Controls of primary productivity and nutrient cycling in a temperate grassland with year-round production

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Abstract　Net primary production (NPP) and nutrient dynamics of grasslands are regulated by different biotic and abiotic factors, which may differentially affect functional plant groups. Most studies have dealt with grasslands that have extremely low or zero production over a significant period of the year. Here we explore the relative importance of a few environmental factors as controls of aerial and below-ground plant biomass production and nutrient dynamics in a grassland that is active throughout the year. We investigate their effect on the response of three main plant functional groups (warm- and cool-season graminoids and forbs). We conducted a factorial experiment in a continuously grazed site in the Flooding Pampa grassland (Argentina). Factors were seasons (summer, autumn, winter and spring), and environmental agents (mowing, shade, addition of phosphorus [P] and nitrogen [N]). N addition had the largest and most extended impact: it tripled aerial NPP in spring and summer but had no effect on below-ground biomass. This positive effect was accompanied by higher N acquisition and higher soil N availability. Mowing increased aerial NPP in winter, increased root biomass in the first 10 cm during autumn and winter and promoted N and P uptake by plants. Shading did not affect aerial NPP, but stimulated N and P uptake by plants. P addition had no effect on aerial NPP, but increased shallow root biomass and its N content in spring, and tripled P accumulation in plant biomass. The three plant functional groups differentially accounted for these ecosystem-level responses. Graminoids explained the greater biomass production of N-fertilized plots and mowing tended to promote forbs. These results suggest that the environmental controls of aerial NPP in this grassland vary among seasons, differentially impact the major floristic groups, and affect the energy and nutrient transfer to herbivores.

Key words: aerial net primary production, grassland, light, mowing, nitrogen, phosphorus.

INTRODUCTION

Net primary production (NPP) and nutrient cycling are key processes of grassland ecosystems. At a regional scale, correlative patterns have shown that both mean annual aerial primary production and nitrogen mineralization rates are closely related with mean annual rainfall (Sala *et al.* 1988; Burke *et al.* 1997). Other factors such as fire, grazing and climatic variability also influence regional-scale variation of aerial primary production, and nutrient dynamics (Burke *et al.* 1997; Oesterheld *et al.* 1999). At the local spatial scale, and the interannual time scale, current-year rainfall and previous-year aerial net primary production accounted for a significant proportion of aerial primary production of a semiarid grassland area (Sala *et al.* 1988; Lauenroth & Sala 1992; Oesterheld *et al.* 2001). In fact, grasslands are

the most responsive ecosystems to fluctuations in rainfall (Knapp & Smith 2001; Knapp *et al.* 2001), and they also respond to variations in rainfall distribution during the growing season (Knapp *et al.* 2002). These interannual fluctuations are also linked to biogeochemical processes (Lauenroth & Sala 1992; Paruelo *et al.* 1998).

However, it would be inadequate to conclude from these patterns that water availability is the only or the most important limiting factor of NPP of any given grassland. These patterns show that interannual variation of productivity is explained by interannual variations in precipitation, which may simply be a consequence of the large variations in precipitation experienced by many grasslands (Knapp & Smith 2001) or the availability of precipitation data compared to other candidate explanatory variables. Moreover, the high correlation between interannual variation of production and precipitation seems to vanish at high values of mean annual precipitation (Oesterheld *et al.* 1999; Huxman *et al.* 2004).

The patterns of growth and resource allocation of individual plants have been shaped by natural selection in such a way that growth is simultaneously limited by an array of environmental factors (Bloom *et al.* 1985; Osmond *et al.* 1987). At a local level, primary productivity may be limited by a number of factors, such as light interception, water availability, and nutrient recycling, which, in turn, are regulated by various factors, such as temperature or precipitation (Knapp & Seastedt 1986; Chapin *et al.* 1987; Chapin *et al.* 2002). Several experiments have shown responses to multiple factors acting either in isolation or interactively (Shaver *et al.* 1998; James *et al.* 2005).

All these studies have been conducted in systems with a strong and extended dormant season and a short period of plant growth, the conditions of most ecosystem types (Chapin *et al.* 2002). In ecosystems with extended or even permanent growing seasons, such as the Flooding Pampa of Argentina, a number of factors may become relevant in determining primary production and nutrient dynamics at different periods of the year. Additionally, the species and/or plant functional groups may be differentially affected both by season and environmental factors. Some plant species or groups of species exhibit idiosyncratic responses to disturbance, stress, and resource enrichment (Shaver *et al.* 2001; Gough & Hobbie 2003). For example, in the Flooding Pampa grassland, flooding had an overall positive effect on aerial net primary production, but this positive response at the community level included a detrimental effect on a group of exotic forbs that was outweighed by a beneficial effect on native graminoids (Insausti *et al.* 1999). Similarly, grazing had a negative general effect on aerial production of the community, but this net outcome resulted from the reduction of grasses and the promotion of exotic and unpalatable forbs (Rusch & Oesterheld 1997). In the case of mineral nutrition, although it is well known that nutrient enrichment is widely beneficial for plants, wild plants diverge in their economy of nutrients (Grime 1979; Chapin 1980; Tilman 1988; van Breemen 1995). In many ecosystems, nutrient addition has benefited certain plant groups and reduced plant diversity (Wedin & Tilman 1996; Shaver *et al.* 2001). In the case of the Flooding Pampa grasslands, even though typical management practices are limited to elementary animal husbandry (Oesterheld *et al.* 1992), fertilisers are widely used (García *et al.* 2002). However, there are few studies aimed at disentangling how nutrient availability limits the different plant functional groups of these grasslands (Ginzo *et al.* 1982, 1986; Collantes *et al.* 1998). On the other hand, changes in plant species composition derived from diverging responses of species or plant groups to external agents such as fertilization or grazing may trigger positive or negative feedbacks between vegeta-tion and nutrient cycling. For example, in the Flooding Pampa grasslands, grazing promotes the growth of forbs with a greater tissue (and litter) quality and accelerates both litter decomposition rate and nitrogen cycling (Semmartin *et al.* 2004). Grazing promotes, within a grass species, more decomposable ecotypes (Semmartin & Ghersa 2006).

The objective of this work was to understand better the environmental factors that control primary productivity and nutrient cycling in the Flooding Pampa grassland throughout the year. We manipulated a group of environmental agents: mowing, light and nutrients and analysed their effects on soil mineral nitrogen, biomass production and its nutrient concentration, within three functional groups: forbs, warm-season graminoids and cool-season graminoids.

