Is plant biomass input driving soil organic matter formation processes in grassland soil under contrasting management?

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Abstract

Grassland management practices vary in intensity (stocking rates, fertilization) and plant removal strategies (grazing versus mowing). They influence organic matter inputs, which were postulated as main controls of soil organic carbon (SOC) sequestration and might therefore control SOC stabilization. The aim of this study was to test this hypothesis by investigating the impacts of grassland harvesting regimes on parameters related to soil organic matter (SOM) formation processes along a plant biomass input gradient. We used a thirteen-year experiment in Central France under contrasting management (unmanaged, grazing with two intensities, mowing, bare fallow), established a plant biomass input gradient based on biomass leftover after harvest and investigated microbial functioning, necromass accumulation, organic matter degradation and SOM accumulation processes. Our experimental approach included soil analyses for amino sugars, microbial biomass C and N, basal respiration, seven enzyme activities and microbial growth kinetics as indicators of microbial functioning and degradation processes.

Response of the parameters to plant biomass input along the gradient could be arranged into four groups. Amino sugar content and microbial C/N ratio showed a linear response. SOC content and SOC-dependent parameters (enzyme activities, active microbial biomass) showed a bell-like response, most likely reflecting input diversity. Microbial activity showed a ripple-like response to decreasing biomass input indicating its dependence on physiochemical conditions and root biomass, while GluN/GalN and qCO_2 were not influenced by grassland harvesting regimes. These results indicate that grassland harvesting management influences microbial activity and functioning not only by changing plant biomass input, but also through its effects on soil (microbial) functioning possibly related to changing physiochemical soil properties. We conclude that management controls SOM formation through its indirect impacts on belowground (microbial) processes in addition to modifying plant biomass inputs.

1. Introduction

Globally, grasslands have a soil organic carbon (SOC) sequestration potential of about 0.2 Gt C $year^{-1}$ and may thus serve as a net sink for atmospheric CO_2 (Conant et al., 2001; Lal, 2004; Randall, 2022) depending on their management (Smith et al., 2016; Whitehead et al., 2018;). Therefore, detailed assessment of management effects is necessary as any agricultural activity can induce positive or negative feedbacks in terms of plant productivity and biogeochemical carbon (C) cycling in grassland systems (Schipper et al., 2017; Smith et al., 2008). In general, managed grasslands are subjected to two contrasting harvesting regimes - grazing and mowing – interacting differently with soil organic matter (SOM) dynamics and microbial functioning (Gilmullina et al., 2020).

Both harvesting regimes lead to continuous carbon removal and also disturbance of soil processes and biogeochemical cycling, through their impact on abiotic (e.g. soil compaction) and biotic soil processes (e.g plant activity). Differences between grazing and mowing are related to the complex relationships between abiotic factors and the quality and quantity of aboveground and belowground litter and their effects on SOM dynamics (Liu et al., 2014; Koncz et al., 2015). In addition to this direct impact on the C cycle, grassland management influences the plant community composition (Louault et al., 2005; Nerlekar and Veldman, 2020), plant physiological traits (Niu et al., 2016) and plant litter composition (Gilmulina et al., 2021; Wang et al., 2022). In particular, defoliation activity and contrasting nutrient sources under grazing and mowing may alter aboveground and belowground input quality (Alber et al., 2014; Bardgett et al., 1998). Light and moderate grazing were reported to enhance belowground C allocation, which in turn promotes microbial functioning and increases SOC contents (Hewins et al., 2015; Wilson et al., 2018), while heavy grazing decreases the SOC content (Han et al., 2008; Wang et al., 2017). The cessation (abandonment) of grazing sites with reduced grazing intensity was reported to cause SOC losses (Peco et al., 2017, 2006) and to decrease microbial metabolic efficiency (Aldezabal et al., 2015), whereas after heavy grazing cessation soil may be improved and thus recover within times of about 25 years under a cold continental climate (Steffens et al., 2008). There are only few studies

74 comparing management practices under similar climatic conditions (Franzluebbers and
75 Stuedemann, 2009; Liu et al., 2014). Recently it was shown that both systems may have contrasting
76 effects on soil nutrient dynamics (Sun et al., 2023; Wang et al., 2022; Liu et al., 2023). Light
77 grazing and mowing (with N addition), may promote C sequestration, however, light grazing can
78 lead to more efficient microbial functioning and thus to higher C sequestration (Gilmullina et al.,
79 2020).

80 Grassland harvesting regimes thus affect microbial degradation processes in soil via their
81 influence on microbial activity (Chuan et al., 2020; Millard and Singh, 2010; Oates et al., 2012;
82 Sayer et al., 2013; Koncz et al., 2015). During plant litter degradation, microorganisms not only
83 produce CO_2 but they also convert plant-derived C into microbial biomass, which can be further
84 stabilized in soil (Liang et al., 2017; Ma et al., 2018). Whereas, the effect of land-use management
85 on microbial SOM degradation processes is broadly studied (Ali et al., 2018; Cui and Holden, 2015;
86 Xu et al., 2017), microbial SOM formation has received less attention (Liang et al., 2016;
87 Kallenbach et al., 2016).

