

Effects of differential grazing on decomposition rate and nitrogen availability in a productive mountain grassland

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Abstract

Background and aims Grazing may influence nutrient cycling in several ways. In productive mountain grasslands of central Argentina cattle grazing maintain a mosaic of different vegetation patches: lawns, grazed intensively and dominated by high quality palatable plants, and open and closed tussock grasslands dominated by less palatable species. We investigated if differences in the resources deposited on soil (litter and faeces) were associated with litter decomposition rates and soil nitrogen (N) availability across these vegetation patches. **Methods** We compared the three vegetation patches in terms of litter and faeces quality and decomposability, annual litterfall and faeces deposition rate. We determined decomposition rates of litter and faeces in situ and decomposability of the same substrates in a common garden using “litter bags”. We determined soil N availability (with resin bags) in the vegetation patches.

Also, we performed a common plant substrates decomposition experiment to assess the effect of soil environment on decomposition process. This technique provides important insights about the soil environmental controls of decomposition (i.e. the sum of soil physicochemical and biological properties, and microclimate), excluding the substrate quality.

Results The litter quality and faeces deposition rate were higher in grazing lawns, but the total amounts of carbon (C) and nitrogen (N) deposited on soil were higher in tussock grasslands, due to higher litterfall in these patches. The in situ decomposition rates of litter and faeces, and of the two common plant substrates were not clearly related to either grazing pressure, litterfall or litter quality (C, N, P, lignin, cellulose or hemicellulose content). In situ litter decomposition rate and soil ammonium availability were correlated with the decomposition rates of both common plant substrates. This may suggest that difference in local soil environment among patch types is a stronger driver of decomposition rate than quality or quantity of the resource that enter the soil.

Conclusions Our results show that, although high grazing pressure improves litter quality and increases faeces input, the reduction in biomass caused by herbivores greatly reduces C and N input for the litter decomposition pathway. We did not find an accelerated decomposition rate in grazing lawns as proposed by general models. Our results point to soil environment as a potential important control that could mask the effect of litter quality on field decomposition rates at local scale.

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Introduction

Large herbivores affect nutrient cycling through the changes they produce on plant community structure and composition, which may either enhance or reduce overall plant litter quality and decomposability. Herbivores can also alter the quantity of resources that enter the soil, through the consumption of plant tissues and their return to soil as faeces and urine. Herbivores influence the physical, chemical or biological characteristics of the soil environment where decomposition takes place (the “soil environment” sensu Eviner and Chapin 2003), through physical disturbance, which modifies properties such as soil temperature or moisture (Bardgett and Wardle 2003; Güsewel et al. 2005; Semmartin et al. 2008).

Grazing is expected to promote positive feedbacks in productive systems and negative feedbacks in unproductive systems (Ritchie et al. 1998; Bardgett and Wardle 2003; Wardle et al. 2004). According to these authors, in grazed areas of productive systems, herbivores promote the growth and dominance of grazing tolerant, palatable plant species and, therefore, the dominance of high nitrogen (N) leaf litter, which accelerates litter decomposition rates (Augustine and McNaughton 1998). Additionally, and as a consequence of foliar consumption by herbivores, plants increase root exudation, which stimulates microbial biomass and activity, thus enhancing soil N availability and plant N acquisition (Denton et al. 1999; Hamilton and Frank 2001). Although consumption reduces litterfall itself, a fraction of the biomass consumed is re-directed to a fast decomposition pathway in the form of urine and faeces, thus providing microorganisms with highly decomposable resources (Ruess and McNaughton 1987; Seagle et al. 1992; Bakker et al. 2004). Increased soil N availability, resulting from faster decomposition, further contributes to sustained high relative growth rates of heavily consumed species, which dominate the plant community (Holland and Detling 1990; Augustine and McNaughton 1998; Olofsson and Oksanen 2002). All these mechanisms are part of the positive feedback between herbivore consumption, soil nutrient content and plant quality, which have been postulated for productive systems under grazing (Wilson and Agnew 1992).

The effects of grazers on nutrient cycling have been studied mostly through the comparison of grazed with ungrazed situations, or through the comparison of areas with different grazing intensities, for example

nil, moderate and high stocking rates of domestic livestock. However, a very common situation in some productive grasslands is that grazing maintains a mosaic of vegetation patches with different physiognomies (Coughenour 1991; Posse et al. 2000; Adler et al. 2001; McIvor et al. 2005). Information concerning nutrient cycling in differentially grazed vegetation patches coexisting in the landscape is still scarce (Güsewel et al. 2005; Augustine and McNaughton 2006; Vaieretti et al. 2010). In some productive grasslands from temperate climates, animals tend to graze on “grazing lawns” (i.e., short grasslands dominated by high quality palatable plants; McNaughton 1979; 1984), and to avoid tall vegetation dominated by less palatable species (Adler et al. 2001; McIvor et al. 2005; McIntyre and Tongway 2005). By selecting grazing lawns, animals contribute to prevent their conversion into tall vegetation (Hobbs and Swift 1988). In addition, the increased input of dung and urine in lawns could increase nutrient availability in soils, sustaining the growth of high quality palatable species, which in turn attract animals and produce highly decomposable litter. Thus, we could expect accelerated nutrient cycling rates in lawns compared to adjacent tall vegetation patches, as an expression of the positive feedback among plant quality, litter decomposition and animal selectivity.

Productive grasslands of central Argentina mountains are an appropriate system to test the hypothesis that grazing by large herbivores leads to positive feedback in natural grazing mosaics. In these grasslands, the differential use by domestic herbivores maintains a mosaic of vegetation patches, mainly grazing lawns and open and closed tall tussock grasslands (Pucheta et al. 1998a; Cingolani et al. 2003, 2010). The initial formation of lawn patches has not been clearly elucidated, but their maintenance has been attributed to herbivory, as indicated by their rapid conversion into closed tussock grasslands when livestock is excluded (Pucheta et al. 1998a, 1998b; Cingolani et al. 2003; Vaieretti 2010). Previous studies in the area have shown that lawns are dominated by plant species with high foliar quality and palatability (Pucheta et al. 1998a; Vendramini et al. 2000; Cingolani et al. 2007), and that they receive an important input of dung (Vaieretti et al. 2010; von Müller 2011). However, in a recent study, Vaieretti et al. (2010) found that instantaneous soil N availability and decomposition of common substrates were similar in lawns and closed tussock grasslands. These results challenged the idea that the selectivity of

lawns by large herbivores, and the higher quality and palatability of their plants, could lead to an accelerated nutrient cycling. Moreover, to our knowledge there are very few studies (e.g. Güsewel et al. 2005; Risch et al. 2007) that specifically test the mechanisms by which herbivory may alter nutrient availability through altering decomposition patterns in the field.

In the present study we further examined in the mechanisms explored by Vaieretti et al. (2010) in the same grassland mosaic maintained by grazing in central Argentina. We investigated how grazing lawns and open and closed tall tussock grasslands differ in the quality and quantity of resources deposited on the soil, and whether those differences were associated with litter decomposition and soil accumulated N. Specifically our objectives were (1) to determine the quality and decomposability of faeces, and of litter produced in the different patch types; (2) to determine the quantity of litter and faeces deposited on the soil in the different patch types; (3) to determine litter and faeces decomposition in the field (in situ decomposition) and soil N availability in the different patch types, and (4) to assess the differences in the environment where decomposition occurs (hereafter “soil environment”) by measuring decomposition rates of two common plant substrates in the different patch types. Common substrates represent an efficient tool to evaluate the environmental conditions for decomposition, specifically excluding litter quality (Tsarik 1975; Piene and Van Cleve 1978; Orwin et al. 2006).