METHODS

In a field experiment, we evaluated over four seasons of a whole year (1997) how fertilization with inorganic nitrogen (N) and phosphorus (P), light intensity reduction, and mowing affected aerial and below-ground biomass production, nitrogen and phosphorus contents in plant biomass, and mineral nitrogen in soil.

Site description

The study was conducted in the Flooding Pampa (province of Buenos Aires, Argentina), a region of six million hectares that is primarily native grasslands. Annual mean precipitation is around 900 mm uniformly distributed among seasons, and mean monthly temperature ranges from approximately 7°C in winter to 22°C in summer. Overall, the year of the study was average in terms of annual rainfall, although it had a particularly humid late winter and spring (35% higher than the mean of the last 45 years). Our study was located in a stand of the most widespread community, a humid mesophytic meadow defined as *Ambrosia tenuifolia, Eclipta bellidioides* and *Mentha pullegium* community (Burkart *et al.* 1990; Perelman *et al.* 2001). The combination of species with C_3 and C_4 photosynthetic pathways determines a seasonal pattern of above-ground productivity with a maximum that occurs from late spring to the beginning of summer. Annual aerial net primary production is approximately 5500 kg ha^{-1} and ranges from a minimum of 4 kg of dry matter ha^{-1} day^{-1} in autumn to a maximum of 30 kg ha^{-1} day^{-1} in spring (Sala *et al.* 1981). At the beginning of the study the grassland had 200 and 80 g m^{-2} of green and standing dead biomass, respectively. This grassland is usually flooded during winter and early spring. The soil is a Typic Natra-quoll with a loamy A horizon (pH = 6.7), with

approximately 3.5% organic carbon and 24% clay, and a thick natric B horizon (Lavado & Taboada 1987).

Specific basal cover showed that each floristic group had a similar diversity although a reduced number of species dominated each. Cool and warm-season graminoids had 21 and 17 species, respectively, and there were 23 forb species. In the case of cool-season graminoids, *Danthonia montevidensis* and *Eleocharis* spp. together accounted for 58–99% of basal cover of this floristic group. The annual grass *Lolium multiflorum* accounted for 8% of basal cover in autumn and winter. Warm-season graminoid basal cover during summer and autumn was largely accounted for by a prostrate grass, *Stenotaphrum secundatum* (25–38%), and two erect grasses, *Leersia hexandra* and *Panicum gouinii* (12 and 30%). Other important species were *P. millioides*, *Bothriochloa laguroides*, *Paspalidium paludivagum*, and *Setaria geniculata*, each accounting for between 4 and 10% of warm-season graminoid basal cover. In the case of forbs, a single rosette, *Leontodon taraxacoides*, accounted for 54–90% of basal cover of the group. Other important forbs (~5–15% cover) were *Mentha pulegium*, *Phyla canescens*, *Plantago lanceolata*, and *Spilanthes* spp.

Experimental design

The experiment was located in a 1 ha area, temporarily fenced to exclude grazing during the study (36°15.6′S; 58°16.2′W). We performed a two-way factorial experiment with seasonality and environmental agents as factors. Seasonality had four levels: summer, autumn, winter and spring, and environmental agents had a control and the manipulation of four different variables: mowing, reduction of incident light, and nitrogen and phosphorus addition. The treatments were randomly located in 5×5 m plots within the fenced area with three replicates per treatment (total $n = 60$). Thus, each season $\times$ environmental agent combination was an independent plot (i.e. the 15 plots corresponding to each season were sampled only once).

The four levels of season were established according to vegetation productivity: summer (15 December 1996 to 14 March 1997) was the season of maximum growth and dispersion of warm-season species. Autumn (15 March to 14 June 1997) coincided with the end of warm-season species activity, the start of cool-season species growth and the occurrence of the first frosts. Winter (15 June to 14 September 1997) was the period of minimum growth of early cool-season species. Finally, spring (15 September to 14 December 1997) accounted for most cool-season species growth and fructification.

Experimental manipulations were as follows. Mowing consisted of a single removal event at the beginning of each season, made with scissors at 5 cm

height. This removed 30% and 40% of aerial biomass in summer and autumn, respectively, and 50% in winter and spring (clipped material was taken out of the plots). Shade consisted of a 50%-reduction of photon flux density, without affecting the red : far-red ratio, obtained by covering plots with shade cloth as in Chapin *et al.* (1995). Both nitrogen and phosphorus were added in their respective plots as single doses of 25 g m^{-2} at the beginning of each season (N was added as ammonium nitrate and P as calcium triple super phosphate). This single fertilization at high addition rates approximates the highest fertilization rates used in the region (0–25 g m^{-2} for N and 0–21 g^{-2} for P (García *et al.* 2002)). Extractable soil P after P addition, estimated in summer and autumn, were ~10 and ~150 p.p.m. for control and fertilized plots, respectively.

Aerial and below-ground plant biomass

At the end of each season, we measured aerial and below-ground biomass. We sampled aerial biomass of each plot from three 5×50 cm sub samples. We used the final biomass of the control plots in the previous season as an estimation of the initial biomass in the following season. For the summer season (the beginning of the experiment), we estimated initial biomass on 15 December 1996 from three additional plots. We classified aerial green biomass into forbs, cool-season graminoids and warm-season graminoids, and we pooled standing dead biomass. We sampled below-ground biomass by three soil cores of 18 cm height $\times$ 6.5 cm of diameter per plot. Previous studies in this community (Soriano 1992) and another related community (Ansín *et al.* 1998) indicated that 65–85% of below-ground biomass was within the top ~20 cm. Below-ground biomass was divided into two depths, 0–10 cm and 10–18 cm. Soil cores were gently washed with water on a mesh of 2 microns and biomass was separated into roots and other organs (bulbs, rhizomes, stolons). Harvested biomass was oven dried, and sub samples of root biomass were ashed to deduct contamination by soil.

Aerial net primary productivity

Aerial net primary productivity (ANPP) was estimated as the positive differences between final and initial biomass of each biomass component (green biomass of forbs, cool-season graminoids, warm-season graminoids and standing dead biomass). When the difference in any green compartment was negative, it was called net senescence and was deducted from standing dead biomass accumulation, with the restriction that

differences between final and initial standing dead biomass were ≥ 0 (Sala *et al.* 1981; Scurlock *et al.* 2002).

