed the guidelines from Landscape Function Analysis (LFA; Tongway and Hindley, 2004). Next to assessing vegetation cover along transects by point intersect transects, the LFA method provides indicators for soil functioning. These indicators were assessed by visual measurements of soil properties that were integrated into three indices representing infiltration ability, surface stability and nutrient cycling. For each transect, we assessed these three LFA indices in four quadrats of 50×50 cm, 2 located on bare soil and 2 underneath a plant patch (Fig. 1). This way we obtained one mean LFA value for soil under patches and one mean LFA value for bare soil.

2.4. Species co-occurrence (C-score)

To assess species co-occurrence we sampled quadrats of 1.5×1.5 m along each transect (ca. 20 quadrats per transect) and we recorded perennial species presence per quadrat (Fig. 1). This information was used to create a presence/absence matrix for all observed species. We performed species co-occurrence analyses by using the Fortran based program PAIRS (Ulrich, 2008) and used the C-score (Stone and Roberts, 1990) as a metric of co-occurrence for each pair of plant species. The C-score is related to the competitive exclusion concept of “checkerboardedness”, i.e. how many of the possible species pairs in a given community never appear together in the same quadrat. So, positive and large values of this index indicate that species are not co-occurring (exclusion) and that

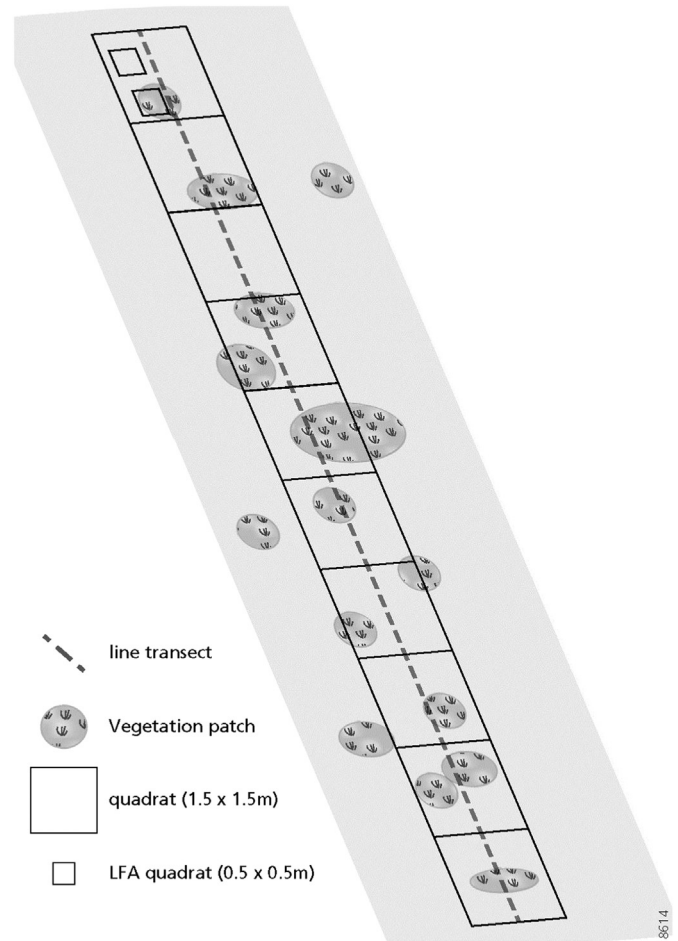


Fig. 1. Sketch of a transect section used to measure vegetation cover (line transect), soil functioning (LFA quadrat) and species co-occurrence (quadrat of 1.5×1.5 m). Note that only a transect section is depicted; a full transect contained 20 quadrats, two LFA quadrats in bare soil and two LFA quadrats underneath a vegetation patch.

competition may be the driving mechanism determining the observed patterns (Gotelli, 2000; Stone and Roberts, 1990). We studied co-occurrence patterns at two levels: at the entire community level and for every pairwise species interaction.

