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Grazing effects on belowground C and N stocks along a network of cattle exclosures in temperate and subtropical grasslands of South America

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[1] We evaluated the effects of grazing on C and N belowground pools by comparing 15 grazing-exclosure pairs across the Río de la Plata grasslands of Uruguay and Argentina. We measured C and N pools of belowground biomass, particulate organic matter (POM), and the mineral associated organic matter (MAOM) in the top meter of the soil. Grazing exclusion in the Río de la Plata grasslands promoted (1) decreased belowground biomass stocks across all sites, (2) increased soil organic carbon (SOC) and soil organic nitrogen (SON) stocks in upland soils, and (3) decreased stocks in shallow and lowland soils. In all cases, SOC and SON variations were largely derived by changes in MAOM stocks that maintained their C:N ratios unchanged. In contrast, stocks of the labile POM fractions changed little, but C:N ratios of these fractions decreased after grazing removal. We hypothesize that changes in soil organic matter (SOM) contents between grazed and ungrazed stands result from the balance between changes in belowground N allocation patterns (root N retention hypothesis) and the ability of the soil to retain the extra N available after the exclusion of herbivores and the cessation of volatilization and leaching from urine and dung patches (N loss hypothesis). On the basis of our results we suggest that the relative importance of these two cooccurring mechanisms will shape grazing effects on SOM stocks, depending on soil properties, including texture, pH and soil depth, and vegetation type, particularly allocation patterns and C:N ratios of different plant species.

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1. Introduction

[2] Grazing is an important disturbance and a critical control of ecosystem functioning in rangelands, a land cover type occupying almost one half of the Earth's lands [*Menke and Bradford*, 1992]. Grazing impacts are complex and vary spatially and temporally, making generalizations difficult [*Brown and Allen*, 1989; *Milchunas and Lauenroth*, 1993; *Oesterheld et al.*, 1999; *Holdo et al.*, 2007]. The biogeochemical effects of grazing, particularly their impact on soil organic carbon (SOC) and soil organic nitrogen (SON) storage, are particularly controversial [*Milchunas and Lauenroth*, 1993; *Schuman et al.*, 2002; *Henderson et al.*, 2004]. Grazing can increase, decrease, or have no effect on soil organic matter (SOM) contents [*Milchunas and Lauenroth*, 1993; *Lavado et al.*, 1996; *Chaneton and Lavado*, 1996; *Frank and Evans*, 1997; *Franzluebbers et al.*, 2000; *Schuman et al.*, 2002; *Henderson et al.*, 2004; *Gill*, 2007; *Wu et al.*, 2008]. Grazing also has variable effects on other ecosystem properties, including primary productivity [*Milchunas and Lauenroth*, 1993; *Oesterheld et al.*, 1999]. The effects of grazing management on soil C fluxes influence C sequestration and the productivity and sustainability of rangeland ecosystems through interactions with soil fertility.

[3] Herbivores may affect SOM storage through mechanisms that alter C and N inputs and outputs from the soil [*Schlesinger*, 1991; *Baisden et al.*, 2002]. Changes in soil C and N *inputs* associated with herbivory may derive from several pathways. First, herbivores may change primary productivity [*Semmartin and Oesterheld*, 1996] and respire large amounts of C, which usually decreases C inputs to the soil [*Piñeiro et al.*, 2006]. Second, grazers may increase or decrease belowground allocation and will consequently increase or decrease C and N inputs from roots to the soil [*Kauffman et al.*, 2004; *Reeder et al.*, 2004]. Third, grazers may change litter quality and decomposition rates potentially

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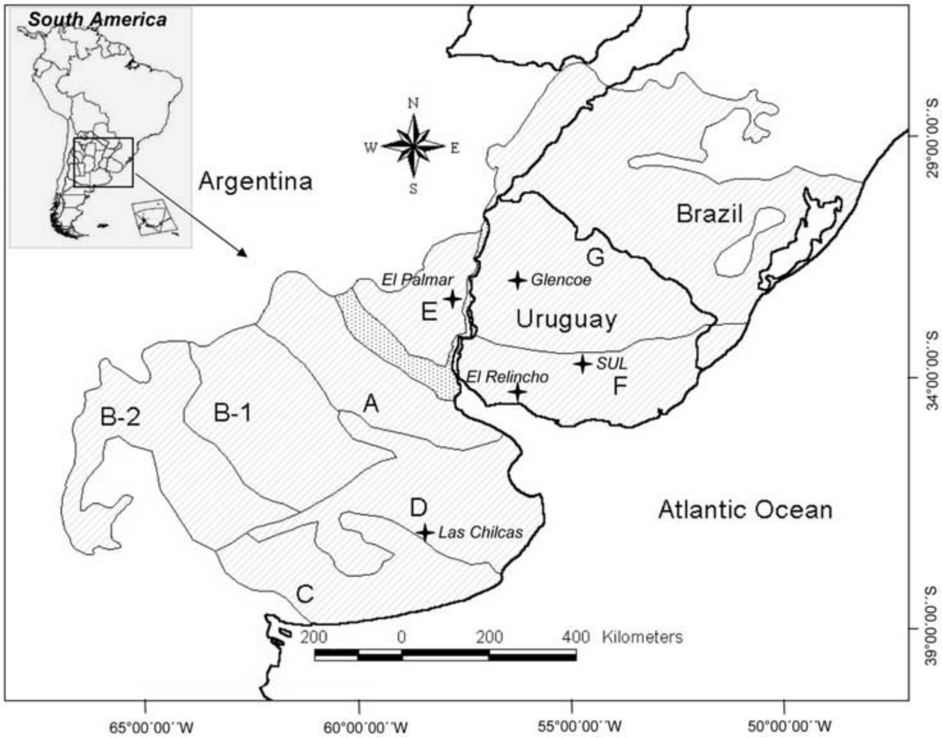

Figure 1. Map of the "Río de la Plata" grasslands and its subdivisions, showing our study locations (black crosses). Las Chilcas has two exclosures, El Relincho has six exclosures, Secretariado Uruguayo de la Lana (SUL) has one exclosure, Glencoe has three exclosures, and El Palmar has three exclosures. The "Rio de la Plata" grasslands subdivisions are: the rolling pampa (A), the inland pampa (B) (with two divisions: (1) the flat pampa and (2) the west pampa), the southern pampa (C), the flooding pampa (D), the mesopotamic pampa (E), the southern campos (F), and the northern campos (G) [*Soriano*, 1992].

altering SOM formation [*Allard et al.*, 2003; *Semmartin and Ghersa*, 2006]. Fourth, changes in legume abundance and N fixation rates may also be affected by grazing, which alters N inputs to the soil [*Allard et al.*, 2003]. Finally, herbivore excretions may accelerate N cycling but decrease N inputs to the soil because of higher N volatilization and leaching from urine and dung patches [*Frank and Evans*, 1997; *Piñeiro et al.*, 2006]. Changes in soil C and N *outputs* associated with herbivory arise mainly from changes in soil organic matter mineralization rates [*Wang et al.*, 2006] or increased erosion [*Neff et al.*, 2005]. Grazing generally decreases litter contents or plant cover and thus may increase SOM mineralization rates because of greater soil temperature fluctuations or soil moisture variability and by increasing aridity [*Lauenroth et al.*, 1994; *Wang et al.*, 2006]. Since SOM contents are controlled by climate, soil texture and vegetation type [*Burke et al.*, 1989; *Jobbágy and Jackson*, 2000], the overall consequences of grazing on SOM accumulation may vary along gradients of these variables.

[4] Among all of the mechanisms determined above, those influencing C inputs and outputs are more likely to affect SOM storage in semiarid grasslands where water availability constrains C cycling [*Burke et al.*, 1998]. SOM storage in subhumid and humid grasslands is, in contrast, more likely to be controlled by nutrient availability, particularly N [*Wedin and Tilman*, 1996; *Burke et al.*, 1998; *Piñeiro et al.*, 2006]. Recently, *Derner et al.* [2006] showed that grazing effects on SOM varied across a precipitation gradient in grasslands of North America and were driven by changes in root partitioning showing that grazing increased nutrient retention. *Piñeiro et al.* [2006] suggested that long-term grazing reduces SOM in South American grasslands and that the magnitude of this reduction is highest in the most fertile sites because of the higher N losses associated with grazing.