Plant nitrogen and phosphorus

We measured total N and P concentration of the aerial biomass fractions and of root biomass between 0 and 10 cm. We milled biomass samples and digested 100 mg sub samples in 2 mL of sulphuric acid at 300°C for 4 h followed by a final digestion with 1 mL of hydrogen peroxide. Digests were diluted to 15 mL with distilled water and colourimetrically analysed with a flow injection autoanalyser (Alpkem Corporation, Wilsonville, Oregon).

Soil mineral nitrogen

We calculated net N mineralization (or immobilization) of 0–10 cm soil by *in situ* and sequential coring of soil using buried PVC tubes as described in Raison *et al.* (1987). Tubes prevented uptake of mineral N by plants during the period measured. Three tubes were placed in the plots approximately 45 days after the beginning of each season. One of the tubes was immediately removed to determine initial N content while the other two remained buried until the end of the season. N mineralized during the incubation period was calculated as the positive changes of NH_4^+-N and NO_3^--N during the incubation period that ranged between 30 and 60 days. Thus, soil N dynamics were estimated for the second half of each season. One of the two tubes had its top covered to prevent leaching and N leaching during the incubation period was calculated as the difference of N between the covered and

the uncovered tube. For analysis, we extracted 10 g of soil from each tube with 50 mL of 2 M KCl and a similar soil sample was oven-dried to determine gravimetric soil moisture. Soil extracts were shaken and filtered and mineral N content was measured colourimetrically as explained above.

Statistical analyses

Data were analysed by two-way ANOVA with season and environmental agent as factors, followed by orthogonal contrasts for environmental agents and control within each season. As we found variance heterogeneity we used the mixed procedure of SAS System, which makes data transformation unnecessary.

RESULTS

Aerial biomass and production

Nitrogen addition was the only treatment that significantly affected green biomass at the end of the season relative to the control, and this effect varied among seasons (Fig. 1, environment–season interaction: $F_{12,40} = 2.00$, $P = 0.05$, significant N *versus* control contrasts in summer and spring, $P < 0.01$). N addition increased green biomass by 40% in summer and by twofold in spring (Fig. 1). Green biomass significantly varied throughout the year with greater values in spring and summer than in autumn and winter (Fig. 1).

The responses of green biomass integrated the effects of the manipulated variables on different biomass fractions (Table 1, Fig. 2). Graminoids and forbs accounted for 50% of green biomass each, but
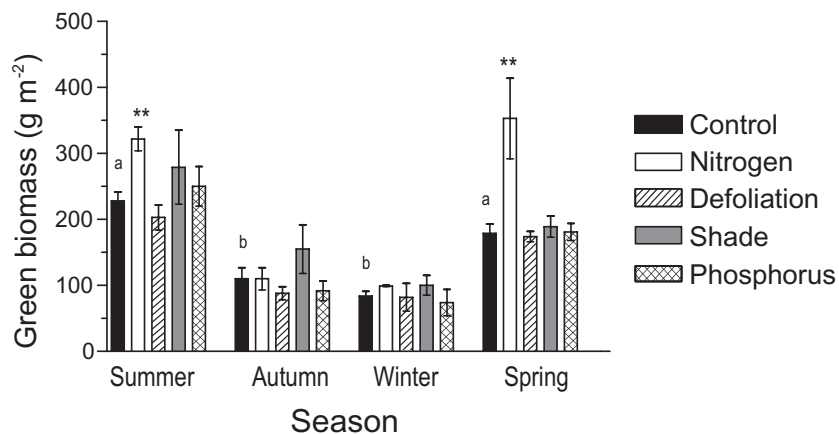

Fig. 1. Effects of environmental agents on green biomass (g m^{-2}) in different seasons. Asterisks indicate the probability level of the orthogonal contrasts of the treatment with respect to the control within season and different letters indicate significant differences in controls across seasons. **$P < 0.01$. Bars denote ±1 standard error.

Table 1.   Results from the two-way ANOVA of plant biomass and plant nitrogen and phosphorus concentration. Figures indicate *F*-values and asterisks the probability level

		Two-way ANOVA							
Source	D.f.	Warm	Cool	Forbs	Standing dead	Roots (0–10 cm)	Roots (10–18 cm)	Other organs	Total below-ground
Plant biomass									
Season	3	22.3***	13.1***	9.3***	16.4***	8.9***	3.7**	6.6**	1.7
Environmental agent	4	2.2*	4.9**	0.6	2.6	3**	0.7	0.3	2.3
Season × Environment	12	1.4	1.4	0.9	1.8	3.4**	0.9	0.9	2.3*
Total	40								
Plant nitrogen									
Season	3	77***	110***	29***	8***	16***	–	–	–
Environmental agent	4	15.6***	5.3**	16.6***	25***	23***	–	–	–
Season × Environment	12	5.6***	1.6	3.0*	5.4***	10.4***	–	–	–
Total	40								
Plant phosphorus									
Season	3	27.6***	51.8***	85***	18***	4.9**	–	–	–
Environmental agent	4	29.5***	32.7***	88***	76***	10.2***	–	–	–
Season × Environment	12	1.6	3.8***	10.5***	9.2***	1.4	–	–	–
Total	40								

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Degrees of freedom correspond to factors and error in the ANOVAs. *Warm* and *Cool* indicate green biomass of warm-season graminoids and cool-season graminoids, respectively.

diverged in their seasonal pattern and in their response to treatments (Table 1, Fig. 2). The positive effects of N addition on green biomass were explained by warm-season and cool-season graminoids (Fig. 2A,B), whereas forbs did not respond to any resource manipulation in any season (Table 1, Fig. 2C). Both groups of graminoids exhibited similar responses to resource availability although they had very well differentiated peaks of biomass production (Fig. 2A,B). Conversely, forbs appeared highly uniform throughout the year, yet had slightly greater values during summer (Table 1, Fig. 2C). Finally, standing dead biomass did not respond significantly to treatments but differed among seasons. It had the greatest accumulation during autumn and winter and the lowest accumulation in spring (Fig. 2D).

Nitrogen addition increased ANPP threefold in summer and spring, and mowing increased it sixfold in winter, the season with the overall lowest productivity (Fig. 3, environment–season interaction: $F_{12,40} = 2.06$, $P = 0.04$, significance level of contrasts indicated in the figure legend). In general, ANPP showed a seasonal pattern that closely tracked that of green biomass with maximum values during spring and summer (Figs 1,3).

