REGULAR ARTICLE

Do soil enzymes respond to long-term grazing in an arid ecosystem?

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Abstract

Background and aims Our objective was to assess the effects of long-term continuous grazing on soil enzyme activities in relation to shifts in plant litter attributes and soil resources in an arid ecosystem, considering both spatial and temporal variations.

Methods We randomly extracted soil samples with the respective litter cover at 5 modal size plant-covered patches (PCP) and the nearest inter-canopy areas (IC) at Patagonian Monte sites with low, medium and high grazing intensity in winter and summer from 2007 to 2009. We analyzed enzyme activities (dehydrogenase, ß-glucosidase, protease, alkaline and acid phosphatase), microbial biomass-C, organic-C, total soil-N, and moisture in soil and mass and quality in plant litter. We assessed faeces density and plant cover in the field.

Results and conclusions Grazing led to reduced grass cover, decreasing plant litter mass with increasing soluble phenolics, and reduced phosphatases, ß-glucosidase

and microbial biomass-C at PCP. A localized nutrient input from animal excreta seems to promote microbial biomass-C, alkaline phosphatase and dehydrogenase activities but only at IC from the site with high grazing intensity. Plant heterogeneous distribution, plant litter quantity and quality, nutrient inputs from grazers and seasonal variation in soil moisture, also affecting soil resources and microbial biomass, modulate soil enzyme responses to long-term grazing in the arid Patagonian Monte.

Keywords Arid ecosystems · Grazing · Plant litter · Soil enzymes · Microbial biomass

Abbreviations

PCP	Modal size plant-covered patches
IC	Inter-canopy areas
L	Low grazing intensity
M	Medium grazing intensity
H	High grazing intensity

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Introduction

Soil enzymes catalyze the breakdown of complex molecules into simpler forms easily assimilable for plants and microorganisms. As a consequence of their importance to preserve soil fertility, soil enzyme activities have been used as soil quality indicators (Caldwell

2005; Burns et al. 2013). Extracellular hydrolases are the enzymes frequently selected for soil studies. Among them, alkaline and acid phosphatases catalyze the transformation of organic phosphorous into inorganic forms available for plants and microorganisms. Proteases hydrolyze peptide bounds rendering peptides and/or amino acids. Protein degradation is associated with ammonium release, a nitrogen source for plants and microorganisms (Alef and Kleiner 1986; Alkorta et al. 2003). β-glucosidase acts in the decomposition of cellulose and other polysaccharides from plant litter, producing glucose which constitutes an energy and carbon source for microbial growth (Tabatabai 1994). In contrast to the extracellular soil enzymes, dehydrogenases are intracellular enzymes involved in oxidative processes of microbial viable cells, therefore its activity is considered a measure of the overall soil microbial activity (Nannipieri et al. 2002). Soil enzyme activities are recognized as sensible and early indicators of soil perturbations resulting from management practices, in part because soil microorganisms which also respond rapidly to environmental changes are the main source of such enzymes (Dick et al. 1996; Blank 2004; Schaller 2009).

The amount and types of organic compounds affect the production of extracellular microbial enzymes by mechanisms not fully understood, but including regulation by the presence of the enzyme substrate (substrate stimulation model) and production of enzymes to acquire limiting nutrients after addition of other limiting resources (resource limitation model), (Hernández and Hobbie 2010). Domestic herbivores usually lead to shifting in plant species composition promoting the dominance of plant species with low nutrient and high secondary defense metabolite contents in tissues which produce leaf litter with low quality (Wardle et al. 2004). On the other hand, nutrients return into soils in the form of animal urine and faeces (Haynes and Williams 1993). Therefore, herbivores may alter nutrient distribution and flow in the ecosystem also affecting soil microbial communities and enzyme activities. However, the knowledge about grazing effects on soil biochemistry and microbial communities is limited, particularly for arid and semiarid regions even though many of them are or have been used for domestic grazer production (Whitford 2002).

There are discrepancies in the results on soil enzyme responses to grazing. Fterich et al. (2012) found a diminution of dehydrogenase, β-glucosidase and acid phosphatase with increasing stocking rates in arid grazed areas of Tunisia, possibly explained by the land degradation and reduced inputs of organic matter and C into the soil. Acosta-Martínez et al. (2010) found higher phosphatase and β-glucosaminidase activities in nongrazed than in grazed pastures and in an integrated cropping-livestock system probably associated with changes in soil P, C, and N cycling; while other enzyme activities such as β-glucosidase and α-galactosidase were not affected by grazing. Higher catalase, urease, and alkaline phosphatase activities were observed in the surface soil after 10-year grazing exclusion compared to continuous grazed areas, indicating that biological activity was improved after livestock exclusion probably due to an increase in the soil organic matter (Yong-Zhong et al. 2005). In contrast, other findings in arid and semiarid ecosystems showed that grazing only sometimes induced a reduction of soil enzyme activities (e.g. urease, asparaginase, alkaline phosphatase, peptidase and/or amidase activities) and attributed enzyme responses to other factors as differences among sites, microsites, and soil depths (Holt 1997; Blank 2004). Since multiple variables, such as environmental conditions (particularly water availability), litter quality and quantity, grazing intensity, and the inherent soil properties may affect soil biochemistry, efforts are needed for a better understanding of how these factors interacts to shape soil enzyme and microbial responses to grazing in arid ecosystems.

In Patagonia, sheep are the predominant domestic herbivores and were introduced at the beginning of the 1900's (Bertiller et al. 2002). Grazing in the Patagonian Monte induces the reduction of grass and total plant covers, shifts in plant species composition and replacements between and within plant life forms, especially the replacement of perennial grasses by evergreen shrubs with high concentration of chemical defenses (Bisigato and Bertiller 1997; Carrera et al. 2008). These changes lead to a reduction in the quantity and quality of plant litter with consequences for decomposition/mineralization processes and soil organic matter dynamics (Carrera et al. 2008; Campanella and Bisigato 2010; Carrera and Bertiller 2013). Plant litter with high concentrations of lignin and soluble phenols may slow down decomposition processes (Vargas et al. 2006; Carrera and Bertiller 2013) and reducing C and N pools in soils (Golluscio et al. 2009).