[5] The objectives of this study were to evaluate the changes promoted by livestock removal across temperate and subtropical grasslands for (1) the amounts and vertical distribution of organic C and N contents in different belowground pools and (2) soil bulk density, as affected by trampling. We also evaluated the association of these changes with climate and soil properties in general. We sampled different SOM fractions (POM-particulate organic matter and MAOM-mineral associated organic mater) and belowground organs in 15 grazing-exclosures pairs along a precipitation and temperature gradient in grasslands of Argentina and Uruguay. We further compared our results against predictions derived from two nonexclusive hypotheses for the effects of grazing on SOM stocks.

2. Materials and Methods

2.1. Study Sites

[6] Our research was conducted along the major soil and climatic gradients of the 70-million-hectare Rio de la Plata

Table 1. Study Sites Description

	Glencoe Experimental Station	El Palmar National Park	SUL Experimental Station[a]	El Relincho Private Reserve	Las Chilcas Farm
Latitude	32° 00′ S	31° 50′ S	33° 52′ S	33°19′ S	36° 30′ S
Longitude	57° 08′ W	58° 17′ W	55° 33′ W	56°58′ W	58° 30′ W
MAP (mm)	1406	1300	1161	1099	861
MAT (°C)	17.3	18.9	16.3	17.4	14.9

	Glencoe Experimental Station			El Palmar National Park			SUL Experimental Station[a]	El Relincho Private Reserve						Las Chilcas Farm	
	G94[b]	G84	G84L	Pa	Pb	Pc	Sa	Ñs	Bl	Bo	Dj	Cc	Lz	Chb	Chc
Exclosure age (years)	8	18	18	30	30	30	13	3	5	9	7	11	11	19	30
Site type	upland	shallow	shallow	upland	upland	upland	upland	shallow	lowland	upland	shallow	upland	upland	lowland	lowland
Soil rocks (%)	3.9	27	6.5	0.50	0.69	0.94	7.8	6.4	6.8	8.0	12	3.2	5.6	0.0	0.0
Soil sand (%)	13	26	26	70	72	44	35	32	25	18	47	36	43	22	18
Soil depth (m)	>1	0.10	0.05	>1	>1	>1	>1	0.30	>1	>1	0.30	>1	>1	>1	>1
Soil pH (0–30)	4.73	4.70	4.81	4.76	4.84	4.77	4.57	4.84	6.42	5.13	4.80	4.46	4.88	7.48	7.24

[a]SUL, Secretariado Uruguayo de la Lana experimental station.
[b]Exclosure name.

grasslands of Argentina and Uruguay (Figure 1) [*Soriano*, 1992; *Paruelo et al.*, 2006]. Mean annual precipitation (MAP) varies from 600 mm in the southwest to 1600 mm in the northeast [*Soriano*, 1992]. Soils of the region developed under grass cover, with trees restricted to riparian areas and rocky outcrops in the north [*Soriano*, 1992]. The Rio de la Plata grasslands were probably grazed sparsely by native herbivores (mainly Pampa deer, *Ozotoceros bezoarticus*) until the sixteenth century, when European settlers introduced livestock. Since then, these grasslands have been grazed by livestock with increasing intensity, particularly in the last 100 years [*Soriano*, 1992; *Piñeiro et al.*, 2006]. Livestock is raised in ranches were cattle moves and forages freely all year-round without having fixed resting places such as small corrals or barns.

[7] To define a network of paired grazed/ungrazed stands we searched for sites where domestic herbivores had been removed by exclosures for at least 3 years. We identified 15 exclosures and their respective grazed pairs in five different locations: in Uruguay, El Relincho Private Nature Reserve (six sites), Secretariado Uruguayo de la Lana (SUL) Experimental Station (one site), and INIA-Glencoe Experimental Station (three sites); in Argentina, El Palmar National Park (three sites) and Las Chilcas (a private ranch) (two sites) (Figure 1 and Table 1). Sites were nearby or separated by several km at each location, but all sites were considered as independent since they were in different paddocks, soil types and/or landscape positions. Soils of the study sites varied in sand content (13% to 72%), the amount of rocks (0% to 27%), pH (4.5 to 7.5) and depth (5 to > 100 cm), capturing most of the physical heterogeneity of the Rio de la Plata grasslands (Table 1). Annual herbivore stocking densities in the grazed stands were not quantified during the exclosure period, but were within normal values for the region (approximately 250 kg of live animal biomass per ha) during the sampling years (2002 to 2003), consuming near ∼45% of annual aboveground net primary production [*Oesterheld et al.*, 1998]. All grazed stands were dominated by C_3 and C_4 grasses, and shrubs were a minor component of each community. Changes in vegetation structure associated with grazing removal were described earlier by *Altesor et al.* [2006]. In synthesis, grazing removal promoted shrub encroachment and a shift from prostrate C_4 to erect C_3 grasses. A more detailed description of the vegetation can be found in the work of *Soriano* [1992] and *Perelman et al.* [2001].

2.2. Field Sampling and Lab Analyses

[8] Root and soil samples were taken inside the exclosures and in the adjacent grazed stands, on average five meters away from the fence to reduce possible edge effects. We also avoided soil changes associated with topography and animal trails. We assured that there were no differences in soil texture between each grazed and ungrazed pair (differences were always less than 3 percent except in one site that was discarded). The top 30 cm of soil were sampled for C, N, texture, rock content and pH using a 2-cm-wide soil corer, with 10 subsamples pooled in each stand. Samples were separated into 0–5, 5–10 and 10–30 cm depth intervals. The 30–100 cm depth range was sampled using three 5.5-cm-wide soil cores that were pooled in each stand and separated into 30–50, 50–70 and 70–100 cm depth intervals. All composite samples were sieved (2 mm) before separating into fractions. Soil organic matter fractions were determined according to *Cambardella and Elliot* [1992]. Briefly, 10 g of 2 mm sieved soil were shaken overnight (18 h) in 50 ml 0.5% hexametaphosphate dispersing solution. The dispersed soil was successively sieved through 500-μm (only 0–5 cm samples) and 53-μm (all samples) sieves and the sand-sized organic material (POM 500 and POM 53) retained on the sieves was thoroughly rinsed, transferred to glass beakers, and oven-dried (60°C) to constant weight. The slurry that passed both sieves contained the mineral associated organic matter (MAOM fraction) and was subject to the same drying process. The MAOM fraction is composed of very small organic matter particles adsorbed to silt and clay. At the 0–5 cm soil interval samples were separated in three fractions: POM

500, POM 53 and MAOM, while in the rest of the soil profile only POM53 and MAOM were separated. Thus, to facilitate the comparison with deep layers, POM 53 C and N amounts reported throughout the paper for the 0–5 cm soil layer include POM 500 C and N amounts. All samples were ground to fine powder using an analytical mill (IKA®, Model A10). Soil C and N concentrations in MAOM and POM fractions were determined with a Carlo Erba Elemental Analyzer at the Duke Environmental Stable Isotope Laboratory (DEVIL). POM 53 at depths greater than 30 cm are not reported here because it was a small fraction of the bulk soil and had very low contents of C and N that were not detectable by the CN analyzer (contents were lower than 0.3% for C and 0.03% for N). Soil carbonates were removed from soil samples (when present) using 0.5M HCl before C and N analysis. The acid was then extracted by adding distilled water and centrifugation at 4000 rpm for 10 min. Water with acid was carefully removed from the sample with a micropipette. This procedure was repeated at least three times.

[9] Soil sand contents were determined by weighing the sand-size fraction obtained in the POM separation procedure while clay contents were determined by the hydrometer method [*Elliot et al.*, 1999] and silt contents by difference. Soil pH was measured in distilled water (15 g of soil in 30 ml) with a pH meter [*Elliot et al.*, 1999]. Four soil bulk-density samples were taken at each site with a 2-cm-wide soil corer, analyzed separately following the approach of *Elliot et al.* [1999], and averaged to give a single value. Bulk density samples were taken to 30-cm depth at the same intervals as for C and N analysis. Soil bulk density from 30 to 100 cm depth was estimated on the basis of SOM and texture contents with an empirical model [*Rawls*, 1983].

[10] Belowground biomass samples were extracted with a 7-cm-wide core, taking three subsamples in the grazed treatments and five subsamples in the exclosures because of the higher plant spatial heterogeneity. Samples were taken at 0–5, 5–10 and 10–30 cm depth. Roots and other belowground organs were carefully separated from the soil by washing, oven-dried at 60°C, and weighed. Each subsample was ground and analyzed for C and N contents separately. Results were then averaged to give a single value for the grazed and exclosured situations at each site. Carbon and N contents in belowground biomass were also measured by dry combustion in a Carlo Erba Elemental Analyzer at Duke University.