Below-ground biomass

The 0–10 cm root biomass was affected by resource availability and seasonality (Table 1, Fig. 4A). Mowing increased root biomass relative to controls in autumn and winter, whereas P addition increased it in spring (Fig. 4A). The root biomass in the 0–10 cm of control plots was significantly lower in summer and had the greatest values in spring. Biomass of other below-ground organs (bulbs, rhizomes, stolons), that were preferentially distributed within the first 10 cm of the soil profile, were only affected by seasonality, with highest values in summer, a pattern opposite to that of roots (Table 1, Fig. 4B). Root biomass at greater depth (10–18 cm) was not influenced by resource availability, and exhibited a seasonal pattern with lower values in summer, a similar pattern to shallow roots (Table 1, Fig. 4C). Total below-ground biomass (the sum of roots and other organs) was interactively affected by season and environmental agents (Table 1, Fig. 4D). The greater root biomass in the first 10 cm of the mowed plots in autumn and winter translated into greater total below-ground biomass (Fig. 4A,D).

Plant nitrogen and phosphorus

Nitrogen and phosphorus in plant biomass were significantly affected by environmental agents and seasonality (Table 1). N addition increased N concentration of aerial biomass in a consistent way, although it did not affect the N concentration of roots. N concentration was higher in roots than in aerial biomass (Table 2). The rest of the environmental manipulations
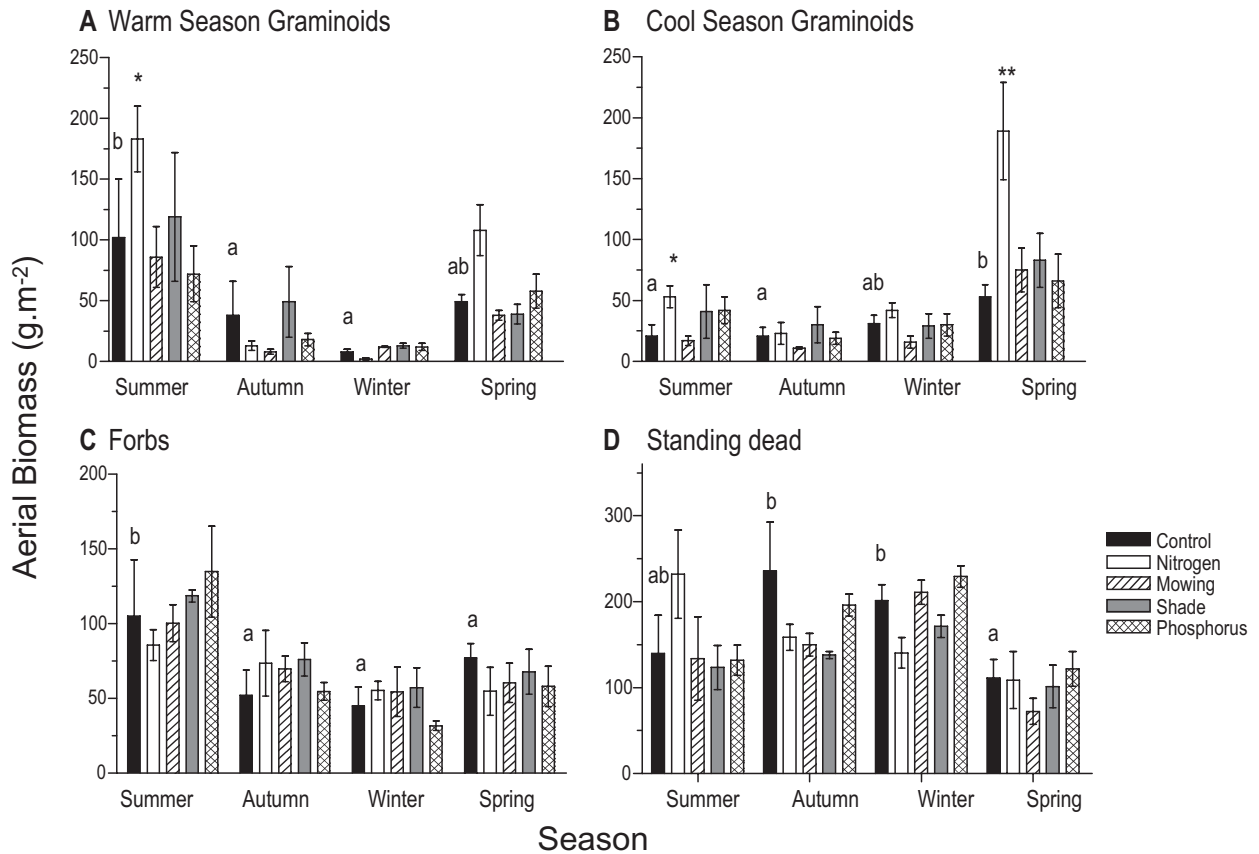
Fig. 2. Effects of environmental agents on green biomass of (A) warm-season graminoids; (B) cool-season graminoids; (C) forbs; and on standing dead biomass (D), in different seasons. Asterisks indicate the probability level of the orthogonal contrasts of the treatment with respect to the control within season and different letters indicate significant differences in controls across seasons. ★★$P < 0.01$, ★$P < 0.05$. Bars denote ±1 standard error. Note different scales on y-axes.
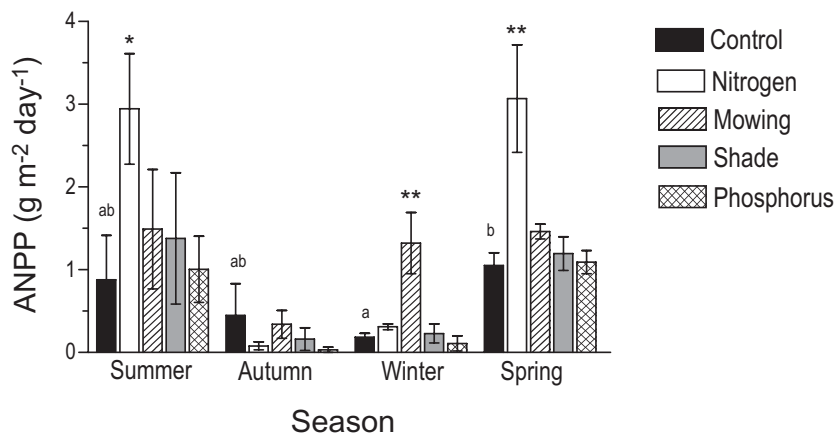

