Ecosystems (2009) 12: 686–697
DOI: 10.1007/s10021-009-9252-6

ECOSYSTEMS

Sheep Grazing Decreases Organic Carbon and Nitrogen Pools in the Patagonian Steppe: Combination of Direct and Indirect Effects

Rodolfo A. Golluscio,[1,4]* Amy T. Austin,[1] Guillermo C. García Martínez,[1] Marina Gonzalez-Polo,[1] Osvaldo E. Sala,[2] and Robert B. Jackson[3]

[1]Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina; [2]Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island, USA; [3]Department of Biology and Nicholas School of the Environment, Duke University, Durham, North Carolina, USA; [4]Department of Animal Production, and IFEVA (Facultad de Agronomía, UBA-CONICET), Av. San Martín 4453, Buenos Aires, Argentina

ABSTRACT

We explored the net effects of grazing on soil C and N pools in a Patagonian shrub–grass steppe (temperate South America). Net effects result from the combination of direct impacts of grazing on biogeochemical characteristics of microsites with indirect effects on relative cover of vegetated and unvegetated microsites. Within five independent areas, we sampled surface soils in sites subjected to three grazing intensities: (1) ungrazed sites inside grazing exclosures, (2) moderately grazed sites adjacent to them, and (3) intensely grazed sites within the same paddock. Grazing significantly reduced soil C and N pools, although this pattern was clearest in intensely grazed sites. This net effect was due to the combination of a direct reduction of soil N content in bare soil patches, and indirect effects mediated by the increase of the cover of bare soil microsites, with lower C and N content than either grass or shrub microsites. This increase in bare soil cover was accompanied by a reduction in cover of preferred grass species and standing dead material. Finally, stable isotope signatures varied significantly among grazed and ungrazed sites, with $\delta^{15}N$ and $\delta^{13}C$ significantly depleted in intensely grazed sites, suggesting reduced mineralization with increased grazing intensity. In the Patagonian steppe, grazing appears to exert a negative effect on soil C and N cycles; sound management practices must incorporate the importance of species shifts within life form, and the critical role of standing dead material in maintaining soil C and N stocks and biogeochemical processes.

Key words: $\delta^{15}N$; $\delta^{13}C$; stable isotopes; semiarid ecosystems; biogeochemistry; shrub–grass steppe; Argentina; desertification; life forms.

Received 15 July 2008; accepted 12 March 2009;

Electronic supplementary material: The online version of this article (doi:10.1007/s10021-009-9252-6) contains supplementary material, which is available to authorized users.

Author Contributions: RAG designed study, performed research, analyzed data, wrote the paper; ATA designed study, wrote the paper; CGGM designed study, performed research, analyzed data; MGP performed research; OES designed study; RBJ designed study, contributed new methods.

**Corresponding author; e-mail:* golluscl@ifeva.edu.ar

INTRODUCTION

Organic matter is the main reservoir of carbon and nitrogen in terrestrial ecosystems, and internal recycling of nutrients is the major source of nutrients determining plant productivity in these ecosystems. The main inputs to the soil organic matter (SOM) pool come from decomposition of above- and belowground plant litter and animal excreta. The main outputs from the SOM pool are the mineralization and leaching of carbon and nitrogen. Interestingly, soil erosion can act as either an input or output flux, depending on the destination of the material and its interaction with the vegetation (Schlesinger 1997).

Grazing by domestic livestock is one of the major human activities in semiarid rangelands around the world (Gillson and Hoffman 2007). Grazing can directly affect soil C and N pools, depending on the relative magnitude of its effect on each flux (Frank and Evans 1997; McNaughton and others 1997). Grazing can also indirectly affect input and output fluxes to SOM because it can induce physiognomic and/or floristic changes in the plant communities (Milchunas and Lauenroth 1993; Burke and others 1997). These structural changes can indirectly alter SOM pools and fluxes through changes in the quantity of senescent material entering SOM pools or changes in litter quality, which can affect carbon and nutrient mineralization rates (Wedin and Tilman 1990; Hibbard and others 2001). In semiarid rangelands the combination of low precipitation and coarse textured soils also increase the probability that grazing affects SOM stocks and dynamics (Burke and others 1997). Scarce precipitation translates to low net primary production, and low SOM concentration, whereas coarse texture is associated with high proportions of active labile SOM fractions (Burke and others 1997).

The use of stable isotopes as integrated measures of ecosystem processes has expanded markedly in the last two decades. The use of the natural abundance of stable carbon and nitrogen isotopes (δ^{13}C and δ^{15}N) have proven particularly useful for interpreting biogeochemical patterns at the regional and site scales (Ehleringer and others 2000; Robinson 2001; Dawson and others 2002). For example, at the regional scale (that is, when comparing different plant communities growing under different edaphic or climatic conditions), δ^{15}N can reflect integrated climatic and biotic processes affecting N cycling (Austin and Vitousek 1998; Amundson and others 2003). On the other hand, at the local scale (that is, when comparing different sites pertaining to the same plant community and growing under the same edaphic and climatic conditions) variation in soil δ^{15}N can indicate differences in: (1) the stable isotope composition of plant litter and roots that potentially enter the SOM pool, (2) the selective degradation of compounds that have different stable isotope signatures, and (3) variation in biological processes that discriminate for the lighter isotopes of N (Dawson and others 2002). As grazing can impact all three local sources of variation, our understanding of how grazing intensity affects ecosystem δ^{15}N signatures to date has not yielded a clear pattern (Schulze and others 1998; Austin and Sala 1999; Cook 2001). However, increased mineralization and losses via ammonia volatilization in urine suggest that enrichment of soil ^{15}N could occur with increased grazing intensity (Frank and Evans 1997; Austin and Sala 1999).

Domestic grazing causes changes in the structure of plant communities in a range of ecosystems (for example, Schuman and others 1999), and the Patagonian steppe is no exception. The history of grazing with domestic herbivores in Patagonia is relatively short, because sheep husbandry began at the late nineteenth century. However, it caused severe degradation in several ecosystems of the region, mainly because of continuous grazing over extensive and highly heterogeneous paddocks (Golluscio and others 1998). Although sheep grazing in this region is widespread, the effects on biogeochemical cycling of these alterations in plant community structure and composition are not well understood. Studies examining grazing effects on vegetation in the region have shown an overall loss of plant cover, but rarely with substantial shifts in the dominant life form (Cipriotti and Aguiar 2005; Bisigato and others 2005), in contrast with other grazed semiarid ecosystems elsewhere (Scholes and Archer 1997; Hibbard and others 2001; Knapp and others 2008). In most cases, grazing decreased the abundance of the low-cover palatable species (that is, primarily herbaceous species and some grasses), increased the relative cover of unpalatable grass species (Perelman and others 1997; Pazos and others 2007), and magnified the patchiness of the vegetation (Cipriotti and Aguiar 2005; Bisigato and others 2005).

