



## Short communication

## Spatial heterogeneity provides organic matter refuges for soil microbial activity in the Patagonian steppe, Argentina

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## ABSTRACT

In arid and semi-arid ecosystems that are frequently classified as water limited, it is unclear how spatial and temporal variability of vegetation and climate could affect microbially-mediated soil processes. Our objective was to determine how aboveground spatial heterogeneity creates characteristic soil conditions that modulate microbial growth and activity in a semi-arid Patagonian steppe. In particular, we explored how micro-environmental and biogeochemical soil characteristics generated by the native vegetation could control soil  $\beta$ -glucosidase activity. Both life-form (shrubs, grasses, mosses and bare soil) and season exerted strong controls on all measured abiotic (soil temperature and gravimetric soil water content, inorganic nitrogen, pH and total C and N) and biotic (microbial biomass C and  $\beta$ -glucosidase activity) soil characteristics. Partial correlation between  $\beta$ -glucosidase activity and extracellular organic C (EOC) was high across seasons ( $r = 0.5$ ;  $P < 0.001$ ) while soil water content did not correlate with soil enzymatic activity ( $r = 0.09$ ;  $P > 0.05$ ). We postulate that labile soil carbon rather than water availability functions as a principal limitation of microbial activity in this semi-arid ecosystem, and the distribution of this carbon is, in large part, determined by the patchy distribution of vegetation.

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Above- and belowground communities are inextricably linked in terrestrial ecosystems; belowground biological processes are modulated by both the quantity and quality of organic material that enters the soil pool from net primary production (Wardle, 2002). In arid and semi-arid ecosystems, vegetation is typically dispersed in a matrix of bare soil creating high resource microsites associated with vegetated patches known as “islands of fertility” (Charley and West, 1975), which have been well-documented in deserts (Schade and Hobbie, 2005; Schaeffer and Evans, 2005). In addition, greater C-degrading and nutrient mineralization enzyme activity under vegetated patches and showing seasonal fluctuations have been reported in a number of low rainfall ecosystems (Pavel et al., 2004; Thompson et al., 2006; Goberna et al., 2007).

Water availability is clearly one of the major limitations of net primary production in semi-arid ecosystems (Austin and Sala, 2002; Muldavin et al., 2008). However, the magnitude of the

water pulse that stimulates microbially-mediated process greatly differs from the ones for vascular plants. Small and short pulses could stimulate soil microbial activity but have no effect on germination of many desert plants (Schwinning and Sala, 2004). The temporal distribution of rainfall, both seasonal variation and size of rainfall events, can regulate microbial processes (Austin et al., 2004). The other dominant regulator of soil microbial processes is temperature, which also has a strong temporal dimension in deserts affecting microbial activity (Fierer et al., 2003) and microbial community composition (Cookson et al., 2007). It is unclear how the constraints of water availability and temperature interact with vegetation patches to determine soil ecosystem processes.

Our objective in this study was to determine how aboveground spatial and temporal heterogeneity generated by the presence of vegetation patches could create characteristic conditions that control soil  $\beta$ -glucosidase activity. We hypothesized that indirect effects of low water availability (low organic matter inputs) may be more important than direct regulation of biological processes as a control on soil microbial activity.

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The study was conducted in a semi-arid steppe (1972 grazing enclosure), in the Río Mayo experimental station of INTA (Instituto Nacional de Tecnología Agropecuaria) (45°41'S and 70°16'W). Mean monthly temperature ranges from 1 °C (July) to 15 °C (January). Precipitation (150 mm) falls primarily in winter (Paruelo and Sala, 1995). Soils are coarse-textured aridisols (Valle, 1998). We established 4 microsites: shrubs ( $n = 18$ ) (*Mulinum spinosum* (Cav.) Pers., *Adesmia volckmanni* Phil. and *Senecio filaginoides*), grasses ( $n = 18$ ) (*Poa ligularis*, *Stipa speciosa*, and *Bromus pictus*), below moss patches ( $n = 6$ ) and in bare soil ( $n = 6$ ). We collected soil (0–5 cm) under each of these microsites with the exception of the moss microsite (0–2 cm) in September 2003 (spring), January 2004 (summer) and May 2004 (fall). We installed 12 plots (5 × 5 m) with four transects of 5 m for vegetative cover measurements. Percent vegetative cover in the study area was calculated using a line-intercept technique (Canfield, 1941).

