

The small scale spatial pattern of C₃ and C₄ grasses depends on shrub distribution

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Abstract At micro-site scale, the spatial pattern of a plant species depends on several factors including interactions with neighbours. It has been seen that unfavourable effects generate a negative association between plants, while beneficial effects generate a positive association. In grasslands, the presence of shrubby species promotes a particular microenvironment beneath their canopy that could affect differently the spatial distribution of plants with different tolerance to abiotic conditions. We measured photosynthetic active radiation, air temperature and wind speed under shrub canopies and in adjacent open sites and analysed the spatial distribution of four grass species (two C₃ and two C₄) in relation to shrub canopy in a grazed sub-humid natural grassland in southern Uruguay. Radiation, air temperature and wind speed were lower under shrubs than in adjacent open sites. The spatial distribution of grasses relative to the shrub canopy varied depending on the photosynthetic metabolism of grasses. C₄ grasses showed a negative association or no correlation with the shrubs, whereas C₃ grasses showed a positive association. Our results highlight the importance of the photosynthetic metabolism of the grasses in the final outcome of interactions between grasses and shrubs. Micro-environmental conditions generated underneath shrubs create a more suitable site for the establishment of C₃ than for C₄ grasses. These results show that facilitation could be more important than previously thought in sub-humid grasslands.

Key words: C₃ and C₄ grasses, negative interactions, positive interactions, shrubs, South America, sub-humid grassland, Uruguay.

INTRODUCTION

In a given community, individuals of different plant species can exhibit a random or non-random spatial pattern (Fortin & Dale 2005). A large number of factors may regulate the spatial pattern of plants, and their relative importance varies with the scale of analysis (Greig-Smith 1983). Over small spatial scales (micro-site), species interactions are among the most important determinants of plant distribution (Fidelis *et al.* 2009). Such interactions comprise a set of negative and positive effects that plants exert on each other (Bertness & Callaway 1994). Negative effects due to competition for light, water and soil nutrients or the release of allelopathic compounds generate repulsion among plants (Rejmánek & Lepš 1996; Kikvidze *et al.* 2005). Positive effects (facilitation) due to the amelioration of harsh abiotic conditions or protection from herbivores generate attraction among plants (Oosterheld & Oyarzabal 2004; Tirado & Pugnaire 2005). It has been hypothesized that the relative

importance of competition and facilitation may vary inversely along a gradient of abiotic stress (Bertness & Callaway 1994). The importance of competition is predicted to increase toward the most benign end of the abiotic gradient, while facilitation is predicted to decrease. Thus, most studies related to facilitation have been conducted in stressful environments, such as water limited ecosystems (Graff *et al.* 2007; Gómez-Aparicio 2009; Holmgren *et al.* 2012).

In plants, photosynthetic metabolism affects the tolerance of individuals to abiotic conditions (Lambers *et al.* 2008). As in the C₄ metabolism photorespiration is virtually eliminated, C₄ plants have higher photosynthetic rates than C₃ plants at high temperatures. Moreover, C₄ plants have higher water use efficiency than C₃ plants because at a given photosynthetic rate they have lower stomatal conductance (Sage 2004). The evolutionary emergence of the C₄ pathway in grasses (Poaceae) has been identified as an adaptation to drier conditions (Pagani *et al.* 1999; Edwards & Still 2008). C₄ grasses rarely colonize forest habitats where less light and lower temperatures prevail, while C₃ grasses are favoured in shady habitats as they tolerate low light intensities (Sage *et al.* 1999). Many studies

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Accepted for publication November 2013.

have shown that, under the canopy of trees and shrubs, soil and air temperatures are lower, wind speed is lower and air humidity is higher than in the open (Geiger 1965; Larcher 1983; Chen *et al.* 1995). In contrast, light availability increases from the canopy to the open sites (Holmgren *et al.* 1997). Thus, the micro-environmental heterogeneity generated by neighbouring plants could promote different responses in co-occurring C₃ and C₄ grasses. So, the interplay between photosynthetic metabolism and species interactions may influence the small scale spatial distribution of grasses.

Grasses and shrubs coexist over a broad range of abiotic conditions, from cool semi-arid steppes to temperate sub-humid grasslands. The presence of shrubs generates a micro-environment underneath its canopy characterized by a reduced solar radiation, air temperature and wind speed compared with open sites (Holmgren *et al.* 1997). This micro-environment has important consequences on the growth and development of grasses. In semi-arid steppes, germination and establishment of individuals are almost restricted to these sites (Aguilar & Sala 1994). The shelter provided by shrubs decreases grass seedling mortality due to a reduction of water stress and photochemical damage (Aguilar & Sala 1994; Armas & Pugnaire 2005). Therefore, these beneficial effects generate a positive association between grasses and shrubs in these sites (Armas & Pugnaire 2005). In contrast, in sub-humid grasslands the presence of shrubs reduces grass growth and productivity (Lett & Knapp 2003; Pezzani *et al.* 2011). These negative effects are due to the reduction of solar radiation that limits the photosynthetic activity of plants (Briggs *et al.* 2005). Although some evidence suggests that shrubs exert a negative effect on grasses productivity, to our knowledge there is no study that has analysed the spatial pattern of grasses and shrubs in sub-humid grasslands.

