Applied Vegetation Science **19** (2016) 557–566

Disentangling grazing effects: trampling, defoliation and urine deposition

Felipe Lezama & José M. Paruelo

Keywords
Campos; Increaser–decreaser; Nitrogen; Plant functional types; Productivity; Selectivity; Soil compaction; Species richness

Abbreviation
ANPP = Above-ground Net Primary Productivity.

Nomenclature
Zuloaga et al. (2008)

Received 20 July 2015
Accepted 21 April 2016
Co-ordinating Editor: Alicia Acosta

Lezama, F. (corresponding author, flezama@fagro.edu.uy)[1],
Paruelo, J.M. (paruelo@agro.uba.ar)[2,3]

[1]Departamento de Sistemas Ambientales, Universidad de la República, Av. E. Garzón 780, Montevideo, Uruguay;
[2]Laboratorio de Análisis Regional y Teledetección and Departamento de Métodos Cuantitativos y Sistemas de Información, Facultad de Agronomía and IFEVA, Universidad de Buenos Aires -CONICET, Av San Martín 4453, 1417 Buenos Aires, Argentina;
[3]IECA, Facultad de Ciencias, Universidad de la República, Iguá 4225, Montevideo, Uruguay

ABSTRACT

Questions: Do the effects of grazing components on vegetation structure differ in their relative importance? Do components interact in their effect on vegetation?

Location: San Jose department, Southern Campos, Uruguay.

Methods: In a manipulative field experiment we simulated three different grazing components: trampling, defoliation and urine deposition, over 3 yr in a natural grassland. Defoliation was analysed through two intensity levels and two procedures: uniform and selective cutting. We evaluated the effects of grazing components on species diversity and composition, and frequency of plant functional types.

Results: All simulated grazing components had at least some effect on vegetation structure. Additionally, both individual and interactive effects on vegetation attributes were detected. Our study indicates that the relative influence of each grazing component varied according to the attribute considered. N addition was the only treatment that affected plant diversity. Plant functional type composition, in turn, was affected mainly by trampling. N addition and trampling were the component that affected the frequency of the largest number of species. Defoliation selectivity showed effects both in terms of plant functional type and species composition. Exclosure treatment and defoliation intensity had slight effects on grassland structure.

Conclusions: This study provides insight on the underlying mechanisms of some observed patterns of grazing on the Campos grasslands. Our results lead us to conclude that all grazing components have to be taken into account to understand vegetation dynamics subjected to grazing. Prevention of woody encroachment by grazing can be attributed to direct and indirect effects of trampling. Trampling should be taken into account to explain increaser species responses. However, mechanisms responsible for other general patterns remain less clear. The importance of selective defoliation in species replacement induced by grazing in these grasslands has yet to be clarified.

Introduction

Grazing impacts of large herbivores involve individual and/or combined effects of different components that occur simultaneously: defoliation, trampling and faeces and urine deposition (Mikola et al. 2009). Each component differs in spatial extent and pattern, frequency and intensity. At the paddock scale, within the same management unit, defoliation is usually patchy, creating a mosaic of repeatedly visited areas with a short canopy and less visited areas where senescent biomass accumulates (McNaughton 1984). Similarly, faeces and urine deposition and trampling also might be concentrated, as in resting areas and trails (Schrama et al. 2013). However, defoliation concentrates on feeding stations of several square meters, while deposition of urine affects a small proportion of these areas (a few cm^2; Augustine & Frank 2001).

At the patch scale ($<10\ m^2$), neighbouring plants of the same or different species are subjected to variable defoliation intensities. This differential defoliation, i.e. selectivity, results from differential expression of evasion mechanisms,

Doi: 10.1111/avsc.12250

which act to reduce plants palatability and/or accessibility (Briske 1991; Augustine & McNaughton 1998). Selective defoliation by herbivores is frequently identified as the principal factor driving long-term species replacement associated with grazing (Brown & Stuth 1993; Moretto & Distel 1999).

Experimental simulation of individual herbivory components has improved our understanding of the mechanisms responsible for grazing effects. Most of these studies have focused on defoliation. Plenty of evidence shows that this component impacts grassland structure and functioning at various levels of organization, from individual plants to ecosystems, both at below- and above-ground level (Milchunas & Lauenroth 1993; Olff & Ritchie 1998; Mikola et al. 2009). However, experiments simulating other components of grazing have demonstrated effects that are not negligible; especially studies on fertilization impacts on diversity, where increased competition for light seems to be the main mechanism of diversity loss (Steinauer & Collins 1995; Hautier et al. 2009). Interactive, synergistic and contrasting effects of the different components have been observed. For example, defoliation and trampling promote prostrate grass species and rosette growth, while fertilization favours tall, light-competing species (Tilman 1993; Striker et al. 2011). Surprisingly, despite recognition of interacting effects, few studies focus on more than two components simultaneously (Mikola et al. 2009; Sørensen et al. 2009).

The vegetation that covers the Uruguayan territory is included in the Campos sub-region of the Rio de la Plata grasslands, one of the most extensive temperate subhumid grasslands areas worldwide (Soriano 1992). These grasslands exhibit a marked heterogeneity in terms of the types of plant community and comprise a diverse set of plant species and plant functional types (Soriano 1992). Physiognomy is primarily determined by perennial grasses, basically of summer growth (C_4), and a secondary group of interstitial species including C_3 grasses and forbs. Usually an upper stratum of dwarf shrubs and tall grasses is present (Soriano 1992). The spectrum of species palatability ranges from grasses and legumes with high nutritional quality to toxic or thorny shrubs and hard grasses.

