Preference for different inorganic nitrogen forms among plant functional types and species of the Patagonian steppe

Laureano A. Gherardi, Osvaldo E. Sala & Laura Yahdjian

Oecologia

Springer

Springer

ECOSYSTEM ECOLOGY - ORIGINAL RESEARCH

Preference for different inorganic nitrogen forms among plant functional types and species of the Patagonian steppe
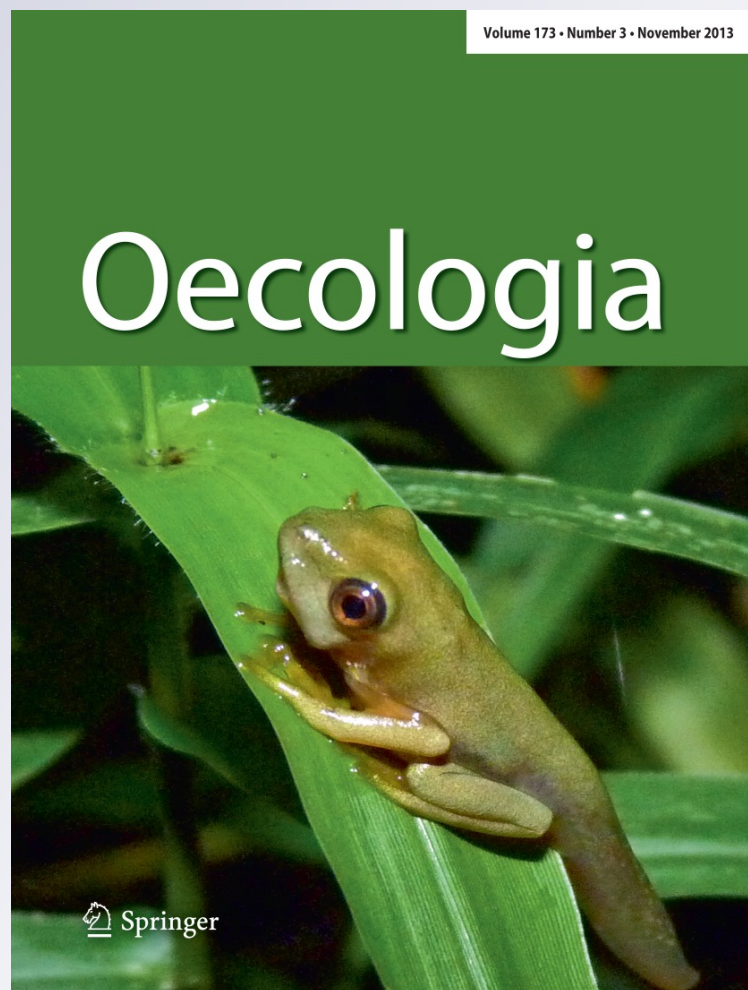
**Laureano A. Gherardi · Osvaldo E. Sala ·
Laura Yahdjian**

Abstract We have explored species–specific preferences for nitrate (NO_3^-) and ammonium (NH_4^+) as an alternative niche separation in ecosystems where nitrogen (N) is present mostly in inorganic forms. The Patagonian steppe is dominated by shrubs and grasses. Shrubs absorb water and nutrients from deep soil layers, which are poor in N, while grasses have the opposite pattern, absorbing most of their water and nutrients from the upper layers of the soil. We hypothesized that the preferences of shrub and grass for inorganic N forms are different and that the rate of potential N uptake is greater in shrubs than in grasses. To test this hypothesis, we grew individuals of six dominant species in solutions of different $NH_4^+:NO_3^-$ concentration ratios. Nitrate uptake was found to be higher in shrubs, while ammonium uptake was similar between plant functional types. The $NH_4^+:NO_3^-$ uptake ratio was significantly lower for shrubs than grasses. Shrubs, which under field conditions have deeper rooting systems than grasses, showed a higher N absorption capacity than grasses and a preference for the more mobile N form, nitrate. Grasses, which had lower N uptake rates than shrubs, preferred ammonium over nitrate. These complementary patterns between grasses and shrubs suggest a more thorough exploitation of resources by diverse ecosystems than those dominated by just one functional type. The loss of one plant functional group or a significant change in its abundance would therefore represent a reduction in resource use efficiency and ecosystem functioning.

