

## RESEARCH ARTICLE

# Differential responses of C<sub>3</sub> and C<sub>4</sub> grasses to shrub effects in a sub-humid grassland of South America

Gastón Fernández<sup>1</sup>  | Alice Altesor<sup>2</sup>

<sup>1</sup>Grupo Multidisciplinario en Ecología para la Agricultura, Centro Universitario Regional del Este, Universidad de la República, Treinta y Tres, Uruguay

<sup>2</sup>Instituto de Ecología y Ciencias Ambientales, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

## Correspondence

Gastón Fernández, Grupo Multidisciplinario en Ecología para la Agricultura, Centro Universitario Regional del Este, Universidad de la República, Treinta y Tres, Uruguay.  
Email: gfernandez@cure.edu.uy

## Funding information

This work was funded by the Agencia Nacional de Investigación e Innovación (BE\_POS\_2009\_719), the Inter-American Institute for Global Change Research (IAI) CRN III 3095, which is supported by the US National Science Foundation (Grant GEO-1128040) and the Programa de Desarrollo de las Ciencias Básicas of Uruguay.

Co-ordinating Editor: Francisco Pugnaire

## Abstract

**Questions:** Plant–plant interactions are key processes that strongly affect the survival, growth and reproduction of individuals in plant communities. In grasslands, the micro-environment generated under the canopy of shrubs could differentially affect co-occurring species with different abiotic requirements. In a C<sub>3</sub>/C<sub>4</sub> grassland with scattered shrubs, we asked the following questions: (a) Does the aerial effect, the below-ground effect, and the net effect of shrubs affect the vegetative and reproductive biomass, the number of tillers, the biomass allocation, and the leaf elongation rate of grasses? and (b) Do these effects differ between C<sub>3</sub> and C<sub>4</sub> grasses?

**Location:** Temperate sub-humid grassland of Uruguay.

**Methods:** We planted one C<sub>3</sub> and two C<sub>4</sub> grasses under a shrub canopy and in adjacent open sites. Half of the grasses were planted with a fabric bag to reduce root competition with the shrub. We measured leaf elongation rate, the number of tillers produced and the biomass of the grasses in every treatment. We also measured photosynthetic photon flux density (PPFD), air temperature and wind speed under shrub canopies and in adjacent open sites.

**Results:** Root biomass, aerial biomass and reproductive biomass, the number of tillers and the leaf elongation rate of the C<sub>4</sub> grasses were negatively affected by the reduction in radiation and probably by below-ground competition with the shrub. On the other hand, the leaf elongation rate of the C<sub>3</sub> grasses was positively affected by the shrub canopy. PPFD, air temperature and wind speed were lower under shrubs than in adjacent open sites.

**Conclusions:** Our results show the interplay between plant interactions and photosynthetic metabolism on the vegetative and reproductive performance of grasses. The micro-environmental conditions generated below shrub canopies create a more appropriate site for the growth of C<sub>3</sub> than for C<sub>4</sub> grasses. These results show that shrubs may differentially affect co-occurring species with different abiotic requirements.

## KEYWORDS

competition, facilitation, manipulative experiments, photosynthetic metabolism, plant interactions, plants performance, Río de la Plata Grasslands, Uruguay

## 1 | INTRODUCTION

Plant–plant interactions are key processes that strongly affect survival, growth and reproduction of individuals in plant communities (Leger & Espeland, 2010). Plants can reduce or enhance the performance of their neighbors, through the simultaneous effects of competition and facilitation, respectively (Malkinson & Tielborger, 2010). The stress gradient hypothesis (SGH) proposed by Bertness and Callaway (1994) predicts that the frequency of facilitation directly increases with abiotic stress. Although the SGH has received empirical support (He, Bertness, & Altieri, 2013), in the last few years their predictions have been questioned. Several authors argue that facilitation often collapses at the extreme end of the stress gradient and that facilitation prevails under moderate rather than extreme conditions (Holmgren & Scheffer, 2010; Michalet, Le Bagousse-Pinguet, Maalouf, & Lortie, 2014). Additionally, shifts from competition to facilitation could be determined by the type and magnitude of the stress gradient (Holmgren & Scheffer, 2010; Maestre, Callaway, Valladares, & Lortie, 2009), the functional traits of the interacting species (Callaway, 2007), their ontogenic stage (Miriti, 2006; Nuñez, Raffaele, Nuñez, & Cuassolo, 2009) and the position of beneficiary plants in the canopy (Al-Namazi, El-Bana, & Bonser, 2017).

The relative frequency of facilitation may also depend on the response of plant species to the set of conditions prevailing in a particular environment (Gross, Liancourt, Choler, Suding, & Lavorel, 2010; Liancourt, Callaway, & Michalet, 2005). As species have different physiological and ecological optima, facilitation is likely to be important only for those species that are constrained by local abiotic conditions (Gross et al., 2010). In grasses, the tolerance of species to abiotic conditions is influenced by their photosynthetic metabolism (Taylor et al., 2014).  $C_3$  grasses have a lower optimum temperature for photosynthesis, lower light intensity required for saturation, lower nitrogen use efficiency and lower photosynthetic water use efficiency (rate of carbon uptake per unit of transpired water) than  $C_4$  species. On the other hand,  $C_4$  species achieve their maximum photosynthetic rates at higher temperatures and higher light intensities than  $C_3$  grasses (Sage, 2004). Thus, microclimate conditions generated under the canopy of a plant could facilitate the growth of  $C_3$  grasses and, at the same time, reduce the growth of the  $C_4$  grasses. However, to our knowledge, the simultaneous effects of competition and facilitation on the growth of plants with different photosynthetic metabolism have not been tested.

Grasses and shrubs coexist across a wide range of ecosystems, from cool semi-arid steppes to temperate sub-humid grasslands. It is known that solar radiation, air temperature and wind speed are lower under shrub canopies than in open sites (Chen, Franklin, & Spies, 1995; Holmgren, Scheffer, & Huston, 1997). The microclimate generated under a shrub canopy affects the survival, growth and development of grasses (Aguar, Soriano, & Sala, 1992; King & Stanton, 2008). In semi-arid steppes, grass germination and establishment are mostly limited to these micro-habitats (Aguar & Sala, 1994). The shelter provided by shrubs decreases the mortality of seedlings due

to the reduction in water stress and photochemical damage (Armas & Pugnaire, 2005). In contrast, in sub-humid grasslands, the presence of shrubs reduces grass growth and productivity due to the reduction in light available for photosynthesis (Briggs et al., 2005; Lett & Knapp, 2003).

Uruguayan Campos belong to the Rio de la Plata Grasslands, one of the largest areas of temperate sub-humid grasslands of South America (Soriano, 1991). These natural grasslands are characterized by a mixture of  $C_3$  and  $C_4$  grasses (Epstein et al., 2002; Paruelo, Jobbágy, Sala, Lauenroth, & Burke, 1998) in which shrubs can be present and may become locally dominant (Paruelo, Jobbágy, Oesterheld, Golluscio, & Aguiar, 2007). The presence of shrubs scattered in a matrix of grasses forms a two-phase mosaic structure with different abiotic conditions that affects the spatial distribution of grasses. In a previous work, we detected a positive spatial association between two  $C_3$  grasses and the shrub *Acanthostyles buniifolius* (Hook. & Arn.) R.M.King & H.Rob. (Asteraceae) and a negative association between two  $C_4$  grasses and the shrub. However, the processes underlying these patterns are still unknown (Fernández, Texeira, & Altesor, 2014). Therefore, the aim of this study was to analyze the effect of the shrub *Acanthostyles buniifolius* on the performance of three species of grasses with different photosynthetic metabolisms and hence different responses to abiotic conditions.