The objective of this article was to evaluate the direct and indirect effects of grazing intensity on soil C and N pools and turnover in the semiarid Patagonian steppe. Our hypotheses were: (1) domestic grazing directly reduces pool sizes of soil C and N and their turnover due to changes in the biogeochemical characteristics below vegetated and unvegetated patches (microsites), and (2) grazing affects soil C and N pools indirectly via changes in the relative importance of different microsites, and, in particular, decreases in plant cover and shifts in the proportion of preferred and unpreferred grass species. Because grazing generally reduces plant cover, we expect that reduced litter inputs to the system (per soil surface unit) would cause a direct negative effect of grazing on SOC (soil organic carbon) and SON (soil organic nitrogen) content. On the other hand, plant traits that promote animal grazing preference are frequently associated with the promotion of decomposer microorganism activity (Wardle and Bardgett 2004). As a consequence, we expect that floristic and structural changes induced by grazing will have an indirect negative effect on the rates of both decomposition and mineralization processes (at the local scale).

Our experimental approach consisted of measuring SOM properties related to C and N stocks and mineralization rates along five replicates of a grazing intensity gradient: Ungrazed (U) versus Moderately grazed (M) versus Intensely grazed (I). Although there are other factors that could affect organic C and N pools, the principal difference among the 15 experimental units was grazing intensity, with soil texture, slope, relief, climate, and soil age being constant across sites. We explored the net effects of grazing on soil C and N pools, resulting from the combination of direct impacts of grazing on biogeochemical characteristics of microsites with indirect effects on relative cover of vegetated and unvegetated microsites. We analyzed the variation in the cover and floristic composition of microsites occupied by grasses, shrubs, and bare soil (indirect effects). We stratified our analyses of grazing effect on soil C and N pools within these three coarse categories (direct effects). Our experimental approach did not allow evaluating the direct effects of grazing on soil properties at the more detailed level of grass or shrub species. As a consequence, some direct effects of grazing on the chemical traits of soil below grasses or shrubs may actually mask indirect effects of grazing on the floristic composition of grass or shrub microsites. Our response variables were the C and N concentrations, as indicative of their pool sizes, and the natural abundance of the heavy isotopes of nitrogen and carbon (δ^{13}C and δ^{15}N) as an integrative measure of direct and indirect effects of grazing on ecosystem processes.

METHODS

Study site

We conducted the study in January 2004 (Southern Hemisphere summer) at the experimental field station of INTA (Instituto Nacional de Tecnología Agropecuaria) of Río Mayo, Southwest Chubut Province (Latitude 45°41′S, Longitude 70°16′W). Average annual precipitation is 150 mm, 70% of which falls in autumn–winter, whereas mean monthly temperature varies from 2 to 7°C (Sala and others 1989). Soils (Petrocalcic Calciorthids, Golluscio and others 1982) have a shallow sandy layer until 40 cm depth and low SOM content (0.4%, Paruelo and others 1988). Below the upper layer is a sandy-clay layer with high gravel and calcium carbonate content (Paruelo and others 1988). Mean aboveground net primary production is 56 g m^{-2} y^{-1}, with half corresponding to grasses and half to shrubs (Jobbágy and Sala 2000).

The sampling areas are representative of the semiarid steppe of the Patagonian Occidental District, which occupies about 30% of the Patagonian phytogeographic province (Golluscio and Oesterheld 2007). The dominant plant community in the area is a shrub–grass steppe, whose dominant shrub species are *Mulinum spinosum* (Cav). Pers, *Adesmia volckmannii* Phil., and *Senecio filaginoides* DC. (Golluscio and others 1982). The first two species have palatable leaves and flowers, but both are defended from herbivores by spines. The third shrub species, *Senecio filaginoides*, is chemically defended against herbivores by secondary metabolites inducible as a response to herbivory (Cavagnaro and others 2003). The dominant tussock grasses are *Stipa speciosa* Trin. et Rupr., *Stipa humilis* Cav., and *Poa ligularis* Nees ap. Steud., locally named ‘‘coirones’’ (Golluscio and others 1982). Among them, only *Poa ligularis* (less than 5% cover) is highly preferred by sheep. Some other low-cover grasses (*Poa lanuginosa* Poir., *Hordeum comosum* Presl., *Bromus pictus* Hook., and *B. setifolius* Presl.) and forbs (*Calceolaria polyrhiza* Cav., *Rodophiala elwesii* (C.H. Wright) Traub, *Leuceria millefolium* Dusén et Skottsb., *Valeriana clarioneifolia* Phil., *Euphorbia collina* Phil., *Cerastium arvense* L., and so on) are also included in the sheep diet (Somlo and others 1997).

Experimental Design and Analysis

In January 2004, we selected five sampling areas, and three sites within each of them subjected to a gradient in grazing intensity: one site fenced to exclude large herbivores (Ungrazed, U), another moderately grazed (Moderate, M: 0.1–0.3 sheep ha^{-1}), and another highly grazed (Intense, I) located in the same paddock but closer to the water point than the other two. All sites were of similar slope, aspect, and topography, as this region of the Patagonian steppe is a flat extensive plain, with very little change in topography or elevation. This experimental design has five true replications for the three levels of grazing intensity (total = 15 experimental units), an important advantage over some studies using grazing exclosures, which are often pseudoreplicated (Hurlbert 1984). However, as in most mensurative experiments, the replicates of the different treatments were not completely comparable. Specifically, the five sampling areas differed in the time of enclosure: 1954 (50 years before sampling), 1972 (32 years), 1983 (21 years), 1994 (10 years), and 1998 (6 years). To account for this problem, we considered the five sampling areas as blocks within a complete randomized block design (15 experimental units, with the mean of 25 samples within each unit being used for the analysis), and, when possible, we included a term in our statistical model to analyze if the effect of grazing on soil properties varied with the age of exclosure.

The distance between the three sites within each sampling area ranged from 50 to 500 m, and the distance among the five sampling areas ranged from 1,000 to 10,000 m. The moderately grazed site was located adjacent to the grazing exclosure. The heavily grazed site was located outside a circle of 500 m radius centered in the water point most closely associated with the grazing exclosure. Within this 500 m circle, the input of feces and urine, and plant mortality are disproportionately high because animals remain longer than expected based only on forage availability and as such, was excluded as part of the sampling area. In each site, we counted sheep fecal pellets along a 100 m-long × 0.2 m-wide line as an independent index of grazing pressure (Lange and Willcocks 1978). On the same 100 m-long line, we measured aerial cover of each plant species, bare soil, and standing dead plant material by using the line interception method, and obtained 25 soil samples (0.05 m diameter × 0.05 m depth) of the top 5 cm every 4 m. For each soil sample, we recorded the respective microsite (bare soil, grass, or shrub). We sampled the first 5 cm of soil to maximize the chances of detecting grazing effects: the concentration of both soil C and N, and the ratio of active-fraction to overall-SOM are highest in the upper layers (Burke and others 1997).