Introduction

Nitrogen (N) availability in arid ecosystems is scarce, spatially and temporally heterogeneous (Noy-Meir 1973; Schimel and Parton 1986) and frequently limits primary production, as shown by meta-analyses of N fertilization studies of arid ecosystems (Yahdjian et al. 2011) and of all terrestrial ecosystems (Elser et al. 2007). Dominant forms of inorganic N in the soil are nitrate (NO_3^-) and ammonium (NH_4^+), which vary in their abundance through space and time (Harris 1977; McKane et al. 1990; Nadelhoffer et al. 1996). Preference for different N forms has important ecosystem consequences because it may allow for niche complementarity, species coexistence, and enhanced resource use efficiency (McKane et al. 2002; Reynolds et al. 2003).

In the Patagonian steppe, inorganic N is concentrated in the uppermost layers of the soil, with 49 % of total inorganic N found in the top 5 cm of the soil profile (Sala et al. 2012), a pattern that is common to most other arid ecosystems (Jobbagy and Jackson 2001). Ammonium is the major form of inorganic N in the upper layers (Armas et al. 2008;

Communicated by Russell Monson.

L. A. Gherardi (✉) · O. E. Sala
School of Life Sciences, Arizona State University, 427 E Tyler
Mall, Tempe, AZ 85287, USA
e-mail: Laureano.Gherardi@asu.edu

O. E. Sala
School of Sustainability, Arizona State University, Tempe, AZ,
USA

L. Yahdjian
Institute for Agricultural Plant Physiology and Ecology (IFEVA),
School of Agronomy, University of Buenos Aires, National
Research Council (CONICET), Buenos Aires, Argentina

Yahdjian et al. 2006) which have an NH_4^+: NO_3^- ratio of 10:1, but nitrate is the dominant form of N in the deeper layers where this ratio is 1:100 (Sala et al. 2012). Nitrate and ammonium interact differently with soil particles because of their chemical structure. Ammonium is a cation that binds easily with negatively charged clay particles and consequently has limited movement in the soil profile relative to nitrate, which as an anion moves easily in the soil matrix (Paul 2007). Different forms of N are also absorbed and transformed at different rates by plants and microbes. Nitrate and ammonium distributions in the soil profile result from differential absorption, leaching, and transformation rates with depth (Yahdjian and Sala 2010).

Vegetation of the Patagonian steppe is dominated by two plant functional types, grasses and shrubs, which have contrasting rooting architecture and phenology (Sala et al. 1989, 1993). Shrubs are deep rooted with a clear annual senescent phase, whereas grasses are shallow rooted and maintain green tissue year round. These differences in structure and phenology result in striking differences in their functioning. Shrubs absorb most of the water from deep layers of the soil, and grasses, in contrast, absorb most of their water from the upper layers of the soil (Sala et al. 1989). A recent study using tracers showed that indeed shrubs and grasses absorb N from where they absorb most of the water, shrubs from lower soil layers and grasses from soil upper layers (Sala et al. 2012). From the N economy point of view, this absorption pattern may put shrubs at a disadvantage with grasses, which absorb N from a relatively N-rich layer of the soil. Also, despite that shrus explore a much poorer soil layer than grasses, they account for the same fraction of aboveground net primary productivity as grasses (Sala et al. 1989).

Patterns of soil N distribution and absorption by shrubs and grasses together with evidence of their long-term coexistence led us to the central questions of this study. (1) Given that nitrate abundance relative to ammonium increases with depth, do shrubs show a preference for nitrate over ammonium? And, vice versa, do grasses have a preference for ammonium over nitrate? (2) Given that shrubs absorb N from a relatively N-poor layer, have they greater capacity to absorb N from the soil than grasses?

To address these questions, we performed a greenhouse experiment that consisted of a hydroponic culture of 180 individuals of the six dominant plant species of the Patagonian steppe, three grasses and three shrubs, growing in three different inorganic-N solutions (just ammonium, just nitrate, or ammonium + nitrate). Our response variables were uptake of total N, nitrate, and ammonium and their ratios by species and plant functional type. In addition, to assess potential confounding factors of different plant growth rates and biomass, we measured relative growth rate, shoot biomass, root biomass, and root:shoot biomass ratio for each species and plant functional type.

Methods

Our hydroponic experiment consisted of plants growing on culture tubes that followed a completely randomized factorial design with 21 treatments: six species plus one control tube with no plants by three solution types. We used ten replicates per treatment, resulting in a total of 210 experimental units [10 replicates × 6 species × 3 solutions + (10 × 3 control solutions) = 210 tubes]. The three solutions contained different NH_4^+:NO_3^- ratios (1:0, 1:1, 0:1) and the same total N concentration (2 mM). This concentration ensured more than enough N supply for the experimental period and allowed us to test for potential N uptake rates. Control treatments consisted of tubes with solutions under identical conditions but without plants. We used the three grass and the three shrub species as replicates of each plant functional type.