Although there is consensus on the value of soil enzymes as early indicators of soil disturbances, little is known about soil enzyme performance in chronically

disturbed ecosystems, such as those with a long grazing history. Our previous work in the Patagonian Monte showed that grazing mostly affected soil physicochemical attributes and enzyme activities through direct and indirect effects of trampling and urine and changes in perennial grass cover but these insights were observed only in one date (Prieto et al. 2011). The effect of changes in perennial grass cover on enzyme activities suggests a link between plant litter variations and soil enzyme activities that we further explored in this paper in addition to the effect of seasonal variations as determinants of soil enzyme activity. Our objective was to assess the effects of long-term continuous grazing on soil enzyme activities in relation to shifts in plant litter attributes and soil resources in an arid ecosystem, considering both spatial and temporal variations. We hypothesized that long-term grazing reduces the plant litter quantity and quality and the availability of soil resources, resulting in a decrease of soil enzyme activities related with microbial activity and nutrient cycling mainly during the drought period.

Materials and methods

Study site and sampling

The study was conducted in a typical Patagonian Monte paddock located in north-eastern Chubut Province, Argentina (42°39′S, 65°23′W, 115 m a.s.l.). Soils are a complex of Typic Petrocalcids–Typic Haplocalcids (del Valle 1998). The vegetation corresponds to the shrubland of *Larrea divaricata* Cav. and *Stipa* spp. (southern portion of the Monte Phytogeographic Province, León et al. 1998), with plants (canopy cover <60 % of the soil) distributed in patches formed by shrubs and grasses on a matrix of bare soil or scarce vegetation (Bisigato and Bertiller 1997). The study was carried out in a paddock of about 2,500 ha with a stocking rate of 0.14 sheep ha^{-1} during the last 23 years. Within the paddock, we selected three sampling sites (about 2 ha each) located at 3,000, 300 and 100 m from an artificial watering point, which according to previous studies correspond to low (L), medium (M) and high (H) grazing intensities, respectively (Bisigato and Bertiller 1997; Bertiller et al. 2002; Prieto et al. 2011). Physical soil properties vary across this gradient of grazing intensity; silt content ranges from 17.5 to 21.6 % at L to 16.8–21.9 at H (Bisigato et al. 2008) and soil bulk density

from 1.2 g cm^{-3} at L to 1.5 g cm^{-3} at H (Prieto et al. 2011). Soils of IC areas have 8 % less sand and 94.6 % more clay than PCP areas (Carrera 2003).

We assessed soil enzyme variability in space and time due to a high spatial environmental heterogeneity induced by plant patches and a clear seasonal pattern of wet (winter) and dry (summer) periods in the upper soils (Coronato and Bertiller 1997; Bisigato and Bertiller 1997). In summer (February) and winter (July) of 2008 and 2009, we randomly selected five modal size (height: > 1 m, diameter 1.5–2.5 m) plant-covered patches (PCP) per site, containing at least the species: *L. divaricata* and *Chuquiraga erinacea* subsp. *hystrix* (Don) Ezcurra in L and M, and *L. divaricata* in H and the respective nearest neighboring modal inter-canopy area of bare soil (IC diameter >1 m). We recorded the mean canopy diameter (m), and visually estimated perennial grass, shrub and dwarf shrub covers at each modal PCP using 1 % cover intervals (Bisigato and Bertiller 1997; Bertiller and Ares 2008; Prieto et al. 2011). We collected three litter and soil sub-samples (0–10 cm depth and 10 cm in diameter) under the south-eastern part of the canopy of each PCP and other three in the middle of the nearest neighboring IC. Each set of three sub-samples was subsequently pooled and immediately transported to the laboratory at 4 °C for further processing. Each pooled soil sample was sieved to 2 mm to separate soil from plant litter which was kept for chemical quality analyses. Sheep faeces were separated from plant litter and counted as an index of local grazing pressure (Bisigato and Bertiller 1997). A soil aliquot was air-dried and used for chemical analyses. Another soil aliquot was sieved to 1 mm in order to remove small litter fragments, stored at 4 °C and used for microbiological and enzyme studies. Soil moisture was gravimetrically evaluated (105 °C, 48 h) and all results were expressed on the basis of dry soil weight. Monthly precipitations were recorded by CENPAT weather station located at 40 km from the sampling sites. In addition, for comparison purposes, soil chemical and enzyme data from a previous sampling performed in July 2007 were also incorporated into this study (Prieto et al. 2011).

Plant litter analyses

The plant litter from PCP and IC areas was cleaned of attached soil particles with a brush, dried at 60 °C for 48 h and weighed. In three additional samples from PCP

at each sampling date and site (L, M, and H), we separated the litter mass into woody litter (woody parts), shrub leaf litter (tall, medium and dwarf shrubs), grass leaf litter, particulate organic material (small size particles which could not be assigned to the previous fractions) and the mass of fine roots (< 2 mm diameter). Each fraction was dried at 60 °C for 48 h and weighed.

The chemical composition of the woody litter, shrub and grass leaf litter fractions was analyzed. The C concentration was determined by dry digestion at 550 °C (Schlesinger and Hasey 1981), soluble phenolics by the Folin-Ciocalteu method with tannic acid as standard (Waterman and Mole 1994), lignin by the acid-detergent digestion technique (van Soest 1963), and total N by semi-micro Kjeldahl (Coombs et al. 1985). The total concentrations of C, N, lignin and soluble phenolics in plant litter were calculated considering the concentration of each chemical species in the plant litter fractions (woody litter, shrub and dwarf shrub leaf litter and grass leaf litter) and the mass of the fractions. The relationships C/N, soluble phenolic/N and lignin/N were also computed.

Soil analyses

Organic soil organic-C was determined by wet combustion (Nelson and Sommers 1982) and total soil-N by semi-micro Kjeldahl (Bremner and Mulvaney 1982). Microbial biomass-C was assessed by the substrate-induced respiration method according to ISO 14240-1 as described in Prieto et al. (2011).