2.3. Stocks Correction to an Equivalent Soil Mass

[11] Soil C and N contents have been expressed in the paper on an equivalent soil mass. *Davidson and Ackerman* [1993] showed that it is indispensable to report soil C and N concentrations on an equivalent mass basis to provide a quantitative measure independent of treatment differences in bulk density. It has been shown that when comparing grazed versus ungrazed paired treatments (or any other land use transition that changes soil bulk density), soil C and N stocks measured at fixed sampling depths may overestimate elements stocks in the more compacted grazed sites [*Mikhailova et al.*, 2000; *Henderson et al.*, 2004]. This is because soil compaction may artificially increase an element concentration in a fixed volume of soil, by increasing the amount of soil mass or soil bulk density. Thus, when soil samples are extracted with a soil corer at fixed sampling depths, soil mass should be corrected or soil samples should be taken at variable depths regarding soil bulk density changes [*Davidson and Ackerman*, 1993].

[12] Soil organic C and N contents at each fraction and depth in the ungrazed sites were corrected to an equivalent soil mass as measured in the grazed treatments. Because bulk density was usually lower in ungrazed treatments, it was necessary to add additional organic C or N mass to each soil layer until reaching an equivalent soil mass as in the grazed treatments. The additional mass of C or N was subtracted from the lower layer, to which an additional mass was added from the subsequent depth until an equivalent soil mass was achieved. This process was repeated for all soil depths, and thus sampling depths reported in the paper are relative to grazing treatments, while layer thickness varied in ungrazed sites to reach an equivalent soil mass. See *Davidson and Ackerman* [1993] and *Henderson et al.* [2004] for a detailed description of the correction.

2.4. Statistical Analysis

[13] Comparisons were based on paired *t* tests and were preformed within each depth range. Sites were grouped as upland (including deep well-drained acid soils with pH lower than 6 and situated in high topographic positions), shallow, which includes shallow acid soils with pH lower than 6 and contact with bedrock at less than 30 cm depth; and lowland, which includes frequently flooded alkaline soils with pH greater than 6. Interactions of grazing effects with these soil classes were tested using a one-way ANOVA, where the variable analyzed was the difference between the grazed and ungrazed stands. Differences were considered significant at $p < 0.05$ and indicated with standard statistical nomenclature (*** = $p < 0.001$, ** = $p < 0.01$ and * = $p < 0.05$). Simple and multiple regression analyses were used to investigate the relationships between changes in belowground C and N stocks (belowground biomass, SOC and SON) after grazing removal with site properties (soil pH, soil texture, soil bulk density, rock content, exclosure age, mean annual precipitation and mean annual temperature). Simple regressions were also performed to evaluate the relationship between SOC, SON and C and N in belowground biomass.

3. Results

3.1. Belowground Biomass

[14] Belowground biomass C storage (roots + other belowground organs) was lower in exclosures than in grazed stands, but only in the top 5 cm of the soil (Figure 2a). The average reduction in the top 5 cm for all sites was 1772 kg ha^{-1}, a loss of 53%. Only one site, *Bl*, had higher root C contents in the exclosure (473 kg ha^{-1}), and the maximum loss for any site was 4511 kg ha^{-1}. Grazing effects on belowground biomass C and N stocks did not vary between site types (upland, shallow and lowland) ($p > 0.73$, $n = 15$). Belowground biomass N content in the 0–5 cm soil layer

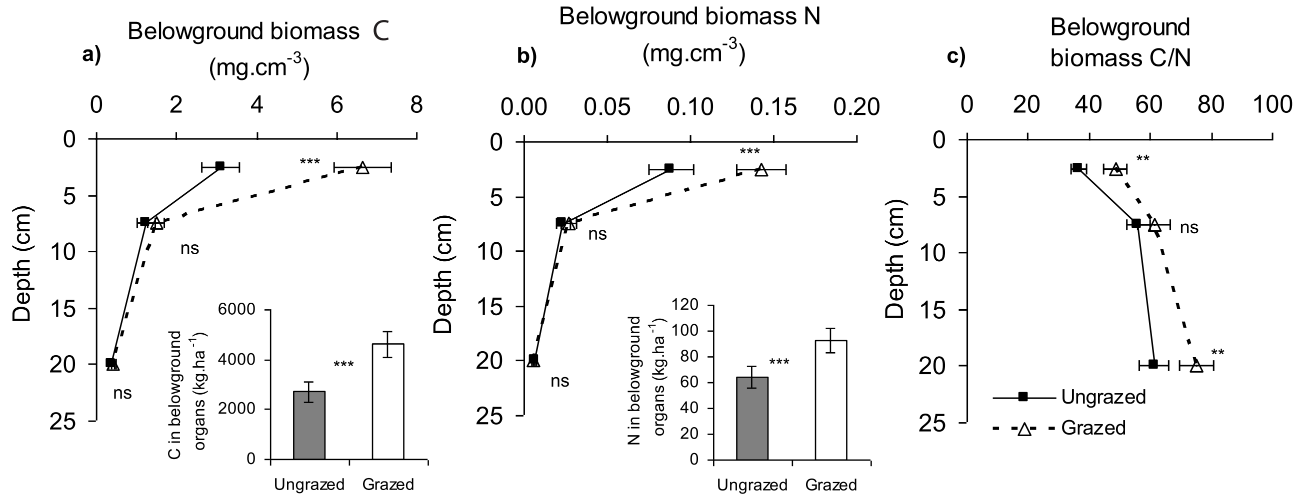

Figure 2. Belowground biomass C, N, and C:N ratio in ungrazed (solid lines) and grazed (dashed lines) stands at 0–5, 5–10, and 10–30 cm depth intervals (average for all sites, $n = 15$). Data is plotted at mean sampling depth. Insets show total C and N in belowground biomass for the first 30 cm of the soil. Error bars show standard errors.

was also lower in exclosures than in grazed stands (average = -27 kg.ha^{-1} or -38%), even though differences were proportionally smaller than for C (Figure 2b). For the top 30 cm of the soil profile, the exclosures also had significantly less belowground biomass C and N (41% and 31%, respectively; Figure 2, insets). Assuming that the exclosures were similar to the grazed stands before being fenced, such changes represented a loss of 128 kg C ha^{-1} year^{-1} and 2.0 kg N ha^{-1} year^{-1}. For the region overall, total C or N contents in belowground biomass were significantly related

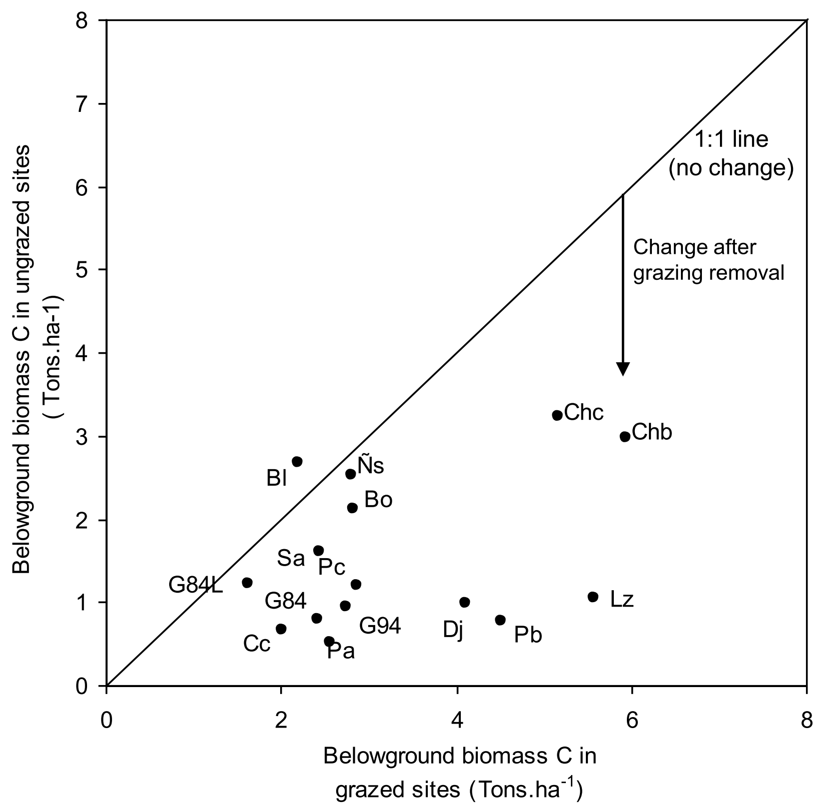

Figure 3. Relationship between C contents in belowground organs in grazed and ungrazed stands. Data is for 0–5 cm depth where the major differences between treatments occurred. Points falling below the 1:1 line indicate that there is more root C in the grazed relative to the ungrazed stands. See site descriptions in Table 1. The correlation between both variables is not significant ($p = 0.21$).

