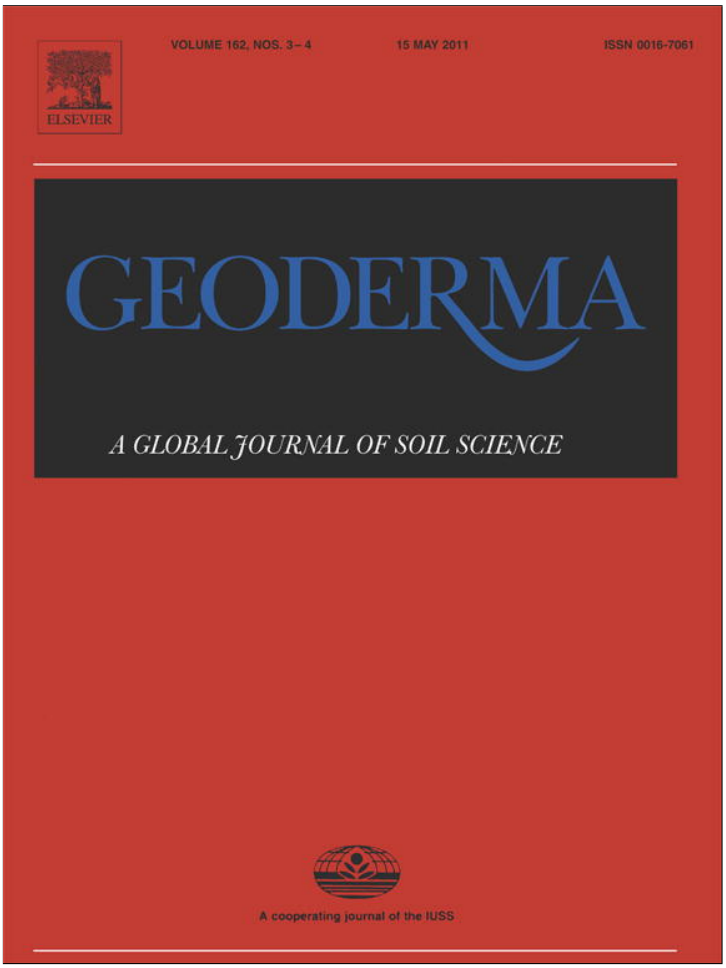

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# Soil enzyme and microbial activities in a grazing ecosystem of Patagonian Monte, Argentina

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ABSTRACT

Grazers exert important effects on the plant–soil system influencing the structure and functioning of the ecosystems. The effect of grazing on soil enzyme and microbial activities in arid ecosystems of the Patagonian Monte (Argentina) was studied by assessing the cover of plant life forms (grasses, shrubs and dwarf shrubs) at five modal plant-covered patches at three sites across a grazing gradient. We hypothesized that grazing mostly affects negatively soil enzyme and microbial activities through its effect on vegetation and on soil chemical and physical parameters. Soil cores were extracted from plant-covered patches and associated inter-canopy areas and analyzed the chemical (organic-C, total-N and pH), physical (bulk density), microbial (microbial biomass-C, and heterotrophic microorganism counts), and enzyme (dehydrogenase, ß-glucosidase, protease, alkaline and acid phosphatase and the geometric media for these activities) soil properties. Grass and dwarf shrub covers declined, and soil pH and bulk density increased with increasing grazing intensity at plant patches and inter-canopy areas. Soil organic-C and soil-N decreased at plant patches and, were positively correlated to grass cover. Soil organic-C was also positively correlated with ß-glucosidases and phosphatases, indicating that a reduction in soil organic-C could be associated with low enzyme activity and consequently with low soil organic matter turnover and nutrient release. Negative effects of grazing on enzyme activities were also detected on protease activity at inter-canopy areas and geometric media of the enzyme activities at plant patches. Increasing grazing intensity was associated with increasing values of microbial biomass-C and heterotrophic microorganism counts mostly at inter-canopy areas, suggesting that inputs of labile N by urine and dung may partially counteract the negative grazing effects. We concluded that grazing mostly affected soil physicochemical and biological parameters through direct and indirect effects of trampling and urine and changes in perennial grass cover, respectively.



## 1. Introduction

The soil is a natural resource whose quality depends on a number of chemical, physical, and biological properties which could be affected by degradation processes triggered by natural events as well as by anthropic activities (Jenny, 1980; Sylvia et al., 2005). Heterotrophic microorganisms are largely responsible for the turnover of soil organic-C (Wick et al., 2002; Winding et al., 2005). These microbes use C-substrates in the synthesis of new cell material with efficiencies of 30 to 50% depending on the complexity of C-sources, environmental conditions (such as nutrient and water availability), and the particular type of microorganism (Sylvia et al., 2005).

In arid and semiarid regions, water controls biotic processes conditioning most of them to sporadic and scarce rainfall events (Collins et al., 2008; Noy-Meir, 1973; Reynolds et al., 2004). Additionally, the patchy nature of vegetation in these ecosystems causes environmental spatial heterogeneity (Carrera et al., 2009; Mazzarino et al., 1996; Tongway et al., 2003) which could in turn further affect microbial activity and soil functioning among other processes. Since arid and semiarid ecosystems cover more than 37% of the global land surface (Lal, 2004), land management impacts should be carefully analyzed to avoid land degradation (Bertiller et al., 2002; Lechmere-Oertel et al., 2005). Most of these ecosystems are used as rangelands with domestic grazers (Whitford, 2002). Long-term grazing disturbance leads directly or indirectly to changes in the aboveground plant components such as the reduction of the total and palatable plant cover or the increase of unpalatable woody plants with high concentrations of secondary compounds (Bardgett et al., 1998; Schlesinger et al., 1990). However, the effects of aboveground grazing disturbance on belowground components such as some soil properties and microbial activities are still not fully understood (Bardgett and Wardle, 2003; Bardgett et al., 1998). There is evidence that aboveground plant changes could affect not only the size of the

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organic matter input to soils but also the quality of the soil organic matter (Bardgett and Wardle, 2003; Bardgett et al., 1998). On the other hand, part of the herbivore ingests returns to the soil in the form of urine and dung increasing labile organic matter and N inputs to soil (Bardgett and Wardle, 2003; Haynes and Williams, 1993).