Grazing impacts on plant communities of the Rio de la Plata grasslands has been extensively studied from enclosure–grazing experiments (i.e. Sala et al. 1986; Chaneton & Facelli 1991; Altesor et al. 2006; Lezama et al. 2014). Several of these articles (Chaneton & Facelli 1991; Rodríguez et al. 2003; Altesor et al. 2006) reported consistent responses in terms of species composition, richness, diversity, canopy structure and plant morphology. For example, overall species richness increased with grazing, mainly due to the increase in number of forbs and prostrate grasses (Rodríguez et al. 2003; Altesor et al. 2006). But contradictory results have also been reported. For instance, Rodríguez et al. (2003) found a marked increase in the relative importance of cool-season grasses after 5 yr of livestock exclusion, while Altesor et al. (2006) did not find changes in the balance of warm– and cool-season grasses.

Altesor et al. (1998) were able to identify sets of species with different long-term (50 yr) responses to grazing, including increaser and decreaser species. Decreaser species included grasses preferred by livestock, while increaser species corresponded to non-preferred grasses and forbs. However, some species did not fit this scheme. *Paspalum dilatatum*, a highly preferred grass, moved from rare to common in the period of 50 yr. Although grazing-induced changes in Campos grasslands have been basically interpreted in terms of defoliation effects (Rodríguez et al. 2003; Altesor et al. 2005), it has also been recognized that trampling may help to explain some of the reported changes (Lezama et al. 2014).

Globally, grazing has been studied in experimental trials with the presence of animals, hence with the combined action of all the grazing components. Few studies simultaneously disentangle the components of grazing (Kohler et al. 2004; Mikola et al. 2009; Sørensen et al. 2009). Our objective in this article was to analyse, through a factorial experiment, the effects of defoliation, nitrogen (N) addition and trampling on structural aspects of a natural grassland. Trampling and fertilization were analysed as simple factors with two levels, while defoliation was analysed through two intensity levels and two simulated herbivory procedures: uniform and selective cutting. As far as we know, selective defoliation has not been evaluated at the community level in manipulative experiments.

In this study we addressed the following questions: do effects of grazing components on vegetation structure differ in their relative importance? Do components interact in their effect on vegetation? Regarding the effects of individual grazing components, we expected that: (1) defoliation, and especially intensive defoliation, will promote grasses and forbs with prostrate habit of growth (Rodríguez et al. 2003); (2) selective defoliation will increase the cover of unpalatable species, especially shrubs (Altesor et al. 1998); (3) trampling will decrease shrub cover (Altesor et al. 2006; Lezama et al. 2014); (4) N addition will decrease species richness (Tilman 1993); (5) trampling and intense defoliation will increase species richness through the generation of vegetation gaps (Rodríguez et al. 2003).

Methods

Study site

The study was conducted at a private ranch located in San Jose department, in the south-central region of Uruguay

Doi: 10.1111/avsc.12250

(34°20′ S, 56°58′ W) covered with natural grasslands grazed by sheep and cattle. The average annual precipitation and temperature for the last 10 yr were 1082 mm and 16.7 °C, respectively (http://www.inia.org.uy/, accessed 1 June 2012). The experiment was carried out in a grazing exclosure (established in 2006) of 5000 m^2 located in a flat area on an Argiudoll soil. The paddock where the exclusion was located was continuously and moderately grazed (0.7 $cows{\cdot}ha^{-1}$) year-round during the 3 yr of the study.

Experimental design

The experiment consisted of the application of trampling, N fertilization, defoliation intensity and defoliation selectivity treatments in a factorial design. Each factor presented two levels: moderate and high intensity defoliation, selective and non-selective defoliation, trampling and non-trampling, and fertilization and no fertilization. These factors were combined in 16 experimental treatments. Additionally a reference treatment (without any manipulation) was established inside the exclosure.

A grid of 112 plots of 2.56 m^2 (1.6 × 1.6 m) was established, where the plots were arranged in four rows of 28 plots separated by walkways of 0.4-m width. A subset of 68 plots was selected based on their structural and floristic homogeneity. The 17 experimental treatments were assigned within this subset following a completely randomized design with four replicates.

Defoliation was carried out seasonally based on a grid of 64 subplots of 20 × 20 cm that covered the entire surface of the plot. A subplot sequence of removal was randomly defined for each season by a new draw. Biomass removal, at 2–3-cm height, was performed following this sequence until reaching a fresh biomass weight of 150 or 300 g for the moderate and high defoliation treatments, respectively. As a consequence, the number of defoliated subplots could vary among plots with the same defoliation intensity level. Removed biomass was oven-dried for 48 h at 50 °C, to quantify dry weight. Each year an average of 210.9 and 105.4 $g{\cdot}m^{-2}$ of dry matter were removed from the high and moderate defoliation treatment respectively. These values correspond roughly to 35.0% and 17.5% of the annual above-ground net primary productivity (ANPP) estimated for these grassland communities (Altesor et al. 2005), which corresponds to low and high levels of livestock harvest indices for this type of grassland (Golluscio et al. 1998). Non–selective defoliation was carried out by removing all the material within each subplot, while selective defoliation left intact non-preferred species.

Trampling and fertilization were applied homogeneously to the entire surface. Trampling was applied seasonally and fertilization once a year during winter (in June). N was added when N mineralization was low and, hence, larger effects of fertilization may be expected. Trampling procedure followed the protocol of Striker et al. (2006): an artificial hoof of 82 cm^2 surface and 22.5 kg weight was dropped from a height of 0.4 m approximately 64 times. Fertilization was made with a sprinkler, applying 200 g urea dissolved in 10 l of water (36 $g\ N{\cdot}m^{-2}$) per plot.

