

Responses of C₃ and C₄ grasses to application of nitrogen and phosphorus fertilizer at two dates in the spring

G. Rubio, F. H. Gutierrez Boem and R. S. Lavado

Cátedra de Fertilidad y Fertilizantes and INBA, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina

Abstract

In grasslands composed of C₃ and C₄ plants, a different pattern of response by the two photosynthetic types to fertilizer may change the floristic composition of the community. In this study, we evaluated the effects of an application of nitrogen (N) and phosphorus (P) fertilizer at two dates in the spring on the aerial growth of C₃ and C₄ grasses. *Danthonia montevidensis* and *Stipa neesiana* (C₃), and *Setaria geniculata* and *Sporobolus indicus* (C₄), were selected from a native grassland of the Flooding Pampa, Argentina. Two consecutive experiments (early and late spring) were conducted in pots filled with the local soil. The C₃ grasses showed no response in aerial biomass, and only minor responses in aerial concentrations of N and P, numbers of leaves and tillers, and nutrient use efficiency to an application of P or N fertilizer. In contrast, their C₄ counterparts showed a consistent positive response. C₄ species performance was considerably influenced by date of fertilizer application in the spring. There was on average a 1.8 and a 2.5 increase in aerial biomass in early and late spring, respectively, to fertilizer application. The later date in spring was associated with higher ambient temperatures and it appeared that temperature rather than fertilizer application was the main limiting factor for C₃ species. It was concluded that the positive response in C₄ species to an application of P and N fertilizer indicates that the differences between C₃ and C₄ species were related to a higher demand to sustain greater growth rates in C₄ species.

Keywords: nitrogen, phosphorus, Flooding Pampa, natural grasslands, photosynthetic groups

Introduction

Metabolic differences between C₃ and C₄ plants result in a variety of responses to environmental conditions which contribute to regulate the structure and function of ecosystems (Ehleringer and Monson, 1993). Natural grassland ecosystems are composed of communities dominated either by a mixture of C₃ and C₄ species or by species belonging to only one photosynthetic type. The proportion of individuals belonging to each group is mainly determined by the environmental conditions in which the particular characteristics of each group confer a competitive advantage (Ode *et al.*, 1980). Comparative studies have usually found that C₄ plants have a requirement for higher temperatures for growth and may suffer metabolic disorganization at lower temperatures that can support acceptable growth of C₃ plants (Ehleringer and Bjorkman, 1977; Ode *et al.*, 1980). This offers an explanation as to why grassland systems occupying low-latitude areas are predominantly composed of C₄ species, whereas C₃ species frequently dominate high-latitude areas and a mixture of both types commonly is found in mid-latitude areas (Teery and Stowe, 1976). The major role of temperature in determining the competitive ability between C₃ and C₄ species has been related to photosynthetic rate. Ehleringer and Bjorkman (1977) and Pearcy *et al.* (1981) observed that, at higher temperatures, C₄ species have a greater photosynthetic rate and quantum yield than C₃ species and that this relative advantage disappears at low temperatures.

The central role of temperature on the performance of C₃ and C₄ plants may affect their response to an application of fertilizer. It is known that the photosynthetic rate per unit of leaf-nitrogen (N) is often higher in C₄ plants because of the CO₂-concentrating

Correspondence to: G. Rubio, Cátedra de Fertilidad y Fertilizantes and INBA, Facultad de Agronomía, Universidad de Buenos Aires. Av. San Martín 4453, C1417DSE, Buenos Aires, Argentina.
E-mail: rubio@agro.uba.ar

Received 2 July 2009; revised 7 October 2009

mechanism leading to CO₂ saturation of Rubisco (Sage and Percy, 1987; Oaks, 1994). However, several studies have shown no differences between C₃ and C₄ plants growing at varying levels of N (e.g. Boryslawsky and Bentley, 1985; Gebauer *et al.*, 1987). Christie and Detling (1982) observed a very low response of both C₃ and C₄ species to applied N when grown at non-optimal temperatures. They concluded that the N efficiency of both groups is highly regulated by temperature, thus responses to N can vary on a seasonal basis. Phosphorus (P), the second limiting nutrient to plant productivity worldwide, possesses additional functions in C₄ plants, derived from its participation in processes unique to the metabolism of C₄ plants (Iglesias *et al.*, 1993). Although C₄ plants have been suggested to have a higher sensitivity to P deprivation (Iglesias *et al.*, 1993), Halsted and Lynch (1996) and Jacob and Lawlor (1991) found that C₃ and C₄ species had similar efficiencies of P use. Ghannoum and Conroy (2007) and Ghannoum *et al.* (2008) also concluded that the advantages of C₄ photosynthesis at the level of the leaf, in terms of nutrient-use efficiency, do not always translate to the level of the whole plant. Comparative studies of the different responses of C₃ and C₄ species to defoliation are abundant (e.g. Woodis and Jackson, 2008), but how responses to P fertilizer in C₃ and C₄ plants are affected by temperature has not been reported.