Soil enzyme activities

The activities of β-glucosidase, acid phosphatase, and alkaline phosphatase were determined colourimetrically as the amount of p-nitrophenol (PNP) produced from p-nitrophenyl-β-D-glucopyranoside and p-nitrophenyl-phosphate, respectively (Tabatabai 1994). Protease (casein-hydrolyzing) activity was determined according to Ladd and Butler (1972). Dehydrogenase activity was measured using 2,3,5-triphenyltetrazolium chloride (TTC) as substrate, and the results were expressed as μg triphenyl formazan (TPF) g^{-1} dry soil h^{-1} (Malkomes 1993). Samples and controls (adding the substrate after stopping the reaction) were analyzed in triplicate and averaged. For dehydrogenase assay, controls were performed with Tris–HCl buffer instead of TTC.

Statistical analyses

The significance of the differences in the mean canopy diameter, perennial grass cover, shrub and dwarf shrub covers, masses of plant litter from different fractions, particulate organic material, fine roots and chemical plant litter attributes among sites was evaluated by one-way ANOVA. Enzyme activities at different dates were also compared by one-way ANOVA. Soil organic-C, total soil-N, alkaline phosphatase and protease activities were analyzed by two-way ANOVA, being the factors the grazing intensity (L, M, H) and the patch type (PCP, IC). Natural logarithmic transformation was used when needed. Fisher Least Significant Difference was used for multiple comparisons. As acid phosphatase, β-glucosidase, and microbial biomass-C data did not accomplish variance homogeneity ANOVA assumption, we only performed multiple comparisons by Tamhane's test. Plant litter and faeces densities and dehydrogenase activity at PCP and IC areas from different sites were analyzed by Kruskal-Wallis (H) test.

The relationships between soil enzyme activities and vegetation and soil attributes at PCP and IC areas were analyzed by Spearman correlation analysis and principal component analysis (PCA). We also tested the functional dependence of soil enzyme activities on soil microbial biomass-C, soil organic-C, total soil-N, soil moisture and plant litter mass by lineal multiple regression procedures. The best combination of predictors was selected by stepwise regression based on the coefficients of determination (R^2) and the significance levels of the calculated regressions ($p<0.01$). Statistical analyses were carried out with SPSS 7.0 package (Norusis 1997).

Results

Faeces counts, plant cover and plant litter properties at the grazing sites

Sheep faeces density significantly increased from L to H sites ($H_5=58.90$, $p<0.01$; Table 1). High grazing intensity induced a reduction in the mean canopy diameter as well as in the perennial grass and dwarf shrub covers ($F_{2,72}=13.13$; $H_2=54.10$ and $F_{2,72}=9.23$, respectively, $p<0.01$; Table 1). In contrast, shrub cover was not affected by grazing intensity ($F_{2,72}=0.005$, $p=0.99$;

Table 1 Mean values ± one standard error of feces, vegetation and plant litter attributes at the modal plant-covered patches by grazing intensity

Variables	Grazing intensity		
	Low	Medium	High
Faeces density (number m^{-2})	194.16±73.65 a	299.20±82.76 b	1862.05±269.03 c
Mean canopy diameter (m)	2.14±0.09 c	1.89±0.06 b	1.57±0.08 a
Perennial grass cover (%)	5.80±0.49 c	2.00±0.20 b	0.70±0.15 a
Dwarf shrub cover (%)	4.68±1.19 b	4.92±1.25 b	0.44±0.16 a
Shrub cover (%)	55.84±2.63 a	56.24±3.47 a	56.28±4.08 a
Grass leaf litter (g m^{-2})	230.05±37.18 b	172.45±43.06 b	43.34±11.79 a
Shrub and dwarf shrub leaf litter (g m^{-2})	175.81±22.83 b	274.71±50.03 b	123.86±19.97 a
Woody litter (g m^{-2})	609.68±107.09 b	214.46±20.37 a	356.86±24.68 b
Particulate organic material (g m^{-2})	235.08±42.01 b	66.05 ±12.06 a	94.67±23.92 a
Fine roots (g m^{-2})	86.27±13.91 b	34.22±6.28 a	26.74±5.58 a
Soluble phenolics (mg g^{-1} woody and leaf litter)	114.58±9.51 a	207.01±29.09 b	400.18±50.37 c
Lignin (mg g^{-1} woody and leaf litter)	158.95±6.30 a	152.45±11.39 a	173.34±6.68 a
C (mg g^{-1} woody and leaf litter)	415.06±9.82 a	428.49±18.50 a	409.04±7.00 a
N (mg g^{-1} woody and leaf litter)	10.72±0.63 ab	9.95±0.50 a	12.68±1.10 b
Soluble phenolic/N (woody and leaf litter)	11.14±1.02 a	20.89±2.71 b	32.79±4.00 c
C/N (woody and leaf litter)	40.37±2.36 b	43.63±1.52 b	34.10±1.71 a
Lignin/N (woody and leaf litter)	15.27±0.78 a	15.45±1.00 a	14.45±0.94 a

Different lowercase letters indicate significant differences among grazing intensities ($p<0.05$)

Table 1). We found the lowest masses of grass and shrub and dwarf shrub leaf litter at H ($F_{2,72}=11.28$ and $F_{2,72}=7.22$, respectively, $p<0.01$) and that of woody litter at M ($F_{2,72}=14.71$, $p<0.01$), (Table 1). The particulate organic material and fine root masses were significantly lower at M and H than at L ($F_{2,72}=10.28$ and $F_{2,72}=10.15$, respectively, $p<0.01$; Table 1).

Regarding the quality of plant litter (Table 1), the concentration of soluble phenolics significantly increased with grazing intensity ($F_{2,42}=33.47$, $p<0,01$). Particularly, soluble phenolic concentrations significantly increased in shrub leaf litter and woody litter fractions from L to H sites (Table 2). Lignin and C concentrations in plant litter did not vary among sites ($F_{2,42}=2.43$, $p=0.10$ and $F_{2,42}=0.40$, $p=0.67$, respectively). We only detected differences in N concentration between M and H, with higher values at the latter ($F_{2,42}=4.06$, $p<0.05$). As a consequence of increased phenolic concentration in plant litter with grazing, the phenolic/N ratio also increased in M and H ($F_{2,42}=22.73$, $p<0.01$; Table 1). The lowest C/N ratio was at H ($F_{2,42}=6.48$, $p<0.01$), while lignin/N did not vary ($F_{2,42}=0.34$, $p=0.71$) among sites (Table 1).