Fig. 3. Effects of environmental agents on above-ground net primary productivity (ANPP) in different seasons. Asterisks indicate the probability level of the orthogonal contrasts of the treatment with respect to the control within season and different letters indicate significant differences in controls across seasons. ★★$P < 0.01$, ★$P < 0.05$. Bars denote ±1 standard error.
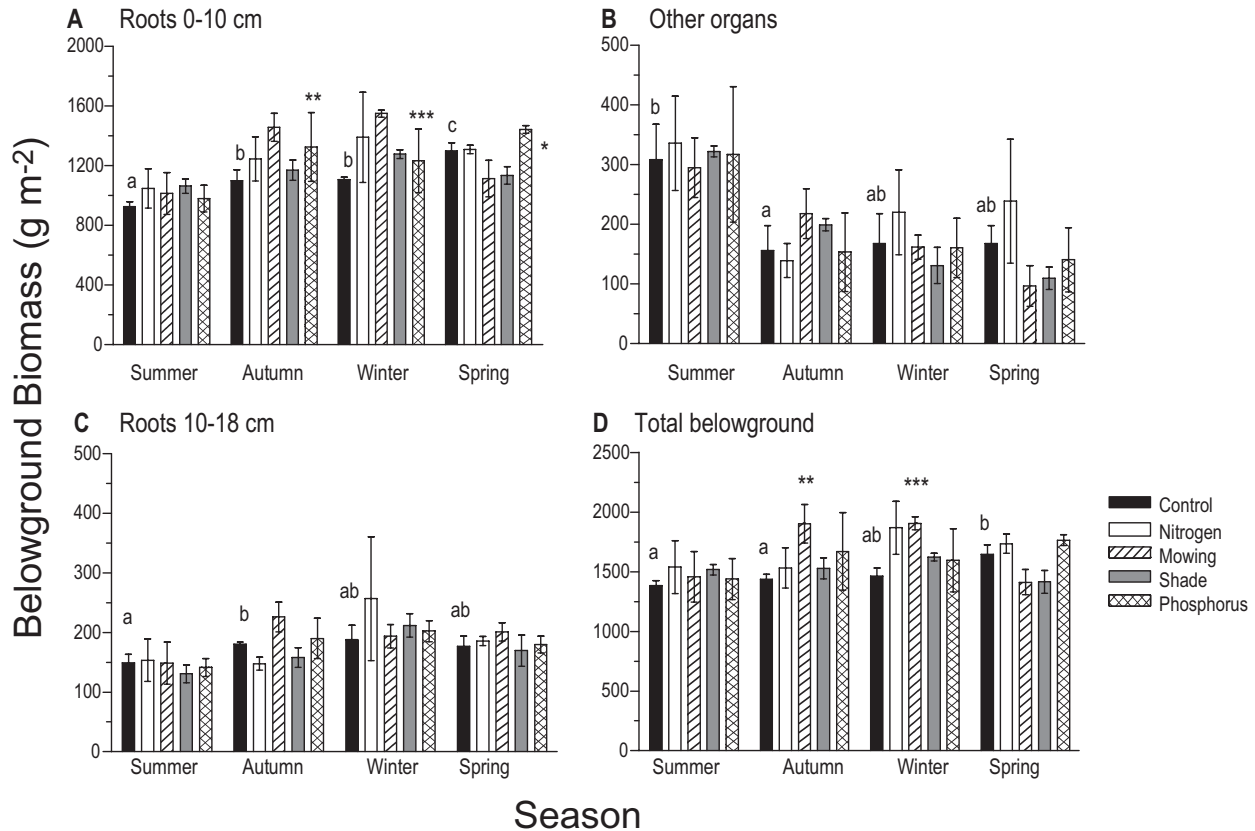
Fig. 4. Effects of environmental agents on below-ground biomass of (A) shallow roots; (B) other below-ground organs (rhizomes, stolons, bulbs, etc.); (C) deep roots; (D) total below-ground (roots + other organs), in different seasons. Asterisks indicate the probability level of the orthogonal contrasts of the treatment with respect to the control within season and different letters indicate significant differences in controls across seasons. $\star\star P < 0.01$, $\star P < 0.05$. Bars denote ± 1 standard error. Note different scales on y-axes.

occasionally affected N concentration. Mowing increased N concentration of warm-season graminoids in summer and autumn, and that of cool-season graminoids and forbs in summer. Shade increased N concentration of green biomass in summer and spring but significantly reduced that of roots in spring and winter. Conversely, P addition only increased N concentration of forbs in winter (Table 2). Overall, N concentration of biomass was greater in autumn and winter and lower in summer and spring (Table 2). N concentration in green biomass of the three functional groups differed (forbs > cool-season graminoids > warm-season graminoids).

Phosphorus concentration in plant biomass was exclusively affected by P addition, except that shade increased P concentration of roots in autumn (Table 3). P fertilization tripled P concentration in aerial biomass and doubled it in root biomass (Table 3). P concentration was greater in forbs than in graminoids, and was lower in spring and summer than in autumn and winter (Table 3).

Soil nitrogen

Soil mineral N, that was almost exclusively in the form of ammonium, consistently responded to N addition ($F_{4,94} = 6$, $P < 0.001$, significant N versus control contrasts $P < 0.05$, Fig. 5A). However, the greater soil N availability was not accompanied by changes in the net N mineralization rates (Fig. 5B). Seasonality affected both soil N contents and mineralization rates. Soil N content was greater in summer and autumn and decreased in winter and early spring (Fig. 5A) and N mineralization rate was significantly lower during autumn (Fig. 5B).