Soils extracts were analyzed for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  using an Alpkem Flow IV® autoanalyzer (O-I Corporation, College Station, TX, USA) (Robertson et al., 1999). Total C and N were determined with an elemental analyzer (LECO) (Robertson et al., 1999). Soil temperature was measured at 10, 12 and 16 h integrating 10 cm depth. Microbial biomass C was measured using a modified chloroform fumigation–extraction technique (Jenkinson and Powlson, 1976), using a cylindrical piece of mesh in order to improve the diffusion efficiency of the chloroform. The C concentration in the unfumigated sample (0.5 M  $\text{K}_2\text{SO}_4$ ), represents the most labile organic matter fraction in the soil, termed extracellular organic C (EOC) (Hofman and Dusek, 2003). The 1–4  $\beta$ -glucosidase activity was assayed using p-nitrophenyl- $\beta$ -glucopyranoside as a substrate dissolved in acetate buffer (pH 5). The reaction was stopped with 0.2 ml of NaOH (0.1 N) (Sinsabaugh et al., 1999). Enzyme activity and microbial biomass were determined within 15 days of sampling.

We evaluated differences in abiotic and biotic characteristics of microsites with two-way ANOVA (log transformed in the case of non-normal distributions). We used a non-parametric Kruskal–Wallis test for total soil C and N data that could not be normalised. Statistical analyses were performed using Statistica 6 software (Stat-soft, Inc., Tulsa, OK, USA). Pearson partial and multiple correlation coefficients were used to evaluate the degree of association between  $\beta$ -glucosidase activity and the other variables for each season and across all seasons. We calculated multiple correlation coefficients and the fifth order partial correlation coefficient between  $\beta$ -glucosidase and each variable named in Table 2 (Sokal and Rohlf, 1995) using a numerical Python script (Ascher et al., 2001) that performed the algebraic calculations over the correlation matrix.

Microsites generated by the different life-forms varied significantly and exerted a strong and significant control on abiotic soil characteristics (Table 1, Figs. 1 and 2). Bare soil had 50% or less carbon and nitrogen than the other microsites (Table 1). Microclimatic soil variables, including thermal amplitude and soil water content showed a significant season–life-form interaction with the exception of pH (Fig. 1). Thermal amplitude was most marked in

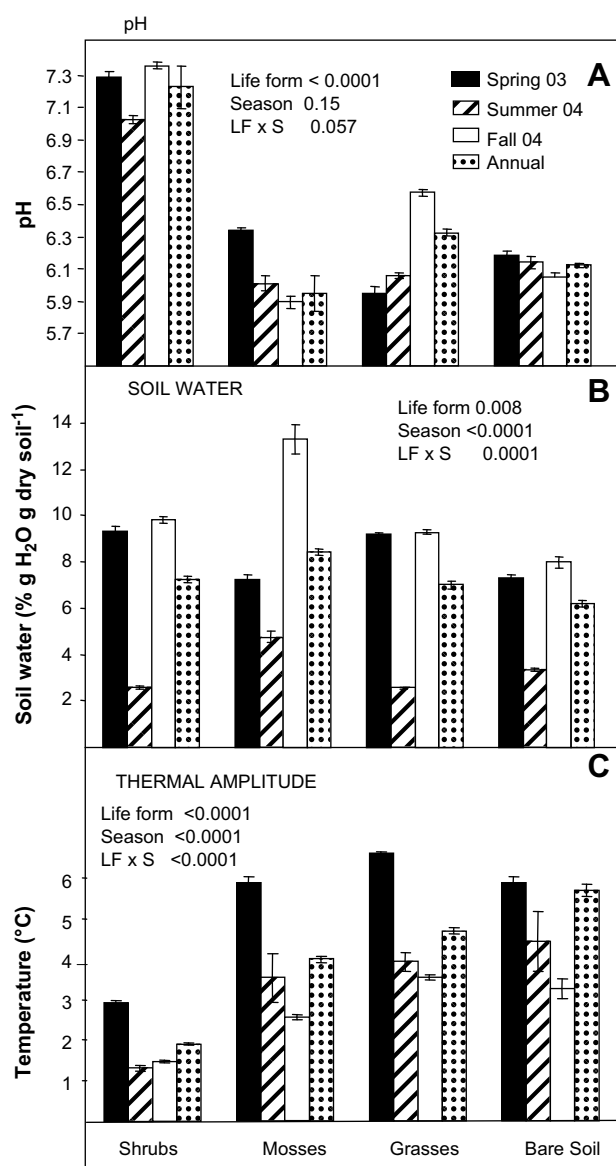


Fig. 1. pH, water content and thermal amplitude in soils under different life-forms for three sampling dates and the annual average. Thermal amplitude was defined as the difference between the maximum and minimum temperature registered during the day. Results for two-way ANOVA of life-form and season are shown. Bars indicate means (+SE), and an alpha of 0.05 was used in all cases.