Uruguayan grasslands belong to Rio de la Plata grasslands, one of the largest areas of sub-humid grasslands of South America (Soriano 1991). These grasslands are a mixture of C₃ and C₄ grasses (Paruelo *et al.* 1998; Epstein *et al.* 2002). Shrubs are also abundant and may become locally dominant (Paruelo *et al.* 2007). The presence of shrubs scattered in a matrix of C₃ and C₄ grasses provides an ideal system to study the role that positive interactions among plants play in community structure.

In this paper we analysed the spatial distribution of the C₃ grasses *Piptochaetium stipoides* and *Melica brasiliana* and the C₄ grasses *Paspalum dilatatum* and *Coelorachis selloana* relative to the shrub *Eupatorium buniifolium* in a grazed sub-humid natural grassland in the south-central region of Uruguay. We expected to observe a positive association between C₃ grasses and shrubs and a negative association between C₄ grasses and shrubs.

METHODS

The study site is located in the Southern Campos of the Rio de la Plata grasslands, in south-central Uruguay (31°54'S, 58°15'W). For the 2000–2009 period, mean annual precipitation was 1368 mm and mean annual temperature was 16.6°C, ranging from a mean of 6.5°C in July to 29°C in January (INIA 2012). In this region, cattle grazing promotes a two strata plant community, a lower and dense stratum of prostrated C₄ grasses and rosette herbs, and a taller one dominated by erect grasses (C₃ and C₄) and shrubs (Soriano 1991; Altesor *et al.* 2006).

Studied species

Eupatorium buniifolium (Compositae) is a perennial, medium-tall (1–2 m height) native shrub which produces many stems from a subterranean woody xilopod. Under grazing, their canopy can be described as an inverted cone with lax foliage at the top of the stems. In this region the mean cover of individuals of this species is 0.15 m², ranging from 0.01 m² to 0.7 m². *Coelorachis selloana* and *P. dilatatum* are perennial warm season erect bunch grasses with C₄ metabolism. Under grazing, bunches of *C. selloana* reach a maximum height of 0.15 m and a mean basal cover of 0.0016 m². *Paspalum dilatatum* adopt a semi-prostrate habit, reaching a maximum height of 0.1 m and a mean basal cover of 0.0064 m². *Melica brasiliana* and *P. stipoides* are perennial cool season erect bunch grasses with C₃ metabolism. Their leaves are narrower and longer than those of *C. selloana* and *P. dilatatum* and are formed by few tillers with a maximum height of 0.3 m and a mean basal cover of 0.0004 m² under grazing. The C₄ grasses used in this study belong to Panicoideae and the C₃ to Pooideae sub-families of Poaceae. Together, these subfamilies account for the 75% of the 253 grass species registered in Uruguayan grasslands (Cayssials & Rodríguez 2013).

Abiotic conditions

To analyse the effect of the presence of shrubs on abiotic conditions, we measured, from February to December of 2010, photosynthetic active radiation (PAR), air temperature and wind speed. All variables were measured at midday above the shrub canopy (2 m height) and at ground level (0.1 m height) in 24 shrubs and in their respective paired open sites. PAR was measured seasonally using a hand-held sensor model Cavadevices. Temperature and wind speed were measured every two months using a hand-held sensor model Kestrel 2000. We also calculated the variation between abiotic conditions above the shrub canopy and at ground level (below and outside shrubs) as follows: (value at 0.1 m – value at 2 m/value at 2 m) × 100. Thus, positive values represent a percentage of increase in radiation, temperature or wind speed at ground level, relative to the value above shrubs, whereas negative values represent a percentage of decrease. Data were compared by Mann–Whitney *U*-test (at a significance level of 5%) using Infostat software package (Di Rienzo *et al.* 2010).

Plant spatial distribution analysis

For a detailed description of the spatial pattern of grasses and shrubs, we delimited a homogeneous area of 180 m² (9 m × 20 m) and determined the coordinates (*x,y*) of all individuals of interest in December of 2010. The bivariate point patterns of plants generated were analysed using Ripley's *L* function (a transformation of Ripley's *K* function, that stabilizes variance and facilitates interpretation) and the *O*-ring statistic (related to the pair correlations function *g(r)*) (Goreaud & Pélissier 2003; Wiegand & Moloney 2004; Baddeley 2010). Both statistics are based on the analysis of the distance between pairs of points (shrub-grass) allowing us to detect the type of spatial association and the distance at which it occurs (Wiegand & Moloney 2004). Ripley's *K*₁₂ function considers each shrub as the centre of a circle of radius '*r*' and counts the number of individuals of a grass species within it. The *O*-ring statistic does not use a ring, but a circle of radius '*r*' and width '*dr*', and therefore considers the individuals of a grass species at a distance '*r*' from the shrub (Wiegand & Moloney 2004). We used the toroidal shift method to correct for the edge effects (Haase 1995). The values obtained were compared against the null model of spatial independence of each shrub-grass pair. To test the null hypothesis, we constructed confidence envelopes obtained from the highest and lowest values of the functions from 1999 Monte Carlo simulations, corresponding to a significance level of 0.001. Thus, we tested whether individuals of a grass species were randomly distributed around shrubs, or present a pattern (positive or negative association). *L(r)* and *O(r)* values above and beneath the derived confidence limits indicate positive and negative association respectively.