We cultivated plants of the six dominant species from the Patagonian steppe: three grass species [*Poa ligularis* Nees apud Steudel, *Poppostipa speciosa* (Trin. et Rupr.) (ex *Stipa speciosa*), and *Pappostipa humilis* (Cav.) (ex *Stipa humilis*)] and three shrub species [*Mulinum spinosum* Cav (Pers), *Adesmia volckmanni* (Philippi), and *Senecio filaginoides* (De Cand) (Ulibarri 1986)]. These dominant species account for 96 % of aboveground net primary production (Golluscio and Sala 1993) and represent 90 % of plant cover (Soriano et al. 1994).

Plants were collected from the Río Mayo Experimental Station (INTA), Chubut, Argentina (45°41′S, 70°16′W) during the spring. Individual shrubs and tussock grasses were brought to a greenhouse at the School of Agronomy, University of Buenos Aires (34°25′S, 58°28′W) and maintained in pots with soil from the field site at 25/15 °C day/night, and 54 % mean relative humidity (RH) for acclimation. After 1 week, 30 individuals of each species (2 or 3 tillers per grass and 1 individual per shrub) were washed with distilled water and transplanted to 50-ml culture tubes separated from each other by 12 cm. We carefully selected young grass tussocks and individual juvenile shrubs that were not in reproductive stages (Moore et al. 1991) in order to minimize N uptake differences due to ontogenetic stages (Imsande and Touraine 1994). Individuals of each species were randomly assigned to tubes, which were filled with distilled water and provided with aeration. After a 5-day acclimation period, we rewashed all roots and tubes and refilled the tubes with nutritive solutions, which were randomized among the 30 individuals of each species. Control tubes were washed and filled with nutritive solutions, which were randomly assigned to each tube.

N concentrations in the solutions were: 2 mM $(NH_4)_2SO_4$ in the ammonium solution (NH_4^+/NO_3^- ratio 1:0), 1 mM $(NH_4)_2SO_4$ and 1 mM Ca $(NO_3)_2$ in the solution with both N forms (NH_4^+/NO_3^- ratio 1:1), 2 mM Ca $(NO_3)_2$ in the nitrate solution (NH_4^+/NO_3^- ratio 0:1). We added 0.02 mM $CuSO_4$ and 0.02 mM H_3Bo_3 to all solutions to inhibit microbial activity and mycorrhizae development and thereby prevent N concentration changes that were not directly related to plant absorption. In addition, all solutions contained the same basic nutrient composition; 2 mM KH_2PO_4, 2 mM K_2SO_4, 1 mM $MgSO_4$, 4 mM $CaSO_4$, and traces of micronutrients to ensure nutrient supply.

After 9 days of cultivation under the same climatic conditions as those of acclimation, we took 20 ml samples from each solution and weighed plants after drying them in a 70 °C oven for 48 hours. Solution samples were stored in a freezer at −5 °C until analysis by flow-injection with a QuickChem® QC8500 Automated Ion Analyzer (Lachat Instruments, Hach Company, Loveland, CO). Ammonium, nitrate, and total inorganic N uptake rates (μg-N mg biomass^{-1} day^{-1}) were calculated as the difference between concentrations in treatment solutions and the average concentration of the respective controls with the same NH_4^+/NO_3^- ratio, divided by the time encompassed and expressed per milligram of dry biomass, as follows:

$$r = \frac{\overline{\ln (w2)} - \overline{\ln (w1)}}{t2 - t1}$$

where $w1$ is the mean plant weight at time 1, and $w2$ is the mean plant weight at the end of the experiment, with weight given in grams. The $t1$ and $t2$ parameters are the time in days at the beginning and end of the experiment. At the end of the experiment, shoots and roots were separated, dried in a 70 °C oven for 48 hours, and weighed for biomass analyses.

Total inorganic N uptake rates were analyzed using a two-way analysis of variance (ANOVA) with species or plant functional types and N treatments as main effects, followed by a Tukey HSD test for multiple comparisons. Nitrate and ammonium uptake rates for plant functional types were analyzed using one-way ANOVA, whereas the same rates for species within functional types were analyzed using the Kruskal–Wallis test because species absorption data were not normally distributed. The $NH_4^+:NO_3^-$ uptake ratio, relative growth rate, and biomass data were analyzed using one-way ANOVA with plant functional type as the main effect. Differences from 1 of plant functional type uptake ratios were tested using the t test. All analyses were performed with R ver. 2.14.2 (R Development Core Team 2012). Dependent variables were normalized with