Soil enzymes are important components for biochemical functioning of soils as they take part in organic matter decomposition and nutrient cycling (Makoi and Ndakidemi, 2008; Patra et al., 2006). They mainly derive from microorganisms but also from plants and animals. In addition, a fraction of some soil enzymes (e.g. β-glucosidase and proteases) may be no longer associated with living cells, and remain in soil as immobilized enzymes adsorbed to clay surfaces or complexed to humic colloids (Alkorta et al., 2003; Makoi and Ndakidemi, 2008). Soil enzyme activities have been used as sensitive indicators of microbial activity since they could respond quickly to changes in environmental conditions, microbial community structure, and disturbances (Dick et al., 1996). Among them, the dehydrogenase activity has been used as a measure of the overall microbiological activity of soil since dehydrogenases catalyze intracellular processes of viable microbial cells (Nannipieri et al., 2002) and are linked to the first stage of organic matter decomposition (Makoi and Ndakidemi, 2008). Other soil enzymes such as hydrolases are involved in C (e.g. β-glucosidase, cellulase, amylase, chitinase), N (e.g. protease and urease), and P (e.g. acid and alkaline phosphatases) cycling. The enzyme β-glucosidase acts in the decomposition of cellulose and other carbohydrate polymers derived from plant litter. Its activity produces sugars, which are used for microbial growth. Thus, β-glucosidase activity reflects the state of soil organic matter (Garcia et al., 1994) and has been considered as an indicator of soil quality (Dodor and Tabatabai, 2005; Saviozzi et al., 2001). Proteases hydrolyse peptide bounds between amino acids of peptides and proteins. They have an important role in the first stages of N mineralization and release, regulating its availability to plants and microorganisms. Phosphatases catalyze the hydrolysis of phosphoric (mono) ester bonds of organic phosphorus producing phosphate which can be assimilated by plants. Plants can promote soil phosphatase activity due to the enhancement of microbial activity or by acid phosphatase secretion in the rizosphere (Hiradate et al., 2007). Some studies showed that these enzymes increase in response to low P availability in soil and decrease after phosphate fertilization (Gil-Sotres et al., 2005; Ndakidemi, 2006). Therefore, microbial and enzyme activities related to C and nutrient mineralization–immobilization have been extensively studied to evaluate the impact of agricultural practices such as cultivation (Bandick and Dick, 1999; Bending et al., 2004; Moscatelli et al., 2007), agrochemical manure and fertilizer application (Patra et al., 2007) or to monitor the bioremediation of polluted soils (Hinojosa et al., 2004; Lee et al., 2008). However, there is scarce knowledge about the effect of grazing on soil enzyme activities related to C and nutrient cycling in natural ecosystems (Craine et al., 2009).

Domestic grazers induce plant cover reduction and species replacement increasing the abundance of long-lived evergreen woody plants with slow growth rates and high concentration of secondary compounds in tissues (Bertiller and Bisigato, 1998; Bisigato and Bertiller, 1997; Carrera et al., 2005). Moreover, grazers affect physical soil properties through trampling (Manzano and Návar, 2000; Steffens et al., 2008; Yates et al., 2000). All those effects are likely to change structure and functioning of microbial communities, further affecting the rates of organic matter decomposition and nutrient cycling, and in consequence other ecosystem processes such as primary production (Bardgett et al., 1998; Rutigliano et al., 2004). Some studies report a decrease of soil enzyme activities with ecosystem degradation (Garcia et al., 2002; Holt, 1997) but there is still scarce evidence on these issues in grazing ecosystems. The aim of this study was to assess soil microbial and enzyme activities and their relationships with plant cover and soil physiochemical properties at sites with increasing grazing intensity in the Patagonian Monte shrublands. In this context, we hypothesized that grazing mostly affects negatively soil enzyme and microbial activities through its effect on plants and soil parameters.

## 2. Material and methods

### 2.1. Study site and sampling

The study area was located in north-eastern Chubut Province, Argentina (42°39'S, 65°23'W, 115 m a.s.l.). The mean annual temperature is 13.8 °C and the mean annual precipitation is 231 mm (10-year average, www.cenpat.edu.ar). Soils are a complex of Typic Petrocalcids–Typic Haplocalcids (del Valle, 1998). Vegetation is representative of the steppe of *Larrea divaricata* Cav. and *Stipa* spp., characteristic of the Patagonian Monte (southern portion of the Monte Phytogeographic Province, León et al., 1998). Plant canopy cover is low (<60% of the soil) and presents a random patchy structure formed by clumps of shrubs and perennial grasses on a matrix of bare soil or sparse vegetation (Bisigato and Bertiller, 1997; Mazzarino et al., 1998). This study was conducted in a typical Patagonian Monte paddock of about 2500 ha, with a stocking rate of 0.14 sheep $ha^{-1}$ during the last 18 years (Bertiller et al., 2002). At this area, we selected three sampling sites (about 2 ha each) located at 3000, 300 and 100 m from an artificial watering point. Based on sheep faecal counts (Bisigato and Bertiller, 1997), perennial grass cover (Rodríguez et al., 2007) and remote sensing analyses (Ares et al., 2003), these sites are characterised by low (L), medium (M) and high (H) grazing intensities, respectively (Bertiller et al., 2002). Additionally, the intensity of grazing in H was augmented by the concentration of all the animals of the paddock at least 1 week per year due to managerial purposes. In July 2007, 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: *Larrea divaricata* and *Chuquiraga erinacea* (Don) Ezcurra in L and M, and *L. divaricata* in H and the respective nearest neighbouring modal inter-canopy area of bare soil (IC, diameter of >1 m) (Bisigato and Bertiller, 1997). Inter-canopy areas lacked aboveground cover of perennial species all year round. However, shallow fine roots of perennial plants of neighbouring PCPs could colonize the IC soil in the depth from 0 to 15 cm (Carrera and Bertiller, 2010).

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 (Bertiller and Ares, 2008; Bisigato and Bertiller, 1997). We collected three soil sub-samples (0–10 cm depth) under the south-eastern part of the canopy (between the basal insertion of the branches and the edge of the patch crown) of each PCP and other three in the middle of the nearest neighbouring IC (Fig. 1). Each set of three sub-samples was subsequently pooled and immediately transported to the laboratory at 4 °C for further

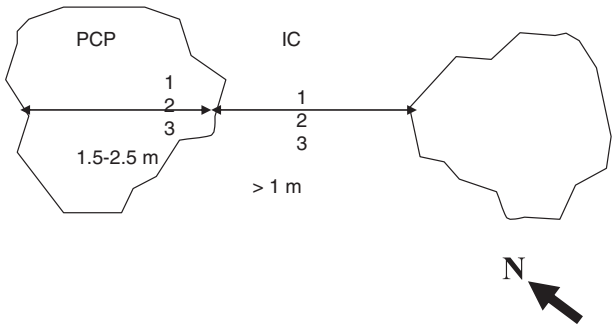


**Fig. 1.** Scheme of sampling at PCP (plant-covered patches) and IC (inter-canopy bare soil) areas. Numbers (1, 2, and 3) indicate the location of sub-samples.

