The contrasted impacts of grasshoppers on soil microbial activities in function of ecosystem productivity and herbivore diet

Sébastien Ibanez[1], Arnaud Foulquier[1], Charles Brun[1,2], Marie-Pascale Colace[1], Gabin Piton[1], Lionel Bernard[1], Christiane Gallet[1], Jean-Christophe Clément[1,3]

1 Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS UMR 5553, LECA, Chambéry, France

2 University of Applied Sciences and Arts Western Switzerland – Land, Nature, Environment Institute, Hepia Geneva, Route de Presinge 150, CH-1254, Jussy, Switzerland

3 Univ. Savoie Mont Blanc, INRAE, CARRTEL, Thonon-Les-Bains, France

Key words: nutrient cycling, primary productivity, grasshoppers, grasslands, ecosystem functioning, plant- soil feedbacks.

Abstract

Herbivory can have contrasted impacts on soil microbes and nutrient cycling, which has stimulated the development of conceptual frameworks exploring the links between below- and aboveground processes. The “productivity model” predicts that herbivores stimulate microbial activities and accelerate nutrient mineralization in productive ecosystems, while they have an opposite effect in less productive ecosystems. In parallel, the “diet model” predicts that herbivores feeding on conservative plants accelerate nutrient cycling while those feeding on exploitative plants decelerate nutrient cycling, due to changes in litter inputs. Since these two frameworks can lead to conflicting predictions in some cases, experimental evidence combining herbivore diet and productivity is required.

During two consecutive years, we conducted an experiment controlling the presence of three grasshopper species consuming either grasses, forbs or both in twelve natural and managed alpine grasslands of contrasted productivities. In order to assess the effects of herbivory on soil microbes, we measured their enzymatic activities, their biomass and the soil potential nitrogen mineralization (PNM). Soil and vegetation characteristics were also determined in order to test if they modulated the effects of herbivory on microbes.

Contrary to the predictions of the diet model, the effects of herbivory on microbial characteristics did not depend on the herbivores diet but relied on ecosystem productivity. The most productive sites were characterized by exploitative plant species which depleted N resources in the soil, and by microbes producing relatively few extracellular enzymes, leading to a lower PNM. Herbivory increased microbial biomass and decreased the production of extracellular enzymes in those sites, possibly through the stimulation of root exudates produced by exploitative species. The least productive sites were characterized by conservative plants, which led to the sequestration of soil C, and by microbes having a resource acquisition strategy (more extracellular enzymes, higher PNM). Herbivory decreased microbial biomass and increased the production of extracellular enzymes in those sites. This pattern can be explained by the loss of carbon associated with insect respiration, which increases the need for microbes to acquire resources and by a lower production of root exudates by conservative species. Therefore, the effects of two years of herbivory on soil microbes were at odds with the productivity model, which focuses instead on longer term effects corresponding to herbivory-induced changes in plant species composition. This highlights the multidimensional feature of the impacts of herbivory on ecosystem functioning, both in space and time.

Introduction

During the last decades the influence of herbivory on terrestrial ecosystem functioning has been highlighted through its effects on matter and energy fluxes linking above and belowground communities (Parker et al. 2017; Kristensen et al. 2020; Sandén et al. 2020). Plant-herbivore interactions influence the quantity and quality of organic matter inputs to soil detrital food webs with important implications on the rate of microbial processes which regulate organic matter decomposition and nutrient recycling and ultimately control the maintenance of soil fertility and carbon sequestration (Bardgett and Wardle 2010). Previous studies have already reported a diversity of impacts of herbivory (Hunter 2001; Bakker et al. 2004), including positive (e.g. Frank et al. 2000; Belovsky and Slade 2000), negative (e.g. Ritchie et al. 1998) or no detectable effects (Singer and Schoenecker 2003) on soil carbon and nutrient cycling. An overarching conceptual model of these contrasting effects is needed in order to understand which ecological variables control the direction and magnitude of the effects of herbivory on soil microbial communities and related ecosystem processes.

Among the different frameworks that have been proposed, one focuses on the contrasting effects of ecosystem productivity (Bardgett and Wardle 2003, 2010; Wardle et al. 2004), and one on the mitigation by herbivore diet (Ritchie et al. 1998; Belovsky and Slade 2000; Hunter 2001). The “productivity model” predicts that in productive ecosystems herbivores consume a high percentage of the net primary production (NPP), rapidly returning organic matter to the soil as easily decomposable fecal material enriched in nutrients (“fast cycle”, McNaughton et al. 1988). Herbivores also promote compensatory plant growth (McNaughton 1983) or nutrient reallocation in leaf tissues of exploitative plant species (Potthast et al. 2021), while they slow down the establishment of conservative plant species which produce more recalcitrant litter (Reich 2014). The combination of these positive effects on the quality of detrital resources leads to an acceleration of nutrient cycling, which further induces a positive feedback loop. Instead, in infertile ecosystems herbivores consume a smaller proportion of the NPP, favoring the accumulation of recalcitrant plant litter (“slow cycle”, McNaughton et al. 1988). This comes along with the promotion of conservative plant species producing even more recalcitrant litter therefore a slower nutrient cycling.

In contrast, the “diet model” focuses on a different axis of variation of herbivore-plant-soil interactions and distinguishes two types of herbivores, those consuming exploitative plants (fast-growing with high leaf nutrient content), and those eating conservative plants. Indeed, although most vertebrate herbivores either feed non-selectively or prefer high-quality plants (Hofmann 1989; Clauss et al. 2003), many insects prefer tougher plants (Ibanez et al. 2013) and perform better on seemingly low-quality diets (Cease et al. 2012; Talal et al. 2020). Herbivores feeding on exploitative plants favor the growth and survival of conservative plants and therefore the accumulation of more recalcitrant litter. This slows down organic matter cycling, as predicted by the productivity model according to which herbivores preferentially feed on higher quality plants in the least productive ecosystems (Bardgett and Wardle 2010). Instead, herbivores feeding on conservative plants transfer low quality litter into the fast cycle, which accelerates decomposition and nutrient cycling, and promotes exploitative plant species (Belovsky and Slade 2000). A comparable framework was also proposed by Tuomi et al (2019) in the particular context of burrow-dwelling rodents. These herbivores feed on exploitative

plants thereby reducing soil N availability; but they can also physically damage unpalatable conservative plants by their burrowing activities, which has opposite effects. At high densities the latter process becomes preponderant, these rodents can therefore accelerate nutrient cycling despite a diet dominated by the more palatable exploitative plants.

During the last 20 years, several experiments controlling for herbivory either by ungulates or insects provided evidence in favor of the diet model. When herbivores fed on the most conservative plants, they increased primary production and/or nutrient availability (McNaughton et al. 1997; Belovsky and Slade 2000, 2018; Garibaldi et al. 2007; Schmitz 2008; Nitschke et al. 2014). In contrast, when herbivores fed on the most exploitative plants they had an opposite effect (Pastor et al. 1993; Ritchie et al. 1998; van Wijnen et al. 1999; Harrison and Bardgett 2004; Schmitz 2008; Belovsky and Slade 2018). The two experiments showing such combination of both effects used polyphagous grasshopper species (*Melanoplus femurrubrum* and *M. sanguinipes*), for which the diet changes either according to the type of predators present (Schmitz 2008) or depending on intraspecific variation of leaf water content (Belovsky and Slade 2018). However, these experiments as well as others (e.g. Deraison et al. 2015) manipulated either the presence or the diets of herbivores in a single type of ecosystem, which hampers the articulation of the diet model with the productivity model.

Furthermore, while the productivity and diet models explicitly consider plant and herbivore resource acquisition strategies, they do not consider the resource acquisition traits of soil microbes. Yet, microbial communities are key regulators of nutrient recycling in the plant-soil system, and recent developments suggest that including microbial communities in a multitrophic functional trait framework could strengthen our mechanistic understanding of ecosystem functioning (Malik et al. 2020; Piton et al. 2020b). Resource acquisition strategies of microbial communities can be partly inferred from the characterization of enzymatic activities involved in C, N and P acquisition through the extracellular depolymerization of organic compounds (Piton et al. 2020b, a). A higher investment in resource acquisition traits (i.e. increased extracellular enzyme activities) is expected along a gradient of decreasing soil resource availability, paralleling a shift in plant resource acquisition strategy along the exploitative-conservative continuum (Piton et al. 2020b). Increased quantity (i.e. soil organic matter, N and P amounts) and quality (i.e. lower soil C/N, higher nitrate, ammonium contents) of available resources from directly assimilable fecal material enriched in nutrients (e.g. Fielding et al. 2013) or increased labile carbon inputs from root exudates (Hamilton and Frank 2001; Paterson et al. 2003; Hamilton et al. 2008) are expected to promote microbial communities (Wardle et al. 2004; Grigulis et al. 2013) with a high yield strategy characterized by a low investment in extracellular enzyme production (Malik et al. 2020; Piton et al. 2020b). On the opposite, a reduction of available resources quality should promote microbial communities with a resource acquisition strategy characterized by a high investment in extracellular enzymes targeting complex polymeric organic matter (Malik et al. 2020). Higher nitrogen availability in the form of labile compounds (amino acids, NH_4^+ or NO_3^-) resulting from herbivory should reduce the microbial demand for this element, resulting in a lower investment in N-acquiring enzymes. Yet, increased availability of labile C from root exudates following herbivory might also increase nutrient limitation of microbial communities, especially if herbivory stimulates plant productivity and associated nutrient acquisition. This would result in higher nutrient immobilization, which is more likely under less fertile conditions that offer low

resource availability for microbes. Considering the microbial resource acquisition strategy, along with herbivore traits, plant traits and soil characteristics is therefore a necessary step to develop a global multitrophic and mechanistic understanding of aboveground-belowground nutrient cycling.

Although the productivity and diet models focus on different axes of variations (ecosystem productivity and herbivore diet), they can be combined. In fertile ecosystems dominated by herbivores feeding on lower quality plants, the predictions of both models are aligned, pointing towards the dominance of high-quality plants and the acceleration of nutrient cycling. For infertile ecosystems populated by herbivores preferring higher quality plants, both models also predict similar outcomes, but this time foresee the dominance of low-quality plants, and the deceleration of organic matter cycling (Bardgett and Wardle 2010). However, in the two remaining cases the two models make opposite predictions. In infertile ecosystems inhabited by herbivores selecting low quality plants, nutrient cycling may be affected in both directions, depending on the relative strength of the various processes at play in the productivity and diet models. Similarly, herbivores feeding on high quality plants in fertile ecosystems may have contrasting effects on matter turn-over. For instance, under fertile conditions herbivores feeding on high quality plants can favor their dominance (Buckland and Grime 2000), presumably because high resource availability allows compensatory growth for these plant species. This may in turn promote nutrient cycling and soil fertility (Bardgett and Wardle 2010). However, if high-quality plants invest in defensive secondary compounds instead of displaying compensatory growth, and if the herbivores can cope with them, slow-growing species may become dominant and nutrient cycling may slow down.

