

Testing the role of biotic stress in the stress gradient hypothesis. Processes and patterns in arid rangelands

Pamela Graff and Martín R. Aguiar

P. Graff (graff@agro.uba.ar) and M. R. Aguiar, Cátedra de Ecología - IFEVA, Facultad de Agronomía, Univ. de Buenos Aires-CONICET, San Martín 4453, Buenos Aires C1417DSE, Argentina.

Since many arid ecosystems are overstocked with domestic herbivores, biotic stress could have a stronger influence in modulating the balance of species interactions than expected from the stress gradient hypothesis (SGH). Here we tested a priori predictions about the effect of grazing on species interactions and fine scale spatial structure of grasses in water-limited ecosystems. We used detailed vegetation mapping and spatial analysis, and performed a field experiment where the direct and indirect components of positive interactions were disentangled to provide evidence of links between process and pattern. We found associational resistance (biotic refuge) to be the dominant process in grazing situations, while competition, instead of direct facilitation, seemed to govern grass spatial patterns when herbivore pressure was relaxed. These results suggest that facilitation between grasses in arid communities may be related to herbivory rather than nurse plant effects. Associational resistance tends to have the strongest effect on spatial aggregation of species at intermediate grazing pressure. Results suggest that contrary to SGH, this physical clustering of species decreased when grazing pressure reached their maximum levels. Positive associations remained significant only when palatability differences between neighbours is large, suggesting that managing stocking rate is a key factor determining the persistence of herbivory refuges. These refuges are potential foci to initiate population recovery of high quality forage species in arid degraded areas.

Interactions among plants are dominant drivers of the structure and dynamics of natural plant populations and communities (Tilman 1982). Therefore, the interplay of positive and negative interactions has become one of the major emerging themes in community ecology (Callaway and Walker 1997, Holmgren et al. 1997, Levine 2000, Bruno et al. 2003). It has been predicted that positive and negative net outcomes of species interactions varied along productivity or abiotic stress gradients determining plant community structure (Bertness and Callaway 1994, Callaway and Walker 1997, Holmgren et al. 1997, Brooker et al. 2008). The stress-gradient hypothesis (SGH), is a general conceptual model based on Bertness and Callaway's seminal paper (1994) that predicts a positive net outcome from species interactions at both extremes of the abiotic stress gradient. Direct facilitation via physical stress amelioration, was predicted to be the dominant positive interaction under greatest stress, whereas indirect facilitation, driven by neighbour amelioration of consumer stress, was predicted to be more intense in productive-benign environments (Bertness and Callaway 1994). Many field-based experiments have been conducted in an attempt to validate these models at the harsh end of the abiotic gradient (Maestre et al. 2005), where the debate over the role of competition and direct facilitation in structuring arid communities remains uncertain (Maestre et al. 2005, 2006, Michalet 2006, Brooker et al. 2008, Malkinson

and Tielbörger 2010). In contrast, neighbour amelioration against consumer (biotic) stress has been relatively less explored (Graff et al. 2007, Smit et al. 2007, 2009).

Inclusion of biotic stress (consumer pressure) as a driver of plant–plant interactions increases complexity (Smit et al. 2007, 2009) and alters the importance of positive–negative interactions in harsh environments (Graff et al. 2007). Moreover, in the presence of herbivores, indirect facilitation can occur (Graff et al. 2007). A plant may gain protection by shared defence or associational resistance (Atsatt and O'Dowd 1976, Milchunas and Noy-Meir 2002, Baraza et al. 2006, Smit et al. 2007) if a nearby unpalatable species reduces the probability of herbivore attack, resulting in a net positive effect between plants.

Little is known about how facilitative interactions between plants change with increasing biotic stress, such as that caused by consumer pressure or herbivory (Smit et al. 2009, Vandenberghe et al. 2009). The few studies that examined associational resistance cover a large range of ecosystems, but have rarely been focused on arid environments (but see McAuliffe 1986, Oesterheld and Oyarzabal 2004, Anthelme and Michalet 2009). Indirect facilitation mediated by herbivores (associational resistance) may be overlooked in arid environments because according to the SGH, it is expected to be more important in benign environments (Bertness and Callaway 1994, Smit et al. 2009). However, since many

arid ecosystems are overstocked with domestic herbivores (Oosterheld et al. 1992, Milchunas and Lauenroth 1993), indirect plant interactions mediated by herbivores may have stronger impacts than expected from the SGH (Graff et al. 2007, Smit et al. 2009)