**Table 1**
Mean values ± one standard error of canopy mean diameter, and perennial grass, shrub and dwarf shrub covers at the modal plant-covered patches by grazing intensity. Different lowercase letters indicate significant differences among grazing intensities ($P \leq 0.05$), $n = 15$.

| Grazing intensity | Mean canopy diameter (m) | Perennial grass cover (%) | Shrub cover (%) | Dwarf shrub cover (%) |
|---|---|---|---|---|
| Low | 2.4 ± 0.2 a | 5.4 ± 0.8 c | 53.0 ± 7.1 a | 10.2 ± 3.9 b |
| Medium | 1.8 ± 0.2 a | 3.0 ± 0.4 b | 57.0 ± 5.3 a | 3.8 ± 1.9 ab |
| High | 1.8 ± 0.1 a | 0.4 ± 0.2 a | 65.2 ± 3.9 a | 0.8 ± 0.5 a |

processing. Each pooled soil sample was sieved to 2 mm to remove plant litter and roots and separated in two aliquots. One aliquot was air-dried and used for chemical and physical analyses and the other was sieved to 1 mm in order to remove small litter fragments, stored at 4 °C and used for microbiological and enzyme studies. All results were expressed on the basis of oven-dried (105 °C, 48 h) soil weight.

### 2.2. Soil chemical and physical analyses

We measured soil organic-C by wet combustion (Nelson and Sommers, 1982), soil-N by semi-micro Kjeldahl (Bremner and Mulvaney, 1982) and soil pH ($pH_{H2O}$ in 1:2.5 ratio). Bulk density was estimated by the core method (Blake, 1965) on separate soil samples (7.23 cm diameter and 4.75 cm depth) taken from each modal PCP and IC at each site.

### 2.3. Soil microbiological analyses

After 4 days of soil pre-incubation at 22 °C and 15% water-holding capacity (Vargas et al., 2006), substrate-induced respiration was performed according to ISO 14240–1 (1997) using glucose (1 mg $g^{-1}$ dry soil) in a static bottle system with alkaline absorption (Beck et al., 1997). The absorbed $CO_2$ was precipitated with 3N $BaCl_2$ and the remaining 0.1N NaOH back titrated with 0.1N HCl using phenolphthalein indicator until a colourless end point. Microbial biomass-C was calculated using the equation below (Eq. (1)). Three and five replicates were analyzed for controls and samples, respectively.

$$X = 40R + 0.37 \quad (1)$$

X is the concentration of soil microbial carbon (μg $g^{-1}$ soil)
R is the rate of $CO_2$ evolution (μl of $CO_2$ $g^{-1}$ soil $h^{-1}$)

Culturable heterotrophic bacteria and fungi were counted by the dilution plate count method (Wollum II, 1982). For each sample, three 5-g replicates of soil were aseptically weighed and transferred to dilution bottles containing 47.5 ml of sterile saline solution. Bottles were shaken for 15 min at 250 rev $min^{-1}$ and allowed to stand for 10 s. The suspensions were serially diluted and plated onto Plate Count Agar (Rand et al., 1975) and Rose Bengal (Martin, 1950) media, for bacterial and fungal counts, respectively. Plates were incubated at 25 °C during 7 days. After the incubation, colonies were counted.

### 2.4. Soil enzyme activities

We assessed β-glucosidase, protease and acid phosphatase activities which could be produced by microorganisms, plants and animals, and alkaline phosphatase activity which derived only from microorganisms and animals (Alef et al., 1995). In addition, we assessed the activity of dehydrogenases. 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). Results of activity were expressed as μg *p*-nitrophenol $g^{-1}$ dry soil $h^{-1}$. Soil protease (casein-hydrolyzing) activity was determined according to Ladd and Butler (1972). Results were expressed as μg tyrosine $g^{-1}$ dry soil $h^{-1}$. Dehydrogenase activity was measured using the method described by Malkomes (1993). The substrate used was 2,3,5 triphenyl tetrazolium chloride (TTC), and the results were expressed as μg triphenyl formazan (TPF) $g^{-1}$ dry soil $h^{-1}$. Samples were analyzed in triplicate and averaged. Controls were prepared adding the substrate after stopping the reaction. For dehydrogenase

