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REGULAR ARTICLE

Grazing-induced changes in plant species composition affect plant and soil properties of grassland mesocosms

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Abstract Grazing-induced floristic changes in plant communities may accelerate or reduce plant and soil processes through changes in litter quality. Here, we intended to elucidate if the joint action of live and senescing plant tissue of palatable and non-palatable species differentially influences soil processes and properties. We conducted a 1-year experiment with mesocosms from a subhumid grassland. Mesocosms were monocultures of palatable or non-palatable species and a multispecific control. Palatable species included a legume and annual and perennial grasses, whereas non-palatable species included a perennial grass and annual and perennial forbs. Palatable monocultures showed greater soil mineral nitrogen, soil bacterial diversity, and lower soil pH than non-palatable monocultures. These differences were not accounted for by differences in plant biomass. The multispecific control treatment only exhibited greater shoot biomass than the monocultures, and lower root biomass than the palatable monocultures. Our results suggest that the whole (live + dead plant tissue) had a specific imprint on soil system even when variation was not very apparent in terms of plant biomass, and that this effect was associated with plant palatability to domestic large herbivores.

Keywords Grazing · Plant species effect · Carbon · Nitrogen · Soil biota

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Introduction

Domestic herbivores influence soil biota and nutrient cycling through a wide variety of indirect and direct mechanisms that act at different levels (Bardgett et al. 1998; Wardle and Bardgett 2004). Among indirect mechanisms, biomass removal by herbivores often alters rhizosphere activity by increasing root exudation of labile compounds (Hamilton and Frank 2001), by promoting ecotypes with different allometry and tissue quality (Olofsson and Oksanen 2002; Semmartin and Ghersa 2006), and by changing species composition, which impact on the litter breakdown dynamics of the plant community (Olofsson et al. 2004a, b; Semmartin et al. 2004; Garibaldi et al. 2007). Herbivores directly influence soil functioning by returning carbon and mineral nutrients in the form of dung and urine deposition (Seagle and McNaughton 1992; Bardgett

et al. 2001) and by trampling, which often reduces soil aeration and moisture and increases soil salinization (Lavado and Taboada 1988; Lavado et al. 1992). These mechanisms may be partially responsible for the greater soil nitrogen content, litter breakdown, soil pH, and bacterial proportion in soil biota in intensively grazed ecosystems (Shariff et al. 1994; Chaneton et al. 1996; McNaughton et al. 1997; Tracy and Frank 1998; Bardgett et al. 2001; Olofsson et al. 2004a, b; Grayston et al. 2004; Altesor et al. 2006).

Grazing-induced changes of plant species composition have variable impact on plant traits and soil processes such as litter quality, litter decomposition, and nutrient mineralization. Increasing evidence from litterbag experiments supports the grazing optimization hypothesis (McNaughton 1979), as they show that herbivores promote plant species with highly nutritious and easily decomposable tissue (Olofsson and Oksanen 2002; Semmartin et al. 2004; Garibaldi et al. 2007). However, a series of studies also revealed a negative impact of grazing on plant decomposability and nutrient cycling (Pastor et al. 1993; Moretto et al. 2001; Moretto and Distel 2002; Wardle et al. 2002). Most of these studies on the effects of species composition on nutrient cycling have focused on litter-mediated effects and paid less attention to the effects mediated by live tissue (Wedin and Tilman 1990, 1996; Zak et al. 2003). Plant phenology, carbon allocation pattern, quantity and quality of root exudates, and nutrient uptake from soil solution are part of living plant traits and processes that vary among species and may also contribute to the species effects on soil functioning (Wardle et al. 2004). Therefore, the effects of plant species on nutrient cycling and soil biota result from the joint action of live and dead plant tissue.

In the Flooding Pampa grassland (Argentina), grazing by cattle promotes dramatic changes in species composition that are associated with changes in several sub-processes of nutrient cycling (Semmartin et al. 2004, 2007, 2008; Garibaldi et al. 2007). Grazing increases plant diversity, and promotes the replacement of native perennial grasses by exotic forbs and annual grasses (Sala et al. 1986; Facelli 1988; Rusch and Oesterheld 1997; Chaneton et al. 2002). Litter of many of these grazing-promoted forbs is richer in nitrogen and is more decomposable than the litter of the grasses they replaced (Semmartin et al. 2004; Semmartin and Ghersa 2006; Garibaldi et al. 2007). The objective of this study was to identify the effects of grazing on soil processes and properties mediated by shifts in plant species composition. We performed a mesocosm experiment with soil and vegetation monoliths obtained from a grazed stand of the Flooding Pampa grassland. We turned these mesocosms in seven types of monocultures, four with palatable and three with unpalatable species, plus an unmodified multispecific control. One year later, we evaluated several plant and soil properties. Therefore, we were able to evaluate to what extent individual species differentially impact on a number of soil traits, and how these impacts were associated with species palatability.