Recently, it has been proposed that the importance of positive interactions may be lower at the extreme ends of the abiotic gradients than predicted by the SGH (Smit et al. 2007, 2009, Malkinson and Tielbörger 2010). Smit et al. (2007) proposed that associational resistance (AR) would show a hump-shaped curve rather than increasing monotonically with increasing biotic stress levels. Smit et al. (2007) suggested that at high grazing pressures, herbivores may be forced to become less selective and feed upon low-quality food plants (Baraza et al. 2006), therefore the protective role of the benefactor species on their protégés is expected to decrease (Graff et al. 2007, Smit et al. 2007). They also postulated that the relative AR effect would be stronger as differences in palatability between benefactor species and beneficiaries increase (i.e. benefactor species being fairly unpalatable and beneficiaries highly palatable) (Atsatt and O'Dowd 1976, Milchunas and Noy-Meir 2002, Baraza et al. 2006, Smit et al. 2007). Although it has been firmly established that characteristics of both benefactor-beneficiary individual plants are important in determining the strength of AR, the two determinants of AR have rarely been combined (Vandenberghe et al. 2009). Curiously, most studies investigating browsing of a protégé plant in relation to associational refuges, have kept the palatability of the beneficiary plant fixed (but see Vandenberghe et al. 2009).

Plant spatial pattern is assumed to be a good test of the importance of species interactions in community organization (Silvertown and Wilson 2000). We tested the following a priori predictions about fine-scale spatial patterns expected from grass-to-grass interactions changes along a grazing gradient (Smit et al. 2007) within the extreme end of the abiotic gradient (arid ecosystem):

- 1) In the absence of grazing. Facilitation via habitat amelioration or competition could be the dominant forces shaping spatial patterns between species. If habitat amelioration is the predominant interaction between species (Bertness and Callaway 1994), we predicted that palatable grasses (potential beneficiaries) would be spatially aggregated at small scales around taller unpalatable species (potential benefactors). Alternatively, if belowground competition dominates, grasses would be spatially segregated. Assuming that grasses of the same rooting depth would show low horizontal overlap (Schenk and Robert 2002), the scale of the segregated pattern should match root distribution patterns (Schurr et al. 2004)
- 2) At moderate grazing pressure. If AR is an important interaction and determines species distributions, a spatial aggregation between palatable and unpalatable species at herbivore's foraging scale is expected. An increment in the proportion of palatable-unpalatable pairs aggregated relative to ungrazed exclosures is also expected due to a reinforcement in positive interactions (Smit et al. 2007). Species' relative palatability will also determine the degree of AR (Baraza et al. 2006, Smit et al. 2007). Stronger aggregations are

expected as difference in species palatability between species increases.

- 3) At high grazing pressure. The protective role the benefactor species on their protégés is expected to decrease (Smit et al. 2007), therefore it is predicted that a decrease in the frequency of species co-occurrence related to moderate grazing situations will occur. Under high grazing pressure, herbivores will intensify their search for palatable species and consume species with lower palatability, therefore, reducing AR effects (Baraza et al. 2006, Smit et al. 2007).

Spatial statistical techniques are inductive (i.e. can only suggest causal relationships) (Murrell et al. 2001, McIntire and Fajardo 2009). Therefore, the same pattern could be derived from different processes. We complemented spatial analysis with a manipulative experiment to disentangle the direct and indirect components of positive interactions under grazing, since aggregation expected could be derived from direct facilitation as well from AR effect. Using this complementary approach, we are in a better position to infer pattern and process relationships (Silvertown and Wilson 2000, Murrell et al. 2001, McIntire and Fajardo 2009).

Methods

Site description, studied species and grazing gradient

Field work was carried out in a grass-shrub steppe typical of the Western District of Patagonia (Leon et al. 1998) in southwestern Chubut, Argentina (45°4'S, 70°3'W, 500 m a.s.l.). Mean monthly temperatures range from 2°C in July and 14°C in January. The climate is arid, with an intense summer drought. Mean annual precipitation is 154 ± 44 mm (mean \pm SD), with a winter and early spring (May to September) rainy season. Strong, dry winds blow mainly from the west throughout the year (Paruelo et al. 1998). This community is co-dominated by grasses and shrubs, which contribute more than 96% of the total plant cover and plant biomass (Fernández et al. 1991). Forbs contribute <1% to plant cover. The vegetation is organized in a two-phase mosaic: Patches of shrubs (*Senecio filaginoides*, *Mulinum spinosum* and *Adesmia volkmanni*) and grasses forming high cover patches, within a matrix of tussock grasses (*Stipa speciosa*, *Poa ligularis*, *Bromus pictus* and *Stipa humilis*) with bare ground between (Soriano et al. 1994). High cover patches represent <25% of vegetation mosaic in both ungrazed and grazed situations (Cipriotti and Aguiar 2005a). In this system, grass seedling recruitment is facilitated by shrub canopies but only when root competition among grasses is avoided, competition being the overwhelming interaction in shrub-grass patches (Aguiar and Sala 1994). In low-cover patches, devoid of shrubs, neighbour interactions occur largely among grasses (Graff et al. 2007). In the field, relative cover of *Bromus* and *Poa* decreases with grazing intensity, whereas *Stipa* cover increases in both the matrix and in shrub patches (Perelman et al. 1997, Cipriotti and Aguiar 2005a). Therefore, the grass-shrub mosaic structure remains essentially the same in grazed and long-term ungrazed fields (Cipriotti and Aguiar 2005a).