Table 2. Average Soil Organic Carbon and Nitrogen Contents in Grazed and Ungrazed Stands at Different Site Types[a]

	Uplands	Shallow	Lowlands
SOC ungrazed (tons ha^{-1})	102	109	60
SOC grazed (tons ha^{-1})	94	118	69
Difference (tons ha^{-1})	8.6	−7.7	−8.9
Difference (%)	10	−15	−13
Change rate (kg ha^{-1} a^{-1})	624	−1192	−800
SON ungrazed (tons ha^{-1})	9.0	9.6	6.4
SON grazed (tons ha^{-1})	8.1	10.0	7.3
Difference (tons ha^{-1})	0.96	−0.47	−0.93
Difference (%)	13	−13	−13
Change rate (kg ha^{-1} a^{-1})	66	−64	−89
Soil C:N ungrazed	11.3	11.2	9.4
Soil C:N grazed	11.6	11.5	9.5

[a]Depth is 0–30 cm. Differences between grazing regimes, within site types, are all significant ($p < 0.01$) for both soil organic carbon (SOC) and soil organic nitrogen (SON). C:N differences between grazing regimes are not significant within each site type.

to MAP ($r^2 > 0.40$), MAT ($r^2 > 0.26$) and soil sand % ($r^2 = 0.17$) in both grazed and nongrazed stands ($n = 15$, $p < 0.05$ in all cases).

[15] The reductions in belowground biomass C in the exclosures were larger than for belowground biomass N and were reflected by changes in the C:N ratios of belowground plant tissues. Nitrogen concentrations in belowground plant tissues at 0–5, 5–10 and 10–30 cm intervals for the grazed and ungrazed stands were 0.89 versus 1.19%, 0.72 versus 0.81% and 0.60 versus 0.78%, respectively, but differences were significant only at the 0–5 and 10–30 cm layers ($p < 0.03$, $n = 15$). Carbon concentrations in belowground plant tissues across treatments remained unchanged, and thus C:N was generally higher in grazed stands, 25% for the 0–5 layer and 18% for the 10–30 layer (Figure 2c). Changes in C:N were probably caused by shifts in plant species composition. It is unclear whether plant species shifts occurred because of the lower N availability under grazing or because of livestock grazing selectivity during consumption.

[16] Differences in belowground biomass C between grazing regimes were higher at sites with higher initial belowground biomass C stocks (Figure 3). After grazing exclusion, belowground biomass C reached a relatively uniform value of ∼1000 kg of C ha^{-1} in most exclosures of upland and shallow soils (Figure 3; the same was true for belowground biomass N, that reached ∼30 kg of C ha^{-1}, data not shown). However, biomass C (and N) stocks in the exclosures at the three lowland sites (*Chc*, *Chb* and *Bl*) arrived at a higher value under grazing conditions, as at the *Bo* site, which had compacted and poorly drained soils, and at *Ñs*, a 3-year-old exclosure where changes are probably still occurring rapidly. Both the magnitude and the proportion of belowground biomass C and N changes after grazing removal were not significantly related to soil properties (pH, texture, bulk density, rock content or soil depth), climate (MAP and MAT) or, surprisingly, exclosure age.

3.2. Soil C and N

[17] The effects of grazing exclusion on SOC and SON interacted strongly with site type ($p < 0.001$, $n = 15$). SOC and SON contents were higher in the exclosures than in the grazed stands in upland soils, but lower in shallow and lowland soils (Table 2). The rates of change after grazing exclusion were similar in magnitude between site types, but differed in sign. Exclosures in deep, well-drained soils gained N at high rates (∼50 Kg ha^{-1} a^{-1}), suggesting high N inputs from atmospheric deposition or asymbiotic fixation (because of the absence of legumes). Most of the changes in total SOC or SON occurred as a consequence of changes in the MAOM fraction (an average rate of change of 1.12% per year), even though we expected greater changes in the POM fractions because of their faster turnover (Figures 4a and 4b). Although POM C and N stocks remained relatively constant, POM C:N ratios were mostly higher in grazed stands, regardless of increases or decreases in total SOM pools (Figure 4c), and mirroring changes in the C:N ratios of belowground biomass. The higher POM C:N of grazed stands suggests a lower N availability for plant growth under grazing and greater N immobilization by soil microbes. In contrast, MAOM C:N ratios did not vary between grazing regimes. Most SOM changes observed after grazing removal occurred in the top 30 cm of the soil (within the A horizon), with smaller changes observed in deeper layers of upland soils.

[18] Carbon and N reductions in the POM 53 fraction after grazing removal were associated with decreases in belowground biomass N contents in the surface layers, while total SOC and SON changes (POM+MAOM) for the entire soil profile were significantly related to soil texture in upland soils and to soil pH when considering all deep soils together (upland and lowland sites). In shallow sites reductions in the C contents of the POM 53 fraction for the entire soil profile were significantly related to N losses in belowground biomass ($r^2 = 0.92$, $p < 0.01$, $n = 4$). The same was true for upland soils, but only for the 0–5 cm layer ($r^2 = 0.51$, $p = 0.03$, $n = 8$). Lowland sites showed a similar, but insignificant pattern, probably due to the low number of replicates available. SOC gains in the MAOM fraction in upland soils were significantly related to clay+silt contents for the entire soil depth, but not in the POM 53 fraction (Figure 5), probably because clay and silt favored organic matter stabilization only in the recalcitrant SOC fraction. Sites with high clay+silt contents had the highest SOC increments in the MAOM fraction after livestock removal. As expected, SON contents showed similar relationships in all cases. When considering only deep soils total

Figure 4. (a) Soil organic carbon, (b) nitrogen, and (c) C:N ratios, of different soil particle size fractions in grazed and ungrazed stands, at different depth and for the three site types (upland soils, $n = 8$; shallow soils, $n = 4$; and lowland soils, $n = 3$). Samples were taken at 0–5, 5–10, 10–30, 30–50, 50–70, and 70–100 cm intervals and are plotted at mean sampling depth. MAOM, mineral associated organic mater; POM 53, particulate organic matter separated using a 53 μm sieve; POM 500 particulate organic matter separated using a 500 μm sieve; see section 2 for details. N stocks in ungrazed sites are expressed in an equivalent soil mass as to the grazed sites.

