

# Differential responses of three grasses to defoliation, water and light availability

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**Abstract** Grazing not only modifies the structure and functioning of grasslands, it also changes micro-environmental conditions that alter the availability of resources. The aim of this study was to analyze the response of grasses with different photosynthetic pathways ( $C_3/C_4$ ), growth forms (prostrate/erect), and grazing responses (increaser/decreaser) to defoliation and resource availability. In a greenhouse, we performed a factorial experiment with three factors: defoliation, light, and water and three species: *Axonopus affinis* ( $C_4$  prostrate, increaser), *Coelorachis selloana*

( $C_4$  erect, decreaser), and *Bromus auleticus* ( $C_3$  erect, decreaser). We measured the relative growth rate (RGR), biomass assignment, and specific leaf area. The RGR of both  $C_4$  species was affected by light availability, while the decreaser  $C_3$  did not respond to any factor. Biomass allocation to leaves and stolons changed with the interaction between light and water in the  $C_4$  prostrate species (increaser). In the  $C_4$  erect grass (decreaser), biomass allocation was more affected by defoliation under low levels of light and water. Low light availability and defoliation reduced the assignment to leaves, while the allocation to rhizomes increased. Species-specific responses to resources availability that are modified by grazing were related to photosynthetic pathway, growth form, and grazing responses. Biomass allocation was related to strategies to avoid and/or tolerate grazing. The investment to leaves was limited by light and water availability in prostrate species, while in erect grasses it was controlled by defoliation and water availability. Our results highlight the importance of species responses to changes in resource availability associated to grazing regimes.

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## Introduction

Grazing by large herbivores is a major determinant of vegetation structure, species composition, and

production in grasslands (Milchunas and Lauenroth 1993). Grazers affect individual plants by defoliation and trampling, which, in turn, change the micro-environmental properties (McNaughton 1979, 1985; Milchunas et al. 1988; Sala 1988; Whicker and Detling 1988), such as the soil moisture, temperature, and light conditions. These changes impact the cycling of materials and thus resource availability. The assessment of species responses to defoliation and resource availability is crucial to understand the mechanism through which grazing modifies community and ecosystem properties.

Several models predict an increase of the impact of grazing on vegetation structure with increasing productivity (Milchunas et al. 1988; Proulx and Mazumder 1998; Cingolani et al. 2005). Recently, Lezama et al. (2014) provided empirical evidence for this hypothesis for South American temperate grasslands. For the sub-humid region, several comparative studies of paired grazed and ungrazed areas reported significant differences in community structure (Facelli 1988; Díaz et al. 1992; Rodríguez et al. 2003; Cingolani et al. 2005; Altesor et al. 2006; Lezama et al. 2014), ecosystem functioning (Oesterheld et al. 1999; Altesor et al. 2005; Lezama et al. 2014), biogeochemical and physical properties of soils (Taboada and Lavado 1988; Milchunas and Lauenroth 1993; Lavado et al. 1995; Altesor et al. 2006; Piñeiro et al. 2009), and environmental conditions (Fernández et al. 2014). Additionally, the results showed that species richness and diversity were higher in grazed than in ungrazed areas (Rodríguez et al. 2003; Altesor et al. 2006). Grazing induced changes in floristic composition, with certain plant species being favored under herbivory (increasers), whereas other species become scarce (decreasers) (*sensu* Dyksterhuis 1949; Noy-Meir et al. 1989). In Uruguayan ungrazed areas, erect C<sub>3</sub> grasses increase their frequency and co-dominate with C<sub>4</sub> grasses (Rodríguez et al. 2003; Altesor et al. 2005). Altesor et al. (2005) showed that aboveground net primary production (ANPP) was higher in grazed than in ungrazed areas. However, grazing simulation inside ungrazed areas increases ANPP.