Moreover, the productivity model focuses on the contrasting effects of herbivory along a gradient of plant productivity, but soil nutrient cycling is ultimately controlled by microbial communities, whose response to herbivory may rather depend on a gradient of soil resources, on which they rely more directly than plant productivity. Even though both gradients are often correlated, leading to productive ecosystems associated with low carbon sequestration (Wardle et al. 2004; Grigulis et al. 2013), decoupling can be observed (Piton et al. 2020b). Hence, the productivity and diet models should include the functional responses of belowground microbial communities in order to provide a completer and more mechanistic picture of herbivore-plant-soil interactions.

Since the existing frameworks provide either congruent or conflicting predictions, this calls for experimental evidence controlling for herbivore diet in ecosystems with contrasting plant productivity and soil resource levels, in order to test the effect of herbivory on microbial communities. In the present contribution, we bridge this gap with a semi-controlled experiment using insect-proof cages, where four types of grasslands (managed or natural, dominated by grasses or by forbs) in 12 montane sites were subjected to herbivory by three grasshopper species which either preferred grasses, forbs or a mixture of the two. Following two years of herbivory, activities of extracellular soil enzymes specialized in carbon, nitrogen and phosphorus acquisition were measured, as well as nitrogen mineralization potential and microbial biomass. We hypothesized that the effect of herbivory on these soil microbial characteristics depends on the interaction between herbivore diet and ecosystem productivity.

Material & methods

Study sites and experimental design

The experiment was conducted in the French Pre-Alps, in North Vercors on the calcareous plateau of Autrans-Méaudre (45°10'N, 5°32'E). Four grassland types were chosen on the base of their botanical composition: extensively managed and fertilized grasslands dominated by the grass *Festuca rubra*, intensively managed and fertilized grasslands dominated by the grass *Lolium perenne*, natural grasslands dominated by forbs characterized by *Heracleum sphondylium* and *Chaerophyllum hirsutum* and natural warm grasslands mainly formed by the grass *Bromus erectus*. The coordinates of the 12 study sites are given in Sup. Tab. 1. All grasslands were composed of a mixture of forbs and grasses, although the dominant functional group depended on the grassland type (Sup. Fig. 2A). Three replicates per grassland type were randomly chosen in the study area, for a total of twelve sites ranging from 1000 to 1300 m.a.s.l. In each site, five 1m² plots (60 plots in total) were selected randomly within a 5m-radius and assigned to one of the five following grasshopper herbivory treatments: *Miramella alpina*, *Pseudochorthippus paralellus*, *Stauroderus scalaris*, an equal mixture of the three species, no grasshoppers (control). The grasshopper species were chosen according to their diet, as *M. alpina* prefers to feed on forbs, *P. parallelus* on grasses and *S. scalaris* mainly on grasses but also on forbs (Ibanez et al. 2013). Plots were covered by a $1m^3$ metallic cage covered by insect-proof mesh (PE 22.30, 920 x 920 µm; DIATEX, Saint Genis Laval, France), into which the adult grasshoppers were introduced. The number of grasshoppers per cage was adjusted to fit 1-g of grasshopper for 300-g of plant aboveground biomass (dry mass), which corresponds to the maximal natural insect densities locally observed in the study area. The dry mass of each species and each sex was also considered, after preliminary measurements. Grasshoppers were present inside the cages during 65 days from the 2^{nd} week of July until the 3^{rd} week of September, during two consecutive years 2016 and 2017. During these periods, the number of grasshoppers per cage was checked every two weeks, including the control cages from which a few grasshoppers were occasionally removed.

Soil characteristics

In mid-September 2017, four 10-g soil samples were collected in each plot at 5-cm depth, and then bulked. The samples were sieved at 5-mm and stored at 4°C before processing within 48h. Subsamples of 5-g of soil were dried at 70°C for 1 week to determine soil moisture, followed by 4h at 550°C to determine soil organic matter (SOM) content. Soil subsamples were air dried and ground to powder to measure total C and N contents using a FlashEA 1112 elemental analyzer (Fisher Scientific Inc., Waltham, MA, USA), and to determine soil pH in a 1:2.5 (soil : distilled water) solution. Solution of 0.5M K_2SO_4 was used to extract soil nitrate (NO_3^-), ammonium (NH_4^+), total dissolved nitrogen (TDN) (Jones and Willett 2006), and phosphate (PO_4^-) on 10-g of fresh soil. N and P concentrations were measured on an automated photometric analyzer using standard colorimetric methods (Gallery Plus: Thermo Fisher Scientific, Waltham, Massachusetts, USA). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and the mineral N ($NO_3^- + NH_4^+$).

Microbial biomass, activities and traits

Microbial biomass nitrogen (MBN) content was based on the difference of soil N content before and after chloroform-fumigation extraction of 10 g of fresh soil (Vance et al. 1987). We then calculated MBN in µgN/g using a correction factor of 0.45 (Jenkinson et al. 2004).
Potential nitrogen mineralization rates (PNM) were estimated after incubation under anaerobic conditions of 10 g of fresh soil for 7 days at 40°C in the dark (Wienhold 2007). Mineralized organic N was accumulated as NH_4^+ during this incubation and PNM rates were calculated based on the difference between NH_4^+ content before and after incubation and expressed as µgN/g dry soil/day.
Microbial resource acquisition strategies were characterized using different microbial community-weighted mean traits (Piton et al. 2020b). We measured the potential activity of seven extracellular enzymes contributing to the degradation of C-rich substrates (α-glucosidase, β-1,4-glucosidase, β-d-cellobiosidase and β-xylosidase), N-rich substrates (β-1,4-N acetylglucosaminidase and leucine aminopeptidase), and P-rich substrates (phosphatase) using standardized fluorimetric techniques (Bell et al. 2013). We homogenized 2.75 g of fresh soil (1 minute in a Waring blender) in 200 ml of sodium acetate buffer solution that was adjusted at the mean soil pH observed in the present study (i.e. 6.2). The resulting soil slurries were added in duplicates to 96-deep-well microplates. We then added a substrate solution for each enzyme. Duplicated standard curves (0–100-µM concentration) were prepared for each soil sample by mixing 800 ml of soil slurry with 200 ml of 4-methylumbellifferone (MUB) or 7-amino-4-methylcoumarin (MUC) in 96-deep-well microplates. Microplates were incubated during 3hrs (dark, 175 rpm, 20°C), and centrifuged at 2,900 g for 3 min. Soil slurries (250 µl) were then transferred into black Greiner flat-bottomed microplate and scanned on a Varioskan Flash (Thermo Scientific) reader using excitation at 365 nm and emission at 450 nm (Bell et al. 2013). For each soil sample, the four enzyme activities degrading C-rich substrates, the two enzymes activities degrading N-rich substrates and all the seven enzymes were summed to obtain extracellular enzyme activity for C-rich substrates (EEC), N-rich substrates (EEN) respectively. Phosphatase activity was used to represent extracellular enzyme activity for P-rich substrates (EEP). EEC, EEN and EEP were calculated per gram of dry soil per hour (global activities, nmol activity/ g dry soil/h). Microbial resource limitation and associated trade-offs between C and N acquisition was assessed through the calculation of the ecoenzymatic ratio EEC:EEN (Sinsabaugh et al. 2009).

Vegetation surveys and plant biomass

In order to quantify the diet of each grasshopper species, a visual estimation of herbivory intensity was conducted in August 2016. Ten individuals belonging to each of the 6 most dominant plant species in each site were inspected for herbivory marks by grasshoppers and the percentage of leaf area eaten was visually estimated.

A botanical survey was conducted at the beginning of the experiment (June 2016) and after one year of controlled herbivory (June 2017). The point quadrat method was used (Levy and Madden 1933; Vittoz and Guisan 2007; Lavorel et al. 2008), with 50 regularly spaced points in each $1m^2$ cage. This method allows to determine the relative abundance of the most common species in each plot, but does not constitute a complete inventory of the specific richness. To assess the effect of herbivory,

we computed the proportion of forbs in 2017 minus the proportion of forbs in 2016 in each cage. At the end of the experiment in September 2017 the aboveground biomass was harvested in each plot, dried 48h at 40°C, sorted into forbs and grasses and weighed. In order to get a community-level measure of leaf C/N ratio, a representative sample of the harvested biomass was grounded to powder, homogenized, and 5 mg of the leaf powder were then analyzed for carbon and nitrogen concentration using a CHN analyser (FlashEA 1112, ThermoFisher Scientific, MA, USA). These measures also provided the amount of nitrogen in aboveground plant biomass per unit area (gN/m², N_B).

Statistical analysis

Linear mixed models in which the random effects corresponded to the 12 sites (with 5 pseudo-replicas per site) were used to predict the logit of the total percentage of leaf biomass eaten in function of grassland type, herbivore identity, plant functional group (forbs *vs* grasses), and the interaction between these three factors.

Coinertia analysis (Dray et al. 2003) was used to quantify the coefficient of correlation (RV coefficient, ranging between 0 and 1) between vegetation characteristics (total aboveground biomass, forbs biomass, N_B, community-level plant C/N ratio, plant Shannon index), soil abiotic characteristics (water content, pH, SOM, TDN, phosphorus content, C/N ratio) and soil microbial characteristics (EEC, EEN, EEP, EEC:ENN ratio, PNM and microbial biomass). Redundancy analysis (RDA, Borcard et al. 1992) was used to quantify the amount of variation of either microbial, soil abiotic and vegetation characteristics explained by either grassland type or site.

In order to quantify the effect of herbivory on the six microbial characteristics, the standardized response to herbivory was calculated as the difference between grasshopper treatments and the control treatment (no grasshoppers) of the same site, divided by the standard deviation of the corresponding site. Coinertia analysis was used to test if the standardized responses depended on soil abiotic and/or on vegetation characteristics.

To test if these effects of herbivory depended on grassland characteristics (either SOM or plant productivity N_B), and on herbivore identity, the standardized responses of microbial characteristics were then used as a response variable in separate linear mixed models including sites as random effects. Since the warm grassland #1 had a particularly high SOM, the same models as above were performed excluding this site to check if it had a disproportionate effect on the results.

In all mixed models, the denominator degrees of freedom were calculated using Satterthwaite's approximation (Satterthwaite 1946). Type III sums of squares were used for models including interactions between factors. For each model, the normality and homoscedasticity hypothesis were visually checked. All statistical analyses were performed with the R software (R Core Team 2019), using the packages lmerTest (Kuznetsova et al. 2017) and ade4 (Dray and Dufour 2007).