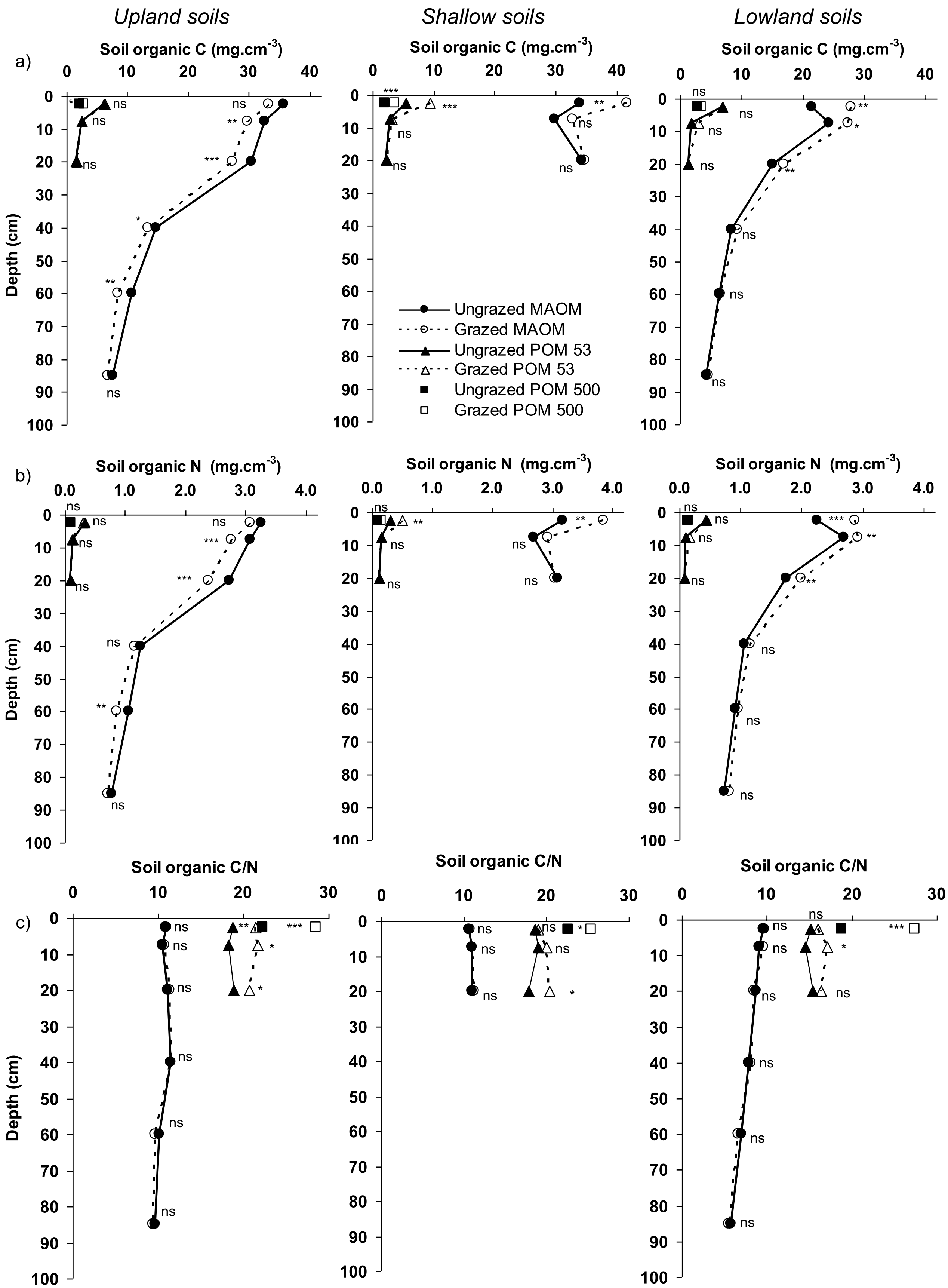
Upland soils
Shallow soils
Lowland soils
a)
b)
c)
Soil organic C (mg.cm^{-3})
Soil organic N (mg.cm^{-3})
Soil organic C/N
Depth (cm)
Ungrazed MAOM
Grazed MAOM
Ungrazed POM 53
Grazed POM 53
Ungrazed POM 500
Grazed POM 500

Figure 4

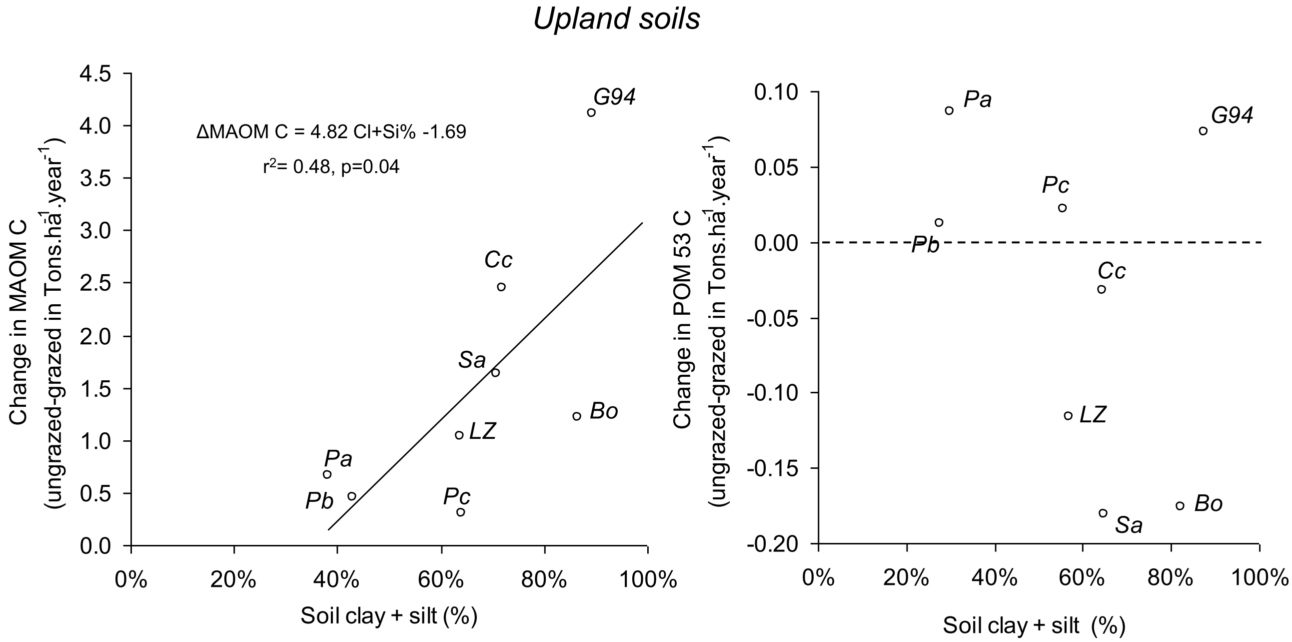

Figure 5. Relationship between annual rates of change in (left) MAOM carbon and (right) POM 53 carbon after grazing removal with soil clay+silt contents for upland soils. MAOM is average for 0–100 cm, and POM 53 is average for 0–30 cm. MAOM, mineral associated organic mater; POM 53, particulate organic matter separated using a 53 μm sieve; see section 2 for details.

SOC and SON changes where significantly related to soil pH (Figure 6). A multiple regression model that included soil pH, soil depth and changes in belowground biomass N, significantly explained SOC changes ($r^2_{adj} = 0.75$, $p < 0.001$, n = 15) and SON changes ($r^2_{adj} = 0.77$, $p < 0.001$, n = 15) across all site types. We did not detect a clear control of climate on the magnitude and sign of the effect of grazing on SOM, probably because the entire area falls within a humid to subhumid climate. SOM changes after grazing removal were not significantly related to exclosure age, although older exclosures tended to have a greater accumulation of SOM in upland sites or less loss of SOM in shallow and lowland sites.

[19] Variations in the C:N of SOM pools after grazing removal were similar across all site types and thus total SOC changes were strongly associated with SON changes across the region (Figure 7 and Table 3). The y intercept of the regression between total SOC and total SON changes after grazing removal was not significantly different from 0 ($p > 0.25$), and the slope suggests that for each g of N lost or gained, ~10.1 g of C were similarly lost or gained. Similar relationships were found for all the soil fractions, supporting

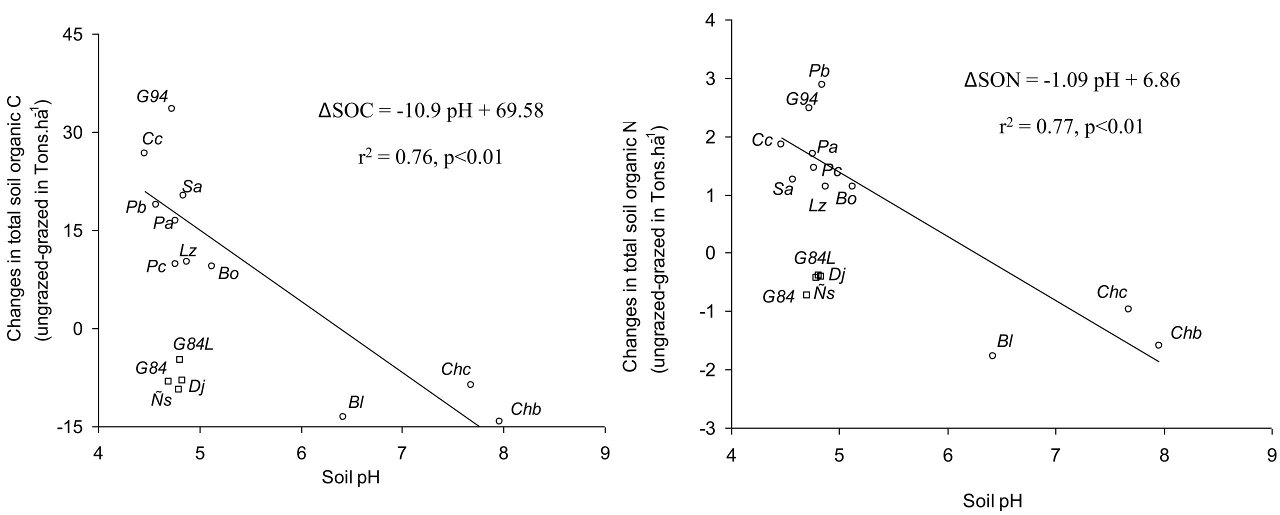

Figure 6. Relationship between (left) changes in total soil organic C and (right) changes in total soil organic N after grazing removal with soil pH in lowland and upland sites (open circles). Shallow sites (open squares) are not included in the regression.