$$NH_4^+ \text{uptake}_{(sample\ 1)} = \left[\mu gN\ \left(as\ NH_4^+\right)_{sample\ 1} - \mu gN\ \left(as\ NH_4^+\right)_{Sol\ control} \right] \text{mg biomass}^{-1} \text{day}^{-1}$$

$$NO_3^- \text{uptake}_{(sample\ 2)} = \left[\mu g\ N\ \left(as\ NO_3^-\right)_{sample\ 2} - \mu gN\ \left(as\ NO_3^-\right)_{Sol\ control} \right] \text{mg biomass}^{-1} \text{day}^{-1}$$

$$\text{Total N uptake} = NH_4^+ \text{uptake} + NO_3^- \text{uptake}$$

We also calculated N uptake expressed per unit of root biomass in order to explore physiological mechanisms explaining N uptake patterns. Ammonium and nitrate uptake were estimated from treatments that included these N forms in the solutions. Nitrate uptake was estimated from the treatments of nitrate only and both N forms; ammonium was estimated from the treatments of ammonium only and both N forms. We estimated total N uptake using all treatments. The $NH_4^+:NO_3^-$ uptake ratio (NH_4^+uptake:NO_3^- uptake) was calculated to evaluate species and life-form preferences for these two inorganic-N sources using only the treatment where both N forms were present in the exact same proportion and equally available to plants, as:

$$NH_4^+ \text{uptake rate}/NO_3^- \text{uptake rate}$$

The unbiased estimator of relative growth rate r was calculated for each plant functional type as the difference between the mean of the natural logarithm-transformed plant weight of each plant functional type at the beginning and at the end of the experiment divided by the duration of the experiment (Hoffmann and Poorter 2002).

$\log_{10}$ when required to meet ANOVA assumptions prior to analysis, but data are presented as the original values in figures and text to facilitate interpretation. In all cases, we used a 5 % significance level.

Results

The $NH_4^+:NO_3^-$ uptake ratio was significantly higher for grasses than for shrubs ($F_{1,4} = 10.71$, $P = 0.03$; Fig. 1). The average ratio for the three grass species was greater than 1 ($t = 2.353$, $P < 0.05$), while the mean N uptake ratio for the three shrub species was lower than 1 ($t = -5.673$, $P = 0.01$), indicating opposite preference for the two inorganic N forms. Grasses showed a preference for ammonium and shrubs for nitrate. The $NH_4^+:NO_3^-$ uptake ratio was estimated in the treatment that had both N forms equally available in order to avoid confounding effects associated with nutrient availability.

The nitrate uptake rate was significantly higher for shrubs than for grasses ($F_{1,8} = 10.25$, $P = 0.01$), but ammonium uptake rates were similar for the two plant
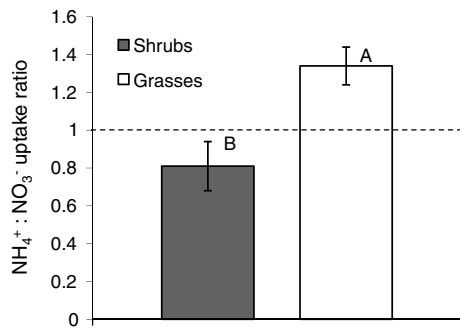
Fig. 1 Ammonium:nitrate (NH_4^+:NO_3^-) absorption ratio, calculated as the ratio between the ammonium and nitrate uptake rate for each plant functional type growing in a solution that contained the same concentration of both N forms. *Bars* are mean values (±standard error, SE). *Different uppercase letters above bars* indicate significant differences between plant functional types ($F_{1,4} = 10.71$, $P = 0.03$). N uptake ratios of both plant functional types were significantly different from 1 ($t_{(3)} = 2.35$, $P < 0.05$; $t_{(3)} = -5.67$, $P = 0.01$)
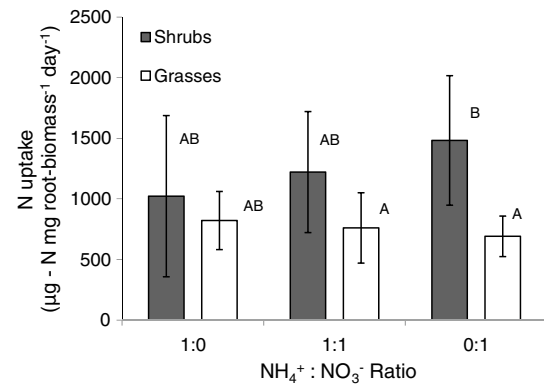

