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ARTICLE

Decoupling facilitative effects in a temperate subhumid grassland: photosynthetic metabolism matters

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ABSTRACT

Background: Abiotic microhabitat amelioration and protection from herbivores are two of the main mechanisms associated with facilitative interactions in plant communities.

Aims: We investigated the effect of the shrub *Acanthostyles buniifolius* on the herbaceous community of a subhumid grassland in order to disentangle the protective role of the shrub from its effect on ameliorating the abiotic conditions of microhabitats.

Methods: In two contiguous areas, one continuously grazed and the other excluded from livestock for 3 years, we determined floristic composition and species cover under the shrub canopy and in paired open sites. We calculated the Relative Interaction Index (RII) for cover of plant functional types and species.

Results: Under grazing, C_3 grass richness was higher under the shrub canopy than in open plots. Also, the RII was higher in the grazed area for most functional types. Inside the exclosure, the cover of C_3 grasses was higher in the presence of the shrub. Conversely, the richness and cover of C_4 grasses was lower under the shrub canopy.

Conclusions: We found evidence of facilitation in a subhumid grassland. The shrub may protect grasses from grazers and may benefit C_3 grasses through microclimatic amelioration. Therefore, photosynthetic pathway should be considered when analysing facilitative interactions in mesic environments.

Introduction

Understanding the role of plant–plant interactions on community structure and dynamic is a central goal in plant ecology (Michalet et al. 2015). It has been widely documented that plants may exert negative or positive effects on their neighbours, through a complex balance that involves competition and facilitation, respectively (Bertness and Callaway 1994; Bruno et al. 2003; McIntire and Fajardo 2014). Historically, most empirical studies and theoretical models have focused on competition as the main driver of plant distribution and community structure (Hairston et al. 1960; Schoener 1983; Craine and Dybzinski 2013). However, during the last few decades, a growing body of descriptive and experimental evidence have emphasised the role of facilitation as an important and ubiquitous process affecting plant community richness, composition, and dynamics (Hunter and Aarssen 1988; Stachowicz 2001; Le Bagousse-Pinguet et al. 2014).

Shrub species are among the best-known examples of benefactor plants (Filazzola and Lortie 2014). A benefactor plant can facilitate the growth and survival of its neighbours through modifications of abiotic conditions (abiotic microhabitat amelioration) and protection from grazing (biotic refuge effects). Abiotic microhabitat amelioration includes the reduction of solar irradiance and wind speed, as well as the buffering of extreme temperatures as compared to surrounding open areas (Xu et al. 2010; Fernández et al. 2014; Mihoč et al. 2016), which ultimately reduce photo-inhibition and water stress for some understorey species (Holmgren et al. 1997). Also, soil conditions could be modified through increases in moisture, nutrients, organic matter and stability of soil structure (Flores and Jurado 2003; Bonanomi et al. 2011). Refuge effects include shelter against defoliation and trampling by large herbivores via physical (thorns, spines) and/or chemical (toxins) defences or concealment (Fidelis et al. 2009; Louthan et al. 2014). Although a benefactor plant can exert both positive effects (microsite amelioration and refuge from grazing) simultaneously, their interactive effect has been poorly evaluated (Soliveres et al. 2012; Filazzola and Lortie 2014).

It has been proposed that the relative importance of facilitation increases with increasing physical stress

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and grazing pressure (Bertness and Callaway 1994; Holmgren et al. 1997; Callaway et al. 2002; Graff et al. 2007; Smit et al. 2007). As a result, the role of facilitative interactions has been commonly tested in stressful environments such as arid, semi-arid, subalpine ecosystems, and salt marshes and has been largely ignored in more productive environments (Michalet and Pugnaire 2016). However, in the last few years, several authors have argued that facilitation often collapses at the harshest end of the abiotic gradient and that facilitative interactions may prevail under moderate rather than extreme conditions (Holmgren and Scheffer 2010; Michalet et al. 2014). In addition, the relative importance of facilitation may depend on the life history of the beneficiary species (Liancourt et al. 2005). Therefore, a benefactor plant may differentially affect co-occurring species with different abiotic requirements (Yang et al. 2010).