Although being threatened by agricultural expansion and commercial afforestation (Paruelo et al. 2006; Gautreau 2013), the Uruguayan Campos, an extensive sub-unit of the Rio de la Plata grasslands (Soriano 1991), is one of the largest areas of the world covered

by sub-humid temperate grasslands with moderate to high productivity (Paruelo et al. 2010). In the Uruguayan Campos, prostrate C<sub>4</sub> grasses, with broad leaves and horizontal growth through stolons or rhizomes, increase their abundance and dominate under grazed conditions, generating a low and dense stratum of maximum 5 cm height (Altesor et al. 1999; Rodríguez et al. 2003). In ungrazed areas, where domestic herbivores were excluded for more than 3 years, the canopy is taller and dominated by erect C<sub>3</sub> and C<sub>4</sub> grasses and shrubs (Altesor et al. 1999, 2005, 2006; Rodríguez et al. 2003). These taller-statured grasses are better adapted to conditions of low irradiance but height makes them more susceptible to herbivory (Coughenour 1985; Milchunas et al. 1988), decreasing their abundance under grazing.

Leoni et al. (2009) documented that increaser and decreaser species are characterized by different traits, which may explain the differences observed in ANPP between grazed and ungrazed conditions. Increaser species were associated with high values of specific leaf area, tillering rate, leaf number, leaf weight ratio, green leaf rate, and root weight ratio. These traits maximize the regeneration of photosynthetically active surfaces and allow for a constant growth rate through time. On the other hand, decreaser species were associated with high values of dead biomass/total biomass, leaf elongation rate, reproductive biomass/total biomass, senescence rate, and total biomass, which led to a reduction in the relative growth rate (RGR) through time. Removing large herbivores (mainly cattle) reduces defoliation, trampling, and feces and urine deposition, which promotes changes in resource availability and environmental conditions. In ungrazed areas, shrub richness and cover increase (Altesor et al. 2006), vegetation increases in height, and great quantity of standing dead biomass accumulates (Altesor et al. 2005). These changes generate a significant reduction in light availability, temperature, and wind speed at ground level (Fernández et al. 2014). These conditions and the higher litter cover in ungrazed areas as compared to grazed areas (Altesor et al. 2006) decrease the evaporative demand and increase soil water availability (Bagnato 2010).

Disturbances associated with grazing are severe at the individual plant level, potentially affecting ecophysiological responses and biomass allocation patterns (Fahnestock and Knapp 1994). Therefore, the

aim of this study was to analyze the responses of two erect decreaser grasses: one  $C_3$  (*Bromus auleticus*) and other  $C_4$  (*Coelorachis selloana*), and an increaser  $C_4$  prostrate grass (*Axonopus affinis*), to defoliation, water and light availability. The availability of both resources is associated with grazing regimes. In ungrazed areas, shrub encroachment and standing dead biomass accumulation generate a reduction in light availability while diminishing the evaporative demand (Altesor et al. 2006; Bagnato 2010). Considering that each species has traits that make it successful under certain disturbance regimes and environmental conditions, we expect that the growth rates of increasers and decreasers, as well as biomass allocation, respond differently not only to defoliation, but also to changes in resource availability. We predict that the reduction of RGR in response to defoliation will be lower in increaser than in decreaser species. The effect of low irradiance will be lower in decreasers than in the increaser species and the opposite effect will be observed for water availability.

## Methods

We performed a complete factorial experiment to evaluate the effects of three factors with two levels each ( $2^3 = 8$  treatments, see Table 1): defoliation (D+/D-), water (W+/W-), and light (L+/L-), on (i) the RGR, (ii) the biomass assignment, and (iii) the specific leaf area (SLA), for *A. affinis*, *C. selloana*, and *B. auleticus*. The species were selected because of the magnitude of the effect of grazing on their cover values (Grazing Response Index, GRI) according to Caussials (2010) and their abundances in the study area. For each species, GRI was calculated from the cover values in paired ungrazed–grazed plots as

$$\text{GRI} = \frac{\sum (G_{\text{cover}} - UG_{\text{cover}})}{\sum (G_{\text{cover}} + UG_{\text{cover}})},$$

where  $G_{\text{cover}}$  is the cover value of a certain species under grazing regime and  $UG_{\text{cover}}$  is the cover value of the same species in excluded area. The index values range from -1 to 1.