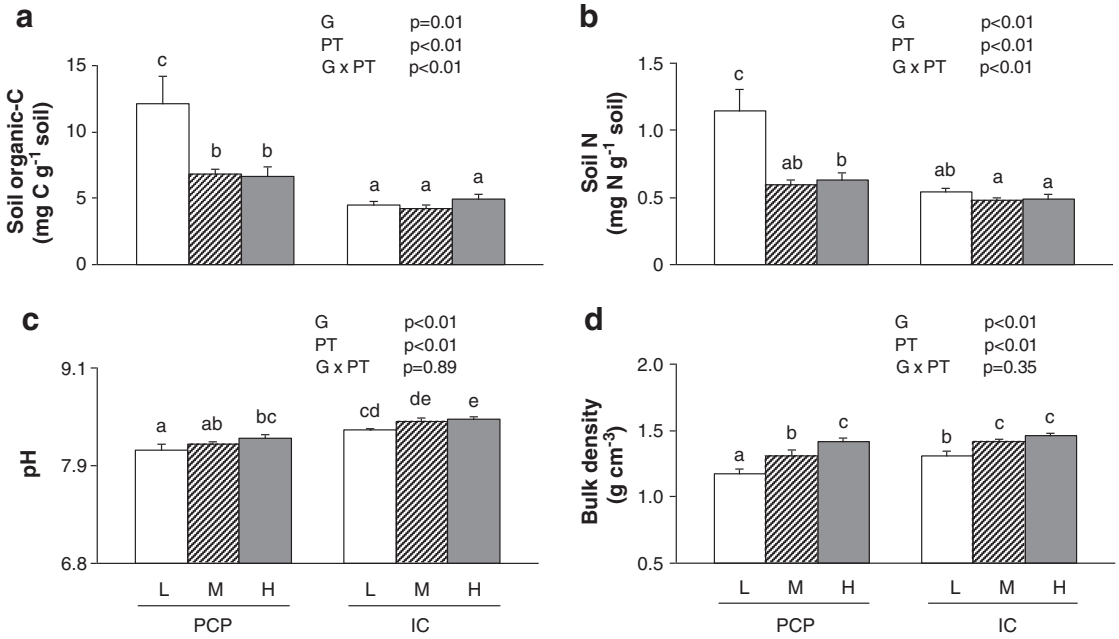


**Fig. 2.** Chemical and physical properties: a. organic-C, b. N, c. pH, and d. bulk density of soils at PCP (plant-covered patches) and IC (inter-canopy) areas with L (low), M (medium) and H (high) grazing intensities, $n = 30$. Vertical lines indicate one standard error. Different lowercase letters indicate significant differences among mean values of different treatments. G, grazing intensity; PT, patch type; and G × PT, grazing intensity × patch type interaction.

assay, controls were performed with Tris–HCl buffer instead of TTC. For each sample, the geometric mean of the assayed enzyme activities was calculated (Eq. (2)). This algorithm was used to assess soil quality (García-Ruiz et al., 2008) and recovery of polluted soils (Hinojosa et al., 2004).

$$GMea = (Glu \times AcP \times AlP \times Pro \times Dehy)^{1/5} \quad (2)$$

Glu, AcP, *AlP*, Pro and Dehy are β-glucosidase, acid phosphatase, alkaline phosphatase, protease and dehydrogenase activities, respectively.

### 2.5. Statistical analyses

The significance of the differences in the mean canopy diameter, and perennial grass, shrub and dwarf shrub covers among sites was evaluated by one-way ANOVA. Soil properties (including the GMea index) were analyzed by two-way ANOVA, being the factors the grazing intensity (L, M, and H) and the patch type (PCP, and IC). Data were checked to meet ANOVA assumptions. Except for the GMea index for which square root transformation was applied, natural logarithmic transformation was used when needed. Fisher Least Significant Difference was used for multiple comparisons. We only performed multiple comparisons in the cases of alkaline and acid phosphatase activities since they did not accomplish ANOVA assumptions. The relationships between the cover of plant life forms and soil biochemical properties at PCP were analysed by correlation analysis. Unless otherwise noted, the significance level was $P \leq 0.05$ throughout this study.

## 3. Results

### 3.1. Patch traits and soil chemical and physical parameters

The diameter of modal plant patches did not vary with grazing intensity. Perennial grass and dwarf shrub cover decreased with increasing grazing intensity while shrub cover did not change at PCPs (Table 1).

We found a significant effect of the interaction between grazing intensity and patch type on soil organic-C and soil-N. Both soil parameters varied with grazing only at PCPs and were significantly higher in L than in M and H (Fig. 2a–b). Soil pH and bulk density were significantly affected by grazing intensity at both patch types. These soil parameters were lower at PCP than at IC areas and they showed an increasing trend from L to H (Fig. 2c–d).

### 3.2. Soil microbiological properties

We observed a significant effect of the interaction between grazing intensity and patch type on microbial biomass-C and fungal counts. Both microbiological properties significantly increased in H in comparison with L and M at IC areas, while at PCPs the highest values were found in L and H (Fig. 3a–b). Heterotrophic bacterial counts did not show significant differences between PCPs and ICs, but they were significantly affected by grazing intensity, being higher in H than in L and M (Fig. 3c).

### 3.3. Soil enzyme activities

Most of the enzyme activities and the GMea index were significantly higher at PCP than at IC areas (Fig. 4). We did not find effects of grazing intensity on dehydrogenase, β-glucosidase, acid phosphatase and alkaline phosphatase activities (Fig. 4a–d). Protease activity decreased from L to H at IC soils (Fig. 4e). Grazing intensity had a marginal effect on the GMea index; having L at PCP the largest value (Fig. 4f).

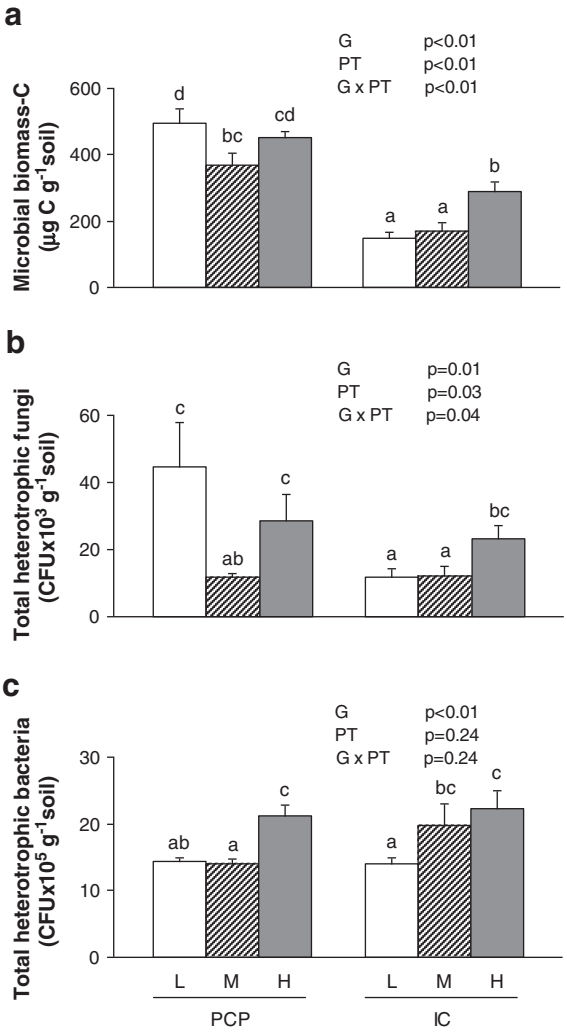


**Fig. 3.** Microbiological properties: a. microbial biomass-C, b. fungal and c. bacterial counts of soils at PCP and IC areas with L, M and H grazing intensities, $n = 30$. Vertical lines indicate one standard error. Different lowercase letters indicate significant differences among mean values of different treatments. G, grazing intensity; PT, patch type; and G × PT, grazing intensity × patch type interaction. Acronyms of patch type and grazing intensity as in Fig. 2.