Sampling procedure

Initial (Dec 2006) and final (Dec 2009) floristic composition was recorded using the point-quadrat method, a non-destructive sampling procedure (Greig-Smith 1983). A permanent quadrat of 80 × 80 cm was located in the centre of each plot; surrounded by a buffer strip of 40 cm. Measurements were done using a metal frame supporting 52 vertical sampling pins. These were arranged in four rows, and introduced into the vegetation stratum from a height of 50 cm.

Soil samples were obtained at the end of experimental period to evaluate treatment effects on bulk density and N content. Three soil bulk density samples were taken from the first 5 cm in each plot with a 2-cm wide soil corer. Samples were analysed separately and averaged to obtain a single value. Additionally, three subsamples were taken from the first 10 cm with a 3-cm wide soil corer to evaluate mineral N. Ammonium and nitrate concentrations were determined with Reflectoquant test strips (Reflectoquant® system) and a RQflex10 reflectometer (Merck, Darmstadt, DE). After 3 yr, soil bulk density of the top 5 cm increased on average 9.2% with trampling (from 0.7198 to 0.7862 $g{\cdot}cm^{-3}$; $t_{62} = -3.574$, $P < 0.01$) and mineral N content was 93.4% higher in fertilized plots (from 7.61 to 14.72 $mg{\cdot}kg^{-1}$; $t_{26} = -5.359$, $P < 0.001$).

Indices and statistical analyses

To analyse the treatment effects on diversity, Richness (S = number of plant species per plot), Shannon–Weiner Index ($H' = -\Sigma p_i \mathrm{In} p_i$, where p_i corresponds to the relative frequency of the ith species; Greig-Smith 1983) and evenness ($E = H'/\mathrm{In}(S)$) were calculated for each plot. To analyse the mechanisms driving species richness changes, colonization and extinction processes, we calculated species gains and losses for each plot comparing 2006 and 2009 data.

We evaluated the response of the most common individual species (those that occurred in at least one-third of the plots). The response at the plant functional type (PFT) level was evaluated using five classes: forbs, cool-season grasses, warm-season grasses, graminoids (sedges and rushes) and shrubs (Altesor et al. 2006). The frequencies of dominant species and PFTs were calculated based on the relative number of pins that hit per quadrat.

The treatment effects on richness, diversity and evenness and on the frequency of PFT and main species were evaluated by four-way ANOVA using the difference between 2009 and 2006 for each plot as response variable. We used paired Student *t*-test to determine if the same variables in the reference treatment changed significantly during the experiment. Extinction and colonization rates were analysed using four-way ANOVA. In all the mentioned analysis, main effects and second-order interactions between grazing components were assessed. Homogeneity of variances was tested using Levene's test and, if necessary, a square root transformation was applied to the response variable. When significant interactions were detected, Tukey's test was applied subsequently to determine the treatment effects. The analyses were performed using SPSS (SPSS, Chicago, IL, US). All results are presented as untransformed means of four replicates ± SE (only statistically significant results are shown).

Results

Species richness, evenness and diversity

Overall, 73 species were recorded in the 2006 and 2009 floristic samplings (Appendix S1). One species was found only at the beginning, and conversely, 12 species were added to the list in 2009.

Species richness was affected by N addition. Fertilized plots had, on average, 3.5 less species than unfertilized ones (a 22.5% lower richness). Fertilization did not affect evenness consequently Shannon diversity index response was identical to the richness (Appendix S2). The response of species richness to fertilization was explained by a 101% increase in species extinction rate. There were no significant changes in the colonization rate ($F_{15,48} = 6.006$, $P = 0.018$; $F_{15,48} = 2.510$, $P = 0.12$, respectively).

Besides fertilization, the other grazing components had no significant effects on richness, diversity or extinction and colonization rates. The reference treatment, meanwhile, did not show any significant effect in any of these attributes (Appendix S3).

Changes in plant functional type (PFT) composition

Graminoids and shrubs showed significant responses to grazing simulation (Table 1). The frequency of graminoids increased in trampled plots regardless of the other grazing components (ca. 44.7%; Fig. 1a). Shrubs were affected by three of the four grazing components studied. The frequency of shrubs was 77.2% higher in selectively defoliated plots than in those uniformly defoliated (Fig. 1b). In addition, trampling and N addition interacted in affecting shrub frequency (Fig. 1c). In comparison to trampled and/or fertilized plots, plots without those treatments showed a higher shrub frequency.

The frequency of forbs, cool-season and warm-season grasses was not affected by treatments (Table 1). As with richness and diversity, the reference treatment did not have effects in PFT frequencies (Appendix S3).

Species responses

Analysis of variance revealed significant effects of grazing components on ten of the 14 species selected (12 Poaceae and two Asteraceae; Table 1). Only one species varied significantly in reference plots between 2006 and 2009: *Bothriochloa laguroides* frequency decreased from an average of 10.6 ± 4.8% to 2.4 ± 2.0% ($t_3 = 3.417$; $P = 0.42$; Appendix S3).

Several dominant species were affected by trampling (Fig. 2a, Table 1). Two warm-season grasses (*Paspalum dilatatum* and *P. notatum*) and one winter grass (*Jarava plumosa*) increased with trampling. Conversely, *Aristida uruguayensis* and *Stenotaphrum secundatum*, both warm-season grasses, were less frequent in plots subjected to trampling (Fig. 2a).