Analyses of soil nutrient content (g kg^{-1} C and g kg^{-1} N) and stable isotopes were completed at the Duke Environmental Stable Isotope Laboratory, using a CarloErba Elemental Analyzer to measure C and N content, and a Finnigan MAT Delta Plus XL mass spectrometer to measure δ^{13}C (standard: Vienna PDB) and δ^{15}N (standard: atmospheric N_2). Soil samples were oven dried at 60°C for 48 h, then ground with mortar and pestle until samples could pass a 40 μm mesh. Soils were not acid-treated prior to analysis as preliminary testing showed a very low level of carbonates ($<1\%$; Paruelo and others 1988) and as such, bulk soils were analyzed without treatment.

Statistical Analysis

To evaluate the statistical significance of *the net effects* of grazing on each response variable we performed an analysis of variance (ANOVA) among grazing levels (Ungrazed, Moderate, and Intense), with sampling areas as blocks, on the means of the 25 values obtained for each sampling site (total $n = 15$). This analysis did not allow us to analyze the significance of the 'grazing × block' interaction because this interaction is the error term of the analysis.

To evaluate the *direct effect* of grazing within each microsite, and the chemical differences among microsites, we performed an ANOVA among grazing levels, with sample areas as blocks, and microsite as the split-plot factor within each grazing level. This analysis was done on the means of each microsite at each site (total $n = 45 = 3$ microsites $\times$ 15 experimental units). In the statistical model we also included a 'grazing × block' interaction term to analyze if the effect of grazing varied with the age of exclosure. When this interaction was statistically significant we explored the linear regression between the response variable of interest and the years of exclosure for each microsite at each grazing level. As the frequency of shrub-occupied microsites was less than 10% in 10 of the 15 experimental units, we only considered these microsites for the analysis of the among microsite differences, but excluded them from the analysis of the direct effect of grazing on the chemical composition of the different microsites.

To evaluate the *indirect effect* of grazing, mediated by the changes in the structure of the plant community, we performed the same analysis on the arc-sine transformed cover of each microsite (grasses, shrubs, herbs, bare soil, and standing dead litter) at each site within each sampling area. When we detected significant site × microsite interaction, we applied partial ANOVAs among the three grazing levels within each microsite (sampling areas = blocks). All data (or transformed data) satisfied the assumptions of homogeneity of variance, with normal distributions. We completed all statistical analyses with Statistica (Statsoft Inc., Tulsa OK, USA) and Infostat (Córdoba University, Argentina) software, and in all cases used a significance level of $\alpha = 0.05$.

Quantification of Direct and Indirect Effects

We described the net effect (NE) of grazing on any variable X as the sum of direct and indirect effects of grazing on the three microsites (Equation 1; see analytical approach in Appendix in Supplementary material):

$$\mathrm{NE_X} = \Sigma\ \mathrm{DET_M} + \ \mathrm{IET_M} \qquad (1)$$

where, M designates the three microsites analyzed (bare soil, grass- and shrub-covered patches); $\mathrm{ET_M}$ is direct effect term on each microsite; and $\mathrm{IET_M}$ is indirect effect term on each microsite.

In turn, both terms can be described by the following equations:

$$\mathrm{DET_M} = \ \mathrm{p_{M,U}}.\ \mathrm{DE_M} \qquad (2)$$

$$\mathrm{IET_M} = \ \mathrm{X_{M,I}}.\ \mathrm{IE_M} \qquad (3)$$

where, $\mathrm{p_{M,U}}$ is the mean frequency of microsite M in ungrazed plots, $\mathrm{X_{M,I}}$ is the mean value of variable X in microsite M in intensely grazed plots, $\mathrm{DE_M}$ is the direct effect of grazing measured on microsite M (that is, the difference between the values of X in microsite M in intensely grazed and ungrazed plots), and $\mathrm{IE_M}$ is the indirect effect of grazing measured on microsite M (that is, the difference between the frequencies of microsite M in intensely grazed and ungrazed plots).

Within this conceptual framework, we analyzed the direct and indirect effect terms at each microsite, and also the overall direct and indirect effect terms, by summing the respective effect terms of the three microsites. Then, we calculated the relative importance of direct and indirect effects on the net effect, both at the scale of individual microsite and entire community, as the ratio between the absolute value of each effect and the sum of the absolute values of all the effects.

RESULTS

Grazing intensity significantly affected ecosystem structure in the Patagonian steppe. Bare soil cover significantly increased and shrub cover showed no change with grazing (Table 1). This increase in bare soil cover was largely due to a significant decrease in herbaceous and standing dead cover, but not to a decrease in grass cover (Table 1). Interestingly, the floristic composition of grass microsites varied with grazing intensity. The cover of the most preferred grass species (*Bromus pictus*, *B. setifolius*, *Poa ligularis*, and *P. lanuginosa*) was significantly higher in ungrazed than in grazed areas, and that of unpreferred species (*Stipa speciosa*, *S. humilis*, and *Festuca argentina* (Speg.) Parodi) increased in moderately and intensely grazed sites (Table 1). Floristic composition of shrub microsites was not affected by grazing intensity (Table 1).

Grazing intensity also significantly affected ecosystem biogeochemistry. Soil organic carbon (SOC) content declined with grazing intensity but not significantly (Figure 1A, $P = 0.16$); whereas soil organic nitrogen (SON) content decreased by 18% in the intensely grazed areas when compared to the

Table 1. Effect of Grazing on Plant Community Structure

Variable	Snedecor's *F*					Microsite class	Grazing intensity		
	G	M	G × M	Block	G × block		U	M	I
Fecal density	<0.01**	–	–	0.55 ns	–	–	0 a	15.1 a	38.9 b
Cover (%)	0.30 ns	<0.01**	0.02*	0.15 ns	0.99 ns	Bare soil	38.3 a	43.68 b	49.8 b
						Grass	22.6 a	20.0 a	19.9 a
						Shrubs	7.3 a	11.2 a	8.1 a
						Herbs	5.1 a	3.4 b	1.9 b
						Litter	26.9 a	21.8 b	20.4 b
Grass cover (%)	0.02*	<0.01**	<0.01**	0.19 ns	0.65 ns	Preferred	13.3 a	6.7 b	1.7 c
						Unpreferred	9.3 a	13.3 b	18.1 c
Shrub cover (%)	0.64 ns	0.02*	0.33 ns	0.08*	0.33 ns	*A. volckmannii*	2.4 a	2.3 a	1.9 a
						M. spinosum	2.8 a	4.6 a	5.0 a
						S. filaginoides	2.8 a	4.2 a	1.2 a