Soil properties at the grazing sites

We observed a significant interaction between the patch type (PCP, IC) and the grazing intensity (L, M, H) on soil organic-C and total soil-N concentrations ($F_{2,144}=20.20$ and $F_{2,144}=23.89$, respectively, $p<0.01$) and total plant litter mass ($H_5=80.76$, $p<0.01$) (Fig. 1a, b and c, respectively). In all cases the highest C, N and plant litter mass values were found at L sites in PCP, while we found a trend of increasing C and N concentrations and total litter with grazing intensity in IC. Soil moisture did not change with grazing intensity at PCP or IC areas ($H_5=7.03$, $p=0.23$; Fig. 1d), but for both it was higher at winter than at summer, excluding 2007 winter when precipitations were lower than the historic media for July (Table 3).

Soil enzyme activities and microbial biomass at the grazing sites: spatial and seasonal variations

In general, the extracellular hydrolases showed higher activity levels at PCP than at IC soils (Fig. 2a, b, c and d). A significant interaction between patch type and

Table 2 Chemical quality of plant litter fractions (mean values ± one standard error) at the modal plant-covered patches by grazing intensity

Plant litter fractions	Grazing intensity		
	Low	Medium	High
Woody litter			
C (mg g^{-1})	407.5±5.8 a	407.7±7.1 a	405.3±5.9 a
N (mg g^{-1})	10.9±0.6 a	9.8±0.3 a	11.0±0.4 a
Lignin (mg g^{-1})	184.9±5.0 a	203.5±5.1 b	200.4±5.6 b
Soluble phenolics (mg g^{-1})	101.0±9.4 a	184.9±22.6 b	251.9±29.4 c
Shrub and dwarf shrub leaf litter			
C (mg g^{-1})	413.7±7.0 b	438.5±4.6 c	382.8±8.2 a
N (mg g^{-1})	11.5±0.5 b	10.0±0.3 a	12.7±0.6 b
Lignin (mg g^{-1})	141.0±2.6 b	123.7±3.5 a	119.9±7.9 a
Soluble phenolics (mg g^{-1})	223.7±22.9 a	277.1±20.2 b	938.1±61.1 c
Grass leaf litter			
C (mg g^{-1})	367.9±12.1 a	327.3±19.4 a	382.3±8.6 a
N (mg g^{-1})	7.9±0.5 ab	6.5±0.4 a	9.1±0.6 b
Lignin (mg g^{-1})	88.3±7.9 b	74.5±3.9 ab	70.0±3.5 a
Soluble phenolics (mg g^{-1})	45.6±4.0 b	33.2±4.8 a	41.6±2.4 ab

Different lowercase letters indicate significant differences among grazing intensities ($p \leq 0.05$)
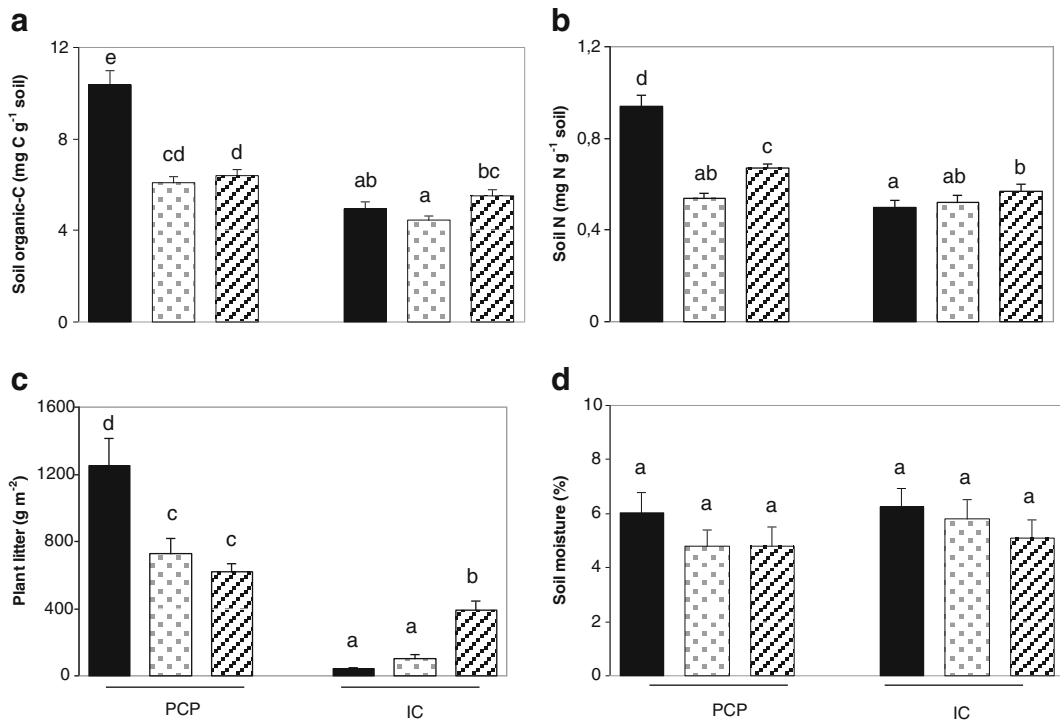

Fig. 1 Properties of the grazing sites: **a**. soil organic-C, **b**. total soil-N, **c**. plant litter mass, and **d**. soil moisture at PCP (plant-covered patches) and IC (inter-canopy) areas of sites with low (*solid black*), medium (*points*) and high (*lines*) grazing intensities.