We selected two grasses with negative GRI values: a  $C_3$  perennial erect grass (*B. auleticus*, GRI = -1, only present in enclosures) and a  $C_4$  perennial erect grass (*C. selloana*, GRI = -0.48). In addition, we selected a grass with a positive GRI value, the  $C_4$

**Table 1** Treatment combinations with two levels (+, -) of the three factors (D defoliation, W water, L light)

Treatment	Abbreviation
Non-defoliated	D-W+L+
Water at field capacity	
High level of light	
Non-defoliated	D-W+L-
Water at field capacity	
Low level of light	
Defoliated	D+W+L+
Water at field capacity	
High level of light	
Defoliated	D+W+L-
Water at field capacity	
Low level of light	
Non-defoliated	D-W-L+
Water restriction	
High level of light	
Non-defoliated	D-W-L-
Water restriction	
Low level of light	
Defoliated	D+W-L+
Water restriction	
High level of light	
Defoliated	D+W-L-
Water restriction	
Low level of light	

perennial prostrate grass *A. affinis* (GRI = 0.76). We collected tillers from two contiguous areas, one of them grazed and the other excluded to domestic herbivory for 15 years, in a natural grassland of the Uruguayan Campos sub-region of the Rio de la Plata grasslands (Soriano 1991). Tillers of *C. selloana* and *B. auleticus* were collected in the enclosure and tillers of *A. affinis* in the grazed area.

The site is located in the south-central region of Uruguay (34°19' S, 57°02' W) on a homogeneous and typical prairie soil (Argiudol). In July, we extracted soil blocks including the selected plant species. Blocks were brought to the greenhouse and the selected species were carefully separated from the rest, washing their roots. Immediately, tillers of the three species were planted in individual pots with 2500 grams of river sand and weekly watered with Hoagland's nutrient solution during 2 months.

After an acclimation period of 1 month, we eliminated the extreme individuals and selected 144 pots with plants of similar size for each species, the necessary number to complete three harvests of six individuals from each of the eight treatments per species. The pots were periodically rotated in order to maintain homogeneous conditions.

To simulate defoliation (D+), plants were defoliated once at the beginning of the experiment by cutting half of the blades of the leaves. Water was supplied at 100 % (W+) and 50 % (W-) of field capacity, representing the two levels of water availability. Water status was gravimetrically monitored every day.

The levels of light availability included full sunlight (L+) and a reduction of the incoming light (L-). Light restriction was generated with a shadow grid which allowed the passage of approximately 50 % of the sunlight, simulating light conditions under grazing exclusion (Fernández et al. 2014). For both levels of light availability, the radiation was measured with a hand radiometer that covers only the photosynthetically active radiation (PAR, 400–700 nm). The average PAR for the full-sunlight condition was 758 and 333  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the low irradiance level.

The first harvest corresponded to the start of the experiment (initial conditions) and the remaining two were carried out every 2 weeks. For each harvest, six individuals per species were randomly selected in each treatment. Harvested individuals were separated into roots, stolons (for *A. affinis*) or rhizomes (for *C. selloana* and *B. auleticus*), green leaves, dead leaves, and inflorescences. Dry weight of each fraction was determined after oven-drying (48 h at 70 °C) the material.

The RGR for each species and treatment was calculated as the slope of the linear regression between the ln of total dry biomass through time (Hoffmann and Poorter 2002; Poorter and Garnier 1996) (milligrams of new biomass produced per gram of pre-existing biomass per day,  $\text{mg g}^{-1} \text{d}^{-1}$ ). In the last harvest, we calculated the biomass allocation to leaves (leaf mass fraction, LMF) and rhizome or stolon mass fraction (Stol-RhMF) depending on the species. SLA was estimated only in the last harvest, on fully expanded leaves of three plants harvested from each treatment. In the middle of leaf blades, a 4-cm piece was cut and the width was measured with a caliper.