Materials and methods

Study area

The study was conducted in the upper belt of Córdoba mountains, in central Argentina (31° 37'S, 64° 48'W). The area comprises different landscape units, including valley bottoms and ravines, as well as plateaus with different degree of dissection. Soils are mostly mollisols, derived from the weathering of the granitic substrate and fine-textured eolian deposits (Cabido et al. 1987). At 2,200 m above sea level, the area has a mean annual temperature of 8 °C with a winter absolute minimum of -15 °C and no frost-free period. Mean annual precipitation is 900 mm (1992–2010), with most rainfall concentrated in the warmer months, between October and April (Colladon et al. 2010). Vegetation is a mosaic of *Polylepis australis* woodlands and grasslands that alternate with erosion

pavements and granite outcrops (Cingolani et al. 2004). Grasslands comprise three distinct physiognomies: lawns, open tussock grasslands and closed tussock grasslands (Cingolani et al. 2003; Vaieretti et al. 2010). Lawn patches are dominated by short (3–10 cm) palatable plants (forbs, annual and perennial short grasses, rushes and sedges) with high foliar N content, high specific leaf area and low leaf toughness, which also suggest high relative growth rates (Pucheta et al. 1998b; Vendramini et al. 2000; Cingolani et al. 2007; Pérez Harguindeguy et al. 2000a). Closed tussock grassland patches are strongly dominated by tall tussock grass species (60–90 cm), such as *Festuca tucumanica* E.B. Alexeev, *Deyeuxia hieronymi* (Hack.) Türpe and *Poa stuckertii* (Hack.) Parodi, whereas open tussock grasslands comprise a mixture of short vegetation and tussock species (Pucheta et al. 1998a; Cingolani et al. 2003; Vaieretti et al. 2010; Online Resource 1). Previous studies in the region determined that aboveground net primary production (ANPP) is 40 % less in grazing lawns than in adjacent closed tussock grasslands protected from grazing, and standing biomass (live plus dead) is six times lower (Pucheta et al. 1998a). From the beginning of the 17th century the main activity in the area has been livestock raising. By the early 20th century, livestock (mainly cattle and sheep) had completely replaced large native herbivores (Díaz et al. 1994). Because of the large size of the paddocks, animals can select among different landforms and plant communities (von Müller 2011).

The present study was conducted in Quebrada del Condorito National Park, where domestic livestock is maintained in some areas to preserve local biodiversity (Cingolani et al. 2010). Sampling was performed on a gently dissected plateau, in a large paddock (1,514 ha) under moderate cattle grazing, i.e., around 0.20 Cattle Equivalents per ha (CE.ha⁻¹) of vegetated land, discounting rock surface. We selected a representative area of about 225 ha within the paddock. In this area we randomly chose 10 plots (replicates) of about 15×15 m, for each of the three patch types (lawns, and open and closed tussock grasslands, total=30). The plots were equally distributed in two different hills within the selected area, at similar altitudes above sea level (2,150–2,200), in order to include the natural spatial variability of this kind of landscapes. Slopes were representative of the gentle topography of the area and were in all cases lower than 5 %. Soil properties of the study area were described in detail in Vaieretti et al. (2010), indicating that the organic carbon (C) content is 2.5–9.0 % (10 % average organic

matter), the total N content is 0.2–0.7 % and pH is 4.5–5.4. Additionally, the authors found that soil physico-chemical properties (texture, total C, total N, P, pH, cation exchange capacity, cations, and depth) were similar in the three patch types (i.e. no significant differences among them; Online Resource 1).

Cattle density

In the sampled paddock, cattle are free to wander and graze among the three grassland patch types, but they prefer and spend most of their time in lawn patches (von Müller 2011). To verify the differences in grazing pressure among patch types, during three consecutive years, from 2006 to 2008, we measured annually the frequency of dung found at each plot in 50 randomly placed 900 cm² squares. Then, we calculated local cattle density (CE.ha⁻¹, Cattle Equivalents per ha) per plot per year through the equation obtained by von Müller et al. (2012) in the same study area (local cattle density = 0.017 * dung frequency). We obtained one value per plot by averaging the values obtained for 2006, 2007 and 2008, as recommended by von Müller et al. (2012).

Soil physical properties

From January to July 2007 we collected a soil compound sample of the upper horizon (0–15 cm) monthly for each plot. We determined soil water content through the gravimetric method (Jarrell et al. 1999). In the laboratory, we weighed all soil samples (fresh weight), we dried them at 105 °C in the oven for 48 h, and then we re-weighed them (dry weight). We calculated soil water content (%) as the percentage weight lost. We calculated the average soil water content, for each patch type, for the two incubation periods of the decomposition experiments: from January to March 2007, and from January to July 2007. We also measured soil impedance by inserting a pocket penetrometer (Forestry Suppliers Inc.) into the soil. In addition, we measured bulk density at all plots, on randomly collected 15 cm depth soil samples dried until constant weight at 60 °C. We calculated bulk density based on total dry weight and sample volume (Elliot et al. 1999).

Litter and faeces quality and decomposability

In September 2006 we collected a sample of naturally mixed litter from the soil surface in each plot and one

sample of fresh faeces from all plots. Faeces were combined into one compound sample because there was no reason to assume that faeces would differ among plots. We oven-dried the samples at 50 °C during 48 h, and then we grinded and homogenized them. The samples were then sent to the National Institute of Agricultural Technology (INTA, Bariloche, Argentina) where total nitrogen (N) and phosphorous (P) were determined with an Autoanalyser (RFA 300-Alpken, Wilsonville, O.R., USA), following O'Neill and Webb (1970) and Westerman (1990), respectively. Lignin, cellulose and hemicellulose content were determined using the technique of Goering and Van Soest (1970). We estimated total carbon (C) as 50 % of ash-free biomass (Gallardo and Merino 1993; McClaugherty et al. 1985). We also calculated C:N ratio, lignin:N ratio and total fibre content (LCH = lignin + cellulose + hemicellulose). For all litter samples and for the faeces sample, determinations were performed for three sub-samples, which were then averaged.

Additionally, we determined the decomposability of the faeces, and litter collected by their simultaneous incubation in a common garden (Cornelissen et al. 1999; Pérez Harguindeguy et al. 2000b). Decomposability measured this way integrates structural and chemical characteristics of the material incubated (e.g. litter and faeces) because it is an expression of the quality of that material as a substrate for microorganisms (Pérez-Harguindeguy et al. 2013). We prepared the samples following the widely used litter bag technique (Cornelissen 1996; Pérez-Harguindeguy et al. 2013). We used the mixed litter and the compound sample of fresh faeces collected in September 2006 to construct 20 bags of litter and 16 bags of faeces (sub-samples) per plot. We filled the bags (0.3 mm nylon mesh size) with 3 g of air-dried litter and faeces. Although the small mesh size reduces access to soil fauna (Seastedt 1984; Bradford et al. 2002), it helped to prevent excessive loss of material during bag manipulation and does not affect the decomposition pattern significantly (Cornelissen et al. 1999; Vaieretti et al. 2010). We built a 4×4 m decomposition bed close to the study area, in a paddock free of livestock. Before incubation, we removed the top 5 cm of soil and we filled the bed with mixed soil collected from the 30 selected plots. We placed the bags randomly on the soil surface and covered them with litter from the area to homogenise physical conditions, and to avoid damage by birds and small mammals (Cornelissen et al. 1999; Pérez Harguindeguy et al. 2000b). We retrieved half of the bags