Natural ecosystems where species of both photosynthetic groups co-exist are suitable to investigate the competitive differences between them. The comparison of co-existing and closely related C₃ and C₄ grasses avoid the confounding effects of taxonomy and habitats (Ghannoum and Conroy, 2007). One example of these ecosystems is the natural grasslands of the Flooding Pampa, Argentina. This region (around 9 million ha) is mostly covered by native grasslands devoted mainly to the production of beef cattle. Species of C₃ and C₄ plants show a temporal separation in the above-ground productivity of these grasslands, the former group being mainly responsible for plant production during the cold season and the latter during the warm season (Facelli, 1988). During spring, the period of highest plant growth, both photosynthetic groups contribute to this growth. Extreme environmental conditions (e.g. soil water-logging in late winter, salinization of top soil and drought during summer) prevents these grasslands being used for cropping or the native communities being replaced by sown grass-legume swards. There is a growing interest in finding alternatives to increase the productivity of these grasslands. Local soils are relatively rich in N but P levels are particularly low (Rubio *et al.*, 2008). As a consequence, the application of P fertilizer is considered as one of the few feasible alternatives for increasing the productivity of these

grasslands and increasing the usually low economic profitability of the use of such grasslands.

When additional nutrients are added to grasslands composed of a mixture of photosynthetic groups, a different pattern of response to the availability of soil nutrients between both metabolic groups may lead to a change in floristic composition. Griffin *et al.* (2002) found that the addition of P allowed the dominance of *Agropyron repens* (C₄ species) at the expense of *Poa pratensis* (C₃ species). If the pattern of response to fertilizer is modified by temperature, the time of the year at which the fertilizers are applied adds a new dimension. To assess the potential impact of enhanced nutrient supply on the native grassland community, the objective of this research was to evaluate the effects of N and P fertilizer at two dates in the spring, when temperature was likely to vary, on the aerial growth of C₃ and C₄ grasses. The approach was to undertake two consecutive pot experiments, the first in early spring and the second in late spring. Two grasses of each photosynthetic group were selected from a native grassland community of the Flooding Pampa. The four grasses can be found growing together and have basically the same growth form. These similarities facilitate the comparison of the role of the photosynthetic pathway on the response to environmental conditions.

Materials and methods

Plant species and soil

Plant species and soil for the pot experiments were collected from a stand of one of the most widespread plant communities of the Flooding Pampa, so called the 'B' community (Burkart *et al.*, 1990). Stands of this community occupy large lowland areas, in which the predominant soil is Typic Natraquoll (General Guido Series). The top horizon (0–15 cm depth) is a silt loam, with a pH (saturation paste) of 6.6. It contained 26.6 g kg⁻¹ organic C (Walkley-Black), 8.7 mg kg⁻¹ of extractable P (Bray 1) and 624 mg kg⁻¹ of extractable K (1 N NH₄OAc). Pots (diameter of 14 cm and with a height of 15 cm) were filled with homogenized material from this horizon.

Danthonia montevidensis and *Stipa neesiana* were selected as representatives of the C₃ group, and *Setaria geniculata* and *Sporobolus indicus* as representatives of the C₄ group. *Danthonia montevidensis* is the dominant species in the studied lowland community but it is not abundant in the neighbouring highland 'A' community (Burkart *et al.*, 1990). Compared with the grassland community 'B', community 'A' occupies well drained and more fertile soils that are not subjected to flooding or extreme summer drought events (Perelman *et al.*,

1982, 2001). *Stipa neesiana*, *S. geniculata* and *S. indicus* are well distributed in both 'A' and 'B' communities. *Sporobolus indicus* is the only selected species that is found in the community 'D' (Burkart *et al.*, 1990), associated with habitats of the Flooding Pampa characterized by very shallow alkaline soils (Perelman *et al.*, 1982, 2001).