2.4.1. Community level

To estimate plant co-occurrence at the community level (the ‘checkerboardedness’ of all species together) we followed the approach used in similar transect-based studies on plant–plant co-occurrence (López et al., 2013; Maestre et al., 2010). We calculated co-occurrence of species by grouping all transects per treatment into one single matrix, because matrix sizes were too small per transect to detect any changes in co-occurrence at the community or the species pair level. For this analysis, aspect was made categorical by grouping deviations till 60° in north-facing slopes and deviations higher than 120° in south-facing slopes. Hence, we used six treatments obtained from three grazing levels crossed with two aspects (north and south). Observed C-scores per treatment were compared with an average for C-scores obtained by 1000 randomly assembled null matrices. Simulated matrices were assembled by Monte Carlo procedures using a fixed-equiprobable algorithm. This type of algorithm is recommended for absence–presence data from transect data (Gotelli, 2000), as it does not constrain the number of plant species in one quadrat and assumes that every quadrat is equal in its probability of expected number of plant species, but

maintains species abundance fixed (i.e., rare species remain rare while abundant species remain abundant). The degree of co-occurrence or exclusion was expressed as the deviation of our observed C-score from simulated C-scores. This deviation is expressed as the standardized effect size (SES). SES is calculated as $(I_{\text{obs}} - I_{\text{sim}})/S_{\text{sim}}$, where I_{obs} is the observed value of the C-score, and I_{sim} and S_{sim} are the mean and standard deviation, respectively, of the C-score values that were obtained from the 1000 randomly created matrices. Positive C-score SES values indicate lower “checkerboardedness” (spatial segregation) than expected by chance, while negative values indicate higher “checkerboardedness” (spatial aggregation) than expected by chance. We calculated the C-score and corresponding SES for every transect at the community level, hence grouping all species together. In addition, we calculated SES for the variance ratio (V ratio) and the number of species pairs, but since both indexes were highly correlated to the C-score SES ($r = 0.96$ and $r = 0.81$ respectively), we only presented the C-score effect sizes.

2.4.2. Species pairs

As the number of species pairs in a matrix increases exponentially with the number of species (in our case, a matrix with 20 species holds 190 species pairs) many ‘significant’ species pairs can be expected by chance at the 1% or 5% error level. To reduce such high false detection error rates, we employed the conservative empirical Bayes mean criterion (Bayes M criterion) developed by Gotelli and Ulrich (2010). In this method, The C-score is calculated for all pairs of species, which are then grouped into 22 evenly spaced bins from 0.0 to 1.0. For each randomized matrix of the 1000 runs, the C-score values are calculated for all species pairs separately, which are grouped into the 22 bins. Then, the mean and confidence limits of the expected number of species pairs within each bin is calculated from these null matrices. Observed species pairs within each bin are ordered according to their observed C-scores and only pairs that fall above the mean (Bayes M criterion) for the expected number of species were considered for further analysis.

2.5. Data analysis

We tested data of vegetation cover, LFA and C-score for normality using Kolmogorov–Smirnov tests and for homogeneity of variance using Levene’s test. We found independence of grazing level and aspect (one way ANOVA: $F_{2, 36} = 0.068$; $p = 0.934$) and a correlation between vegetation cover and aspect ($R^2 = 0.406$), and between LFA values and aspect (R^2 ranging from 0.095 for infiltration at bare soil to $R^2 = 0.386$ for stability at bare soil). Therefore, to assess the relation between vegetation cover and grazing and the relation between the six separate LFA variables and grazing, we performed in these cases ANCOVA tests with aspect as a continuous covariable and grazing as a fixed categorical variable. We performed Tukey HSD post-hoc tests to further test for contrasts between the three grazing treatments. All analyses were performed in R, version 2.15.1 (R Core Team, 2013). For all linear models we calculated partial effect sizes (η^2), which is the partial variance explained by any significant factor.

3. Results

3.1. Vegetation cover and soil functioning (LFA)

Vegetation cover ranged from 60% at ungrazed north-facing slopes to 4% on south-facing slopes with high grazing pressure. Full effects ANCOVA revealed significant effects of aspect ($F_{1, 33} = 31.111$; $p < 0.001$, $\eta^2 = 0.373$) and grazing ($F_{2, 33} = 7.163$;

$p = 0.003$; $\eta^2 = 0.173$) on vegetation cover (Fig. 2). Vegetation cover significantly decreased with increasing aspect deviation from the north. Furthermore, vegetation cover was significantly lower at high grazing pressure than at ungrazed slopes, with an average decline of 14.5% in cover from no grazing to high grazing pressure. There was no significant difference between no grazing and low grazing pressure (Fig. 2). Low grazing pressure only resulted in reduced vegetation cover for south-facing slopes, although the grazing-aspect interaction was non-significant ($F_{2,33} = 2.033$, $p = 0.147$).