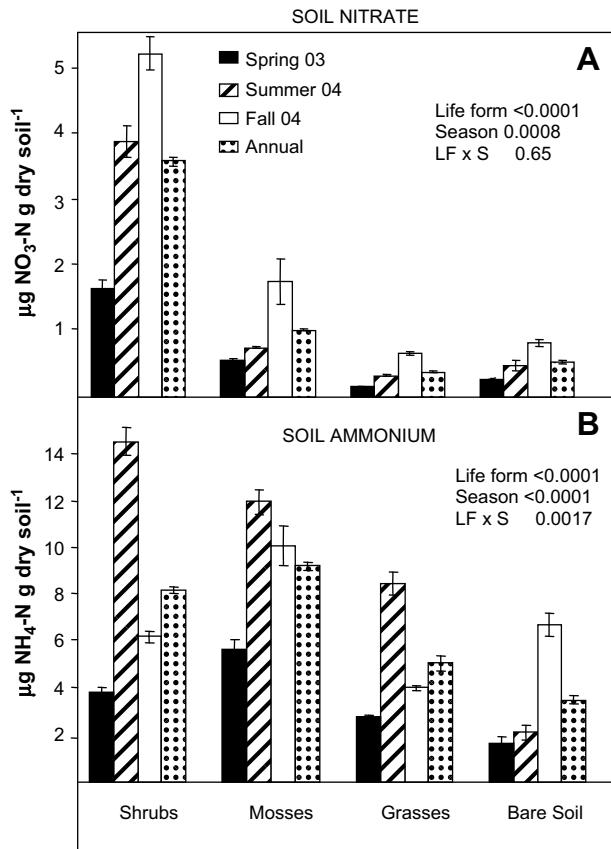
spring, and the presence of shrubs appeared to buffer temperature extremes (Fig. 1C). Soil nitrate under shrubs was approximately 3-fold greater than in the rest of the life-forms ( $P < 0.001$ , Fig. 2A), consistently across seasons. By contrast, soil ammonium showed peaks of high concentration under particular life-forms depending on the season (Fig. 2B).

Soil microbial biomass C demonstrated both seasonal and spatial variation, except for the moss microsite that was constant and elevated throughout the year (Fig. 3A). EOC was greater beneath shrubs in all seasons, but additionally, there were pulses of EOC in the moss microsites in summer and fall (Fig. 3B).  $\beta$ -Glucosidase activity in soils under shrubs was greatest, and was similar to the moss microsite throughout the year ( $P < 0.05$ , Fig. 3C); fall was the season with lowest  $\beta$ -glucosidase activity across microsites (Fig. 3C). Bare soil showed minimum values of enzymatic activity

Table 1

Means (SE) for vegetation and bare soil cover and total soil C, N, and C/N ratios for all soil microsites in the semi-arid Patagonian steppe. Different letters indicate significant differences ( $P < 0.05$ ) among soils collected under dominant shrub and grasses species, mosses and bare soil (Kruskal–Wallis test).

Life-form	% Cover	n	Soil %C	Soil %N	Soil C/N ratio
Shrubs	11.8	18	1.4 (0.04) <sup>a</sup>	0.11 (0.002) <sup>a</sup>	12.2 (0.11) <sup>a</sup>
Grasses	16	18	0.7 (0.01) <sup>bc</sup>	0.06 (0.008) <sup>bc</sup>	9.9 (0.06) <sup>bc</sup>
Mosses	1.4	6	0.8 (0.04) <sup>ac</sup>	0.06 (0.003) <sup>ac</sup>	12.4 (0.30) <sup>ac</sup>
Bare soil	51.4	6	0.4 (0.01) <sup>b</sup>	0.04 (0.001) <sup>b</sup>	9.6 (0.32) <sup>b</sup>