Although facilitation is known to have consequences at the community level, its effects have received more attention and are better understood at the individual and population levels (Cavieres and Badano 2009). However, the importance of facilitative interactions as a driver of community structure cannot be inferred from pair-wise interactions due to the variety of responses that can be found in a multispecies assemblage (Soliveres and Maestre 2014). An alternative way to assess the role of facilitation at the community level is to group species into plant functional types (PFTs). Given that species belonging to different functional types should differ in timing, source, and efficiency of resource use, facilitation intensity could vary among four functional types of temperate grasslands, defined by life form and photosynthetic metabolism of the species: C_3 (cool-season) grasses, C_4 (warm-season) grasses, forbs, and non-grass monocots.

The C_4 metabolism is a modification of the ancestral C_3 pathway and has evolved more than 60 times in at least 18 families of flowering plants (Christin et al. 2011). The C_4 photosynthetic pathway is especially common among monocots, such as grasses and sedges, but infrequent among dicots. The ecological success of C_4 species is associated with the expansion of open biomes dominated by herbaceous plants, such as savannas and grasslands (Christin et al. 2008; Edwards and Smith 2010). C_4 plants generally tolerate higher temperatures and solar irradiance, drier conditions, greater seasonality, and lower atmospheric CO_2 levels than C_3 species and rarely colonise forest habitats where less light and lower temperatures prevail (Sage 2004). Conversely, the C_3 pathway greatly decreases photosynthetic performance in situations that promote photorespiration, typically high-temperature and low-CO_2 environments (Edwards and Smith 2010).

Here, we evaluated the role of the shrub *Acanthostyles buniifolius* (Hook. & Arn.) R. M. King & H. Rob. on the community structure of a temperate subhumid grassland in Uruguay. Using an experimental design with a 3-year herbivore exclosure, we aimed to unravel the abiotic microsite effects from the grazing refuge effects of the shrub on community richness, cover of PFTs, and cover of individual species. We expected a stronger positive effect of the shrub under grazing than in the exclosure, since both mechanisms (abiotic microhabitat amelioration and herbivore protection) would be operating in the presence of grazers. In addition, we predicted that species with C_3 metabolism would benefit most from the presence of *A. buniifolius*, since the alleviated microclimatic conditions found beneath the shrub canopy may reduce their photorespiratory activity.

Materials and methods

Study site

The study was conducted in the Southern Campos of the Río de la Plata grasslands, east of Uruguay (33° 11′S, 54°33′W). This region encompasses an area of 2.5 Mha, of which 66% is covered by natural grasslands dominated by *Piptochaetium montevidense, Richardia humistrata, Chevreulia sarmentosa, Dichondra sericea*, and *Paspalum notatum* (Lezama et al. 2011). These 'old-growth' grasslands (Veldman et al. 2015) have been continuously grazed since the introduction of cattle at the beginning of the seventeenth century. Cattle grazing affects plant traits and community composition. Grazed communities are dominated by prostrate, warm season (C_4) grasses that are replaced by erect, cool season (C_3) grasses in grazing exclosures (Cayssials and Rodríguez 2018). Forbs conform a subordinate group of perennial C_3 species that occupy the spatial interstices among dominant grasses, and their dynamics seem to be regulated by processes other than grazing (Rodríguez et al. 2003). Grasslands respond rapidly to grazing removal such that major changes in community structure occur after 2–3 years of exclosure (Rodríguez et al. 2003). For the period 1991–2016, mean annual precipitation was 1,400 mm and mean annual temperature was 17°C (http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico).

Benefactor species

A. buniifolius (Asteraceae) is a perennial, medium-tall (1–2 m height) shrub native to Argentina, Uruguay, Brazil, Paraguay, and Bolivia (Grossi et al. 2011). Its life span does not exceed 20 years. It flowers typically during the austral summer and produces large quantities of achenes that disperse freely by the wind (Galíndez et al. 2013). Additionally, it has the capability to resprout after fire or physical damage from a woody xylopodium (Biganzoli et al. 2009). It is usually profusely branched, with lax foliage at the tips of the branches. Although the species lacks thorns or toxins, it has low or no nutritional value (Ríos 2007), and thus it is scarcely consumed by cattle in its adult phase and may protect smaller neighbouring plants under its canopy from herbivores.

A. buniifolius is the most common shrub in Uruguayan grasslands, but its distribution is far from uniform. Its cover ranges from 0% to 100% depending upon grazing management, the history of tillage, or natural variability (Baeza 2016). In the study site, it grows scattered in the herbaceous matrix, covering 5–10% of the area. Previous studies have suggested that *A. buniifolius* can benefit some C_3 grasses under its canopy (Fernández 2011; Fernández et al. 2014). However, these studies focused on pair-wise interactions with a limited number of beneficiary species and did not compare grazed with ungrazed areas.