### 3.4. Relationship between the cover of plant life forms and soil biochemical properties at PCPs

Shrub and dwarf shrub covers were not significantly correlated to soil biochemical properties (data not shown). Perennial grass cover was positively correlated with soil organic-C, soil-N, and acid phosphatase activity (Table 2). Soil organic-C was positively correlated with soil-N, acid and alkaline phosphatases, and β-glucosidase. A marginal correlation was observed between soil organic-C and microbial biomass-C. Enzyme activities were always correlated among them (Table 2).

## 4. Discussion

Long-term grazing disturbance leads directly or indirectly to changes in the plant cover, soil physicochemical properties and soil biological activities (Bardgett and Wardle, 2003; Bardgett et al., 1998; Schlesinger et al., 1990; Yates et al., 2000). In our study, increasing grazing intensity (low, medium, and high) was associated to a raise in soil pH and bulk density at both plant-covered patches (PCP) and inter-canopy (IC) areas. This is consistent with higher trampling (Manzano and Návar, 2000; Steffens et al., 2008; Yates et al., 2000)

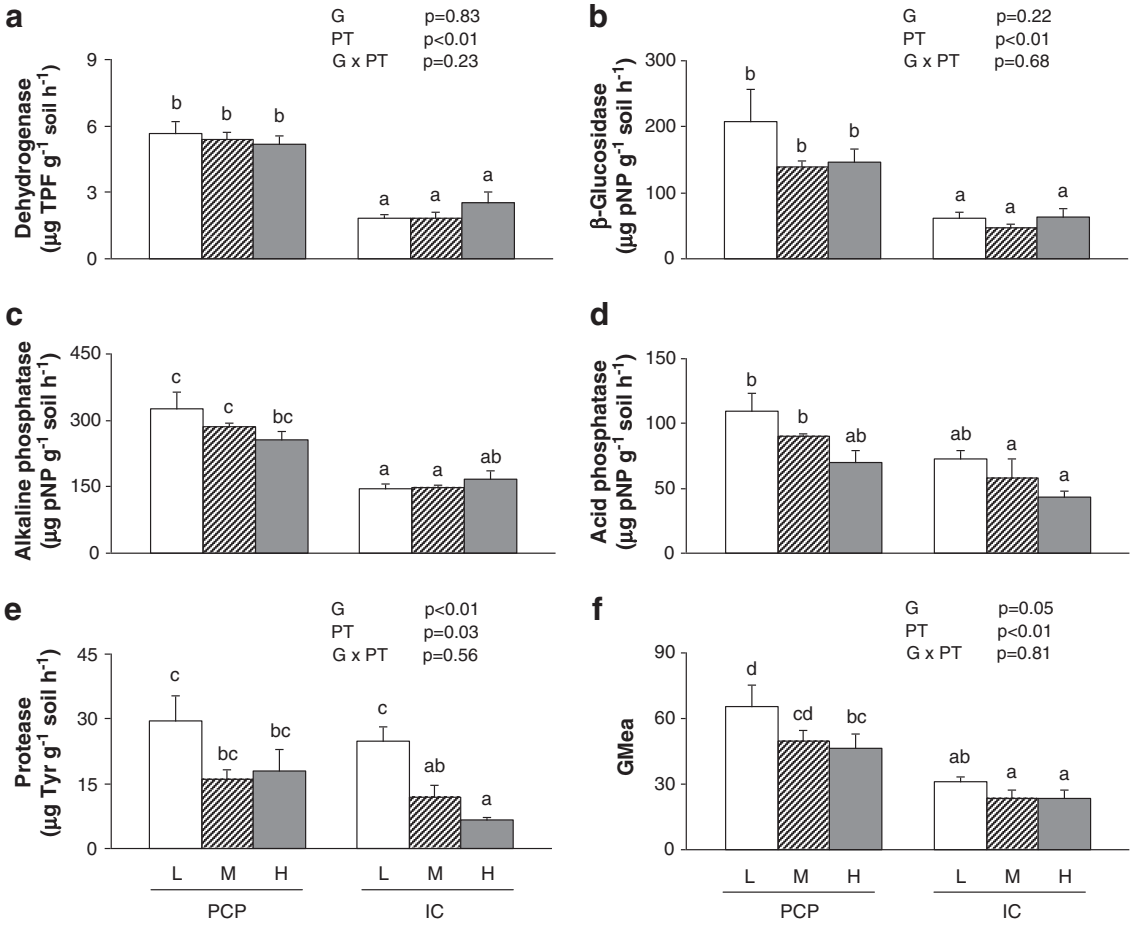


**Fig. 4.** Enzyme activities: a. dehydrogenase, b. ß-glucosidase, c. alkaline phosphatase, d. acid phosphatase, e. protease, and f. GMea (geometric mean for the assayed enzyme activities) at PCP and IC areas with L, M and H grazing intensities, $n = 30$. Vertical lines indicate one standard error. Different lowercase letters indicate significant differences among mean values of different treatments. G, grazing intensity; PT, patch type; and G × PT, grazing intensity × patch type interaction. Acronyms of patch type and grazing intensity as in Fig. 2.

and contribution of sheep urine inputs in H than in L. Urine inputs could increase soil pH through the release of $OH^-$ during the hydrolysis of urea to $NH_4^+$ rising also inorganic N in soil (Ma et al., 2007; Shand et al., 2002). Moreover, the increase of microbial biomass-C and heterotrophic microbial counts registered for H at most IC areas, could be considered as a response of heterotrophic microorganisms to the increase of inorganic N in soil mediated by urine inputs. Heterotrophic microorganisms are recognized as fast growing degraders of labile-C and could be favoured by the source of nutrients (e.g. urea-N) derived from grazer's dejections under intense grazing (Wardle, 2002). However, the positive effects of fecal-urine inputs on soil biota may be highly localized in unproductive ecosystems (Bardgett and Wardle, 2003) resulting in a limited response of plant productivity to those inputs at the ecosystem scale.