after 75 days (from January to March 2007) while the remaining bags were retrieved after 198 days (from January to July 2007). After each incubation period, we stored the bags at $-14\text{ }^{\circ}\text{C}$ until processing. Once defrosted, we removed adhering soil, soil fauna and other extraneous materials by brushing or swiftly rinsing with water. We oven-dried all cleaned bags for at least 48 h at $60\text{ }^{\circ}\text{C}$, and then weighed them. To estimate initial dry mass before incubation, we calculated air-dried water content by drying sub-samples of naturally mixed litter and faeces at $60\text{ }^{\circ}\text{C}$ during 48 h. Water content (%) was calculated from the weight loss in sub-samples after drying. We estimated decomposition of each litter and faeces bag as the percentage of dry weight loss at the end of the incubation period (Cornelissen 1996; Cornelissen et al. 1999). We obtained one value of decomposability per plot per period by averaging the dry weight loss of 10 litter bags. Also, we obtained one value of decomposability of faeces per period of incubation by averaging the dry weight loss of eight faeces bags.

Litter and faeces quantity

We measured annual litterfall and faeces deposition rate using four to six fixed squares (1 m^2) randomly distributed within each plot. We cleared each square of all litter and faeces at the beginning of autumn (April 2006) and collected accumulated litter and faeces from each square (sub-samples) in August 2006, October 2006 and May 2007. At the laboratory, we dried litter and faeces at $60\text{ }^{\circ}\text{C}$, until constant weight was attained. Additionally, we visually identified and estimated the proportion of six different plant growth forms in the litter sub-samples; forbs, perennial graminoids, annual graminoids, thick tussock grasses, thin tussock grasses, and *Eryngium agavifolium* (a forb very different in leaf characteristics from the others, Díaz and Cabido 1997; Díaz et al. 1998). Assuming that there was no significant difference in density of litter from different plant sources, we calculated the dry weight of each growth form by multiplying its proportion by the total dry weight of the litter sub-sample. We averaged values of all sub-samples to obtain one value per growth form per plot. Finally, we calculated annual litterfall of each growth form by summing up the values of all dates, and the total annual litterfall by summing up the values of all growth forms. We also calculated annual faeces deposition rate by averaging the weight accumulated per plot for each date, and then summing up all the dates. In

addition, based on annual litterfall and faeces deposition rate, and on their C and N content, we calculated the C and N annual deposition rate for each plot.

Litter and faeces decomposition in the field

We measured litter decomposition at each patch type by incubating naturally mixed litter at the same sites where the litter was collected (hereafter “in situ litter decomposition”). We used the same naturally mixed litter collected in September 2006, used for the decomposability assessment, to construct 20 litter bags (0.3 mm nylon mesh size) with 3 g of air-dried litter per plot. We incubated these litter bags during the same two periods (75 and 198 days, 10 litter bags per plot per period of incubation) as the decomposability assessment. We incubated the litter bags above the soil surface, fixed to the ground with stakes and protected with barbed wire to avoid damage by cattle and small animals. To determine in situ faeces decomposition we constructed 20 bags (0.3 mm nylon mesh size) per plot. We filled the bags with 3 g of air-dried of the same fresh faeces collected in September 2006. We incubated the faeces bags simultaneously with litter bags (10 faeces bags per plot per period of incubation). Substrates (litter and faeces) were not re-moistened prior to incubation in the field. We processed all litter and faeces bags after incubation, and we calculated decomposition in the same way as explained for the decomposability assessment.

Soil N availability

To measure accumulated available soil inorganic N (NH_4^+ , NO_3^-), we placed three 5 g-ion exchange resin bags (Amberlite IRN-150) packed in 0.3 mm double nylon mesh at each sampling plot (Sibbeson 1977; Binkley and Hart 1989; Frank et al. 2000). We buried a set of three resin bags into the soil (10 cm deep) at each plot, which were incubated from January to March 2007 (75 days). During the period of incubation, ionic resins absorb available nutrients in the same way plant roots do; therefore, resins may realistically reflect nutrient availability for plants during a period of time (Binkley 1984a). After incubation, we collected the resin bags and rinsed them in distilled water, we then extracted ions in NaCl (2 N) for 12 h and, subsequently we analysed the content of N-NH_4^+ and N-NO_3^- in a continuous flow analyser (Frank et al. 2000; Bakker et al. 2009).

Common plant substrates decomposition

Finally, to assess the (herbivores-mediated) differences in the soil environment among patch types, excluding the effect of litter quality, we incubated two common plant substrates at each plot. The common substrates technique is widely used (Binkley 1984b; O'Lear et al. 1996) because it provides important insights about the soil environmental controls of decomposition, excluding effects of substrate quality (Tsarik 1975; Piene and Van Cleve 1978; Orwin et al. 2006). Soil environment evaluated this way integrates soil physicochemical and biological properties, as well as microclimate (Swift et al. 1979; Adl 2003; Eviner and Chapin 2003; Bardgett 2005). We selected as common plant substrates the litter of two abundant species in the study area: a native annual grass, *Muhlenbergia peruviana* (P Beauv.) Steud. and a native perennial grass, *Poa stueckertii* (Hack.) Parodi. The differences in litter qualities of these species suggest that decomposition would be relatively fast in *M. peruviana* and relatively slow in *P. stueckertii* (Pérez Harguindeguy et al. 2000b; Vendramini et al. 2000; Vaieretti 2010). For each species, we randomly collected the litter across all plots within the study area and then we pooled it. We constructed 10 bags (per plot and per substrate) of 0.3 mm nylon mesh size, filled with 3 g of each air-dried substrate. We incubated the bags within the plots simultaneously with in situ litter and faeces bag incubations (five sub-samples of each common plant substrate per plot per period of incubation, 75 and 198 days respectively).

Additionally, we performed a laboratory decomposition experiment to exclude the effect of microclimate from that of soil physicochemical and biological properties (Berg and Laskowski 2006). We collected soil samples from each plot (at 15 cm in depth), and sieved them (2 mm mesh) to remove rocks and plant material. We placed soil samples in jars, covered them with a 0.3 mm nylon mesh circle, and then we placed 0.25 g of *P. stueckertii* litter, as common plant substrate, on top of mesh circles (Wardle et al. 1998). We put the jars into an incubation chamber at constant air temperature (25 °C) and moisture (50 %). Once a week, we moistened the jars with distilled water and changed their position within the chamber to avoid any difference in temperature and/or moisture within the chamber. We incubated the common plant substrate in the jars during two periods: 36 and 67 days. In both cases, sample

processing after incubation and decomposition calculations were performed in the same way as explained for the decomposability assessment.

Data analyses

We compared local cattle density and soil physical properties (water content for each period separately—from January to March 2007, and from January to July 2007, bulk density, compaction), among the three patch types, with mixed-effect ANOVA. We used patch type as a fixed factor (three levels) and hill as a random factor (two levels).

We used a similar mixed-effect ANOVA to compare litter quality parameters (C, N, P, lignin, cellulose, hemicellulose, C:N ratio, lignin:N ratio, LCH) and litter decomposability among the three patch types. Additionally, we explored differences in quality parameters and decomposability between faeces and litter of each patch type through one-sample t-tests (because we had only one value of each parameter for faeces). Also, to corroborate that litter and faeces decomposability reflects litter and faeces quality, we performed Pearson correlations between litter and faeces decomposability, and their respective quality parameters ($n = 31$; i.e. 30 litter samples plus one faeces samples).