Experimental design

In the spring of 2016, we selected two contiguous areas located within the same soil unit, one continuously grazed at a moderate stocking rate (<0.5 animals/ha) and the other a 3-year fenced livestock exclosure of 2,500 m^2. In both grazing treatments, we randomly selected five shrubs and five paired open sites placed as close as possible to these shrubs as locations for 20 plots of 1 m^2 (10 plots under the grazing treatment and 10 plots in the exclosure). Shrubs were of similar size (1.5 m height and projected canopy cover of 1 m^2) and were separated from each other by at least 3 m and up to 5 m from the edge of the exclosure. The design was not replicated for the grazed–ungrazed contrasts. However, the exclosure was located in a way that plant community structure inside and outside the exclosure was similar at the beginning of the exclusion period. Thus, we believe that the grazing treatment is the major cause for the eventual differences inside vs. outside the exclosure.

Floristic composition was determined through the point-intercept method (Greig-Smith 1983), using a metal frame of 1 m^2 supporting 81 vertical pins (separated 10 cm) which were inserted into the vegetation from a height of 50 cm. All the species in contact with the pins were registered. Species were sorted into four functional types: C_3 (cool-season) grasses, C_4 (warm-season) grasses, forbs, and non-grass monocots (including Cyperaceae, Hypoxidaceae and Iridaceae). The absence of C_4 forbs in Uruguayan grasslands, as well as taxonomic uncertainties in monocots that we could not solve, prevented the separation of these two latter groups into C_3 and C_4 species.

Incident light, soil temperature and soil moisture were measured in the 20 sampling plots, at around midday. All variables were measured seasonally (four measurements per year) over 3 years (2014–2016). Environmental measurements of the cooler seasons (autumn, winter) and those of the warmer seasons (spring, summer) were pooled together. Incident light was measured at ground level (0.1 m height) using a digital light meter (Tondaj LX1010B). Soil surface temperature was measured using an infrared thermometer (Nicety ST530) and soil moisture was measured at 0.1 m depth with a soil moisture meter (Extech MO750).

Statistical analyses

Differences in total species richness and PFT richness among treatments (open/shrub × grazed/exclosure) were analysed using one-way ANOVA followed by Tukey's post hoc test ($\alpha = 0.05$). Prior to analysis, we carried out data normality tests and evaluated homoscedasticity. All data followed normality assumptions.

Species cover was calculated as: YX/n; where YX is the number of pins that hit the species X and n is the total number of pins (Damgaard et al. 2011). The cover of plant functional types (C_3 grasses, C_4 grasses, forbs and non-grass monocots) was calculated as the sum of the cover of all individual species belonging to that PFT.

In order to evaluate the effect of the shrub on the cover of species and PFTs, we calculated the Relative Interaction Index (RII) following Armas et al. (2004):

$$RII = (X_{S+} - X_{S-})/(X_{S+} + X_{S-})$$

where X_{S+} and X_{S-} are the cover of the target species or PFT in the presence and absence of shrubs. The index is symmetrical around zero (neutral effect)

and ranges from +1 (facilitation) to −1 (competition). We used t-tests to determine significant deviation from zero.

Species richness and RII of functional types were analysed including all registered species. For pairwise interaction analyses, only species that occurred in at least three paired plots were considered.

The effect of the shrub on incident light, soil temperature, and soil moisture was analysed using one-way ANOVA coupled with Tukey's test when main effect was significant. Data followed normality assumptions. All statistical analyses were made using InfoStat (Di Rienzo et al. 2017).

Results

Species richness

In total, 64 species were found in all plots. Of these, 13 were C_3 grasses, 24 were C_4 grasses, 23 were forbs, and 3 were non-grass monocots (Table S1).

In the grazed area, total species richness was higher under the shrubs than in the surrounding open area ($F_{3,16} = 14.97$, $P < 0.0001$). Of the functional types, only the grasses responded to the presence of the shrub, but the response varied between metabolic pathways. C_3 grass richness was 70% higher under the shrub canopy in the grazed area ($F_{3,16} = 7.28$, $P = 0.003$), but was unaffected in the exclosure by the presence of the shrub. Alternatively, C_4 grass richness did not differ under grazing, but was 22% lower under shrubs when livestock were excluded ($F_{3,16} = 6.60$, $P = 0.004$) (Figure 1).