308

309

Results

Leaf quality and consumption

The percentage of leaf area eaten depended on a large part on the interaction between plant functional group and herbivore identity ($p<0.001$), indicating that the selected grasshoppers had contrasted diets as expected. *M. alpina* preferred forbs over grasses (7% of leaf area consumed *vs* 3.2%), *S. scalaris* preferred grasses over forbs (7.2% *vs* 3%), while *P. parallelus* ate almost exclusively grasses (11.3% *vs* 0.9%, Sup. Fig. 1A). When these three species were combined, grasses were slightly more impacted than forbs (7.4% *vs* 4.8%). The overall effect of grassland type on the leaf area eaten was not significant ($p=0.50$), which reflects the fact that the number of introduced grasshoppers in each cage depended on the estimated plant standing biomass. However, there was a significant interaction between grassland type and plant functional group ($p<0.001$), because in forbs dominated communities grass species were much more heavily impacted than forb species (15.3% *vs* 2.7%), whereas in the other grassland types both functional groups were consumed in similar proportions (Sup. Fig. 1B). The p-value of the three-way interaction between plant functional group, grassland type and grasshopper identity equaled 0.58, which indicates that the diet of each grasshopper species did not depend on grassland type. The elementary analysis of fresh plant material showed that the leaf C/N ratio was higher for grasses than for forbs, whatever the grassland type ($p<0.001$ in all cases). However, the magnitude of the difference depended on grassland type (interaction between plant functional group and grassland type, $p<0.001$). The largest difference was in warm grasslands (forbs: 25, grasses: 38) and the smallest in communities dominated by forbs (forbs: 22, grasses: 27, Sup. Fig. 2). The botanical survey conducted after one year of herbivory revealed that the proportion of forbs did not vary in most treatments ($p>0.4$), except in the cages containing the forbs-feeding *M. alpina* where forbs declined by 7.2% (Sup. Fig. 3, $p=0.013$).

Relations between vegetation, soil microbial and abiotic characteristics

The coinertia analysis between six microbial soil characteristics (nitrogen mineralization potential, EEN, EEC, EEP, microbial biomass, EEC/EEN ratio) and six abiotic soil characteristics (water content, pH, SOM, TDN, phosphorus content, C/N ratio) had a RV coefficient of 0.59 ($p<0.001$). One axis of covariation between the two types of variables corresponds to an anticorrelation between soil pH and soil phosphorus content on the one hand, and EEP on the other hand, as well as EEC/EEN ratio to a lesser extent (Figure 1A). Therefore, in acidic soils having low phosphorus content, microbes invested more in phosphorus acquisition enzymes and slightly more on carbon than nitrogen acquisition enzymes. Another axis of covariation was between the abiotic characteristics TDN, SOM and water content, and the microbial characteristics EEC and PNM. The RDAs indicate that grassland types were heterogeneous with respect to soil characteristics, grassland type explaining 28% of the variation for the six soil abiotic characteristics and only 9% for the six microbial

characteristics (Sup. Fig. 3). In contrast, the soil characteristics were homogeneous at the site level (Sup. Fig. 3).

Turning to vegetation, the coinertia analysis between the six abiotic soil characteristics and the five vegetation characteristics (total aboveground biomass, forbs biomass, N_B, community-level plant C/N ratio, plant Shannon index) had a RV coefficient of 0.22 ($p<0.001$). Ecosystem productivity was negatively associated with SOM, pH and soil phosphorus content, and was not linked to TDN (Figure 1B). The RV coefficient of the coinertia between five vegetation characteristics and the six microbial characteristics was lower (0.29, $p<0.001$), which suggested that the microbial soil characteristics were more related to soil abiotic characteristics than to vegetation characteristics. The EEC/EEN ratio covaried with ecosystem productivity (aboveground biomass and N_B) while EEC, EEN and PNM was associated with the leaf C/N ratio and the proportion of grasses (Figure 1C). The RDA indicates that grassland types were homogeneous with respect to vegetation characteristics with 53% of variation explained by grassland type (Sup. Fig. 3). Indeed, warm grasslands were characterized by high leaf C/N ratio, forb-dominated grassland by a high proportion of forbs and a high Shannon diversity, intensive grasslands by a high biomass and nitrogen content, while extensive grasslands had intermediate vegetation characteristics. Means ± sd of each vegetation, soil microbial and abiotic characteristics are given for each of the 12 sites in Sup. Tab. 1.

Effect of herbivory on microbial characteristics.

The coinertia analysis revealed that standardized responses of microbial characteristics to herbivory were related to soil abiotic characteristics (RV=0.22, $p<0.001$). The coinertia analysis (Figure 2) and the linear mixed models (Figure 3) both indicate that the responses of PNM and EEN to herbivory increase with SOM, as well as EEC and EEP although to a lesser extent (see Figure 3 for the p-values of the linear mixed models). More specifically, below 10% of SOM herbivory decreased PNM and soil enzymatic activities while above 15% of SOM herbivory had a positive effect on these variables. The response of microbial biomass to herbivory did not depend on SOM ($p=0.75$) but varied according to TDN. In sites with TDN below 30 µgN/g, herbivory increased microbial biomass, while in sites with TDN above 50 µgN/g herbivory decreased microbial biomass (Figure 3E, $p=0.014$). Finally, the response of EEP to herbivory was positively related to pH ($p=0.007$). In contrast, the effect of herbivory on microbial characteristics did not depend on vegetation characteristics (RV=0.09, $p=0.17$). In particular, neither plant biomass nor productivity (N_B) were related to changes in the standardized responses of microbial characteristics (details not shown, $p>0.05$). In all cases, the effects of herbivory on microbial characteristics depended neither on herbivore diet nor on the interaction between herbivore diet and ecosystem characteristics such as SOM, TDN or productivity (details not shown, $p>0.05$).

Discussion

Covariations between ecosystem properties

We first explore the covariations between vegetation, soil biotic and soil abiotic characteristics, in order to better identify the ecological differences between the studied grasslands. In the next sections, we will discuss how these differences conditions different types of responses to herbivory. The comparison of the 12 study sites indicated that, in line with the productivity model, soil C sequestration measured by SOM was low in sites having the higher plant biomass (Figure 1B). However, there was a positive relationship between SOM and TDN (Figure 1A), and no relationship between TDN and plant biomass (Figure 1B). TDN was also highly correlated with dissolved organic nitrogen, as in e.g. Chu & Grogan (2010). Since DON is mostly issued from SOM (Van Kessel et al. 2009), this explains the correlation between SOM and TDN. The decoupling between TDN and plant biomass might be explained by management, since extensive and intensive grasslands are fertilized each year; intensive grasslands being the most fertilized and productive ones (Piton et al. 2020b & Sup. Fig. 3). Nutrients available after fertilization are likely rapidly assimilated by the fast growing and productive plant species, resulting in both high productivity and low measured nutrient availability. After plant growth, mowing exports nutrients away from these grasslands, while fertilization reintroduces the lacking nutrients. Therefore, in managed grasslands a high proportion of annual primary production is consumed and indirectly returned to the soil through subsequent fertilization, as are natural fertile ecosystems in the productivity model (Bardgett and Wardle 2010).

Turning to microbes, those living in soils with high SOM and TDN were more active and accelerated the nutrient cycle (high PNM and ECC, Figure 1A). However, microbial biomass did not increase with SOM, which implies that high SOM comes along with higher mass-specific extracellular activities for carbon uptake and higher mass-specific nitrogen mineralization, which corresponds to a resource acquisition (A) strategy (sensu Malik et al. 2020). Microbial biomass did not covary with any soil characteristic (Figure 1 A & C), as in Farrell et al. (2011, see their Table 1) and Chu & Grogan (2010 see their Tables 1 & 2). This may reflect the fact that microbial biomass results from the complex interplay between potentially opposing factors such as microbial resource use efficiency, resource acquisition and resource availability, itself depending on SOM and plant input quantity and quality (e.g. root exudates). Moreover, microbial biomass depends on the whole soil trophic network (Calderón-Sanou et al. 2021). This result stresses the importance to consider the resource acquisition strategy of soil microbes and not only their biomass to understand their response to environmental gradients and their effect on nutrient recycling in soil (Piton et al. 2020a). Recent literature has proposed that microbes adopt an A strategy either when resources are scarce (Malik et al. 2019, 2020; Piton et al. 2020b), or when resources are abundant (Wood et al. 2018). Since microbes rely on two main carbon sources, not only soil organic matter but also those exuded by plants in the rhizosphere, it is necessary to explore the relationships between the traits of the microbial and plant communities. We found that the A strategy comes along with plant communities having higher leaf C/N ratios (Figure 1C), which corresponds to conservative plant resource use strategy. Since exploitative plants produce more root exudates (Williams et al. 2022), a labile carbon source would be available to microbes in exploitative plant communities, favoring a high yield (Y)

strategy characterized by low enzyme production, according to Malik et al. (2020). In contrast, in conservative plant communities, microbes would lack labile carbon source and rely more on soil organic matter. This implies an A strategy characterized by the production of extracellular enzymes related to carbon acquisition, which ultimately releases nitrogen from soil organic matter (Figure 1C).

In summary, the pattern of covariations between ecosystem properties suggests a distinction between two types of grasslands. On the one hand, the most productive sites (>400 g/m²) are characterized by exploitative plants, by soils having relatively low SOM (<10%) and TDN (<40 µg/g) contents, and by Y-strategist microbes producing fewer extracellular enzymes, possibly because of higher C supply in the rhizosphere (Table 1, left column). On the other hand, in the least productive dominated by conservative plants, the SOM and TDN contents are higher and the microbial community is characterized by an A strategy since microbes need to produce extracellular enzymes to get access to SOM resources (Table 1, right column).