Experiments

Two pot experiments were carried out to test the effects of the environmental conditions (mainly temperature) on plant responses to nutrient enrichment. Experiment 1 was performed in the early spring (September to October) and Experiment 2 in late spring (November to December). During the first week of August (Experiment 1) and October (Experiment 2), juvenile individuals of similar size of the selected species were transplanted into pots. To mimic field conditions, soil was extracted from the same habitat as the individuals of the four species. After a 4-week acclimation period, plants were randomly assigned to the fertilizer treatments and there were the same number of tillers in each pot. No cuts were made to the plants before imposing the fertilizer treatments. Pots were placed in the open air and, during rainfall events, they were covered with a plastic rain shelter to prevent uncontrolled soil wetting. Each experiment consisted of a $4 \times 2 \times 2$ factorial design with six replicates per treatment. The factors were species (the four species already described), N application rate (0 and 150 mg N per pot) and P application rate (0 and 46 mg P per pot). Nitrogen was added as urea and P as triple superphosphate. Nutrients were added at the beginning of September in Experiment 1 and at the beginning of November in Experiment 2. Pots were maintained at around 0.75–1.00 of field capacity by daily weighing and watering.

Measurements

Plants were harvested 60 d after fertilizer application. Above-ground material was dried for 48 h at 60°C and weighed. The number of tillers and leaves were also recorded. Nitrogen concentration in leaves was measured by the Kjeldahl method. Phosphorus concentration was evaluated colorimetrically after a perchloric-nitric acid digestion.

Statistical analyses

Data of each experiment were statistically analysed by a factorial ANOVA, after testing the variables for normality and for homogeneity of variance. Variances were stabilized when necessary using an empirical power transformation (Kuehl, 1994). When there was a

significant effect of treatment, contrasts (Student's *t*-test) were used to compare the mean values. Nutrient-use efficiency was calculated as the amount of aerial biomass produced per unit of nutrient in the same pot. Nutrient dilution curves were calculated by plotting nutrient concentration of aerial biomass as a function of aerial biomass accumulation, and fitting a function for each nutrient level and C₃ or C₄ group using the data from both experiments. The function used was $y = ax^b$, where *y* is the nutrient concentration and *x* is the aerial biomass.

Results

In the experiments, temperature and relative air humidity reproduced well conditions in early and late spring in the grassland where these plants naturally grow (Figure 1). The mean daily temperature was 17.1°C in Experiment 1 and 21.3°C in Experiment 2 whereas average relative air humidity was very similar in both experiments, viz. 72.4% and 71.7% respectively.

C₄ plants accumulated significantly ($P < 0.001$) more aerial biomass than their C₃ counterparts in both experiments (Figure 2). Aerial biomass was higher in Experiment 2 (late spring) than in Experiment 1 (early spring) (Figure 2). On average, *S. indicus* had 2.3 times more aerial biomass in late spring (Experiment 2) than in early spring (Experiment 1) and *S. geniculata*, the other C₄ species, 3.0 times more. The aerial biomass of *D. montevidensis* was similar in both experiments whereas *S. neesiana*, the other C₃ plant, had almost 2.0 the aerial biomass in late spring (Figure 2).

Plant responses to fertilizer were different between experiments and within experiments were affected by species and fertilizer, as well as there being significant interactions among them. Fertilizer application by N and P, or together, resulted in non-significant effects on

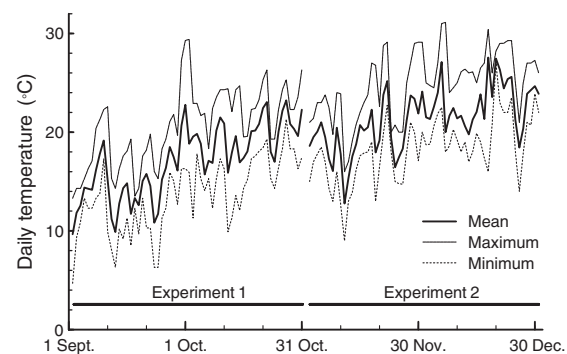
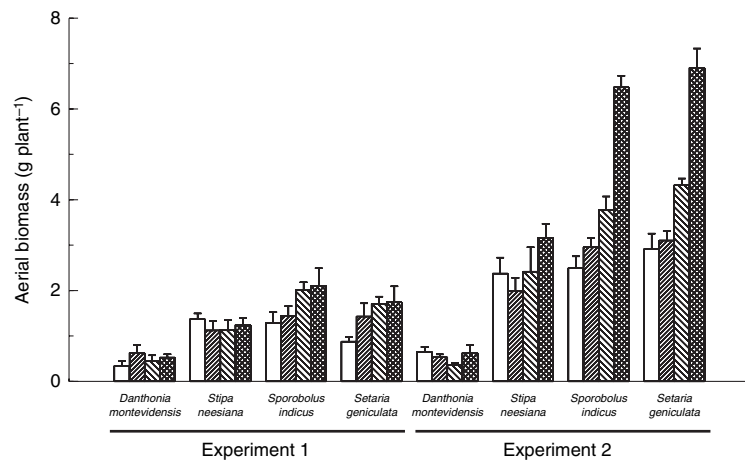