Specifically, we evaluated the aerial effect, the below-ground effect, and the net effect of the shrub *Acanthostyles buniifolius* on the vegetative and reproductive performance of the  $C_4$  grasses *Axonopus fissifolius* (Raddi) Kuhl., *Mnesithea selleana* (Hack.) de Koning & Sosef, and the  $C_3$  grass *Bromus auleticus* Trin. ex Nees through a manipulative experiment in a productive and species-rich grassland of Uruguay.

We expect that the performance of  $C_3$  and  $C_4$  grasses will be differentially affected by changes in the availability of resources and conditions promoted by the shrub. We predict that the reduction in light availability and temperature under the shrub canopy will adversely affect the performance of  $C_4$  grasses while having a positive effect (i.e., facilitation) on the performance of  $C_3$  grasses due to reduced evaporative demand. The effect of below-ground competition will be lower than aerial effects due to abundant rainfall and soil fertility. As the net result will be determined by the relative strength of facilitation and competition, we expect the predominance of positive effects on  $C_3$  grasses and of negative effects on  $C_4$  grasses.

## 2 | METHODS

The study site is located in San José (34°20' S, 56°58' W) in the South Central region of Uruguay. Rainfall is evenly distributed throughout the year with an annual mean of 1,118 mm during the 1980–2009 period. For the same period, mean annual temperature was 16.8°C, ranging from a minimum of 2.3°C in June to a maximum of 31.8°C in January. During the study period, monthly mean rainfall was 95 mm, which was rather similar to historical values

(<http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico>, last accessed July 1, 2017).

Grasslands of this region have been continuously grazed by cattle since the beginning of the seventeen century. Cattle grazing promotes a vegetation physiognomy with two layers: a dense lower stratum, no more than 10 cm high, principally composed of prostrate grasses and rosette forbs, and a taller stratum of erect grasses and shrubs (Altesor et al., 2006; Rodríguez, Leoni, Lezama, & Altesor, 2003). Under grazing, the prostrate  $C_4$  grasses with broad leaves and horizontal growth through stolons or rhizomes (e.g., *Axonopus fissifolius*, *Paspalum notatum* Flügge and *Stenotaphrum secundatum* (Walter) Kuntze) become increasingly abundant and dominant. In contrast, caespitose  $C_3$  and  $C_4$  grasses (e.g., *Bromus auleticus*, *Nassella neesiana* (Trin. & Rupr.) Barkworth, *Piptochaetium* spp. and *Mnesithea selloana*) become less abundant. Caespitose grasses dominate in ungrazed sites, which are unusual in Uruguayan grasslands (Altesor, Oesterheld, Leoni, Lezama, & Rodríguez, 2005; Rodríguez et al., 2003). Although shrub richness and cover increase under exclosures, *Acanthostyles buniifolius* may also become abundant under grazing conditions (Altesor et al., 2006).

## 2.1 | Selected species

*Axonopus fissifolius* is a stoloniferous species with a high tillering rate and a high specific leaf area typical of fast-growing species. *Mnesithea selloana* and *Bromus auleticus* are caespitose species with low tillering rate and low specific leaf area typical of slow-growing species (Leoni, Altesor, & Paruelo, 2009).

## 2.2 | Experimental design

In October 2008, we collected bunches of the grasses *Bromus auleticus* ( $C_3$ ), *Axonopus fissifolius* ( $C_4$ ), and *Mnesithea selloana* ( $C_4$ ) from a 15-year-old exclosure. Bunches were separated into individuals with three to five tillers, planted in plastic pots (0.1 m diameter and 0.2 m height) with soil of the same site and kept during six months in a common garden for acclimatization. After this period, 30 individuals of *Axonopus fissifolius* and 21 individuals of *Mnesithea selloana* and *Bromus auleticus* were chosen for the field experiment. In April 2009, we selected 24 individuals of the shrub *Acanthostyles buniifolius* inside an area (ca. 1,000 m<sup>2</sup>) that was fenced to avoid livestock. We selected shrubs of similar height (~1.5 m) and canopy cover (~1 m<sup>2</sup>), and which were separated at least five meters from each other. Under each shrub, we planted two individuals of the same species in holes of 0.1 m diameter by 0.3 m deep (individuals with aerial effect,  $A^+$ ). Individuals were planted at the mid-point between the base of the stems and the edge of the shrub canopy. One individual was planted with its roots enclosed in a fabric bag to significantly reduce root competition without modifying the movement of water (individuals with aerial effect and without below-ground effect;  $A^+B^-$ ). Another individual was planted without the bag (individuals with aerial effect and with below-ground effect;  $A^+B^+$ ). A third individual was planted in

an open site, three meters away from the shrub, with a bag around its roots (individuals without aerial and without below-ground effects;  $A^-B^-$ ). In order to guarantee a correct establishment of the grasses, the plants were watered weekly for two months before the beginning of the experiment, since an unusual drought affected the region. Every shrub and the three related grasses were considered a block. Thus, we installed 10 blocks in the case of *Axonopus fissifolius* and seven blocks in the case of *Mnesithea selloana* and *Bromus auleticus*. At the beginning of the experiment, there were no differences in the number of tillers of *Axonopus fissifolius* [ $F(2, 27) = 0.31, p = 0.73$ ]; *Mnesithea selloana* [ $F(2, 18) = 1.7, p = 0.22$ ] and *Bromus auleticus* [ $F(2, 18) = 0.07, p = 0.93$ ]. When individuals were transplanted and throughout the experiment, the herbaceous vegetation around treatments was carefully removed by hand to avoid interactions with other species.

## 2.3 | Vegetative and reproductive variables

In order to estimate the aerial effect, the below-ground effect, and the net effect of the shrub on the performance of the grasses, we measured the final number of tillers of the grasses, the plants' biomass (roots, tillers, stolons, and reproductive), and their leaf elongation rate. In order to look for changes in the allocation of biomass among treatments, we calculated the leaf mass fraction (leaf dry mass over total dry mass) and root mass fraction (root dry mass over dry mass; Poorter et al., 2012).

We evaluated the aerial effect, the below-ground effect, and the net effect of the shrub on the performance of grasses using the Relative Interaction Index (RII) following Armas, Ordiales, and Pugnaire (2004). The RII graphs allowed us to indicate the sign and magnitude of shrub effects.

$$RII = \frac{(X_a - X_b)}{(X_a + X_b)}$$

where  $X_a$  and  $X_b$  are the biomass of grasses (root, aerial and reproductive) or the number of tillers of individuals  $A^+B^-$  and  $A^-B^-$  for the aerial effect;  $A^+B^+$  and  $A^+B^-$  for the below-ground effect; and  $A^+B^+$  and  $A^-B^-$  for the net effect.