DISCUSSION

This work provided empirical evidence that N addition, mowing and light reduction affected productivity and the acquisition of N and P by plants, whereas P addition had a more limited effect. Mowing effects

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Table 2. Effects of environmental agents on plant nitrogen concentration (%) in different seasons

Season	Biomass fraction		Control		Nitrogen		Mowing		Shade		Phosphorus	
Summer	Warm graminoids	a	0.89	0.10	1.50	0.13*	1.04	0.23*	1.23	0.04***	1.08	0.13
	Cool graminoids	b	1.19	0.12	1.74	0.14***	0.36	0.09*	1.57	0.12*	1.17	0.16
	Forbs	b	1.40	0.12	2.13	0.09***	1.67	0.17***	2.24	0.10***	1.51	0.19
	Standing dead	a	0.91	0.02	1.27	0.07***	0.94	0.03	1.21	0.16	0.97	0.08
	Roots (0–10 cm)	a	1.49	0.13	1.91	0.02	1.73	0.17	1.56	0.02	1.54	0.05
Autumn	Warm graminoids	b	1.15	0.08	2.22	0.05***	1.58	0.06***	1.45	0.05**	1.28	0.09
	Cool graminoids	c	1.87	0.22	2.31	0.32	2.27	0.25	2.01	0.31	1.70	0.14
	Forbs	c	1.63	0.28	2.82	0.29**	2.12	0.06	2.01	0.18	1.83	0.04
	Standing dead	ab	1.05	0.16	1.54	0.04**	1.08	0.01	1.09	0.03	0.97	0.07
	Roots (0–10 cm)	b	2.09	0.10	2.27	0.21	1.95	0.13	1.72	0.04**	1.98	0.18
Winter	Warm graminoids	b	1.54	0.05	2.04	0.06**	1.31	0.01	1.75	0.13	1.82	0.06
	Cool graminoids	c	1.78	0.00	2.19	0.07*	1.65	0.06	1.77	0.06	2.03	0.05
	Forbs	bc	1.57	0.04	2.43	0.08***	1.62	0.06	1.88	0.18	2.05	0.18*
	Standing dead	b	0.94	0.05	1.49	0.09***	1.16	0.12	1.39	0.01***	1.07	0.12
	Roots (0–10 cm)	b	2.13	0.18	2.21	0.16	2.11	0.20	1.65	0.02***	2.33	0.06
Spring	Warm graminoids	a	0.86	0.06	1.17	0.26*	1.00	0.05	1.11	0.06*	0.92	0.10
	Cool graminoids	a	0.80	0.03	1.37	0.11***	0.89	0.08	0.96	0.06*	0.91	0.04*
	Forbs	a	1.19	0.06	1.71	0.02**	1.18	0.04	1.56	0.07***	1.30	0.02
	Standing dead	a	0.88	0.07	1.20	0.05**	1.01	0.01	1.09	0.04*	0.88	0.05
	Roots (0–10 cm)	ab	1.99	0.20	1.78	0.13	1.79	0.05	1.56	0.02*	2.12	0.03

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Asterisks indicate the probability level of the treatments with respect to the control within season and different letters in the third column indicate significant differences of controls across seasons for each biomass fraction. Standard errors in italics.

Table 3. Effects of environmental agents on plant phosphorus concentration (%) in different seasons

Season	Biomass fraction		Control		Nitrogen		Mowing		Shade		Phosphorus	
Summer	Warm graminoids	b	0.09	0.01	0.09	0.01	0.09	0.01	0.11	0.01	0.27	0.03***
	Cool graminoids	b	0.10	0.01	0.10	0.01	0.11	0.01	0.13	0.01	0.25	0.01***
	Forbs	b	0.13	0.01	0.12	0.01	0.14	0.01	0.18	0.01	0.36	0.01***
	Standing dead	bc	0.11	0.03	0.08	0.02	0.05	0.01	0.10	0.01	0.23	0.01***
	Roots (0–10 cm)	a	0.09	0.01	0.10	0.02	0.10	0.01	0.11	0.01	0.15	0.02**
Autumn	Warm graminoids	c	0.11	0.01	0.12	0.01	0.13	0.01	0.12	0.01	0.29	0.03***
	Cool graminoids	c	0.13	0.01	0.12	0.01	0.16	0.03	0.15	0.02	0.35	0.01***
	Forbs	b	0.12	0.03	0.15	0.01	0.14	0.01	0.15	0.01	0.43	0.03***
	Standing dead	b	0.09	0.01	0.11	0.02	0.09	0.02	0.08	0.01	0.2	0.03***
	Roots (0–10 cm)	b	0.09	0.02	0.14	0.01	0.13	0.02	0.15	0.01*	0.18	0.03***
Winter	Warm graminoids	d	0.14	0.01	0.15	0.01	0.12	0.01	0.13	0.01	0.41	0.05**
	Cool graminoids	c	0.13	0.01	0.12	0.01	0.13	0.02	0.13	0.01	0.48	0.07***
	Forbs	b	0.14	0.02	0.14	0.01	0.14	0.01	0.15	0.02	0.45	0.03***
	Standing dead	c	0.09	0.01	0.12	0.01	0.11	0.02	0.11	0.01	0.27	0.03***
	Roots (0–10 cm)	ab	0.12	0.01	0.09	0.02	0.13	0.01	0.12	0.01	0.19	0.02**
Spring	Warm graminoids	a	0.05	0.01	0.05	0.01	0.06	0.01	0.06	0.01	0.18	0.03**
	Cool graminoids	a	0.06	0.02	0.06	0.01	0.08	0.02	0.05	0.01	0.12	0.01***
	Forbs	a	0.08	0.01	0.08	0.01	0.09	0.02	0.10	0.01	0.15	0.01***
	Standing dead	a	0.07	0.01	0.07	0.02	0.09	0.02	0.09	0.01	0.14	0.01***
	Roots (0–10 cm)	a	0.12	0.01	0.09	0.02	0.09	0.01	0.10	0.01	0.15	0.01**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Asterisks indicate the probability level of the treatments with respect to the control within season and different letters in the third column indicate significant differences of controls across seasons for each biomass fraction. Standard errors in italics.
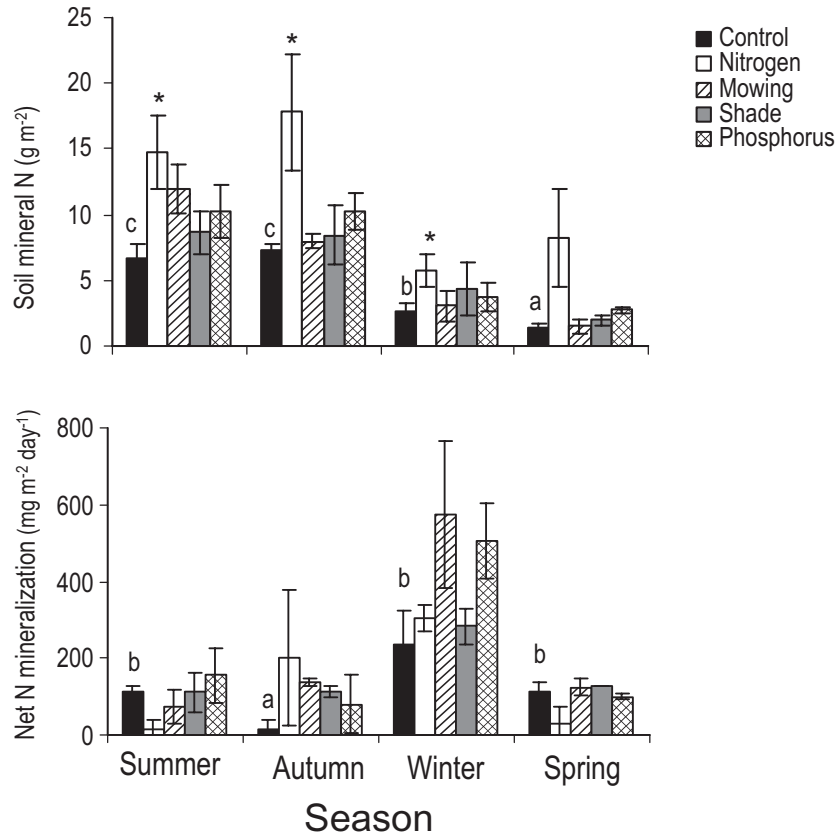
Fig. 5. Effects of environmental agents on (A) soil mineral N content; (B) net N mineralization rate, in different seasons. Asterisks indicate significant differences with respect to the control within season and different letters indicate significant differences in controls across seasons. *$P < 0.05$. Bars denote ±1 standard error.