Figure 1 Daily minimum and maximum temperatures during Experiments 1 and 2.

Figure 2 Aerial biomass of two C₃ (*Danthonia montevidensis* and *Stipa neesiana*) and two C₄ (*Sporobolus indicus* and *Setaria geniculata*) grass species in Experiment 1 (early spring) and Experiment 2 (late spring) as affected by no fertilizer (□) nitrogen (right diagonal hatching), phosphorus (left diagonal hatching) and combined (cross-hatching) fertilizer treatments. Error bars represent the standard error of the mean.



aerial biomass (Figure 2), and number of tillers or leaves of C₃ plants (Table 1), in both experiments. The addition of N did not change the N status of these plants, as indicated by the similar N dilution curves of fertilized and non-fertilized plants (Figure 3). Application of P fertilizer resulted in a significant ($P < 0.001$) increase in the P concentration of C₃ plants (Figure 3; Table 1).

The application of N and P fertilizer resulted in a notable impact on C₄ plants. Responses in the biomass of aerial shoots to fertilizer varied between experiments and within experiments were affected by species and fertilizer (Table 1). In Experiment 1, the response of both C₄ species in aerial biomass, and number of tillers and leaves, to the addition of nutrients was similar: no significant effect of N fertilizer but significant ($P < 0.001$) effects of P fertilizer. Application of P fertilizer resulted in a higher increase in the shoot biomass of *S. geniculata* than in *S. indicus*: proportionately by 0.95 and 0.55 respectively. These values were very similar to those observed when evaluating the differences in aerial biomass between the control and the combined N and P fertilizer treatments: proportionately an increase of 1.0 in *S. geniculata* and 0.62 in *S. indicus* (Figure 2). Nutrient-use efficiency was less affected by fertilizer application than the other variables. However, addition of P significantly ($P < 0.001$) reduced the efficiency of P use in both C₄ species.

In Experiment 2, both C₄ species showed positive responses in aerial biomass (Figure 2) to both nutrients. Responses to P fertilizer were greater than responses to N fertilizer. The response to the N fertilizer when added as a single nutrient was modest: an increase of 0.18 and 0.06 for *S. indicus* and *S. geniculata* respectively. Response to application of P fertilizer, however, resulted in an increase of 0.51 and 0.48 in the dry

weight of aerial biomass of *S. indicus* and *S. geniculata* respectively. The combined effects of N and P fertilizer resulted in a dramatic increase in dry weight of aerial biomass: proportionately 1.59 and 1.37 in *S. indicus* and *S. geniculata* respectively. Increases in above-ground growth promoted by P fertilizer were accompanied by simultaneous increases in number of tillers and number of leaves in *S. geniculata* (Table 1). As was observed in Experiment 1, a significant ($P < 0.001$) decrease in efficiency of P use after application of P fertilizer was observed in both C₄ species. Nitrogen-dilution curves of C₄ plants showed slightly lower values than C₃ plants and a small but noticeable effect of N fertilizer (Figure 3). In contrast, P dilution curves of C₄ plants showed higher values than C₃ plants (Figure 3).

Discussion

The results showed a similar pattern of response to the application of N and P fertilizers within each photosynthetic group and important differences between them. Whereas no responses to applications of N or P were found in most growth variables for C₃ grass species, C₄ species showed clear responses to N and P fertilizer. In the experiments, both soil and plants were obtained from the same habitat to reproduce, to some extent, the conditions that these species face in the field. Availability of N and P was not a main limiting growth factor in C₃ species in both experiments. Thus, the interaction of fertilizer and temperature would have little relevance for the performance of this group. Rubio *et al.* (1997b) observed, in a field study performed in a neighbouring grassland community, a lack of response to N and P fertilizer during winter, the period in which C₃ plants were the main contributor to biomass production. Those results are consistent with the data presented here and suggest that fertilizer is not a useful tool to

Table 1 Plant traits of four grass species grown in two seasons with contrasting nutrient enrichment treatments. Factors were: season, species (Sp), nitrogen supply (N) and phosphorus supply (P).

