



Impact of grazing abandonment on plant and soil microbial communities in an Atlantic mountain grassland



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ABSTRACT

Livestock grazing is a global land-use activity with multiple social, cultural, and environmental impacts. However, it is expected to decrease progressively in the Cantabrian Mountain areas as notable changes in livestock management systems have been observed, such as shorter stay in mountain, lower stocking rates, and less shepherd control. This study aimed to evaluate the effect of grazing abandonment on microbial function and diversity through changes promoted in aboveground vegetation and soil properties. In 2005, we erected permanent fences around two 50 × 50-m plots (excluded plots) in a temperate mountain grassland that has historically supported livestock managed by an extensive grazing system. A grazed plot was defined around each excluded plot. After 5 years, grazing abandonment induced shifts in floristic composition, decreased soil compaction at 0–10 cm soil depth, and reduced soil temperature in the summer due to a thicker plant layer. Consequently, microorganisms reduced soil enzymatic activity and microbial biomass, and increased CO₂ emissions and metabolic quotient, which indicated a lower metabolic efficiency of soil processes in excluded plots. These results suggest that soil microbial community function was very sensitive to the impacts of livestock grazing exclusion. The bacterial community was more diverse compared to the fungal community, but no significant difference in bacterial species richness was found between excluded and grazed plots. Microbial genetic diversity was not directly correlated with aboveground vegetation diversity and no clear pattern emerged as a response to grazing abandonment, probably because soil microbial diversity depends on site attributes that operate at a very fine spatial scale.

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1. Introduction

Livestock grazing is a global land-use activity with multiple social, cultural, and environmental impacts including those that affect soil health, plant productivity, and grassland structure (Greenwood and McKenzie, 2001; Yang et al., 2013). Soil microorganisms play basic roles in soil health (Hill et al., 2000; Pankhurst et al., 1997), but the question of whether microbial functioning and diversity may be modified by livestock grazing remains poorly explored.

Progressive decrease in livestock is one of the most expected changes in land use in Atlantic agroecosystems (Rounsevell et al., 2006), especially in mountain areas affected by the removal of the European Union subsidies for marginal grazing land (Strijker,

2005; Taylor, 2006). Permanent grasslands, which are the main natural resources for livestock maintaining in extensive grazing systems, have experienced a decreasing trend (Pe'er et al., 2014). Additionally, remarkable management changes – shorter stay in mountain, lower stocking rate, and less shepherd control – have been detected in the extensive livestock systems of the Cantabrian Mountains, where historically shepherded livestock used to graze from May to November. If these management trends continue, a progressive medium- or long-term grazing abandonment could be expected.

When livestock is removed from historically grazed grasslands such as those on the Cantabrian Mountains, important processes driven directly by grazing (mainly defoliation, trampling, and fertilization) disappear, changes occur in the soil environment, and consequently, changes in the soil microbial function and diversity are expected. Defoliation reduces plant aerial biomass, leading to a reduction in organic matter inputs to soil via litter, and modifies the floristic composition by changing the competitive relationships

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between plants (Bakker et al., 1983; Medina-Roldán et al., 2012; Milchunas and Lauenroth, 1993). Guitian and Bardgett (2000) showed that defoliation led to an increase in soil microbial biomass and carbon use efficiency in the root zone of several grass species of a semi-natural *Agrostis-Festuca* grassland. Furthermore, since defoliation diminishes the thickness of the plant layer, it also influences fluctuations in soil temperature (Odrizola et al., 2014; Zhao et al., 2011). Trampling promotes soil compaction, which additionally affects plant root development by reducing root depth and increasing root density near the soil surface (Chaieb et al., 1996; Fernández et al., 2011). These impacts on soil structure and root development determine not only the physical distribution and size of soil pores (inhabited by microorganisms) but also affect the soil microbiota since they determine the availability of oxygen and root exudates (Rodríguez et al., 1995). Furthermore, these belowground effects of trampling alter the productivity and composition of the aboveground plant community. When trampling is continuous and heavy, gaps may appear in dense grassland and provide suitable conditions for more opportunistic non-grass species to develop (Milchunas et al., 1988). Consequently, belowground competition affects the floristic composition of aboveground vegetation. Meanwhile, fertilization from animal faeces and urine may increase nitrogen availability in the soil through enhancing the rates of nitrogen mineralization and may alter other soil abiotic factors including pH and oxidative organic matter availability, which affect microorganisms' biomass, activity rate (i.e. function), and diversity (Bardgett et al., 1997). Nevertheless, other studies have reported that the different grazing treatments did not result in differences in plant N content nor in quality of litter (Bakker et al., 2004). Therefore, these authors concluded that herbivores create a shortcut in the N cycle, but it does not mean that enhancement of the N cycle is necessarily the net outcome, so this question is still debated.