We worked with one unpalatable grass, *Stipa speciosa* (hereafter *Stipa*) and two highly palatable grasses, *Poa ligularis* (hereafter *Poa*) and *Bromus pictus* (hereafter *Bromus*). *Stipa* is generally avoided by sheep (Bonvissuto et al. 1983). Of the three species, *Stipa* has the highest C: N ratio, highest lignin and lowest crude protein content (Adler et al. 2004). *Poa* has higher leaf quality than *Stipa* (Adler et al. 2004) and it is one of the two species preferred by sheep. Finally, *Bromus* is the most palatable of the preferred species, showing the highest levels of leaf N content (Adler et al. 2004).

The biotic gradient was represented by three levels of sheep grazing pressure (ungrazed, moderate and intense grazing). Each grazing level was represented in nine paddocks located within a 15 000 ha study area. Ungrazed paddocks (UN) were three exclosures of different age (28, 37 and 56 years old). Moderately grazed (MG) and intensively grazed (IG) replicates were paddocks (>200 ha) that had been continuously grazed for at least 35 years at moderate (~0.2 sheep ha⁻¹, three paddocks), and high stocking rates (~0.4 sheep ha⁻¹, three paddocks), respectively. All sites had the same soil type and topographic position. Ungrazed and moderately grazed paddocks were in the Rio Mayo Experimental Station; intensively grazed plots were in privately owned paddocks adjacent to the Experimental Station.

Vegetation mapping

Eight fine-scale maps were recorded in each of the nine paddocks. The area of each map was 1 m². These maps were located within a 50-ha area in each paddock. To avoid areas of concentrated grazing pressure, we selected the 100-ha area away from water points and fences.

The maps were randomly located, but because density of both palatable species in moderate and intense grazing is very low, some random points did not have *Poa* and *Bromus* individuals, so we followed the next point randomly assigned until eight maps were recorded (with *Poa* and *Bromus* individuals present) for each of the nine paddocks (72 maps recorded in total). At the assigned location, the grid was placed within the low cover matrix of tussock grasses (tussock grasses and bare soil, lacking shrubs) in order to record spatial patterns associated exclusively with interactions among grasses. Maps had a resolution of 2 × 2 cm, related to the grain required to account for a plant-centred perspective (sensu Purves and Law 2002) and to explore changes in spatial distributions due to grass species interactions (Schurr et al. 2004). All plants were mapped accounting for their real shape and size of canopy cover. Signs of recent herbivory (defoliated plants) were also indicated as a category (grazed or non-grazed). Maps were digitized with image software (IDRISI32 environment, 1987–2000) using three categories for dominant grass species: *Bromus*, *Poa* and *Stipa*, and two sub-categories to distinguish between grazed and non-grazed individual plants. Data were transformed from vector into raster format with a resolution of 2 × 2 cm to perform the spatial analysis.

Spatial pattern analyses

To test predictions we analysed *Bromus*–*Stipa* (maximum differences in palatability), *Poa*–*Stipa* (intermediate differences

in palatability) fine scale spatial patterns across the grazing gradient. We also tested the prediction related to spatial pattern under moderate and intense grazing (outcomes guided mostly by associational resistance), by analyzing other patterns in grazed paddocks: *Bromus* (grazed plants)–*Stipa*, *Bromus* (non-grazed plants)–*Stipa*, *Poa* (grazed plants)–*Stipa*, and *Poa* (non-grazed plants)–*Stipa*.

Spatial patterns of palatable (potential beneficiaries or protégés) relative to non-palatable (benefactor) species were analyzed using bivariate O-ring statistics ($O_{12}(r)$ Wiegand and Moloney 2004) after testing that the distribution and abundance of the species studied were not spatially autocorrelated. The $O_{12}(r)$ -ring is a point-pattern statistic that considers the mean number of neighbours from pattern 2 in a ring of radius r and ring width of dr around an individual of pattern 1, thus isolating specific distance classes. In this case, bivariate O-ring statistics measure the density of neighbouring palatable plants as a function of distance from the centres of an average unpalatable *Stipa* tussock, as detailed in Wiegand and Moloney (2004).