RII ranges from -1 to +1, with negative values indicating a negative (competitive) effect of the shrub on grass performance and positive values indicating a positive (facilitative) effect.

Leaf elongation rate (LER) of *Bromus auleticus* and *Mnesithea selloana* was calculated 12 times from August 2010 to January 2011. In each individual, two new healthy leaves were marked and measured with a ruler ( $Lt_0$ ). Three days after, the length of every marked leaf was measured again ( $Lt_1$ ). LER was calculated as  $\{[(Lt_1 - Lt_0)/Lt_0]/\text{time}\} \times 100$ .

The LER of *Axonopus fissifolius* was not measured because the plants started producing stolons and growing horizontally towards the edge of the canopy. Therefore, they were harvested in August 2010 before the start of the LER measurements.



Variable	Period	Microsite		p-Value
		Open sites	Below shrubs	
PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Annual	843 $\pm$ 34	378 $\pm$ 73	<0.0001
Air temperature ( $^{\circ}\text{C}$ )	Spring–Summer	34.1 $\pm$ 0.9	31.6 $\pm$ 0.7	0.04
	Fall–Winter	10.65 $\pm$ 0.2	10.67 $\pm$ 0.25	0.98
Wind speed (m/s)	Annual	2.3 $\pm$ 0.3	0.74 $\pm$ 0.07	0.0002

**TABLE 1** Effects of shrubs canopy on abiotic conditions. Mean ( $\pm$ SE) of the incoming photosynthetic photon flux density (PPFD), air temperature and wind speed measured below the shrub canopy and in open sites ( $n = 24$ ). PPFD and wind speed were measured seasonally and values were averaged. The Mann–Whitney  $U$  test was performed in all cases

*Mnesithea selloana* and *Bromus auleticus* were harvested in January 2011, immediately after the last LER measurement. After harvest, the plants were separated into roots, leaves and inflorescences. Dry weight of each fraction was determined on oven-dried (72 hr at  $70^{\circ}\text{C}$ ) material.

## 2.4 | Abiotic conditions

We characterized the micro-environmental conditions below shrubs and in open sites through the photosynthetic photon flux density (PPFD), air temperature and wind speed. All variables were measured around midday during 2010, at ground level (0.1 m height) under the canopy of shrubs and in their respective paired open sites. PPFD was measured seasonally using a hand-held sensor (Cavadevices, Buenos Aires, Argentina). Temperature and wind speed were measured every two months using a hand-held sensor (model 2000, Kestrel, Boothwyn, PA). The effect of shrubs on PPFD, temperature and wind speed was analyzed through the Mann–Whitney  $U$  test. PPFD and wind speed values were averaged as the same pattern was found on all measurement dates. The effect of shrubs on abiotic conditions is summarized in Table 1.

## 2.5 | Data analysis

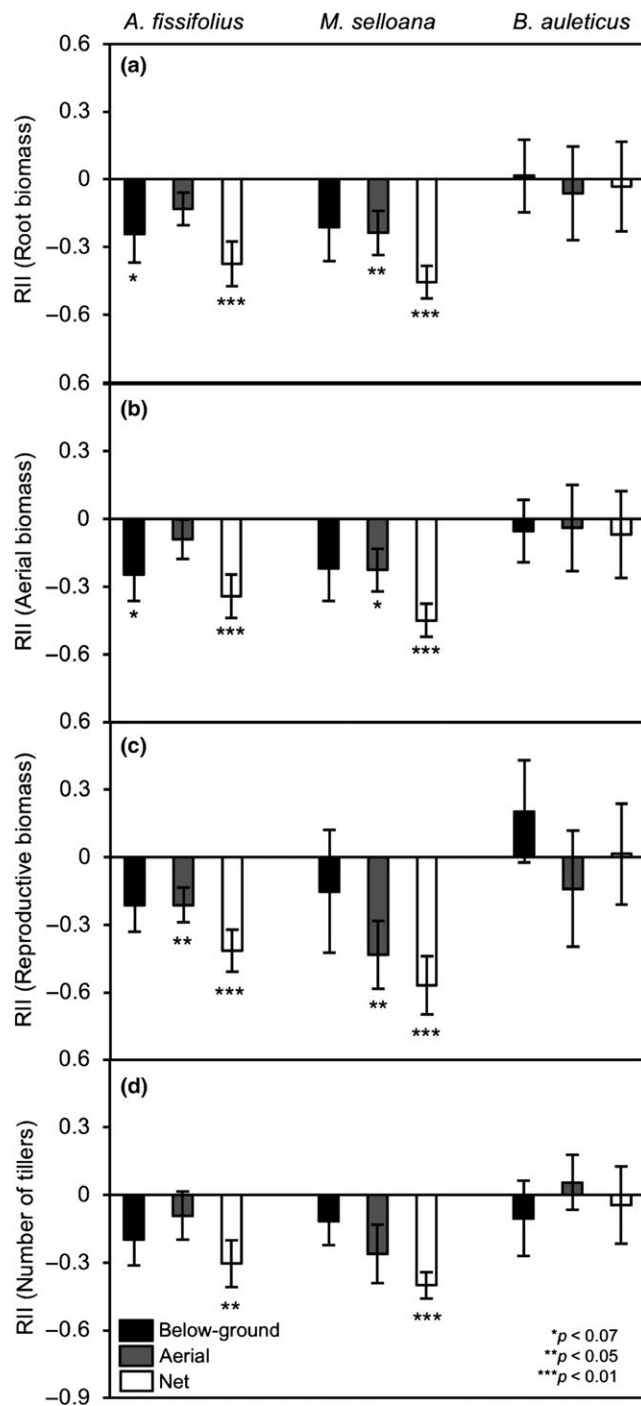
To determine whether shrub effects were significantly different from zero, statistical tests (Student's  $t$ ) were performed on RILs. Treatment and block effects on root biomass, aerial biomass, reproductive biomass, number of tillers, root mass fraction, and leaf mass fraction of grasses were analyzed using one-way ANOVA. Aerial biomass corresponds to tiller biomass in the case of *Mnesithea selloana* and *Bromus auleticus* and tillers plus stolons in the case of *A. fissifolius*. The effect of treatments on the rate of leaf elongation of *Bromus auleticus* and *Mnesithea selloana* was compared using repeated measures ANOVA with treatments as a between-subjects factor and time as a within-subjects factor. When the ANOVA showed significant differences between treatments, the Fisher LSD post-hoc analysis was performed. Prior to the analysis, the assumptions of normality and homogeneity of variance were verified. The effect of shrubs on grass performance was determined using the post-hoc contrast derived from ANOVA between  $A^+B^-$  and  $A^-B^-$  for aerial effects; between  $A^+B^+$  and  $A^+B^-$  treatments for the below-ground effect; and between  $A^+B^+$  and  $A^-B^-$  for the net effect. All analyses were performed using InfoStat. All analyses were performed using InfoStat (Di Rienzo et al., 2017).