We performed a mixed-effect ANOVA to assess differences in the quantity of organic material deposited on the soil surface among the three patch types (annual litterfall, annual faeces deposition rate, and C and N deposition rates). As in previous analyses, we used patch type as a fixed factor and hill as a random factor.

We performed a repeated measures ANOVA to test differences in litter and faeces in situ decomposition among the three patch types. We used plot as subject variable ($n = 30$), substrate (litter and faeces) as within-factor, and patch type and hill as between-factors. We used mixed-effect ANOVAs to compare soil N-NH_4^+ and N-NO_3^- availability among patch types, with patch type as a fixed factor and hill as a random factor. Additionally we performed Pearson correlation analyses of in situ litter decomposition and faeces decomposition with soil N-NH_4^+ and N-NO_3^- availability. We used decomposition results for 75 days of incubation because we did not have data of soil accumulated N available for 198 days of incubation ($n = 27$, because the resin bags were lost in three plot).

We also performed a repeated measures ANOVA to test differences in both common substrates decomposition

rates among the three patch types (field experiment). We used plot ($n=30$) as subject variable, substrate (*M. peruviana* and *P. stueckertii*) as within-factor, and patch type and hill as between-factors. To compare decomposition of *P. stueckertii* on soils from different plots under controlled conditions (laboratory experiment), we used mixed-effect ANOVA, with patch type as a fixed factor and hill as a random factor.

For all analyses, we tested homoscedasticity and normality of residuals with Levene and Kolmogorov-Smirnov tests, respectively. In some cases, data were log-transformed to meet the statistical assumptions. Post-hoc comparisons of means were based on Tukey's test at a significant level of $P<0.05$, except for local cattle density comparison that was based on post-hoc Duncan test. When the interaction term between factors was not significant, it was discarded from the models.

We performed Spearman Correlations analysis to explore the relationship between in situ litter decomposition and the C and N deposition rates from litter and faeces (as parameters of the quantity of resources deposited on soil), litter decomposability (as an expression of the quality of litter as a substrate for microorganisms), and the decomposition of common substrates, average of *M. peruviana* and *P. stueckertii* decomposition (as indicator of the soil environment effect) ($n=30$). As common substrates showed a very similar decomposition pattern ($r=0.72$, $p<0.001$) we used their average as a more general expression of the soil environment effect. Additionally we explored the relationship between soil N availability (N-NH_4^+ and N-NO_3^-) and the C and N deposition rates, litter mixtures decomposability, and decomposition of common substrates ($n=27$). We used decomposition results after 75 days of incubation for these analyses.

Finally, to explore which proportion of the variability in "in situ litter decomposition" can be explained by litter decomposability, and which proportion can be explained by soil environment, we carried out a multiple regression analysis. We used decomposition after the whole incubation period (198 days) for this analysis. We used in situ litter decomposition as the dependent variable. Decomposability of litter (as an expression of the quality of litter as substrate for microorganisms), and decomposition of two common plant substrates (both averaged, as an expression of the soil environment effect) were used as independent variables. For all the statistical analyses reported above we used the package SPSS Inc v17.0.

Results

Cattle density

As expected, local cattle density, calculated from dung frequency, was highest in grazing lawns (0.61 ± 0.08 CE ha^{-1} ; mean \pm SE), intermediate in open tussock grasslands (0.39 ± 0.05 CE. ha^{-1}) and lowest in closed tussock grasslands (0.13 ± 0.02 CE ha^{-1}) ($F_{2,27}=21.29$, $p<0.001$). Cattle density did not differ between hills.

Soil physical properties

Soil water content was not significantly different among patch types for either of the two periods analysed (January-March 2007: $F_{2,26}=2.08$, $p=0.15$; January-July 2007: $F_{2,26}=2.14$, $p=0.14$; Online Resource 1). In turn, soil water content was different between hills for the two periods (January-March 2007: $F_{2,26}=23.64$, $p<0.001$; January-July: $F_{2,26}=25.99$, $p<0.001$). Plots located on the northern hill showed higher soil water content (38.1 ± 1.6 and 38.1 ± 1.4 %) than plots located on the southern hill (28.2 ± 1.4 and 28.4 ± 1.4 %) for the same periods, respectively.

Soil impedance (mean \pm SE) at 0.7 cm depth, was significantly higher ($F_{2,27}=6.28$, $p=0.006$) in lawns (2.65 ± 0.22 kg.cm^{-2}) and open tussock grasslands (2.60 ± 0.11 kg.cm^{-2}) than in closed tussock grasslands (1.87 ± 0.17 kg.cm^{-2}). Soil bulk density (0–15 cm depth) did not differ among patch types ($F_{2,27}=1.71$, $p=0.20$; Online Resource 1), nor between hills.

Litter and faeces quality and decomposability

Litter quality was significantly different among the three patch types (Online Resource 2). Litter of lawns showed higher N and lignin content, lower cellulose and hemicellulose content, C:N ratio, and LCH (total fibre content) than litter of both tussock grasslands (mixed-effect ANOVA; $p<0.05$). We did not find significant differences in litter quality between hills. Additionally, faeces showed significant differences in all quality parameters measured when compared with litter from open and closed tussock grasslands (one sample t -test; Online Resource 3). In turn, when comparing faeces quality with litter from lawns, we found that faeces showed similar content of cellulose, N, C:N ratio and LCH ($p>0.05$) as lawn litters. However, faeces showed higher content of lignin and P, as well as

higher lignin:N ratio ($p < 0.05$), and lower C and hemicellulose content ($p < 0.05$) than lawn litter (one sample t -test; Online Resource 3). Litter and faeces decomposability should reflect the quality of these substrates. According to this postulation we found that decomposability was positively correlated with N content, and negatively correlated with cellulose, hemicellulose, LCH content and C:N ratio (data not shown). We found higher decomposability of lawns' litter compared to both tussock grasslands' litter (75 days, $F_{2,27} = 9.40$, $p = 0.001$; 198 days, $F_{2,27} = 5.43$, $p = 0.01$; Fig. 1a, b respectively). Faeces decomposability was significantly higher than litter decomposability for all patch types (Online Resource 2 and 3).

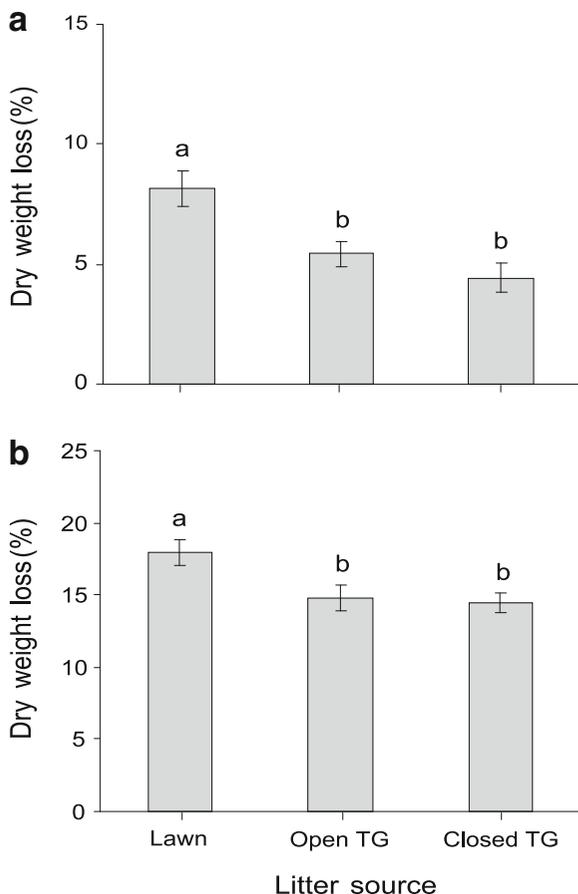


Fig. 1 Percentage of dry weight loss of litter belonging to the three vegetation patch types (mean \pm SE, $n = 10$) incubated in a common garden at (a) 75 days, and (b) 198 days. The differences among treatments were tested with mixed-effect ANOVA. Different letters indicate significant differences among patch types at $p < 0.05$. TG = tussock grasslands