## Data analysis

For each species, we performed paired comparisons between levels of treatments (i.e., 28 different pairs of comparisons). For that, we tested for differences between the slopes of linear regression models of the ln-transformed total dry mass of the six harvested individuals versus time. Because replicates were used to estimate the slopes of the ln-biomass versus time (i.e., RGR), we could not perform an ANOVA. ANOVA based on the ln of total biomass including time as a factor yielded the same results (Table S1).

SLA and biomass allocation to leaves (LMF) and to rhizomes or stolons (Stol-RhMF) were analyzed using three-way ANOVAs, with Defoliation, Light, and Water as factors. Data were checked for normality and variance homogeneity. All analyses were performed using InfoStat version 2016.

## Results

The three species registered the highest RGR in the treatment without defoliation and with abundant resources (D-W+L+) (Table 2). In *A. affinis*, the RGR differed significantly between most of the treatments in which light availability was reduced (9 of the 16 comparisons in which the opposite light conditions were tested; Table 3). In *C. selloana*, the RGR differed significantly between all the treatments in which light availability was reduced (Table 3). Defoliation significantly decreased RGR in *C. selloana* when the level of the other resources was held constant and high (Table 3). In *B. auleticus*, none of the 28 comparisons of RGR among treatments showed significant differences.

Defoliation and the level of resources led to a different response among species, with regard to biomass allocation to leaves (LMF) and stolons or rhizomes (Stol-RhMF) (Fig. 1). The interaction between light and water had a significant effect on both leaves and stolon mass fractions in *A. affinis* (Fig. 1). Under shaded conditions, leaves assignment was higher than under high irradiance, but well-watered plants assigned 13 % more biomass to leaves than plants under water restrictions ( $F = 5.1$ ,  $df = 1$ ,  $P = 0.029$ ). Under low irradiance, the Stol-RhMF was

**Table 2** RGR calculated as the slope of the linear regression model through the ln-transformed total dry mass versus time for each treatment and species

Treatment	<i>Axonopus affinis</i>		<i>Coelorachis selloana</i>		<i>Bromus auleticus</i>	
	RGR $\pm$ SE (mg g <sup>-1</sup> d <sup>-1</sup> )	r <sup>2</sup>	RGR $\pm$ SE (mg g <sup>-1</sup> d <sup>-1</sup> )	r <sup>2</sup>	RGR $\pm$ SE (mg g <sup>-1</sup> d <sup>-1</sup> )	r <sup>2</sup>
D-W+L+	43.28 $\pm$ 8.27	0.63***	37.94 $\pm$ 4.6	0.81***	28.89 $\pm$ 7.67	0.47**
D-W+L-	14.61 $\pm$ 9.86	0.12	11.39 $\pm$ 5.00	0.25*	18.28 $\pm$ 8.34	0.23*
D+W+L+	22.39 $\pm$ 8.80	0.29*	25.44 $\pm$ 4.02	0.72***	20.00 $\pm$ 7.81	0.29*
D+W+L-	3.39 $\pm$ 10.21	0.007	9.11 $\pm$ 6.84	0.10	9.33 $\pm$ 7.06	0.10
D-W-L+	37.39 $\pm$ 9.18	0.51***	30.44 $\pm$ 4.77	0.72***	20.44 $\pm$ 8.47	0.27*
D-W-L-	1.17 $\pm$ 9.07	0.37	7.17 $\pm$ 4.79	0.12	10.11 $\pm$ 6.19	0.14
D+W-L+	35.06 $\pm$ 9.08	0.48***	33.89 $\pm$ 7.28	0.58***	24.39 $\pm$ 6.82	0.45**
D+W-L-	9.89 $\pm$ 9.05	0.07	9.72 $\pm$ 4.84	0.20	22.44 $\pm$ 5.03	0.55***

Significant regression lines are indicated by asterisks: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