It should be noted that the below-ground effect of the shrub was estimated in plants affected by the shrub canopy (i.e., through the difference between the  $A^+B^+$  and  $A^+B^-$  treatments), and therefore, it was an indirect estimate. Consequently, conclusions derived from the below-ground effect of the shrub on grasses performance were taken with caution.

## 3 | RESULTS

Overall, we detected mostly negative effects of the shrub on the performance of both  $C_4$  grasses and mostly neutral effects in the case of the  $C_3$  grass. There was a negative aerial effect that reduced the root biomass of *Mnesithea selloana* by 35% and the reproductive biomass of *Axonopus fissifolius* and *M. selloana* by 40% and 57%, respectively. The below-ground effect reduced the root biomass by 38% and the aerial biomass of *Axonopus fissifolius* by 45%. Net effect reduced the root biomass, the aerial biomass, the reproductive biomass and the number of tillers of both  $C_4$  grasses. Root biomass was reduced by 40% and 61%, aerial biomass by 56% and 63%, reproductive biomass by 57% and 61%, and number of tillers by 54% and 59% in *Axonopus fissifolius* and *Mnesithea selloana*, respectively. On the contrary, the shrub had neutral effects on the biomass and the number of tillers of *Bromus auleticus* (Figure 1; Table 2). We did not find significant differences among treatments in the root mass fraction or the leaf mass fraction in any of the three species (Table 2). We found a significant block effect only in the reproductive biomass of *Axonopus fissifolius* (Table 2). We detected positive effects of the shrub on the LER of *Bromus auleticus* [ $F(2, 18) = 9.44$ ;  $p = 0.0016$ ]. The aerial effect and the net effect of the shrubs increased the elongation rate of the leaves with 38% and 15%, respectively. In addition, there was a temporal variation on the below-ground effect from neutral on 10 measurement dates to negative on two dates (3 August and 14 January). LER was significantly affected by time [ $F(11, 198) = 32.425$ ;  $p < 0.0001$ ] and by the interaction between treatment and time [ $F(22, 198) = 1.8$ ;  $p = 0.018$ ; Figure 2a].

The LER of *Mnesithea selloana* was marginally affected by the shrub [ $F(2, 18) = 3.41$ ;  $p = 0.055$ ]. We detected a negative below-ground effect that reduced the LER by 11.5% and a negative net effect that reduced the LER by 11%. LER was significantly affected by time [ $F(11, 198) = 24.88$ ;  $p < 0.0001$ ], but not by the interaction between treatment and time [ $F(22, 198) = 1.26$ ;  $p = 0.2$ ; Figure 2b].



**FIGURE 1** Relative Interaction Index (RII) for the performance of grasses. Mean ( $\pm$ SE) RIIs for the below-ground effect, aerial effect and net effect of *Acanthostyles buniifolius* on the root biomass (a); aerial biomass (b), reproductive biomass (c) and the number of tillers (d) of the  $C_4$  grasses *Axonopus fissifolius* and *Mnesithea selloana* and the  $C_3$  grass *Bromus auleticus*. Asterisks indicate that the value is significantly different from zero (neutral effect) according to Student's *t* test

## 4 | DISCUSSION

Our results provide evidence of negative but also positive effects exerted by a shrub species on the performance of three grasses

in a temperate sub-humid grassland of South America. Moreover, the sign of these effects seems to be related to the photosynthetic metabolism of the grasses. The reduction in solar radiation and temperature under the canopy of *Acanthostyles buniifolius*, and probably the below-ground competition with the shrub, negatively affected the performance of the  $C_4$  grasses *Axonopus fissifolius* and *Mnesithea selloana*. On the contrary, the reduction in solar radiation and temperature had a positive effect but only on the LER of the  $C_3$  grass *Bromus auleticus*. Thus, we partially confirmed our predictions.

Although the shift from competition to facilitation has been proposed to depend on abiotic conditions (Bertness & Callaway, 1994; Holmgren & Scheffer, 2010), our result support the idea that the tolerance of individual species to the prevailing abiotic conditions may also affect the net outcome of a plant–plant interaction (Gross et al., 2010). In this case, these differential effects of the shrub on the performance of grasses could be explained by the differential response to temperature and light availability of  $C_3$  and  $C_4$  grasses. In general, higher temperatures and high solar radiation favor the growth of  $C_4$  grasses over that of the  $C_3$  grasses (Sage, 2004). Thus, most  $C_4$  grasses dominate in warm, dry and sunny conditions. In contrast, the  $C_3$  grasses are typical of cold, wet and shady environments (Pau, Edwards, & Still, 2013). Living in a micro-environment with reduced solar radiation and temperature may benefit  $C_3$  grasses by reducing photorespiratory activity (Sage, 2004).

Both  $C_4$  species were negatively affected by the net effect of the shrub, suggesting an overwhelming response of  $C_4$  grasses. This negative net effect reflects the reduction in solar radiation and probably the below-ground competition with the shrub. A reduction in the relative growth rate of *Axonopus fissifolius* and *Mnesithea selloana* due to the reduction in light availability was also found in a greenhouse experiment (Altesor, Leoni, Guido, & Paruelo, 2017). 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). For example, Lett and Knapp (2003) found that the reduction in light under the shrub *Cornus drummondii* C.A.Mey. was the main constraint for the growth of the  $C_4$  grass *Andropogon gerardii* Vitman. The negative effect of shade on the performance of  $C_4$  grasses may be due to the high energetic cost for  $CO_2$  fixation related to  $C_4$  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).

The reduction in the reproductive biomass of the  $C_4$  grasses could be due to the lower number of tillers produced by plants growing under the canopy of shrubs. Additionally, tillers need to reach a certain threshold size to initiate the transition from the vegetative to the reproductive stage (Ott & Hartnett, 2011). Perhaps, as a result of the limitation of light, the tillers of the plants that grew under the shrubs did not reach this threshold size and, therefore, were not large enough to flower. A reduction in reproductive biomass was also observed for  $C_4$  grasses growing under natural forest (Naumburg, DeWald, & Kolb, 2001), under shrubs (Schramm & Ehrenfeld, 2010), and under artificial shade (Lee & Cavers, 1981).