PFT and species cover

For most functional types, the Relative Interaction Index, estimated from species cover values, was always higher in the grazed area compared to the exclosure. The C_3 grasses was the only functional type that showed positive values of RII in both
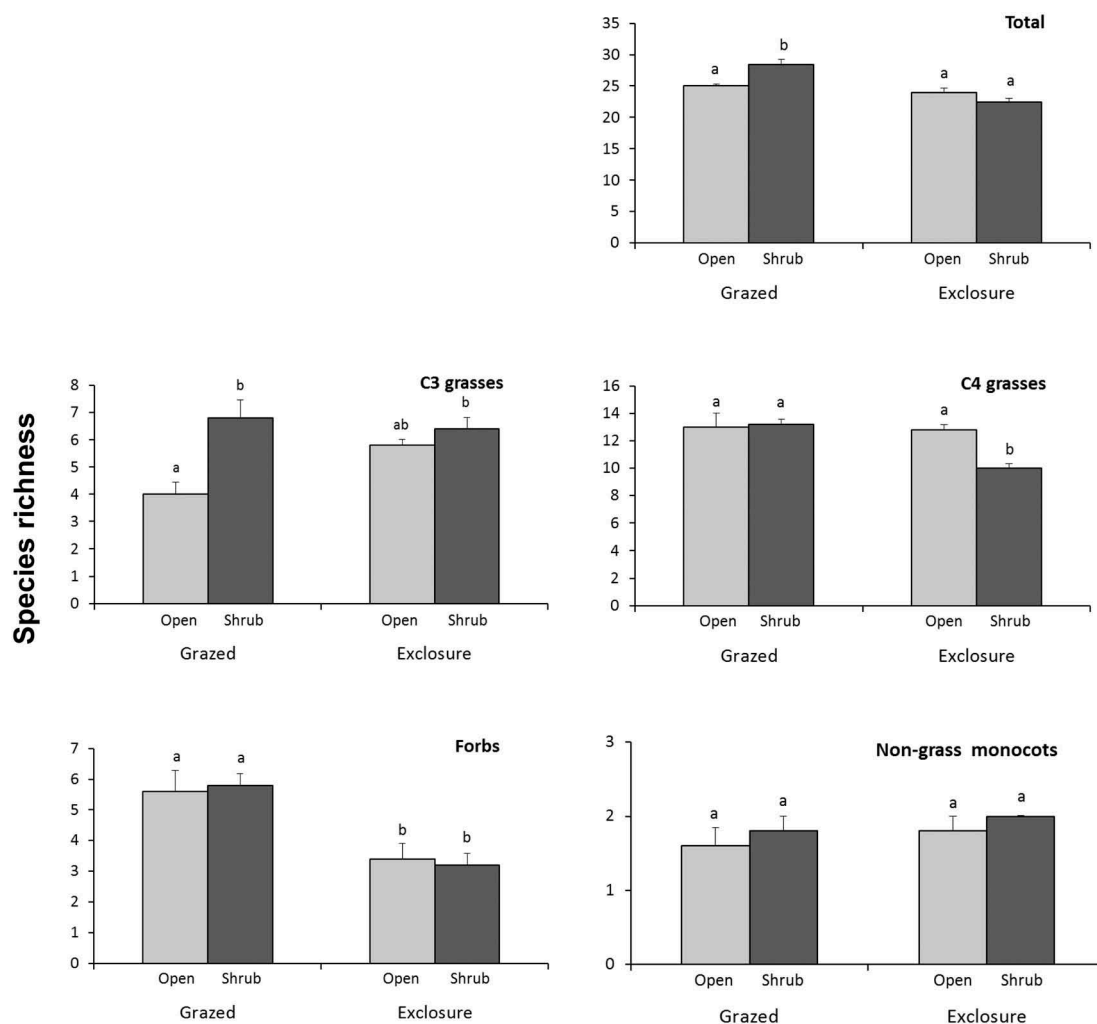

Figure 1. Total species richness and by functional type of the herbaceous vegetation growing under the canopy of the shrub *Acanthostyles buniifolius* and in the surrounding open area, inside and outside a grazing exclosure ($n = 5$ for each treatment). All registered species were included in the analyses. Values are means (± SE). Different letters indicate significant differences among microsites ($P < 0.05$) in a post-ANOVA Tukey test. Boldface type highlights comparisons that were statistically different.

grazing treatments, whereby the effect of the shrub doubled in the grazed area. C_4 grasses and non-grass monocots showed negative values of RII inside the exclosure and neutral values under grazing. Forbs were indifferent to the presence of the shrub, showing values that did not differ significantly from zero in both grazing treatments (Figure 2).