17.8 % lower in watered plants than in those with low water availability. On the contrary, with high irradiance, irrigated plants assigned 51.6 % more biomass to Stol-RhMF than those under water restrictions ( $F = 10.63$ ,  $df = 1$ ,  $P = 0.002$ ) (Fig. 1). In *C. selloana*, light-by-defoliation interaction modified significantly both LMF ( $F = 7.29$ ,  $df = 1$ ,  $P = 0.01$ ) and Stol-RhMF ( $F = 3.933$ ,  $df = 1$ ,  $P = 0.05$ ; Fig. 1). Under low irradiance conditions, defoliation decreased the LMF by 16 % and, on the contrary, the Stol-RhMF increased by 31.6 %. Under water restrictions, LMF was lower in defoliated plants ( $F = 3.95$ ,  $df = 1$ ,  $P = 0.05$ ) (Fig. 1). In *B. auleticus*, the interaction between defoliation and water availability affected LMF (Fig. 1). With low water availability, defoliation reduced LMF by 10.4 %, but when well watered, defoliation increased LMF by 11.6 % ( $F = 6.11$ ,  $df = 1$ ,  $P = 0.02$ ).

In all three species, SLA was significantly affected by light availability. Under low irradiance, SLA was 65 % higher in *A. affinis* ( $F = 68.38$ ,  $df = 1$ ,  $P < 0.0001$ ), 49 % higher in *C. selloana* ( $F = 30.04$ ,  $df = 1$ ,  $P < 0.0001$ ), and 79 % higher in *B. auleticus* ( $F = 35.23$ ,  $df = 1$ ,  $P < 0.0001$ ) than its values under high irradiance conditions. Only for *A. affinis*, we detected a significant interaction between water and defoliation ( $F = 7.05$ ,  $df = 1$ ,  $P = 0.02$ ). Watered plants of *A. affinis* increased their SLA by 17 % when defoliated (Fig. 2).

## Discussion

### Relative growth rate responses

Our results suggest that species respond differently to defoliation and the changes in resource availability induced by grazing, confirming partially our predictions. Species-specific responses, in terms of RGR, were more associated to the photosynthetic pathway ( $C_3/C_4$ ) than to the response to herbivory (increaser/decreaser). Changes in light availability affected growth rates in the two  $C_4$  species (*A. affinis* and *C. selloana*). Both species had significantly higher relative growth under high irradiance condition, whereas the RGR of the  $C_3$  species (*B. auleticus*) did not differ under different light levels. The response of RGR to irradiance of *C. selloana* was more linked to its photosynthetic pathway ( $C_4$ ) than to its growth form (erect). In the case of *A. affinis*, and regardless the level of resources, defoliated plants grew as fast as undefoliated ones, showing a compensation response to clipping (Ferraro and Oosterheld 2002). Several hypotheses have been proposed to explain compensatory growth after biomass removal. Some of them are as follows: (i) photosynthesis rate increases; (ii) apical dominance is released, thus promoting tillering; and (iii) mineral nutrient availability is increased due to nutrient cycling (McNaughton 1983). The lack of compensatory growth in *C. selloana* with high level of light and water may explain its negative GRI.

**Table 3** Comparisons of slopes of the linear regression models of the ln-transformed total dry mass versus time among the treatments (*D* defoliation, *W* water, *L* light) for *Axonopus affinis* and *Coelorachis selloana*