Litter and faeces quantity

Annual litterfall was significantly different among patch types ($F_{2,27} = 207.88$, $p < 0.001$), but not between hills. In closed tussock grasslands annual litterfall was twice as high as that recorded in open tussock grasslands, and one order of magnitude higher than in lawns (Fig. 2a). In lawns, a combination of forbs, perennial graminoids and annual graminoids comprised 70 % of the litter, which was consistent with the floristic composition of the established vegetation (Vaieretti et al. 2010; Online Resource 1). In open tussock grasslands, 75 % of the litter was composed by thin tussock grasses, whereas in closed tussock grasslands litter was dominated both by thick and thin tussock grasses, mainly *Poa stueckertii* and *Deyeuxia hieronymi* (Fig. 3).

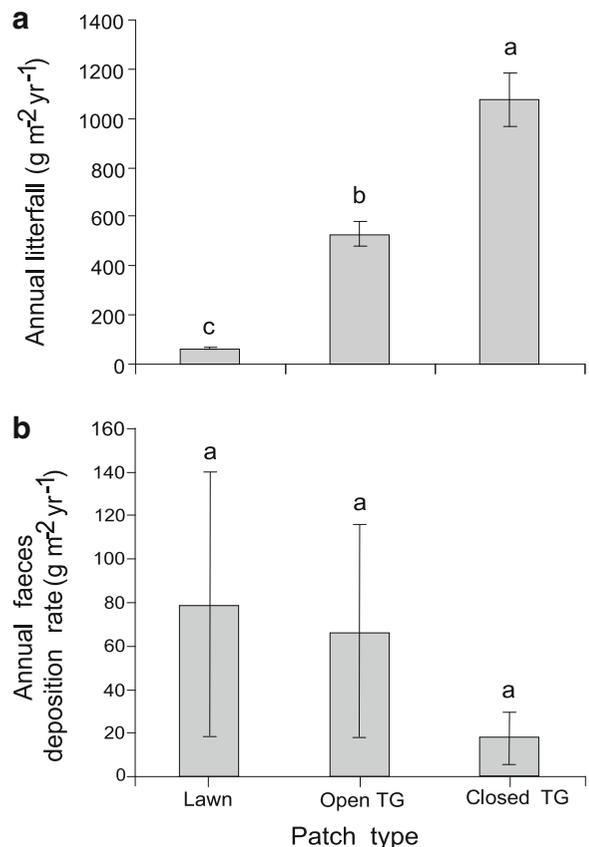


Fig. 2 Annual litterfall (a), and faeces deposition rate (b) in the three vegetation patch types (mean \pm SE, $n = 10$). The differences among treatments were tested with mixed-effect ANOVA. Different letters indicate significant differences among patch types at $p < 0.05$. TG = tussock grasslands

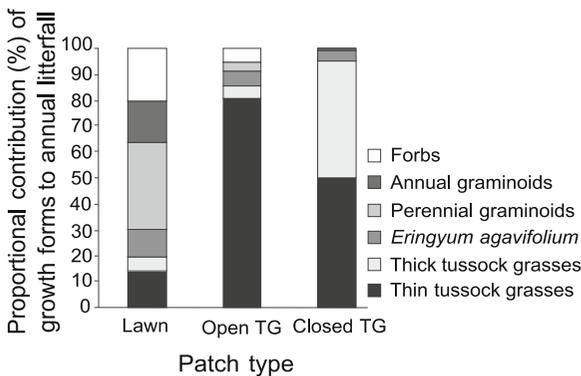


Fig. 3 Proportional contribution (%) of different plant growth forms to annual litterfall in the three selected vegetation patch types. TG = tussock grasslands

Annual faeces deposition rate was higher in lawns and open tussock grasslands, and lower in closed tussock grasslands (Fig. 2b), following a similar trend as shown for dung frequency. But, in this case the trend was not significant ($F_{2,27}=2.60$, $p=0.093$), probably due to the high variability of this measurement (data were highly variable between plots within the same patch type). We did not find significant differences in annual faeces deposition rate between hills.

Deposition rates of C and N (mean±SE) through litter were significantly higher ($F_{2,27}=53.4$, $p<0.001$ for C; $F_{2,27}=38.8$, $p<0.001$ for N) in closed tussock grasslands (501 ± 43.2 gC m⁻² yr⁻¹; and 10.5 ± 1.24 gN m⁻² yr⁻¹), intermediate in open tussock grasslands (245 ± 26.4 gC m⁻² yr⁻¹; and 4.92 ± 0.53 gN m⁻² yr⁻¹) and lower in lawns (30 ± 2.4 gC m⁻² yr⁻¹; and 0.86 ± 0.08 gN m⁻² yr⁻¹). Contrarily, we did not find significant differences in the deposition rates of C and N from faeces among the patch types (34.3 ± 11.7 gC m⁻² yr⁻¹ and 1.03 ± 0.35 gN m⁻² yr⁻¹ in lawns, 29 ± 9.4 gC m⁻² yr⁻¹ and 0.87 ± 0.28 gN m⁻² yr⁻¹ in open tussock grasslands, and 7.9 ± 2.3 gC m⁻² yr⁻¹ and 0.24 ± 0.07 gN m⁻² yr⁻¹ in closed tussock grasslands). Total deposition rates of C and N (i.e. through litter plus faeces) were the highest in closed tussock grasslands and the lowest in lawns, because they were mainly determined by litter input.

Litter and faeces decomposition in the field

In situ litter decomposition was significantly different among patch types (Table 1). We found the highest and lowest in situ litter decomposition in lawns and in open tussock grasslands, respectively,

whereas litter decomposition in closed tussock grasslands was intermediate and not significantly different from either of the other two patch types (Online Resource 4). Decomposition of faeces was not different among patch types (Fig. 4). Also, we found a significant interaction between patch type and substrate. Specifically, litter decomposed faster than faeces in lawns, but in open and closed tussock grasslands faeces decomposed at a similar rate than litter (Table 1; Fig. 4). We found no differences in in situ decomposition of litter, nor in decomposition of faeces between hills. The patterns found were similar for both incubation periods (75 and 198-days, Fig. 4a and b respectively).

Soil N availability and its relationship with in situ litter and faeces decomposition

Soil N-NH₄⁺ captured by ion exchange resins during 75 days of incubation (Fig. 5a) was significantly higher in closed tussock grasslands than in open tussock grasslands ($F_{2,24}=5.46$, $p=0.01$). In lawns, soil N-NH₄⁺ was intermediate and not significantly different from that of the other two patch types. Soil N-NO₃⁻ captured by ion exchange resins during 75 days of incubation was significantly lower in lawns than in any of the tussock grasslands ($F_{2,24}=7.99$, $p=0.002$; Fig. 5b). In both cases, no significant differences were found between hills.