Materials and methods

The Flooding Pampa grassland and plant species

We collected plant and soil monoliths from a humid meadow located nearly 200 km south of Buenos Aires city (36°30′S, 58°30′W). The climate is temperate subhumid, with mean monthly temperatures ranging from 7°C in July to 22°C in January. Mean annual precipitation is 990 mm, uniformly distributed throughout the year. The landscape exhibits an extremely flat topography over Typical Natraquolls, with a 15-cm-deep loamy A horizon (pH 6.7) containing 3.5% organic carbon and 24% clay, and a thick natric B horizon (Lavado and Taboada 1988). Vegetation corresponds to one of the most widespread community type in the central Flooding Pampa (Perelman et al. 2001; Aragón and Oesterheld 2008) which comprises a mix of approximately 60 species evenly distributed among dicotyledonous forbs and C3 and C4 graminoids (Semmartin et al. 2007); woody components are virtually absent in this grassland. The combination of species with C3 and C4 photosynthetic pathways determines a seasonal pattern of aboveground productivity with a maximum from late spring to the beginning of summer (Semmartin et al. 2007; Aragón and Oesterheld 2008). Annual aerial net primary production is approximately 550 gm^{-2} and ranges from a minimum of 0.35 $gm^{-2}day^{-1}$ during autumn and winter to 1 $gm^{-2}day^{-1}$ during spring and summer (Semmartin et al. 2007). The site where vegetation and soil monoliths were collected has been continuously grazed at moderate intensities (~0.5 cows per hectare) by domestic cattle for ca.100 years and has never been ploughed.

The seven studied plant species corresponded to four palatable species: *Lolium multiflorum*, *Festuca arundinacea*, *Paspalum dilatatum*, and *Lotus tenuis*, and three non-palatable species: *Stenotaphrum secundatum*, *Leontodon taraxacoides,* and *Ambrosia tenuifolia* (Table 1). The species were chosen based on their importance within the community, and on their individual preference by domestic grazing, as inferred from long-term exclosure experiments and local knowledge (Facelli 1988; Chaneton et al. 2002; Rusch and Oesterheld 1997). In addition, each group displays a variety of life forms, growth habit, origin, etc. (Table 1). Although the palatable *Lotus tenuis* and the unpalatable *Ambrosia tenuifolia* do not show the expected response to grazing (Table 1), the former has been widely described as a valuable forage species (Sevilla et al. 1996; Agnusdei and Mazzanti 2001), and the latter as a widespread and problematic weed (Sala et al. 1981; León and Burkart 1998).

Experimental design and mesocosm set-up

At the end of spring 2004, we collected 24 'soil + vegetation' monoliths (hereafter mesocosms) from a grazed paddock of the grassland described above. Mesocosms were prisms of 45×45 cm and 20 cm depth, and were collected from a ~2,500-m^2 grazed stand. They included 20 cm of top soil and the vegetation rooted in it. The mesocosms were selected as follows. For each of the seven species (Table 1), we selected three mesocosms (replicates) containing individuals of that species, hereafter 'the target species'. We achieved this by walking a fixed number of steps and choosing the nearest patch that had at least a few individuals of the target species. Additionally, we randomly collected three mesocosms that were assigned to a multispecific control treatment. By using this systematic method for collection, we assured the independency among the experimental units. We carried and cultured the mesocosms to a common experimental garden at the Faculty of Agronomy campus in Buenos Aires, where climatic conditions are relatively similar to those at the field site. We randomly distributed them in a grid of 50 m^2, and watered them for 4 weeks to allow for plant recovery from transplant stress. In February (summer), we manually removed aerial and belowground organs of all non-target species so that 21 of the 24 mesocosms turned into monospecific vegetation units. In the three multispecific controls, we removed an amount of biomass similar to that removed in the monospecific mesocosms in order to visually balance their initial biomass. Mesocosms were grown throughout a year, and were weeded and watered weekly.