RESULTS

Abiotic conditions

Abiotic conditions under shrub canopies were different from those in adjacent open sites. Plants growing underneath shrubs received 55% less radiation than those growing in open sites. Air temperature at ground level (0.1 m height) increased in relation to air temperature above the shrub canopy (2 m height), but this increment was higher in open sites (5.83%) than

under shrubs (3.13%). Differences in air temperature between micro-sites were significant during spring and summer months. On the other hand, wind speed at ground level decreased in both sites, in relation to wind speed above the shrub canopy, but it decreased more under shrubs than in open sites (76.44% vs. 66.26% respectively) (Table 1).

Plant spatial distribution analysis

A total of 2569 individuals were mapped: 94 individuals (0.52 individuals per m²) of *E. buniifolium*; 1680 individual bunches (9.33 individuals per m²) of *C. selloana*; 233 (1.29 individuals per m²) of *P. dilatatum*; 116 (0.64 individuals per m²) of *M. brasiliiana* and 446 (2.47 individuals per m²) of *P. stipoides*. Bivariate spatial point patterns of the four grasses and the shrub are shown in Appendix S1.

As we expected, the spatial distribution of grasses relative to the shrub canopy differed between C₃ and C₄ grasses. At small distances, C₄ grasses were negatively associated or randomly distributed relative to the shrubs, whereas C₃ grasses were positively associated.

The spatial association found between *C. selloana* and *E. buniifolium* varied according to the distance analysed. Ripley's *L* function showed that there was a negative association from the centre of the shrub up to 40 cm and from 50 cm to 90 cm and randomness at larger distances (Fig. 1a). The *O*-ring statistic also showed a negative association, but from 15 to 25 cm, with a peak at 25 cm, and randomness at smaller and larger distances (Fig. 1c). The spatial distribution of *P. dilatatum* relative to *E. buniifolium* using Ripley's *L* function indicated a random distribution at all distances analysed (Fig. 1b). On the other hand, the *O*-ring statistic indicated a marginal positive association at distances between 155 and 160 cm and randomness at smaller and larger distances (Fig. 1d).

The spatial association found between *M. brasiliiana* and *E. buniifolium* varied according to the distance analysed. Ripley's *L* function, showed positive association from a distance of around 10–40 cm and

Table 1. Effects of shrub canopy on abiotic conditions

Variable	Period compared	Site		<i>P</i> -value
		Under shrubs	Without shrubs	
PAR (mmol m ⁻² s ⁻¹)	Annual	378 ± 72.8	843 ± 34.2	<0.0001
Air temperature (°C)	Spring-summer	31.6 ± 0.66	34.1 ± 1.0	0.044
	Autumn-winter	11.2 ± 0.18	11.3 ± 0.25	0.89
Wind speed (m s ⁻¹)	Annual	0.8 ± 0.08	1.1 ± 0.1	0.009

Mean (± SE) of the incoming photosynthetic active radiation (PAR), air temperature and wind speed measured at the herbaceous layer (0.1 m height) below shrub canopy and in paired open sites (*n* = 24). Mann–Whitney *U*-test were performed and significant differences (*P* < 0.05) are shown in bold.