Vertical lines indicate one standard error. *Different lowercase letters* indicate significant differences among mean values of different sites

Table 3 Soil moisture at plant-covered patches (PCP) and inter-canopy areas (IC)

Different lowercase letters indicate significant differences among sampling dates ($p<0.05$)

[a] CENPAT-CONICET meteorological station

Sampling dates	PCP soil moisture (%)	IC soil moisture (%)	Total precipitation in the month (mm)[a]	Historic precipitation media of the month 1982–2002 (mm)[a]
July 2007	2.43±0.15 b	3.70±0.15 b	1.5	15.8
February 2008	1.64±0.07 a	1.72±0.08 a	5.1	20.3
July 2008	8.41±0.42 d	8.74±0.30 c	10.2	15.8
February 2009	3.40±0.30 c	4.34±0.33 b	13.6	20.3
July 2009	9.61±0.25 d	10.14±0.35 c	20.0	15.8

grazing intensity was found on alkaline phosphatase activity ($F_{2,144}=14.90$, $p<0.01$). At PCP this activity decreased at M and H sites, while it increased with grazing intensity at IC soils (Fig. 2a). Acid phosphatase and β-glucosidase activities also decreased with grazing intensity at PCP (Fig. 2b–c). At IC, acid phosphatase was the only enzyme activity that diminished with grazing intensity (Fig. 2b). β-glucosidase and protease activities did not vary with grazing at IC areas, neither did protease at PCP (Fig. 2c–d).

As hydrolases, dehydrogenase activity and microbial biomass-C were also higher at PCP than at IC soils (Fig. 3a–b). Both variables increased with grazing intensity at IC soils (Fig. 3a–b). On the other hand, dehydrogenase activity did not change with grazing intensity at PCP (Fig. 3a) and the lowest microbial biomass-C was found at H site (Fig. 3b).

Except for protease activity, we found significant positive correlation coefficients among enzyme activities and microbial biomass-C, soil organic-C and total soil-N at PCP soils (Table 4). At IC, excluding acid phosphatase, all the enzyme activities correlated positively to microbial biomass-C and soil organic-C, while only dehydrogenase and protease activities correlated to total soil-N (Table 4). We only registered significant correlations to faeces density at IC areas, which were positively related to alkaline phosphatase and dehydrogenase activities, and negative with acid phosphatase activity (Table 4).

In addition, β-glucosidase and deshydrogenase were positively correlated to plant litter mass at PCP and IC areas, while acid and alkaline phosphatases showed positive correlation with plant litter mass only at PCP and IC areas, respectively (Table 4). We also observed a significant positive correlation between perennial grass cover and all the hydrolase activities and between fine root mass and phosphatase and β-glucosidase activities (Table 4). Soluble phenolic concentrations in plant litter showed a significant negative correlation to both phosphatases and β-glucosidase activities at PCP (Table 4). In contrast, plant litter C, N and lignin concentrations did not showed significant correlations with the enzyme activities (Table 4).

The comparisons of enzyme activities and microbial biomass-C values at different sampling dates (winters 2007, 2008 and 2009; summers 2008 and 2009) for each site did not show a common seasonal pattern of enzyme activity even when we detected significant differences in soil enzyme activities and microbial biomass among dates. These variations depended on particular enzymes and sites (Figs. 2 and 3). Particularly, several of the highest enzyme activities registered at IC areas occurred at winters 2008 and/or 2009 (e.g. alkaline phosphatase at M, Fig. 2a; acid phosphatase at L, Fig. 2b; β-glucosidase at L and M, Fig. 2c; protease at all sites, Fig. 2d; dehydrogenase at M, Fig. 3a).

Except for dehydrogenase, all the enzyme activities and microbial biomass-C (Spearman coefficient 0.31, $p<0.01$) correlated to soil moisture at IC soils, and only alkaline phosphatase and protease at PCP (Table 4).

The PCA linking soil biological and chemical properties at different sites and dates showed that the first axis (PC1) explained 62.5 % of the total variance, and clustered IC soils at negative values while PCP soils were grouped at positive values on this axis (Online Resource 1). PCP, particularly those of L site, had higher values of soil organic-C, total soil-N, microbial biomass-C, plant litter mass, and enzyme activities than IC areas. The second component (PC2) explained 16.8 % of the total variance and grouped samples according to soil moisture. Along axis 2 samples were separated in two groups, 2008 and 2009 winters with higher soil moisture (Table 3) at positive values and 2008 and 2009 summers along with 2007 winter at negative values with lower soil moisture (Online Resource 1).