Fig. 2 Nitrate and ammonium uptake rates for grasses and shrubs. Nitrate uptake (μg-N mg biomass^{-1} day^{-1}) was calculated as the difference in nitrate concentration between treatment and control in the two treatments where nitrate was present (only NO_3^- and NO_3^- + NH_4^+). Ammonium uptake was calculated in the same way but only in solutions that contained ammonium (only NH_4^+ and NO_3^- + NH_4^+). *Bars* are mean values (±SE). *Double asterisks* mean significant differences ($F_{1,8} = 10.25$, $P = 0.01$), *NS* means no significant differences ($F_{1,8} = 1.85$, $P = 0.21$), between shrubs and grasses

functional types ($F_{1,8} = 1.85$, $P = 0.21$; Fig. 2). The nitrate uptake rate was estimated from treatments with the solutions containing just nitrate and nitrate plus ammonium, and the ammonium uptake rate was estimated using solutions with just ammonium and ammonium plus nitrate.

Nitrogen uptake expressed per unit of root biomass differed between plant functional types across solutions with different NH_4^+:NO_3^- ratios (Fig. 3). Plant functional types showed no significant difference when ammonium was the only N source ($F_{1,4} = 1.207$, $P = 0.33$), a marginal difference when both forms were present ($F_{1,4} = 6.22$, $P = 0.07$), and a significant higher N uptake by shrubs than grasses when nitrate was the only N source ($F_{1,4} = 20.5$, $P = 0.01$). These rates were estimated in solutions with

Fig. 3 Inorganic N uptake (μg-N mg root-biomass^{-1} day^{-1}) for grasses and shrubs in solutions with different NH_4^+:NO_3^- ratios. *Bars* are mean values (±SE). *Different uppercase letters above bars* indicate significant differences at $P < 0.05$ between plant functional types within each solution treatment
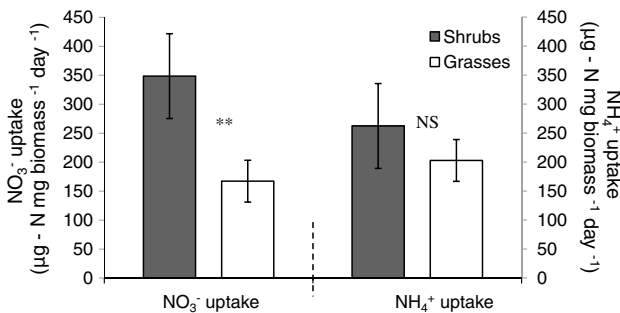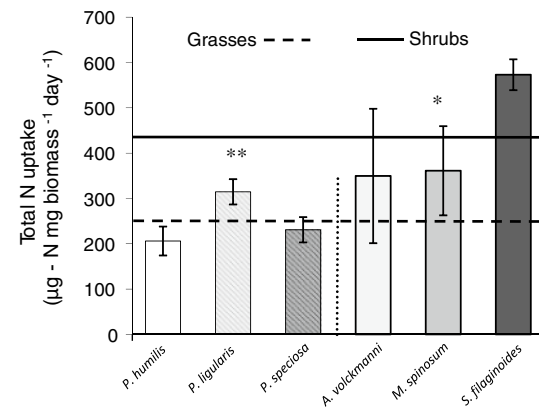

Fig. 4 Total inorganic N uptake calculated as the difference in N concentration between treatments and the control solution for three shrub species (*Adesmia volckmanni*, *Mulinum spinosum*, and *Senecio filaginoides*) and three grass species (*Pappostipa. humilis*, *Poa ligularis*, and *Pappostipa speciosa*). *Bars* are means (±SE) for each species ($n = 10$). *Asterisk* indicates significant difference among shrub species ($F_{2,81} = 8.4$, $P < 0.05$), *double asterisks* indicate significant difference among grass species ($F_{2,81} = 3.61$, $P < 0.05$). *Solid and dashed lines* indicate mean total inorganic N uptake rates by shrubs and grasses that were significantly different ($F_{1,12} = 14.51$, $P < 0.01$) and calculated from all solution treatments combined

only ammonium, nitrate plus ammonium, and only nitrate, respectively.

The total inorganic N uptake rate was significantly higher for shrubs than for grasses ($F_{1,8} = 14.51$, $P < 0.01$; Fig. 4, solid and dashed lines). Species within each plant functional type showed different total N uptake rates, but these differences were smaller than those between functional types (Fig. 4). Among the grasses, *P. ligularis* had the highest inorganic N uptake rate ($F_{2,81} = 3.61$, $P < 0.05$; Fig. 4), and among the shrubs *S. filaginoides* had the highest inorganic N uptake rate ($F_{2,81} = 8.4$, $P < 0.05$; Fig. 4).