**TABLE 2** Mean ( $\pm$ SE) root biomass, aerial biomass, reproductive biomass, number of tillers, and root mass fraction and leaf mass fraction of *Axonopus fissifolius* (A. fiss), *Mnesithea selloana* (M. sell), and *Bromus auleticus* (B. aul); A<sup>+</sup>B<sup>+</sup>: plants below shrub canopy with below-ground effects; A<sup>+</sup>B<sup>-</sup>: plants below shrubs without below-ground effects; A<sup>-</sup>B<sup>-</sup>: plants in open sites without below-ground effects

Variable	Species	A <sup>+</sup> B <sup>+</sup>	A <sup>+</sup> B <sup>-</sup>	A <sup>-</sup> B <sup>-</sup>	Treatment		Block	
					F	p	F	p
Root biomass (g)	A. fiss	2.2 $\pm$ 0.4 a	3.5 $\pm$ 0.4 b	3.7 $\pm$ 0.4 b	4.08	0.035	0.89	0.56
	M. sell	6.5 $\pm$ 1.4 a	10.6 $\pm$ 1.9 a	16.7 $\pm$ 2.6 b	10.7	0.002	2.1	0.13
	B. aul	1.9 $\pm$ 0.46 a	3.0 $\pm$ 0.35 a	2.8 $\pm$ 0.46 a	1.94	0.18	0.99	0.48
Aerial biomass (g)	A. fiss	4.0 $\pm$ 0.7 a	7.1 $\pm$ 1.2 b	9.1 $\pm$ 1.5 b	4.3	0.03	0.73	0.67
	M. sell	4.7 $\pm$ 1.5 a	8.6 $\pm$ 2.5 ab	12.7 $\pm$ 3.3 b	5.1	0.03	1.88	0.17
	B. aul	9.8 $\pm$ 2.6 a	8.9 $\pm$ 2.3 a	10.7 $\pm$ 2.6 a	0.12	0.89	0.68	0.67
Reprod. biomass (g)	A. fiss	1.4 $\pm$ 0.3 a	2.0 $\pm$ 0.3 a	3.3 $\pm$ 0.54 b	8.24	0.003	2.6	0.04
	M. sell	0.7 $\pm$ 0.36 a	0.77 $\pm$ 0.3 a	1.8 $\pm$ 0.5 b	4.25	0.04	1.52	0.25
	B. aul	7.6 $\pm$ 2.7 a	4.5 $\pm$ 1.4 a	5.0 $\pm$ 1.4 a	0.71	0.51	1.1	0.41
Number of tillers	A. fiss	39.3 $\pm$ 6.9 a	64 $\pm$ 12 ab	85 $\pm$ 17.3 b	4.2	0.03	0.8	0.62
	M. sell	12.6 $\pm$ 1.6 a	20 $\pm$ 5.6 ab	31 $\pm$ 5.9 b	7.15	0.009	2.97	0.05
	B. aul	16 $\pm$ 3.27 a	21.0 $\pm$ 5.4 a	17.7 $\pm$ 5.4 a	0.24	0.78	0.34	0.9
Root mass fraction	A. fiss	0.29 $\pm$ 0.03 a	0.29 $\pm$ 0.02 a	0.24 $\pm$ 0.02 a	1.7	0.16	1.93	0.17
	M. sell	0.59 $\pm$ 0.03 a	0.57 $\pm$ 0.03 a	0.55 $\pm$ 0.02 a	0.26	0.77	0.71	0.65
	B. aul	0.12 $\pm$ 0.05 a	0.28 $\pm$ 0.08 a	0.17 $\pm$ 0.05 a	2.3	0.14	0.85	0.56
Leaf mass fraction	A. fiss	0.46 $\pm$ 0.02 a	0.46 $\pm$ 0.03 a	0.44 $\pm$ 0.02 a	1.88	0.12	0.51	0.61
	M. sell	0.37 $\pm$ 0.03 a	0.39 $\pm$ 0.03 a	0.39 $\pm$ 0.03 a	0.21	0.81	0.99	0.47
	B. aul	0.53 $\pm$ 0.03 a	0.51 $\pm$ 0.04 a	0.54 $\pm$ 0.03 a	0.21	0.81	0.93	0.51

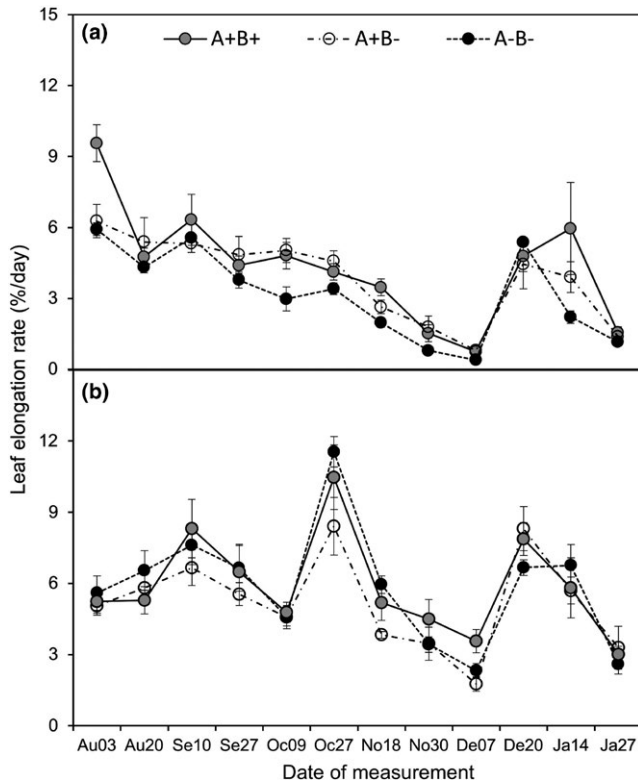
Different letters indicate significant differences among treatments ( $p < 0.05$ ).

We also detected negative effects on the root biomass and on the aerial biomass of *Axonopus fissifolius* and on the LER of *Mnesithea selloana*. These negative effects could be due to the reduction in water availability beneath the shrub canopy. We have evidence that the availability of water in the top 20 cm of soil profile is 20% lower under the shrub than in open sites (Fernández, 2008). Additionally, it has been observed that the improvement in water availability increases the biomass (Fay, Carlisle, Knapp, Blair, & Collins, 2003; Heaton, Voigt, & Long, 2004) and the LER of the C<sub>4</sub> grasses (Passioura & Munns, 2000). The reduction in water could be due to root competition with the shrub. Several studies have shown a negative effect on the growth of C<sub>4</sub> grasses due to competition for soil resources with shrubs (Maestre, Bautista, & Cortina, 2003; Peltzer & Köchy, 2001) and trees (Dohn et al., 2013; Ludwig, Dawson, Prins, Berendse, & Kroon, 2004; Scholes & Archer, 1997), suggesting that competition is an important interaction between C<sub>4</sub> grasses and woody plants in grasslands. The net negative effects exerted by the shrub on the growth of C<sub>4</sub> grasses could explain the negative association found between the spatial distribution of two C<sub>4</sub> grasses (*Mnesithea selloana* and *Paspalum dilatatum*) and the shrub *Acanthostyles buniifolius* in these grasslands (Fernández et al., 2014).

While the canopy of the shrub reduces the growth of the C<sub>4</sub> grasses, it increases the LER of the C<sub>3</sub> grass *Bromus auleticus*. These aerial and net positive effects on the LER of *Bromus auleticus* could be explained by the reduction in solar radiation and temperature

below the shrub canopy. As we did not find an increase in the biomass or the leaf mass fraction of *Bromus auleticus*, we suspect that the higher LER could be due to changes in the size of leaves to cope with reduced radiation (Altesor et al., 2017). It is well documented that plants can increase their specific leaf area (leaf area per unit biomass) to increase the interception of light (Carlucci, Streit, Duarte, & Pillar, 2012). In this sense, a positive correlation between LER and specific leaf area and a negative correlation between LER and leaf dry matter content have been observed in grasses (Arredondo & Schnyder, 2003). Additionally, the micro-environment generated under the shrub may have reduced transpiration demand and hence improved the water status of this species below the shrub canopy (Holmgren et al., 1997). Some studies have shown an increase in leaf size and LER due to higher plant water status (Colabelli, Assuero, & Durand, 2004; Pedrol, Ramos, & Reigosa, 2000). These results could explain the positive spatial association found in these grasslands between *Acanthostyles buniifolius* and the C<sub>3</sub> grasses *Melica brasiliana* and *Piptochaetium stipoides* (Trin. & Rupr.) Hack. & Arechav. (Fernández et al., 2014).