Fig. 2 Enzyme activities:
a. alkaline phosphatase, **b**. acid
phosphatase, **c**. ß-glucosidase,
d. protease at PCP and IC areas of
sites with low (*solid black*),
medium (*points*) and high (*lines*)
grazing intensities at different
sampling dates (1: winter 2007, 2:
summer 2008, 3: winter 2008, 4:
summer 2009, 5: winter 2009).
Vertical lines indicate one
standard error. *Uppercase letters*
indicate significant differences
among mean values of different
sites and *lowercase letters* among
different sampling dates at each
site. Acronyms of patch type and
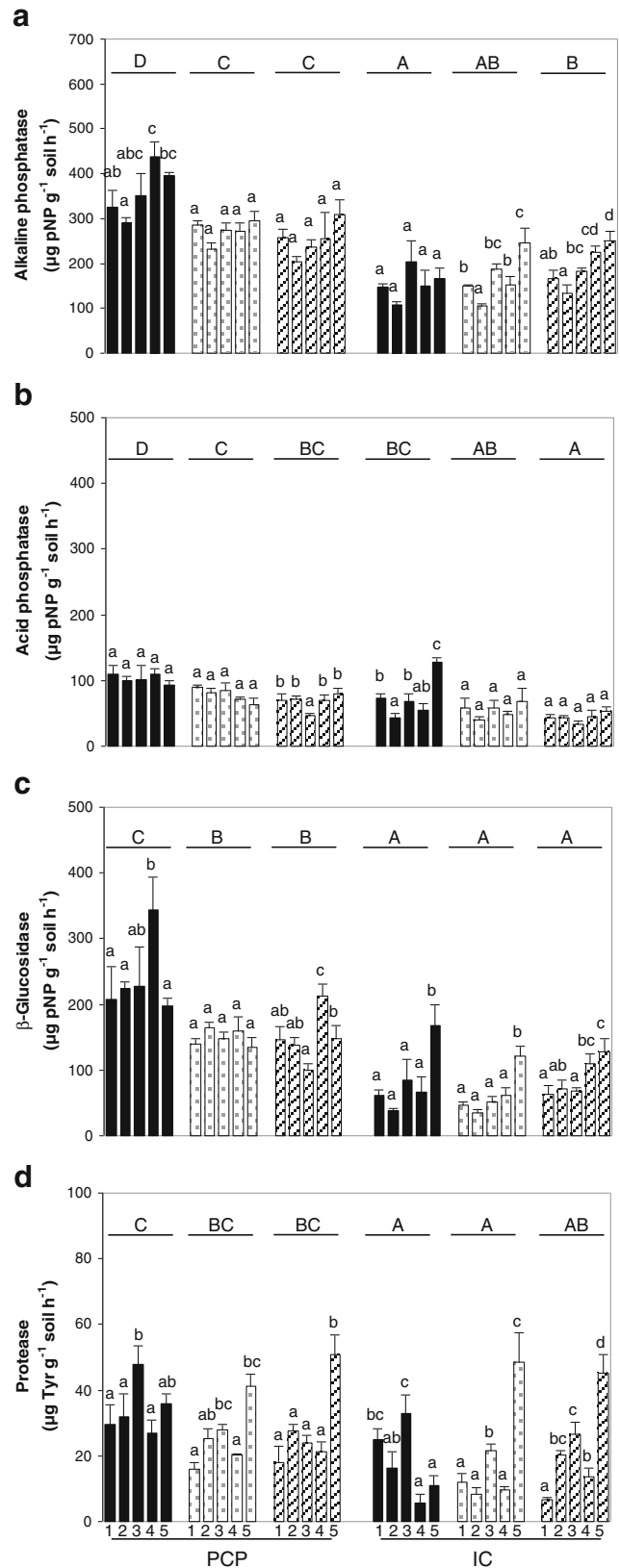grazing intensity as in Fig. 1

Fig. 3 Microbial activity and biomass: **a**. dehydrogenase, **b**. microbial biomass-C at PCP and IC areas of sites with low (*solid black*), medium (*points*) and high (*lines*) grazing intensities at different sampling dates (1: winter 2007, 2: summer 2008, 3: winter 2008, 4: summer 2009, 5: winter 2009). *Vertical lines* indicate one standard error. *Uppercase letters* indicate significant differences among mean values of different sites and *lowercase letters* among different sampling dates at each site. Acronyms of patch type and grazing intensity as in Fig. 1
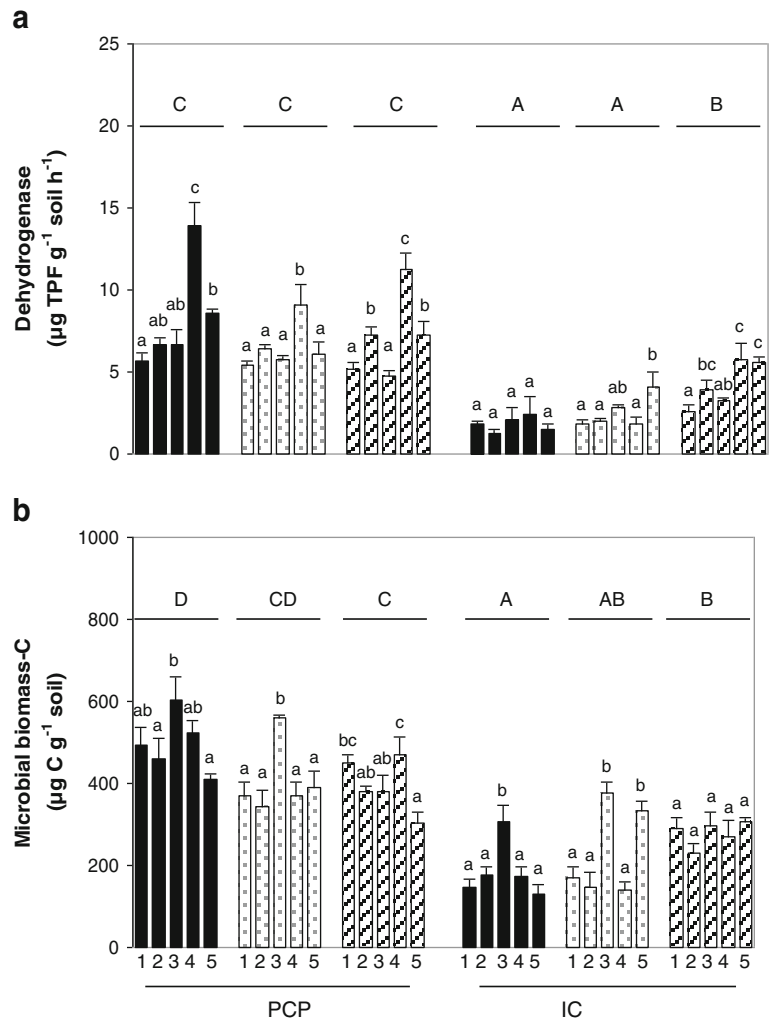
Best predictors of enzyme activities

The soil organic-C and the microbial biomass-C were the most frequent best predictors of soil enzyme activities either in combination between them or with soil moisture (Table 5). Soil organic-C and microbial biomass-C explained 65 % of the observed variation of β-glucosidase activity, while arranged inversely explained 53 % of dehydrogenase activity variation (Table 5). These predictors in addition to soil moisture explained the largest percentage of alkaline phosphatase variation (68 %), (Table 5). The best predictors for acid phosphatase activity were soil organic-C and soil moisture (Table 5). On the other hand, soil moisture in combination with microbial biomass-C and total soil-N explained 47 % of protease activity variation (Table 5).

Plant litter mass was not retained as significant predictor of enzyme activity.