The positive effect of *A. buniifolius* on the cover of C_3 grasses was more evident when the species were analysed individually. About 70% (six out of nine) of the C_3 grass species used in pair-wise analyses were recorded in both grazing treatments. In the grazed area, all species increased their cover in the presence of the shrub. Inside the exclosure, more than half showed a positive trend in RII, although only *Danthonia montevidensis* registered positive values significantly different from zero. Otherwise, *D. rhizomata* was the only species registered with negative values (Figure 3).

Of the 16 C_4 grass species used for pair-wise analyses, only 6 (37.5%) were recorded in both grazing treatments, showing a significant trend in the RII in at least one of them. Essentially, the effect of the shrub on these six C_4 grass species was neutral or positive under grazing but negative inside the exclosure. Three species (*Coelorachis selloana*, *Paspalum notatum* and *P. plicatulum*) shifted from neutral values under grazing to negative values inside the exclosure. The remaining species showed positive values under grazing and positive (*Schizachyrium tenerum*), negative (*Eragrostis bahiensis*) and neutral (*Aristida murina*) values inside the exclosure (Figure 3).

Forbs showed no clear trends in their response to the presence of *A. buniifolius*. Only three species were recorded in both grazing treatments, with significant values of RII in at least one of them (Figure 3). Most non-grass monocots were rare species (present in less than three plots) and were not analysed individually.

The list of species registered with RII values that did not differ significantly from zero (neutral effect), as well as those recorded in only one of the grazing treatments, are shown in Table S2.

Abiotic measurements

In both grazing treatments, light availability about half below the canopy of *A. buniifolius* compared to the surrounding open area ($F_{3,16} = 66.4$ and $F_{3,16} = 80.7$ for cold and warm-season respectively, $P < 0.0001$). Soil temperature was significantly lower under the shrubs during the warmest months ($F_{3,16} = 28.7$; $P < 0.001$). In contrast, there were no significant differences in soil moisture between the shrub and open microsites (Table 1).

Discussion

We found evidence of positive effects of the shrub *A. buniifolius* on the richness and cover of the dominant life-form (i.e. grasses) of a temperate subhumid grassland. According to the stress-gradient hypothesis (SGH) the frequency of facilitative interactions is more common in conditions of high abiotic stress
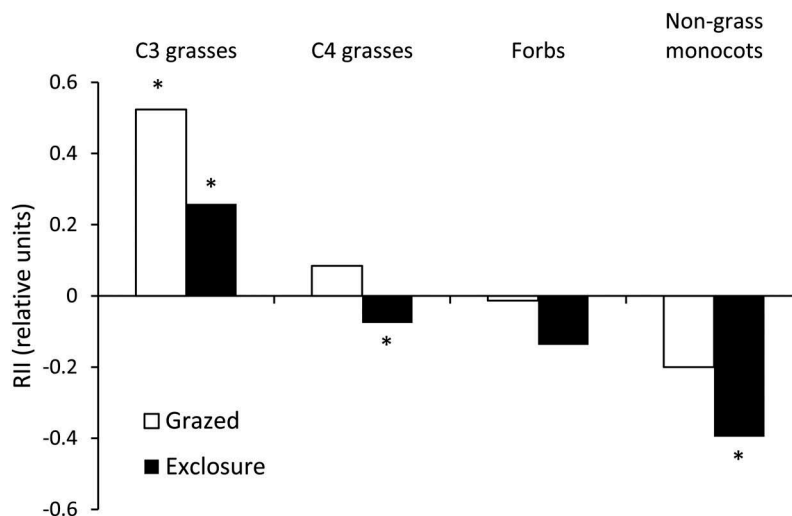