Herbivory effects on enzymatic activities

The present section explores if the effect of herbivory on microbial communities depend on the grassland types described above. We found a covariation between SOM and the responses of soil enzymatic activities and PNM to herbivory (Figure 2). Herbivory had a negative effect on soil enzymatic activities and PNM when SOM was below 10%, and a positive effect when SOM was above 15% (Figure 3). This means that herbivory can increase or decrease nutrient cycling depending on soil conditions, in relation with microbial strategies. In soils rich in SOM, A strategists invested even more in extracellular enzymes in response to herbivory. Herbivory provides fresh organic matter (FOM such as green fall, feces, cadavers) to the soil, which contains nutrients that are more easily available than SOM but which nevertheless needs to be depolymerized before assimilation (e.g. chitin). Since in sites rich in SOM, A strategists possess the enzymatic traits that give them access to this resource, this would explain why A strategists invest more in extracellular enzymes following herbivory. Consequently, the enhancement of extracellular enzymatic activities might have cascaded on PNM, as expected according to the key role of organic matter depolymerization in N mineralization (Schimel and Bennett 2004). In contrast, in low SOM soils Y strategists decrease even more their investment in extracellular enzymes. A possible explanation is that litter inputs are not the only consequences of herbivory on ecosystem functioning (Hunter et al. 2012). In particular, herbivory stimulates root exudation (Holland 1995; Holland et al. 1996), which increases microbial activity in the rhizosphere and subsequently favors compensatory plant growth (Hamilton and Frank 2001; Hamilton et al. 2008). Williams et al. (2022) found that root exudation is more intense in exploitative than in conservative plants, but it is not known if the effect of herbivory on root exudation depends on plant functional groups. If the stimulation of root exudation by herbivory is more intense for exploitative than for conservative plants, this would explain the finding that herbivory decreased the production of extracellular enzymes in sites having low SOM, since they are dominated by exploitative plants. However, although the effect of herbivory on soil enzymatic activities and PNM depended on SOM (Figure 3), it did not directly depend on the mean leaf C/N ratio (details not shown, $p>0.05$). Root exudation following herbivory has been found to enhance NMP for the benefit of plants (Hamilton and Frank 2001), while our results suggest the opposite.

However, inputs of available C may also result in N immobilization in the microbial biomass (Lovett and Ruesink 1995), which is this time in line with our results. This calls for both qualitative and quantitative characterization of root exudation in future works to further elucidate how soil and plant properties jointly control ecosystem response to herbivory.

Following the productivity model, we initially postulated that in more productive and fertilized sites having low SOM content, herbivory would enhance nitrogen mineralization. We found the opposite, presumably because of the stimulation of root exudation, as discussed above. Furthermore, according to the productivity model when herbivores accelerate plant succession this favors conservative plants with higher leaf C/N ratio, thereby reducing nutrient mineralization and enhancing soil C sequestration (Wardle et al. 2004). Therefore, herbivory would deccelerate nitrogen mineralization in sites having high SOM content. The grasshopper *M. alpina* negatively impacted forbs during the experiment, whatever the sites (-7.2% between 2016 and 2017), but this was not followed by a particular effect of this species on soil properties. The two other species of insect did not change plant community composition (Sup. Fig. 2). Plant community shifts induced by herbivory are more likely a longer-term effect of herbivory than the two-years duration of the experiment. Herbivory may also deccelerate nitrogen mineralization through the induction of recalcitrant plant secondary compounds (Schultz and Baldwin 1982; Rhoades 1985; Agrawal 1999) which subsequently slows down litter decomposition (Findlay et al. 1996; Hattenschwiler and Vitousek 2000). However, we did not find any effect of herbivory on litter decomposition (Ibanez et al. 2021).

Turning to microbial biomass, herbivory had a positive effect in sites having low TDN, and a negative effect when TDN was high (Figure 3E), but contrary to the enzymatic activities the effect of herbivory on microbial biomass did not depend on the SOM content (Figure 2A). In previous studies, herbivory increased microbial biomass through an input of labile C contained in the feces (Lovett and Ruesink 1995; Van Der Wal et al. 2004). Herbivory was also found to decrease microbial biomass when plants' C inputs to the soil are instead reduced due to herbivores respiration (Sankaran and Augustine 2004). Since TDN was positively linked with mean leaf C/N ratio (Figure 1B), itself being negatively correlated to root exudates (Williams et al. 2022), we hypothesize that in sites with low TDN, herbivory triggered an input of labile C into the rhizosphere, thereby stimulating the growth of microbial biomass, whereas in sites with high TDN, herbivory reduced the plants C inputs, with a negative impact on microbial biomass.

No effect of herbivore diet

The proportion of forbs in the diet of *M. alpina* was the highest, followed by the mixed feeder *S. scalaris* and then by the grass feeder *P. parallelus* (Sup. Fig. 1A), in line with previous studies (Ibanez et al. 2013). Since the grasshoppers' diet did not depend on the grasslands they were introduced in, it was possible to test if the effects of herbivory on soil enzymatic activities depended on their diet. The diet model predicts that herbivores feeding on high quality plants should favor the accumulation of poor-quality litter and thereby slow down nutrient cycling, and that herbivores feeding on low quality plants should favor the accumulation of high-quality litter, with an accelerating effect on nutrient cycling (Belovsky and Slade 2000).

In the present study, high quality plants characterized by low leaf C/N ratio (Sup. Fig. 2) were consumed preferentially by the forb feeder *M. alpina* (Sup. Fig. 1A), which decreased their relative abundance by about 7% (Sup. Fig. 3). In warm and forb-dominated prairies, litter decomposition during winter was faster for forbs (28% of mass loss) than for grasses (17%), although in extensive and intensive prairies winter decomposition was similar for both plant functional groups, due to the intermittent presence of snow cover (Ibanez et al. 2021). In any case, this suggests that the year-round decomposition rate is higher for forbs than for grasses, as it is generally the case (Tilman 1988). Given that *M. alpina* modifies the balance between forbs and grasses, this should affect the overall decomposition rate, with potential impacts on soil microbial communities. However, *M. alpina* did not have any contrasted effect on soil microbial characteristics, in comparison to the other grasshopper species. Perhaps this would have required a heavier impact on the relative abundance of forbs, rather than only 7%.

Lower quality plants (high leaf C/N ratio) were consumed preferentially by the mixed feeder *S. scalaris* and almost exclusively by the grass feeder *P. parallelus* (Sup. Fig. 1A), without any subsequent variation of the proportion of forbs and grasses in the plant communities (Sup. Fig. 3). This might be explained by plant compensatory growth, especially in the case of exploitative grasses (Barton 2016). Instead conservative grass species are less tolerant to herbivory (Avanesyan 2016), however in communities dominated by conservative grasses (e.g. *Bromopsis erecta*) grass feeders did not decrease the relative abundance of grasses, presumably because grasses were highly dominant in these communities. In any case, grass feeders did not have any distinguishable effect on soil microbes, relatively to the other grasshopper species. Previous short-term experiments using similar insect loads have reported that the effects of grasshoppers on soil processes depended on their diet type (e.g. Schmitz 2008; Belovsky and Slade 2018). Our findings question the generality of these results, since we found that the effect of herbivory on enzymatic activities did not depend on grasshopper species identity, nor on the interaction between grasshopper species and ecosystem characteristics such as SOM content.

Conclusion

Previous work showed that herbivory has contrasted impacts on ecosystem functioning in general (Brown and Gange 1992; Bardgett 2005; Schmitz 2008), and on soil microbes in particular (Denton et al. 1998; Stark and Grellmann 2002), in function of the environmental conditions and of the diet of herbivores. The motivation of this work was to observe such contrasted impacts in a single experiment across different types of grasslands, using herbivores having different diets, in order to better understand the pathways which create this apparently idiosyncratic pattern. We did not find any interaction between ecosystem productivity and herbivore diet on soil microbial characteristics, contrary to our expectation. However, the effects of herbivory on soil microbes depended on several properties of the 12 study sites. Table 1 summarizes our main findings and can provide some lines of interpretation, although its dichotomic schematization does not fully represents the multidimensionality of the results (Figure 1-3). On the one hand, the most productive sites were characterized by a higher biomass of exploitative plant species which depleted N resources in the

soil, and by yield-strategists microbes having a smaller investment in extracellular enzymes. Since exploitative plant species tend to produce more root exudates (Williams et al. 2022), we postulate that herbivory increased C supply in the rhizosphere (e.g. Holland et al. 1996; Hamilton and Frank 2001), which would explain the observed increase in microbial biomass and the lesser production of extracellular enzymes. On the other hand, the least productive sites were characterized by a lower biomass of conservative plants, which led to the sequestration of soil C, and by acquisition-strategists microbes having a larger investment in extracellular enzymes. We hypothesize that, in those sites, the consumption of plants results in lower soil C inputs due to herbivore respiration (Sankaran and Augustine 2004), which would explain why herbivory eventually decreased microbial biomass and increased the investment in extracellular enzymes in these less productive sites. Although the framework presented in Table 1 has some similarities with the productivity model (Bardgett and Wardle 2010), our findings point towards an acceleration of N cycling in less productive sites and a deceleration in more productive sites, in opposition with the productivity model. However, both frameworks do not consider the same time scales. The present experiment was conducted over two years and focuses on physiological time scales (root exudation, enzyme production), while the productivity model encompasses plant community dynamics, which occurs on longer time scales. In any case, we are convinced that none of these frameworks fully grasp the complex relationships between plants, soil, microbes and the effects of herbivory, which are most likely multidimensional. At the very least, these frameworks have some heuristic value and can be used for the design of future experiments.

Figures & Tables

Figure 1

Coinertia of (A) soil microbial and abiotic characteristics, (B) soil abiotic and vegetation characteristics and (C) microbial and vegetation characteristics. tdn=total dissolved nitrogen, hum=soil water content, som=soil organic matter, CN=soil C/N ratio, pho=soil phosphorus content, PNM=potential nitrogen mineralization, mbn=microbial biomass, EEN, EEC, EEP= extracellular enzyme activities related to either nitrogen, carbon or phosphorus, ratio=EEC/EEN, plantCN=community-level plant C/N ratio, biom=total aboveground plant biomass, forbs=forbs biomass (g), N_B=nitrogen content in total aboveground plant biomass (N_B), shan=Shannon diversity index of plants.