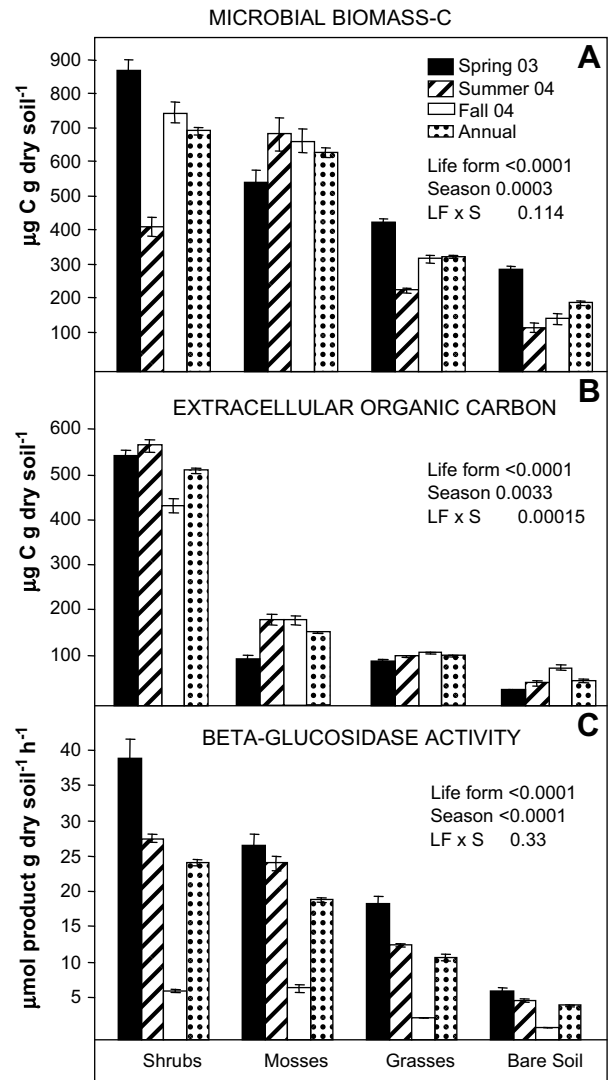


**Fig. 2.** Nitrate and ammonium concentration in soils under different life-forms for three sampling dates and the annual average. Results for two-way ANOVA of life-form and season are shown. Bars indicate means (+SE), and an alpha of 0.05 was used in all cases.

and was significantly different from all other microsites except for the grasses microsite in fall ( $P < 0.05$ , Fig. 3C).

Both seasonal climatic fluctuations and the vegetation matrix affected microbial activity, and it appears that the control on  $\beta$ -glucosidase activity stems from the direct effect of vegetation patches on soil organic matter and the modulation of the abiotic and biotic characteristics in these soil microsites (Table 1, Fig. 3). Although there were significant differences in soil water content among microsites and seasons (Fig. 1), soil water did not correlate with  $\beta$ -glucosidase activity in any season when partial correlations were considered (Table 2). Moreover, across all the seasons, EOC showed the greatest partial correlation with  $\beta$ -glucosidase activity (Table 2), suggesting labile C limitation for  $\beta$ -glucosidase activity. These results coincide with the idea that microbial lysis during rewetting events may be a principal energy source for microbial activity in low rainfall ecosystems (Austin et al., 2004) and that low bioavailability of C may be a major control on microbial activity (Nadeau et al., 2007).

These results contrast with other studies that have shown positive correlations between water availability and enzymatic activity in semi-arid conditions conducted at the plot scale, when microsites were not considered (Sardans and Peñuelas, 2005; Bastida et al., 2008), but other studies have demonstrated that water pulse size did not predict rates or duration of soil respiration pulses (Sponseller, 2007) or that changes in precipitation input did not affect annual soil net N mineralization (Yahdjian et al., 2006). The discrepancies in these studies



**Fig. 3.** Microbial biomass, extracellular organic carbon and  $\beta$ -glucosidase activity in soils under different life-forms for three sampling dates and the annual average. Results for two-way ANOVA of life-form and season are shown. Bars indicate means (+SE), and an alpha of 0.05 was used in all cases.

highlight a need to broaden our understanding of the interaction of water availability with other ecosystem characteristics, which appear to play important roles in the modulation of enzymatic activity.

In the Patagonian steppe, organic matter inputs are determined by spatially and temporally heterogeneous net primary production (Jobbágy and Sala, 2000). At the same time, aboveground carbon loss appears to be driven, in part, by abiotic losses due to photo-degradation (Austin and Vivanco, 2006). Shrub patches do appear to be a hotspot for microbial activity, but due to buffered environmental conditions and protection of organic matter in 'refuges' for microbial activity, and not due to differences in soil water availability. It appears that the capacity to respond to changing soil moisture is highly constrained by other variables in this ecosystem, which stems from the spatial distribution of carbon and nutrient resources. As such, the dependence of biogeochemical cycles in semi-arid ecosystems on spatial distribution of the vegetation suggests that any activity that modifies spatial heterogeneity could have quantitative effects on ecosystem functioning (Aguar and Sala, 1999).

**Table 2**

Correlation and partial correlation coefficients between  $\beta$ -glucosidase activity and soil characteristics from different microsites. Significant differences (after Bonferroni correction for 6 comparisons) marked with asterisks as follows: \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ).

	Spring 2003		Summer 2004		Fall 2004		Three seasons	
	Correlation	Partial correlation	Correlation	Partial correlation	Correlation	Partial correlation	Correlation	Partial correlation
EOC	0.75***	0.45**	0.57***	-0.26	0.70***	0.20*	0.52***	0.50***
Microbial biomass C	0.68***	0.29	0.26	-0.01	0.57***	0.06	0.40***	0.29***
N extractable	0.54***	0.46**	0.49***	0.39**	0.67***	0.13	0.17*	-0.08*
Temperature	-0.64***	0.02	-0.62***	-0.27	-0.50**	0.08	0.14	0.27***
Thermal amplitude	-0.68***	-0.13	-0.69***	-0.35*	-0.60***	0.01	-0.10	0.21*
Soil water content	0.26	0.32	0.05	0.25	0.31*	0.06	-0.08	0.09

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