Table 1 Mean dry biomass for two plant functional types

Plant part	Grass	Shrub
Root	100.56 (35.5)[a]	42.57 (10.2)[b]
Shoot	169.64 (43.7)[a]	68.72 (20.2)[b]
Root:shoot	0.64 (0.064)[a]	0.80 (0.034)[a]

Data are presented as the mean (standard error). Values followed by different lowercase letters are significantly different ($P < 0.05$) between plant functional types

These specific responses were not modified by the form of N source within either the grasses ($F_{2,81} = 0.63$, $P = 0.53$) or the shrubs ($F_{2,81} = 0.31$, $P = 0.73$).

Root and shoot dry mass was greater in grasses than in shrubs (root $F_{1,12} = 6.74$, $P = 0.02$; shoot $F_{1,12} = 12.12$, $P < 0.01$; Table 1), but the root:shoot ratio of both plant functional types was similar ($F_{1,12} = 3.94$, $P = 0.09$, Table 1). The different solution treatments had no effect on either root biomass ($F_{1,12} = 0.07$, $P = 0.93$) or shoot biomass ($F_{1,12} = 0.37$, $P = 0.69$). There was also no interaction between solution treatment and plant functional type root biomass or between treatment and plant functional type shoot biomass ($F_{1,12} = 0.024$, $P = 0.98$ and $F_{1,12} = 0.029$, $P = 0.97$, respectively). We did not find a significant difference between plant functional types in relative growth rate ($F_{1,4} = 5.47$, $P = 0.10$). The mean rates for grasses and shrubs were 0.013 and 0.0094 g g^{-1} day^{-1}, respectively.

Discussion

In response to question (1) (Given that nitrate abundance relative to ammonium increases with depth, do shrubs show a preference for nitrate over ammonium? And, vice versa, do grasses have a preference for ammonium over nitrate?), our results show that the shrubs tested in our study preferred nitrate over ammonium whereas the grasses preferred ammonium over nitrate. The difference between plant functional types in terms of the $NH_4^+:NO_3^-$ uptake ratio from a solution in which both N forms were equally available shows that shrubs absorbed on average more N as nitrate than as ammonium and that grasses absorbed more N as ammonium than as nitrate (Fig. 1). Shrubs had higher nitrate absorption rates than grasses but a similar ammonium uptake rate (Fig. 2), suggesting that the adaptation which explains higher total N uptake rates in shrubs than in grasses may be associated with rapid nitrate absorption kinetics. Although nitrate has higher uptake and assimilation costs than ammonium (Bloom et al. 1992), it can be stored in vacuoles, while ammonium accumulation is limited by its toxicity (Britto and Kronzucker 2002).

Our results regarding question (2) (Given that shrubs absorb N from a relatively N-poor layer, have they greater capacity to absorb N from the soil than grasses?) indicate that the shrubs tested here, which in their natural ecosystem have deep roots and explore relatively N-poor soil layers, had a greater potential N uptake capacity than the shallow-rooted grasses. Shrubs also showed their highest uptake rates when nitrate was the dominant available form (Fig. 3), which was expected as nitrate is characteristic of deep soil layers exploited by shrub roots. The relative growth rate was similar between plant functional types, suggesting that observed differences in N uptake were the result of physiological characteristics of the N-absorption process and not due to different growth rates, as it has been suggested for other systems (Levang-Brilz and Biondini 2003). N uptake is controlled by the movement of N through the soil to the root surface and by the kinetics of its absorption in root cells (Chapin et al. 2002). The experimental growing conditions of our hydroponic culture, which included a nutrient-rich solution constantly stirred by air bubbling, provided constant N availability on the root surface. Thus, the higher N uptake per unit of root biomass in shrubs compared to grasses must result from a higher shrub affinity for inorganic N (Fig. 3). High N affinity has a high metabolic cost; 30–50 % of the plant's carbon budget goes into supporting nutrient absorption (Chapin et al. 2002). Therefore, a high N affinity can be interpreted as an evolutionary adaptation by a plant species exploring N-poor soil layers. Shrubs show a greater capacity than grasses to absorb N, which is scarce and may be sporadically available while it is moving through the soil profile. On the other hand, grasses explore a soil layer where the N content is relatively large and they have less affinity to N than shrubs.