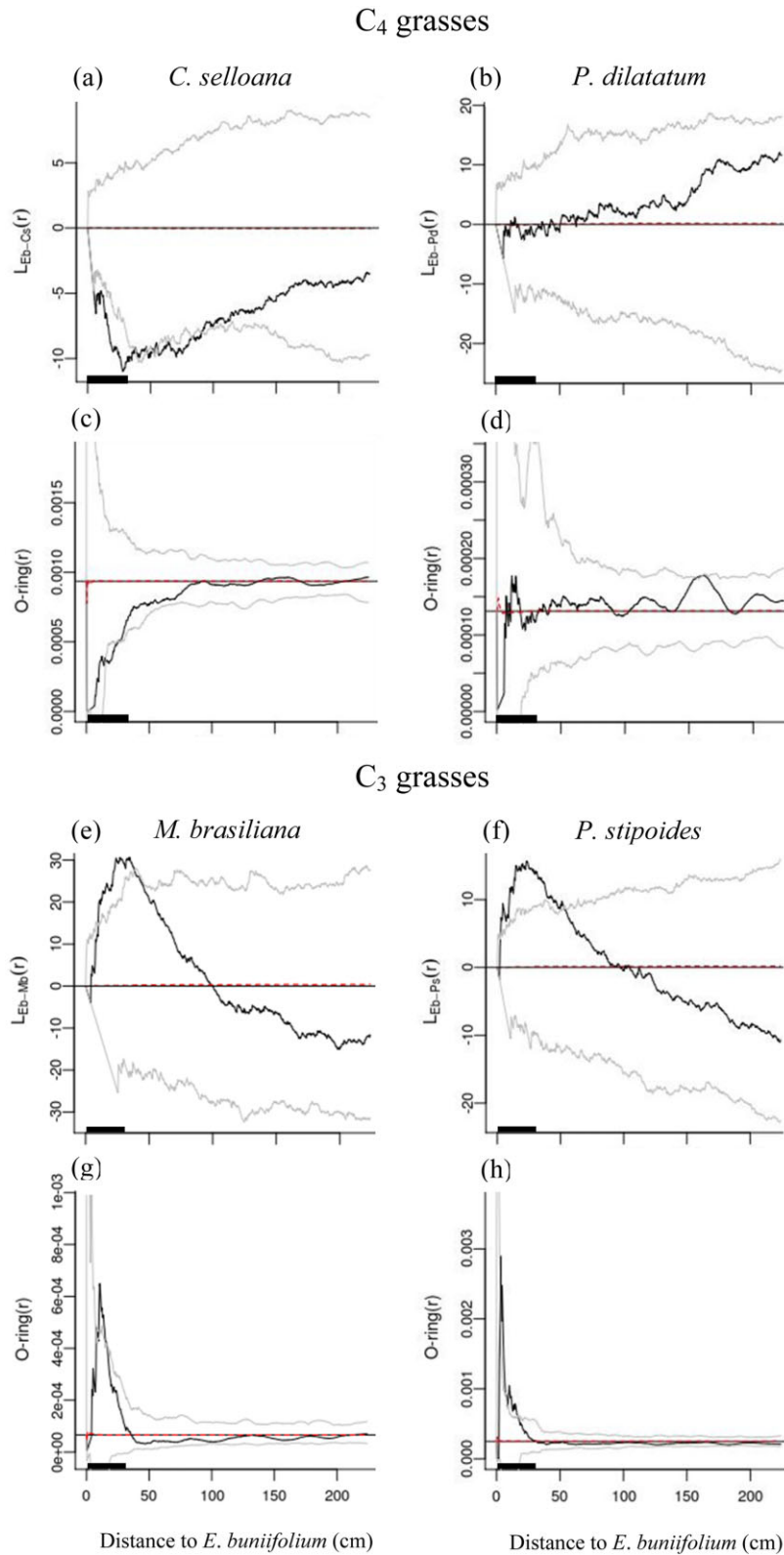


Fig. 1. Spatial association found between the C₄ grasses *C. selloana* and *P. dilatatum* (a–d) and the C₃ grasses *M. brasiliana* and *P. stipoides* (e–h) with the shrub *Eupatorium buniifolium* in a sub-humid grassland of Uruguay. Grey lines indicate 99.9% upper and lower confidence limits. Values above upper limits indicate positive association between the grass species and the shrub, values between upper and lower confidence envelope indicate no spatial association and values below lower limits of the confidence limit indicate negative association. Black rectangle indicates the medium size (radii) of shrub canopy.

randomness at smaller and larger distances (Fig. 1e), while the results obtained with *O*-ring statistic showed positive association but for distances between 8 and 15 cm, with a peak at 15 cm and randomness at smaller and larger distances (Fig. 1g). The spatial distribution of *P. stipoides* relative to *E. buniifolium* also varied according to the distance analysed. Ripley's *L* function showed positive association from 3 to 55 cm and randomness for smaller and larger distances (Fig. 1f), while the results from the *O*-ring statistic showed a positive association for distances up to 20 cm, with a peak at 15 cm, and randomness for distances larger than 20 cm (Fig. 1h).

DISCUSSION

Our results provide evidence that positive interactions among plants are also important for community structure in temperate sub-humid grasslands. At short distances, the net outcome of the interaction between shrubs and grasses depends strongly on the photosynthetic pathway of the grasses. The negative association or random distribution that we found between *C. selloana* and *P. dilatatum* (C_4 grasses) and shrubs, suggests either a net negative or neutral effect of shrubs on C_4 grasses. In contrast, *M. brasiliiana* and *P. stipoides* (C_3 grasses) were positively associated with the shrubs, suggesting a facilitation mechanism.