Soil available N-NH₄⁺ was positively correlated with in situ litter decomposition ($r=0.62$, $p=0.0005$, $n=27$). However, in situ litter decomposition was not associated to soil N-NO₃⁻ availability ($r=-0.31$, $p=0.12$, $n=27$). In addition, we did not find a significant correlation between faeces decomposition and soil N-NH₄⁺ ($r=0.08$, $p=0.68$, $n=27$), or N-NO₃⁻ availability ($r=-0.33$, $p=0.10$, $n=27$).

Common plant substrates decomposition

The decomposition of common plant substrates differed among patch types and between substrates (Table 1). At 75 days of incubation we found a significant patch type x substrate interaction (as a consequence, the differences were calculated among six treatments resulting from the combination of three patch types x two substrates). Specifically, *M. peruviana* decomposed faster in closed tussock grasslands and *P. stuckertii* decomposed slower in open tussock grasslands (Table 1; Fig. 6a). At 198 days, the interaction between patch type and substrate was not significant (Table 1). The two common plant substrates decomposed significantly faster in closed tussock

Table 1 Results of repeated measures ANOVAs examining the effect of patch type and substrate type on in situ decomposition rates of litter and faeces, and on two common plant substrates*(Mulenbergia peruviana* and *Poa stueckertii*). Columns show the statistical parameters (*df*, *F* and *p*) for both analyses and both incubation periods (75 and 198 days, respectively)

Effect	Litter and faeces decomposition			<i>M. peruviana</i> and <i>P. stueckertii</i> decomposition		
	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
				75 days		
Patch type	2,27	4.3	0.024	2,27	5.2	0.012
Substrate	2,27	13.9	0.001	2,27	75.3	< 0.001
Patch x Substrate	2,27	6.6	0.005	2,27	4.3	0.025
				198 days		
Patch type	2,27	9.2	0.04	2,27	6.3	0.006
Substrate	2,27	10.2	0.004	2,27	88.8	<0.001
Patch x Substrate	2,27	3.6	0.001	2,27	0.2	0.85

Patch type=between subject

Substrate=within subject

“Substrate” refers to litter and faeces in the first case, and *M. peruviana* and *P. stueckertii* in the second case

grasslands, intermediate in lawns, and slower in open tussock grasslands (Fig. 6b; Online Resource 4). In the laboratory incubation, *P. stueckertii* showed similar decomposition rate when incubated on soils from different patch types (36 days, $F_{2,26}=0.41$, $p=0.67$; 67 days, $F_{2,27}=1.24$, $p=0.30$).

Decomposition and N availability in relation to litter and faeces quality and quantity

In situ litter decomposition was not correlated to any litter quantity parameter measured, nor to litter decomposability, but, it was positively correlated to common plant substrates decomposition (Table 2; Online Resource 5). When we explored through multiple regression which proportion of the variability of “in situ litter decomposition” is explained by different controls, we found that soil environment effect (tested by the average of common substrates decomposition rate) explained 40 % of variance ($t=4.89$, $p<0.0001$) while litter decomposability effect (as an expression of the quality of litter as a substrate for microorganisms) explained 15 % of variance ($t=2.68$, $p=0.012$; $R^2=0.52$, $p<0.0001$).

Available soil $N-NH_4^+$ was also correlated to common plant substrates decomposition and, to annual C and N deposition rate from faeces as well, but it was not correlated to litter decomposability. In addition, soil $N-NO_3^-$ availability was correlated to the annual C and N deposition rate from litter, and also to litter decomposability (Table 2).

Discussion

What is the effect of grazing pressure on quality and quantity of resources deposited on soil?

Even though cattle is free to wander and graze anywhere within the paddock under study, we found that herbivore density is higher in lawn patches compared to tussock grassland patches. As expected, we found the highest quality (higher N content, and lower LCH content) and hence decomposability in litter produced in lawn patches. Pucheta et al. (1998a) have shown in similar lawn patches that herbivores promote the dominance of forbs and short graminoids, which have higher nutrient content in their green tissues, and higher litter quality than tussock grasses. These patterns are also consistent with results obtained in other grasslands not limited by nutrients (Olofsson and Oksanen 2002; Semmartin et al. 2004; Güsewel et al. 2005, among others). Annual litterfall was highest in closed tussock grasslands, and annual faeces deposition rate was slightly higher in lawns (Fig. 2). Total annual amounts of C and N deposited on the soil were highest in closed tussock grasslands and lowest in lawns, as these values were mainly determined by the differences in litterfall between both patch types. Herbivores have been frequently found to reduce litter biomass (Bazely and Jefferies 1986; Sirotiak and Huntly 2000) at the same time as they increase the quality of litter entering the soil (Bakker et al. 2009).

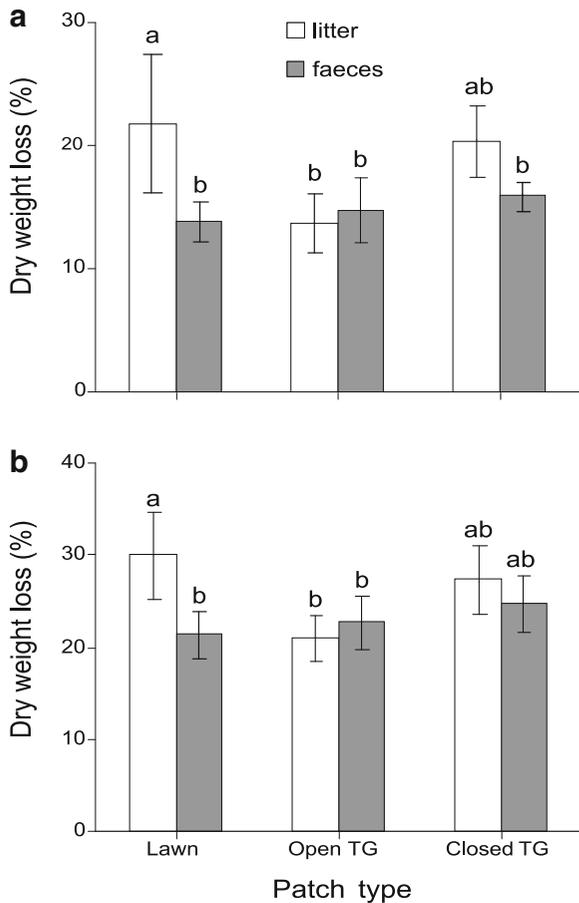


Fig. 4 Percentage of dry weight loss (mean \pm SE, $n = 10$) of litter and faeces incubated in situ at (a) 75 days, and (b) 198 days in the three vegetation patch types. The differences among treatments were tested with repeated measures ANOVA. For both incubation periods the interaction between patch type and substrate factors was significant (c.f. Table 1). Different letters indicate significant differences in dry weight loss among treatments (combination of patch type \times substrate) at $p < 0.05$. TG = tussock grasslands

We found that changes in litter quality produced by higher herbivory in lawn patches, and higher faeces input (and probably urine input as well), are not enough to counterpart the negative effect of herbivory in litterfall, and the result is a net decrease in C and N input in lawn patches compared to tussock grasslands.

Do quality and quantity of litter and faeces determine decomposition rates in the field?