Calculations were performed with the software Programita version 2010 (<www.thorsten-wiegand.de/towi_programita.html/>, Wiegand and Moloney 2004). We used the real shape and size of all plants to perform the analyses (Wiegand et al. 2006). To detect possible facilitation or competition between species, we contrasted our data with a null model (Wiegand and Moloney 2004) that randomizes the locations of the palatable species (e.g. *Poa* or *Bromus*) and keeps the locations of the *Stipa* grasses fixed. Ninety-five percent confidence envelopes per each grid were calculated from the highest and lowest values of $O(r)$ taken by 19 simulations of the null model (Bailey and Gatrell 1995). In these analyses, if at a given distance r , $O_{12}(r)$ is above the upper (or below the lower) limit of the confidence envelope, we say that pattern 2 (e.g. *Poa* or *Bromus*) is positively (or negatively) associated with pattern 1 (e.g. *Stipa*) at the distance r . In order to analyze the vegetation data, we combined data from all eight maps per paddock into a single weighted O-ring statistic, resulting in a weighted O-ring statistic per each of the nine paddocks (<www.oesa.ufz.de/towi/pdf/ProgramitaCombineReplicates.pdf/>). Since $O_{12}(r)$ is a density function, values varied between grazing treatments because of the reduction in palatable species with grazing pressure. Therefore, to compare the degree of association between species at each grazing condition, for each paddock we calculated the difference between the weighted O-ring statistic value obtained and the upper (or lower) confidence limit for the weighted values that presented a positive (or negative) deviance from the null model (represented by the confidence interval) at specific distance classes as follows:

- if $O_{12}(r) > \text{upper 95\% confidence limit (CL}^+)$ then $y = (O_{12}(r) - \text{CL}^+) / \text{CL}^+ > 0$
- if $O_{12}(r) < \text{upper 95\% confidence limit (CL}^+)$ and $> \text{lower 95\% confidence limit (CL}^-)$, then $y = 0$
- if $O_{12}(r) < \text{lower 95\% confidence limit (CL}^-)$, then $y = (O_{12}(r) - \text{CL}^-) / \text{CL}^- < 0$

Mean and range values of relative aggregation or segregation patterns for each distance class (0 to 50 cm) were calculated for the three grazing treatments.

Field experimental design

We performed a two-year field experiment using cages and canopy meshes on adult established plants to disentangle the direct and indirect components of positive interactions under grazing. We also assessed if the absence of a direct facilitative effect and a strong AR effect detected previously on transplants (Graff et al. 2007) persisted in established adult plants.

Neighbour canopy and the accessibility of herbivores to two palatable species (*Bromus* and *Poa*) were manipulated in a factorial design. We conducted the experiment in a paddock that had been continuously grazed for at least 35 years at moderate stocking rates (~ 0.2 sheep ha⁻¹). Unfortunately, we could not establish the experiment along the grazing gradient because of the low probability of finding enough palatable plants at intensively grazed areas that matched the selection criteria (Graff et al. 2007).

Individual tussocks of *Bromus* ($n = 20$) and *Poa* ($n = 20$) growing in the proximity of *Stipa* (touching crowns and intermixed canopies) were selected following these criteria: *Bromus* and *Poa* plants had more than 20 living tillers (adult plants), similar height, similar vigour (60% live mass) and no signs of herbivory. The experiment was established for each palatable species with two factors arranged in a complete randomized design. The factor 'neighbour canopy' had two levels: intact *Stipa* canopies (I) and canopies bent away from the palatable species down to ground level (B). *Stipa* canopies were bent using a coarse plastic mesh of a dark colour to camouflage it with the vegetation and the ground. The factor 'accessibility of herbivores' had two levels: no cage excluding herbivores (NC) and cage excluding herbivores (C). Cages (40 × 40 × 50 cm) were constructed with a 4 × 4 cm steel mesh. These cages prevented sheep herbivory but did not affect the physical environment (light and wind).

Treatments were applied in May when the initial size (measured as the number of living tillers per plant) of all palatable plants was recorded (and then used as the initial value and co-variable). In general, these treatments neither modified the conditions of the ground surface, nor caused mortality of *Stipa* plants, therefore we assumed that only aerial interactions were manipulated (underground competition was present in all treatments).

The palatable plants were then measured in October and January of the first year; May and October of the second year, and in January of the third year. At each date, we registered palatable plant height (from soil surface to the tallest expanded leaf (panicles excluded), total number of green tillers and total number of panicles. We also recorded sheep grazing. Plants were considered 'grazed' when they showed clear bite marks and had heights lower than one-half of the height recorded in the previous census. Herbivory was expressed as the percentage of plants grazed per treatment. In addition, aboveground tissue of target plants was harvested on the last sampling date and dried at 70°C for 48 h, then weighed for biomass determination.

Field experiment analysis

ANCOVA tests were used to examine the effects of vegetation (intact (I) or bent (B) *Stipa* canopy treatments) and the

herbivores (no cage (NC) or caged (C) treatments) on final tiller number, panicle number and total aerial biomass per plant at the end of the experiment, after a two-year period. Because we did not harvest the plants at the onset of the experiment, total tiller number at the onset of the experiment was used as the covariable in the analysis. Treatments were compared with Tukey post-hoc tests ($\alpha = 0.05$) (InfoStat ver. 2009, Di Rienzo et al. 2009).