The negative association found between *C. selloana* and *E. buniifolium* indicated a net negative effect of shrubs on this species at micro-site scale (Rejmánek & Lepš 1996). This negative effect could be due to two non-exclusive causes. One of them is the reduction of solar radiation underneath the shrub canopy. Light limitation has been invoked as one of the major factors that limit growth and productivity of herbaceous plants in sub-humid grasslands (Briggs *et al.* 2005; Pezzani *et al.* 2011). For example, Lett and Knapp (2003) found that the reduction of incident light under the shrub *Cornus drummondii* was the main constraint for the growth of the C_4 grass *Andropogon gerardii*. The intolerance of *C. selloana* to shade may be due to the high energetic cost for CO_2 fixation related to C_4 metabolism, compared with C_3 metabolism. This high energetic requirement would be offset by a high rate of photosynthesis, which could be reached in sites that are well exposed to sunlight (Sage 2004). In a greenhouse experiment (unpubl. data), we observed that for the two C_4 grasses evaluated (*Axonopus affinis* and *C. selloana*) the relative growth rate (RGR) was particularly sensitive to a reduction in light availability. For *C. selloana*, the RGR under simulated shadow was 68% lower than under full irradiance. Another explanation could be competition for soil resources such as water. Different studies have shown the negative effect of competition for soil resources between grasses and

shrubs (Sala *et al.* 1989; Peltzer & Köchy 2001; Maestre *et al.* 2003) and between grasses and trees (Picon-Cochard *et al.* 2001; Ludwig *et al.* 2004; Simmons *et al.* 2008), suggesting that belowground competition could be an important interaction between grasses and woody plants.

On the other hand, the distribution of *P. dilatatum* showed a lack of correlation with *E. buniifolium*. This random distribution suggests a neutral net effect of the shrub on the growth of this species (Kikvidze *et al.* 2005). As in the case of *C. selloana*, the reduction of solar radiation could be an important negative effect of shrubs on the growth of this grass species. In a shrub removal experiment installed at the same sampling site, Rossado (2011) found that the density of *P. dilatatum* increased in plots where shrubs had been removed. Moreover, McDaniel and Ostertag (2010) found that the RGR of *P. dilatatum* was directly dependent on light availability. These results suggest that light availability is a limiting factor to the growth of *P. dilatatum*. However, there must be a positive effect of the shrub that counteracts the negative effect of the reduction of solar radiation. We conjecture that this positive effect could be the reduction of temperature and wind speed under shrubs that lowers the water demand of individuals growing underneath it (Holmgren *et al.* 1997). Although we do not have a direct measure of the water status of this species, individuals of *Paspalum notatum* growing associated with *E. buniifolium*, had higher midday leaf water potential than individuals growing in open sites (Fernández 2008). Another explanation for the differences in the spatial distribution of *C. selloana* and *P. dilatatum* could be related to the protection exerted by shrubs against frosts. In this sense, Cavaco *et al.* (2003) found that long term exposure of *P. dilatatum* plants to low temperatures reduced to almost half the RGR and biomass production compared to control plants.

In contrast to the spatial patterns found for the C_4 species, *P. stipoides* and *M. brasiliiana* (C_3 grasses) showed a positive association with *E. buniifolium* at small scale (micro-site). This pattern indicates a net positive effect of the shrub on the growth of these grasses. This result agrees with the observation that the density of *P. stipoides* decreased in plots where shrubs were removed (Rossado 2011). This positive effect could be promoted by the reduction of solar radiation, air temperature and wind speed under the shrub canopy that ameliorates abiotic conditions for C_3 grasses (Holmgren *et al.* 1997; Armas & Pugnaire 2005). In grazed sites, solar radiation, air temperature and wind speed at ground level are higher, while water availability is lower than in ungrazed sites where C_3 grass species dominate (Altesor *et al.* 2006). These changes occur because cattle grazing 'open' the canopy of vegetation, allowing the solar radiation to reach the soil surface, generating an increase in temperature and

wind speed. So, abiotic conditions of grazed sites could be very stressful for the C_3 grasses, particularly under high summer temperatures, and the shelter provided by shrubs could be important for their growth and survival. The attenuation of the stressful conditions under the shrub canopy could have two positive effects on C_3 grasses. One of them is a reduction of photorespiration (loss of CO_2 fixed during Calvin cycle) and the other is a reduction of water demand (Sage 2004). The amelioration of abiotic conditions has been previously described in arid and semi-arid environments, where the shelter provided by shrubs enhances grasses survival and growth (Callaway 1995; Callaway & Walker 1997). Although, it has been argued that facilitation dominates in harsh environments, our results support the idea that facilitation could be more important than previously thought in more benign environments (Holmgren & Scheffer 2010).