As expected, our results showed that litter quality and litter decomposability were strongly related. These results are in agreement with those obtained by other authors for

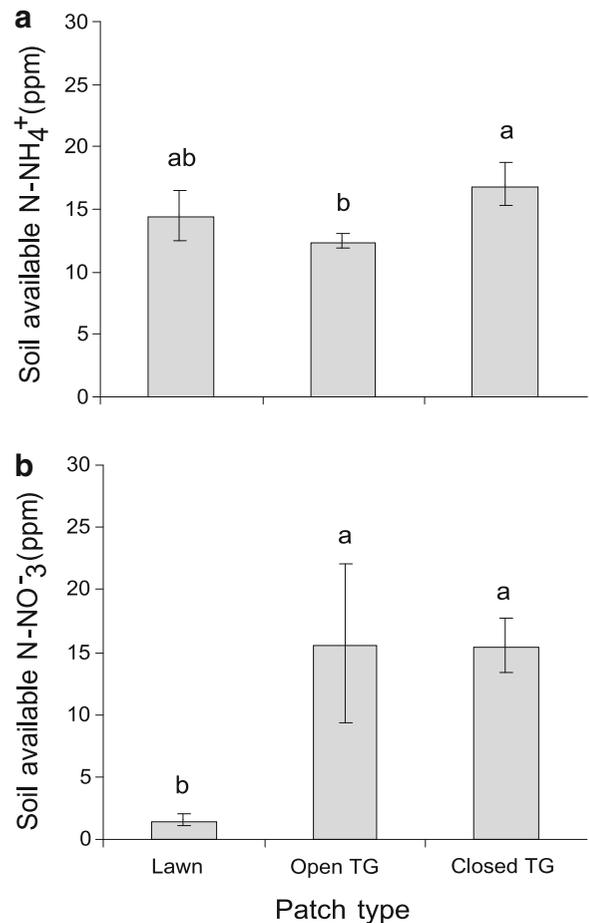


Fig. 5 Soil N available as (a) N-NH₄⁺, and (b) N-NO₃⁻ captured by ion exchange resin bags during 75 days of incubation in the three vegetation patch types. Bars indicates means \pm SE, Lawns, $n = 8$; Open TG, $n = 9$; Closed TG, $n = 10$, because the resin bags were lost in three plots. The differences among treatments were tested with mixed-effect ANOVA. Different letters indicate significant differences among patch types at $p < 0.05$. TG = tussock grasslands

litter mixtures and individual species (Cornelissen et al. 1999; Garnier et al. 2004; Cornwell et al. 2008). Plants are known to drive decomposition rates through the ‘afterlife’ effects of their attributes on litter quality (Pérez-Harguindeguy et al. 2013). As an expression of the quality of a substrate for microorganisms litter decomposability is usually related to litter N and lignin content, among other chemical attributes (Cornelissen et al. 1999). Through the changes in species composition and abundance and their consequent change in litter quality, herbivores are expected to affect litter decomposition rates in different patch types (Augustine and McNaughton 2006).

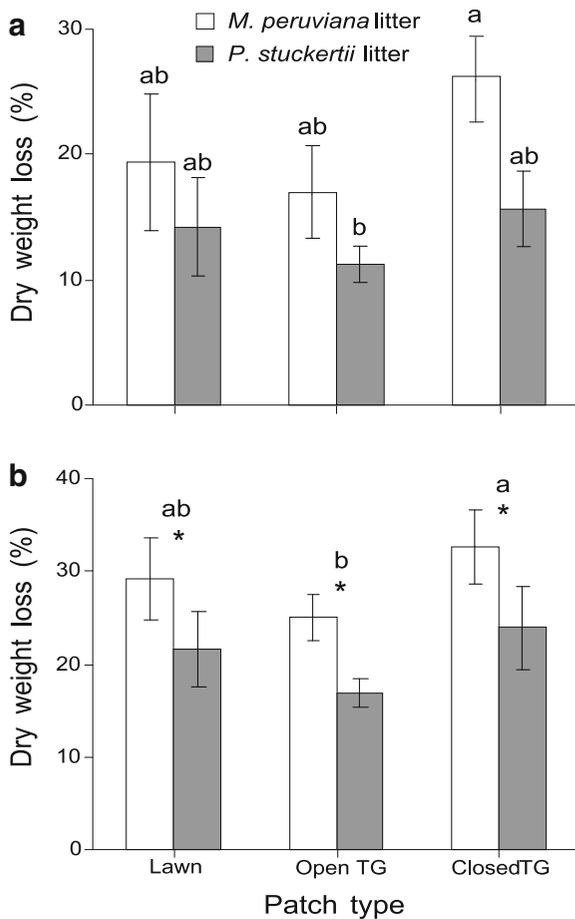


Fig. 6 Percentage of dry weight loss of *M. peruviana* and *P. stuckertii* incubated in the field in the three vegetation patch types (mean \pm SE, $n=10$). The differences among treatments were tested with repeated measures ANOVA. **(a)** at 75 days of incubation, patch type \times substrate interaction was significant (c.f. Table 1), and different letters indicate significant differences in dry weight loss among treatments (combination of patch type \times substrate) at $p < 0.05$. **(b)** at 198 days of incubation, patch type \times substrate interaction was not significant (c.f. Table 1). Thus, different letters indicate significant differences in dry weight loss among patch types, and asterisks (*) indicate significant differences in dry weight loss between the common plant substrates at $p < 0.05$. TG = tussock grasslands

Surprisingly, in situ litter decomposition rates were not associated with litter decomposability, nor with the amount of C and N entering the soil as litter or faeces. However, in situ litter decomposition rates were associated with common plant substrates decomposition rates. The weakness of association between decomposability of litter and faeces with its in situ decomposition rates, suggest that litter decomposability (and quality) would be less important for in situ decomposition, at

the local scale of this study, than previously proposed in general models (Lavelle et al. 1993). We by no means suggest that litter decomposability and quality are themselves not important, as this has been proven to be a strong control of decomposition in several wide comparisons (Cornelissen et al. 1999; Cornwell et al. 2008). However, the relevance of this control on field decomposition may depend on how large the differences in litter decomposability and quality among patch types are. Vegetation changes (composition and physiognomy) associated to herbivory in the patches analysed here modified decomposability in a consistent way, but the magnitude of differences was about 3–5 % in mass loss. In other studies where litter quality was indeed reflected in the in situ decomposition, litter from lightly grazed patches had less than half decomposability compared to the litter from highly grazed patches (e.g. 21 % vs. 82 % mass loss in lightly grazed and highly grazed patches, respectively, in Güsewel et al. 2005). Further evidence in this sense is shown by in situ faeces decomposition. Faeces decomposed faster than all litter types when they were incubated in a common garden, but decomposed similarly or even slower than the same litter when incubated in each patch type (in situ decomposition). The small differences in decomposability between faeces and litter of each patch type could be the cause of the inconsistent patterns of decomposition of faeces.

Our results suggest that aspects of soil environment others than litter quality (e.g. soil physicochemical and biological properties, and microclimate together) could be driving field decomposition rates at the scale studied, and could be masking the particular effect of litter quality. Multiple regression analysis showed that soil environment effect explained 40 % of the variance of in situ decomposition rates, while litter decomposability effect explained only 15 % of the variance. We knew from previous results that some soil physicochemical properties (i.e. total C, N, pH, sand, clay, etc.) were similar across patches (Vaieretti et al. 2010). In the current study, also soil bulk density was similar among patch types and was not related to the differences in litter decomposition.

