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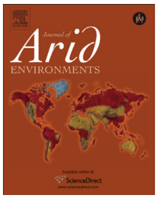

Short communication

Grasses have larger response than shrubs to increased nitrogen availability: A fertilization experiment in the Patagonian steppe

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ABSTRACT

Nitrogen limits plant growth in almost all terrestrial ecosystems, even in low-precipitation ecosystems. Vegetation in arid ecosystems is usually composed of two dominant plant-functional types, grasses and shrubs, which have different rooting and water acquisition patterns. These plant-functional types may respond differently to N availability because they have different strategies to absorb and retranslocate N. We hypothesized that grasses are more N limited than shrubs, and consequently will show higher responses to N addition. To test this hypothesis, we added 50 kg N ha^{-1} $year^{-1}$ as NH_4NO_3 during two years in the Patagonian steppe, Argentina, and we evaluated the responses of aboveground net primary production and N concentration of green leaves of the dominant grass and shrub species. Grass biomass significantly ($P = 0.007$) increased with increased N availability whereas shrub biomass did not change after two years of N addition. Shrubs have higher nitrogen concentration in green leaves than grasses, particularly the leguminous *Adesmia volkmanni*, and showed no response to N addition whereas foliar N concentration of grasses significantly increased with N fertilization ($P < 0.05$). Grasses may have a larger response to increase N availability than shrubs because they have a more open N economy absorbing up to 30% of their annual requirement from the soil. In contrast, shrubs have a closer N cycle, absorbing between 7 and 16% of their annual N requirement from the soil. Consequently shrubs depend less on soil N availability and are less responsive to increases in soil N.

Nitrogen limits plant growth in almost all terrestrial ecosystems (LeBauer and Treseder, 2008; Vitousek and Howarth, 1991), even in low-precipitation ecosystems such as deserts and steppes (Yahdjian et al., 2011). In a recent meta-analysis of 68 N-fertilization experiments in arid to sub-humid ecosystems, nitrogen addition increased aboveground net primary production across all studies by 50%, with increasing absolute responses along a range of 50–650 mm of annual precipitation (Yahdjian et al., 2011).

In arid ecosystems, N availability for plants and microbes is quite dynamic and changes from year to year as a response to changes in water availability and N deposition. Water availability affects N availability by differentially altering two processes, N immobilization and N mineralization. Whereas plant immobilization is sensitive to water availability, mineralization is not, and consequently in some arid ecosystems mineral soil N content decreases as water availability increases (Reichmann et al., 2013; Yahdjian et al., 2006). Independently, N deposition is expected to increase worldwide, even in arid regions where long-term trends have already shown increasing annual rates of atmospheric N inputs (Báez et al., 2007).

Although we have a good understanding of the dynamics of N availability in arid soils, we still have an incomplete picture of the response of different plant species and plant-functional types to changes in N availability. The dominant plant-functional types of arid ecosystems are grasses and shrubs that may respond differently to N availability because they have different strategies to absorb and retain N (Aerts and Chapin, 2000; Gherardi et al., in press; Sala et al., 2012). However, of the 68 experiments assessing the effect of nitrogen fertilization on productivity included in a recent meta-analysis, very few distinguished the response to N fertilization among plant life forms or functional types (Yahdjian et al., 2011).

Grasses and shrubs may have a different response to N availability because they have different rooting patterns, growth rates, and water (Sala et al., 1989) and nutrient capture strategies (Sala

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et al., 2012). Grasses have shallow and finely ramified roots that allow for thorough exploitation of relatively nutrient-rich soil layers, whereas shrubs have deep roots with few ramifications and explore relatively nutrient-poor layers (Jackson et al., 1996). In the Patagonian steppe, grasses absorb N predominantly from upper soil layers and shrubs from lower soil layers, mirroring globally described patterns of root distributions of shrubs and grasses (Jackson et al., 1996). Although their root patterns are different, both grasses and shrubs in the Patagonian steppe achieve similar N-use efficiencies because of contrasting patterns in N productivity and N residence time (Sala et al., 2012). Grasses have higher N productivity and lower N residence time than shrubs (Sala et al., 2012). Additionally, shrubs show higher N-absorption capacity than grasses, and both plant-functional types show opposite preference for inorganic N forms, strategies that maximize N uptake (Gherardi et al., in press). Grasses prefer ammonium over nitrate and have relatively low N-uptake capacity whereas shrubs prefer nitrate over ammonium and have relatively high N-uptake capacity (Gherardi et al., in press). Although there are many differences among plant-functional types in the N cycle, we do not know if these two contrasting plant functional types would show the same response to increased nitrogen availability.