In light of these aboveground–belowground interactions, we hypothesise that grazing abandonment will influence microbial function and diversity through changes that such abandonment causes in aboveground vegetation and soil properties. The aboveground effects have been fairly well studied, but studies are not conclusive and discrepant results have been reported (Ren et al., 2012). This indicates that vegetation responses are dependent on local experimental conditions, i.e. soil type, nutrient contents, initial vegetation attributes, productivity level, herbivore size, and grazing intensity (Bakker et al., 2006; Milchunas et al., 1988; Ren et al., 2012), and therefore, should be addressed in situ. Similarly, the possible impact of grazing abandonment on soil properties, particularly on microbial responses, is not well known (Smith et al., 2003). Recent studies that do address the effects of grazing on soil microbial properties have been commonly restricted to the function–activity level, focusing on microbial biomass, respiration, and/or enzyme activities (Ford et al., 2013; Mijangos et al., 2010). In consequence, community level associations between vegetation and microbial assemblages are less understood (Grayston et al., 2004). Few studies involve the quantification of soil microbial diversity, especially by molecular methods that do not depend on cultivable microorganisms, and its role in biological functions related to grazing.

Specifically, our goals in this study were (i) to verify whether medium-term grazing removal significantly altered vegetation composition, plant diversity, and structure (plant layer thickness), and if it did, to quantify the magnitude and direction of these changes; (ii) to test whether medium-term grazing removal affected soil properties such as soil compaction, temperature regime, and organic matter; and (iii) to analyse the effect of grazing exclusion on soil microbial function (community level physiological profiles, enzymatic activity, microbial biomass, and CO₂ emissions) and diversity (genetic diversity of bacteria and fungi,

independently), linking the observed microbial responses to changes promoted by grazing removal. To address these questions, we conducted an experiment by simulating 5 years of grazing exclusion in a temperate mountain grassland. We expected a decrease in plant diversity and accumulation of plant material in excluded plots, which reduces soil temperature and slows down nutrient cycling, negatively affecting both the abundance and activity of soil microbial communities and altering their functional and genetic diversity.

2. Materials and methods

2.1. Study area and experimental design

The study area is located in the Aralar Natural Park (42°59'48"N and 2°06'51"W), in an 11,000-ha protected area in the Basque Country (Northern Spain). The area has oceanic climate, with the mean annual temperature of 12.4°C and the mean annual precipitation of more than 1400 mm. The vegetation in the park comprises a mosaic of gorse–heather shrublands and grasslands that support livestock (mainly 18,000 dairy sheep of the Latxa breed) managed in an extensive grazing system. The area, traditionally used by livestock (beef cattle, dairy sheep, and horses) from May to the end of October, occupies 2077 ha (18.9% of the park area) and is dominated by native grasslands included in the Habitats Directive (European Commission, 2006). The most relevant vegetation type for livestock maintenance is the *Jasiono-Danthonietum* grassland (code 6230, subtype a), primarily comprising perennial graminoids such as *Festuca rubra* s.l., *Agrostis capillaris* L., and *Luzula campestris* (L.) DC., and herbaceous dicotyledons such as *Galium saxatile* L., *Trifolium repens* L., and *Cerastium fontanum* Baumg.

Our experiment had a completely randomized two-factor design. In June 2005, two experimental sites (Oidui and Alotza) were established in the *Jasiono-Danthonietum* grassland, both located on flat terrain. In each site, we erected permanent fences around two 50 × 50-m plots for excluding livestock ('excluded' plot). Around each fenced plot, we defined a 'grazed' plot, where sheep, cattle, and horses were allowed to graze continuously during the vegetative period, from the beginning of May to the end of October. Thus, one factor is experimental site (with two levels: Oidui and Alotza) and the other factor is treatment (with two levels: grazed and excluded). The two experimental sites differed in altitude (Oidui: 876 m a.s.l.; Alotza: 1224 m a.s.l.) and soil type (Oidui: silty clay loam texture, with 33.8% of sand; Alotza: clayey loam, with 20.2% of sand). The two soils are classified as calcic luvisol and hortic luvisol (FAO, 1981) and were similar in soil pH (5.0 ± 0.1) and C/N ratio (13.1 ± 1.1). At the beginning of the experiment, the relative abundance of functional groups in the aboveground vegetation differed slightly between the sites; in Oidui, 70.8 ± 2.3% of vegetation comprised graminoids, 23.8 ± 1.0% forbs, and 5.4 ± 1.3% mosses, while in Alotza, 49.0 ± 1.7% were graminoids, 27.5 ± 0.5% forbs, and 23.5 ± 1.2% mosses. The grazing intensity varies during the summer period and ranges from 2.4 to 4.5 LU (h day⁻¹), which is considered a moderate-to-high intensity in temperate grasslands. In June 2005, at the start of the exclusion experiment, we conducted a vegetation sampling into each site, following the sampling procedure explained in Section 2.3, and using redundancy analysis, verified that floristic composition between grazed and excluded plots was not significantly different (data not shown).

2.2. Vegetation sampling

For determining the floristic composition of the experimental plots, we conducted a vegetation survey using the quadrat cover

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