The average uptake rates for grass species can be ordered as: *P. ligularis* > *P. speciosa* > *P. humilis*, which matches root vertical distribution (Fig. 4; Soriano et al. 1987). Grass species with the highest N uptake have the deepest rooting system, which is a pattern that agrees with the plant functional type rationale described above. On the other hand, the patterns of shrub N uptake rates in our study did not follow their root distribution. The shrub-uptake ranking may be modified by the fact that one of the shrub species, *A. volckmanni*, is a N-fixing species (Golluscio et al. 2006).

Plants growing in infertile soils generally have a high capacity to absorb soil ions with high mobility but have a relatively low capacity to absorb less mobile ions (Aerts and Chapin 2000). The rationale for this behavior is that ion movement through the soil matrix limits the uptake of less mobile ions, while the kinetics of absorption into root cells limits the uptake of mobile ions. Indeed, Jackson and Caldwell (1996) found that root proliferation always led to an increase in the net uptake of phosphate, while higher uptake kinetics was the factor that increased nitrate uptake.

Our study contributes a novel perspective to the understanding of the complementary use of N forms by different plant functional types because it focuses on the preference for nitrate or ammonium, which are by far the dominant N forms available for plant uptake (Schlesinger 1997). On the contrary, previous studies have focussed the complementary use of organic versus inorganic N forms in various environments, from the arctic tundra (McKane et al. 2002) and alpine communities (Miller and Bowman 2003; Raab et al. 1996) to low productivity grasslands (Weigelt et al. 2003, 2005). All of these sites are strongly N limited (Schimel and Bennett 2004), and they have high concentrations of dissolved soil organic N, which are rare among ecosystems across the globe. The only study looking at N-form preferences at a site where dissolved inorganic N is the dominant form of N available for plants did not distinguish between nitrate and ammonium (Harrison et al. 2007). Consequently, this study did not find evidence of species-specific N-form preference. Our results suggest that at sites where the main source of N is inorganic, most of the differences in N-form preference among species occur between nitrate and ammonium.

The results of our study show that, in the Patagonian steppe, grasses which have fibrous and shallow roots are adapted to absorb inorganic N mainly as ammonium, the main inorganic N form in the upper soil (Armas et al. 2008; Yahdjian and Sala 2006), which has limited mobility in the soil profile. In contrast, shrubs that explore deep and N-poor soil layers and have non-fibrous roots have kinetic adaptations to preferentially take up nitrate. Such differences allow for the complementary use of N between grasses and shrubs and suggest a more thorough exploitation of resources by diverse ecosystems than those dominated by just one functional type. The loss of one group or a significant change in its abundance would therefore represent a reduction in resource-use efficiency and ecosystem functioning. For example, overgrazing that usually reduces the grass cover relative to shrubs (Sala and Paruelo 1997) may result in a total reduction in N absorption at the ecosystem level since the ammonium freed by the reduction of grass cover may not be necessarily available for shrubs. From another point of view, the differential preference of inorganic N forms by shrubs and grasses and their different N acquisition strategies (Sala et al. 2012) may constrain the possibility of dramatic shifts in the relative abundance of these two life forms. It should be noted that shrub encroachment, which is a global phenomenon (Eldridge et al. 2011), is rare in the Patagonian steppe. It is possible that deep nitrate availability limits the expansion of shrubs in this ecosystem.

References

Aerts R, Chapin FS III (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:2–45

Armas C, Pugnaire FI, Sala OE (2008) Patch structure dynamics and mechanisms of cyclical succession in a Patagonian Steppe (Argentina). J Arid Environ 72:1552–1561

Bloom AJ, Sukrapanna SS, Warner RL (1992) Root respiration associated with ammonium and nitrate absorption and assimilation by barley. Plant Physiol 99:1294–1301

Britto DT, Kronzucker HJ (2002) NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Chapin FS, Matson PA, Mooney HA (eds) (2002) Principles of terrestrial ecosystem ecology. Springer, New York

Eldridge D, Bowker M, Maestre F, Roger E, Reynolds J, Whitford W (2011) Impacts of shrub encroachment on ecosystem structure and functioning; towards a global synthesis. Ecol Lett 14:709–722

Elser JJ,Bracken MES, Cleland EE, Gruner DS,Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, SmithElser JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142

Golluscio RA, Sala OE (1993) Plant functional types and ecological strategies in Patagonian forbs. J Veg Sci 4:839–846

Golluscio R, Faigón A, Tanke M (2006) Spatial distribution of roots and nodules, and δ^{15}N evidence of nitrogen fixation in *Adesmia volckmanni*, a Patagonian leguminous shrub. J Arid Environ 67:328–335

Harris GA (1977) Root phenology as a factor of competition among grass seedlings. J Range Manag 30:172–177

Harrison KA, Bol R, Bardgett RD (2007) Preferences for different nitrogen forms by coexisting plant species and soil microbes. Ecology 88:989–999