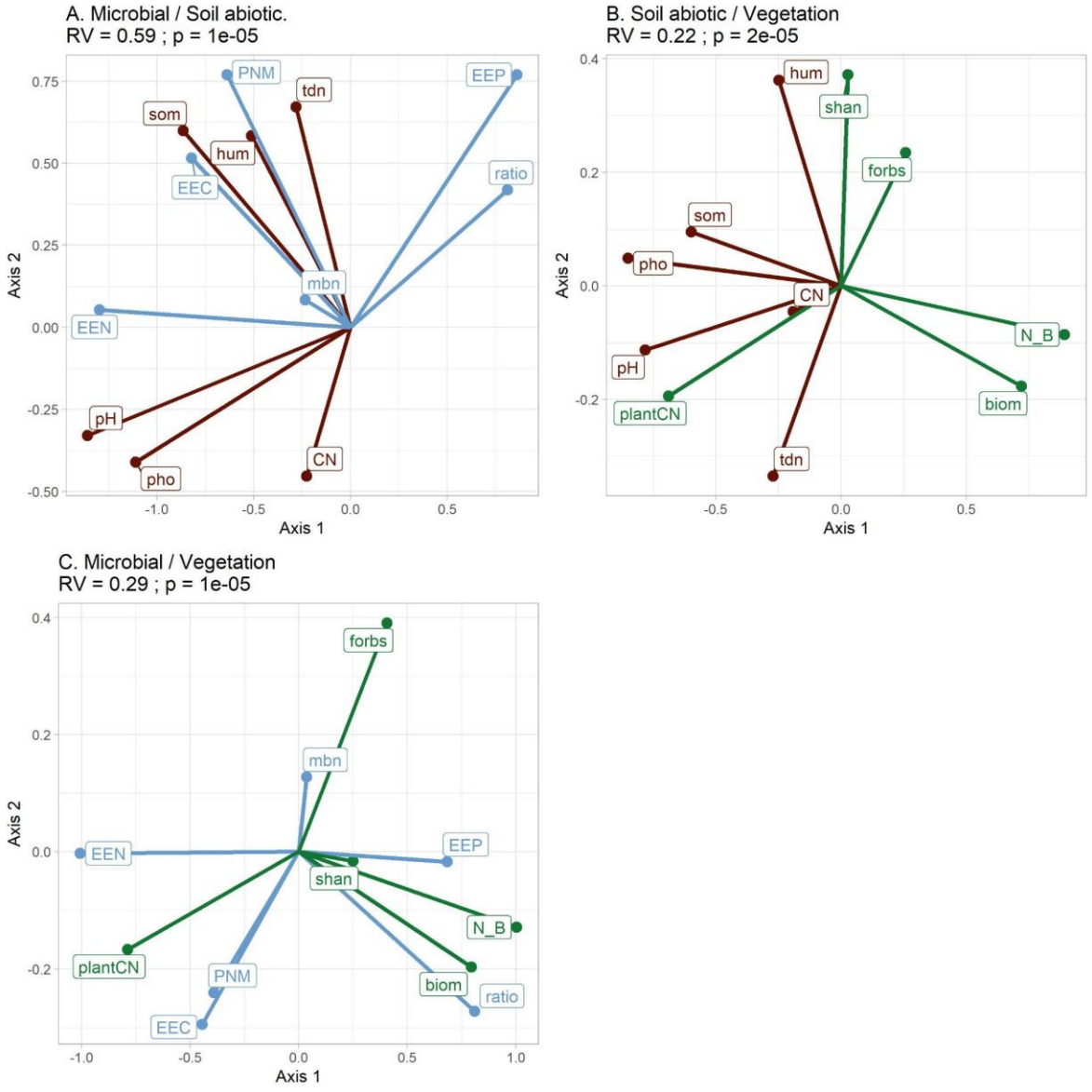


Figure 2

Coinertia of soil abiotic characteristics and the standardized response to herbivory of microbial characteristics. (A) Canonical weights of soil abiotic characteristics (in brown) and standardized response to herbivory of microbial characteristics (in purple). (B) The 48 arrows correspond to all the plots containing grasshoppers. The soil abiotic characteristics are at the beginning of the arrows, the standardized response to herbivory of microbial characteristics are at their end. tdn=total dissolved nitrogen, hum=soil water content, som=soil organic matter, CN=soil C/N ratio, pho=soil phosphorus content, PNM=potential nitrogen mineralization, mbn=microbial biomass, EEN, EEC, EEP= extracellular enzyme activities related to either nitrogen, carbon or phosphorus, ratio=EEC/EEN.

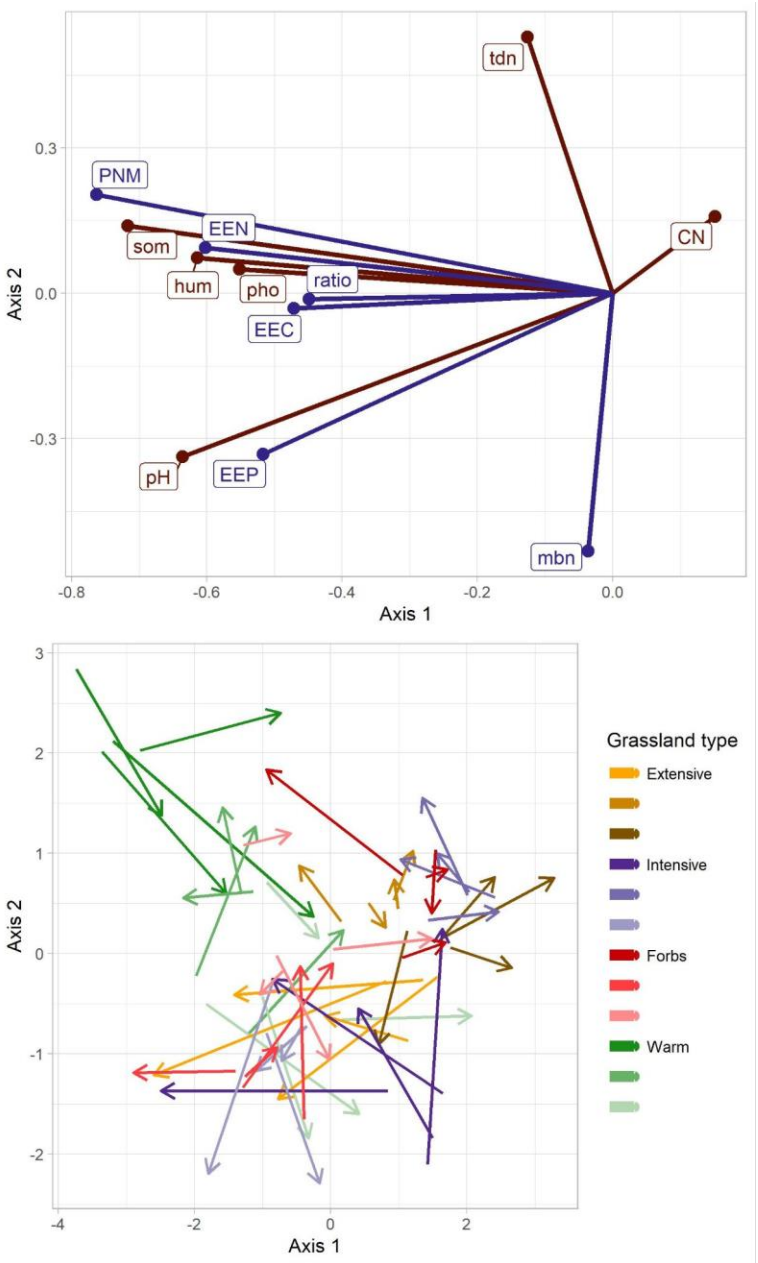


Figure 3

Relationship between soil organic matter and the standardized response to herbivory, calculated as the difference between each grasshopper treatment and the control treatment (no grasshoppers) of the same site, divided by the standard deviation of this site. p is the p-value of the linear mixed model including all sites (continuous regression line), while p' excludes the warm grassland #1 having SOM values >30 (dashed regression line).

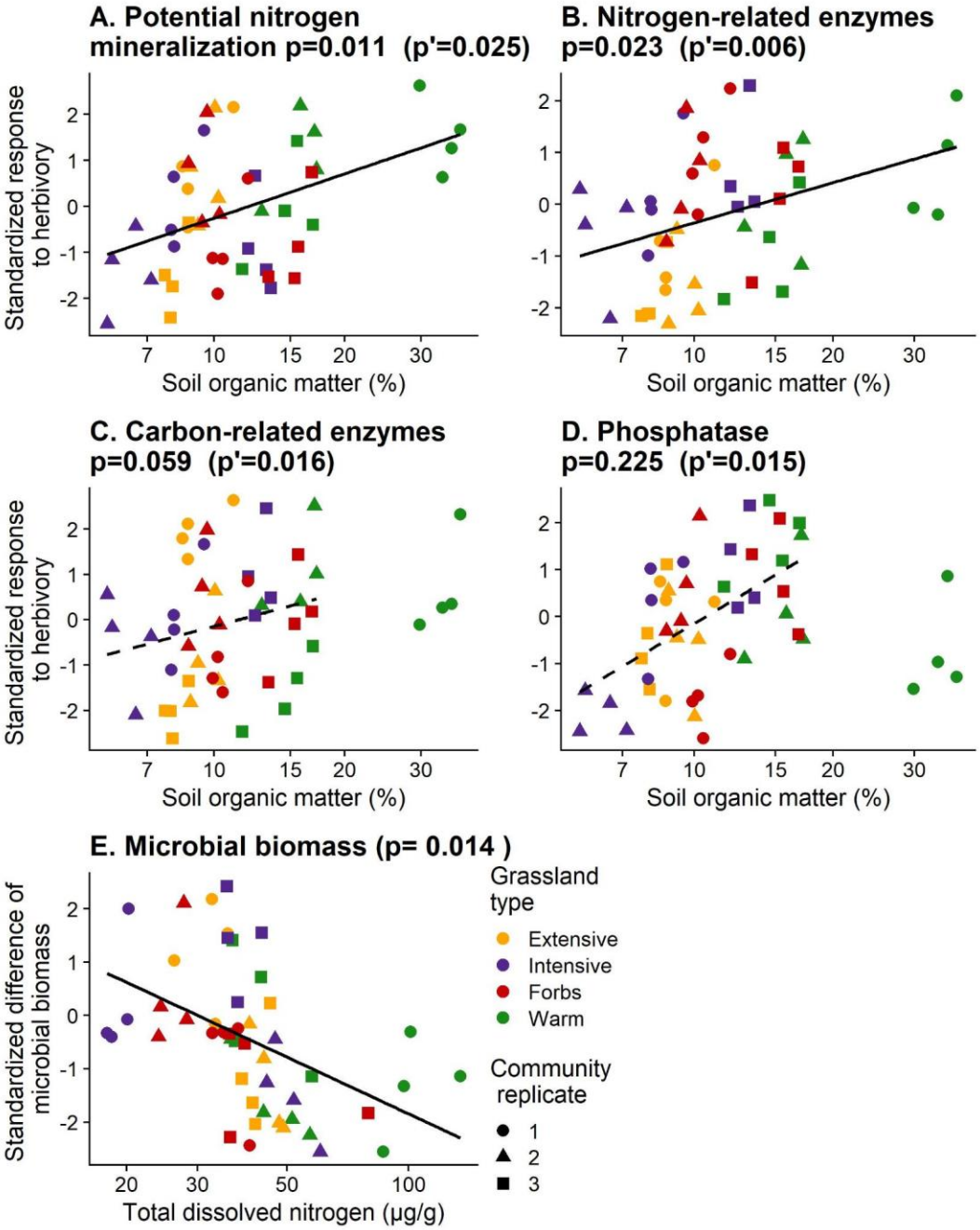


Table 1

Schematic representation of the main results, and their articulation with previous works.