Figure 2. Mean relative interaction indices (RII) calculated for cover of four functional types growing under the canopy of the shrub *Acanthostyles buniifolius* and in the surrounding open area, inside and outside a grazing exclosure (*n* = 5 for each treatment). Cover of all registered species were included in the analyses. Values higher or lower than zero indicate positive (facilitation) or negative (competition) effects of the shrub, respectively. Asterisks indicate values that significantly differ from zero in a *t*-test (*P* < 0.05).
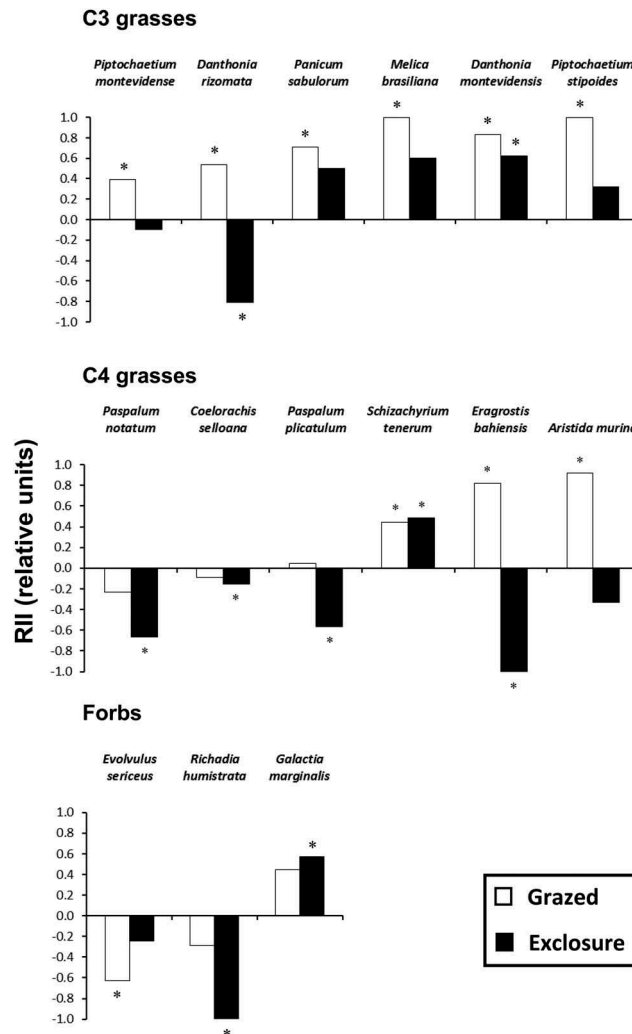
C3 grasses

C4 grasses

Forbs

Figure 3. Mean relative interaction indices (RII) calculated for cover of species growing under the canopy of the shrub *Acanthostyles buniifolius* and in the surrounding open area, inside and outside a grazing exclosure (*n* = 5 for each treatment). RII values range from 1 (facilitation) to −1 (competition). Asterisks indicate significant deviation from zero using *t*-test (*P* < 0.05). Only species recorded in both grazing regimes with values significantly different from 0 in at least one of them are shown.

Table 1. Light availability, soil temperature, and soil moisture measured under the canopy of the shrub *Acanthostyles buniifolius* and in the surrounding open area, inside and outside a grazing exclosure (*n* = 5 for each treatment). Variables were measured seasonally over 3 years (2014–2016).

Variable	Season	Grazed		Exclosure		$F_{(3, 16)}$	P
		Shrub	Open	Shrub	Open		
Light availability (luxes)	Autumn/winter	**379.4ᵃ**	**517.3ᵇ**	**279.5ᶜ**	**492.8ᵇ**	**66.4**	**<0.0001**
	Spring/summer	**420.4ᵃ**	**839.5ᵇ**	**329.7ᵃ**	**792.4ᵇ**	**80.7**	**<0.0001**
Soil temperature (ºC)	Autumn/winter	**12.9ᵃ**	**13.05ᵃ**	**13.3ᵃᵇ**	**14.95ᵇ**	**5.9**	**0.006**
	Spring/summer	**27.85ᵃ**	**31.25ᵇ**	**25.1ᶜ**	**30ᵇ**	**28.7**	**<0.0001**
Soil moisture (%)	Autumn/winter	9.9	9.7	10.1	9.8	0.24	0.87
	Spring/summer	8.0	8.1	8.3	7.5	2.26	0.12

Different letters indicate significant differences among microsites (*P* < 0.05) in a post-ANOVA Tukey test. Boldface type highlights comparisons that were statistically different.