Discussion

Grazing intensity induced an increase of the concentration of soluble phenolics and soluble phenolics/N ratio in plant litter due to high contribution of shrub litter with high concentration of these secondary metabolites at M and H sites. We also observed an increase of N and a diminution of C/N ratio in the plant litter of H. These results are consistent with the dominance of *L. divaricata* in shrub canopies at H, producing leaf litter with low C/N ratio, low lignin concentration and high concentrations of N and soluble phenolics (Vargas

Table 4 Spearman correlation coefficients between enzyme activities and soil chemical and biological properties at plant-covered patches (PCP) and inter-canopy areas (IC)

	β-Glucosidase	Alkaline phosphatase	Acid phosphatase	Dehydrogenase	Protease
PCP					
Microbial biomass-C	0.514**	0.434**	0.397**	0.230*	0.186
Soil organic-C	0.701**	0.562**	0.672**	0.464**	0.152
Soil-N	0.498**	0.374**	0.535**	0.278*	0.165
Soil moisture	−0.083	0.408**	−0.047	0.071	0.521**
Faeces density	0.117	−0.158	−0.103	0.217	0.163
Plant litter mass	0.512**	0.189	0.413**	0.328**	−0.102
Fine root mass	0.461**	0.286**	0.578**	0.069	−0.093
Perennial grass cover	0.289*	0.461**	0.425**	0.087	0.241*
Plant litter-C	−0.100	0,078	0.053	−0.269	−0.085
Plant litter-N	−0.244	−0.159	−0.126	−0.168	−0.118
Plant litter-C/N	0.139	0.162	0.158	−0.015	−0.030
Plant litter-Lignin	−0.026	0.014	−0.218	0.102	0.032
Plant litter-Soluble phenolics	−0.299*	−0.500**	−0.342*	−0.198	−0.120
Plant litter- Soluble phenolics/N	−0.279	−0.528**	−0.345*	−0.154	−0.045
IC					
Microbial biomass-C	0.291*	0.604**	−0.076	0.623**	0.502**
Soil organic-C	0.252*	0.399**	−0.016	0.519**	0.282*
Soil-N	0.076	0.180	−0.196	0.389**	0.359**
Soil moisture	0.464**	0.527**	0.388**	0.080	0.481**
Faeces density	0.267	0.377*	−0.399**	0.631**	0.158
Plant litter mass	0.354*	0.466**	−0.155	0.632**	0.265

*$p \leq 0.05$, **$p \leq 0.01$

et al. 2006). This shrub also produces antimicrobial compounds, some of them polyphenols, against Gram negative and positive bacteria and fungi (Anesini and Perez 1993; Amani et al. 1998; Quiroga et al. 2001; Zampini et al. 2007).

At PCP, alkaline and acid phosphatase and β-glucosidase activities diminished with increasing grazing intensity. Such response seems to be explained by a reduction of substrates available for decomposers along with an increased inhibitory role of soluble phenolics in

Table 5 Best predictors, sample numbers (n), coefficients of determination for multiple stepwise regression (R^2) and significance levels (p) for regressions among enzyme activities as dependent variables and microbial biomass-C, soil organic-C, total soil-N and soil moisture as independent variables

Dependent variable	Independent variables	Equations	n	R^2	p
β-Glucosidase	Soil organic-C, microbial biomass-C	β-Glucosidase=−45.42+18.34*soil organic-C+0.18*microbial biomass-C	150	0.65	< 0.01
Dehydrogenase	Microbial biomass-C, soil organic-C	Dehydrogenase=1.07+0.10*microbial biomass-C+0.43*soil organic-C	150	0.53	< 0.01
Alkaline phosphatase	Microbial biomass-C, soil organic-C, soil moisture	Alkaline phosphatase=0.22+0.26*microbial biomass-C+17.19*soil organic-C+7.06*soil moisture	150	0.68	< 0.01
Acid phosphatase	Soil organic-C, soil moisture	Acid phosphatase=18.98+6.57*soil organic-C+1.78*soil moisture	150	0.34	< 0.01
Protease	Soil moisture, microbial biomass-C, total soil-N	Protease=−6.62+2.14*soil moisture+0.03*microbial biomass-C+16.32*total soil-N	150	0.47	< 0.01

plant litter. These enzyme activities were positively correlated with perennial grass cover and fine root biomass (acid phosphatase and β-glucosidase also with plant litter mass), soil organic-C, total soil-N and microbial biomass-C diminishing at high grazing intensity in line with results from other studies in grazed semiarid ecosystems (Raiesi and Asadi 2006; Shrestha and Stahl 2008). No correlation was observed among the concentrations of C, N and lignin and C/N ratio in plant litter with enzyme activities. The diminution of plant litter C/N ratio under high grazing did not result in an increase of enzyme activities possibly because of the recalcitrant/inhibitory effects of such litter (high soluble phenolics and soluble phenolics/N ratio) in combination with a significant reduction of plant litter mass which could have limited substrate availability for microorganisms. Moreover, alkaline and acid phosphatase and β-glucosidase activities negatively correlated to soluble phenolics, agreeing with a negative relationship between phenolics in plant litter and the activity of soil hydrolytic enzymes, which in turn could limit organic matter decomposition (Hättenschwiler and Vitousek 2000; Reyes-Reyes et al. 2003; Yao et al. 2009; Tian et al. 2010). Thus, the increase of soluble phenolics and the soluble phenolic/N ratio in the plant litter at high grazing intensities may lead to a retard in plant litter decomposition and N release contributing to the diminution in total soil-N, soil organic-C and enzyme-producing microbial biomass registered at PCP. Furthermore, the diminution of fine root biomass with grazing intensity was possibly associated with the reduction of grass and dwarf shrub cover, as they have shallower roots than tall and medium shrubs (Schulze et al. 1996; Bisigato and Bertiller 1997; Schenk and Jackson 2002). This diminution in root biomass could explain the decrease of acid phosphatase activity with grazing intensity, as these enzymes are also released from plant roots (Hiradate et al. 2007; Prieto et al. 2011).