	Experiment 1				Experiment 2			
	Control	Fertilizer	Treatment	N + P	Control	Fertilizer	Treatment	N + P
		N	P			N	P	
No. tillers								
<i>D. montevidensis</i>	16.2	12.5	20.2	20.4	31.0	21.1	16.0	25.6
<i>S. neesiana</i>	13.4	8.0	12.6	15.8	27.6	22.2	22.2	34.3
<i>S. indicus</i>	8.4	10.0	17.0	17.4	13.0	16.2	12.6	29.5
<i>S. geniculata</i>	12.0	19.0	24.4	18.7	15.1	20.5	22.5	36.8
s.e. of mean		0.786				1.087		
No. leaves								
<i>D. montevidensis</i>	67.4	58.1	86.6	77.0	111.1	82.8	56.0	106.8
<i>S. neesiana</i>	52.0	40.2	51.4	62.2	91.3	97.2	79.7	117.0
<i>S. indicus</i>	42.2	44.0	68.8	79.5	58.7	73.0	60.5	109.0
<i>S. geniculata</i>	86.8	115.8	146.8	123.7	96.6	128.2	151.3	252.1
s.e. of mean		4.271				5.947		
N-use efficiency (g g ⁻¹)								
<i>D. montevidensis</i>	28.0	26.9	26.9	28.5	30.4	25.8	28.1	27.6
<i>S. neesiana</i>	36.8	30.2	35.2	34.9	32.6	35.0	51.0	36.6
<i>Sporobolus indicus</i>	49.8	45.5	50.1	37.7	53.4	38.3	49.6	51.5
<i>Setaria geniculata</i>	30.3	36.3	32.3	31.4	62.4	35.7	61.7	36.1
s.e. of mean		1.17				2.20		
P-use efficiency (g g ⁻¹)								
<i>D. montevidensis</i>	421	471	393	402	672	589	529	560
<i>S. neesiana</i>	518	465	444	377	772	819	568	547
<i>S. indicus</i>	376	450	340	313	642	789	405	375
<i>Setaria geniculata</i>	401	375	291	318	941	756	592	619
s.e. of mean		8.9				24.5		
N conc. of shoots (mg g ⁻¹ DM)								
<i>D. montevidensis</i>	35.6	37.2	37.1	35.3	33.1	40.8	36.5	37.3
<i>S. neesiana</i>	27.4	33.3	28.5	28.7	31.5	28.8	19.9	27.3
<i>S. indicus</i>	21.0	22.0	20.0	26.5	20.3	26.3	21.9	21.0
<i>S. geniculata</i>	35.5	27.8	31.0	31.8	17.1	28.1	18.0	27.8
s.e. of mean		0.9				1.3		
P conc. of shoots (mg g ⁻¹ DM)								
<i>D. montevidensis</i>	2.43	2.15	2.55	2.50	1.50	1.73	1.90	1.80
<i>S. neesiana</i>	1.93	2.16	2.27	2.66	1.30	1.26	1.76	1.86
<i>S. indicus</i>	2.66	2.23	2.95	3.20	1.56	1.30	2.46	2.66
<i>S. geniculata</i>	2.50	2.66	3.43	3.16	1.10	1.33	1.73	1.63
s.e. of mean		0.06				0.07		

increase herbage production of the C₃ species of these communities.