Although no below-ground effects were detected on the biomass of *Bromus auleticus*, the below-ground effect of the shrub reduced the LER of this species on two dates of measurement. This result provides evidence for a temporal shift, from neutral to negative, maybe as a consequence of below-ground competition. Temporal shifts in the outcome of plant-plant interactions,



**FIGURE 2** The effect of shrubs on the leaf elongation rate (LER) of C<sub>3</sub> and C<sub>4</sub> grasses. Mean percentage change ( $\pm$ SE) in the daily LER of the C<sub>3</sub> grass *Bromus auleticus* (a) and the C<sub>4</sub> grass *Mnesithea selloana* (b) planted in open sites with a bag (A<sup>-</sup>B<sup>-</sup>), under shrub with a bag (A<sup>+</sup>B<sup>-</sup>) and under shrub without a bag (A<sup>+</sup>B<sup>+</sup>). The LER was measured from 3 August (Au3) to 27 January (Ja27)

due to ontogeny or seasonal changes of abiotic conditions, have also been observed (Le Roux, Shaw, & Chown, 2013; Soliveres, DeSoto, Maestre, & Olano, 2010). We speculate that the effect of the shrub on the performance of *Bromus auleticus* depends on the balance between the aerial positive effect and the negative below-ground effect. In the short term, this balance could have been shifted from positive to negative depending on the temporal fluctuations in environmental conditions (Wright, Schnitzer, & Reich, 2014). However, in the long term, we found only neutral effects on the biomass and the number of tillers of *Bromus auleticus* because positive effects and negative effects compensated each other.

Although plants were affected by aerial and probably by below-ground competition with the shrub, we did not observe any change in the allocation of biomass in any of the three species. This is especially intriguing in the case of both C<sub>4</sub> grasses that were the most responsive to the aerial and belowground effect of the shrub. We expected changes in the allocation of biomass in response to a decrease in resource availability. The “functional equilibrium” theory predicts that plants respond to a decrease in light with increased allocation to leaves, whereas they respond to a decrease in soil resources with increased allocation to roots (Poorter & Nagel, 2000). The lack of changes in biomass allocation

could reflect temporal fluctuations in the aerial and soil resources that prevent the allocation of more biomass to leaves or to roots in the long term.

Previous studies provided evidence of the importance of the metabolic pathway as a predictor of species’ response to environmental changes induced by grazing (Altesor et al., 2017). Our results also highlight the role that the metabolic pathway plays in the outcome of interactions between grasses and shrubs. Several studies have shown the differential growth response of C<sub>3</sub> and C<sub>4</sub> grasses to light (Zhu, Long, & Ort, 2008), water (Ripley, Frole, & Gilbert, 2010), and temperature manipulation (Yamori, Hikosaka, & Way, 2014). These differences have been used to explain the distribution pattern of grasses at a global scale (Ehleringer, 2005). We found that C<sub>3</sub> and C<sub>4</sub> grasses also responded differently to the micro-environment generated by a shrub species.

In summary, our study contributes to the knowledge of the role of plant–plant interactions in mesic environments. We showed that shrubs have negative effects on the performance of the C<sub>4</sub> grasses *Axonopus fissifolius* and *Mnesithea selloana* and mostly neutral effects on the performance of the C<sub>3</sub> grass *Bromus auleticus*. The C<sub>4</sub> grasses cannot cope with the reduced light environment while the C<sub>3</sub> grass modifies some leaf traits to intercept more light and benefit from the reduction in temperature under the shrub’s canopy. The micro-environment generated underneath shrubs creates a more suitable site for the growth of C<sub>3</sub> than of C<sub>4</sub> grasses.

## ACKNOWLEDGEMENTS

We thank Guillermo Macció and Mabel Bertolotto, owners of the study site, for their logistical support. We thank Claudia Rodríguez for her comments and suggestions on the manuscript. B. Costa, S. García, E. Leoni, F. Lezama, L. López, G. Parodi and J. M. Paruelo kindly helped us with field work. We also thank three anonymous reviewers for their helpful comments on an earlier version of the manuscript.

## AUTHOR CONTRIBUTIONS

GF and AA designed the research, collected the data and wrote the manuscript.

## ORCID

Gastón Fernández  <https://orcid.org/0000-0002-3866-6428>

## REFERENCES

- Aguiar, M. R., & Sala, O. E. (1994). Competition, facilitation, seed distribution and the origin of patches in a Patagonian steppe. *Oikos*, 70, 26–34. <https://doi.org/10.2307/3545695>
- Aguiar, M. R., Soriano, A., & Sala, O. E. (1992). Competition and facilitation in the recruitment of seedlings in the Patagonian steppe. *Functional Ecology*, 6, 66–70. <https://doi.org/10.2307/2389772>