and/or high consumer pressure (Bertness and Callaway 1994). Species of temperate grasslands of Uruguay are not exposed to critical abiotic stressors, but since the introduction of livestock by European settlers, about 400 years ago, they have been subjected to moderate/high grazing pressure. Consistent with our expectations, we found that in the grazed plots the cover of grass species was higher beneath the shrub canopy than outside of it. Additionally, we found that C₃ grasses were also favoured by the presence of the shrub inside the exclosure, suggesting more adequate abiotic conditions for this functional type, and supporting the idea that positive interactions may be more

common in mesic environments than previously thought (Holmgren and Scheffer 2010). However, we may not altogether rule out that the interaction observed reflects the legacy of previous herbivory before fencing 3 years prior to the study.

Photosynthetic metabolism was found to be a key attribute for understanding the facilitative interactions in this temperate grassland. Inside the exclosure (where the biotic refuge effect would not be operating), the positive effects of the shrub were mainly associated with the C_3 grasses. Compared to their C_4 counterparts, C_3 leaves have no biochemical CO_2 concentrating mechanisms. At high temperatures and low atmospheric CO_2, the key C_3 photosynthetic enzyme rubisco does not distinguish completely between CO_2 and O_2. The O_2 uptake leads to the less efficient ($\leq 40\%$) carbon-gain photorespiration process (Edwards et al. 2010). In order to reduce photorespiration, C_3 plants can increase internal leaf CO_2 concentration simply by opening stomates (Sage 1999). However, this poses a problem for C_3 species growing in open habitats, like natural grasslands, where species are exposed to intense solar radiation and high temperatures. Increasing stomatal conductance comes at a high cost in terms of transpiration and risks of severe wilting and even death (Sage 1999; Gurevitch et al. 2006). Living in a micro-environment with reduced solar radiation and temperature, like that found under the canopy of *A. buniifolius*, may benefit C_3 grasses by reducing photorespiratory activity. Abiotic stress amelioration constitutes the core mechanism underlying the SGH (Bertness and Callaway 1994; Holmgren et al. 1997; Bruno et al. 2003) and although it has been widely documented in xeric environments (Cavieres and Badano 2009; Filazzola and Lortie 2014), our results indicate that C_3 grasses of subhumid grasslands also benefit from this mechanism.

Most C_4 grass species were negatively affected by the presence of the shrub inside the exclosure. Contrary to C_3 species, which can acclimate to shade through structural, biochemical, and physiological changes of the leaves (Sage and McKown 2006), C_4 species do not thrive well in low-light environments (Ehleringer 1978). Although C_4 plants have substantial acclimation potential, certain features unique to C_4 photosynthesis (e.g. the mesophyll-bundle sheath complex and associated structural and physiological traits) must be maintained in order to function efficiently, reducing the potential for photosynthetic acclimation to shade (Sage and McKown 2006). Our results are consistent with findings for particular C_4 grass species, both in natural

systems (Lett and Knapp 2003) and greenhouse experiments (Altesor et al. 2017).

Despite the opposite effects of the shrub on C_3 and C_4 grasses inside the exclosure, the shrub would be protecting both functional types from grazing by herbivores. Herbivore protection is a well-known mechanism that promotes facilitative interactions (Barbosa et al. 2009; Smit et al. 2009; Filazzola and Lortie 2014) and several studies have shown that unpalatable plants reduce the grazing pressure on their palatable neighbours (Oesterheld and Oyarzabal 2004; Bossuyt et al. 2005; Callaway et al. 2005; Graff et al. 2007; Noëll-Estapé et al. 2013). The protective role of *A. buniifolius* may be due to visual concealment from grazers, since the shrub lacks spines or toxicity (Louthan et al. 2014). After 3 years of livestock exclusion, we assumed that grazing protection was negligible inside the exclosure, but appears to function simultaneously with the abiotic amelioration mechanism under grazing. For C_3 grasses, it seemed that the two mechanisms (microclimatic amelioration and herbivore protection) converge, both leading to positive effects. This may explain the two-fold increase in the intensity of facilitation when livestock were present. Alternatively, the two mechanisms seem to work in opposite directions for C_4 grasses. The negative effect of the shrub on C_4 grass cover inside the exclosure shifted to neutral in the grazed area. In the latter case, the positive effect of the shrub mediated by grazing protection may offset the negative effect induced by shading, leading to a neutral net outcome.