It is important to point out that grazing could directly affect the spatial pattern of the C_3 grasses too. For example, the spatial association found between *M. brasiliana* and *P. stipoides* with the shrub, could be the result of amelioration of abiotic conditions and protection against grazing. It has been reported that cattle grazing dramatically reduces C_3 grasses cover in Uruguayan natural grasslands (Rodríguez *et al.* 2003; Altesor *et al.* 2005) and several studies have shown that under grazing unpalatable plants reduce the grazing pressure on its palatable neighbors (Oesterheld & Oyarzabal 2004; Graff *et al.* 2007; Fidelis *et al.* 2009). However, we believed that *E. buniifolium* is not a good 'anti-grazing barrier'. In general, unpalatable plants have some type of defence mechanism such as thorns, leaf toughness or toxicity (Callaway *et al.* 2000, 2005; Fidelis *et al.* 2009) but *E. buniifolium* has none of these features and is even grazed by cattle when young (Altesor *et al.* 2006). Moreover, the spatial pattern found between *C. selloana* with the shrub was negative, although this grass species is highly palatable to cattle and its density decreased at grazed sites (Altesor *et al.* 2005). This evidence suggests that the modification of abiotic conditions under the shrub is the main mechanism that affects the spatial patterns of grasses in these grasslands. To critically evaluate the role of shrubs as herbivory refuges, the spatial analysis performed in this study should be repeated within a grazing enclosure. However the structural changes that took place in the first 2–3 years of such an enclosure (increase in the density and cover of shrubs, Rodríguez *et al.* 2003; Altesor *et al.* 2006; Lezama *et al.* 2014) precluded such study because of the virtual absence of patches without shrubs within the enclosure. Low radiation, air temperature and wind speed (relative to above the canopy) are the prevalent conditions in ungrazed areas. As a general pattern the relative

importance of C_3 grasses increases in ungrazed areas (Rodríguez *et al.* 2003; Altesor *et al.* 2006; Lezama *et al.* 2014).

Our results show the interplay between plant interactions and photosynthetic metabolism on the spatial distribution of plants. The heterogeneity generated by the presence of shrubs, would make the micro-environment under shrubs more suitable for C_3 than for C_4 grasses. By facilitating the growth of C_3 grasses, shrubs could generate a 'mass effect' that promotes the establishment of grasses in sites where environmental conditions are not favourable, increasing the local richness of species. This effect could be very important in natural grasslands, as the loss of quality forage is a serious problem for cattle production and C_3 grasses provide high quality forage due to their low C/N relationship.

Our results have both theoretical and applied implications. From an ecological theory perspective, they highlight the importance of grasses photosynthetic metabolism in the final outcome of interactions between grasses and shrubs. The net effect of a plant on another has been predicted to change along an environmental gradient of abiotic stress (Bertness & Callaway 1994; Holmgren *et al.* 1997), grazing pressure (Graff *et al.* 2007) and at different life stages (Aguiar & Sala 1998). However, under the same abiotic conditions and grazing pressure, the net effect of a plant on another could also depend on ecophysiological features of the plants (e.g. photosynthetic metabolism). From a management point of view, our study stresses the importance of shrubs on the distribution of C_3 grasses under grazing conditions. Shrubs are considered undesirable plants in grasslands and their removal is a very usual management practice because people believe that they have a negative effect on growth of the grasses. While C_4 grasses have a tendency to 'avoid' shrubs, caution must be taken in this belief, because C_3 grass growth is associated with them. In these grasslands, total eradication of shrubs will promote an environmental homogeneity that could result in the loss of richness and high quality forage species.

ACKNOWLEDGEMENTS

This work was partially supported by the Agencia Nacional de Investigación e Innovación (ANII) and the Programa de Desarrollo de las Ciencias Básicas (PEDECIBA). We thank to Guillermo Macció and Mabel Bertolotto, owners of the study site, for their hospitality and logistical support. To Claudia Rodríguez and Federico Gallego for helped us with field work. We thank to Claudia Rodríguez and José M. Paruelo for useful suggestions and comments on the manuscript. We thank anonymous reviewers for

comments on previous drafts of the manuscript. Juan M. Rosa Machado kindly helped us with English translation.