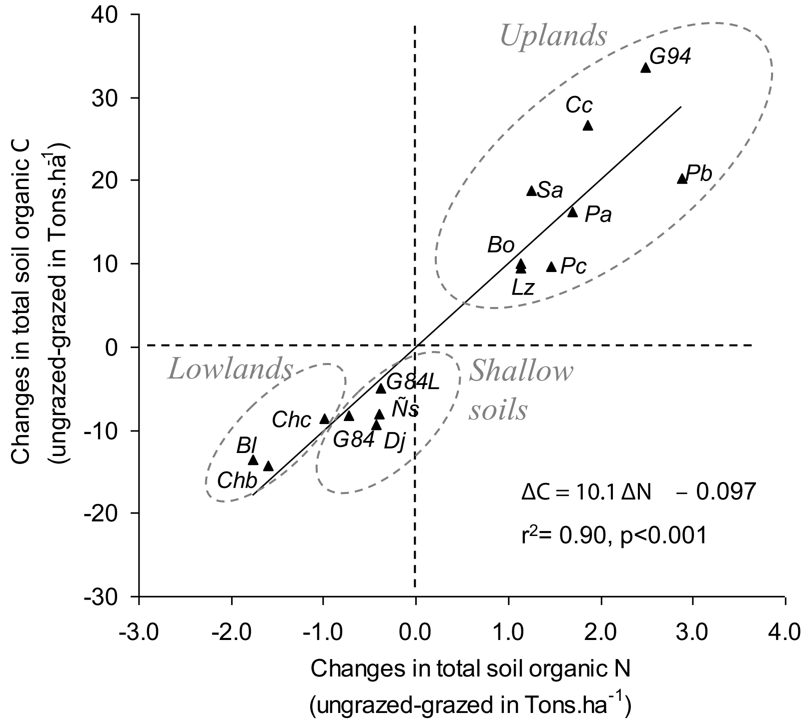

Figure 7. Relationship between changes in total soil organic C and N stocks associated with grazing removal across sites for the entire soil profiles. Changes in the different soil fractions (MAOM, POM 53 and POM 500), and annual rates of change showed a similar pattern with respect to total soil organic stocks; see Table 3. Dashed circles show different site types.

a strong link between SON and SOC dynamics (Table 3). Slopes were higher for the more labile fractions, reflecting higher C:N ratios; regression coefficients (r^2) were highest in the MAOM fraction, reflecting a tighter association between SOC and SON contents (less variable C:N among sites) in this recalcitrant soil fraction (Table 3).

[20] Total SOC stocks (instead of their changes after grazing removal) were tightly associated with total SON stocks, in both grazed ($r^2 = 0.77$, $p < 0.001$, $n = 15$) and ungrazed stands ($r^2 = 0.84$, $p < 0.001$, $n = 15$) across the *Rio de la Plata* grasslands. Total SOC and SON stocks, either in grazed or ungrazed stands, were significantly related to regional variations in soil depth ($r^2 > 0.45$, $p < 0.01$, $n = 15$), soil bulk density ($r^2 > 0.35$, $p < 0.01$, $n = 15$) and surface 0–30 rock contents ($r^2 > 0.27$, $p < 0.01$, $n = 15$), but not with soil texture, MAP, MAT or soil pH. However, when considering only uplands ($n = 8$), the amount of SOC in grazed or ungrazed stands was related to MAP ($r^2 > 0.50$, $p < 0.05$), while SON contents were related to MAT ($r^2 > 0.53$, $p < 0.05$) and surface 0–30 rock contents ($r^2 > 0.62$, $p < 0.05$), suggesting that SOM controls differ somewhat among site types. Multiple regression models for total SOC and SON stocks for the entire region were marginally significant and did not improve much simple model estimations.

3.3. Soil Bulk Density

[21] Changes in soil bulk density after livestock removal were tightly associated with soil sand contents (Figure 8). Grazing removal decreased soil bulk density in sites with high sand contents, but had no effect in sites with low sand contents, regardless of the age of the exclosure. These results suggest that changes in soil bulk density probably occurred in the first few years after grazing removal. Considering that soil expanded after grazing removal because of the cessation of trampling, we estimated soil expansion in the exclosures on the basis of changes in soil bulk density. Soil expansion in the exclosures, was highest in the upper layers, increasing as much as 4.9 cm in height in the coarser soils (an average decrease in soil bulk density of -0.20 g cm^{-3} for the first 30 cm of the soil).

4. Discussion

4.1. Belowground Biomass

[22] Our results support the idea of a unique influence of grazing on belowground biomass stocks across the whole region. Changes in root biomass observed in our study

Table 3. Regression Parameters Between Soil Organic C and N for Different Soil Fractions[a]

Soil Fraction	Slope	y Intercept	r^2	p Value	Depth Considered (cm)
POM 500	20.0	−0.25	0.71	<0.001	0–5
POM 53	15.2	−0.62	0.84	<0.001	0–30
MAOM	9.89	0.86	0.89	<0.001	0–100
Total Soil	10.1	0.097	0.90	<0.001	0–100

[a]With n = 15.

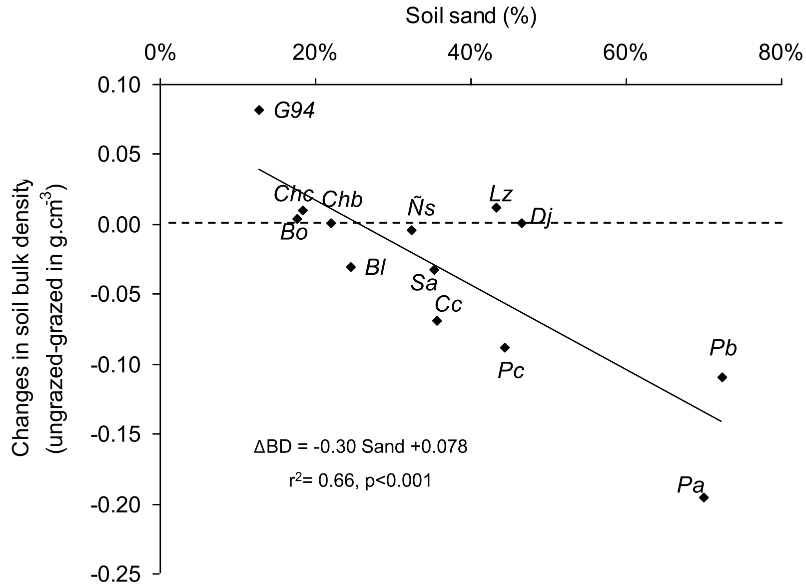

Figure 8. Relationship between changes in soil bulk density after grazing removal and soil texture. Data is average for the first 30 cm of the profile. The two sites (G84 and G84L) with lithic contact at < 10 cm were not included. In these two sites, reductions of soil bulk density were much greater (-0.20 g cm^{-3}) than for deeper soils with similar sand contents (~26%).

agree with other local studies that show decreases in root biomass after grazing removal [*Doll and Deregibus*, 1986; *Pucheta et al.*, 2004]. However, our results contrast with studies preformed in some other regions where grazing reduced belowground biomass. A detailed analysis of published data showed lower root biomass in grazed sites compared to ungrazed sites at mesic locations (~400 to 850 mm of mean annual precipitation) [*Schuman et al.*, 1999; *Fuhlendorf et al.*, 2002; *Kauffman et al.*, 2004; *Xie and Wittig*, 2004; *Derner et al.*, 2006; *Gao et al.*, 2008], but higher root biomass in grazed sites at humid and dry locations [*Smoliak et al.*, 1972; *Doll and Deregibus*, 1986; *McNaughton et al.*, 1998; *Frank et al.*, 2002; *Pucheta et al.*, 2004; *Reeder et al.*, 2004; *Cui et al.*, 2005]. Thus, community level responses to grazing seem to vary across a precipitation gradient although pot level experiments usually show reductions in belowground biomass after clipping, but do not incorporate potential changes in species composition [*Ferraro and Oesterheld*, 2002]. In our humid sites, shifts in species composition could have altered not only belowground biomass stocks but also their C:N ratio, that tend to be higher under grazing. The higher basal cover and the greater abundance of prostrate C_4 species, with large roots and rhizome biomass in the surface soil, were probably responsible for the observed increases in belowground biomass at our grazed sites [*Altesor et al.*, 2006].