Grazing negatively affected the perennial grass cover. This change was associated with the reduction in soil organic-C leading to a decrease of ß-glucosidase and phosphatase activities (Table 2). Results suggest that grazing influenced the potential of organic matter decomposition and nutrient cycling resulting in low soil organic matter turnover and nutrient release. This was supported by an overall decrease in the hydrolytic enzyme activity (i.e. GMea index, Fig. 4f) at PCP of intense grazed sites. A decline in the activity of hydrolytic enzymes such as ß-glucosidases and phosphatases at PCPs may be explained by the fact that perennial grasses supply a more labile C-rich substrate of cellulolytic nature than woody plants, which usually produce recalcitrant litter for decomposers (Aerts and Chapin, 2000; Carrera et al., 2005). In this sense, Badiane et al. (2001) found higher ß-glucosidase activity in non-grazed plots dominated by herbaceous plants than in grazed plots with preponderance of woody plants. Besides, perennial grass cover was positively related to acid phosphatase activity (Table 2) indicating that the reduction of perennial grass cover in H sites may induce a reduction of hydrolyzed organic P to cope with P-stressed conditions. Further, soil protease activity significantly decreased with grazing intensity at ICs suggesting that N mineralization potential may be also highly affected by grazing. This may be explained by the inhibition of protease synthesis or changes in soil microorganism composition associated with the

**Table 2**
Pearson correlation coefficient between the perennial grass cover and soil biochemical properties at PCPs. C: soil organic-C, N: soil-N, Dehy: dehydrogenase, AlP: alkaline phosphatase, AcP: acid phosphatase, Glu: β-glucosidase, Pro: protease, and Mb: microbial biomass-C, respectively. *P<0.05, **P<0.01, ***P<0.001.

| | Grass cover | C | N | Dehy | AlP | AcP | Glu | Pro | Mb |
|---|---|---|---|---|---|---|---|---|---|
| Grass cover | 1.00 | | | | | | | | |
| C | 0.58* | 1.00 | | | | | | | |
| N | 0.53* | 0.85*** | 1.00 | | | | | | |
| Dehy | 0.02 | 0.41 | 0.26 | 1.00 | | | | | |
| AlP | 0.30 | 0.54* | 0.41 | 0.84*** | 1.00 | | | | |
| AcP | 0.53* | 0.68** | 0.55* | 0.70** | 0.91*** | 1.00 | | | |
| Glu | 0.09 | 0.66** | 0.52 | 0.82*** | 0.89*** | 0.77*** | 1.00 | | |
| Pro | 0.27 | 0.45 | 0.41 | 0.57* | 0.79*** | 0.64** | 0.76*** | 1.00 | |
| Mb | 0.03 | 0.51 | 0.39 | 0.41 | 0.31 | 0.32 | 0.51 | 0.36 | 1.00 |

local N-urine input from animals (Nunan et al., 2006; Patra et al., 2005). Protease activity can also vary independently of microbial activity due to the formation of inorganic and organic colloids in soil (Makoi and Ndakidemi, 2008). Since N is a limiting nutrient in arid ecosystem (Mazzarino et al., 1996, 1998), the reduction of protease activity could negatively affect N availability and hence plant growth mainly at IC areas in intensively grazed sites.

Lastly, our findings highlighted that PCP soil had higher values of microbial biomass-C, fungal counts, and enzyme activities (dehydrogenase, ß-glucosidase, phosphatases, and proteases) than soil of IC areas at all grazing intensities. These results are in agreement with findings of other studies on hydrolase activities in semiarid soils (Garcia et al., 2005) and measurements of ß-glucosidase activity reported by Gonzalez-Polo and Austin (2009) in other ecosystems of Patagonia. These differences between PCP and IC areas were attributed to the direct effects of plants on soil organic matter and on abiotic and biotic conditions under their canopies.

Effects of grazing on soil organic-C and soil-N were larger at PCP than at IC areas and these changes strongly affected enzyme activities. These findings highlight the role of plant canopies on soil processes (Burke et al., 1999; Carrera et al., 2003, 2009; Mazzarino et al., 1998). Accordingly, our results emphasize the importance of conserving and maintaining plant-covered patches for microbial and enzyme activities related to C and nutrient cycling (Adler et al., 2001).

## 5. Conclusion

We concluded that grazing mostly affected soil physicochemical and biological parameters through the direct effect of trampling and urine inputs and indirectly through its effects on the perennial grass cover, which supplies a more labile C-rich substrate than shrubs. Our results demonstrated a reduction of perennial grass cover and soil organic-C along with a decline in ß-glucosidase, phosphatase, and protease activities with increasing grazing. Thus, the reduction of perennial grass cover induced by grazing intensity affected negatively soil enzyme and microbial activities related with C, P, and N cycling. In addition, increasing grazing intensity was associated with increasing values of microbial biomass-C and heterotrophic microorganism counts mostly at IC areas, suggesting that inputs of labile N by urine and dung may partially counteract the negative grazing effects.