<i>Axonopus affinis</i>	D–W–L+	D+W–L+	D+W+L+	D+W–L–	D+W+L–	D–W+L–	D–W–L–
D–W+L+	$F = 0.236$ $P = 0.63$	$F = 0.449$ $P = 0.507$	$F = 2.999$ $P = 0.093$	<b><math>F = 7.410</math></b> <b><math>P = 0.01</math></b>	<b><math>F = 9.21</math></b> <b><math>P = 0.005</math></b>	<b><math>F = 4.956</math></b> <b><math>P = 0.033</math></b>	<b><math>F = 11.78</math></b> <b><math>P = 0.002</math></b>
D–W–L+		$F = 0.030$ $P = 0.863$	$F = 1.377$ $P = 0.249$	<b><math>F = 4.521</math></b> <b><math>P = 0.041</math></b>	<b><math>F = 6.097</math></b> <b><math>P = 0.019</math></b>	$F = 2.833$ $P = 0.102$	<b><math>F = 7.86</math></b> <b><math>P = 0.009</math></b>
D+W–L+			$F = 1.003$ $P = 0.324$	$F = 3.842$ $P = 0.059$	<b><math>F = 5.355</math></b> <b><math>P = 0.027</math></b>	$F = 2.318$ $P = 0.138$	<b><math>F = 6.97</math></b> <b><math>P = 0.013</math></b>
D+W+L+				$F = 0.977$ $P = 0.33$	$F = 1.978$ $P = 0.169$	$F = 0.345$ $P = 0.561$	$F = 2.820$ $P = 0.103$
D+W–L–					$F = 0.225$ $P = 0.639$	$F = 0.124$ $P = 0.727$	$F = 0.464$ $P = 0.5$
D+W+L–						$F = 0.621$ $P = 0.437$	$F = 0.028$ $P = 0.869$
D–W+L–							$F = 1.008$ $P = 0.323$
<i>Coelorachis selloana</i>	D–W–L+	D+W–L+	D+W+L+	D+W–L–	D+W+L–	D–W+L–	D–W–L–
D–W+L+	$F = 1.302$ $P = 0.26$	$F = 0.22$ $P = 0.642$	<b><math>F = 4.198</math></b> <b><math>P = 0.048</math></b>	<b><math>F = 17.94</math></b> <b><math>P = 0.000</math></b>	<b><math>F = 12.29</math></b> <b><math>P = 0.001</math></b>	<b><math>F = 15.31</math></b> <b><math>P = 0.000</math></b>	<b><math>F = 21.69</math></b> <b><math>P = 0.000</math></b>
D–W–L+		$F = 0.161$ $P = 0.69$	$F = 0.064$ $P = 0.43$	<b><math>F = 9.34</math></b> <b><math>P = 0.005</math></b>	<b><math>F = 6.558</math></b> <b><math>P = 0.015</math></b>	<b><math>F = 7.616</math></b> <b><math>P = 0.009</math></b>	<b><math>F = 11.99</math></b> <b><math>P = 0.002</math></b>
D+W–L+			$F = 1.032$ $P = 0.317$	<b><math>F = 7.668</math></b> <b><math>P = 0.009</math></b>	<b><math>F = 6.16</math></b> <b><math>P = 0.018</math></b>	<b><math>F = 6.501</math></b> <b><math>P = 0.016</math></b>	<b><math>F = 9.476</math></b> <b><math>P = 0.004</math></b>
D+W+L+				<b><math>F = 6.288</math></b> <b><math>P = 0.017</math></b>	<b><math>F = 4.256</math></b> <b><math>P = 0.047</math></b>	<b><math>F = 4.82</math></b> <b><math>P = 0.035</math></b>	<b><math>F = 8.664</math></b> <b><math>P = 0.006</math></b>
D+W–L–					$F = 0.004$ $P = 0.949$	$F = 0.058$ $P = 0.81$	$F = 0.145$ $P = 0.706$
D+W+L–						$F = 0.069$ $P = 0.79$	$F = 0.06$ $P = 0.807$
D–W+L–							$F = 0.381$ $P = 0.541$

In *Bromus auleticus*, no significant differences were observed among treatments (data not shown)