χ^2 -tests were used to examine only the effects of herbivores on plants that were damaged despite the protection of neighbour's canopy treatments (*Stipa* intact canopies or bent away canopies). To analyze grazing frequency on palatable plants, the GENMOD procedure was used (SAS 1996). The model used a binomial distribution, a type 3 analysis and a 'logit' link function, since the response was binary (grazed, not grazed).

Results

Vegetation maps and spatial pattern analysis

Poa–Stipa and *Bromus–Stipa* patterns along the grazing gradient were different. At ungrazed paddocks (UN), *Poa–Stipa* showed random patterns at fine scales (<10 cm) and weak segregated patterns (i.e. negative departures from zero values) at intermediate distances (10–20 and 36–42 cm) (Fig. 1a). In contrast, *Bromus–Stipa* showed strong positive associations (i.e. positive departures from zero values) at fine scales (<14 cm) and random patterns at larger distances (Fig. 1b).

Under moderate grazing pressures (MG), the pattern of positive association (i.e. aggregation) between beneficiary and benefactor grasses was significant for both species to <14 cm (Fig. 1a–b). Maximum values of species clustering depended also on grazing pressure and differences in palatability of the pair examined as predicted. *Poa* plants were four times more aggregated with *Stipa* tussocks than when ungrazed, and *Bromus* showed the maximum degree of positive association, two times more clustered to *Stipa* than *Poa* at fine scales (<6 cm) (Fig. 1a–b).

Under intensive grazing (IG), positive associations decreased compared with moderate grazing in *Poa–Stipa* and *Bromus–Stipa* (Fig. 1a–b). For *Poa–Stipa*, association with *Stipa* at fine scales was slightly positive up to 10 cm (Fig. 1a). For *Bromus–Stipa*, aggregations remained strongly significant up to 16 cm (Fig. 1b).

Non-consumed plants were more aggregated with *Stipa* than expected from the null model, consistent with AR effects (Fig. 2). Non-grazed plants of both species were more abundant at short distances (<16 cm) from *Stipa* (Fig. 2a–b). In contrast, the grazed plants generally displayed random patterns (i.e. no departures from zero values) (Fig. 2a–b). Ungrazed plants of *Bromus* showed positive associations with *Stipa* that were four times (MG) and three times (IG) stronger than for *Poa* plants.

Field experiment

The exclusion of sheep using cages did not have significant effects on *Poa* (Fig. 3). Treatments did not have significant

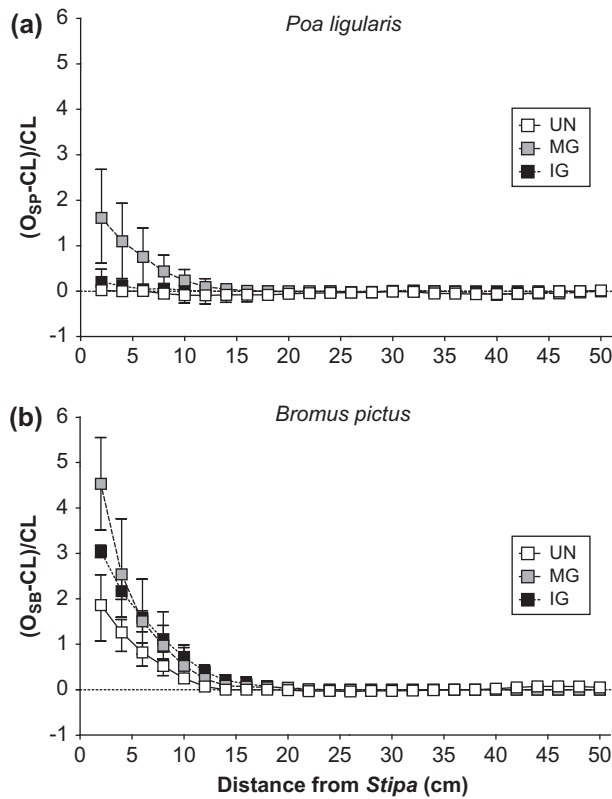


Figure 1. Spatial association of beneficiary (*Bromus* and *Poa*) and benefactor species (*Stipa*) at three grazing pressures. UN, MG and IG indicate ungrazed, moderately and intense grazing paddocks, respectively ($n = 3$). Data are expressed as the mean and range ($n = 3$) of the relative dispersion value of the O_{12} -ring index from the 95% null model confidence envelopes (CL) at each grazing condition as $(O_{12-CL})/CL$. Antecedent condition null model (Wiegand and Moloney 2004) randomizes the locations of *Poa* and *Bromus* (pattern 2) and keeps the locations of the *Stipa* grasses fixed (pattern 1). Points above zero values indicate aggregation (values exceeded upper limits of the 95% confidence envelope), zero values indicate no spatial association (between upper and lower confidence limits) and negative values indicate segregation (values below lower limits of the 95% confidence envelope). An increment in the y axis indicates that the relative abundance of beneficiary species in the presence of benefactors increases. P = *Poa*; B = *Bromus*; S = *Stipa*.