Nitrogen addition produced negative responses in three warm season grasses (*Botriochloa imperatoides, B laguroides* and *S. secundatum,* and a dwarf shrub (*Baccharis trimera*), while the frequency of *Cynodon dactylon* (a warm-season grass) was significantly higher in fertilized plots (Fig. 2b).

The factors related to defoliation (intensity and selectivity) showed slight effects on main species frequencies. Selective defoliation affected, regardless of the other grazing components, the frequency of two species in opposite directions; It reduced *A. uruguayensis* and promoted *B. trimera* frequencies (Fig. 2c). On the other hand, defoliation intensity affected only one main species: the grass *S. secundatum* responded negatively to intense defoliation (Fig. 2d).

Three interactions among grazing components were detected at species level (Table 1, Fig. 2e–g). The combination of N addition and selective defoliation increased the frequency of *P. dilatatum*, while no changes occurred when these components acted individually (Fig. 2e). *Eryngium horridum* showed identical response to trampling and N addition than shrubs (Fig. 2g). Additionally, defoliation intensity and selectivity interacted on *E. horridum*. Selective defoliation in combination with intense defoliation increased its frequency (Fig. 2f).

Discussion

Our results indicate that the relative influence of each grazing component varied according to the attribute

Table 1. Results of ANOVA for plant functional type and main species frequency differences between 2009 and 2006 as a function of defoliation intensity (I), defoliation selectivity (S), trampling (T), N addition (N) and their interactions.

		I	S	T	N	I × S	I × T	I × N	S × T	S × N	T × N
Plant functional type											
Cool-season grasses		0.111	0.297	0.033	0.206	1.615	0.004	0.008	1.539	2.289	0.132
Warm-season grasses		0.332	1.903	2.989	0.222	0.008	1.369	0.418	0.037	2.869	0.464
Shrubs		1.023	__12.950**__	__27.019***__	__16.557***__	2.337	1.439	3.112	1.554	2.337	__8.099*__
Forbs		2.780	3.218	0.022	1.347	2.506	0.485	0.075	0.092	0.004	0.022
Graminoids		0.608	0.139	__4.564*__	4.279	0.838	0.056	2.540	0.139	0.719	0.010
Main species											
Cool-season grasses	Jp	0.001	0.219	__6.263*__	1.056	0.013	0.878	0.107	3.592	0.799	0.29
	Nc	0.31	1.105	1.105	0.031	0.491	1.105	2.486	2.486	0.123	1.105
	Nn	2.877	2.558	2.719	0.866	1.15	0.001	3.031	0.001	0.543	0.044
	Ps	0.822	1.205	1.012	1.823	1.452	1.012	0.261	3.375	0.05	0.048
Warm-season grasses	Am	0.283	2.768	0.04	1.579	0.005	0.037	3.71	0.069	3.423	0.072
	Au	0.31	__6.512*__	__4.620*__	3.283	0.108	0.274	0.868	0.924	0.00	2.694
	Bi	0.475	0.359	0.012	__23.753***__	2.233	1.595	0.012	0.146	0.059	0.364
	Bl	0.18	0.36	0.162	__3.887*__	0.792	0.263	3.119	0.885	0.910	1.000
	Cd	0.131	0.033	0.037	__4.157*__	0.702	0.564	0.175	0.316	0.379	0.224
	Pd	1.754	1.218	__15.783***__	__12.471**__	2.387	3.118	0.195	0.438	__5.894*__	1.754
	Pn	1.341	0.080	__8.044**__	2.724	1.051	0.115	0.001	0.165	0.929	2.333
	Ss	__4.757*__	0.015	__3.982*__	__12.107**__	1.128	0.007	3.309	0.402	0.619	0.183
Shrubs	Bt	0.619	__6.516*__	3.372	__4.414*__	0.008	1.720	3.061	3.061	1.523	1.968
	Eh	0.021	__3.911*__	__14.917***__	__7.953**__	__4.529*__	0.002	0.333	0.057	1.344	__4.308*__

Am, *Aristida murina*; Au, *Aristida uruguayensis*; Bt, *Baccharis trimera*; Bi, *Botriochloa imperatoides*; Bl, *Botriochloa laguroides*; Cd, *Cynodon dactylon*; Eh, *Eryngium horridum*; Jp, *Jarava plumosa*; Nc, *Nassella charruana*; Nn, *Nassella neesiana*; Pd, *Paspalum dilatatum*; Pn, *Paspalum notatum*; Ps, *Piptochaetium stipoides*; Ss, *Stenotaphrum secundatum*.

F-values of ANOVAs are presented. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns $P > 0.05$.

Numbers in bold denote significant effects ($P < 0.05$).

considered. N addition and trampling were the components that affected the frequency of the largest number of species. Life-form composition was affected mainly by trampling. Diversity, in turn, was affected only by N addition. The comparison of our results with previous grazing simulation studies shows similarities and also differences in the ranking of importance of individual components. While Sørensen et al. (2009) identified trampling as the most important factor in a subarctic grassland, Kohler et al. (2004) and Mikola et al. (2009) found an overriding effect of defoliation in controlling community structure in mountain grasslands and pastures, respectively. Differences between studies may be related to specific characteristics of simulated herbivores and grasslands, but also with the specific characteristics of each experimental layout. For instance, our study incorporated, in a novel way, selective defoliation as an experimental factor, which ultimately proved to be a relevant grazing component on structuring grassland communities.