Table 1 Plant species characteristics grouped by its preference to domestic herbivores, palatable and non-palatable, used in the mesocosms experiment

	Life form	Growth habit	Peak growth	Origin	Life history	Relative cover (%)[a]	
						Grazed	Ungrazed
Palatable							
Lolium multiflorum	Grass, C_3	Erect	Spring	Exotic	Annual	8	48
Festuca arundinacea	Grass, C_3	Erect	Spring	Exotic	Annual	0.95	14.9
Paspalum dilatatum	Grass, C_4	Semi-prostrate	Summer	Native	Perennial	2.5	3
Lotus tenuis	Legume, C_3	Semi-prostrate	Spring	Exotic	Perennial	0.75	0
Non-palatable[b]							
Stenotaphrum secundatum	Grass, C_4	Prostrate	Summer	Native	Perennial	30.2	0.01
Leontodon taraxacoides	Forb, C_3	Rosette	Spring	Exotic	Perennial	13.5	0.02
Ambrosia tenuifolia	Forb, C_3	Erect	Summer	Native	Perennial	0.41	1.9

[a] Mean relative basal cover of species in each study site for the period 1985–2004 (data from Chaneton et al. 2002; Semmartin et al. 2007)

[b] Species' responses to grazing determined from comparisons of grazed and ungrazed (exclosure) sites, after Chaneton et al. (1988, 2002), Facelli (1988), Rusch and Oesterheld (1997)

Plant and soil analyses

Both at the beginning (February 2005) and at the end of the experiment (February 2006), we estimated shoot and root biomass, soil mineral nitrogen, soil pH, and the soil bacterial community-level physiological profiles (CLPP). At the end of the experiment, we also measured, in situ, heterotrophic plus root respiration, after a destructive harvest of plant shoot biomass. Shoot and root biomass were quantified on the basis of destructive harvests of biomass. Initial shoot biomass of mesocosms was not known directly, but as we quantified all the biomass removed, we could indirectly test for differences in initial biomass by testing for differences in removed biomass. The assumption was that all mesocosms originally had similar biomass. Final shoot biomass was quantified on the basis of a destructive harvest of the total biomass in each mesocosm. Harvested biomass was classified into live and dead tissue by species. We sampled belowground biomass by two soil cores (20 cm deep, 1.88 cm diameter) per mesocosm. Cores were divided into two depths, 0–5 cm and 6–20 cm, and gently washed with 5% sodium hexametaphosphate solution as dispersant agent. Above- and belowground biomass was dried at 60°C for 48 h. Initial and final mineral nitrogen content of soil solution were quantified by extracting ~15 g of fresh soil samples in 50 ml KCl 2 M. Ammonium and nitrate contents of filtered extracts were measured with a flow injection autoanalyzer (Alpkem, Wilsonville, Oregon). Initial and final soil pH were measured in a 1:2.5 soil:water solution.

The functional composition of the heterotrophic bacterial community was studied by substrate utilization patterns (adapted from Garland and Mills 1991; and see Naiman et al. 2009). Initial and final soil bacterial community-level physiological profiles were quantified based on the color development of soil inocula on 23 carbon sources: alanine, arginine, asparagine, benzoic acid, cellobiose, dextrose, phenylalanine, fructose, glycerol, histidine, itaconic acid, lactic acid, lactose, levulose, malic acid, manitol, piruvic acid, proline, putrescine, ramnose, salicylic acid, tween 80, xylose, and a blank with sterile distilled water. Each sterile microplate contained 96 wells where samples were distributed. Each well received 50 µl of a standard basal media and 50 µl of tetrazolium violet, which develops color under CO_2 production. Each well was inoculated with soil aliquots of 50 µl from 10^{-4} soil suspensions and were incubated at 25°C for a maximum of 96 h. Well color development was measured at 24, 48, 72, and 96 h (only 48-h measurements are shown), recorded as optical density at 590 nm with a plate reader (Multiskan; Labsystems, Helsinki, Finland). The average well color development of the 23 carbon sources of each sample was calculated and used to transform individual well values to eliminate variation in color development caused by different cell densities (Garland 1996). At the end of the experiment, after a destructive harvest of shoot biomass, we measured C-CO_2 emissions from soil biota and plant roots. Each mesocosm was sampled by three 1-min-long subreplicates with an infrared gas analyzer (Licor 6200; Lincoln, NE, USA) adapted to a 6-l cylindrical PVC chamber (Hall et al. 1990).

Data analyses

We performed independent analyses on the initial and final datasets. We expected to find a relative homogeneity for plant and soil variables of the different mesocosms at initial time. In contrast, at final time, we expected to find differences among groups of species according to their preference by grazers. Therefore, for the five measurements recorded at initial time (removed shoot biomass, root biomass, soil pH, soil mineral N content, and soil bacterial CLPP), we performed analyses of variance with *species* factor (8 levels, 7 species, and 1 multispecific control). At final time, these variables were analyzed by covariance analyses, including initial values as covariable (except for respiration, only measured at final time). In addition, the ANCOVA model for respiration, soil mineral nitrogen, pH, CLPP, and bacterial richness and diversity, also included final plant biomass as a co-variable, in order to isolate biomass as a source of variation (Zak et al. 2003). Univariate ANOVA and ANCOVA tests were followed by a priori established mean contrasts between: (1) palatable versus non-palatable species, (2) palatable species versus control, and (3) non-palatable species versus control. Initial and final analyses of CLPP consisted of two independent principal component analysis (PCA) followed by an ANOVA of the position of each sample along the first discriminant axis. Those variables not fulfilling variance homoge-