At IC areas, we found that total soil-N increased with grazing intensity, possibly due to the N input from grazer's urine and faeces, particularly at the H site placed just 100 m away from the watering point. Coincidently, alkaline phosphatase and dehydrogenase activities and microbial biomass-C increased in IC soils at H. These enzyme activities correlated to faeces density at IC areas. From herbivore ingest, 60 to 99 % of the nutrients return to the soil in the form of dung and urine (Haynes and Williams 1993), causing a nutrient redistribution and propitiating local changes in the soil

chemistry such as increasing of N and P (Moleele and Perkins 1998; Shand et al. 2000). At arid and semi-arid rangelands of Iran, Shahriary et al. (2012) observed that soil around watering points (100 m) had higher soil-N than at farther distances (1,000 and 10,000 m). Similarly, soil-N concentration increased in the vicinity from the water-points (i.e. 0–75 m) in commercial cattle ranches in a semi-arid savanna, in South Africa (Smet and Ward 2006). Urine enters N into the soils in the form of urea which is rapidly transformed to ammonia, and then to nitrate. Dehydrogenase activity corresponds to intracellular enzymes from viable cells (Nannipieri et al. 2002) and alkaline phosphatase is exclusively attributed to microorganisms (Tabatabai 1994), therefore it is expectable that these activities respond rapidly to N input from urine. Furthermore, the increase of plant litter mass in IC soils at H, possibly due to the animal traffic promoting plant litter dispersion from patch sources, physical fragmentation and incorporation into the soil, could have contributed to enhance microbial activity (Schuman et al. 1999). In this context, dehydrogenase and alkaline phosphatase activities increased with grazing-addition of N and possibly C (from plant litter dispersion) together, supporting a response consistent with the resource limitation model at IC areas (Hernández and Hobbie 2010). Such model also considers that nutrient additions might moderate the activity of enzymes involved in acquiring such nutrients, which is coincidently with no variations of β-glucosidase and protease activities at IC. In contrast, acid phosphatase activity decreased with grazing intensity at IC, which could be related with lower secretion of such enzymes from plant roots. Nevertheless, the positive effect of grazing over alkaline phosphatase and dehydrogenase activities and microbial biomass-C at IC soils of H was localized, as it was not observed at L site and these activities were always lower than in PCP soils. Thus, as in the Patagonian Monte soil resources are heterogeneously distributed, mainly associated with vegetated patches (Mazzarino et al. 1996), a localized input of animal excreta may accentuate even more such heterogeneity promoting belowground microsites with higher microbial and enzyme activity but at a local scale.

Even though a seasonal pattern of wet (winter) and dry (summer) periods in the upper soil was described for the Patagonian Monte (Coronato and Bertiller 1997), rain events may greatly vary intra and inter-seasonally. In winter 2007 the precipitations were scarce and the soil moisture was smaller than at winters 2008 and 2009.

Except for dehydrogenase, all the enzyme activities and the microbial biomass-C correlated to soil moisture at IC areas and several of the highest enzyme activity levels occurred at winters 2008 and/or 2009. Only alkaline phosphate and protease activities correlated to soil moisture at PCP. Inter-canopy areas with scarce o without vegetation are more exposed to evaporation and desiccation than soils under canopies which contribute to moderate the temperature and preserve soil moisture (Noe and Abril 2008).

Even though different mechanisms seems to affect enzyme activities at PCP and IC areas and individual enzymes responded differently, considering all the results together soil organic-C and microbial biomass-C were the most frequent best predictors of soil enzyme activities either in combination between them or with soil moisture, explaining more that 50 % of the observed variation of dehydrogenase, alkaline phosphatase and β-glucosidase activities. This agrees with other studies that associated soil organic-C and microbial biomass-C with these enzyme activities (Wick et al. 2002; Bastida et al. 2006; Paudel et al. 2012). Soil moisture also contributed to explain alkaline phosphatase variations as in some soil studies (Harrison 1983; Speir and Cowling 1991). Acid phosphatase variations were poorly explained by soil organic-C and moisture, this activity could have better related to soil P and the processes that affect its availability in the soil such as plant growth (Raghothama 1999). The highest level of dehydrogenase activity was detected at PCP in summer 2009. An increase of soil dehydrogenase activity in summer was also found in other studies postulating that even though microbial cells suffer stress by higher soil temperature and lower humidity, they have to maintain their organic matter oxidation machinery at a high level thus accounting for the high soil dehydrogenase activity levels (Li and Sarah 2003; Bastida et al. 2006; Gil et al. 2010). In contrast, soil protease activity depended on soil moisture in combination with microbial biomass-C and total soil-N. Thus, dry periods with low humidity and low N-input may restrict soil proteolysis and N availability (Watanabe and Hayano 1996; Wick et al. 2002).

Conclusions

We demonstrated that long-term grazing led to reduced grass cover, and decreasing plant litter mass with increasing soluble phenolics which were associated with a negative effect on soil enzyme activities (particularly those related to C and P acquisition) and microbial biomass at PCP. In contrast to our hypothesis, a different mechanism seems to control the response of some enzyme activities to grazing intensity at IC areas. There, microbial biomass-C and soil enzyme activities closely related to microbial metabolism (dehydrogenase and alkaline phosphatase), increased at the site with high grazing intensity, presumably by a localized nutrient input from animal excreta which promotes belowground microsites with higher soil microbial and enzyme activity in agreement with the resource limitation model. Nonetheless, this grazing positive effect on some soil enzyme activities was limited to the IC areas of the site with high grazing intensity and these activities were always lower than in PCP soils.

Soil organic-C and microbial biomass-C either combined between them or with soil moisture showed to be the most frequent best predictors of soil enzyme activities. This pointed out the importance of microbial biomass as source of soil enzymes and soil organic-C as substrate for microorganisms and enzymes, and of soil moisture particularly at IC areas.

Altogether our results suggest that plant heterogeneous distribution, plant litter quantity and quality, nutrient inputs from grazers and seasonal variation in soil moisture, also affecting soil resources and microbial biomass, modulate soil enzyme responses to long-term grazing in the arid Patagonian Monte.

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