Underlying mechanisms of grazing effects on community structure

Our first prediction stated that selective defoliation would increase non-preferred species at the expense of preferred species (Altesor et al. 1998). Our results partially agree with this prediction. Only *Eryngium horridum*, one of four non-preferred species, increased with selective defoliation (when it occurred in combination with intense defoliation). Interestingly, *E. horridum*, which has been identified as a long-term increaser (Altesor et al. 1998), did not increase in unclipped plots (reference plots), which suggests that defoliation of neighbour plants had a positive effect. This suggests that the results were not only derived from the simple fact of not being cut.

On the other hand, we found contrasting responses to selectivity among preferred species: from negative (e.g. *A. uruguayensis*), neutral (e.g. *Piptochaetium stipoides*) to positive (e.g. *P. dilatatum*). A remarkable case is *P. dilatatum* that presented the highest response under a combination of selective defoliation and N addition, while it did not benefit from selectivity without N fertilization. This species has been identified as an increaser in spite of being a grazing-preferred species (Altesor et al. 1998).

Our experimental approach does not allow elucidation of the relative importance of defoliation tolerance from selective defoliation on species replacement. However, the results indicate that, in these grasslands, dominant species with different strategies may coexist. Simple models stating

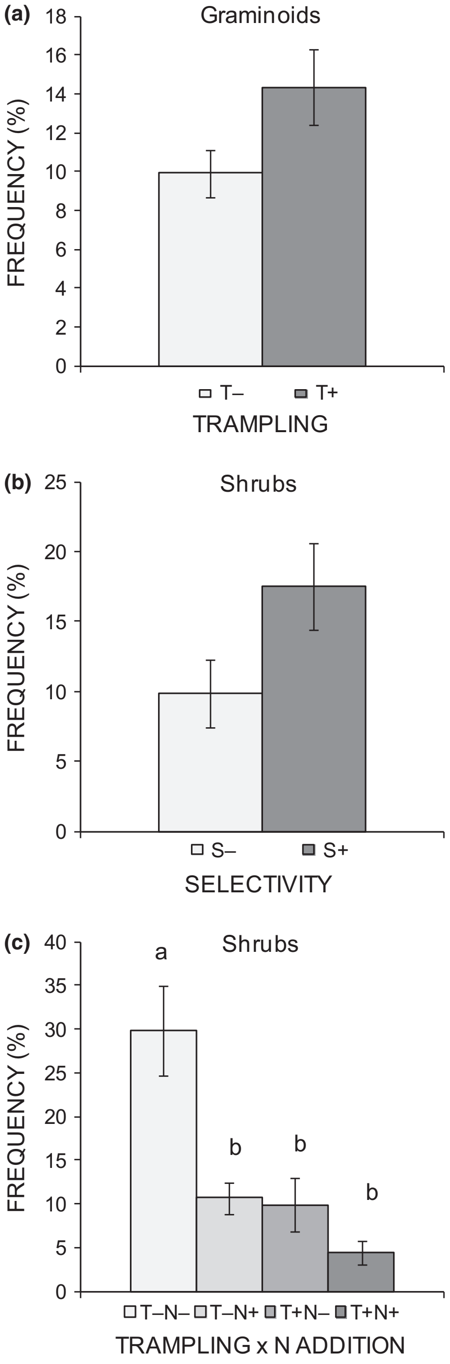

Fig. 1. PFT frequencies under different treatments of grazing components at the end of a 3-yr field experiment (Mean ± SE). Only significant effects are shown: (**a**) Graminoids; (**b**) and (**c**) Shrubs. S: defoliation selectivity, T, trampling; N, N addition. Different letters indicate significant differences ($P < 0.05$) among treatments based on Tukey's test (only for interacting effects).

that unpalatable species would be promoted by selective grazing (Moretto & Distel 1999), or that increaser species has typically high grazing tolerance (Del-Val & Crawley 2005) do not account for the vegetation changes observed in these sub-humid grasslands.

Contrary to expectations, the two species identified as increasers that were present in our study, both grasses with a prostrate habit, were not promoted by intense defoliation. In the case of *B. laguroides* the species persisted in treatments that included different levels of defoliation, with or without trampling, but was negatively affected by N addition. In the reference treatment, without either defoliation or trampling, we observed a marked decrease in this species in accordance with previous grazing exclosure studies. Consequently, we cannot disentangle whether increaser response is attributable to defoliation, to trampling or to both. On the other hand, the species *P. notatum* was promoted by trampling independent of defoliation intensity. In summary, our results lead us to conclude that both grazing components must be taken into account to understand increaser response to grazing, and not only defoliation as is usually assumed.

As expected, trampling provoked a pronounced reduction of shrub importance in the community. Our study suggests that both direct and indirect effects of trampling are responsible for this impact. On one hand, direct physical damage to woody sprouts was observed in the field. In addition, trampling promoted *P. dilatatum*, a trampling tolerant grass with root anatomical features that allow it to resist physical damage (Striker et al. 2011). On the other hand, the marked increase in sedges and rushes and the soil compaction observed support the idea of a change in water regime, and hence an indirect effect of trampling that may affect shrubs (Cole 1995). The results help to explain why, in these grasslands, grazing reduces shrub dominance instead of promoting encroachment, as found in other parts of the world (Lezama et al. 2014). Woody encroachment is identified as a global threat to grasslands and, in a recent review, Ratajczak et al. (2014) highlight the role of altered fire regime in the generation of this problem for tall-grass prairies of North America, a similar vegetation type to the Campos. A model of shrub encroachment for the Campos grasslands must consider large herbivore grazing as a major promoting factor.