Overall, LFA values significantly varied with both grazing pressure and aspect. For bare soils, aspect had a bigger effect on all three indices than grazing pressure (Table 1). Higher aspect deviation from the north significantly reduced values for stability, infiltration, and nutrient cycling functions, while higher grazing significantly reduced LFA values for stability and nutrient cycling but not for infiltration. Tukey HSD tests for stability revealed significant contrasts between no grazing and low grazing (Mean_{nograzing} = 60.6 Mean_{lowgrazing} = 53.47, $t = -3.374$, $p < 0.01$) but no contrast between low and high grazing. Nutrient cycling was significantly lower in high grazing pressure compared to no grazing (Mean_{nograzing} = 15.42 Mean_{highgrazing} = 11.50, $t = -3.320$, $p < 0.01$).

For soils underneath vegetation patches none of the three indices was significantly affected by grazing pressure, while higher aspect deviation from the north reduced the values for all the three indices (Table 1). Also we found a significant interaction between grazing and aspect, with high grazing pressure exacerbating the negative impact of aspect within patches.

3.2. Plant–plant interactions at the community level

For north-facing slopes SES values were positively deviating from null models for both ungrazed slopes ($p < 0.001$) and slopes with high grazing pressure ($p < 0.001$), indicating exclusion

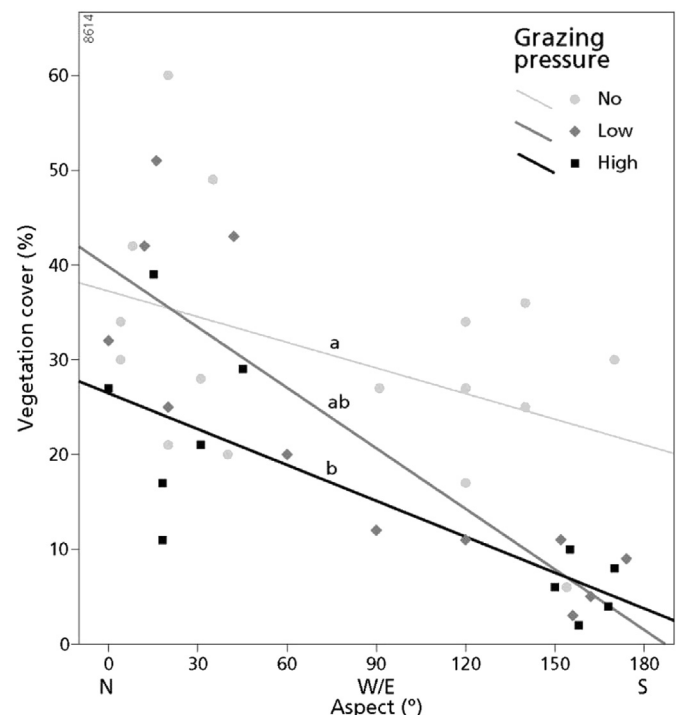


Fig. 2. Mean vegetation cover along the sampled transects as a function of grazing pressure and aspect. Aspect is expressed as degrees of deviation from north. Different letters indicate significant differences obtained from Tukey HSD tests at $p < 0.01$.

Table 1

F, *p* and η^2 (partial effect sizes) values for the two main factors and their interactions for the three LFA indices for bare soils and soil underneath vegetation patches.

	Grazing			Aspect			Grazing:Aspect		
	<i>F</i>	<i>p</i>	η^2	<i>F</i>	<i>p</i>	η^2	<i>F</i>	<i>p</i>	η^2
Bare soil									
Stability	7.511	0.002	0.207	27.062	<0.001	0.37	0.187	0.830	0.052
Infiltration	0.058	NS	0.030	3.878	0.0582	0.099	2.481	0.101	0.124
Nutrients	6.085	0.006	0.172	26.658	<0.001	0.371	1.333	0.278	0.037
Patch									
Stability	2.471	NS	0.087	8.562	0.006	0.152	6.445	0.005	0.229
Infiltration	1.791	NS	0.076	5.356	0.027	0.113	4.129	0.026	0.175
Nutrients	1.768	NS	0.063	6.373	0.017	0.117	7.17	0.002	0.264

between species being dominant. At north-facing slopes with low grazing pressure SES were not significantly deviating from null models, indicating neutral interactions to be dominant. At south-facing slopes SES were significantly positive at ungrazed slopes ($p = 0.005$), indicating exclusion between species being dominant. However, SES shifted to being significantly negative at slopes with low grazing pressure ($p = 0.030$), hence indicating a shift towards co-occurrence being dominant. At slopes with high grazing pressure SES were not significantly deviating from null models (Fig. 3).