- Al-Namazi, A. A., El-Bana, M. I., & Bonser, S. P. (2017). *Competition and facilitation structure plant communities* under nurse tree canopies in extremely stressful environments. *Ecology and Evolution*, 7, 2747–2755. <https://doi.org/10.1002/ece3.2690>
- Altesor, A., Leoni, E., Guido, A., & Paruelo, J. M. (2017). Differential responses of three grasses to defoliation, water and light availability. *Plant Ecology*, 218, 95–104.
- Altesor, A., Oesterheld, M., Leoni, E., Lezama, F., & Rodríguez, C. (2005). Effect of grazing on community structure and productivity of a Uruguayan grassland. *Plant Ecology*, 179, 83–91. <https://doi.org/10.1007/s11258-004-5800-5>
- 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. *Journal of Vegetation Science*, 17, 323–332. <https://doi.org/10.1111/j.1654-1103.2006.tb02452.x>
- Armas, C., Ordiales, R., & Pugnaire, F. I. (2004). Measuring plant interactions: a new comparative index. *Ecology*, 85, 2682–2686. <https://doi.org/10.1890/03-0650>
- Armas, C., & Pugnaire, F. I. (2005). Plant interactions govern population dynamics in a semi-arid plant community. *Journal of Ecology*, 93, 978–989. <https://doi.org/10.1111/j.1365-2745.2005.01033.x>
- Arredondo, J. T., & Schnyder, H. (2003). Components of leaf elongation rate and their relationship to specific leaf area in contrasting grasses. *New Phytologist*, 158, 305–314. <https://doi.org/10.1046/j.1469-8137.2003.00745.x>
- Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology and Evolution*, 9, 191–193. [https://doi.org/10.1016/0169-5347\(94\)90088-4](https://doi.org/10.1016/0169-5347(94)90088-4)
- Briggs, J. M., Knapp, A. K., Blair, J. M., Heisler, J. L., Hoch, G. A., Lett, M. S., & McCarron, J. K. (2005). An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. *BioScience*, 55, 243–254. [https://doi.org/10.1641/0006-3568\(2005\)055\[0243:AEITCA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0243:AEITCA]2.0.CO;2)
- Callaway, R. M. (2007). *Positive interactions and interdependence in plant communities*. Dordrecht, The Netherlands: Springer.
- Carlucci, M. B., Streit, H., Duarte, L. D. S., & Pillar, V. D. (2012). Individual-based trait analyses reveal assembly patterns in tree sapling communities. *Journal of Vegetation Science*, 23, 176–186. <https://doi.org/10.1111/j.1654-1103.2011.01339.x>
- Chen, J., Franklin, J. F., & Spies, T. A. (1995). Growing-season microclimatic gradients from clearcut edges into old-growth Douglas-fir forests. *Ecological Applications*, 5, 74–86. <https://doi.org/10.2307/1942053>
- Colabelli, M., Assuero, S., & Durand, J. L. (2004). Water status and leaf elongation of C<sub>3</sub>- and C<sub>4</sub> grasses of Flooding Pampa grassland. *Journal of Vegetation Science*, 15, 817–822. [https://doi.org/10.1658/1100-9233\(2004\)015\[0817:wsaleo\]2.0.co;2](https://doi.org/10.1658/1100-9233(2004)015[0817:wsaleo]2.0.co;2)
- Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2017). InfoStat versión 2017. Grupo InfoStat de Córdoba, Argentina: FCA, Universidad Nacional de Córdoba. Retrieved from <http://www.infostat.com.ar>
- Dohn, J., Dembélé, F., Karambé, M., Moustakas, A., Amévor, K. A., & Hanan, N. P. (2013). Tree effects on grass growth in savannas: competition, facilitation and the stress-gradient hypothesis. *Journal of Ecology*, 101, 202–209. <https://doi.org/10.1111/1365-2745.12010>
- Ehleringer, J. R. (2005). On the influence of atmospheric CO<sub>2</sub>, temperature, and water on the abundances of C<sub>3</sub>/C<sub>4</sub> taxa. In J. R. Ehleringer, T. E. Cerling, & M. D. Dearing (Eds.), *A history of atmospheric CO<sub>2</sub> and its effect on plants, animals, and ecosystems* (pp. 214–231). New York, NY: Springer Verlag.
- 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. *Journal of Biogeography*, 29, 875–888. <https://doi.org/10.1046/j.1365-2699.2002.00701.x>
- Fay, P. A., Carlisle, J. D., Knapp, A. K., Blair, J. M., & Collins, S. L. (2003). Productivity responses to altered rainfall patterns in a C<sub>4</sub>-dominated grassland. *Oecologia*, 137, 245–251. <https://doi.org/10.1007/s00442-003-1331-3>
- Fernández, G. (2008). *Efecto de los arbustos sobre el estrato graminoso y comportamiento hídrico de los Tipos Funcionales de Plantas en pastizales naturales*. Unpublished bachelor thesis, Facultad de Ciencias, Universidad de la República, Uruguay.
- Fernández, G., Texeira, M., & Altesor, A. (2014). The small scale spatial pattern of C<sub>3</sub> and C<sub>4</sub> grasses depends on shrub distribution. *Austral Ecology*, 39, 532–539. <https://doi.org/10.1111/aec.12113>
- Gross, N., Liancourt, P., Choler, P., Suding, K. N., & Lavorel, S. (2010). Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions. *Perspectives in Plant Ecology, Evolution and Systematics*, 12, 9–19. <https://doi.org/10.1016/j.ppees.2009.09.001>
- He, Q., Bertness, M. D., & Altieri, A. H. (2013). Global shifts towards positive species interactions with increasing environmental stress. *Ecology Letters*, 16, 695–706. <https://doi.org/10.1111/ele.12080>
- Heaton, E., Voigt, T., & Long, S. P. (2004). A quantitative review comparing the yields of two candidate C<sub>4</sub> perennial biomass crops in relation to nitrogen, temperature and water. *Biomass and Bioenergy*, 27, 21–30. <https://doi.org/10.1016/j.biombioe.2003.10.005>
- Holmgren, M., & Scheffer, M. (2010). Strong facilitation in mild environments: The stress gradient hypothesis revisited. *Journal of Ecology*, 98, 1269–1275. <https://doi.org/10.1111/j.1365-2745.2010.01709.x>
- Holmgren, M., Scheffer, M., & Huston, M. (1997). The interplay of facilitation and competition in plant communities. *Ecology*, 78, 1966–1975. [https://doi.org/10.1890/0012-9658\(1997\)078\[1966:tiofac\]2.0.co;2](https://doi.org/10.1890/0012-9658(1997)078[1966:tiofac]2.0.co;2)
- King, E. G., & Stanton, M. L. (2008). Facilitative effects of *Aloe* shrubs on grass establishment, growth, and reproduction in degraded Kenyan rangelands: implications for restoration. *Restoration Ecology*, 16, 464–474. <https://doi.org/10.1111/j.1526-100x.2007.00310.x>
- Le Roux, P. C., Shaw, J. D., & Chown, S. L. (2013). Ontogenetic shifts in plant interactions vary with environmental severity and affect population structure. *New Phytologist*, 200, 241–250. <https://doi.org/10.1111/nph.12349>
- Lee, S. M., & Cavers, P. B. (1981). The effects of shade on growth, development, and resource allocation patterns of three species of fox-tail (*Setaria*). *Canadian Journal of Botany*, 59, 1776–1786. <https://doi.org/10.1139/b81-237>
- Leger, E. A., & Espeland, E. K. (2010). The shifting balance of facilitation and competition affects the outcome of intra- and interspecific interactions over the life history of California grassland annuals. *Plant Ecology*, 208, 333–345. <https://doi.org/10.1007/s11258-009-9710-4>
- Leoni, E., Altesor, A., & Paruelo, J. M. (2009). Explaining patterns of primary production from individual level traits. *Journal of Vegetation Science*, 20, 612–619. <https://doi.org/10.1111/j.1654-1103.2009.01080.x>
- Lett, M. S., & Knapp, A. K. (2003). Consequences of shrub expansion in mesic grassland: Resource alterations and graminoid responses. *Journal of Vegetation Science*, 14, 487–496. <https://doi.org/10.1111/j.1654-1103.2003.tb02175.x>
- Liancourt, P., Callaway, R., & Michalet, R. (2005). Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology*, 86, 1611–1618. <https://doi.org/10.1890/04-1398>
- Ludwig, F., Dawson, T. E., Prins, H. H., Berendse, F., & Kroon, H. (2004). Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. *Ecology Letters*, 7, 623–631. <https://doi.org/10.1111/j.1461-0248.2004.00615.x>
- Maestre, F. T., Bautista, S., & Cortina, J. (2003). Positive, negative and net effects in grass–shrub interactions in Mediterranean semiarid grasslands. *Ecology*, 84, 3186–3197. <https://doi.org/10.1890/02-0635>
- Maestre, F. T., Callaway, R. M., Valladares, F., & Lortie, C. J. (2009). Refining the stress-gradient hypothesis for competition and