Aside from a lack of studies in the Uruguayan grasslands, strong evidence from all over the world indicates that N addition often reduces species richness through the promotion of tall light-competing species that exclude small species (Lepš 2014). Our results indicate that N addition markedly reduced species richness, and the fact that extinction rate and not colonization rate was affected by N addition suggests that this factor influenced competitive relationships, as proposed elsewhere (Tilman 1993; Hautier et al. 2009). Remarkably, however, we did not find a clear promotion of erect grasses at the expense of prostrate plants after N addition. In fact, the species that benefitted

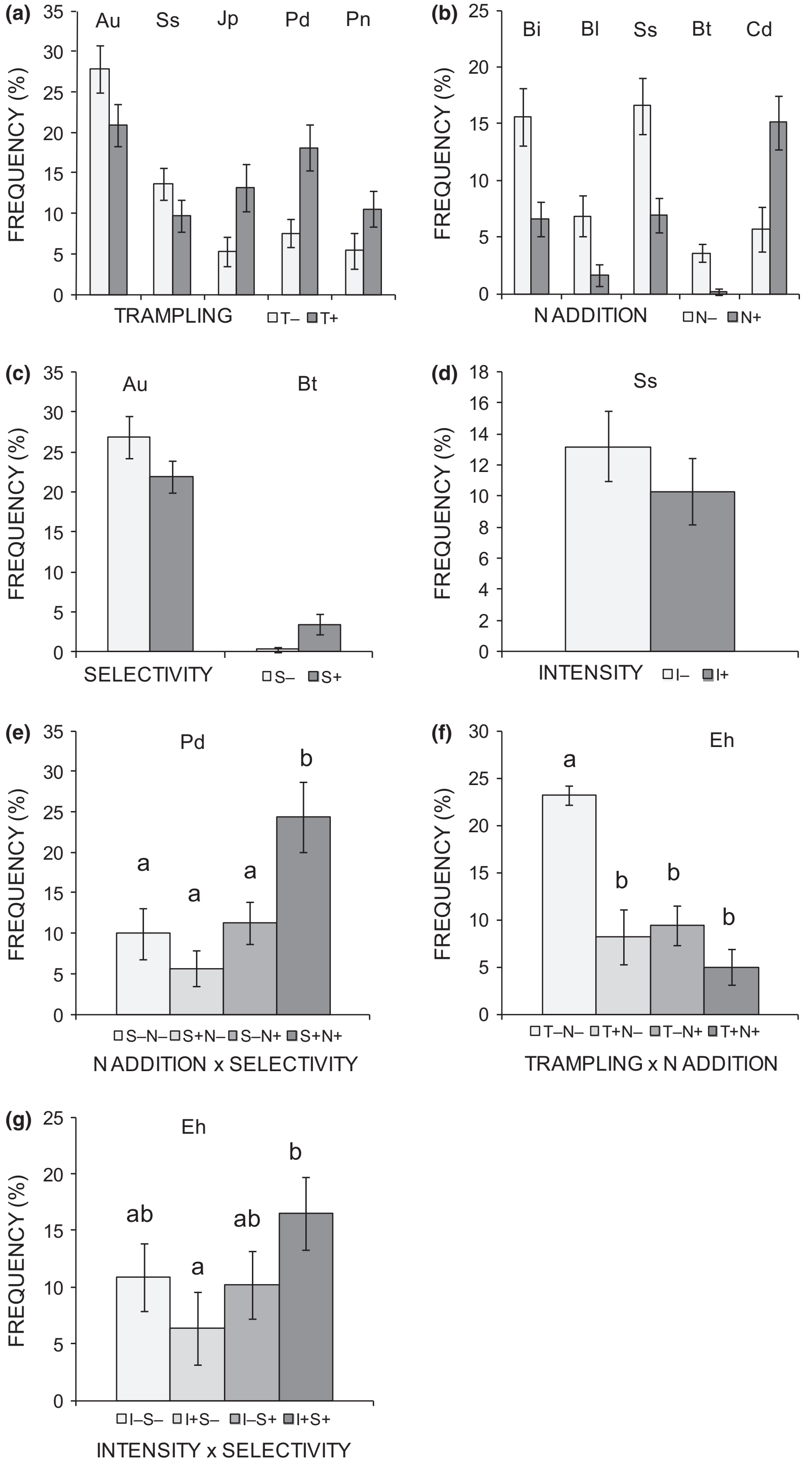

Fig. 2. Main species frequencies under different treatments of grazing components at the end of a 3-yr field experiment (Mean ± SE). Only significant effects are shown: (**a**) T: trampling, (**b**) N: N addition, (**c**) S: defoliation selectivity, (**d**) I: defoliation intensity, (**e**) N addition × selectivity, (**f**) trampling × N addition, (**g**) intensity × selectivity. Au, *Aristida uruguayensis*; Bt, *Baccharis trimera*; Bi, *Botriochloa imperatoides*; Bl, *Botriochloa laguroides*; Cd, *Cynodon dactylon*; Jp, *Jarava plumosa*; Pd, *Paspalum dilatatum*; Pn, *Paspalum notatum*; Ss, *Stenotaphrum secundatum*. Different letters indicate significant differences ($P < 0.05$) among treatments based on Tukey's test (only for interacting effects).

from N addition was *Cynodon dactylon,* a versatile invasive grass that occurs widely in Campos grasslands (Bresciano et al. 2014). Our finding is added to the growing evidence of the threat represented by N enrichment to grassland preservation.