4.2. Soil C and N

[23] Our result showed that grazing can either increase or decrease belowground SOC and SON stocks depending on several characteristics of the site analyzed. Several studies showed variable effects of grazing on SOM storage [*Milchunas and Lauenroth*, 1993; *Henderson et al.*, 2004; *Derner et al.*, 2006], but very few attempt to explain their results on the basis of potential mechanisms underling the observed patterns. Here, we will discuss plausible hypotheses for the effects of grazing on SOM, and compare their predictions against our results. Prior modeling results showed that SOM stocks (both SOC and SON stocks) were mainly determined by N dynamics within the *Rio de la Plata* grasslands [*Piñeiro et al.*, 2006]. Nitrogen additions have been shown to increase SOM formation in other humid environments [*Studdert and Echeverría*, 2000; *Neff et al.*, 2002]. Our results also support a tight connection between C and N dynamics in the region, and support the notion that N could be constraining SOC accumulation under grazing since organic matter inputs to the soil contain relatively less N [*Baisden and Amundson*, 2003; *Fabrizzi et al.*, 2003], as shown by the higher C:N of belowground organs and the soil POM 500 fraction in the surface layers.

[24] On the basis of the assumption that N limits SOM accumulation, the effect of grazing on SOM pools may arise from two mechanisms. The first one, the "*N loss hypothesis*," states that grazing primarily increases N losses by concentrating N in urine and dung patches that eventually is lost through volatilization or leaching, decreasing total N stocks and constraining SOM formation and storage. Nearly 20% of N deposited in urine and dung can be released by volatilization or leaching, depending on factors such as soil pH, soil depth, NH_4 or NO_3 concentration in soil, cation/anion exchange capacity, temperature, moisture and climate [*Lockyer and Whitehead*, 1990; *Scholefield et al.*, 1993; *Whitehead and Raistrick*, 1993; *Frank and Evans*, 1997]. Higher N losses will decrease N stocks and limit SOM formation and SOC sequestration at ecological timescales [*Johnson and Matchett*, 2001; *Conant et al.*, 2005; *Piñeiro et al.*, 2006]. Consequently, N and SOM pools will increase under grazing exclosure. However, on the basis of our results, we propose that the suppression of N losses in ungrazed

Table A1. Carbon and Nitrogen Contents in Belowground Biomass at Different Soil Depths for Each Site[a]

	Pa	Pb	Pc	Bo	Cc	Lz	Sa	G94	Dj	Ñs	G84L	G84	Bl	Chb	Chc
							ROOT C Ungrazed								
0–5	0.51	0.78	1.20	2.12	0.66	1.06	0.95	1.61	0.98	2.52	0.80	1.22	2.68	2.98	3.23
5–10	0.57	0.89	0.48	0.46	0.24	0.39	0.30	0.39	0.37	1.47	0.38		0.29	1.43	1.02
10–30	0.20	0.49	0.29	2.64	0.70	0.31	0.86	0.55					0.24	1.75	0.52
							ROOT C Grazed								
0–5	2.57	4.53	2.87	2.82	2.02	5.57	2.74	2.43	4.10	2.81	2.42	1.64	2.20	5.94	5.18
5–10	0.80	0.83	0.80	0.65	0.36	0.63	0.67	0.57	0.87	0.58	0.50		0.71	1.98	0.49
10–30	0.38	0.31	0.40	0.85	0.70	1.30	1.23	1.13					0.52	1.25	1.05
							ROOT N Ungrazed								
0–5	0.018	0.030	0.039	0.042	0.015	0.024	0.046	0.043	0.022	0.046	0.023	0.045	0.103	0.076	0.089
5–10	0.012	0.024	0.012	0.006	0.004	0.007	0.003	0.008	0.008	0.020	0.007		0.005	0.030	0.018
10–30	0.006	0.005	0.006	0.032	0.011	0.005	0.021	0.007					0.005	0.030	0.007
							ROOT N Grazed								
0–5	0.028	0.072	0.068	0.062	0.041	0.084	0.075	0.054	0.084	0.059	0.068	0.059	0.051	0.132	0.133
5–10	0.013	0.013	0.014	0.005	0.013	0.009	0.010	0.008	0.019	0.008	0.009		0.014	0.041	0.009
10–30	0.004	0.004	0.005	0.007	0.016	0.013	0.021	0.013					0.011	0.021	0.015

[a]Units are tons ha^{-1} $depth^{-1}$.

stands will affect SOM pools only in sites where soils are able to transform the greater N availability into SOM. Such capacity is associated with soil depth and soil texture, since the adsorption of organic matter to clay and silt particles is an important determinant of SOM stabilization [*Hassink*, 1997; *Jobbágy and Jackson*, 2000].

[25] The second mechanism, the "root-N retention hypothesis," states that grazing increases C and N allocation to belowground biomass, enhancing C inputs to the soil and N conservation, leading to SOM accumulation. Greater belowground C production under grazing may increase C inputs to the soil [*Milchunas and Lauenroth*, 1993; *Hui and Jackson*, 2006], since biomass allocated to aboveground tissues is not fully incorporated into the soil because of herbivory, surface respiration by decomposers and photodegradation [*Austin and Vivanco*, 2006]. It has been shown that SOM accumulation in surface layers is mainly affected by the quantity and quality of roots and their turnover [*Jobbágy and Jackson*, 2000]. Increased root allocation not only increases soil C inputs, but also N retention within the soil [*Heckathorn and Delucia*, 1996; *Dell et al.*, 2005]. Conservation of N in root tissues and tight cycling within the root zone has been suggested as mechanisms that reduce N leaching [*Dell et al.*, 2005; *Stewart and Frank*, 2008], potentially enhancing SOM accumulation [*Bird and Torn*, 2006]. In contrast to the N-loss hypothesis, the root-N retention hypothesis suggests reduced SOM pools in areas where grazing is suppressed. Such reductions should be greater in surface layers, where the highest decline in root biomass would be expected [*Jackson et al.*, 1996].

[26] We propose that both mechanisms (N loss and root-N retention hypotheses) are not mutually exclusive and operate simultaneously, but the relative importance of each mechanism may explain the differences in SOM changes observed after grazing removal across different site types. In upland soils the relative importance of both mechanisms may vary with soil depth. In the surface soil layers, the potential higher N availability of the exclosures (due to lower volatilization and leaching losses), was offset by the lower root biomass, resulting in no changes in surface SOM stocks. In deeper layers, where root biomass did not change, the retention of SOM by clay and silt in the MAOM fraction could have prevailed, resulting in the higher SOM contents observed in the exclosures. Overall, in grazed sites N losses were probably higher than the retention of N promoted by roots in surface soil layers and thus total SOM stocks were greater in the exclosures.

[27] Exclosures of shallow and lowland soils did not accumulate SOM in deep soil layers and thus root-N retention maintained higher SOM stocks in grazed stands. In shallow soils we propose that SOM retention in depth is blocked by bedrock, and thus surface reductions in belowground biomass would shape SOM changes after grazing removal. The lower surface root biomass in the ungrazed stands would decrease SOM stocks, while in the grazed stands N is efficiently retained within the root zone [*Smoliak et al.*, 1972; *Frank et al.*, 1995, 2000]. In these soils high losses of dissolved organic nitrogen in leaching waters are expected after grazing removal [*Jackson et al.*, 2006]. As *Dell et al.* [2005, p. 1280] stated, "Conservation of N by plants and tight cycling of N within the root zone suggest mechanisms by which prairie can be a highly productive ecosystem despite limited N availability." Similarly, grazed stands in lowland sites had higher SOM stocks than ungrazed stands and root-N retention prevailed. At these sites, the mechanism by which N is preserved in the grazed sites is less clear. Possibly, the interaction of grazing and flooding may be causing the observed pattern (both grazed and ungrazed stands at lowland sites remain flooded annually during ~3 months). However, the mechanism by which exclosures lose SON and SOC in lowland sites requires future study.