Effect of grazing intensity (U: ungrazed versus M: moderately grazed versus I: intensely grazed sites) on the fecal density (pellets m^{-2}*), the cover of preferred (Bromus pictus, B. setifolius, Poa ligularism, and P. lanuginosa) versus unpreferred grass species (Stipa speciosa, S. humilis, and Festuca argentina), and the cover of the main shrub species (Adesmia volkmanni, Mulinum spinosum, and Senecio filaginoides). The first four columns summarize the results of the ANOVA tests, showing the P-values for the different sources of variation: G = Grazing, M = Microsite type (corresponding to the Microsite Class column), block = sampling area (ns = not significant, * = 0.05 > P > 0.01, ** = P < 0.01). The last columns represent mean values recorded for each variable in each class and at each site. When significant grazing × microsite interactions were detected, different letters indicate significant differences among grazing intensities within each class of microsite.*

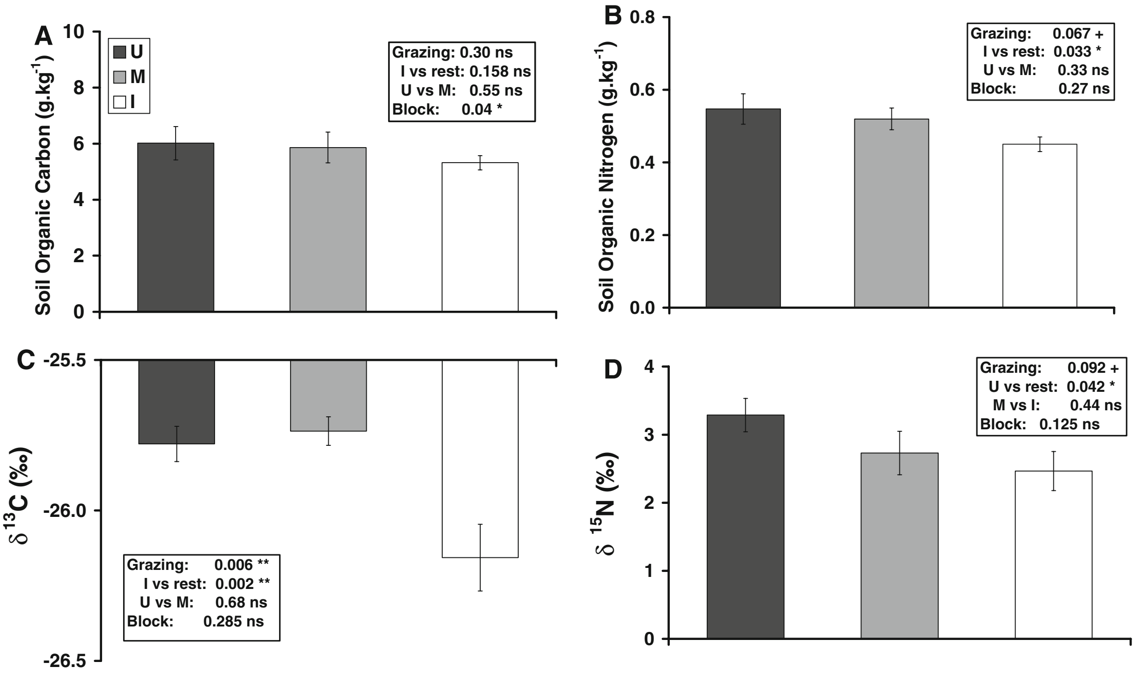

Figure 1. Net effect of grazing on the soil content of C (**A**), N (**B**), soil δ^{13}C (**C**), and δ^{15}N (**D**). *Black bars*: Ungrazed sites, *Gray bars*: Moderately grazed sites, *White bars*: Intensely grazed sites. *Vertical lines* indicate the standard errors ($n = 5$). Tables indicate the *P*-values resulting from the ANOVA with orthogonal contrasts among grazing levels, and sampling areas as blocks (ns = not significant, $^+ = 0.10 > P > 0.05$, $* = 0.05 > P > 0.01$, $** = P < 0.01$).

moderately grazed or ungrazed sites (Figure 1B, $P < 0.05$), ranging from 0.55 g kg^{-1} on average in ungrazed sites to 0.45 g kg^{-1} in intensely grazed sites. Soil organic matter (SOM) was more ^{13}C enriched in the ungrazed or moderately grazed sites than in the intensely grazed sites (Figure 1C), and more ^{15}N enriched in the ungrazed areas than in the grazed ones (Figure 1D).

The detection of indirect effects via changes in vegetative structure and composition depends on the existence of biogeochemical differences among microsites in all grazing intensities. Biogeochemical differences among microsites were evident for soil C and N contents, but not for δ^{15}N and δ^{13}C isotope signatures (Figure 2). SOC and SON concentrations below shrubs were higher than below grasses than in the bare soil microsites ($P < 0.05$, statistical analysis not shown; Figure 2A, B). Instead, all the microsites showed similar δ^{15}N and δ^{13}C values ($P > 0.10$, statistical analysis not shown; Figure 2C, D).

Soil variables changed directly with grazing intensity in the grass and bare soil microsites (shrubs excluded from the statistical analysis of direct grazing effects because of their low number of subsamples per site). The C content of soil below grasses was higher, and the N content in the bare soil microsites was lower, in the intensely grazed sites than in the moderately or ungrazed ones (significant grazing × microsite interaction effect; Figure 2A, B). Both grasses and bare soil microsites were more enriched with ^{13}C and ^{15}N in the ungrazed sites than in the intensely grazed sites (Figure 2C, D).

The effect of grazing on the soil properties was not affected by the age of exclosure, except in the case of the natural abundance of ^{15}N, where we detected a significant grazing × block interaction (Figure 2). In concordance with the comparison between grazed and ungrazed areas, δ^{15}N increased with the age of exclosure for the three microsites analyzed, and this correlation was only observed in the ungrazed areas (Table 2).