	Most productive sites	Least productive sites	Source
Plants	High biomass	Low biomass	Figure 1
	Low leaf C/N	High leaf C/N	Figure 1
	Exploitative plants	Conservative plants	Figure 1
	High C supply in the rhizosphere	Low C supply in the rhizosphere	Williams et al. 2020
Soil	Low soil organic matter	High soil organic matter	Figure 1
	Low total dissolved nitrogen	High total dissolved nitrogen	Figure 1
Microbes	Low extracellular enzymes	High extracellular enzymes	Figure 1
	High yield strategy Y	Resource acquisition strategy A	Malik et al. 2020
Effects of herbivory on microbes	Decreases extracellular enzymes	Increases extracellular enzymes	Figures 2 & 3
	Decreases mineralization	Increases mineralization	Figures 2 & 3
	Increases microbial biomass	Decreases microbial biomass	Figures 2 & 3
	Increases C supply is the rhizosphere (root exudates)[1]	Herbivore respiration decreases C inputs to the soil[2]	[1]: e.g. Hamilton & Frank 2001 [2]: e.g. Sankaran & Augustine 2004
Prediction of the productivity model	Herbivory retards plant succession, which accelerates N cycling	Herbivory favors plant succession, which decelerates N cycling	Bardgett & Wardle 2010

Acknowledgements

This work was funded by the ECO-SERVE project through the 2013–2014 BiodivERsA/FACCE-JPI joint call for research proposals, with the national funders ANR, NWO, FCT (BiodivERsA/001/2014), MINECO, FORMAS and SNF. This work was also funded by the Alpine Ecology Lab. We thank the municipality of Autrans-Méaudre, the local farmers and the Refuge de Gève for their authorization to access the study sites, Jonathan Crison for his hospitality. We are grateful to Jean-Noël Avrillier, Annie Millery, Hugo Girard, Matteo Tolosano, Louise Maris, Pablo Raguet, Océane Guillot, Jessica Barbe & Alison Dillien for their assistance during field and lab work.

References

Agrawal AA (1999) Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80:1713–1723

Avanesyan A (2016) Tolerance of native and exotic prairie grasses to herbivory by Melanoplus grasshoppers: application of a nondestructive method for estimating plant biomass changes as a response to herbivory1. J Torrey Bot Soc 144:15–25

Bakker ES, Olff H, Boekhoff M, et al (2004) Impact of herbivores on nitrogen cycling: contrasting effects of small and large species. Oecologia 138:91–101. https://doi.org/10.1007/s00442-003-1402-5

Bardgett R (2005) The biology of soil: a community and ecosystem approach. Oxford university press

Bardgett RD, Wardle DA (2010) Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press Oxford

Bardgett RD, Wardle DA (2003) Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268

Bell CW, Fricks BE, Rocca JD, et al (2013) High-throughput fluorometric measurement of potential soil extracellular enzyme activities. JoVE J Vis Exp e50961

Belovsky GE, Slade JB (2000) Insect herbivory accelerates nutrient cycling and increases plant production. Proc Natl Acad Sci 97:14412–14417. https://doi.org/10.1073/pnas.250483797

Belovsky GE, Slade JB (2018) Grasshoppers affect grassland ecosystem functioning: Spatial and temporal variation. Basic Appl Ecol 26:24–34

Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73:1045–1055

Brown VK, Gange AC (1992) Secondary plant succession: how is it modified by insect herbivory? Vegetatio 101:3–13

Buckland SM, Grime JP (2000) The effects of trophic structure and soil fertility on the assembly of plant communities: a microcosm experiment. Oikos 91:336–352. https://doi.org/10.1034/j.1600-0706.2000.910214.x

Calderón-Sanou I, Münkemüller T, Zinger L, et al (2021) Cascading effects of moth outbreaks on subarctic soil food webs. Sci Rep 11:15054. https://doi.org/10.1038/s41598-021-94227-z

Cease AJ, Elser JJ, Ford CF, et al (2012) Heavy Livestock Grazing Promotes Locust Outbreaks by Lowering Plant Nitrogen Content. Science 335:467–469. https://doi.org/10.1126/science.1214433

Chu H, Grogan P (2010) Soil microbial biomass, nutrient availability and nitrogen mineralization potential among vegetation-types in a low arctic tundra landscape. Plant Soil 329:411–420. https://doi.org/10.1007/s11104-009-0167-y

650 Clauss M, Lechner-Doll M, Streich WJ (2003) Ruminant diversification as an adaptation to the
651 physicomechanical characteristics of forage. A reevaluation of an old debate and a new
652 hypothesis. Oikos 102:253–262

653 Denton CS, Bardgett RD, Cook R, Hobbs PJ (1998) Low amounts of root herbivory positively
654 influence the rhizosphere microbial community in a temperate grassland soil. Soil Biol
655 Biochem 31:155–165

656 Deraison H, Badenhausser I, Loeuille N, et al (2015) Functional trait diversity across trophic levels
657 determines herbivore impact on plant community biomass. Ecol Lett 18:1346–1355

658 Dray S, Chessel D, Thioulouse J (2003) Co-inertia analysis and the linking of ecological data tables.
659 Ecology 84:3078–3089

660 Dray S, Dufour A-B (2007) The ade4 package: implementing the duality diagram for ecologists. J
661 Stat Softw 22:1–20

662 Farrell M, Hill PW, Farrar J, et al (2011) Seasonal variation in soluble soil carbon and nitrogen across
663 a grassland productivity gradient. Soil Biol Biochem 43:835–844.
664 https://doi.org/10.1016/j.soilbio.2010.12.022

665 Fielding DJ, Trainor E, Zhang M (2013) Diet influences rates of carbon and nitrogen mineralization
666 from decomposing grasshopper frass and cadavers. Biol Fertil Soils 49:537–544

667 Findlay S, Carreiro M, Krischik V, Jones CG (1996) Effects of damage to living plants on leaf litter
668 quality. Ecol Appl 6:269–275

669 Frank DA, Groffman PM, Evans RD, Tracy BF (2000) Ungulate stimulation of nitrogen cycling and
670 retention in Yellowstone Park grasslands. Oecologia 123:116–121

671 Garibaldi LA, Semmartin M, Chaneton EJ (2007) Grazing-induced changes in plant composition
672 affect litter quality and nutrient cycling in flooding Pampa grasslands. Oecologia 151:650–662

673 Grigulis K, Lavorel S, Krainer U, et al (2013) Relative contributions of plant traits and soil microbial
674 properties to mountain grassland ecosystem services. J Ecol 101:47–57.
675 https://doi.org/10.1111/1365-2745.12014

676 Hamilton EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply?
677 Evidence from a grazing tolerant grass. Ecology 82:2397–2402

678 Hamilton EW, Frank DA, Hinchey PM, Murray TR (2008) Defoliation induces root exudation and
679 triggers positive rhizospheric feedbacks in a temperate grassland. Soil Biol Biochem
680 40:2865–2873

681 Harrison KA, Bardgett RD (2004) Browsing by red deer negatively impacts on soil nitrogen
682 availability in regenerating native forest. Soil Biol Biochem 36:115–126.
683 https://doi.org/10.1016/j.soilbio.2003.08.022

684 Hattenschwiler S, Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient
685 cycling. Trends Ecol Evol 15:238–243

686 Hofmann RR (1989) Evolutionary steps of ecophysiological adaptation and diversification of
687 ruminants: a comparative view of their digestive system. Oecologia 78:443–457

688 Holland JN (1995) Effects of above-ground herbivory on soil microbial biomass in conventional and
689 no-tillage agroecosystems. Appl Soil Ecol 2:275–279

690 Holland JN, Cheng W, Crossley D (1996) Herbivore-induced changes in plant carbon allocation:
691 assessment of below-ground C fluxes using carbon-14. Oecologia 107:87–94

692 Hunter MD (2001) Insect population dynamics meets ecosystem ecology: effects of herbivory on soil
693 nutrient dynamics. Agric For Entomol 3:77–84

694 Hunter MD, Reynolds BC, Hall MC, Frost CJ (2012) Effects of herbivores on terrestrial ecosystem
695 processes: the role of trait-mediated indirect effects. In: Trait-mediated indirect interactions:
696 ecological and evolutionary perspectives. Takayuki Ohgushi, Oswald Schmitz, Robert D. Holt,
697 Cambridge University Press, pp 339–370

698 Ibanez S, Brun C, Millery A, et al (2021) Litter and soil characteristics mediate the buffering effect of
699 snow cover on litter decomposition. Plant Soil. https://doi.org/10.1007/s11104-020-04803-x

700 Ibanez S, Lavorel S, Puijalon S, Moretti M (2013) Herbivory mediated by coupling between
701 biomechanical traits of plants and grasshoppers. Funct Ecol 27:479–489.
702 https://doi.org/10.1111/1365-2435.12058

703 Jenkinson DS, Brookes PC, Powlson DS (2004) Measuring soil microbial biomass. Soil Biol Biochem
704 36:5–7. https://doi.org/10.1016/j.soilbio.2003.10.002

705 Jones DL, Willett VB (2006) Experimental evaluation of methods to quantify dissolved organic
706 nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol Biochem 38:991–999

707 Kristensen JÅ, Rousk J, Metcalfe DB (2020) Below-ground responses to insect herbivory in
708 ecosystems with woody plant canopies: A meta-analysis. J Ecol 108:917–930

709 Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest Package: Tests in Linear Mixed
710 Effects Models. J Stat Softw 82:1–26. https://doi.org/10.18637/jss.v082.i13

711 Lavorel S, Grigulis K, McIntyre S, et al (2008) Assessing functional diversity in the field –
712 methodology matters! Funct Ecol 22:134–147. https://doi.org/10.1111/j.1365-
713 2435.2007.01339.x

714 Levy EG, Madden EA (1933) The point method of vegetation analysis. N Z J Agric 46:267–279

715 Lovett GM, Ruesink AE (1995) Carbon and nitrogen mineralization from decomposing gypsy moth
716 frass. Oecologia 104:133–138. https://doi.org/10.1007/BF00328577

717 Malik AA, Martiny JBH, Brodie EL, et al (2020) Defining trait-based microbial strategies with
718 consequences for soil carbon cycling under climate change. ISME J 14:1–9.
719 https://doi.org/10.1038/s41396-019-0510-0

720 Malik AA, Puissant J, Goodall T, et al (2019) Soil microbial communities with greater investment in
721 resource acquisition have lower growth yield. Soil Biol Biochem 132:36–39.
722 https://doi.org/10.1016/j.soilbio.2019.01.025

McNaughton SJ (1983) Compensatory plant growth as a response to herbivory. Oikos 329–336