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## References

Adler, P.B., Raff, D.A., Lauenroth, W.K., 2001. The effect of grazing on the spatial heterogeneity of vegetation. Oecologia 128, 465–479.
Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1–67.
Alef, K., Nannipieri, P., Trazar-Cepeda, C., 1995. Enzyme activities. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 311–373.
Alkorta, I., Aizpurua, A., Riga, P., Albizu, I., Amezaga, I., Garbisu, C., 2003. Soil enzyme activities as biological indicators of soil health. Rev. Environ. Health 18, 65–73.
Ares, J., Bertiller, M., Bisigato, A., 2003. Modeling and measurement of structural changes at a landscape scale in dryland areas. Environ. Model. Assess. 8, 1–13.
Badiane, N.N.Y., Chotte, J.L., Patea, E., Masse, D., Rouland, C., 2001. Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. Appl. Soil Ecol. 18, 229–238.
Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471–1479.
Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84, 2258–2267.
Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biol. Biochem. 30, 1867–1878.
Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., Scheu, S., 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol. Biochem. 29, 1023–1032.
Bending, G.D., Turner, M.K., Rayns, F., Marx, M.C., Wood, M., 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biol. Biochem. 36, 1785–1792.
Bertiller, M.B., Ares, J.O., 2008. Sheep spatial grazing strategies at the arid Patagonian Monte, Argentina. Rangeland Ecol. Manag. 6, 38–47.
Bertiller, M.B., Bisigato, A.J., 1998. Vegetation dynamics under grazing disturbance. The state and transition model for the Patagonian Steppes. Ecología Austral. 8, 191–199.
Bertiller, M.B., Ares, J.O., Bisigato, A.J., 2002. Multi-scale indicators of land degradation in the Patagonian Monte, Argentina. Environ. Manage. 30, 704–715.
Bisigato, A.J., Bertiller, M.B., 1997. Grazing effects on patchy dryland vegetation in Northern Patagonia. J. Arid Environ. 36, 639–653.
Blake, G.R., 1965. Bulk density. In: Black, C.A., Evans, D.D., White, J.L., Ensminger, L.E., Clark, F.E. (Eds.), Methods of Soil Analysis, Part 1, Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling. American Society of Agronomy, Madison, pp. 374–390.
Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp. 595–624.
Burke, I.C., Lauenroth, W.K., Riggle, R., Brannen, P., Medigan, B., Beard, S., 1999. Spatial variability of soil properties in the shortgrass steppe: the relative importance of topography, grazing, microsite, and plant species in controlling spatial patterns. Ecosystems 2, 422–438.
Carrera, A.L., Bertiller, M.B., 2010. Relationships among plant litter, fine roots and soil organic-C and N across an aridity gradient in northern Patagonia, Argentina. Ecoscience 17, 276–286.
Carrera, A.L., Bertiller, M.B., Sain, C.L., Mazzarino, M.J., 2003. Relationship between plant nitrogen conservation strategies and the dynamics of soil nitrogen in the arid Patagonian Monte, Argentina. Plant Soil 255, 595–604.
Carrera, A.L., Vargas, D.N., Campanella, M.V., Bertiller, M.B., Sain, C.L., Mazzarino, M.J., 2005. Soil nitrogen in relation to quality and decomposability of plant litter in the Patagonian Monte, Argentina. Plant Ecol. 181, 139–151.
Carrera, A., Mazzarino, M., Bertiller, M., del Valle, H., Martinez-Carretero, E., 2009. Plant impacts on nitrogen and carbon cycling in the Monte Phytogeographical Province, Argentina. J. Arid Environ. 73, 192–201.
Collins, S.L., Sinsabaugh, R.L., Crenshaw, C., Green, L.E., Porras-Alfaro, A., Stursova, M., Zeglin, L.H., 2008. Pulse dynamics and microbial processes in aridland ecosytems. J. Ecol. 96, 413–420.
Craine, J.M., Ballantyne, F., Peel, M., Zambatis, N., Morrow, C., Stock, W., 2009. Grazing and landscape controls on nitrogen availability across 330 South African sites. Aust. Ecol. 34, 731–740.
del Valle, H.F., 1998. Patagonian soils: a regional sintesis. Ecol. Aust. 8, 103–125.
Dick, R.O., Breakwell, D.P., Turco, R.F., 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran, J.W., Jones, A.J. (Eds.), Methods for Assessing Soil Quality, Special Publication 49. Soil Science Society of America, Madison, pp. 247–271.
Dodor, D.E., Tabatabai, M.A., 2005. Glycosidases in soils as affected by cropping systems. J. Plant Nutr. Soil Sci. 168, 749–758.
Garcia, C., Hernández, T., Costa, F., 1994. Microbial activity in soils under Mediterranean environmental conditions. Soil Biol. Biochem. 26, 1185–1191.
Garcia, C., Hernández, T., Roldán, A., Martín, A., 2002. Effect of plant cover decline on chemical and microbiological parameters under Mediterranean climate. Soil Biol. Biochem. 34, 635–642.
Garcia, C., Roldan, A., Hernandez, T., 2005. Ability of different plant species to promote microbiological processes in semiarid soil. Geoderma 124, 193–202.
García-Ruiz, R., Ochoa, V., Hinojosa, M.B., Carreira, J.A., 2008. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol. Biochem. 40, 2137–2145.
Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M.C., Seoane, S., 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biol. Biochem. 37, 877–887.
Gonzalez-Polo, M., Austin, A.T., 2009. Spatial heterogeneity provides organic matter refuges for soil microbial activity in the Patagonian steppe, Argentina. Soil Biol. Biochem. 41, 1348–1351.
Haynes, R.J., Williams, P.H., 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Adv. Agron. 49, 119–199.
Hinojosa, M.B., García-Ruíz, R., Viñegla, B., Carreira, J.A., 2004. Microbiological rates and enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill. Soil Biol. Biochem. 36, 1637–1644.
Hiradate, S., Furubayashi, A., Uchida, N., Fuji, Y., 2007. Adsorption of 2,4-dichlorophenoxyacetic acid by an andosol. J. Environ. Qual. 36, 101–109.
Holt, J.A., 1997. Grazing pressure and soil carbon, microbial biomass and enzyme activities in semi-arid northeastern Australia. Appl. Soil Ecol. 5, 143–149.
ISO 14240–1, 1997. Soil quality – determination of soil microbial biomass, part 1: substrate-induced respiration method. International Organization for Standardization, Geneva, Switzerland.
Jenny, H., 1980. The soil resource: origin and behavior. In: Billings, W.D., Lange, O.L., Remmert, K. (Eds.), Ecological Studies No. 37. Springer, New York.

Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using protein and dipeptide derivatives as substrates. Soil Biol. Biochem. 4, 19–30.
Lal, R., 2004. Carbon sequestration in dryland ecosystems. Environ. Manage. 33, 528–544.
Lechmere-Oertel, R.G., Cowling, R.M., Kerley, G.I.H., 2005. Landscape dysfunction and reduced spatial heterogeneity in soil resources and fertility in semi-arid succulent thicket, South Africa. Aust. Ecol. 30, 615–624.
Lee, S.H., Oh, B., Kim, J., 2008. Effect of various amendments on heavy mineral oil bioremediation and soil microbial activity. Bioresour. Technol. 99, 2578–2587.
León, R.J.C., Bran, D., Collantes, M., Paruelo, J.M., Soriano, A., 1998. Grandes unidades de la vegetación de la Patagonia extra andina. Ecol. Aust. 8, 125–144.
Ma, X., Wang, S., Jiang, G., Haneklaus, S., Schnug, E., Nyren, P., 2007. Short-term effect of targeted placements of sheep excrement on grassland in inner Mongolia on soil and plant parameters. Commun. Soil Sci. Plant Anal. 38, 1589–1604.
Makoi, J.H.J.R., Ndakidemi, P.A., 2008. Selected soil enzymes: examples of their potential roles in the ecosystem. Afr. J. Biotechnol. 7, 181–191.
Malkomes, H.P., 1993. Eine modifizierte Methode zur Erfassung der Dehydrogenaseaktivität (TTC-Reduktion) im Bodem nach Herbizidanwendung. A modified method to examine the dehydrogenase activity (TTC reduction) in soil after herbicide treatments. Nachrichtenbl. Dtsch. Pflanzenschutzdienst 45, 180–185 (In German, with English Abstract).
Manzano, M.G., Návar, J., 2000. Processes of desertification by goats overgrazing in the Tamaulipan thornscrub (matorral) in north-eastern Mexico. J. Arid Environ. 44, 1–17.
Martin, J.P., 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69, 215–232.
Mazzarino, M.J., Bertiller, M.B., Sain, C.L., Laos, F., Coronato, F.R., 1996. Spatial patterns of nitrogen availability, mineralization, and immobilization in northern Patagonia, Argentina. Arid Soil Res. Rehabil. 10, 295–309.
Mazzarino, M.J., Bertiller, M.B., Sain, C., Satti, P., Coronato, F., 1998. Soil nitrogen dynamics in northeastern Patagonia Steppe under different precipitation regimes. Plant Soil 202, 125–131.
Moscatelli, M.C., Di Tizio, A., Marinari, S., Grego, S., 2007. Microbial indicators related to soil carbon in Mediterranean land use systems. Soil Tillage Res. 97, 51–59.
Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns, R.G., Dick, R.P. (Eds.), Enzymes in the Environment. Activity, Ecology and Applications. Marcel Dekker, New York, pp. 1–33.
Ndakidemi, P.A., 2006. Manipulation legume/cereal mixtures to optimize the above and below ground interactions in the traditional African cropping systems. Afr. J. Biotechnol. 5, 2526–2533.
Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp. 539–579.
Noy-Meir, I., 1973. Desert ecosystems: environment and producers. Annu. Rev. Ecol. Syst. 4, 25–52.
Nunan, N., Singh, B., Reid, E., Ord, B., Papert, A., Squires, J., Prosser, J.I., Wheatley, R.E., McNicol, J., Millard, P., 2006. Sheep-urine-induced changes in soil microbial community structure. FEMS Microbiol. Ecol. 56, 310–320.
Patra, A.K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S.J., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J.I., Richaume, A., Le Roux, X., 2005. Effects of grazing on microbial functional groups involved in soil N dynamics. Ecol. Monogr. 75, 65–80.
Patra, A.K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S.J., Guillaumaud, N., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J.I., Le Roux, X., 2006. Effects of management regime and plant species on the enzyme activity and genetic structure of N-fixing, denitrifying and nitrifying bacterial communities in grassland soils. Environ. Microbiol. 8, 1005–1016.
Patra, A.K., Le Roux, X., Grayston, S.J., Loiseau, P., Louault, F., 2007. Unraveling the effects of management regime and plant species on soil organic carbon and microbial phospholipid fatty acid profiles in grassland soils. Bioresour. Technol. 99, 3545–3551.
Rand, M.C., Greenberg, A.E., Taras, M.J., Franson, M.A., 1975. Standard Methods for Examination of Water and Wastewater. American Public Health Association, Washington, D.C.
Reynolds, J.F., Kemp, P.R., Ogle, K., Fernandez, R.J., 2004. Modifying the 'pulse-reserve' paradigm for deserts of North America: precipitation pulses, soil water and plant responses. Oecologia 141, 194–210.
Rodríguez, M.V., Bertiller, M.B., Sain, C.L., 2007. Spatial patterns and chemical characteristics of root biomass in ecosystems of the Patagonian Monte disturbed by grazing. J. Arid Environ. 70, 137–151.
Rutigliano, F.A., D'Ascoli, R., Virzo De Santo, A., 2004. Soil microbial metabolism and nutrient status in a Mediterranean area as affected by plant cover. Soil Biol. Biochem. 36, 1719–1729.
Saviozzi, A., Biasci, A., Riffaldi, R., Levi-Minzi, R., 2001. Long-term effects of farmyard manure and sewage sludge on some biochemical characteristics. Plant Soil 233, 251–259.
Schlesinger, W.H., Reynolds, J.F., Cunningham, G.L., Huenneke, L.F., Jarrell, W.M., Virginia, R.A., Whitford, W.G., 1990. Biological feedbacks in global desertification. Science 247, 1043–1048.
Shand, C.A., Williams, B.L., Dawson, L.A., Smith, S., Young, M.E., 2002. Sheep urine affects soil solution nutrient composition and roots: differences between field and sward box soils and the effects of synthetic and natural sheep urine. Soil Biol. Biochem. 34, 163–171.
Steffens, M., Kölbl, A., Totsche, K.U., Kögel-Knabner, I., 2008. Grazing effects on soil chemical and physical properties in semiarid steppe of Inner Mongolia (P.R. China). Geoderma 143, 63–72.
Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A., 2005. Principles and applications of soil microbiology, Second ed. Pearson Education, Upper Saddle River, New Jersey.
Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, M.A., Wollum, A. (Eds.), Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. SSSA Book Series 5, Madison, pp. 775–833.
Tongway, D.J., Sparrow, A.D., Friedel, M.H., 2003. Degradation and recovery processes in arid grazing lands of central Australia. Part 1: soil and land resources. J. Arid Environ. 55, 301–326.
Vargas, D.N., Bertiller, M.B., Ares, J.O., Carrera, A.L., Sain, C.L., 2006. Soil C and N dynamics by leaf-litter decomposition of shrubs and perennial grasses of the Patagonian Monte. Soil Biol. Biochem. 38, 2401–2410.
Wardle, D.A., 2002. Communities and ecosystems. Linking the aboveground and belowground components. Princeton University Press, New Jersey.
Whitford, W., 2002. Decomposition and nutrient cycling. In: Whitford, W. (Ed.), Ecology of Desert Systems. Academic Press, San Diego, pp. 235–274.
Wick, B., Kühne, R.F., Vielhauer, K., Vlek, P.L.G., 2002. Temporal variability of selected soil microbiological indicators under different soil quality conditions in south-western Nigeria. Biol. Fertil. Soils 35, 155–167.
Winding, A., Hund-Rinke, K., Rutgers, M., 2005. The use of microorganisms in ecological soil classification and assessment concepts. Ecotoxicol. Environ. Saf. 62, 230–248.
Wollum II, A.G., 1982. Cultural methods for soil microorganisms. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp. 781–802.
Yates, C.J., Norton, D.A., Hobbs, R.J., 2000. Grazing effects on plant cover, soil and microclimate in fragmented woodlands in south-western Australia: implications for restoration. Aust. Ecol. 25, 36–47.