neity (removed shoot biomass, root biomass, and CLPP) were log transformed to reduce heterogeneity. We calculated bacterial functional richness, based on the number of oxidized carbon substrates, and diversity through the Shannon-Weaver index (*H*), which combines richness and evenness, as follows $H = \Sigma \text{pi} * (\ln \text{pi})$, where pi is the ratio between the activity on each substrate (the optical density developed) and the sum of all activities on all substrates. We used an optical density of 0.25 as a threshold for positive response (Garland 1996). In order to explore for possible initial differences in the original plant community structure, we evaluated initial species richness and eventual initial plant species composition. We performed an ANOVA of initial plant species richness and a principal component analysis, based on presence of non-target species in each mesocosm. Then, we performed an ANOVA using the position of each sample along the first discriminant axis, as described for CLPP.

Results

Species richness of the mesocosms in their original composition did not differ among mescosm types ($P_{7,\ 16}$=0.2, data not shown). Total richness was 41 plant species, with an average of 11 species per mesocosm. In addition, plant species composition did not differ among mesocosm types (first PCA axis only explained 14% of variation in species composition of mesocosms, data not shown). The most abundant species, based on the removed shoot biomass, were *Stenotaphrum secundatum*, *Paspalidium paludivagum*, and *Paspalum vaginatum* (33, 15, and 12% of total removed biomass, respectively).

Both shoot biomass removed at initial time, and initial root biomass were similar among species (Table 2, Fig. 1). In contrast, at final time, remaining shoot biomass was greater in the multispecific controls than in the monospecific mesocosms, with no difference between palatable and non-palatable mesocosms (Table 2, Fig. 1 left panel). Final root biomass was significantly lower in palatable than in non-palatable mesocosms (Table 2, Fig. 1 right panel). Initial biomass did not account for variation in final biomass (*P* of co-variable=0.64).

Soil mineral nitrogen did not differ among species at initial time, but did differ at final time (Table 2), when palatable species showed greater nitrogen contents than non-palatable species (*P* of contrast= 0.003; Fig. 2). Initial soil mineral nitrogen content did not account for significant variation in final contents (*P* of co-variable=0.26), whereas total final plant biomass negatively co-varied with final soil nitrogen content (*P* of co-variable=0.01).

Soil pH did not differ among species at initial time, but marginally differed at final time (*P*=0.08; Table 2) when pH of mesocosms cultivated with palatable species was lower than that of mesocosms cultivated with non-palatable species (Fig. 3). Neither initial pH nor final root biomass accounted for significant variation in final soil pH (*P* of co-variable=0.8 and 0.9, respectively).

The CLPP did not differ among species at any time (Table 2, Fig. 4). Additionally, initial CLPP did not account for significant variation of final CLPP (*P* of co-variable=0.23). At both sampling times, the first

Table 2 ANOVA (*F* values) results for the effects of three plant groups (multispecific control, monospecific palatable, and non-palatable species) on initial and final variables; plants, soil, and microorganisms; shoot and root biomass (0-5 cm); nitrogen; soil pH; microbial richness and diversity (Shannon-Weaver index); CLPP (community level physiological profile, position along the first axis of the PCA test); and soil + roots respiration. Note that analyses of monospecific mesocosms at initial time correspond to a situation where both palatable and non-palatable species were still growing in a mixture of other species, whereas analyses at final time correspond to actual monocultures

Experimental time	Plants		Soil		Microorganisms			
	Shoot[a]	Root	pH	Nitrogen	Richness	Diversity	CLPP	Soil + roots respiration
Initial time	0.30	0.81	0.32	1.61	1.80	0.91	0.63	-
Final time	4.00*	2.50†	2.27†	4.34**	0.57	2.19†	1.02	0.66

Numerator and denominator degrees of freedom were 7 and 16, respectively, except for nitrogen at final time (1, 14, respectively)

[a] Shoot biomass at initial time corresponds to removed biomass, and at final time corresponds to remaining biomass