3.3. Plant–plant interactions at the species pair level

Overall, the patterns at species pair level were in line with the results from the community wide C-score analysis. At north-facing slopes that were either ungrazed or grazed at high pressure, we found more significantly excluding pairs than co-occurring pairs (Tables 2 and 3). On the slopes with low grazing pressure we found no significantly co-occurring or excluding species pairs and at north-facing slopes with high grazing pressure we found co-occurring pairs to be dominant. Also at ungrazed south-facing slopes, most significant C-score SES indicated exclusion except for five species pairs. However, at the south-facing slopes with low grazing pressure we only found significantly co-occurring species pairs. At high grazed south-facing slopes we found one species pair to be co-occurring and one pair to be excluding each other.

The analysis of pairs revealed two abundant species pairs that made shifts from being significantly positively associated to significantly negatively associated, or the other way around, with increased grazing pressure or drought stress associated to aspect.

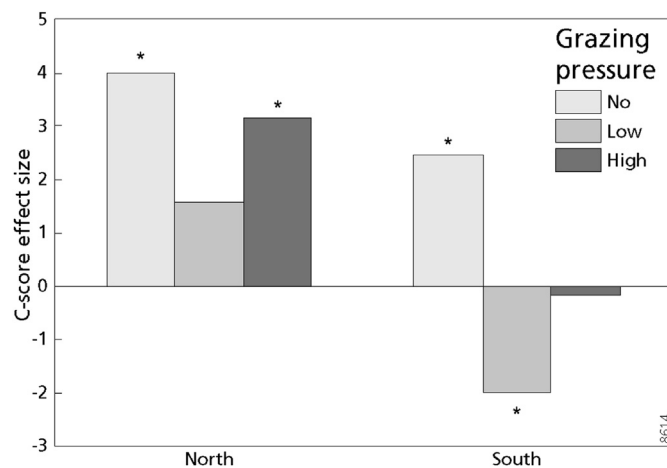


Fig. 3. C-score standardized effect sizes (SES) along three levels of grazing pressure at north- and south-facing slopes (reflecting drought stress). Positive values indicate exclusion of species. Negative values indicate co-occurrence of species. * indicate significant deviation from the simulated null models at $p < 0.05$.

On north-facing slopes, *S. tenacissima* and *A. cytisoides* were significantly excluding each other under low grazing pressure (Table 3), whereas they were significantly co-occurring under high grazing pressure. Furthermore, at the ungrazed slopes, *S. tenacissima* and *Salsola genistoides* switched from excluding each other at south-facing slopes to co-occurring at north-facing slopes. These three species were all highly abundant with occurrences of 38% (*A. cytisoides*), 31% (*S. tenacissima*) and 19% (*S. genistoides*) of the sampled quadrats.

4. Discussion

The aim of this study was to investigate how plant–plant interactions shift along a combined environmental gradient of drought stress (slope aspect) and grazing pressure, and to assess how changes at species level are related to changes at the community level. Grazing pressure and aspect significantly affected vegetation cover and soil functioning, with overall decreasing cover and soil functioning with either an increase in grazing pressure or an increase in aspect deviation from the north. Together, the variation in vegetation cover and LFA indices showed a clear two-factor gradient in the functional/degradation status of the site, with stress increasing from no grazing on north-facing slopes to high grazing pressure on south-facing slopes. However, different patterns arose on this gradient, depending on whether species interactions were studied at the community level or at the species pair level. At the community level, on north-facing slopes, species were excluding each other mostly over the whole grazing gradient. However, for one abundant species pair (*S. tenacissima* and *A. cytisoides*) we found a shift from exclusion to co-occurrence along the same grazing gradient from ungrazed to highly grazed north-facing slopes, highlighting the importance of assessing pairwise interactions next to changes in interactions at the community level. At south-facing slopes, species interactions at the community level shifted from being excluding at ungrazed slopes, to co-occurring at

Table 2

Percentage of significant co-occurring species pairs and percentage of significant excluding species pairs expressed as the total of nr of possible species pairs used in the pairwise C-score analysis.