In summary, the net negative effect of grazing on SOC and SON was more associated with direct effects on SON than on SOC (60% vs. 34% of the net effect, respectively). This can be explained because in the two variables the sign of the direct effect of grazing on bare soil and shrub patches was negative, as that of the net effect, whereas the direct effect on grasses had the opposite sign. However, the magnitude of the positive direct effect on grasses was higher on SOC than on SON, compensating more for the negative direct effects of grazing on bare soil and shrub-occupied patches in C than in N pools (Table 3). The net effect of

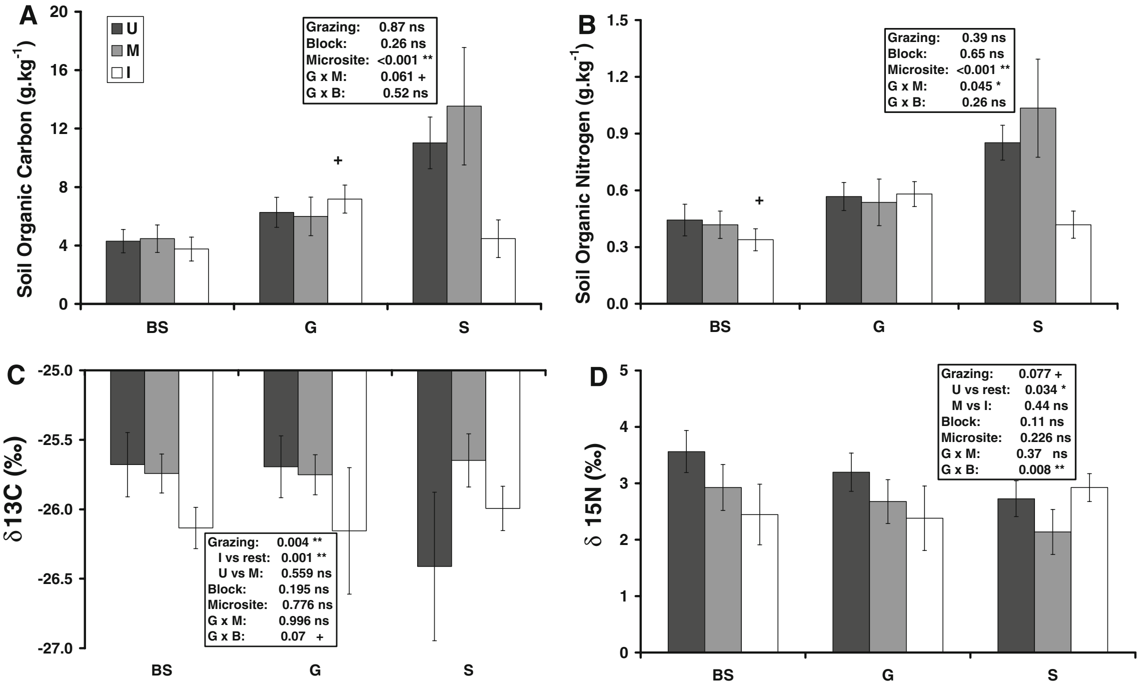

Figure 2. Direct effect of grazing on the soil content of C (**A**), N (**B**), δ^{13}C (**C**), and δ^{15}N (**D**), within three different patch types (BS = Bare soil, G = Grass, S = Shrub). *Bar colors* indicate different grazing intensities as in Figure 1. *Vertical lines* indicate the standard errors ($n = 5$). Tables indicate the P-values resulting from the ANOVA among grazing levels with sampling areas as blocks, and plots split into grass and bare soil microsites (ns = not significant, $^{+} = 0.10 > P > 0.05$, $* = 0.05 > P > 0.01$, $** = P < 0.01$). When significant Grazing × Microsite interactions were detected, symbols above bars indicate which grazing intensity was the highest within each microsite $^{+} = 0.10 > P > 0.05$, $* = 0.05 > P > 0.01$, $** = P < 0.01$). Shrub microsites were excluded from this ANOVA because of the scarce number of samples within some parcels.

Table 2. Effect of the Time Under Exclosure on Soil C and N Pools and Isotopic Signatures

Soil variable	Ungrazed		Moderately grazed		Intensely grazed	
	Bare soil	Grass	Bare soil	Grass	Bare soil	Grass
C (g kg^{-1})	0.05	0.42	0.16	0.61	0.46	0.02
N (g kg^{-1})	0.28	0.05	0.12	0.66	0.5	0.02
δ^{13}C (‰)	0.32	0.18	0.07	0.22	0.36	0.02
δ^{15}N (‰)	**0.78**	**0.67**	0.45	0.13	<0.01	0.01

Coefficient of determination (r^2) of the linear regression between the four soil variables analyzed and the years since the installation of the exclosure for grass and bare soil microsites under the three grazing levels compared. Values in bold indicate that the regression in statistically significant ($P < 0.05$; $n = 5$ in all cases). Shrub microsites excluded because of the scarce number of samples within some parcels.

grazing on the isotopic signature of SOC and SON was almost exclusively due to direct effects (92 and 96% of the overall net effect, respectively) because the indirect effects of bare soil and grasses compensated almost entirely: due to the high chemical similarity between grass and bare soil microsites, the indirect grazing effects had essentially the same magnitude but opposite sign on both microsites (Table 3).

DISCUSSION

Direct and Indirect Effects of Grazing Intensity on Soil C and N Pools

The overall net effect of domestic grazing on carbon and nutrient pools in the Patagonian steppe was a decrease in C and N pools, which was observed principally in the intensely grazed sites (Hypothesis 1) (Figure 1). These decreases in SOC and SON

Table 3. Importance of Direct and Indirect Effects of Grazing on the Four Soil Variables Analyzed

Microsite	Effect term	C (g kg^{-1})		N (g kg^{-1})		δ^{13}C (‰)		δ^{15}N (‰)	
		Effect	% of net effect	Effect	% of net effect	Effect	% of net effect	Effect	% of net effect
Bare Soil	IET	0.69	18.4	0.06	21.1	−4.78	39.8	0.45	23.0
	DET	−0.21	5.5	−0.04	13.5	−0.18	1.5	−0.43	21.9
Grass	IET	−1.57	42.0	−0.13	43.4	5.75	47.9	−0.52	26.8
	DET	0.47	12.6	0.01	2.3	−0.28	2.3	−0.43	21.8
Shrub	IET	0.16	4.3	0.02	5.2	−0.94	7.8	0.11	5.5
	DET	−0.64	17.1	−0.04	14.4	0.08	0.7	0.02	1.0
Total	IET	−0.72	65.9	−0.05	40.0	0.03	7.9	0.03	3.7
	DET	−0.37	34.1	−0.08	60.0	−0.37	92.1	−0.83	96.3
	NE	−1.10		−0.13		−0.34		−0.80	

Absolute and relative values of Indirect and Direct Effect Terms of grazing (IET and DET, respectively, see Equations 1–3) on the four soil variables analyzed at the three microsites and at the entire community (see analytical approach in Appendix in Supplementary material).

translated to decreases in soil C and N content of 616 and 86 kg ha^{-1}, respectively (in top 5 cm of soil; calculated from Figure 1A, B respectively, under the assumption of a constant dry bulk density of 1.77 kg dm^{-3} [CV% = 4.7%, $n = 10$]; Paruelo and others 1988). However, the real decrease of soil C and N contents may have been slightly lower because grazing commonly causes an increase in dry bulk density.