**$P \leq 0.01$; *$P \leq 0.05$; †$P \leq 0.1$

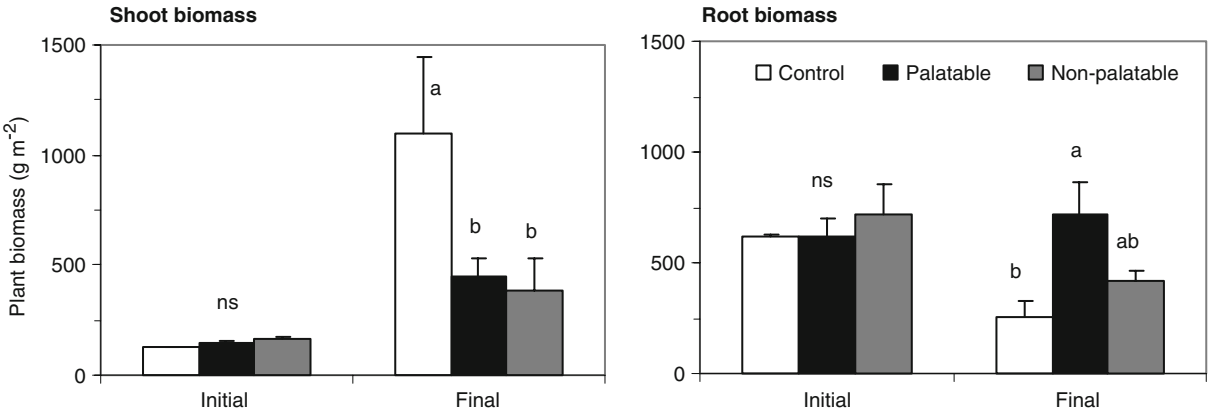

Fig. 1 Shoot (live + standing dead) and root (0–5 cm) biomass of multispecific controls, and monospecific mesocosms cultivated with palatable and non-palatable grassland species, at the beginning (*initial*) and after 1 year (*final*) of cultivation in an experimental garden. Values represent removed biomass at initial time and remaining biomass at final time (see "Materials and methods" for further explanation). Note that monospecific mesocosms at initial time correspond to a situation where both palatable and non-palatable species were still growing in a mixture of other species, whereas analyses at final time correspond to actual monocultures. Different *letters* indicate significant differences of contrasts among the three groups of species: control, palatable, and non-palatable species; *ns* non-significant differences among groups of species. *Vertical bars* ±1 SE

PCA axis explained a low proportion of variance and the carbon sources that best correlated with them varied with the time (Fig. 5). The Shannon-Weaver diversity index of microbes did not differ among species at initial time, whereas it showed marginal differences at final time, when non-palatable mesocosms were less diverse than the rest (Table 2, Fig. 5). Neither initial diversity nor plant biomass accounted for significant variation of final diversity (P of covariable=0.77 and 0.53, respectively). Soil microbial plus root respiration at the end of the experiment ranged between 630 and 850 mg C-CO_2 m^{-2} h^{-1}, and did not differ among species (Table 2). Root biomass

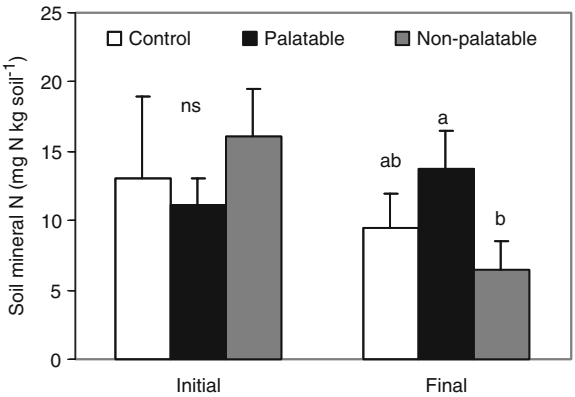

Fig. 2 Soil mineral N content of multispecific controls. and monospecific mesocosms cultivated with palatable and non-palatable grassland species, at the beginning (*initial*) and after 1 year (*final*) of cultivation in an experimental garden. Note that monospecific mesocosms at initial time correspond to a situation where both palatable and non-palatable species were still growing in a mixture of other species, whereas analyses at final time correspond to actual monocultures. Different *letters* indicate significant differences of contrasts among the three groups of mesocosms: control, palatable and non-palatable species; *ns* non-significant differences among groups of mesocosms (P>0.05). *Vertical bars* ±1 SE