Treatment	Nr of species	Nr of poss. sp. comb.	Sign. co-occurring species (%)	Sign. excluding species (%)
North slopes				
No grazing	17	136	2.94	6.61
Low grazing	13	78	—	—
High grazing	11	55	1.89	10.91
South slopes				
No grazing	15	105	4.76	7.61
Low grazing	11	55	7.27	0
High grazing	8	28	3.57	3.57

Table 3

P-values for all significant species pairs that are either excluding or co-occurring in the matrix. Co-occurring species (negative SES) are highlighted in italics. In bold we highlight species pairs that made a shift in association direction with either grazing or slope aspect. Species acronyms: AnCi: *Anthyllis cytisoides*, ArBa: *Artemisia barrelieri*, BrRe: *Brachypodium retusum*, CiAl: *Cistus albidus*, CiCl: *Cistus clusii*, FuEr: *Fumana ericoides*, FuTh: *Fumana thymifolia*, GlCo: *Globularia alypum*, HeSt: *Helianthemum stoechas*, HeAp: *Helianthemum apenninum*, OnTr: *Ononis tridentata*, RoOf: *Rosmarinus officinalis*, SaGe: *Salsola genistoides*, StTe: *Stipa tenacissima*, TePo: *Teucrium polium*, TePs: *Teucrium pseudochaemepitys*, ThHi: *Thymelaea hirsuta*, ThVu: *Thymus vulgaris*.

Treatment	Species 1	Species 2	Obs. C-score	Exp. C-score	C-score SES	p-value
North Slopes						
No grazing	RoOf	ArBa	0.415	0.219	4.84	0.000
	RoOf	HeAp	0.866	0.338	3.20	0.001
	AnCi	StTe	0.465	0.291	2.90	0.003
	AnCi	SaGe	0.483	0.300	2.94	0.003
	TePo	FuEr	0.353	0.227	3.72	0.000
	BrRe	ArBa	0.496	0.287	4.30	0.000
	BrRe	HeAp	0.496	0.295	4.02	0.000
	FuEr	ArBa	0.622	0.347	4.76	0.000
	FuEr	CiCl	0.685	0.446	3.31	0.000
	StTe	FuTh	0.258	0.415	-2.65	0.007
	StTe	SaGe	0.289	0.544	-2.91	0.003
	AnCi	HeAp	0.125	0.243	-2.72	0.006
	RoOf	FuEr	0.113	0.210	-2.85	0.004
	High grazing	RoOf	0.285	0.167	3.55	0.000
		RoOf	0.420	0.219	4.74	0.000
		RoOf	0.712	0.328	3.50	0.000
		AnCi	0.517	0.299	2.85	0.004
		StTe	0.600	0.350	3.52	0.000
		BrRe	0.658	0.425	2.73	0.006
	AnCi	StTe	0.156	0.406	-3.00	0.002
South Slopes						
No grazing	StTe	ThVu	0.541	0.320	2.78	0.005
	StTe	HeAp	0.776	0.362	3.80	0.000
	StTe	TePo	0.658	0.286	6.04	0.000
	StTe	SaGe	0.584	0.323	3.14	0.002
	StTe	ArBa	0.559	0.244	6.87	0.000
	RoOf	ArBa	0.675	0.431	3.37	0.000
	ArBa	CiCl	0.812	0.538	3.10	0.002
	ArBa	TePo	1.000	0.613	3.06	0.002
	AnCi	HeAp	0.203	0.663	-3.49	0.000
	AnCi	TePo	0.312	0.530	-2.71	0.000
	ArBa	HeAp	0.157	0.600	-3.27	0.000
	ArBa	FuEr	0.250	0.576	-3.07	0.002
	ArBa	TePo	0.133	0.497	-4.81	0.001
	Low grazing	TePo	0.156	0.393	-2.63	0.008
		ArBa	0.156	0.403	-2.76	0.005
		BrRe	0.337	0.712	-2.93	0.004
	AnCi	GlCo	0.156	0.403	-2.76	0.005
High grazing	RoOf	SaGe	0.792	0.409	3.90	0.000
	FuEr	SaGe	0.792	0.404	-2.68	0.007

slopes with low grazing pressure, while interactions were neutral at south-facing slopes with high grazing pressure.

4.1. Vegetation cover and soil degradation

We found that vegetation cover was affected by grazing and aspect, but aspect appeared to be the stronger driver of decreases in cover. Our grazing-driven decline in vegetation cover (14.4%) is in line with other studies on plant interactions along a livestock gradient in semi-arid ecosystems, with reported declines in vegetation cover of 10 percent (Alados et al., 2004; Saiz and Alados, 2012) and 12 percent (Bisigato and Bertiller, 1997; Bisigato et al., 2005). Aspect had a bigger impact on vegetation cover in our study, with an average decline of 17.2% from north- to south-facing slopes. The impact of low grazing pressure appears to have a bigger effect on south-facing slopes compared to north-facing slopes, but this interactive effect was not statistically significant.