The lack of differences in decomposition of common substrates in the laboratory experiment, suggest that under the same microclimatic conditions (temperature and humidity), the activity of microbial communities associated to soil would not differ among patch types. We cannot rule out that some properties of the

Table 2 Spearman correlation coefficients of in situ litter decomposition and soil N availability (N-NH₄⁺ and N-NO₃⁻), with amount of C and N deposited on soil through litter and faeces (as parameters of the quantity of resources deposited on soil), litter decomposability (as an expression of the quality of litter as a

substrate for microorganisms), and the decomposition of common plant substrates (both substrates average - *M.peruviana* and *P. stuckertii* - as indicator of the soil environment effect). * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$; † $p < 0.1$. % DWL=percentage of dry weight loss

	Litter decomposition (% DWL)	Soil N availability (ppm)	
		Soil N-NH ₄ ⁺ availability	Soil N-NO ₃ ⁻ availability
Litter-C return (gC m ⁻² yr ⁻¹)	-0.06	0.21	0.69***
Faeces-C return (gC m ⁻² yr ⁻¹)	-0.12	-0.38*	0.01
Litter-N return (gN m ⁻² yr ⁻¹)	-0.06	0.25	0.62***
Faeces-N return (gN m ⁻² yr ⁻¹)	-0.12	-0.38*	0.01
Litter decomposability (% DWL)	0.14	0.07	-0.50*
Common plant substrates decomposition (% DWL)	0.62**	0.36†	0.17

soil not measured in the standard soil analyses may have been modified with the laboratory manipulation, and thus can be influencing decomposition. However, we consider the most plausible explanation to be an effect of microclimate determining soil environment effect in the field.

Microclimatic conditions (e.g. soil temperature and moisture, irradiation) have been proved to be strongly driven by vegetation structure (Seastedt et al. 1988; LeCain et al. 2000; Posse et al. 2000; Yates et al. 2000). Microclimatic conditions can be a strong control of in situ decomposition (Stark et al. 2000; Risch et al. 2007). In our system, there are indeed significant differences in vegetation structure among patch types (Pucheta et al. 1998a; Cingolani et al. 2004) produced by herbivores. It is probable that these differences result in different microclimatic conditions (e.g. maximum and minimum soil temperature, and its daily or seasonal variation, as well as variation in soil moisture) affecting the decomposition process in the field. The effect of microclimate could be mediated by its influence on microbial communities' composition, abundance and/or metabolic rates, among other mechanisms (Swift et al. 1979; Chapin et al. 2002). Further measurements related to microclimatic parameters and microbial communities characteristics are needed to elucidate this issue.

Do quality and quantity of litter and faeces determine nutrient soil availability in the field?

Grazing is expected to increase soil N availability, stimulating N mineralization by microorganisms

(Denton et al. 1999; Hamilton and Frank 2001). However, we found higher availability of soil accumulated N-NH₄⁺ and N-NO₃⁻ in patches less grazed. We also found that the availability of soil accumulated N-NH₄⁺ was associated with in situ litter decomposition rates (and common substrates decomposition rates). In contrast, availability of soil accumulated N-NO₃⁻ was strongly associated with the quantities of C and N entering the soil as litter. The relationships between soil NH₄⁺ availability and in situ litter and common plant substrates decomposition are not surprising since ammonium is the initial product of the decomposition process (Chapin et al. 2002). Mineralization rates are controlled by multiple factors, including availability of C and different forms of inorganic N (Chapin et al. 2002). The higher input of litter in closed tussock grasslands may have lead to a higher total release of C and N entering the soil which, in turn, could enhance nitrification rates in this patch type in relation to grazing lawns (van Winjen et al. 1999; Olofsson et al. 2007).

Grazing may have both, positive or negative effects on mineralization rates, and consequently on N availability, through several mechanisms (Kiehl et al. 2001). The N loss hypothesis (Frank and Evans 1997; Piñeiro et al. 2006) suggests that N could be more easily volatilised (e.g. NH₃) or leached (e.g. NO₃) from faeces. Alternatively, the root-N retention hypothesis (Stewart and Frank 2008; Piñeiro et al. 2009) suggests that N could be more promptly absorbed by the higher biomass of roots in highly grazed patches. Considering the relatively low pH of these grasslands, the N loss hypothesis could be less plausible as conversion to NH₃ is less frequent in acidic soils (Bussink and Oenema 1998).

Then, lower nutrient losses in the herbivore-waste pathway (or fast cycle) together with higher nutrient input through the litterfall and the decomposition pathway (or slow cycle) could explain the higher N-NH_4^+ and N-NO_3^- availability in tussock grasslands (Bakker et al. 2004). Similar results have been found by Olofsson et al. (2007) in long term rabbit exclusions.

In our grasslands system, we consider more plausible that N is being absorbed fast by the roots in grazing lawns. Previous works within the same study area have reported fine root productivity being twice as high in grazed sites compared to ungrazed sites, with root growth peaking in summer-autumn season, when the present experiment was performed (Pucheta et al. 2004). The greater fine root biomass in lawns could be quickly incorporating N-NH_4^+ , and even competing with microorganisms for this N source, at least in the long term (Stark and Kitöviita 2006). As a consequence, there would be less N-NH_4^+ available to microorganisms to be transformed into N-NO_3^- . Alternatively, the input of labile sources of C from faeces and probably from root exudates stimulated by grazing, could promote gross mineralization (Frank et al. 2000; Bakker et al. 2004). This would increase immobilization of N by microorganisms which would further reduce nitrification rates (Tracy and Frank 1998). However, even though the microorganisms show substantially faster initial uptake of all N forms, that makes them short-term (few days) winners in the competition for nutrients, the short life cycle of microorganisms facilitates the reallocation of N from microorganisms to roots, which make plants to become winners over the long-term (weeks-months) (Hodge et al. 2000; Kuzyakov and Xu 2013). Consistently, soil accumulated N-NO_3^- was lower in grazing lawns, in which root retention may lead to a 'tight' N cycling within the root zone (Piñeiro et al. 2006). In addition, the lower concentrations of N-NO_3^- in the highly grazed lawn could be related to lower nitrification, which has been suggested as a mechanism for lower nitrate concentrations in salt marshes grazed by cattle (Olsen et al. 2011). As a result of its more compacted soils grazing lawns may be flooded during part of the warm rainy season, and anoxic conditions may impede nitrification (Kiehl et al. 2001; Bakker et al. 2004). We do not consider that this mechanism is likely to be responsible for the low N-NO_3^- of our lawn patches as they did not showed higher water content compared to tussock grasslands. In spite of the likeliness or unlikeliness of any of the mechanisms, further

experiments are necessary to pinpoint the exact mechanism responsible for the differences observed here.

Conclusions

Several mechanisms, not mutually exclusive and operating simultaneously, may lead to positive and negative relationships between herbivory and nutrient cycling parameters within the same grassland (de Mazancourt et al. 1998; Bakker et al. 2009). Grazing increased litter quality and the quantity of faeces in grazing lawns. However, the reduction in biomass caused by herbivore consumption greatly reduced C and N input through the slow litter decomposition pathway. The decomposition rates of litter and faeces, and that of two common plant substrates were not clearly related to either grazing pressure, nor litter quantity or quality (C, N, P, lignin, cellulose or hemicellulose content). Our results therefore do not agree with previous general grasslands models that suggest accelerated decomposition rates in grazing lawns (e.g. Ritchie et al. 1998; Bardgett and Wardle 2003; Wardle et al. 2004). We propose that the soil environmental conditions and microclimate may have a much stronger role in the control of field decomposition than previously considered in herbivore-plant-soil studies models.

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