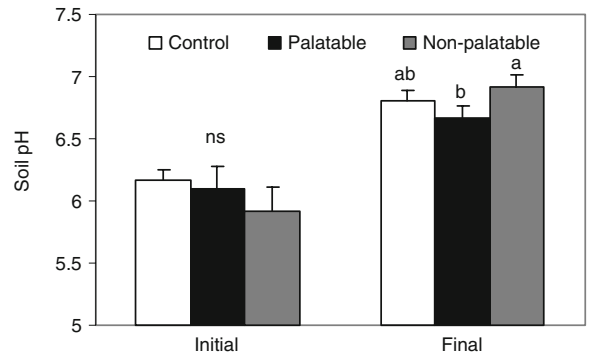

Fig. 3 Soil pH multispecific controls, and monospecific mesocosms cultivated with palatable and non-palatable grassland species, at the beginning (*initial*) and after 1 year (*final*) of cultivation in an experimental garden. Note that monospecific mesocosms at initial time correspond to a situation where both palatable and non-palatable species were still growing in a mixture of other species, whereas analyses at final time correspond to actual monocultures. Different *letters* indicate significant differences of contrasts among the three groups of mesocosms: control, palatable and non-palatable species; *ns* non-significant differences among groups of mesocosms (P> 0.05).*Vertical bars* ±1 SE

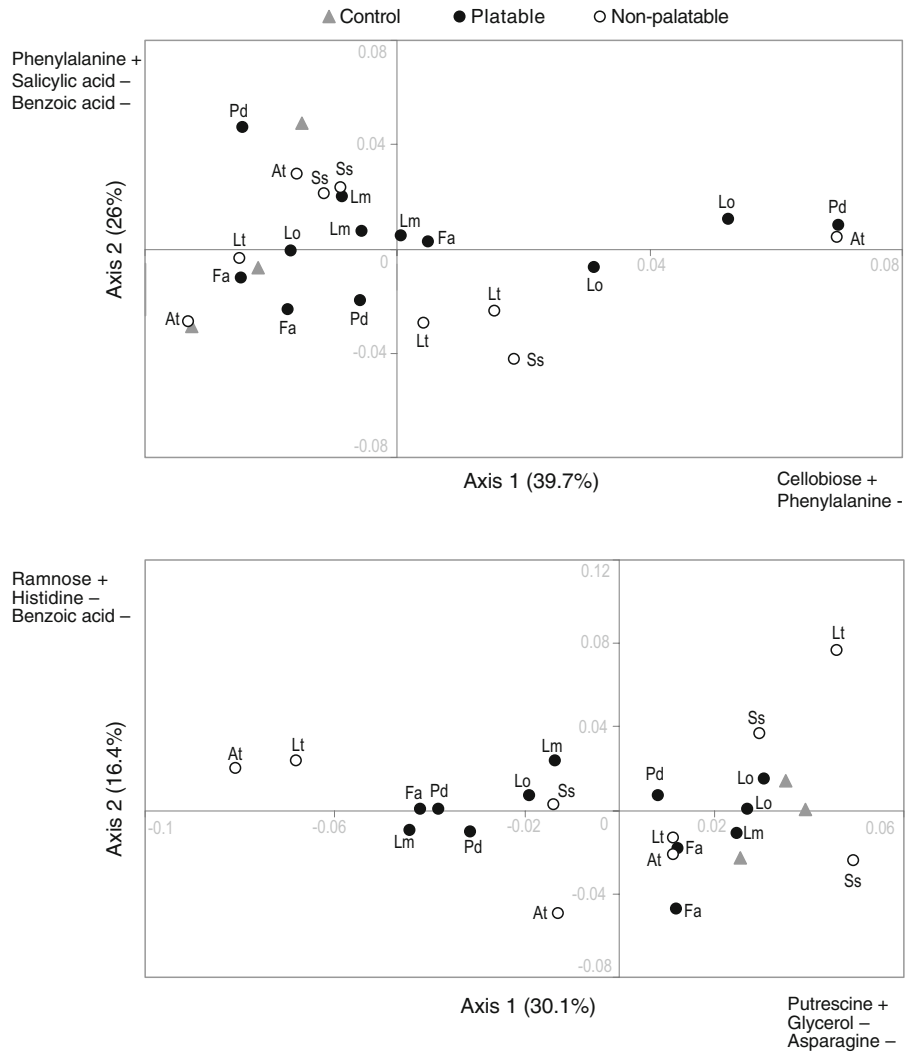

Fig. 4 Principal components on standardized data of microbial activity on 23 carbon sources of soils cultivated with monospecific palatable, non-palatable, and multispecific control grassland species, at the beginning (*upper panel*) and after one year (*lower panel*) of cultivation in an experimental garden. Note that monospecific mesocosms at initial time correspond to a situation where both palatable and non-palatable species were still growing in a mixture of other species, whereas analyses at final time correspond to actual monocultures. Palatable: *Festuca arundinacea* (Fa), *Lolium multiflorum* (Lm), *Lotus tenuis* (Lo), *Paspalum dilatatum* (Pd). Non-palatable: *Ambrosia tenuifolia* (At), *Leontodon taraxacoides* (Lt), *Stenotaphrum secundatum* (Ss). Carbon sources in both axes indicate those that accounted for greater variation in the bacterial activity pattern

at final time did not influence this pattern (P of covariable=0.52).