We expected that trampling and/or intense defoliation would increase species richness through gap creation, which increases colonization by new species due to the increased microsite availability for seedling recruitment (Pywell et al. 2007). Our results showed that the disturbance generated by trampling or biomass removal did not affect plot richness or colonization rates. Contradictory results on the consequences of biomass removal on grassland richness have been reported (Kohler et al. 2004; Lepš 2014). Several authors state that disturbance effects on plant species richness are dependent on gap size; when gaps are not sufficiently large to allow new species establishment they are vegetatively recolonized by resident species (Burke & Grime 1996) and, consequently, the chance for arrival of new species is low. The disturbed area in our defoliation treatments consisted in gaps of at least 400 cm^2 (even more when defoliated subplots were adjacent). It is not likely that in our study gap size represented a limitation to the establishment of new species (Burke & Grime 1996).

Very little information is available on trampling effects on plant species diversity. In fact, the only previous study was that of Kohler et al. (2004) who, as in our study, did not find effects of simulated trampling on mountain grasslands. Gaps created by trampling are different from those after cutting; biomass is not removed but concentrated on the soil surface, and soil is compacted making the establishment of new plants more difficult. Actually, Bakker (1989) suggested that soil compaction of wet soils and trampling by large herbivores may inhibit plant diversity.

Reference plots provide additional clues to understand the effect of grazing on richness. Surprisingly, untreated plots did not reduce their species richness after 3 yr. This contradicts previous results on grazing exclosures in the Campos grasslands (Rodríguez et al. 2003; Lezama et al. 2014), which found that after 2–3 yr of exclosure there was a marked reduction in richness due to competitive exclusion. Perhaps the particular environmental conditions during part of the experiment (severe drought in parts of 2008 and 2009, see Appendix S4) could have delayed or even prevented competitive exclusion. Drought reduction of aerial biomass may have overridden the differential effects of defoliation intensities and trampling on gap generation, explaining the absence of effects of these components on species richness. Another plausible explanation could be that species richness is limited by propagule availability in this system (Foster 2001). Indeed, annual species are scarce in the Campos species pool and propagation is mainly vegetative, based on stolons and rhizomes.

Grazing effects as a result of the balance and interactions among components

In this study we described a broad spectrum of individual and interactive effects, both direct and indirect, of grazing components on structural attributes of a natural grassland. Our analyses clearly showed that interpretation of grazing effects based on the isolated action of a single component (i.e. defoliation) might be too simplistic (see also Kohler et al. 2004; Mikola et al. 2009; Sørensen et al. 2009; Carmona et al. 2013; Schrama et al. 2013).

High variability of grazing effects has been a central topic of debate in grassland ecology (Milchunas & Lauenroth 1993). In the Campos grasslands several attributes responds in an inconsistent manner. Our results provide clues to explain these divergences: there is no single grazing effect (not even a few), but several depending on the balance of grazing components. Given that components balance, and therefore overall grazing effect may vary both in magnitude and direction, conflicting results should be expected (Milchunas & Lauenroth 1993; Lezama et al. 2014). To consider and quantify the importance of each component would increase our ability to predict species and PFT responses to grazing, a key aspect in the management of native forage resources (Bullock et al. 2001).

Acknowledgements

This research was partly funded by grants from INIA FPTA 175 (Uruguay) and Comisión Sectorial de Investigación Científica (Uruguay). This work was carried out with the aid of a grant from the Inter-American Institute for Global Change Research (IAI) CRN3095, which is supported by the US National Science Foundation (Grant GEO-1128040). A. Altesor, S. Baeza, V. Cayssials, S. García, A. Guido, G. Fernández, E. Leoni, L. López, A. Mello, M. Oyarzábal, F. Pezzani, C. Rodríguez, A. Rossado and E. Vega helped us with field tasks.

References

Altesor, A., Di Landro, E., May, H. & Ezcurra, E. 1998. Long-term species change in a Uruguayan grassland. *Journal of Vegetation Science* 9: 173–180.

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.

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.

Augustine, D.J. & Frank, D.A. 2001. Effects of migratory grazers on spatial heterogeneity of soil nitrogen properties in a grassland ecosystem. *Ecology* 82: 3149–3162.

Augustine, D.J. & McNaughton, S.J. 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *Journal of Wildlife Management* 62: 1165–1183.

Bakker, J.P. 1989. Nature management by grazing and cutting. *Geobotany* 14: 1–400.

Bresciano, D., Rodríguez, C., Lezama, F. & Altesor, A. 2014. Patrones de invasión de los pastizales de Uruguay a escala regional. *Ecología Austral* 24: 83–93.

Briske, D.D. 1991. Developmental morphology and physiology of grasses. In: Heitschmidt, R.K. & Stuth, J.W. (eds.) *Grazing management. An ecological perspective*, pp. 85–108. Timber Press, Portland, OR, US.

Brown, J.R. & Stuth, J.W. 1993. How herbivory affects grazing tolerant and sensitive grasses in a central Texas grassland: integrating plant response across hierarchical levels. *Oikos* 67: 291–297.

Bullock, J.M., Franklin, J., Stevenson, M.J., Silvertown, J., Coulson, S.J., Gregory, S.J. & Tofts, R. 2001. A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology* 38: 253–267.

Burke, M.J. & Grime, J.P. 1996. An experimental study of plant community invasibility. *Ecology* 77: 776–790.

Carmona, C.P., Azcárate, F.M. & Peco, B. 2013. Does cattle dung cause differences between grazing increaser and decreaser germination response? *Acta Oecologica* 47: 1–7.