effects on tiller number, panicle number or aerial biomass (Supplementary material Appendix 1 Table 1). However, the interaction between cage \times canopy factors was significant for *Bromus* ($p < 0.05$) (Fig. 3). Inside cages, plants had more panicles, tillers and biomass when the canopy of *Stipa* was bent away to the ground than when growing near *Stipa* with the canopy in its normal (intact) position ($p < 0.05$). Outside cages, the bent canopy of *Stipa* neighbours resulted in *Bromus* plants with less biomass and fewer living tillers (Tukey $\alpha < 0.05$, Fig. 3).

The percentage of grazed plants was lower when the neighbouring canopy remained intact than when it was bent away (Fig. 4). *Stipa*'s fully-intact canopy afforded full protection against sheep grazing to *Poa* plants (0% damaged), although the relationship between damaged plants behind intact and bent *Stipa* canopies was only significant (χ^2 -test $p = 0.0172$) at the end of the experiment. For *Bromus*,

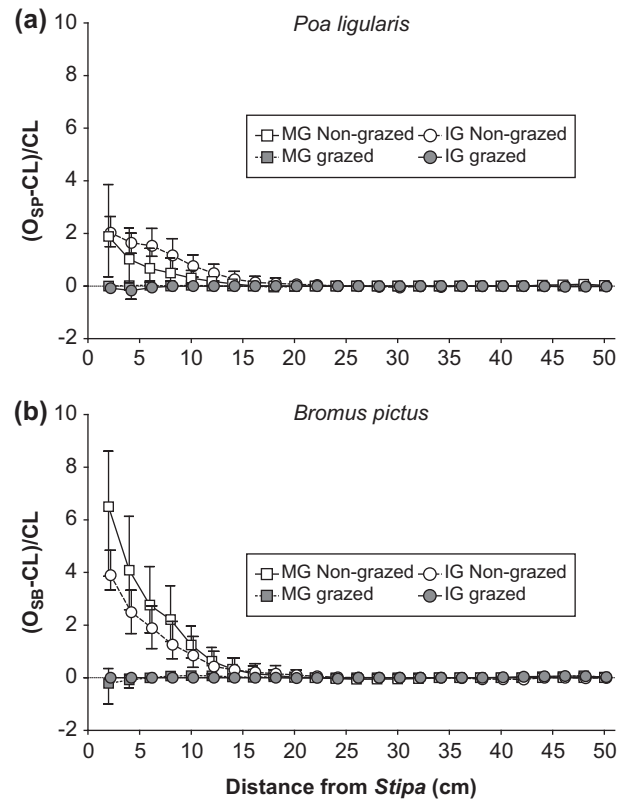


Figure 2. Spatial relationships of non-grazed and grazed palatable plants of *Bromus* (B) and *Poa* (P) related to benefactors (S) in moderately-grazed (MG) and intensively-grazed (IG) paddocks indicate moderate and intense grazing, respectively ($n = 3$). Data is expressed as the mean and range ($n = 3$) of the relative dispersion value of the O_{12} -ring index from the 95% null model confidence envelopes (CL) at each grazing condition as $(O_{12-CL})/CL$. Antecedent condition null model (Wiegand and Moloney 2004) randomizes the locations of grazed and non-grazed plants (pattern 2) and keeps the locations of the *Stipa* grasses fixed (pattern 1). Points above zero values indicate positive association (values exceeded upper limits of the 95% confidence envelope), zero values indicate no spatial association (between upper and lower confidence limits) and negative values indicate segregation (values below lower limits of the 95% confidence envelope). An increment in the y axis indicates that the relative abundance of beneficiary species around benefactors increases.

the difference between plants growing below intact versus modified canopies was significant throughout the experiment (χ^2 -test $p = 0.042$) and the removal of *Stipa* canopy resulted in 100% of *Bromus* plants grazed.

Discussion

Our findings from the experiment and the pattern analysis reinforced the little empirical evidence to date that grazing has a substantial impact on the interactions between plants in arid communities (Oesterheld and Oyarzabal 2004, Bisigato et al. 2005, Anthelme and Michalet 2009) and that it is a major driver of fine-scale plant distribution in arid environments (Bisigato et al. 2005). Grazing transformed competing grass neighbours into refuges (Fig. 3) and increased spatial aggregation amongst grass species (Fig. 1).