Discussion

We showed that in a very short term (1 year), individual species exerted an idiosyncratic imprint on plant, soil, and bacterial components of the plant-soil system which, in turn, was associated with plant palatability for large herbivores (Table 2). After 1 year of cultivation of monospecific mesocosms, the heterogeneous set of the seven species studied displayed, for most of the analyzed traits, a significant variation that was explained by their preference to herbivores. Hence, the palatable species, which included annual and perennial grasses, and a legume, showed higher soil mineral nitrogen, higher soil bacterial diversity, and lower soil pH than the non-palatable species, which included annual and perennial forbs, and a perennial grass. Furthermore, these differences among both groups of species took place in the absence of differences in plant biomass production. The multispecific control treatment exhibited a greater final shoot biomass than the monospecific ones, but did not differentiate from monocultures in any other soil features.

Palatability to herbivores did not account for significant variation in shoot or root biomass, whereas multispecific controls had greater shoot plant biomass than monocultures. The greater plant biomass in multispecific mesocosms agrees with the general

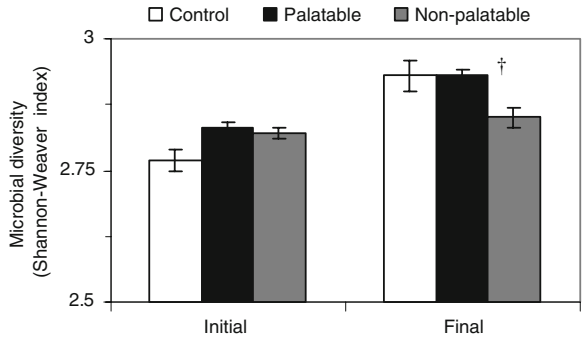

Fig. 5 Microbial diversity based on 23 carbon-based sources metabolized by heterotrophic bacterial biota of soils cultivated with monospecific palatable, non-palatable and multispecific control grassland species, at the beginning (initial) and after one year (final) of cultivation in an experimental garden. Note that monospecific mesocosms at initial time correspond to a situation where both palatable and non-palatable species were still growing in a mixture of other species, whereas analyses at final time correspond to actual monocultures. † Significant differences of non-palatable with respect to palatable and control mesocosms (P=0.09).*Vertical bars* ±1 SE

notion of a positive relationship between primary productivity and plant diversity (Hector et al. 1999; Tilman et al. 2001), which mainly relies on the temporal niche complementariness of plant species (Tilman et al. 1996; Caldeira et al. 2005) together with a greater probability of having more productive species in the mix (Tilman et al. 1997, 2001; Hector et al. 2002). In contrast, the lack of differences among palatable and non-palatable species disagrees with previous evidence revealing that, in the Flooding Pampa grasslands, long-term domestic herbivory reduces aerial net primary productivity (Rusch and Oesterheld 1997). The hypothesis for this negative effect proposes that this is the consequence of the floristic replacement triggered by grazing, in which a low productive group of cool season exotic forbs displaces the most productive, warm season native grasses (Rusch and Oesterheld 1997; Chaneton et al. 2002). In our experiment, the non-palatable group of species included one cool season forb, *Leontodon taraxacoides*, which, in fact, had the lowest biomass accumulation during the experimental period (501 gm^{-2} of final total biomass); but it also included *Ambrosia tenuifolia*, a warm season perennial forb that was among the most productive species (1,330 gm^{-2} of final total biomass). On the other hand, the palatable species displayed a similar variation in biomass production, since *Lolium multiflorum* and *Festuca arundinacea* accumulated 440 and 2,130 gm^{-2} of total biomass, respectively. Although our study does not reflect exactly what takes place in the field, and considered a relatively small number of species, our results suggest that differences found by Rusch and Oesterheld (1997) might be only partially accounted for by traits inherent to the species, and that other grazing effects such as trampling might also explain this negative effect (Taboada and Lavado 1993).