The reduction in SOC and SON pools arose from a combination of direct and indirect effects of grazing on soil biogeochemistry (Hypothesis 2). The direct effect was associated with a significant decline in soil N concentrations in bare soil patches, whereas, the indirect effects of grazing were associated with increased cover of bare soil patches (Table 1), which in turn had the lowest C and N content (Figure 2A, B). The increase in cover of bare soil patches was explained by a reduction in the cover of herbs and standing dead litter, accompanied by a replacement of preferred by unpreferred grass species (Table 1).

The significant decline in soil C and N pools (Figure 1A, B) was in part due to the direct effect of grazing intensity on N concentration below shrub-covered and bare soil patches. However, in soil below grass microsites, N pools did not decline, and C pools even increased with grazing (Figure 2A, B). The increase in C content below grasses in the intensely grazed sites (Figure 2A, B) may be associated with an increase in the belowground allocation of carbon in response to grazing, as hypothesized by Soriano and others (1987) and observed in other semiarid ecosystems (Schuman and others 1999). Additionally, the observed changes in the C content below grass microsites with intense grazing may be due to the alteration of litter quality associated with the floristic change induced by grazing (Semmartin and others 2004; Vivanco and Austin 2006). The replacement of preferred by unpreferred grasses in the intensely grazed sites (Table 1) could increase C concentration due to reduced SOM turnover. In this case, the direct effect of grazing on SOC detected at the coarse microsite level, may mask indirect effects of grazing at the more detailed level of species. However, this direct positive effect of grazing on soil C stock below grasses did not compensate for the combination of the increase in the cover of the C-poor bare soil patches and the overall decrease of C stocks induced by grazing in the bare soil microsites. Notwithstanding, the increased SOC content below grasses in the intensely grazed sites may explain the smaller net decrease of C than that of N pools with grazing (Figure 1A versus 1B), and also the higher importance of direct effects of grazing on SON than on SOC (Table 3).

The increase in cover of bare soil patches as grazing intensity increased was not accompanied by an increase in the cover of shrub-occupied microsites, which has occurred in more mesic Patagonian ecosystems (León and Aguiar 1985), and in arid and semiarid ecosystems elsewhere (Scholes and Archer 1997). Instead, the increased importance of bare soil patches was accompanied by a reduction in cover of standing dead senescent material and in the cover of herbs (Table 1), which are important in the diet of Patagonian domestic herbivores (Somlo and others 1997). In addition, grazing was associated with a floristic change of the grass-covered microsites: the relative importance of preferred grasses decreased, and that of unpreferred grasses increased in spite of the fact that total grass cover was unchanged (Table 1). Such physiognomic and floristic changes may result from the selective grazing habit of sheep increasing mortality of preferred species and removal of aboveground biomass, combined with the very low rates of establishment of these species in re-colonizing bare soil patches (Aguiar and Sala 1997). As indicated above, species shift due to grazing effects that alter above- and belowground allocation patterns could impact soil C and N pools and their effect on decomposition and soil organic matter pools (Reeder and Schuman 2002).

Increased bare soil cover could have other consequences on carbon stocks in these intensively grazed areas. As photodegradation of plant litter appears to be an important vector of carbon loss in this ecosystem (Austin and Vivanco 2006), the reduction in cover could reduce protection of plant litter, which could otherwise be lost to photodegradation when exposed to solar radiation. Shrubs, standing dead material, and grass tussocks could all function as a protection against photodegradative losses, and these protected microsites appear to be important components in maintaining soil organic matter pools and microbial activity (López and others 2003; González-Polo and Austin 2009). Additionally, the increased cover of bare soil microsites suggest that the connectedness between patches is reduced, which could reduce local scale positive interactions and forecast desertification in these intensely grazed sites (Kéfi and others 2007).

Grazing Intensity and Stable Isotope Signatures

The more depleted soil δ^{13}C and δ^{15}N observed in intensely grazed areas than in grazing exclosures (Figure 1C, D) provides indirect evidence that mineralization was reduced with increased grazing intensity. The net effect of grazing on the isotopic discrimination was associated with direct effects, because the grasses and bare soil patches showed higher δ^{13}C and δ^{15}N values in ungrazed than in intensely grazed areas (Figure 2C, D). Indirect effects of grazing did not affect soil isotope signatures (Table 3) because grasses and bare soil patches had essentially the same isotopic signatures. This similarity may be explained because in the first 5 cm of soil the main SOM input to bare soil patches comes principally from grass roots (Soriano and others 1987).

In contrast to other studies that did not show changes in soil δ^{13}C with grazing (Han and others 2008), we found that the soil organic matter was increasingly depleted in ^{13}C in intensively grazed sites, although these differences were relatively small in magnitude (Figure 1C). Variation in soil δ^{13}C values may be due to changes in the isotopic signature of inputs or changes in the proportion of compounds of organic matter with variable isotopic signatures and turnover (Ehleringer and others 2000). All species in the temperate Patagonian steppe are of the C_3 photosynthetic pathway, such that differences in species-specific signatures are not substantial (Golluscio and Oesterheld 2007). In addition, the plant δ^{13}C of ungrazed (-27.0 ± 0.29; mean ± S.E.), moderately grazed (-26.7 ± 0.72), and intensely grazed sites (-26.6 ± 0.23) were not statistically different (calculated as the cover-weighted average of all the species found in each site from the values obtained by Golluscio and Oesterheld (2007) in ungrazed areas). This suggests that there are no substantial differences in the input signature; however, this assumption must be carefully considered because it must be demonstrated that several factors affecting δ^{13}C (for example, discrimination during decomposition, soil water content, mycorrhizal status, and nutrient availability) did not vary with grazing intensity (Dawson and others 2002).