4.3. Soil Bulk Density

[28] Soil texture controlled the effects of grazing on soil bulk density. The relationship between sand content and changes in bulk density reported here helps to reconcile earlier contradictory results that showed no effects of grazing on soil bulk density in fine textured soils [*Taboada and Lavado*, 1988], but high effects in more coarse textured

Table A2. Soil Organic Carbon Contents in Different Soil Fractions in Depth for Each Site[a]

Soil Fraction	Depth (cm)	Pa	Pb	Pc	Bo	Cc	Lz	Sa	G94	Dj	Ñs	G84L	G84	Bl	Chb	Chc
							Ungrazed									
POM 53	0–5	3.78	2.09	3.64	2.53	3.91	3.55	1.97	2.71	2.52	2.68	2.55	3.14	3.57	0.75	6.01
	5–10	1.02	0.86	0.67	1.68	1.94	2.00	0.84	0.87	1.30	2.04		0.84	1.77	0.49	0.32
	10–30	2.57	2.26	1.83	2.63	6.38	4.76	1.73	2.59	3.40	5.27			3.57	1.27	2.88
MAOM	0–5	21.39	18.96	17.99	12.55	16.78	17.26	14.16	23.86	16.09	22.25	13.60	15.88	10.31	11.73	10.23
	5–10	18.53	19.72	16.67	10.51	16.52	17.64	12.08	18.40	13.77	21.94		9.03	8.59	11.57	16.20
	10–30	59.03	67.56	61.32	40.79	61.13	75.74	40.42	80.55	53.36	83.62			28.83	29.38	32.34
	30–50	24.95	30.44	37.45	19.49	24.53	29.43	22.51	45.55					14.07	18.60	17.55
	50–70	17.32	23.15	34.14	12.77	18.39	19.24	14.49	32.44					11.77	16.30	10.50
	70–100	19.55	24.29	40.57	15.81	17.97	12.05	14.30	37.10					9.15	17.42	11.97
							Grazed									
POM 53	0–5	1.76	2.69	2.58	3.45	3.24	5.69	3.59	1.93	4.69	4.87	4.72	4.39	4.07	1.19	5.08
	5–10	0.75	0.65	1.02	2.04	1.19	1.60	1.14	1.28	1.56	2.38		0.71	2.18	2.01	0.35
	10–30	2.27	1.51	1.90	2.92	8.16	4.30	2.16	2.37	6.01	3.21			3.26	2.35	2.12
MAOM	0–5	18.04	18.83	18.41	12.22	15.58	18.34	11.87	19.75	18.73	26.20	16.36	22.14	13.05	13.64	15.25
	5–10	16.35	16.48	16.45	9.53	13.05	16.08	9.75	21.40	14.35	24.98		9.82	10.99	12.42	17.74
	10–30	52.37	58.78	61.21	35.11	52.19	73.60	30.82	72.49	54.54	84.22			30.77	35.12	35.15
	30–50	25.12	29.49	35.31	18.41	16.31	28.04	21.46	41.12					16.96	22.64	16.98
	50–70	13.81	22.06	27.44	9.80	13.99	11.50	12.00	23.84					13.15	15.79	10.77
	70–100	21.34	18.54	40.24	15.86	17.24	12.40	10.84	26.35					10.76	16.73	13.26

[a]Units are tons ha^{-1} depth^{-1}.

soils [*Reeder et al.*, 2004]. Fine textured soils could be offsetting grazing compaction by expansion of clay particles, which are common in these grasslands soils. Our results also suggest that compaction by trampling can be extraordinarily high in shallow soils with bedrock at less than 10 cm deep.

5. Conclusions

[29] In summary, ungrazed stands had significantly lower belowground biomass C in the topsoil. Total SOM stocks (POM+MAOM) were significantly higher in ungrazed than in grazed stands of upland soils and lower in shallow and lowland soils. Surprisingly, differences were largely associated with changes in MAOM pools rather than POM pools. However, the C:N ratio of belowground biomass and soil POM tended to be higher in grazed stands compared to the exclosures, while MAOM C:N was unchanged. We propose that consequences of grazing on SOM stocks in the Río de la Plata grasslands are mainly controlled by soil properties, and vegetation allocation patterns acting through N retention. These two factors should be taken into account when designing management strategies for sustainable production of these and other grasslands.

Appendix A

[30] Measured values of carbon and nitrogen contents in belowground biomass for different depths at each site are shown in Table A1. Measured values of soil organic carbon in different soil fractions and depths at each site are presented in Table A2, and nitrogen contents are presented in Table A3.

Table A3. Soil Organic Nitrogen Contents in Different Soil Fractions in Depth and at Each Site[a]

Soil Fraction	Depth (cm)	Pa	Pb	Pc	Bo	Cc	Lz	Sa	G94	Dj	Ñs	G84L	G84	Bl	Chb	Chc
							Ungrazed									
POM 53	0–5	0.28	0.13	0.21	0.12	0.21	0.17	0.10	0.12	0.15	0.13	0.15	0.16	0.22	0.05	0.39
	5–10	0.07	0.05	0.04	0.07	0.12	0.11	0.05	0.03	0.08	0.10		0.04	0.08	0.04	0.03
	10–30	0.16	0.13	0.10	0.12	0.48	0.26	0.09	0.11	0.24	0.25			0.21	0.09	0.19
MAOM	0–5	2.13	1.94	1.69	1.22	1.51	1.51	1.39	1.62	1.51	1.92	1.41	1.49	1.00	1.27	1.11
	5–10	1.82	2.06	1.51	1.10	1.48	1.57	1.23	1.51	1.34	1.90		0.79	0.89	1.32	1.85
	10–30	5.41	7.09	5.29	4.23	5.20	6.29	4.16	5.96	5.20	7.12			2.92	3.63	3.93
	30–50	2.02	2.80	2.95	2.03	2.17	2.84	2.24	3.12					1.75	2.36	2.29
	50–70	1.87	2.43	2.87	1.49	2.01	2.45	1.69	2.00					1.81	2.01	1.67
	70–100	2.50	2.88	3.45	1.96	2.04	2.15	1.61	2.09					1.90	2.77	1.93
							Grazed									
POM 53	0–5	0.10	0.14	0.11	0.18	0.17	0.27	0.14	0.08	0.26	0.24	0.29	0.20	0.21	0.08	0.34
	5–10	0.04	0.03	0.05	0.09	0.05	0.09	0.05	0.04	0.09	0.11		0.03	0.10	0.11	0.03
	10–30	0.12	0.07	0.09	0.12	0.62	0.26	0.09	0.09	0.28	0.16			0.20	0.16	0.12
MAOM	0–5	1.80	1.95	1.56	1.17	1.40	1.52	1.19	1.69	1.79	2.18	1.65	2.08	1.31	1.48	1.53
	5–10	1.71	1.75	1.36	0.86	1.21	1.49	1.02	1.66	1.38	2.12		0.89	1.00	1.40	1.99
	10–30	4.88	5.93	4.96	3.40	4.61	6.17	3.28	4.94	5.16	7.01			3.41	4.20	4.33
	30–50	2.09	2.61	2.70	2.24	1.53	2.57	2.20	2.60					1.96	2.75	2.23
	50–70	1.71	2.23	2.23	1.28	1.69	1.71	1.54	1.38					1.95	2.11	1.69
	70–100	2.13	1.91	3.60	1.87	2.09	2.14	1.83	1.60					2.39	2.85	2.11

[a]Units are tons ha^{-1} depth^{-1}.

Notation

SOC Soil Organic Carbon.
SON Soil Organic Nitrogen.
SOM Soil Organic Matter.
POM Particulate organic matter.
POM 500 Particulate organic matter separated using a 500 μm sieve.
POM 53 Particulate organic matter separated using a 53 μm sieve.
MAOM Mineral associated organic matter.
MAP Mean annual precipitation.
MAT Mean annual temperature.

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