Aspect influenced all soil functional indices (stability, infiltration, nutrients) for both bare soils and soils underneath vegetation patches, with soil functioning indices decreasing on slopes more oriented to the south. Grazing only influenced the stability and nutrient cycling indices at bare soil, probably because processes such as trampling may have a bigger effect in bare soils than in vegetation patches. This higher sensitivity of degradation processes in bare soil interpatches compared to vegetation patches is in line with previous studies (Mayor and Bautista, 2012). The lack of an effect of grazing on the infiltration index is surprising, as this index has been reported to be particularly sensitive to small changes in soil condition (Mayor and Bautista, 2012). Overall, increasing drought stress associated to higher aspect deviation from the north seems the biggest driver of a decrease in vegetation cover and soil functioning in our system.

4.2. Plant interaction at the community level

At both ungrazed north- and south-facing slopes we found exclusion between plant species to be the dominant interaction. This finding is in line with a similar field study performed in the south east of Spain where the plant community had an overall negative pattern of association (Saiz and Alados, 2012). At slopes with intermediate grazing pressure plants shifted to being more positively associated on south-facing slopes, while on north-facing slopes they were not significantly deviating from the null model. On both aspects we thus found an increase in nestedness in the plant community (or a decrease in exclusion) from no grazing pressure to low grazing pressure. At high grazing pressure, species associations shifted back to neutral for south-facing slopes and to negative associations at north-facing slopes. These patterns partly support the idea that intermediate grazing pressure can increase the amount of positive associations (or decrease the amount of negative associations in the case of north-facing slopes), due to a higher importance of associational resistance, while positive associations might disappear again with even higher grazing pressure (Saiz and Alados, 2012; Verwijmeren et al., 2013). Moreover, we did only observe a shift in the direction of species association from negative to positive with grazing pressure at the south-facing slopes, while at the north-facing slopes we did not observe such a shift. This observation is in contrast with the idea that the effect of grazing on plant interactions is lower at higher drought stressed slopes. Our findings more support the idea that grazing particularly affects plant–plant interactions when drought stress is already limiting plant growth to a larger extent (Gómez-Aparicio et al., 2008; Soliveres et al., 2012).

4.3. Plant interactions at species pair level

The percentages of co-occurring species pairs over our different treatments are in line with the results from the community wide analysis: we found the highest percentage of co-occurring species pairs at south-facing slopes with low grazing pressure. The total percentages of co-occurring species showed a slight increase from north-facing slopes compared to more drought stressed south-facing slopes and thus partly support predictions from the original SGH (Bertness and Callaway, 1994; Callaway, 1995; Callaway and Walker, 1997). However, with higher grazing pressure we did not observe a straightforward increase in frequency of positive interactions, which is contrasting predictions from the original SGH. The observed percentages of positive associations are lower than reported by similar studies in semi-arid grazed ecosystems of 18% (Soliveres et al., 2012) and 10–25% (Saiz and Alados, 2012). This is probably due to the lower number of species observed in our

study, but may also have to do with the different sampling techniques between studies.

We found that two abundant species pairs (*S. tenacissima* – *A. cytisoides* and *S. tenacissima* – *S. genistoides*) did show a shift in the direction of interactions, highlighting the importance of focussing also on pairwise interactions next to interactions at the community level. *A. cytisoides* was negatively associated to *S. tenacissima* at ungrazed north-facing slopes. This may indicate competition from *S. tenacissima* on recruitment of this species, which is in line with earlier studies showing that *S. tenacissima* is a strong competitor for resources resulting in competitive exclusion of shrub species (Alados et al., 2003). Likewise, studies from semi-arid ecosystems in Spain showed increasing negative effects of *S. tenacissima* on planted shrub seedlings (*Pistacia lentiscus*) in semi-arid ecosystems with an increase in drought stress (Maestre and Cortina, 2004). However, at high grazing pressure the association between *S. tenacissima* and *A. cytisoides* shifted to being significantly positive in our study. As *A. cytisoides* is preferred by goats (pers. obs.), probably due to high nutrient (N) content, preferential feeding by goats outside the patches may thus explain this outcome. In agreement with this, results from another experimental study showed more feeding on planted palatable shrubs by rabbits outside *S. tenacissima* patches (Soliveres et al., 2011a).