The soil solution of palatable mesocosms had a greater mineral nitrogen concentration than non-palatable mesocosms. A general model on the nutritional outcome of plant–herbivore interactions in fertile grasslands, such as those studied here, proposes that grazing should accelerate nutrient cycling (Wardle et al. 2004). This would be a consequence of not only the transformation of a significant proportion of primary productivity in highly labile and nutritious animal excretions, but also the promotion of short lived and high quality plant species (Sala et al. 1986; Semmartin et al. 2004). Accordingly, indirect evidence on these grasslands suggested that, in general, domestic grazing accelerated nutrient cycling by direct effects, associated with dung and urine deposition, plant biomass removal, and trampling (Chaneton et al. 1996). Moreover, we have evidence that grazing promotes plant species with a highly decomposable and nutrient-rich litter (Semmartin et al. 2004, 2007; Garibaldi et al. 2007). Our results, however, contrast with those findings since soil mesocosms cultivated with non-palatable species (those promoted by intensive grazing) exhibited lower soil nitrogen contents than those of the palatable species. We believe that this lack of consistency might be accounted for by the species selected for each study. Here, the group of grazing-preferred species included not only *Lolium multiflorum*, an annual grass of high palatability and rapid decomposition (Semmartin and Ghersa 2006; Garibaldi et al. 2007; Semmartin et al. 2008), but also *Lotus tenuis*, a legume with high tissue nitrogen concentration and easily decomposable litter (Craine et al. 2002). In contrast, our previous studies only included grasses as palatable species, which turned this group into one of lower overall decomposability, in agreement with other findings on forb and graminoid species (Grime et al. 1996). In any case, this lack of consistency points out the importance to expand research to a greater number of processes than just nutrient dynamics during litter breakdown.

Soil of mesocosms cultivated with the non-palatable species had a marginally higher pH and lower microbial diversity than that of mesocosms cultivated with palatable species. Soil pH has a strong influence on soil biota structure and functioning at a continental scale (Fierer and Jackson 2006). Furthermore, at the local scale, intensive grazing by large herbivores was invoked as a cause for the high soil pH documented in intensively grazed areas (Bardgett et al. 1996, 2001). In these cases, urine deposition would contribute to raise soil pH (Haynes and Williams 1992) and, in turn, might be responsible for the greater bacterial:fungi community composition and the lower microbial biomass and evenness of intensively grazed grasslands (Bardgett et al. 1996, 2001; Sankaran and Augustine 2004). For our system, we have evidence that intensively grazed areas, associated with water points, have greater soil pH than moderately grazed or ungrazed areas (C. Feola, unpublished data). The rhizosphere has a major ecological function in any particular ecosystem (Bolton et al. 1993; Ehrenfeld 2003). Although soil pH increased in all mesocosms during the experimental period, the slightly lower soil pH of palatable mesocosms might be related to their slightly greater root biomass at the end of the experiment. These mesocosms may have had a greater rhizosphere activity (Paterson et al. 1997). Although we are aware that plant species may affect soil pH (Kourtev et al. 1998), to our knowledge this is the first study showing that the indirect effects of grazing mediated by changes in floristic composition also contribute to foster the effects of grazer excreta on pH and microbial diversity (Fig. 3). On the other hand, non-palatable species reduced soil bacterial diversity (Fig. 5). This trend was independent of plant biomass and coincided with lower mineral nitrogen content, a feature usually connected with diversity of soil biota (Griffiths et al. 2000), and seems to be evidence of the indirect control of herbivores on ecosystem function (Bardgett et al. 1998). Finally, we did not find differences in root and soil carbon respiration among species, in agreement with the accepted idea of a high redundancy in soil biota (Whitman et al. 1998; Nannipieri et al. 2003).

The plant species effects documented here were not associated with changes in plant biomass. However, most evidence has shown that plant species effects on soil correlate with plant biomass (Zak et al. 2003). Moreover, the greater plant diversity of the multispecific controls only translated into higher shoot remaining biomass. Thus, the effect of plant diversity on soil biota is more related with species identity than with species number, as shown by other studies (Hector et al. 2000; Knops et al. 2001, 2007; Porazinska et al. 2003).

As we proposed, the short-term, individual cultivation of different herbaceous species influenced soil biogeochemistry, and that influence correlated with plant palatability to domestic grazers. Although we did not discriminate between live and senescent plant influence on decomposition, we believe that 1 year of cultivation was long enough to allow the imprint of both the ecophysiological influence of live individuals and the decomposition dynamics of their senescent tissue. The influence of plant species on carbon and nutrient dynamics has been largely documented during the past years (Wedin and Tilman 1990; Hobbie 1992; Enriquez et al. 1993; Cornelissen and Thompson 1997; Bardgett et al. 1999). However, some studies revealed that differences among plant species do not translate into concomitant changes in other soil processes (Bardgett et al. 1999; Porazinska et al. 2003; Garibaldi et al. 2007; Semmartin et al. 2008). Here, our results showed that it is possible to detect plant species imprint on the soil subsystem even when variation is not very apparent in terms of plant biomass. Furthermore, this species effect can be associated with plant palatability to domestic large herbivores.

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