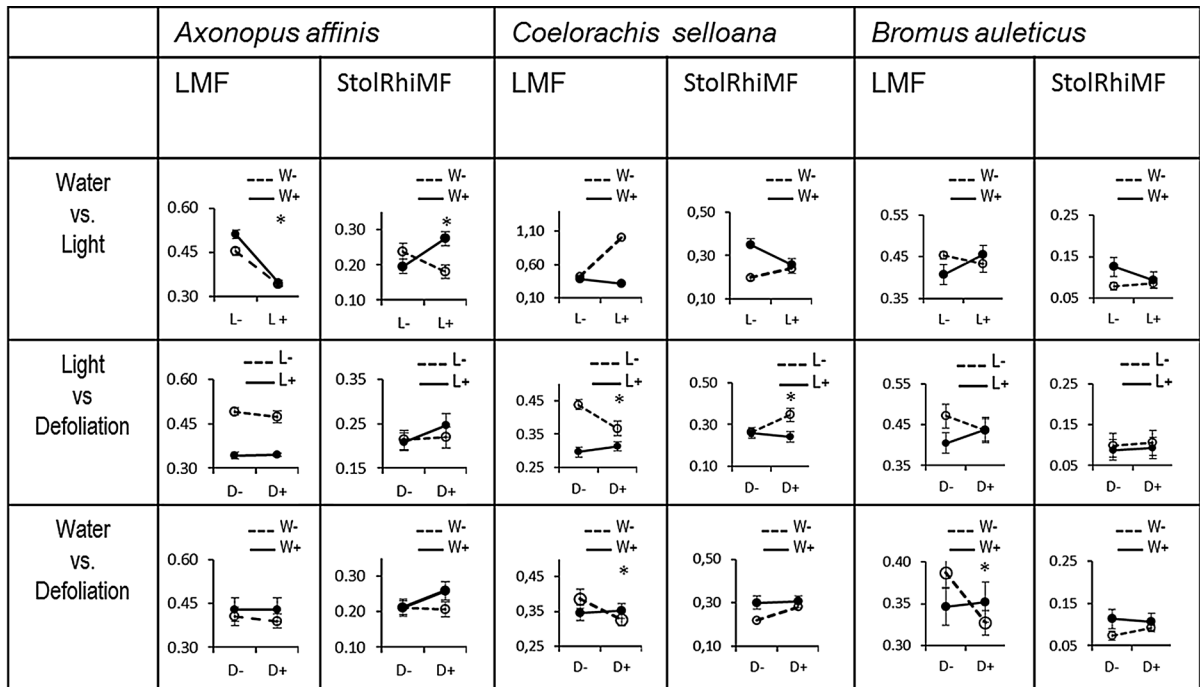
**Bold letters** indicate significant differences between treatments

### Biomass allocation and specific leaf area responses

Both  $C_4$  species (*A. affinis* and *C. selloana*) showed significant changes in biomass allocation induced by irradiance. However, the magnitude depended on the interaction either with water (*A. affinis*) or defoliation (*C. selloana*). Our results show that leaf and stolon allocation of the increaser species (*A. affinis*) is controlled by the interaction between light and water

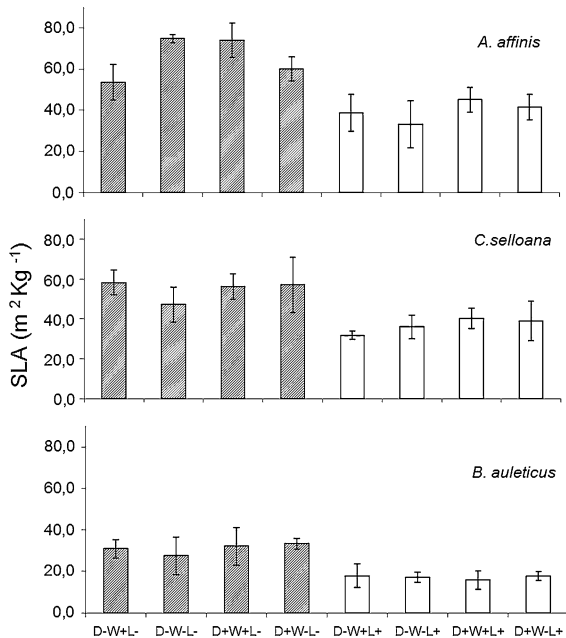
availability. As expected, leaf allocation was higher in light-limiting conditions (Poorter and Nagel 2000), but such response was lower in water-restricted conditions. Grazing reduces soil moisture through changes in vegetation structure (Bagnato 2010). The availability of water becomes a limiting factor, even in sub-humid temperate grasslands (Sala 2001). Under high resource availability, the high proportion of biomass allocated to stolons would allow *A. affinis*, a





**Fig. 1** Two-way interactions of water (W), light (L), and defoliation (D) on the biomass allocation on leaves (LMF) and stolon or rhizome mass fraction (Stol-RhMF) for the species: A.