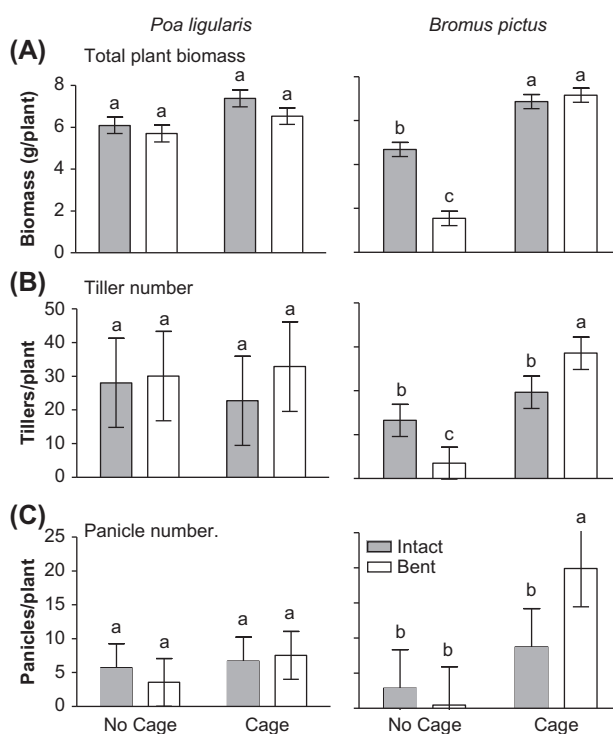


Figure 3. Performance of palatable species (*Poa* and *Bromus*) under treatments that combine the manipulation of benefactor canopy (intact and bent canopies) and herbivores (cage or no cage present). (A) total plant biomass, (B) tiller number and (C) panicle number. Data are given as adjusted means and 1 SE from ANCOVAs including all four treatment groups. The tiller number of target plants at the start of the experiment was used as a covariate (Table 1 in Supplementary material Appendix 1) Treatments with the same letter were not statistically different from one another (Tukey tests $\alpha < 0.05$)

However, as hypothesized, this effect was contingent on the differences in palatability of the interacting species.

Under grazing, associational resistance (AR) was the main interaction between species and the effect depended on the difference in palatability between benefactor and beneficiary species. Both palatable species (*Bromus* and *Poa*) were grazed less frequently when *Stipa* canopies remained intact than when canopies were bent away from them (Fig. 4). These results provided evidence of anti-herbivore protection being provided to palatable species (Graff et al. 2007). As predicted, maximum differences in palatability between species

resulted in a stronger AR effect. The beneficial indirect effect of *Stipa* was stronger for the most palatable species, *Bromus*. When exposed to grazing, the intact canopy of *Stipa* (benefactor) had a positive effect on *Bromus* growth, but not on *Poa*, presumably because *Bromus* had higher palatability than *Poa* (Bonvissuto et al. 1983, Graff et al. 2007).

Due to AR, a high proportion of palatable plants was found to be positively associated with less palatable neighbours in grazed paddocks (Fig. 1). In line with these patterns, plants located far from less palatable neighbours were most frequently grazed, while the plants that showed aggregation with less palatable neighbours were the ones that showed no signs of grazing damage (Fig. 2). For *Bromus* and *Poa*, the pattern of aggregation was intensified in moderately grazed paddocks according to the predictions (Fig. 1).

These positive interactions are not sufficient to counter the overall fact that herbivory had deleterious effects on plants (Belsky 1986). In Patagonian steppes, palatable species have declined as a result of over-stocking (Perelman et al. 1997, Cipriotti and Aguiar 2005a) which has led to a reduction in carrying capacity, and has compromised the sustainability of sheep production in this region (Texeira and Paruelo 2006). Palatable grasses have been lost from the system because they are not adapted to repeated defoliation (Graff and Aguiar unpubl.). *Bromus* is unable to recover its biomass after three defoliations and *Poa* could tolerate only a single defoliation within the growing season. Therefore, managing stocking rate is a key aspect for the existence of potential herbivory refuges.

Supporting the results of Smit et al. (2007) and Graff et al. (2007), positive indirect interactions did not increase with increased biotic stress (grazing intensity) as expected from the SGH. The results of pattern analysis supported the proposed hump-shaped curve, with AR being more important at intermediate grazing pressures. Aggregations between benefactor and beneficiary species increased in moderately grazed paddocks and decreased in intensively grazed paddocks as predicted. Our interpretation is that with more intensive grazing pressure, herbivores change their foraging behaviour, intensifying their search for palatable species and/or consuming species with lower palatability, reducing the AR effect (Baraza et al. 2006, Smit et al. 2007). The decline in species aggregations at the intensive grazing pressure depended also on species' relative palatability. Contrary to Smit et al. (2007) who predicted a convergence to zero of