In contrast to the C₃ species, C₄ species showed a great capacity to increase herbage production after application of fertilizer. Temperature, perhaps combined with irradiance, appeared to limit growth early in the spring, as indicated by the higher biomass accumulation found in late spring (Experiment 2) than early spring (Experiment 1). Interestingly, the response to

fertilizer in C₄ plants was much higher in late spring (Experiment 1) than in early spring (Experiment 1). Whereas application of N and P fertilizer resulted in a 1.6- and 2.0-fold increase in aerial biomass of *S. indicus* and *S. geniculata*, respectively, in early spring (Experiment 1), these values rose to 2.6- and 2.4-fold, respectively, in late spring (Experiment 2). These values also show that the response of both C₄ species was not uniform. The response in *S. geniculata* was greater than

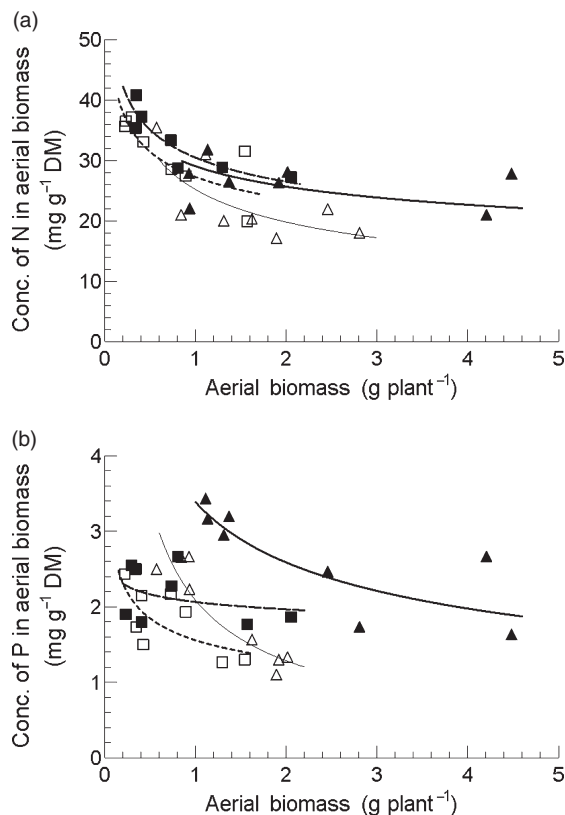


Figure 3 Nitrogen and phosphorus dilution curves of two C₃ (*Danthonia montevidensis* and *Stipa neesiana*) and two C₄ (*Sporobolus indicus* and *Setaria geniculata*) grass species as affected by application of (a) nitrogen and (b) phosphorus fertilizer. The dilution curves are presented for C₃ species with no fertilizer (□, dotted line), C₃ species with fertilizer (■, dashed line), C₄ species with no fertilizer (△, thin solid line) and C₄ species with fertilizer (▲, thick solid line). Data from both Experiments 1 and 2 are included.

in *S. indicus* from the application of fertilizer in early spring (Experiment 1) than in late spring (Experiment 2) but the differences between C₄ species were small in comparison to the differences between C₃ and C₄ species. Thus, photosynthetic type was the major factor which discriminated plant responses to the N and P fertilizer and temperature regimes used.

In a previous experiment, a positive response to N and P fertilizer was observed in the above-ground biomass of *Paspalum dilatatum*, another dominant C₄ species from the community studied here, whereas growth of the C₃ species, *D. montevidensis*, was unaffected by fertilizer treatments (Rubio and Lavado, 1999). In this study, these observations were extended, demonstrating that: (i) the higher capacity of C₄ species, compared with their C₃ counterparts, to respond to

fertilizer, especially P, is confirmed in other co-occurring grass species, such as *S. indicus* (C₄ species), *S. geniculata* (C₄ species) and *S. neesiana* (C₃ species); and (ii) the response to fertilizer by each photosynthetic group is consistent under different temperature regimes.