Hoffmann WA, Poorter H (2002) Avoiding bias in calculations of relative growth rate. Ann Bot 90:37–42

Imsande J, Touraine B (1994) N demand and the regulation of nitrate uptake. Plant Physiol 105:3–7

Jackson R, Caldwell M (1996) Integrating resource heterogeneity and plant plasticity: modeling nitrate and phosphate uptake in a patchy soil environment. J Ecol 84:891–903

Jobbagy EG, Jackson RB (2001) The distribution of soil nutrients with depth: global patterns and the imprint of plants. Biogeochemistry 53:51–77

Levang-Brilz N, Biondini ME (2003) Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. Plant Ecol 165:117–144

McKane RB, Grigal DF, Russelle MP (1990) Spatiotemporal differences in ^{15}N uptake and the organization of an old-field plant community. Ecology 71:1126–1132

McKane RB, Johnson LC, Shaver GR,Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE,Kielland K, Kwiatkowski BL, Laundre JA, Murray G (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature 415:68–71

Miller AE, Bowman WD (2003) Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen. Plant Soil 250:283–292

Moore KJ, Moser LE, Vogel KP, Waller SS, Johnson BE, Pedersen JF (1991) Describing and quantifying growth stages of perennial forage grasses. Agron J 83:1073–1077

Nadelhoffer K, Shaver G, Fry B, Giblin A, Johnson L, McKane R (1996) ^{15}N natural abundances and N use by tundra plants. Oecologia 107:386–394

Noy-Meir I (1973) Desert ecosystems: environment and producers. Annu Rev Ecol Syst 4:25–52

Paul EA (2007) Soil microbiology, ecology, and biochemistry, 3rd edn. Academic Press, Burlington

R Development Core Team (2012) R: a language and environment for statistical computing, version: 2.14.2 edn. R Foundation for Statistical Computing, Vienna

Raab TK, Lipson DA, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. Oecologia 108:488–494

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281–2291

Sala OE, Paruelo JM (1997) Ecosystem services in grasslands. In: Daily GC (ed) Nature's services: societal dependence on natural ecosystems. Island Press, Washington D.C., pp 237–252

Sala OE, Golluscio RA, Lauenroth WK, Soriano A (1989) Resource partitioning between shrubs and grasses in the Patagonian Steppe. Oecologia 81:501–505

Sala OE, Lauenroth WK, Golluscio RA (1993) Arid and semiarid plant functional types. In: Smith TM, Shugart HH, Woodward FI (eds) Plant functional types. Cambridge University Press, Cambridge, pp 217–233

Sala OE, Golluscio RA, Lauenroth WK, Roset PA (2012) Contrasting nutrient-capture strategies in shrubs and grasses of a patagonian arid ecosystem. J Arid Environ 82:130–135

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Schimel D, Parton W (1986) Microclimatic controls of nitrogen mineralization and nitrification in shortgrass steppe soils. Plant Soil 93:347–357

Schlesinger WH (1997) Biogeochemistry: an analysis of global change. Academic Press, San Diego

Soriano A, Golluscio RA, Satorre E (1987) Spatial heterogeneity of the root system of grasses in the Patagonian arid steppe. Bull Torrey Bot Club 114:103–108

Soriano A, Sala OE, Perelman SB (1994) Patch structure and dynamics in a patagonian arid steppe. Vegetatio 111:127–135

Ulibarri E (1986) Species of Adesmia of the series Microphyllae (Leguminosae, Papilionoideae). Darwiniana 27:315–388

Weigelt A, King R, Bol R, Bardgett RD (2003) Inter-specific variability in organic nitrogen uptake of three temperate grassland species. J Plant Nutr Soil Sci 166:606–611

Weigelt A, Bol R, Bardgett RD (2005) Preferential uptake of soil nitrogen forms by grassland plant species. Oecologia 142:627–635

Yahdjian L, Sala OE (2006) Vegetation structure constrains primary production response to increased water availability in the Patagonian Steppe. Ecology 87:952–962

Yahdjian L, Sala OE (2010) Size of precipitation pulses controls nitrogen transformation and losses in an arid Patagonian ecosystem. Ecosystems 13:575–585

Yahdjian L, Sala OE, Austin AT (2006) Differential controls of water input on litter decomposition and nitrogen dynamics in the Patagonian Steppe. Ecosystems 9:128–141

Yahdjian L, Gherardi L, Sala OE (2011) Nitrogen limitation in arid-subhumid ecosystems: a meta-analysis of fertilization studies. J Arid Environ 75:675–680