- facilitation in plant communities. *Journal of Ecology*, 97, 199–205. <https://doi.org/10.1111/j.1365-2745.2008.01476.x>
- Malkinson, D., & Tielbörger, K. (2010). What does the stress-gradient hypothesis predict? Resolving the discrepancies. *Oikos*, 119, 1546–1552. <https://doi.org/10.1111/j.1600-0706.2010.18375.x>
- Michalet, R., Le Bagousse-Pinguet, Y., Maalouf, J.-P., & Lortie, C. J. (2014). Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. *Journal of Vegetation Science*, 25, 609–613. <https://doi.org/10.1111/jvs.12123>
- Miriti, M. (2006). Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology*, 94, 973–979. <https://doi.org/10.1111/j.1365-2745.2006.01138.x>
- Naumburg, E., DeWald, L. E., & Kolb, T. E. (2001). Shade responses of five grasses native to southwestern U.S. *Pinus ponderosa* forests. *Canadian Journal of Botany*, 79, 1001–1009. <https://doi.org/10.1139/b01-0801.1139/cjb-79-9-1001>
- Núñez, C. I., Raffaele, E., Núñez, M. A., & Cuassolo, F. (2009). When do nurse plants stop nursing? Temporal changes in water stress levels in *Austrocedrus chilensis* growing within and outside shrubs. *Journal of Vegetation Science*, 20, 1064–1071. <https://doi.org/10.1111/j.1654-1103.2009.01107.x>
- Ott, J. P., & Hartnett, D. C. (2011). Bud production and dynamics of flowering and vegetative tillers in *Andropogon gerardii* (Poaceae): the role of developmental constraints. *American Journal of Botany*, 98, 1293–1298. <https://doi.org/10.3732/ajb.1000264>
- Paruelo, J. M., Jobbágy, E. G., Oesterheld, M., Golluscio, R. A., & Aguiar, M. R. (2007). The grasslands and steppes of Patagonia and the Río de la Plata plains. In T. T. Veblen, K. R. Young, & A. R. Orme (Eds.), *The physical geography of South America* (pp. 232–248). Oxford, UK: Oxford University Press.
- 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. *Ecological Applications*, 8, 194–206. [https://doi.org/10.1890/1051-0761\(1998\)008\[0194:fascot\]2.0.co;2](https://doi.org/10.1890/1051-0761(1998)008[0194:fascot]2.0.co;2)
- Passioura, J. B. & Munns, R. (2000). Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. *Australian Journal of Plant Physiology*, 27, 941–948. <https://doi.org/10.1071/pp99207>
- Pau, S., Edwards, E. J., & Still, C. J. (2013). Improving our understanding of environmental controls on the distribution of C<sub>3</sub> and C<sub>4</sub> grasses. *Global Change Biology*, 19, 184–196. <https://doi.org/10.1111/gcb.12037>
- Pedrol, N., Ramos, P., & Reigosa, M. J. (2000). Phenotypic plasticity and acclimation to water deficits in velvet-grass: a long-term greenhouse experiment. Changes in leaf morphology, photosynthesis and stress-induced metabolites. *Journal of Plant Physiology*, 157, 383–393. [https://doi.org/10.1016/s0176-1617\(00\)80023-1](https://doi.org/10.1016/s0176-1617(00)80023-1)
- Peltzer, D. A., & Köchy, M. (2001). Competitive effects of grasses and woody plants in mixed-grass prairie. *Journal of Ecology*, 89, 519–527. <https://doi.org/10.1046/j.1365-2745.2001.00570.x>
- Poorter, H., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO<sub>2</sub>, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology*, 27, 595–607. <https://doi.org/10.1071/pp99173>
- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193, 30–50. <https://doi.org/10.1111/j.1469-8137.2011.03952.x>
- Ripley, B., Frole, K., & Gilbert, M. (2010). Differences in drought sensitivities and photosynthetic limitations between co-occurring C<sub>3</sub> and C<sub>4</sub> (NADP-ME) Panicoid grasses. *Annals of Botany*, 105, 493–503. <https://doi.org/10.1093/aob/mcp307>
- Rodríguez, C., Leoni, E., Lezama, F., & Altesor, A. (2003). Temporal trends in species composition and plant traits in natural grasslands of Uruguay. *Journal of Vegetation Science*, 14, 433–440. <https://doi.org/10.1111/j.1654-1103.2003.tb02169.x>
- Sage, R. F. (2004). The evolution of C<sub>4</sub> photosynthesis. *New Phytologist*, 161, 341–370. <https://doi.org/10.1111/j.1469-8137.2004.00974.x>
- Scholes, R. J., & Archer, S. R. (1997). Tree–grass interactions in savannas. *Annual Review of Ecology and Systematics*, 28, 517–544. <https://doi.org/10.1146/annurev.ecolsys.28.1.517>
- Schramm, J. W., & Ehrenfeld, J. G. (2010). Leaf litter and understory canopy shade limit the establishment, growth and reproduction of *Microstegium vimineum*. *Biological Invasions*, 12, 3195–3204. <https://doi.org/10.1007/s10530-010-9711-1>
- Soliveres, S., DeSoto, L., Maestre, F. T., & Olano, M. J. (2010). Spatio-temporal heterogeneity in abiotic factors can modulate multiple ontogenetic shifts between competition and facilitation. *Perspectives in Plant Ecology, Evolution and Systematics*, 12, 227–234. <https://doi.org/10.1016/j.ppees.2010.02.003>
- Soriano, A. (1991). Río de la Plata Grasslands. In R. T. Coupland (Ed.), *Natural grasslands. Introduction and western hemisphere* (pp. 367–407). Amsterdam, The Netherlands: Elsevier.
- Taylor, S. H., Ripley, B. S., Martin, T., De-Wet, L.-A., Woodward, F. I., & Osborne, C. P. (2014). Physiological advantages of C<sub>4</sub> grasses in the field: a comparative experiment demonstrating the importance of drought. *Global Change Biology*, 20, 1992–2003. <https://doi.org/10.1111/gcb.12498>
- Wright, A., Schnitzer, S. A., & Reich, P. B. (2014). Living close to you neighbors: the importance of both competition and facilitation in plant communities. *Ecology*, 95, 2213–2223. <https://doi.org/10.1890/13-1855.1>
- Yamori, W., Hikosaka, K., & Way, D. A. (2014). Temperature response of photosynthesis in C<sub>3</sub>, C<sub>4</sub>, and CAM plants: Temperature acclimation and temperature adaptation. *Photosynthesis Research*, 119, 101–117. <https://doi.org/10.1007/s11120-013-9874-6>
- Zhu, X.-G., Long, S. P., & Ort, D. R. (2008). What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Current Opinion in Biotechnology*, 19, 153–159. <https://doi.org/10.1016/j.copbio.2008.02.004>

**How to cite this article:** Fernández G, Altesor A. Differential responses of C<sub>3</sub> and C<sub>4</sub> grasses to shrub effects in a sub-humid grassland of South America. *J Veg Sci.* 2019;30:203–211. <https://doi.org/10.1111/jvs.12715>