McNaughton SJ, Banyikwa FF, McNaughton MM (1997) Promotion of the cycling of diet-enhancing nutrients by African grazers. Science 278:1798–1800

McNaughton SJ, Ruess RW, Seagle SW (1988) Large Mammals and Process Dynamics in African Ecosystems. BioScience 38:794–800. https://doi.org/10.2307/1310789

Nitschke N, Wiesner K, Hilke I, et al (2014) Increase of fast nutrient cycling in grassland microcosms through insect herbivory depends on plant functional composition and species diversity. Oikos

Parker TC, Sadowsky J, Dunleavy H, et al (2017) Slowed biogeochemical cycling in sub-arctic birch forest linked to reduced mycorrhizal growth and community change after a defoliation event. Ecosystems 20:316–330

Pastor J, Dewey B, Naiman RJ, et al (1993) Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. Ecology 74:467–480

Paterson E, Thornton B, Sim A, Pratt S (2003) Effects of defoliation and atmospheric CO 2 depletion on nitrate acquisition, and exudation of organic compounds by roots of Festuca rubra. Plant Soil 250:293–305

Piton G, Foulquier A, Martínez-García LB, et al (2020a) Disentangling drivers of soil microbial potential enzyme activity across rain regimes: An approach based on the functional trait framework. Soil Biol Biochem 148:107881. https://doi.org/10.1016/j.soilbio.2020.107881

Piton G, Legay N, Arnoldi C, et al (2020b) Using proxies of microbial community-weighted means traits to explain the cascading effect of management intensity, soil and plant traits on ecosystem resilience in mountain grasslands. J Ecol 108:876–893. https://doi.org/10.1111/1365-2745.13327

Potthast K, Meyer S, Tischer A, et al (2021) Grasshopper herbivory immediately affects element cycling but not export rates in an N-limited grassland system. Ecosphere 12:e03449. https://doi.org/10.1002/ecs2.3449

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reich PB (2014) The world-wide 'fast–slow'plant economics spectrum: a traits manifesto. J Ecol 102:275–301

Rhoades DF (1985) Offensive-defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. Am Nat 125:205–238

Ritchie ME, Tilman D, Knops JMH (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. 79:13

Sandén H, Mayer M, Stark S, et al (2020) Moth outbreaks reduce decomposition in subarctic forest soils. Ecosystems 23:151–163

759 Sankaran M, Augustine DJ (2004) Large herbivores suppress decomposer abundance in a semiarid
760 grazing ecosystem. Ecology 85:1052–1061

761 Satterthwaite FE (1946) An approximate distribution of estimates of variance components. Biom Bull
762 2:110–114

763 Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology
764 85:591–602

765 Schmitz OJ (2008) Effects of predator hunting mode on grassland ecosystem function. Science
766 319:952–954

767 Schultz JC, Baldwin IT (1982) Oak Leaf Quality Declines in Response to Defoliation by Gypsy Moth
768 Larvae. Science 217:149–151. https://doi.org/10.1126/science.217.4555.149

769 Singer FJ, Schoenecker KA (2003) Do ungulates accelerate or decelerate nitrogen cycling? For Ecol
770 Manag 181:189–204

771 Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic
772 nutrient acquisition in soil and sediment. Nature 462:795–798.
773 https://doi.org/10.1038/nature08632

774 Stark S, Grellmann D (2002) Soil microbial responses to herbivory in an arctic tundra heath at two
775 levels of nutrient availability. Ecology 83:2736–2744

776 Talal S, Cease AJ, Youngblood JP, et al (2020) Plant carbohydrate content limits performance and
777 lipid accumulation of an outbreaking herbivore. Proc R Soc B 287:20202500

778 Tilman D (1988) Plant strategies and the dynamics and structure of plant communities Princeton. N J
779 Princet Univ Press 26–28

780 Tuomi M, Stark S, Hoset KS, et al (2019) Herbivore Effects on Ecosystem Process Rates in a Low-
781 Productive System. Ecosystems 22:827–843. https://doi.org/10.1007/s10021-018-0307-4

782 Van Der Wal R, Bardgett RD, Harrison KA, Stien A (2004) Vertebrate herbivores and ecosystem
783 control: cascading effects of faeces on tundra ecosystems. Ecography 27:242–252

784 Van Kessel C, Clough T, van Groenigen JW (2009) Dissolved organic nitrogen: an overlooked
785 pathway of nitrogen loss from agricultural systems? J Environ Qual 38:393–401

786 van Wijnen HJ, van der Wal R, Bakker JP (1999) The impact of herbivores on nitrogen mineralization
787 rate: consequences for salt-marsh succession. Oecologia 118:225–231

788 Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial
789 biomass C. Soil Biol Biochem 19:703–707

790 Vittoz P, Guisan A (2007) How reliable is the monitoring of permanent vegetation plots? A test with
791 multiple observers. J Veg Sci 18:413–422

792 Wardle D A, Bardgett Richard D, Klironomos John N, et al (2004) Ecological Linkages Between
793 Aboveground and Belowground Biota. Sci Wash DC 304:1629–1633

794 Wienhold BJ (2007) Comparison of laboratory methods and an in situ method for estimating nitrogen
795 mineralization in an irrigated silt-loam soil. Commun Soil Sci Plant Anal 38:1721–1732

796 Williams A, Langridge H, Straathof AL, et al (2022) Root functional traits explain root exudation rate
797 and composition across a range of grassland species. J Ecol 110:21–33

798 Wood JL, Tang C, Franks AE (2018) Competitive traits are more important than stress-tolerance
799 traits in a cadmium-contaminated rhizosphere: a role for trait theory in microbial ecology.
800 Front Microbiol 9:121

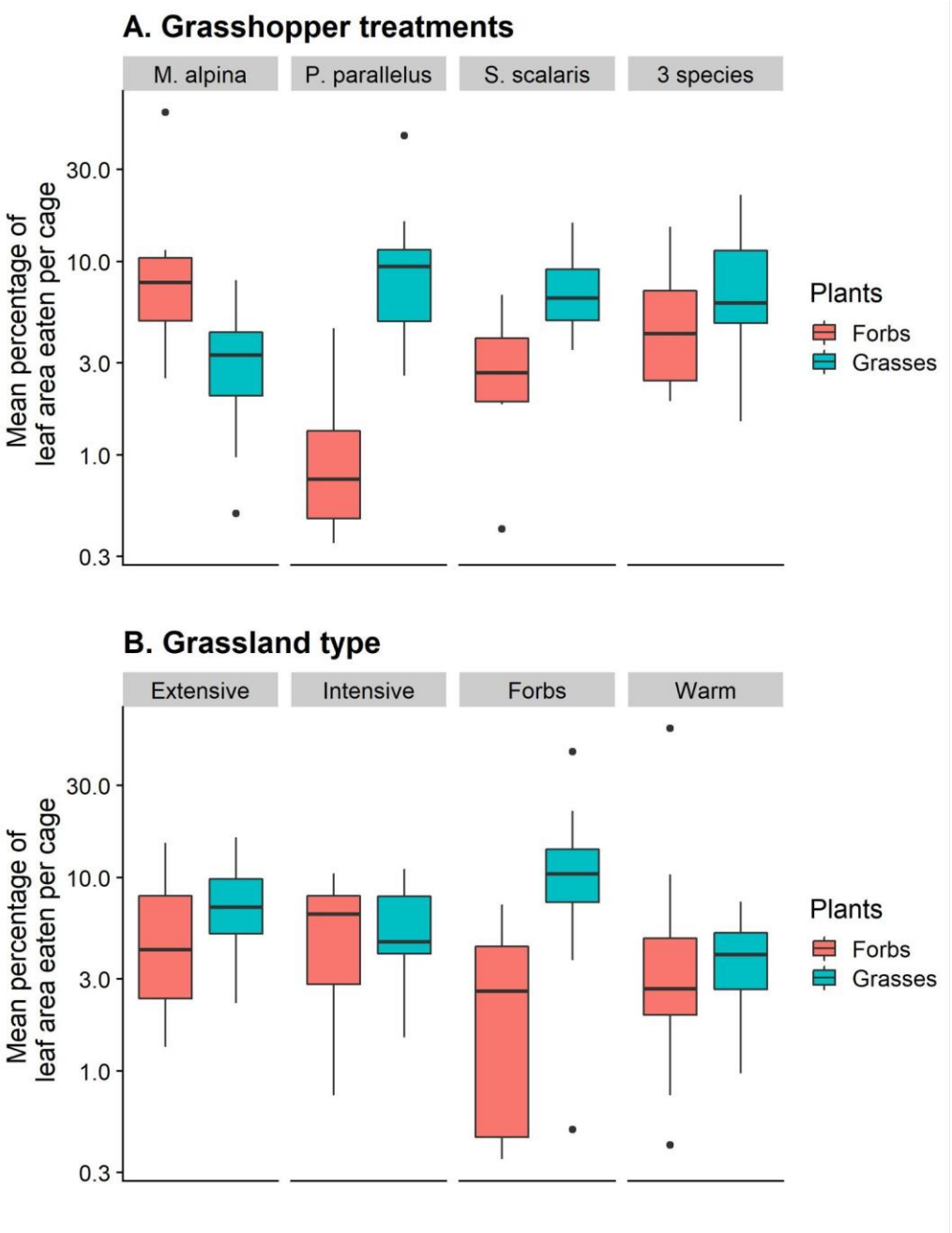
801
802

803

804 # Supplementary Figures & Tables

805 ## Sup. Fig. 1

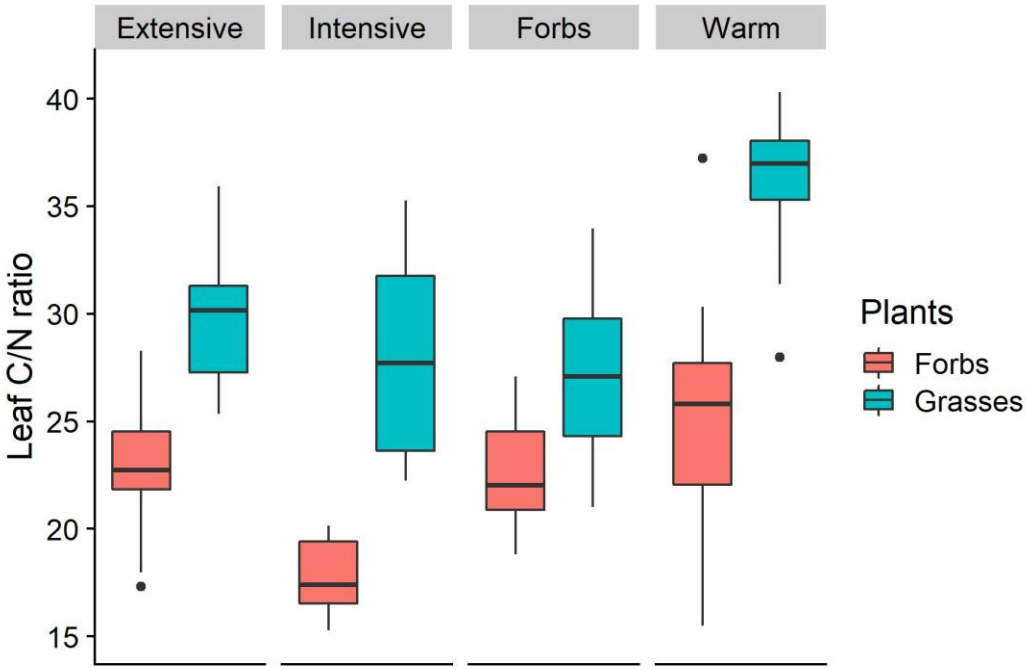
806 Mean percentage of leaf area eaten for each plant functional group, in function of (A) grasshopper
807 treatment, and (B) grassland type. The percentage of leaf area eaten was estimated from
808 observations (mid July 2017) of the dominant plant species in each plot.
809