The fact that P but not N was the first nutrient-limiting nutrient in this study is not surprising since several studies with native local grasses have reported similar results (Rubio and Lavado, 1999; Rubio *et al.*, 1999). It was also observed that C₄ grasses of this grassland maintain a high capacity to grow after application of P fertilizer during the severe drought conditions that these plants experience in the field during most of the summer (Rubio *et al.*, 1997b). Moreover, the response by C₄ plants to P fertilizer was verified also under water-logging conditions that affect the grassland studied, sometimes from winter to late spring (Rubio *et al.*, 1997a). The soil used in this study was relatively high in N but low in P, providing a good scenario to evaluate the differential responses of C₃ and C₄ plants when plants of species from both groups were grown under the same environmental conditions. Results showed that the C₄ species have a higher growth potential compared with their C₃ counterparts and, consequently, a higher nutrient requirement. Some studies have shown that C₄ species have lower shoot N concentration (i.e. higher N-use efficiency) values than C₃ species, which is presumably related to a lower content of photosynthetic proteins (Gastal and Lemaire, 2002; Levang-Brilz and Biondini, 2003). The results of this study are partly consistent with those observations. *Danthonia montevidensis* had a higher N concentration in aerial biomass but the other C₃ plant species, *S. neesiana*, had a similar N concentration to that of *S. geniculata* ($P = 0.47$) but lower than that of *S. indicus* ($P < 0.01$). The N-dilution curves and the lack of responses to N clearly showed that the N content of the soil was sufficient to satisfy the requirements of both C₃ plants. The addition of N caused almost negligible effects on the measurements made on the plants belonging to this group, and no luxury N consumption (i.e. increase in N concentration of aerial biomass without a parallel increase in biomass) was found. On the other hand, the dilution curves indicated that the low but noticeably response of C₄ plants to fertilizer-N was determined by their higher growth potential rather than by their higher N-use efficiency. In the case of P, the observed higher response of C₄ species to P fertilizer was also determined by their higher growth potential rather than by the efficiency of use variables, as indicated by the P-dilution curves. C₄ species are expected to have a higher P requirement for several reasons (Ghannoum and Conroy, 2007). No clear trends emerged when comparing shoot P

concentrations of the four species, hence no conclusions about the performance of the different metabolic types can be reached. However, the P dilution curves revealed luxury consumption of P in fertilized plants from both groups which can be beneficial in terms of adaptation to grazing. In a recent study with grasses from the same grassland community, Oyarzabal and Oesterheld (2009) suggested that accumulation of P above levels that promote growth can be considered as a form of reserve to support future growth when the nutrient needs subsequently to be mobilized. Therefore, the high levels of P reserves in fertilized plants can confer tolerance to defoliation by promoting compensatory growth under P deficiency.

The fact that only C₄ plants responded positively to N and P fertilizer indicates that the observed differences between metabolic types were not related to a particular nutrient but to a higher demand to sustain greater growth rates. The results suggest that the application of fertilizer in late spring to C₄ components of C₃/C₄ grasslands, like those studied here, could lead to an increase in the productivity of the grassland and lead to significant changes in the floristic composition of the grassland.

Acknowledgments

This research was partially supported by CONICET (PIP 5432), University of Buenos Aires (UBACYT G622) and ANPCYT (PICT 931). The authors wish to thank two anonymous referees and the editors for helpful comments.