The species pair *S. tenacissima* and *S. genistoides* also showed a significant shift, but not with increased grazing pressure but rather with a change in aspect. At ungrazed north-facing slopes both species were co-occurring, while at ungrazed south-facing slopes both species were significantly excluding each other. At north-facing slopes *S. tenacissima* might increase recruitment of *S. genistoides*, while with increasing drought stress this interaction might wane again. As *S. genistoides* is an important species in retaining soil shear strength and thus preventing erosion in degraded areas (De Baets et al., 2007), a lack of recruitment of *S. genistoides* could result in further degradation by increased gully erosion.

Our pairwise interaction analysis showed the special importance of the tussock grass *S. tenacissima* as a facilitator of shrubs. Both at increased grazing pressure and at relatively low drought stress, this tussock grass seems to act as a facilitator for recruitment. The highly abundant *S. tenacissima* proved to shift towards co-occurring with the very abundant shrub *A. cytisoides* with increased grazing pressure.

4.4. Conclusion and perspectives

Although changes in aspect were the biggest driver of reduced vegetation cover and soil functioning, grazing had a large effect on plant interactions at the community level, causing a significant shift from negative to positive species associations at south-facing slopes from no grazing to low grazing pressure. At north-facing slopes we did not find a significant shift in the direction of species association along the grazing gradient. However, our study showed that results at the species-pair level contrasted these findings from the community level approach, depending on the species studied. This is because species pairs that may be negatively associated to almost all other species may still be an important facilitator to a single, but very abundant species. This may have important implications in certain stages of succession in which a facilitated species is a weak competitor or is non-resistant to grazing. Hence, our results show the importance of assessing species association strength at both the species pair level and the community level. For understanding the impacts of stress at plant–plant interactions, both levels (community wide changes and species dependent changes) should be assessed simultaneously. Follow-up experimental studies can then disentangle the underlying mechanisms behind the shifts from positive to negative

associations for key species along gradients consisting of multiple stressors. Such work is important for building knowledge that may help to prevent further degradation of semi-arid ecosystems under current ongoing global environmental change (Verwijmeren et al., 2013).

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References

- Alados, C.L., et al., 2003. Quantitative characterization of the regressive ecological succession by fractal analysis of plant spatial patterns. *Ecol. Model.* 163, 1–17.
- Alados, C.L., et al., 2004. Change in plant spatial patterns and diversity along the successional gradient of Mediterranean grazing ecosystems. *Ecol. Model.* 180, 523–535.
- Armas, C., Pugnaire, F.I., 2005. Plant interactions govern population dynamics in a semi-arid plant community. *J. Ecol.* 93, 978–989.
- Bertness, M.D., Callaway, R., 1994. Positive interactions in communities. *Trends Ecol. Evol.* 9, 191–193.
- Bisigato, A.J., Bertiller, M.B., 1997. Grazing effects on patchy dryland vegetation in northern Patagonia. *J. Arid Environ.* 36, 639–653.
- Bisigato, A.J., et al., 2005. Effect of grazing on plant patterns in arid ecosystems of Patagonian Monte. *Ecography* 28, 561–572.
- Brooker, R.W., et al., 2006. Transient facilitative effects of heather on Scots pine along a grazing disturbance gradient in Scottish moorland. *J. Ecol.* 94, 637–645.
- Bulleri, F., et al., 2011. Crossing gradients of consumer pressure and physical stress on shallow rocky reefs: a test of the stress-gradient hypothesis. *J. Ecol.* 99, 335–344.
- Callaway, R.M., 1995. Positive interactions among plants. *Bot. Rev.* 61, 306–349.
- Callaway, R.M., 2007. Positive Interactions and Interdependence in Plant Communities. Springer, Dordrecht.
- Callaway, R.M., Walker, L.R., 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78, 1958–1965.
- De Baets, S., et al., 2007. Root characteristics of representative Mediterranean plant species and their erosion-reducing potential during concentrated runoff. *Plant and Soil* 294, 169–183.
- Goldberg, D.E., et al., 1999. Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. *Ecology* 80, 1118–1131.
- Gómez-Aparicio, L., et al., 2004. Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecol. Appl.* 14, 1128–1138.
- Gómez-Aparicio, L., et al., 2008. Facilitation of tree saplings by nurse plants: microhabitat amelioration or protection against herbivores? *J. Veg. Sci.* 19, 161–172.
- Gotelli, N.J., 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81, 2606–2621.
- Gotelli, N.J., Ulrich, W., 2010. The empirical Bayes approach as a tool to identify non-random species associations. *Oecologia* 162, 463–477.
- Graff, P., et al., 2007. Shifts in positive and negative plant interactions along a grazing intensity gradient. *Ecology* 88, 188–199.
- Grime, J.P., 1979. *Plant Strategies and Vegetation Processes*. Wiley and Sons, Chichester.
- Hay, M.E., 1986. Associational plant defenses and the maintenance of species diversity: turning competitors into accomplices. *Am. Nat.* 128, 617–641.
- He, Q., et al., 2013. Global shifts towards positive species interactions with increasing environmental stress. *Ecol. Lett.* 16, 695–706.
- Le Bagousse-Pinguet, Y., et al., 2012. Release from competition and protection determine the outcome of plant interactions along a grazing gradient. *Oikos* 121, 95–101.
- Levenbach, S., 2009. Grazing intensity influences the strength of an associational refuge on temperate reefs. *Oecologia* 159, 181–190.
- López, R.P., et al., 2013. Co-occurrence patterns along a regional aridity gradient of the subtropical Andes do not support stress gradient hypotheses. *PLoS ONE* 8.
- Maestre, F.T., Cortina, J., 2004. Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. *Proc. R. Soc. B Biol. Sci.* 271, S331–S333.
- Maestre, F.T., et al., 2005. Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *J. Ecol.* 93, 748–757.
- Maestre, F.T., et al., 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J. Ecol.* 97, 199–205.