810

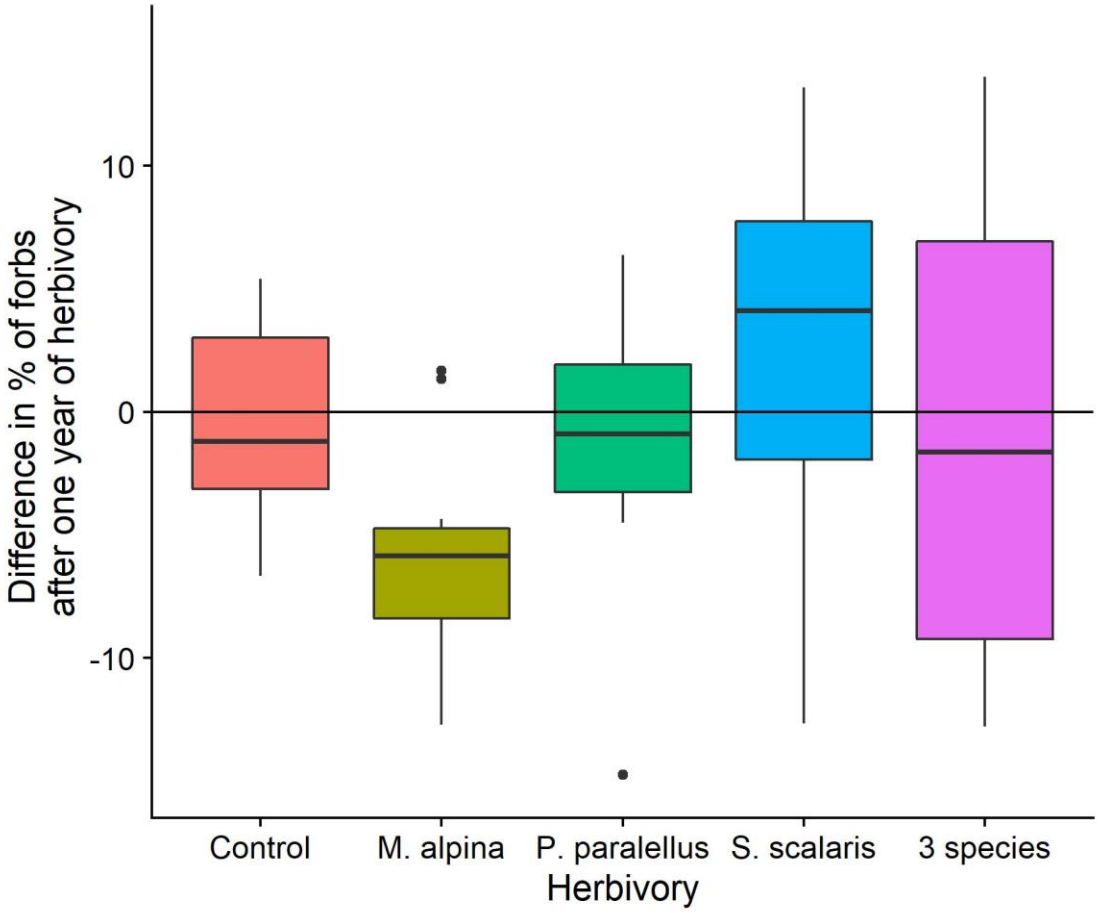
Sup. Fig. 2

Leaf C/N ratio of forbs and grasses in the four grassland types.



Sup. Fig. 3

Variation of the percentage of forbs estimated by the point quadrat method after one year of herbivory, in function of the herbivory treatment.



Sup. Tab. 1

Mean±sd of each of the 12 sites (with 5 plots per site). SWC: Soil water content (%), SOM: Soil organic matter (%), TDN: Total dissolved nitrogen (µg/g), PNM: Potential nitrogen mineralization (µgN/g dry soil/day), MBN: Microbial biomass nitrogen (µg/g), EEN: Nitrogen-related enzymes (nmol activity/ g dry soil/h), EEC: Carbon-related enzymes, EEP: Phosphorous-related enzymes, Biomass: Plant biomass (g/m²), N_B: Nitrogen in aboveground plant biomass (g/m²), Forbs: Forb biomass (g/m²). Geographical coordinates are also given.

	Extensive prairies			Intensive prairies			Forb-dominated grasslands			Warm grasslands		
	EX1	EX2	EX3	IN1	IN2	IN3	MG1	MG2	MG3	BR1	BR2	BR3
SWC	24.6 ± 2.3	27.3 ± 2.5	24.1 ± 1.6	21.2 ± 2.2	19.9 ± 3.4	33.7 ± 1.8	30.5 ± 0.6	29.1 ± 1.3	35.6 ± 1.2	45.9 ± 2.3	27.3 ± 2.2	26.6 ± 4.9
SOM	9.09 ± 1.11	9.55 ± 0.58	8.07 ± 0.39	8.45 ± 0.63	6.35 ± 0.61	12.7 ± 0.6	10.8 ± 0.9	9.53 ± 0.57	15.4 ± 1.3	32.6 ± 4	15.9 ± 1.8	14.8 ± 2
pH	5.21 ± 0.33	5.14 ± 0.1	5.04 ± 0.04	6.24 ± 0.31	4.98 ± 0.03	7.07 ± 0.1	4.15 ± 0.07	7.83 ± 0.1	6.45 ± 0.64	6.51 ± 0.16	7.69 ± 0.29	7.89 ± 0.09
TDN	31.6 ± 3.5	44.8 ± 3.5	42.2 ± 2.7	19 ± 1.1	48.5 ± 8.2	38.3 ± 3.2	36.5 ± 3	26 ± 1.9	46.5 ± 18.6	98.3 ± 23.1	46.3 ± 8	43.3 ± 8.5
Soil C/N	18.2 ± 2.3	18.2 ± 1.3	18.2 ± 2	23 ± 6.4	21.5 ± 5.6	15.4 ± 1.3	25 ± 10	23.3 ± 3.1	19 ± 3.4	16.7 ± 3.8	30.5 ± 13.3	27 ± 9.8
PNM	8.84 ± 5.41	9.76 ± 2.24	11.4 ± 4.9	9 ± 2.27	8.16 ± 1.51	8.14 ± 3.68	6.43 ± 1.45	4.72 ± 1.4	10.1 ± 1.6	28.2 ± 8.8	15 ± 3.8	16.6 ± 4.9
MBN	42.8 ± 5.6	47.7 ± 5	39.9 ± 4	49.8 ± 2.6	28.8 ± 6.9	68.3 ± 3.9	41.6 ± 18.8	54.8 ± 3.8	60.3 ± 4.7	43.9 ± 22.7	29.7 ± 14.7	50.2 ± 9.5
EEN	673 ± 68	466 ± 48	337 ± 65	451 ± 147	336 ± 50	728 ± 382	228 ± 44	1356 ± 136	637 ± 100	1155 ± 132	1155 ± 459	1429 ± 254
EEC/EEN	447 ± 52	351 ± 88	262 ± 79	511 ± 196	352 ± 98	742 ± 333	279 ± 78	483 ± 92	390 ± 130	1038 ± 705	593 ± 369	481 ± 128
EEP	1165 ± 229	1248 ± 236	1131 ± 190	838 ± 254	742 ± 125	480 ± 116	1505 ± 167	105 ± 40	651 ± 186	1827 ± 210	164 ± 127	135 ± 32
EEC/EEN	0.67 ± 0.1	0.75 ± 0.17	0.77 ± 0.1	1.12 ± 0.08	1.03 ± 0.17	1.04 ± 0.14	1.25 ± 0.34	0.35 ± 0.04	0.6 ± 0.12	0.86 ± 0.47	0.7 ± 0.81	0.34 ± 0.06
Biomass	388 ± 66	464 ± 26	465 ± 84	491 ± 89	645 ± 129	702 ± 110	440 ± 31	225 ± 47	412 ± 48	351 ± 98	391 ± 82	274 ± 55
Leaf C/N	28.1 ± 2.4	27.3 ± 2.4	28.7 ± 3.4	23.1 ± 2.2	27.8 ± 5.2	31.3 ± 2.3	23.8 ± 1.8	25.8 ± 1.9	20.5 ± 1.1	32.9 ± 3.8	34.4 ± 2.2	40.6 ± 3.8
N_B	7.35 ± 1.31	9.33 ± 0.74	8.5 ± 1.88	11.4 ± 2.6	12 ± 2.2	11.3 ± 2	9.78 ± 0.92	4.19 ± 1.03	9.94 ± 1.58	6.2 ± 2.44	6.34 ± 1.97	3.47 ± 0.62
Shannon	1.8 ± 0.16	1.69 ± 0.19	1.66 ± 0.2	2.37 ± 0.09	1.46 ± 0.2	1.98 ± 0.13	1.96 ± 0.21	2.28 ± 0.27	2.68 ± 0.03	2.07 ± 0.53	1.63 ± 0.2	0.91 ± 0.41
Forbs	161 ± 44	167 ± 58	126 ± 51	123 ± 70	36.8 ± 37.6	4.69 ± 3.31	369 ± 47	203 ± 47	376 ± 47	64.5 ± 47.9	118 ± 42	37.7 ± 33.3
Lat	45.1589114	45.1665751	45.1590965	45.1509678	45.15276	45.1630496	45.2170777	45.1687254	45.2172083	45.2185605	45.1690667	45.1693922
Long	5.52397504	5.53694229	5.53318494	5.55091331	5.52717685	5.52653451	5.55924632	5.56912743	5.55883236	5.56011453	5.56860468	5.56848548