Chaneton, E.J. & Facelli, J.M. 1991. Disturbance effects on plant community diversity: spatial scales and dominance hierarchies. *Vegetatio* 93: 143–155.

Cole, D.N. 1995. Experimental trampling of vegetation. II. Predictors of resistance and resilience. *Journal of Applied Ecology* 32: 215–224.

Del-Val, E.K. & Crawley, M.J. 2005. Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology* 93: 1005–1016.

Foster, B.L. 2001. Constraints on colonization and species richness along a grassland productivity gradient: the role of propagule availability. *Ecology Letters* 4: 530–535.

Golluscio, R.A., Deregibus, V.A. & Paruelo, J.M. 1998. Sustainability and range management in the Patagonian steppes. *Ecología Austral* 8: 265–284.

Greig-Smith, P. 1983. *Quantitative plant ecology*, 3rd edn. Blackwell, Oxford, UK.

Hautier, Y., Niklaus, P.A. & Hector, A. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* 324: 636–638.

Kohler, F., Gillet, F., Gobat, J.M. & Buttler, A. 2004. Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. *Journal of Vegetation Science* 15: 143–150.

Lepš, J. 2014. Scale- and time-dependent effects of fertilization, mowing and dominant removal on a grassland community during a 15-year experiment. *Journal of Applied Ecology* 51: 978–987.

Lezama, F., Baeza, S., Altesor, A., Cesa, A., Chaneton, E.J. & Paruelo, J.M. 2014. Variation of grazing-induced vegetation changes across a large-scale productivity gradient. *Journal of Vegetation Science* 25: 8–21.

McNaughton, S.J. 1984. Grazing lawns: animals in herds, plant form, and coevolution. *The American Naturalist* 126: 863–886.

Mikola, J., Setälä, H., Virkajärvi, P., Saarijärvi, K., Ilmarinen, K., Voigt, W. & Vestberg, M. 2009. Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. *Ecological Monographs* 79: 221–244.

Milchunas, D.G. & Lauenroth, W.K. 1993. A quantitative assessment of the effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* 63: 327–366.

Moretto, A.S. & Distel, R.A. 1999. Effects of selective defoliation on the competitive interaction between palatable and unpalatable grasses native to a temperate semi-arid grassland of Argentina. *Journal of Arid Environments* 42: 167–175.

Olff, H. & Ritchie, M.E. 1998. Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution* 13: 261–265.

Pywell, R.F., Bullock, J.M., Tallowin, J.B., Walker, K.J., Warman, E.A. & Masters, G. 2007. Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. *Journal of Applied Ecology* 44: 81–94.

Ratajczak, Z., Nippert, J.B., Briggs, J.M. & Blair, J.M. 2014. Fire dynamics distinguish grasslands, shrublands and woodlands as alternative attractors in the Central Great Plains of North America. *Journal of Ecology* 102: 1374–1385.

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.

Sala, O.E., Oesterheld, M., León, R.J.C. & Soriano, A. 1986. Grazing effects upon plant community structure in subhumid grasslands of Argentina. *Vegetatio* 67: 27–32.

Schrama, M., Heijning, P., Bakker, J.P., van Wijnen, H.J., Berg, M.P. & Olff, H. 2013. Herbivore trampling as an alternative pathway for explaining differences in nitrogen mineralization in moist grasslands. *Oecologia* 172: 241–243.

Sørensen, L.I., Mikola, J., Kytöviita, M.M. & Olofsson, J. 2009. Trampling and spatial heterogeneity explain decomposer abundances in a sub-arctic grassland subjected to simulated reindeer grazing. *Ecosystems* 12: 830–842.

Soriano, A. 1992. Rio de la Plata grasslands. In: Coupland, R.T. (ed.) *Natural grasslands. Introduction and Western Hemisphere*, pp. 367–407. Elsevier, Amsterdam, NL.

Steinauer, E.M. & Collins, S.L. 1995. Effects of urine deposition on small-scale patch structure in prairie vegetation. *Ecology* 76: 1195–1205.

Striker, G.G., Insausti, P., Grimoldi, A.A. & Leon, R.J.C. 2006. Root strength and trampling tolerance in the grass *Paspalum dilatatum* and the dicot *Lotus glaber* in flooded soil. *Functional Ecology* 20: 4–10.

Striker, G.G., Mollard, F.P.O., Grimoldi, A.A., León, R.J.C. & Insausti, P. 2011. Trampling enhances the dominance of graminoids over forbs in flooded grassland mesocosms. *Applied Vegetation Science* 14: 95–106.

Tilman, D. 1993. Species richness of experimental productivity gradients: how important is colonization limitation? *Ecology* 74: 2179–2191.

Zuloaga, F., Morrone, O. & Belgrano, M.J. (eds.). 2008. Catálogo de las plantas vasculares del cono sur (Argentina, sur de Brasil, Chile, Paraguay y Uruguay). *Monographs in Systematic Botany from the Missouri Botanical Garden* 107: 3486 pp.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of species recorded.
Appendix S2. Results of ANOVA for species richness, eveness and diversity difference between 2009 and 2006 as a function of defoliation intensity, defoliation selectivity, trampling, N addition and their interactions.
Appendix S3. Results of Student's *t*-tests for species richness, plant functional type and main species frequency difference between 2009 and 2006 in reference treatment.
Appendix S4. Rainfall for the experimental period (2007–2009) and average rainfall for the 2000–2009 period in south-central Uruguay.