References

- BORYSLAWSKY Z. and BENTLEY B.L. (1985) The effect of nitrogen and clipping on interference between C₃ and C₄ grasses. *Journal of Ecology*, **73**, 113–121.
- BURKART S.E., LEON R.J.C. and MOVIA C.P. (1990) Phytosociological description of the Salado Basin grassland (Prov. Bs. As.) in an area representative of their main environments. *Darwiniana*, **30**, 27–69.
- CHRISTIE E.K. and DETLING J.K. (1982) Analysis of interference between C₃ and C₄ grasses in relation to temperature and soil nitrogen supply. *Ecology*, **63**, 1277–1284.
- EHLERINGER J.R. and BJORKMAN O. (1977) Quantum yields for CO₂ uptake in C₃ and C₄ plants: dependence on temperature, CO₂ and O₂ concentration. *Plant Physiology*, **59**, 86–90.
- EHLERINGER J.R. and MONSON R.K. (1993) Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Plant Physiology*, **24**, 411–439.
- FACELLI J.M. (1988) Response to grazing after nine years of cattle exclusion in a Flooding Pampa Grassland, Argentina. *Vegetatio*, **78**, 21–25.
- GASTAL F. and LEMAIRE G. (2002) N uptake and distribution in crops: an agronomical and eco-physiological perspective. *Journal of Experimental Botany*, **53**, 789–799.
- GEBAUER G., SCHUBERT B., SCHUHMACHER M.I., REHDER H. and ZIEGLER H. (1987) Biomass production and nitrogen content of C₃ and C₄ grasses in pure and mixed culture with different nitrogen supply. *Oecologia*, **71**, 613–617.
- GHANNOUM O. and CONROY J.P. (2007) Phosphorus deficiency inhibits growth in parallel with photosynthesis in a C₃ (*Panicum laxum*) but not two C₄ (*P. coloratum* and *Cenchrus ciliaris*) grasses. *Functional Plant Biology*, **34**, 72–81.
- GHANNOUM O., PAUL M.J., WARD J.L., BEALE M.H., COROL D. and CONROY J.P. (2008) The sensitivity of photosynthesis to phosphorus deficiency differs between C₃ and C₄ tropical grasses. *Functional Plant Biology*, **35**, 213–221.
- GRIFFIN T., GIBERSON E. and WIEDENHOEF M. (2002) Yield response of long-term mixed grassland swards and nutrient cycling under different nutrient sources and management regimes. *Grass and Forage Science*, **57**, 268–278.
- HALSTED M. and LYNCH J.P. (1996) Phosphorus responses of C₃ and C₄ species. *Journal of Experimental Botany*, **47**, 497–505.
- IGLESIAS A.A., PLAXTON W.C. and PODESTA F.E. (1993) The role of inorganic phosphate in the regulation of C₄ photosynthesis. *Photosynthesis Research*, **35**, 205–211.
- JACOB J. and LAWLOR D.W. (1991) Stomatal and mesophyll limitations of photosynthesis in phosphate-deficient sunflower, maize and wheat plants. *Journal of Experimental Botany*, **42**, 1003–1011.
- KUEHL R.O. (1994) *Statistical principles of research design and analysis*. Belmont, CA, USA: Duxbury Press.
- LEVANG-BRILZ N. and BRONDINI M. (2003) Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. *Plant Ecology*, **165**, 117–144.
- OAKS A. (1994) Efficiency of nitrogen utilization in C₃ and C₄ cereals. *Plant Physiology*, **106**, 407–414.
- ODE D.J., TIESZEN L.L. and LERMAN J.C. (1980) The seasonal contribution of C₃ and C₄ plant species to primary production in a mixed prairie. *Ecology*, **61**, 1304–1311.
- OYARZABAL M. and OESTERHELD M. (2009) Phosphorus reserves increase grass regrowth after defoliation. *Oecologia*, **159**, 717–724.
- PEARCY R.W., TUMOSA N. and WILLIAMS K. (1981) Relationships between growth, photosynthesis and competitive interactions for a C₃ and a C₄ plant. *Oecologia*, **48**, 371–376.
- PERELMAN S., LEON R.J.C. and DEREGIBUS V.A. (1982) Aplicacion de un metodo objetivo al estudio de las comunidades de pastizal de la depresion del Salado (Please provide English translation of title). *Revista de la Facultad de Agronomia UBA*, **3**, 27–40.

- PERELMAN S.B., LEON R.J.C. and OESTERHELD M. (2001) Cross-scale vegetation patterns of Flooding Pampa grasslands. *Journal of Ecology*, **89**, 562–577.
- RUBIO G. and LAVADO R.S. (1999) Accumulation and allocation of resources in two water-logging tolerant grasses. *New Phytologist*, **143**, 539–546.
- RUBIO G., OESTERHELD M., ALVAREZ C.R. and LAVADO R.S. (1997a) Mechanisms for the increase in phosphorus uptake of water-logged plants: soil phosphorus availability, root morphology and uptake kinetics. *Oecologia*, **112**, 150–155.
- RUBIO G., TABOADA M.A., LAVADO R.S., RIMSKI-KORSAKOV H. and ZUBILLAGA M.S. (1997b) Biomass, nitrogen and phosphorus accumulation on a fertilized natural grassland of the Northern Flooding Pampas. *Ciencia del Suelo*, **15**, 48–50.
- RUBIO G., RIMSKI-KORSAKOV H. and LAVADO R.S. (1999) Uptake of soil mineral phosphorus and fertilizer-³²P in water-logging tolerant plants. *Current Topics in Plant Physiology*, **19**, 338–340.
- RUBIO G., CABELLO M.J., GUTIÉRREZ BOEM F.H. and MUNARO E. (2008) Estimating available soil-P increases after P additions in Mollisols. *Soil Science Society of America Journal*, **72**, 1721–1727.
- SAGE R. and PEARCY R.W. (1987) The nitrogen-use efficiency of C₃ and C₄ plants. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L) and *Amarantus retroflexus* (L). *Plant Physiology*, **84**, 959–963.
- TEERY J. and STOWE L.G. (1976) Climatic patterns and the distribution of C₄ grasses in North America. *Oecologia*, **23**, 1–12.
- WOODIS J.E. and JACKSON R.D. (2008) The effects of clipping height and frequency on net primary production of *Andropogon gerardii* (C₄ grass) and *Bromus inermis* (C₃ grass) in greenhouse experiments. *Grass and Forage Science*, **63**, 458–466.