*affinis*, *C. selloana*, and *B. auleticus*. Significant interactions ( $P < 0.05$ ) are indicated by an asterisk



**Fig. 2** Specific leaf area (SLA) values for the eight treatments of the factorial experiment of three factors with two levels: defoliation (D+/D-), water (W+/W-), and light (L+/L-), for *A. affinis*, *C. selloana*, and *B. auleticus*. Open bars indicate high level of light and striped bars shadowed conditions

species mainly reproduced vegetatively (Leoni et al. 2009), to spread horizontally. In the erect decreaser  $C_4$  species (*C. selloana*), defoliation was important in determining allocation to leaves, mainly under low levels of water and light. Under water restriction, defoliated plants reduced transpiration losses, favoring its persistence in grazing conditions (GRI = -0.48). These results are consistent with the ‘functional equilibrium’ theory (Poorter and Nagel 2000), which suggest that plants respond to a decrease in aboveground resources with increased allocation to aerial parts, whereas they respond to a decrease in below-ground resources with increased allocation to roots.

The  $C_3$  species (*B. auleticus*) presented a GRI value of -1, which means that it is restricted to non-grazed areas (Caussials 2010). Leaf allocation ratio in *B. auleticus* varied with defoliation level depending on water availability. Under water restriction, a common situation in grazed areas, non-defoliated plants allocated more biomass to leaves than those defoliated. If water is not a limiting resource, defoliation promoted an increase in the leaf biomass fraction. Such reduction in leaf allocation would reduce the photosynthetic

capacity of the whole plant (Nowak and Caldwell 1984; Meyer 1998), providing a possible explanation for the negative GRI.

Responses to low levels of light resulted in an increase of specific leaf area in the three species. It is well documented that plants are able to modulate the leaf area per unit biomass by altering their anatomy, enabling an increase in light interception per unit of leaf biomass (Boardman 1977; Carlucci et al. 2012). The two  $C_4$  grasses showed higher values of SLA than the  $C_3$  species, *B. auleticus*. Low SLA is generally related to high fiber content and a large investment in supporting structures which makes erect species dominant in light-limited environments, such as ungrazed areas (Konings 1989; Leoni et al. 2009). Low SLA is also associated with longevity (Reich et al. 1997; Evans and Poorter 2001). When well watered, plants of *A. affinis* responded to defoliation increasing SLA, and thus increasing light interception per unit leaf biomass. The SLA and the fraction of plant biomass allocated to leaves (leaf mass fraction, LMF) determine the total amount of leaf area displayed per unit plant biomass, which are both important determinants of RGR (Poorter et al. 2009).

Grazing may impact the aboveground net primary production both directly, through defoliation, trampling, and dung (Schlesinger et al. 1990; Aguiar et al. 1996; Hobbs et al. 1996), and indirectly by changing resource availability or factor levels. Altesor et al. (2005) proposed that both the canopy structure (height, dead/live tissues ratio) and species composition controlled ANPP responses to grazing in temperate sub-humid grasslands. Leoni et al. (2009) explored the potential effect of species composition on primary production by analyzing differences in RGR and in traits for species with different GRI values (increasers and decreasers). Our study goes a step forward by focusing on the species responses to changes in the micro-environmental conditions associated with the structural modifications promoted by grazing.

## Conclusions

Low light levels affected the RGR of both  $C_4$  species, but only the increaser prostrate species showed compensatory growth in response to defoliation. This

provides support to the idea that metabolic pathway would be a predictor of species response to environmental changes induced by grazing (response–effect traits; Lavorel and Garnier 2002; Suding et al. 2008). The differences in growth form of the  $C_4$  species may contribute to explain the distinct responses to defoliation. While the increaser prostrate  $C_4$  species tolerate herbivory by compensatory growth and horizontal spread by stolons, the erect one is more sensitive to defoliation.

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