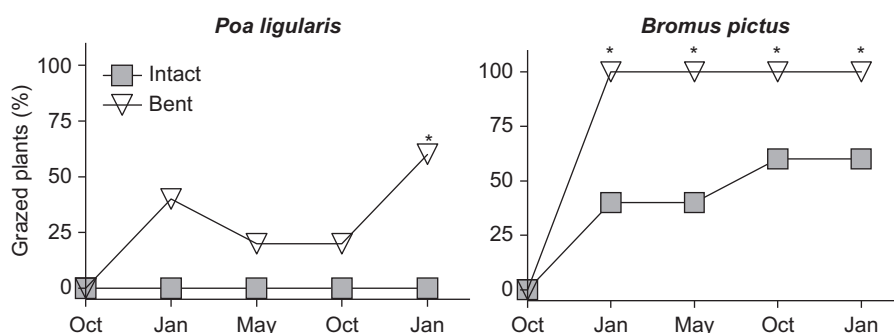


Figure 4. Effect of the *Stipa* canopy treatment on the proportion of grazed *Poaligularis* and *Bromuspictus* plants during the experimental period. Data are shown as the percentage of grazed plants per treatment. Asterisks show significant differences in the number of grazed plants between treatments at any one time (χ^2 -test, $p < 0.05$).

AR effects for all species, in our study, aggregations remained significant under intensive grazing for *Bromus–Stipa* (large difference in palatability), whereas the positive associations tended to disappear for *Poa–Stipa* (small difference in palatability) (Fig. 2). These results in spatial patterns are also consistent with the variation in the strength of indirect facilitation experienced by these species in a manipulative experiment (Graff et al 2007), where the AR effect of *Stipa* on transplanted *Bromus* plants remained significant at intense grazing pressures, whereas it disappeared for transplanted *Poa* plants.

The experiment reported here supported the presence of aboveground competition between grasses. Inside cages (herbivores absent), *Bromus* plants growing close to *Stipa* plants that has their canopy bent away, produced more tillers and panicles than plants growing close to *Stipa* with an intact canopy (Fig. 3). Aboveground competition is not usually considered important in arid ecosystems (Tilman 1982) and a previous experiment with transplants did not show aboveground competition with these same species (Graff et al. 2007). Since species interactions can change with the ontogenic stage of species (Schiffers and Tielbörger 2006), statements arising from this study about aboveground competition effects are limited to mature plants. A recent study reveals that in 47% of the reviewed experiments, the interaction balance between phylogenetically related taxa change from positive to negative with the advancing ontogenic stage of plants (Schiffers and Tielbörger 2006). This points to the importance of using different and complementary research protocols (i.e. different ontogenic stage of tested plants) for drawing valid conclusions about species' interaction effects on community dynamics and spatial distribution patterns (Schiffers and Tielbörger 2006).

Since direct facilitation was not demonstrated as an important interaction between grasses in the steppe, we suggest that other mechanisms may provide explanations for the aggregation between species found in exclosures. A phenomenon described in communities with sparse vegetation is the wind-driven accumulation of seed beneath tussock grasses, which could explain species aggregations (Aguilar and Sala 1997, Oesterheld and Oyarzabal 2004). This mechanism was also invoked to explain the existence of patches with high grass cover associated with shrubs in Patagonia (Aguilar and Sala 1994, 1997). It could also explain the high proportion of *Bromus* plants living in close proximity to tussock grasses after decades of grazing exclusion (Fig. 1, Oesterheld and Oyarzabal 2004), even when seedling survival is strongly constrained by competition (Cipriotti and Aguilar 2005b). We ran a test (data not shown) confirming that in exclosures, *Bromus* was also aggregated with other palatable grasses (*Poa*) whereas in grazing paddocks *Bromus–Poa* were randomly distributed. This ad-hoc analysis suggests that seed can accumulate around any type of tussock, and sheep grazing is able to erase this pattern (Oesterheld and Oyarzabal 2004).

From a rangeland conservation and management perspective, an important issue is the restoration of natural capital. In general, native forage species have decreased in intensively grazed pastures at the same time as less palatable species have increased in biomass and abundance (Milchunas and Lauenroth 1993). We found robust evidence about the positive role of AR in maintaining high quality for-

age species in grazed communities (i.e. refuge effect). However, the effect was contingent on grazing stress and difference between beneficiary species. Positive associations remained significant at high grazing pressures only when palatability differences between neighbours were large. Furthermore, AR could play a key role in restoration of threatened species as benefactor species can function as loci for propagules and recruitment of beneficiary species (Anthelme and Michalet 2009). Therefore, ranchers may consider that less palatable grass may reduce secondary production but, at the same time, may be fundamental in a low energy input plan for natural capital restoration (Aguilar and Román 2007).

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Supplementary material (available online as Appendix O19059 at <www.oikosoffice.lu.se/appendix>). Appendix 1.