The trends in ^{15}N natural are not consistent with increased N losses due to increased grazing intensity (Frank and Evans 1997; Austin and Sala 1999). However, the lack of a general pattern of ^{15}N variation with respect to grazing (Cook 2001; Han and others 2008) may also indicate the complexity of the response of the N cycle to livestock grazing. In this study, soil δ^{15}N was most enriched in ungrazed sites, and soil was increasingly ^{15}N enriched with time under grazing exclusion. These differences could be interpreted, with caution, as indirect evidence that N mineralization rates may be higher in ungrazed than in grazed sites. Most of the biological processes nested in the mineralization process discriminate against the heavy isotopes of N (Dawson and others 2002). Although the SOM δ^{15}N is also affected by the magnitude of leaching, volatilization, and denitrification, which also are biologically-mediated processes that discriminate against the heavy isotope, they only act on the small mineral fraction of soil N (Burke and others 1997; Dawson and others 2002). However, increased residence time of nitrogen in SOM in ungrazed sites could also explain more enriched δ^{15}N signatures, as frequently is observed with soil depth (Robinson 2001), and both increased mineralization and increased N retention over time could enrich ^{15}N signatures in these sites (Austin and Vitousek 1998). Finally, biotic interactions between plants and mycorrhizae or N-fixing bacteria also modify the isotopic signature of plant and soil N (Dawson and others 2002). As a consequence, it must not be discarded as a hypothetical effect of grazing on the isotopic signature of litter input, mediated by their effects on soil microbial communities.

Grazing Intensity and Biogeochemical Consequences

Our results contrast with a number of other studies of grazing impact in semiarid ecosystems, where grazing caused an increase in pools of C and N indirectly due to shrub encroachment (Hibbard and others 2001) or shifts in floristic composition (Schuman and others 1999). In addition, increased rates of mineralization have been observed as direct effects of grazing in North American and African grasslands (Frank and Evans 1997; McNaughton and others 1997). Net nitrogen mineralization in this semiarid steppe site is low, with annual rates of below 0.3 g N m^{-2} y^{-1} (Austin and others 2006) and with little response in net mineralization to changes in incoming precipitation or inhibition of nitrification (Austin and others 2006; Yahdjian and others 2006). Given these low rates of net mineralization, our indirect isotopic evidence suggests that nitrogen mineralization was altered, and perhaps slowed, due to increasing grazing pressure. It is interesting to note that there does not seem to be a generalized biogeochemical response to grazing in terrestrial ecosystems, and it seems clear that the net effects of livestock grazing result from complex interactions among plant floristic composition, potential life form shifts, abiotic constraints and grazing type and intensity. In the case of the Patagonian steppe, the curious lack of a successful shrub invader lends supports to the idea that grazing impacts on SOM will be negative, as removal of live and dead biomass inevitably increases bare soil patches. In addition, the decreases in decomposability of plant litter due to selective grazing by livestock (Semmartin and others 2004; Vivanco and Austin 2006) could be reducing rates of C and N turnover in this ecosystem.

A number of studies demonstrate significant effects of grazing only when very high stocking rates are simulated or tested (for example, Biondini and others 1998). In our study, the reduction of SOM and mineralization rates could explain the virtual lack of response in physiognomy and ANPP to grazing exclusion found in the Patagonian steppe (Soriano and others 1980), and perhaps in several other arid zones of the world (Milton and others 1994). It has been suggested that these ecosystems have experienced irreversible changes in degradation associated with losses due to soil erosion (Milton and others 1994), a possibility that cannot be discarded completely. However, the maintenance of biogeochemical cycling and SOM pools under moderate grazing, in spite of the shift from preferred to unpreferred grass dominance, also suggests a positive feedback between a reduction of N availability due to the increasing dominance of stress-tolerant species, producing recalcitrant litter and thereby maintaining SOM pools. Supporting this model, in another semiarid ecosystem of Argentine, Moretto and Distel (1999) found that under high N availability, palatable species dominate, but that the inverse occurs under low N availability. This model is analogous to that proposed in more humid systems, where the floristic shift toward a community dominated by low-productive species was associated with a decrease in biogeochemical cycling (Wedin and Tilman 1990; Berendse 1994). Such compensatory mechanisms that reduce grazing impacts suggest that these ecosystems may be more resilient than previously thought. However, when threshold effects are reached due to heavy stocking rates, that is, when shifting from moderately to intensely grazed sites, SOM pools severely decrease (Figure 1A, B), and it is not clear whether these alterations in soil C and N pools are reversible. Sound management practices must incorporate the range of factors, and their interactions, which determine grazing impacts in these ecosystems to maintain ecosystem functioning and preserve biogeochemical processes in actively managed ecosystems. Specifically, the maintenance and/or promotion of preferred grass species and litter cover may be suitable management objectives for a sustainable grazing system. The use of resting periods during the growing season may help in achieving these objectives (Golluscio and others 1998).

ACKNOWLEDGEMENTS

We thank Ana Srur and Fernando Cavagnaro, who helped us with the field and laboratory work. John Karr performed the laboratory analyses for C, N, and stable isotopes in the Duke University laboratory. Special thanks to INTA, for permission to work in the Río Mayo experimental station, and three anonymous reviewers who allowed us to improve the manuscript. We acknowledge ANPCyT (PICTs 15124/03, 21247/04, 31970/05, and 00463/08), University of Buenos Aires (G044, G062, G090 and G812), Fundación Antorchas of Antorchas (Early Career Award, ATA) and CONICET (PIP 5963/04) for financial support during this study.

REFERENCES

Aguiar MR, Sala OE. 1997. Seed distribution constrains the dynamics of the Patagonian steppe. Ecology 78:93–100.

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT. 2003. Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochem Cycles 17:1–10.

Austin AT, Vitousek PM. 1998. Nutrient dynamics on a precipitation gradient in Hawaii. Oecologia 113:519–29.

Austin AT, Sala OE. 1999. Foliar δ^{15}N is negatively correlated with rainfall along the IGBP transect in Australia. Aust J Plant Physiol 26:293–5.

Austin AT, Sala OE, Jackson RB. 2006. Inhibition of nitrification alters carbon turnover in the Patagonian steppe. Ecosystems 9:1257–65.

Austin AT, Vivanco L. 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. Nature 442:555–8.

Berendse F. 1994. Litter decomposability—a neglected component of plant fitness. J Ecol 82:187–90.

Bisigato A, Bertiller M, Ares JO, Pazos GE. 2005. Effect of grazing on plant patterns in arid ecosystems of Patagonian Monte. Ecography 28:561–72.

Biondini ME, Patton BD, Nyren PE. 1998. Grazing intensity and ecosystem processes in a northern mixed-grass prairie, USA. Ecol Appl 8:469–79.

Burke IC, Lauenroth WK, Milchunas D. 1997. Biogeochemistry of managed grasslands in Central North America. In: Paul E, Pauchan K, Elliot E, Cole CV, Eds. Soil organic matter in temperate ecosystems. Long-term experiments in North America. New York: CRC Press. p 85–102.

Cavagnaro FP, Golluscio RA, Wassner DF, Ravetta DA. 2003. Caracterización química de arbustos patagónicos con diferente preferencia por los herbívoros. Ecología Austral 13:215–22.

Cipriotti P, Aguiar MR. 2005. Effects of grazing on patch structure in a semi-arid two-phase vegetation mosaic. J Veg Sci 16:57–66.