REFERENCES

- Aguilar M. R. & Sala O. E. (1994) Competition, facilitation, seed distribution, and the origin of patches in a Patagonian steppe. *Oikos* **70**, 26–34.
- Aguilar M. R. & Sala O. E. (1998) Interactions among grasses, shrubs and herbivores in Patagonian grass-shrub steppes. *Ecol. Aust.* **8**, 201–10.
- Altesor A., Oesterheld M., Leoni E., Lezama F. & Rodríguez C. (2005) Effect of grazing on community structure and productivity of a Uruguayan grassland. *Plant Ecol.* **179**, 83–91.
- Altesor A., Piñeiro G., Lezama F., Jackson R. B., Sarasola M. & Paruelo J. M. (2006) Ecosystem changes associated with grazing in subhumid South American grasslands. *J. Veg. Sci.* **17**, 323–32.
- Armas C. & Pugnaire F. I. (2005) Plant interactions govern population dynamics in a semi-arid plant community. *J. Ecol.* **93**, 978–89.
- Baddeley A. (2010) Analysing spatial point patterns in R. Workshop notes. CSIRO Online Technical Publication. [Cited December 2012.] Available from URL: <http://www.csiro.au/resources/Spatial-Point-Patterns-in-R>
- Bertness M. D. & Callaway R. M. (1994) Positive interactions in communities. *Trends Ecol. Evol.* **9**, 191–3.
- Briggs J. M., Knapp A. K., Blair J. M. *et al.* (2005) An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. *Bioscience* **55**, 243–54.
- Callaway R. M. (1995) Positive interactions among plants. *Bot. Rev.* **61**, 306–49.
- Callaway R. M., Kikodze D., Chiboshvili M. & Khetsuriani L. (2005) Unpalatable plants protect neighbors from grazing and increase plant community diversity. *Ecology* **86**, 1856–62.
- Callaway R. M., Kikodze D. & Kikvidze Z. (2000) Facilitation by unpalatable weeds may conserve plant diversity in overgrazed meadows in the Caucasus Mountains. *Oikos* **89**, 275–82.
- Callaway R. M. & Walker L. R. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* **78**, 1958–65.
- Cavaco A., Bernardes da Silva A. & Arrabaça M. C. (2003) Effects of long-term chilling on growth and photosynthesis of the C₄ gramineae *Paspalum dilatatum*. *Physiol. Plant.* **119**, 87–96.
- Cayssials V. & Rodríguez C. (2013) Functional traits of grasses growing in open and shaded habitats. *Evol. Ecol.* **27**, 393–407.
- Chen J., Franklin J. F. & Spies T. A. (1995) Growing-season microclimatic gradients from clearcut edges into old-growth Douglas-fir forests. *Ecol. Applic.* **5**, 74–86.
- Di Rienzo J. A., Casanoves F., Balzarini M. G., González L., Tablada M. & Robledo C. W. (2010) InfoStat versión 2010. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
- Edwards E. J. & Still C. J. (2008) Climate, phylogeny and the ecological distribution of C₄ grasses. *Ecol. Lett.* **11**, 266–76.
- Epstein H. E., Gill R. A., Paruelo J. M., Lauenroth W. K., Jia G. J. & Burke I. C. (2002) The relative abundance of three plant functional types in temperate grasslands and shrublands of North and South America: effects of projected climate change. *J. Biogeogr.* **29**, 875–88.
- Fernández G. (2008) Efecto de los arbustos sobre el estrato gramíneo y comportamiento hídrico de los Tipos Funcionales de Plantas en pastizales naturales (Thesis Licenciatura en Ciencias Biológicas). Facultad de Ciencias (UDELAR), Montevideo, Uruguay.
- Fidelis A., Overbeck G. E., Pillar V. D. & Pfadenhauer J. (2009) The ecological value of *Eryngium horridum* in maintaining biodiversity in subtropical grasslands. *Austral Ecol.* **34**, 558–66.
- Fortin M.-J. & Dale M. (2005) *Spatial Analysis: A Guide for Ecologists*. Cambridge University Press, Cambridge.
- Geiger R. (1965) *The Climate Near the Ground*. Harvard University Press, Cambridge, MA.
- Gómez-Aparicio L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta analysis across life-forms and ecosystems. *J. Ecol.* **97**, 1202–14.
- Goreaud F. & Péliissier R. (2003) Avoiding misinterpretation of biotic interactions with the intertype K_{12} -function: population independence vs. random labelling hypotheses. *J. Veg. Sci.* **14**, 681–92.
- Graff P., Aguiar M. R. & Chaneton E. J. (2007) Shifts in positive and negative plant interactions along a grazing intensity gradient. *Ecology* **88**, 188–99.
- Greig-Smith P. (1983) *Quantitative Plant Ecology*, 3rd edn. Blackwell, Oxford.
- Haase P. (1995) Spatial pattern analysis in ecology based on Ripley's K -function: introduction and methods of edge correction. *J. Veg. Sci.* **6**, 575–82.
- Holmgren M., Gómez-Aparicio L., Quero J. L. & Valladares F. (2012) Non-linear effects of drought under shade: reconciling physiological and ecological models in plant communities. *Oecologia* **169**, 293–305.
- Holmgren M. & Scheffer M. (2010) Strong facilitation in mild environments: the stress gradient hypothesis revisited. *J. Ecol.* **98**, 1269–75.
- Holmgren M., Scheffer M. & Huston M. A. (1997) The interplay of competition and facilitation in plant communities. *Ecology* **78**, 1966–75.
- INIA (2012) Instituto Nacional de Investigación Agropecuaria. [Cited December 2012.] Available from URL: <http://www.inia.org.uy/online/site/106664I1.php>
- Kikvidze Z., Pugnaire F. I., Brooker R. W. *et al.* (2005) Linking patterns and processes in alpine plant communities: a global study. *Ecology* **86**, 1395–408.
- Lambers H., Chapin F. S. I. & Pons T. L. (2008) *Plant Physiological Ecology*, 2nd edn. Springer, New York.
- Larcher W. (1983) *Physiological Plant Ecology*, 2nd edn. Springer-Verlag, New York.
- Lett M. S. & Knapp A. K. (2003) Consequences of shrub expansion in mesic grassland: resource alterations and graminoid responses. *J. Veg. Sci.* **14**, 487–96.
- Lezama F., Baeza S., Altesor A., Cesa A., Chaneton E. J. & Paruelo J. M. (2014) Variation of grazing-induced vegetation changes across a large-scale productivity gradient. *J. Veg. Sci.* **25**, 8–21.
- Ludwig F., Dawson T. E., Prins H. H. T., Berrendes F. & de Kroon H. (2004) Below ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. *Ecol. Lett.* **7**, 623–31.
- McDaniel S. & Ostertag R. (2010) Strategic light manipulation as a restoration strategy to reduce alien grasses and encourage native regeneration in Hawaiian mesic forests. *Appl. Veg. Sci.* **13**, 280–90.