were more apparent in the below-ground portion of the vegetation and shade mainly affected nutrient acquisition by plants. As we expected, the magnitude of these effects depended on the season. N addition tripled ANPP in summer and spring and doubled N contents of aerial biomass throughout the year. Mowing increased ANPP in winter by a factor of six, but this increase was observed during the season with lowest productivity. Mowing also increased the 0–10 cm root biomass in winter and autumn by more than 30%, with concomitant effects on N dynamics. Light reduction did not influence productivity, but increased both N concentration and N contents in aerial biomass, whereas it reduced N contents of below-ground biomass during winter and spring. There were no negative effects of mowing or light reduction on NPP. Furthermore, the influence of the factors studied on biomass and N dynamics of grassland appeared to be mediated by subtle differential effects on the constituent functional groups: warm- and cool-season graminoids and forbs. For example, while N addition mainly promoted growth and N uptake by graminoids, mowing promoted the N acquisition by forbs.

The magnitude of each resource limitation to grassland productivity changed throughout the year and integrated the different responses of the plant functional groups. The effects of resource manipulation on primary production have been extensively documented in the last decades for many grassland ecosystems (Lauenroth & Dodd 1978; Knapp *et al.* 1998; Shaver *et al.* 1998). However, probably due to the seasonal functioning of most temperate grasslands, those studies seldom evaluated the relative importance of different factors throughout the year. Our results provide evidence addressing this point in particular, which is relevant for systems such as the Flooding Pampa grasslands in two respects. First, these ecosystems exhibit a year-round production mediated by the phenological complementarity of different plant groups (Sala *et al.* 1981; León & Bertiller 1982). Second, these plant groups, in addition to phenological differences, diverge in life form (i.e. graminoids, forbs). Here, we have demonstrated that N addition had a stronger influence on grassland functioning than mowing, light reduction or P addition. N effects were strongest during the maximum growing period (spring and summer) and particularly benefited the

graminoids (Fig. 2). This ensures a greater transfer to herbivores as this group constitutes the most palatable fraction of the grassland. This positive short-term response of graminoids coincides with previous evidence documented for a related community after three years of fertilization (Ginzo *et al.* 1982; Collantes *et al.* 1998) whereas it contrasts with evidence from a grassland that, in addition, had been periodically mown (Ginzo *et al.* 1986). In addition, the short-term beneficial effect on graminoids suggests that in a long-term N fertilization scenario plant diversity, and particularly functional diversity, would be reduced, a trend that has been documented for other ecosystems (Wedin & Tilman 1996; Shaver *et al.* 2001). In the case of mowing, although it stimulated productivity of the different plant groups equally, its effects on N acquisition benefited forbs in particular. As pointed out in the case of N fertilization, this short-term differential N uptake of forbs might alter the competitive interactions between groups and result in an increasing dominance of forbs. This is undesirable in terms of secondary production since many of these forbs exhibit secondary compounds that deter herbivory (Semmartin *et al.* 1999).

Both the promotion and the unaffected biomass production of mowed plants involved compensatory responses at the community level. The extent to which plants can compensate for tissue removal by increased growth per unit biomass has been debated among ecologists during the past two decades (McNaughton 1983; Belsky 1986). Global analyses have revealed a broad diversity of responses, which include more negative responses at both the individual and the community level (Oesterheld *et al.* 1999; Ferraro & Oesterheld 2002). Compensatory responses to mowing have been previously found in the Flooding Pampa grasslands (Semmartin & Oesterheld 1996, 2001) although in the present study, positive responses took place during autumn and winter, a particularly critical period in terms of forage availability for grazers in these grasslands (Sala *et al.* 1981; Soriano 1992). Beneficial effects of mowing in winter agree with observations by Knapp and Seastedt (1986), who suggested that removing biomass increased soil temperature and accelerated plant growth and N mineralization. However, extrapolation of these responses to situations with actual grazing should be cautious. Mowing does not include other influential components of grazing such as selective biomass removal, trampling and dung deposition (Oesterheld & McNaughton 2000). Particularly, the similar biomass responses to mowing of the three plant groups should be carefully interpreted since grazers usually feed selectively on grass species.

Phosphorus addition did not increase aerial grassland productivity although it has widely been proposed as one of the most important limiting factors of these grasslands. The chronic P shortage reported for the Flooding Pampa soils (García *et al.* 2002), together with the extremely low cover of legumes (Burkart *et al.* 1990) suggested that P availability plays a key role as a limiting factor of primary production. However, we only documented a positive effect of P addition on the 0–10 cm-root biomass, during spring and a threefold increase of P concentration in plant biomass. These results, on the one hand, confirm actual P absorption by roots. On the other hand, they suggest luxury consumption by the three functional groups (Chapin 1980) as previously reported for this grassland (Ginzo 1983; Rubio & Lavado 1999). Furthermore, our findings agree with the predictions made by Koerselman and Meuleman (1996), who developed a simple tool to determine the nature of the nutritional limitation in grasslands. According to their work, communities with N : P ratios in plant biomass above 16 would be P limited, whereas those falling below 14 would be N limited, with intermediate values reflecting a co-limitation. Therefore, Flooding Pampa grasslands should be N limited since we estimated an average N : P ratio in aerial biomass of ~13. Moreover, the only period in when we found an average ratio of ~17 (in spring), we also found a positive effect of P addition on shallow roots (Fig. 4A). Our results cast doubt on the rationale underlying P fertilization practices that are so strongly widespread in the Flooding Pampa region and are exclusively based on the evaluation of the extractable P in soil (García *et al.* 2002).