In addition to the biomass response, plants may increase foliar N as a response to N addition (Drenovsky and Richards, 2004). Most studies examining nutrient use or nutrient response efficiencies in relation to soil fertility compare different sites that differ in many aspects such as annual precipitation, annual temperature, and soil texture (Knops et al., 1997; Yuan et al., 2005). Here we examined, in a field experiment, the differential response to nitrogen availability of grasses and shrubs. The specific hypothesis that guided our study was that grasses are more frequently N limited than shrubs because grasses have a more open N economy and a lower N-absorption capacity than shrubs.

To test this hypothesis, we performed a nitrogen fertilization experiment during two years in the Patagonian steppe in the south of Argentina, and we evaluated the response regarding aboveground net primary production and foliar N concentration of green leaves of the dominant grass and shrub species.

The study was carried out in an arid ecosystem with predominantly winter precipitation and dominated by C_3 species. The site is located near Río Mayo, Chubut (45° 41′ S, 70° 16′ W), in the Patagonian region of Argentina, where mean monthly temperatures range from 1 °C in July to 15 °C in January, and mean annual rainfall recorded over the last 23 years was 174 mm and ranged between 98 and 275 mm. Precipitation is mainly rainfall concentrated during fall and winter periods (March–September). Soils are coarse textured with pebbles, which account for 47% of its weight, and have a cemented calcareous layer at a depth of about 0.4 m (Paruelo et al., 1988). Dominant plant species evaluated in this study account for 96% of aboveground net primary production. Tussock grasses have a basal cover of 25% and are represented mainly by *Pappostipa speciosa* Trin et Ruprecht (ex *Stipa speciosa*), *Pappostipa humilis* Cav. (ex *Stipa humilis*), *Poa ligularis* Nees ap. Steud, and with lower cover *Bromus pictus*. Shrubs cover 12% of the area and are represented mainly by the deciduous species *Mulinum spinosum* (Cav.) Pers. and *Adesmia volkmanni* Philippi (ex- *Adesmia campestris*), and the evergreen *Senecio filaginoides* DC (Golluscio et al., 1982).

We increased nitrogen availability in plots of natural vegetation by adding 50 kg N ha^{-1} $year^{-1}$ during two years. We established 20 plots of 9 m^2 each, to guarantee the presence of the dominant shrub and grass species in each plot and randomly assigned plots to fertilized and control treatments ($N = 10$). At the beginning of the experiment, plant cover per species estimated with the line-intercept method was similar between treatments ($P > 0.05$). Fertilizer was applied as NH_4NO_3 diluted in 2 l of water (equivalent to a 0.22 mm rain pulse) uniformly distributed on the soil surface with a sprayer in three application events during the growing season, in October, December, and January (or the equivalent to 0.66 mm/yr). Non-fertilized plots received 0.66 mm/yr of water only, applied with the same protocol.

We evaluated treatment effects after two years by measuring vegetation cover by species at peak biomass which is a good estimator of aboveground net primary production (ANPP) in ecosystems with pronounced seasonality (Sala and Austin, 2000). We transformed plant cover per species into biomass employing specific allometric-calibration curves constructed for this study site (Flombaum and Sala, 2007). We estimated vegetation cover using the line-intercept method, recording the crowns that intercepted the line to the nearest 1 cm. We installed two parallel 3-m lines in each plot, 1 m apart from each other and from the border of the plot, and we determined the percent cover of the plant community at the peak biomass of the second experimental year in each plot. We averaged the percent cover of the two lines to obtain one value per plot.

To assess foliar N concentration of green leaves of the dominant grasses and shrubs, we sampled several leaves of each species in each plot in January of the second year, during the vegetative growth peak. We collected leaves from individuals near the center of the plot. The species sampled were all present in each plot, except *B. pictus* that was absent in two plots of each treatment. Leaves were kept refrigerated until return to the laboratory, where they were ground and analyzed for N with an elemental analyzer TrueSpec-LECO (LECO®, St. Joseph, Mich., USA).

We sampled soil inorganic nitrogen in bare soil and vegetation patches to a depth of 10 cm. We sieved fresh soils to pass a 2-mm mesh, and we extracted a subsample of 10 g of soil in 50 ml 2 M KCl for determination of inorganic nitrogen (NH_4^+ and NO_3^-). We kept soil samples refrigerated until return to the laboratory and filtered extracts as soon as possible. We analyzed soil extracts using an Alpkem® autoanalyzer (O-I Corporation, College Station, Texas, USA), which uses a colorimetric analysis of inorganic nitrogen in liquid extracts. We placed a subsample of soil in a drying oven at 105 °C for 48 h for determination of gravimetric soil water content. We corrected soil nitrogen concentrations for soil water content. Soil inorganic nitrogen increased significantly ($P < 0.001$) with fertilization. On average, total soil inorganic nitrogen in the first 10 cm of the soil profile was 5.36 ± 0.2 and 43.6 ± 8.9 µg/g dry soil in control and in fertilized plots respectively during the two experimental years ($n = 10$).