Forbs did not show a consistent response to the presence of *A. buniifolius*. As a group, the effect of the shrub on forb richness and cover was neutral in both grazing treatments. However, when the species were analysed separately, one benefited from growing under the shrub canopy, while two responded negatively, irrespective of the grazing condition. Photosynthetic metabolism cannot account for the heterogeneity of responses, as all registered species had C_3 photosynthesis. C_4 photosynthesis is relatively uncommon in dicotyledonous plants, occurring only in some families (Ehleringer et al. 1997). In natural grasslands of Uruguay, forbs are a phylogenetically heterogeneous group of species that occupy interstitial spaces left by the matrix-forming grasses (Rodríguez et al. 2003; Lezama and Paruelo 2016). The species studied are small-stature perennials, and except for *Sommerfeltia spinulosa* and *Cuphea glutinosa* that can grow up to 40 cm, their biomass is generally concentrated close to the ground. Having small prostrate leaves offers a two-fold advantage in this system: (1) species can avoid being consumed by grazers (Díaz

et al. 1992) and (2) small leaves have a thinner boundary layer, which allows greater convective cooling, usually with a net reduction in water loss through evapotranspiration (Vogel 1968; Givnish 1979; Cunningham et al. 1999). It is likely that forbs of these grasslands are not dependent on beneficial associations with shrubs in order to cope with grazing and high-light environments, and their response is mediated by other mechanisms not considered in this study.

Taken together, non-grass monocots decreased their cover under the shrub canopy. The non-grass monocot functional type includes the sedge family (Cyperaceae), which is the second richest family in C_4 species (ca. 1330 species, 27% of all species in the family), following the grasses (Sage 2004). Therefore, the sedge family is a good system to assess facilitative interactions mediated by photosynthetic metabolism. However, in our study most species of this functional type were rare, present in less than three plots, and therefore were discarded from the individual analysis. More data would be needed in order to discuss facilitative interactions within this functional type.

Our research highlights the convenience of using individual and community approaches as complementary analyses in order to explore facilitative effects in a highly diverse community. The use of a PFT approach allowed us to unravel the effects of microclimatic amelioration and herbivore protection in C_3 and C_4 grasses. An increment in the abundance of C_3 species was also observed under woody species of fynbos (Cowling 1983), savannas (Scholes and Archer 1997), artificially afforested grasslands (Nordenstahl et al. 2011) and natural grasslands (Fernández et al. 2014; Bernardi et al. 2016). However, these studies did not compare grazed with ungrazed areas, and consequently failed to determine the exact mechanism underlying the facilitative effect of the benefactor species. On the other hand, pair-wise interaction analyses revealed heterogeneous behaviour among forbs, suggesting that grouping multiple lineages into a single functional type may mask underlying variation in other traits that could explain the varied responses to the presence of the shrub.

Conclusions

Our study contributes to the knowledge of facilitative interactions, providing evidence that shrubs of a temperate subhumid grassland may protect grasses from grazers and that they can also benefit C_3 grasses by alleviating stressful conditions of the open environment. Nonetheless, we acknowledge

that our findings cannot be generalised to other subhumid grasslands. Community richness and composition, grazing regime and resource availability vary across landscapes and regions, and in turn, plant–plant interaction outcomes may change. Additionally, the limited number of replicates (five) used in our study created a conservative comparison by reducing the chances of finding significant differences among treatments. Further analyses including more sampling sites and replicates are needed. The generalisation of our results is important because shrubs like *A. buniifolius* are undesirable and usually removed by land managers in order to increase the effective grazing area. However, shrubs may play an important role in maintaining the richness and abundance of species of grasslands, especially of those most appreciated by grazers. Although we did not measure reproductive performance, it is also possible that grasses growing under the shrub canopy have higher reproductive success, as already noted by other authors (e.g. Bossuyt et al. 2005). Therefore, shrubs may provide resilience to this highly diverse community in the face of natural and anthropogenic disturbances.

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

Gastón Fernández is an associate professor with research interests in plant–plant interactions and the ecophysiology of grasses.

Felipe Lezama is an associate professor with experience in phytosociological studies of grassland vegetation.

Claudia Rodríguez is an associate professor with a main interest in the effects of grazing by large herbivores on grassland community structure.

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