- Maestre F. T., Bautista S. & Cortina J. (2003) Positive, negative, and net effects in grass–shrub interactions in Mediterranean semiarid grasslands. *Ecology* **84**, 3186–97.
- Oesterheld M. & Oyarzabal M. (2004) Grass-to-grass protection from grazing in a semi-arid steppe: facilitation, competition, and mass effect. *Oikos* **107**, 576–82.
- Pagani M., Freeman K. H. & Arthur M. A. (1999) Late Miocene atmospheric CO₂ concentrations and the expansion of C₄ grasses. *Science* **285**, 876–9.
- Paruelo J. M., Jobbágy E. G., Oesterheld M., Golluscio R. A. & Aguiar M. R. (2007) Grasslands and steppes of Patagonia and the Río de la Plata plains. In: *The Physical Geography of South America* (eds T. T. Veblen, K. R. Young & A. R. Orme) pp. 232–48. Oxford University Press, Oxford.
- Paruelo J. M., Jobbágy E. G., Sala O. E., Lauenroth W. K. & Burke I. C. (1998) Functional and structural convergence of temperate grassland and shrubland ecosystems. *Ecol. Applic.* **8**, 194–206.
- Peltzer D. & Köchy M. (2001) Competitive effects of grasses and woody plants in mixed- grass prairie. *J. Ecol.* **89**, 519–27.
- Pezzani F., Baeza S. & Paruelo J. M. (2011) Efecto de los arbustos sobre el estrato herbáceo de pastizales. In: *Bases Ecológicas y Tecnológicas Para el Manejo de Pastizales, Serie: FPTA N°26* (eds A. Altesor, W. Ayala & J. M. Paruelo) pp. 195–207. INIA, Montevideo, Uruguay.
- Picon-Cochard C., Nsourou-Obame A., Collet C., Guehl J. M. & Ferhi A. (2001) Competition for water between walnut seedlings (*Juglans regia*) and rye grass (*Lolium perenne*) assessed by carbon isotope discrimination and δ¹⁸O enrichment. *Tree Physiol.* **21**, 183–91.
- Rejmánek M. & Lepš J. (1996) Negative association can reveal interspecific competition and reversal of competitive hierarchies during succession. *Oikos* **76**, 161–8.
- Rodríguez C., Leoni E., Lezama F. & Altesor A. (2003) Temporal trends in species composition and plant traits in natural grasslands of Uruguay. *J. Vég. Sci.* **14**, 433–40.
- Rossado A. (2011) Efecto del arbusto *Eupatorium buniifolium* sobre la composición florística del estrato herbáceo en un pastizal natural (Thesis Licenciatura en Ciencias Biológicas). Facultad de Ciencias (UDELAR), Montevideo, Uruguay.
- Sage R. F. (2004) The evolution of C₄ photosynthesis. *New Phytol.* **161**, 341–70.
- Sage R. F., Wedin D. A. & Li M. R. (1999) The biogeography of C₄ photosynthesis: patterns and controlling factors. In: *C₄ Plant Biology* (eds R. F. Sage & R. K. Monson) pp. 313–73. Academic Press, San Diego.
- Sala O. E., Golluscio R. A., Lauenroth W. K. & Soriano A. (1989) Resource partitioning between shrubs and grasses in the Patagonian steppe. *Oecologia* **81**, 501–5.
- Simmons M. T., Archer S. R., Teague W. R. & Ansley J. R. (2008) Tree (*Prosopis glandulosa*) effects on grass growth: an experimental assessment of above- and belowground interactions in a temperate savanna. *J. Arid Environ.* **72**, 314–25.
- Soriano A. (1991) Río de la Plata grasslands. In: *Natural Grasslands. Introduction and Western Hemisphere* (ed. R. T. Coupland) pp. 367–407. Elsevier, Amsterdam.
- Tirado R. & Pugnaire F. I. (2005) Community structure and positive interactions in constraining environments. *Oikos* **111**, 437–44.
- Wiegand T. & Moloney K. A. (2004) Rings, circles, and null-models for point pattern analysis in ecology. *Oikos* **104**, 209–29.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Spatial point pattern of four grasses and a shrub species in a grassland study plot.