We analyzed N fertilization effect on aboveground net primary production and foliar N concentration with a nested one-way ANOVA, with species nested into plant-functional types. Data were log transformed when necessary to accomplish statistical analysis assumptions. All statistical analyses were performed using PROC GLM in SAS version 6.12 packages (SAS Institute, Cary, NC, USA). Significance was assumed at $P < 0.05$.

Grasses and shrubs showed different responses to N addition. Grass ANPP significantly ($F_{1,4} = 20.20$; $P = 0.007$) increased with N availability increase, whereas shrub ANPP did not change after two years of N addition (Fig. 1, inset). Among grass species, *P. humilis* and *B. pictus* were the most responsive (Fig. 1). None of the shrub species evaluated showed a significant response to N addition (Fig. 1).

Leaf N concentration significantly ($F_{1,3} = 204.3$; $P = 0.001$) increased as a result of N fertilization in all grass species (Fig. 2) but did not change for shrubs ($F_{1,2} = 6.2$; $P > 0.05$) with the exception of *S. filaginoides*, which is the shrub with lowest leaf N concentration (Fig. 2). Foliar N responses were significantly higher for grasses than shrubs ($F_{1,4} = 19.30$; $P = 0.01$; Fig. 2, inset). Differences among grass species were not statistically significant ($P > 0.05$). Shrubs from the Patagonian steppe have higher foliar nitrogen

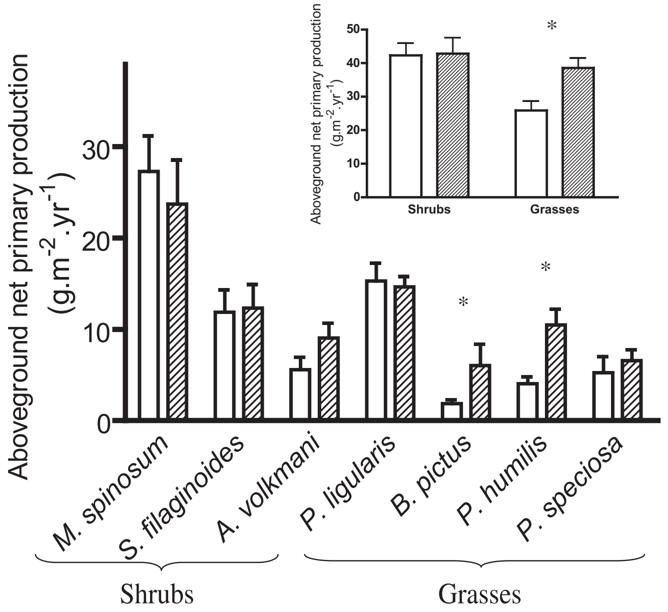

Fig. 1. Aboveground net primary production of shrub and grass functional types (inset) and species in control and fertilized plots, after the second year of N addition. Bars represent mean biomass in plots of natural vegetation that have been fertilized with NH_4NO_3 during two years (dashed bars) and in control plots (empty bars) (+1 SE, $n = 10$). (*) means significant differences between treatments at $P < 0.05$.

concentration in green leaves than grasses ($F_{1,4} = 18.33$; $P = 0.001$), particularly *A. volkmanni*, a leguminous shrub (Fig. 2). *S. filaginoides* showed the lowest foliar N concentration of shrubs and similar to that of grasses (Fig. 2). Foliar N responses to N addition were inversely related to the N concentration of green leaves in the controls ($P = 0.07$).