- Maestre, F.T., et al., 2010. Do biotic interactions modulate ecosystem functioning along stress gradients? Insights from semi-arid plant and biological soil crust communities. *Philos. Trans. R. Soc. B: Biol. Sci.* 365, 2057–2070.
- Mayor, A.G., Bautista, S., 2012. Multi-scale evaluation of soil functional indicators for the assessment of water and soil retention in Mediterranean semiarid landscapes. *Ecol. Indic.* 20, 332–336.
- Michalet, R., et al., 2014. Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. *J. Veg. Sci.* 25, 609–613.
- Pugnaire, F.I., et al., 2011. Positive plant interactions in the Iberian Southeast: mechanisms, environmental gradients, and ecosystem function. *J. Arid Environ.* 75, 1310–1320.
- R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>.
- Saiz, H., Alados, C.L., 2012. Changes in semi-arid plant species associations along a livestock grazing gradient. *PLoS ONE* 7.
- Smit, C., et al., 2007. Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. *Oecologia* 152, 265–273.
- Smit, C., et al., 2009. Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. *J. Ecol.* 97, 1215–1219.
- Soliveres, S., et al., 2011a. Temporal dynamics of herbivory and water availability interactively modulate the outcome of a grass-shrub interaction in a semi-arid ecosystem. *Oikos* 120, 710–719.
- Soliveres, S., et al., 2011b. Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Perspectives in plant ecology. Evol. Syst.* 13, 247–258.
- Soliveres, S., et al., 2012. Nurse plant effects on plant species richness in drylands: the role of grazing, rainfall and species specificity. *Perspectives in plant ecology. Evol. Syst.* 14, 402–410.
- Soliveres, S., et al., 2014. Moving forward on facilitation research: Response to changing environments and effects on the diversity, functioning and evolution of plant communities. *Biol. Rev.* (in press).
- Stone, L., Roberts, A., 1990. The checkerboard score and species distributions. *Oecologia* 85, 74–79.
- Tielbörger, K., Kadmon, R., 2000. Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology* 81, 1544–1553.
- Tilman, D., 1988. *Plant Strategies and the Dynamics and Structure of Plant Communities*. Princeton University Press, Princeton, NJ.
- Tongway, D.J., Hindley, N.L., 2004. *Landscape Function Analysis: Procedures for Monitoring and Assessing Landscapes*. CSIRO Publishing, Brisbane.
- Ulrich, W., 2008. Pairs – a FORTRAN Program for Studying Pair-wise Species Associations in Ecological Matrices. www.uni.torun.pl/~ulrichw.
- Veblen, K.E., 2008. Season- and herbivore-dependent competition and facilitation in a semiarid savanna. *Ecology* 89, 1532–1540.
- Verwijmeren, M., et al., 2013. Interspecific facilitation and critical transitions in arid ecosystems. *Oikos* 122, 341–347.