Cook GD. 2001. Effects of frequent fires and grazing on stable nitrogen isotope ratios in northern Australia. Austral Ecol 26:630–6.

Dawson T, Mambelli S, Plamboeck AH, Templer PH, Tu KP. 2002. Stable isotopes in plant ecology. Annu Rev Ecol Syst 33:507–59.

Ehleringer JR, Buchmann N, Flanagan LB. 2000. Carbon isotope ratio in belowground carbon cycle processes. Ecol Appl 10(2):412–22.

Frank DA, Evans RD. 1997. Effects of native grazers on grassland N cycling in Yellowstone National Park. Ecology 78:2238–48.

Gillson L, Hoffman M. 2007. Rangeland ecology in a changing world. Science 315:53–4.

Golluscio RA, León RJC, Perelman SB. 1982. Caracterización fitosociológica de la estepa del Oeste de Chubut: su relación con el gradiente ambiental. Boletín de la Sociedad Argentina de Botánica 21:299–324.

Golluscio RA, Deregibus VA, Paruelo JM. 1998. Sustainability and range management in the Patagonian steppes. Ecología Austral 8(2):265–84.

Golluscio RA, Oesterheld M. 2007. Water use efficiency of 25 co-existing Patagonian species growing under different soil water availability. Oecologia 154:207–17.

González-Polo M, Austin AT. 2009. Spatial heterogeneity provides organic matter refuges for soil microbial activity in the Patagonian steppe, Argentina. Soil Biol Biochem. doi:10.1016/j.soilbio.2009.03.008.

Han GD, Xiying HY, Mengli ZL, Mingjun WJ, Ellert BH, Walter W, Wang MJ. 2008. Effect of grazing intensity on carbon and nitrogen in soil and vegetation in a meadow steppe in Inner Mongolia. Agric Ecosyst Environ 125:21–32.

Hibbard KA, Archer S, Schimel DS, Valentine DW. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. Ecology 82:1999–2011.

Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments. Ecol Monogr 54:187–211.

Jobbágy EG, Sala OE. 2000. Controls on grass and shrub aboveground production in the Patagonian steppe. Ecol Appl 10:541–9.

Kéfi S, Rietkerk M, Alados CL, Pueyo Y, Papanastasis VP, El Aich A, de Ruiter PC. 2007. Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. Nature 449:213–7.

Knapp AK, Briggs JM, Collins SL, Archer SR, Bret-Harte MS, Ewers BE, Peters DP, Young DR, Shaver GR, Pendall E, Cleary MB. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. Glob Chang Biol 14:615–23.

Lange RT, Willcocks MC. 1978. The relation between sheep-time spent and egesta accumulated within an arid zone paddock. Aust J Exp Anim Husb 18:764–7.

León RJC, Aguiar MR. 1985. El deterioro por uso pasturil en estepas herbáceas patagónicas. Phytocoenologia 13:181–96.

López N, Austin AT, Sala OE, Méndez B. 2003. Controls on nitrification in a water-limited ecosystem: experimental inhibition of ammonia-oxidising bacteria in the Patagonian steppe. Soil Biol Biochem 35:1609–13.

McNaughton S, Banyikwa F, McNaughton M. 1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. Science 278:1798–800.

Milchunas DG, Lauenroth WK. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. Ecol Monogr 63:327–66.

Milton S, Dean W, Plessis M, Siegfried W. 1994. A conceptual model of arid rangeland degradation. Bioscience 44:70–6.

Moretto AS, Distel RA. 1999. Effects of selective defoliation on the competitive interactions between palatable and unpalatable grasses native to a temperate semi-arid grassland of Argentina. J Arid Environ 42:167–75.

Paruelo JM, Aguiar MR, Golluscio RA. 1988. Soil water availability in the Patagonian arid steppe: gravel content effect. Arid Soil Res Rehab 2:67–74.

Pazos G, Bisigato A, Bertiller M. 2007. Abundance and spatial patterning of coexisting perennial grasses in grazed shrublands of the Patagonian Monte. J Arid Environ 70:316–28.

Perelman SB, León RJC, Bussacca JP. 1997. Floristic changes related to grazing intensity in a Patagonian shrub steppe. Ecography 20:400–6.

Reeder JD, Schuman GE. 2002. Influence of livestock grazing on C sequestration in semi-arid mixed-grass and short-grass rangelands. Environ Pollut 116:457–63.

Robinson D. 2001. δ^{15}N as an integrator of the nitrogen cycle. Trends Ecol Evol 16(3):153–61.

Sala OE, Golluscio RA, Lauenroth WK, Soriano A. 1989. Resource partitioning between shrubs and grasses in the Patagonian steppe. Oecologia 81:501–5.

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

Scholes R, Archer S. 1997. Tree–grass interactions in savannas. Annu Rev Ecol Syst 28:517–44.

Schulze E-D, Williams RJ, Farquhar GD, Schulze W, Langridge J, Miller JM, Walker BH. 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. Aust J Plant Physiol 25:413–25.

Schuman GE, Reeder JD, Manley JT, Hart RH, Manley WA. 1999. Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. Ecol Appl 9:65–71.

Semmartin M, Aguiar MR, Distel RA, Moretto AS, Ghersa CM. 2004. Litter quality and nutrient cycling affected by grazing-induced species replacements along a precipitation gradient. Oikos 107:148–60.

Somlo R, Pelliza A, Willems P, Nakamatsu V, Manero A. 1997. Atlas Dietario de Herbívoros Patagónicos. Bariloche, Argentina: PRODESAR-INTA-GTZ. 109 p.

Soriano A, Golluscio RA, Satorre EH. 1987. Spatial heterogeneity of the root systems of grasses in the Patagonian arid steppe. Bull Torrey Bot Club 114:103–8.

Soriano A, Sala OE, León RJC. 1980. Vegetación actual y potencial en el pastizal de coirón amargo (Stipa spp.) del SW de Chubut. Boletín de la Sociedad Argentina de Botánica 19:309–14.

Vivanco L, Austin AT. 2006. Intrinsic species' effects on leaf litter and root decomposition: a comparison of temperate grasses from North and South America. Oecologia 150:97–107.

Yahdjian L, Sala OE, Austin AT. 2006. Differential controls of water input on litter decomposition and nitrogen dynamics in the Patagonian steppe. Ecosystems 9:128–41.

Wardle DA, Bardgett RD. 2004. Human-induced changes in large herbivorous mammal density: the consequences for decomposers. Front Ecol Environ 2:145–53.

Wedin DA, Tilman D. 1990. Species effects on nitrogen cycling: a test with perennial grasses. Oecologia 84:433–41.