Contrary to our expectations, shading did not diminish grassland production throughout the year (Figs 3,4). Light availability is considered an important restriction for NPP in humid and subhumid grasslands such as the Flooding Pampa grasslands (Milchunas *et al.* 1988; Lane *et al.* 2000). Even though there is little empirical evidence on the influence of light at the community level (Chapin *et al.* 1995; Shaver *et al.* 1998), the positive responses of ANPP to mowing have been frequently associated with greater light availability within the canopy (Fahnestock & Knapp 1994; Semmartin & Oesterheld 2001) or with changes in the light quality, particularly the red: far red ratio (Deregibus *et al.* 1994). In these grasslands, we previously documented that mowing increased the photon flux density at the soil surface to 65% of incident light, compared with 25% in the unmown controls (Semmartin & Oesterheld 2001). Therefore, grazing would have relieved the vegetation from a self-imposed stress from the accumulation of standing dead biomass. Likewise, management practices such as intensive grazing events at the beginning of autumn are based on the notion that enhanced light availability promotes grasses growth and tillering during the following season (Deregibus *et al.* 1994; Jacobo *et al.* 2000). In contrast to the negative effects of light reduction in tundra habitats (Chapin *et al.* 1995), our findings suggest that in the Flooding Pampa grasslands, in

any season, vegetation is not light limited (Figs 3,4). However, our extended light-reduction may have interacted with water dynamics and temperature, as well as with the greater N concentration of aerial biomass (Table 2) that might have counteracted the lower photon flux density. We believe that further research must attempt to discriminate between the direct effect of light intensity and other potential confounding factors.

Mowing increased shallow root biomass during autumn and winter (Fig. 4A) and these increments would have resulted in greater N content below-ground as the nutrient concentration was unaffected (Table 2). Both positive and neutral effects of mowing on below-ground biomass agree with results reported in a small number of studies recently performed in grassland ecosystems (McNaughton *et al.* 1998; Frank *et al.* 2002; Pucheta *et al.* 2004). Nevertheless, these studies compared grazed and neighbouring long-term ungrazed sites, whereas our results revealed positive and neutral short-term effects of mowing on below-ground production. Moreover, our results suggest a ~32% reduction of growth in clipped plants relative to control, that was estimated in a recent meta analysis of experiments on individual plants, that in turn widely agrees with the common notion of the detrimental effects of grazing on the below-ground subsystem (Ferraro & Oesterheld 2002), should not be extrapolated to the community level. On the other hand, positive effects of mowing on below-ground biomass during winter appear to be associated with greater net N mineralization rates in soil (Fig. 5B). This observation coincides with results from pot experiments in which clipped *Poa pratensis* individuals produced significant amounts of carbon labile exudates that promoted microbial activity and N mineralization in the rhizosphere (Hamilton & Frank 2001). However, this stimulating short-term effect of mowing should be evaluated in light of the concomitant positive effects on root biomass. The lower decomposability of root biomass compared with aerial biomass might neutralize the greater short-term mineralization by an increasing microbial immobilization, hence, resulting in an enhanced carbon and mineral nutrients retention in soil of grazed systems (Semmartin *et al.* 2004).

Changes in water availability produced by interannual variation of rainfall may alter the response to other environmental manipulation. As Lauenroth and Dodd (1978) reported for semiarid grasslands, the response to N addition may strongly interact with water availability. Thus, the responses to our manipulations might have differed if rainfall during the year of the experiment had been much lower or higher. For example, a four-year experiment in a similar grassland showed a slightly greater effect of N or P fertilization on ANPP in the more humid years (Collantes *et al.* 1998). On the other hand, in this same grassland, we found different responses to mowing when comparing an average year with a flooded one, but in this case, with lower compensatory responses during the flooded year (Semmartin & Oesterheld 1996). At a seasonal level, the greater influence of N addition on green biomass production in spring relative to summer, might be due to the relatively higher soil moisture during the spring.

The differential effects of N addition and mowing on plant functional groups has ecosystem-level implications far beyond the direct effects on biomass availability of a floristic group. The influence of N addition being particularly beneficial for graminoids is expected to produce an amplified impact on carbon and nutrient transfer to secondary producers, since graminoids usually constitute the most palatable items in temperate grasslands. Therefore, both the evenness of the impact of N addition on plant groups and the nature of the plant group specifically promoted should be regarded as important features accounting for variation in the total effect of greater N availability on primary and secondary production. In addition, differences among plant groups in carbon and nitrogen kinetics during decomposition will also differentially impact on nutrient cycling and availability. In the Flooding Pampa grasslands, for example, grasses and forbs exhibit dramatic differences in carbon and N dynamics during litter breakdown. This points out the importance of less apparent feedbacks among vegetation and disturbances such as grazing or N loading (Semmartin *et al.* 2004).

In conclusion, the present study aimed at disentangling the relative importance of the most evident limiting factors of NPP and nutrient cycling of subhumid grasslands throughout the year. N emerged as the main limiting factor during the period of maximum growth (spring and summer) evidenced by positive effects on ANPP of graminoids that were accompanied by greater plant quality (i.e. N concentration), whereas mowing significantly stimulated aerial and below-ground production and N uptake during winter, particularly in forbs, and also increased below-ground production during autumn. Positive effects of N addition on productivity were not accompanied by greater N concentration, but by a greater absolute N uptake. P addition had the opposite main effect. There was no response in terms of biomass production (except shallow roots in spring) and a general large increase of P concentration. We are aware of the necessity of evaluating the potential interactions among factors as well as how these interactions behave over years and, thus, we consider these results also useful for the better selection of the combinations of factors and seasons to evaluate in future research. Additionally, the lack of positive effects to N addition during autumn and the null response to P addition on aerial biomass throughout the year suggest that fertilization practices should be re-evaluated.

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