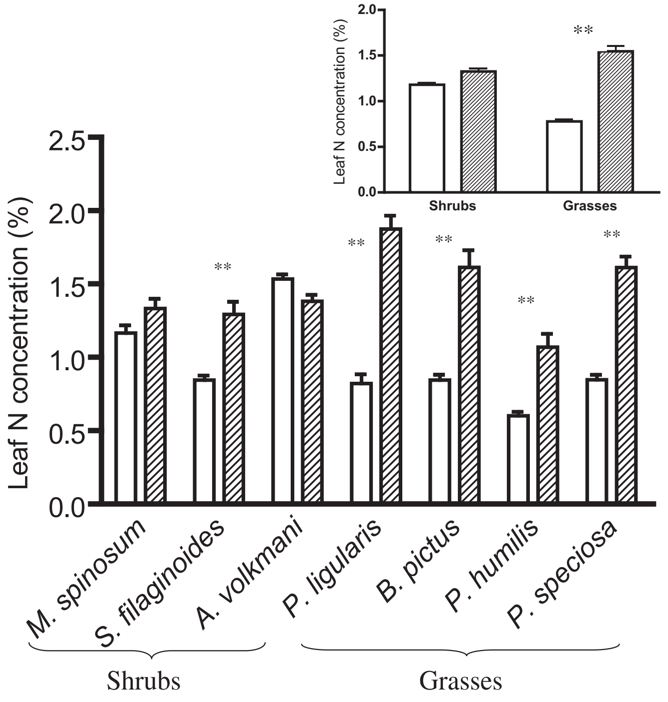

Fig. 2. Nitrogen concentration of green leaves of shrub and grass functional types (inset) and species in control and fertilized plots, after the second year of N addition. Bars represent mean values in plots of natural vegetation that have been fertilized with NH_4NO_3 during two years (dashed bars) and in control plots (empty bars) (+1 SE for $n = 10$). (**) means significant differences between treatments at $P < 0.001$.

Our results support our hypothesis stating that grasses are more N limited than shrubs in the arid Patagonian steppe. This was evident by grass biomass increment with N fertilization that was not observed for shrubs. Nitrogen limitation of vegetation production in arid and semiarid ecosystems seems a widespread phenomenon (Yahdjian et al., 2011), and based on our results we can hypothesize that this is as a result of grass N limitation.

The lack of shrub response to N fertilization may be related to the nature of their relatively close N economy compared to grasses (Sala et al., 2012). Shrubs in the Patagonian steppe absorb annually between 7 and 16% of their annual N requirement with the rest being internally retranslocated. In contrast, grasses have a relatively open N economy and annually absorb from the soil 30% of their N requirements (Sala et al., 2012). Species adapted to low nutrient conditions are physiologically less capable of responding to increased nutrient availability due to their low relative growth rate and lower tissue nutrient requirements (Aerts and Chapin, 2000). Also, many perennial desert shrubs are slow-growing, having strategies adapted to low soil moisture and poor nutrient conditions (Aerts and Chapin, 2000). Shrubs in the Patagonian steppe not only have lower N uptake per year and per unit of biomass than grasses but some of them also fix N episodically (Golluscio et al., 2006).

Within the grass functional group, the differences in N responses among species (from a 60% biomass increase for *B. pictus* to no changes for *P. ligularis*, $F_{1,3} = 2.96$; $P = 0.04$) were higher than differences among shrubs (no significant changes, $P > 0.05$). The two more responsive species were *B. pictus* and *P. humilis*, which are in both extremes of the relative growth rates, being *B. pictus* the grass species with highest relative growth rate and drought plasticity (Couso and Fernández, 2012).

Grass response to N fertilization in the Chihuahuan desert was different from that in the Patagonian steppe since there was no response in productivity to N additions in the Chihuahuan system although grasses in both ecosystems showed a response in foliar N (Reichmann et al., 2013). Shrubs in both locations, Chihuahuan desert and Patagonian steppe, showed no response to fertilization. The differential grass response between these two arid ecosystems highlights the complexity of the issue of plant-functional response to N availability.

Values of foliar N concentration in green leaves of plant species in the present study were similar to values reported for the same site in previous studies (Sala et al., 1989, 2012). Shrub foliar N concentration in our study (1.14%) was lower than the mean N of shrubs from 11 desert ecosystems (2.2%) (Killingbeck and Whitford, 1996). However, N concentration in green leaves of Patagonian shrubs were similar to *Artemisia tridentata* (1.3%) (Schlesinger et al., 1989), a shrub from the Great Basin, which is a desert with climatic similarities to the Patagonian steppe. Among shrubs, *S. filaginoides* the only evergreen shrub, showed the lowest values which is in accordance with the general pattern of lower N concentration in leaves of perennials compared with deciduous species (Aerts and Chapin, 2000).

Grasses and shrubs represent two distinct functional types in arid ecosystems, with different rooting patterns and nutrient use strategies. Here, we demonstrated that these plant functional types are also differently limited by nitrogen and respond differently to nitrogen availability in terms of productivity and foliar nitrogen concentration. Grasses are more N limited than shrubs and respond to N addition increasing its biomass and its foliar N concentration. The different responses of grasses and shrubs to N addition may cause changes in the distribution of these functional types as a consequence of future increases in availability of reactive N resulting from global N deposition or reduced water availability. This in turn may have consequences for rangelands management and trophic interactions.

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