88 While management affects aboveground biogeochemical cycling *directly* through altering
89 aboveground biomass input, and *indirect* through its belowground impact by altering habitat and
90 substrate availability for soil microbial communities. Both processes may affect SOC sequestration
91 through their impact on SOM formation. However, in the recent literature, carbon input was
92 identified as the main control of SOC sequestration (Fujisaki et al., 2018; Chenu et al., 2019),
93 although SOC stabilization is most likely controlled by microbial necromass formation and turnover
94 (Bhattacharyya et al., 2022). Therefore, in this study, we investigated different grassland
95 management practices (mowing and two different grazing intensities), which we conceptualized as
96 a gradient in terms of plant biomass input. In accordance with the recent literature, we hypothesized
97 that management intensity through its impact on plant-derived organic matter (OM) input controls
98 the processes leading to SOM formation. We aimed to investigate SOM formation by using this
99 gradient because investigation of changing (soil) properties along a gradient may have more power
100 to elucidate mechanisms than investigation of contrasting differences between the treatments

(Kreyling et al., 2018). We used a combination of microbial and biogeochemical analysis, enabling to identify simultaneously microbial functioning, plant litter degradation products and microbial residues.

We examined a thirteen-year experiment in temperate climate with four treatments including (1) unmanaged, (2), high grazing intensity, (3) low grazing intensity, (4) mowing and (5) bare fallow. These different treatments represent a plant biomass input gradient, which we established based on aboveground biomass removal. We investigated SOC content, pH, and amino sugars composition. Microbial functioning was characterised by microbial biomass C and N content, fraction of active microorganisms, specific growth rate, basal respiration, metabolic quotient (qCO_2) and specific enzyme activities. We hypothesised that SOC content would decrease linearly along the input gradient together with microbial activity leading to decreased enzyme activities and increased qCO_2 with decreasing plant C input.

2. Materials and methods

2.1. Site description

The study was conducted at the long-term experimental observatory (SOERE) Agroecosystems, Biogeochemical Cycles and Biodiversity (ACBB) in western France. The site is characterized by semi-continental climate with mean annual temperature of 8.7 °C and mean annual precipitation of 770 mm. Before the start of the experiment in 2005, the grassland management at the site was a mixed regime of fertilized mowing and grazing systems. The soil type at the site is Eutric Cambisol developed on granitic bedrock. Each grassland management practice was established on two blocks giving two field replicates. The initial soil general parameters before the beginning of experiment are presented in the table 1.

We focused on three grassland management practices (low and high intensity cattle grazing and mowing) representing a plant-derived OM input gradient, and two endmembers (unmanaged site and bare fallow). At the unmanaged site, all plant biomass is returned to soil and this treatment may, thus, be considered as a positive control in terms of plant-derived OM input. In grazing and

mowing systems, plant biomass is exported at increasing levels. As a negative control in terms of plant-derived OM input we considered bare soil. On plots under mowing, there were three cutting events per year and NPK fertilization to replace nutrients following aboveground plant biomass removal (264 kg N ha^{-1}, 33 kg P ha^{-1} and 189 kg K ha^{-1}, applied in 3 splits for N, early spring, after the first and the second cuts and in 2 first splits for P and K). Under grazing treatments, plots were rotationally grazed during the same times five times per year, with a full (high intensity grazing) or partial (low intensity grazing) utilization of the grassland resulting from a modification of the stocking density (respectively 13.8 and 6.9 LSU ha^{-1}) but having the same duration of grazing. On unmanaged plots, no agricultural activity took place since 2005. In addition, a bare fallow plot was considered, where vegetation was removed in 2005 and kept clean since then. Each treatment was replicated twice (2x4 = 8 plots) except bare soil with only one plot, and having a plot size of 2200 m^2 (for grazing treatments), 400 m^2 (for unmanaged and mowing treatments) and 30 m^2 for bare soil by mechanical means.

The grassland management practices may be placed along a carbon input gradient based on aboveground plant biomass input, which represents the leftover after harvest (APBL) (Table 2). The level of APBL presents plant material (i.e. shoots, stubble, litter), which is left after harvesting events (grazing and mowing). APBL is the difference between the aboveground net primary production (ANPP) and the biomass removed by grazing or mowing. It is important to note that there is also loss of plant biomass under mowing occurring during mechanical removal, which may comprise up to 20% of plant biomass. We consider this loss equal to the losses during grazing by trampling without passing through livestock digestion system (about 30%) (Sanaullah et al., 2010), thus, these losses are not included in our calculations. ANPP, in g DM m^{-2} $year^{-1}$, is the sum of the successive biomass accumulation along the year. The ANPP was measured for all grazing and mowing treatments. For the unmanaged treatment, we used the assumption that ANPP is equal to ANPP of low intensity grazing (Damien et al., 2015; Wu et al., 2019). For grazing and mowing treatments, the ANPP was measured on four 0.6*0.6 m plots. The biomass was determined after cutting at a height of 5.5 cm five times per year in grazed plots (i.e. at the beginning of each grazing

event) and three times per year at each harvest in mowing plots. At the beginning of each vegetation period, the residual standing biomass was removed in the sampling plots and in addition in the grazed plots, a fence was placed to avoid animal defoliation. The harvested biomass was estimated in grazed plot based on the daily animal intake, which was calculated according to animal weight and the number of animal grazing days per year per plot. In mowed plots, harvested biomass was calculated based on the harvested forage yield. For unmanaged and bare fallow treatments, the harvested biomass was set to zero (0). According to APBL we conceptualized the plant biomass input gradient in the order of **Unmanaged > low intensity grazing > high intensity grazing > mowing > bare fallow** (Fig.1).

2.2. Soil sampling

In late October 2018, soil was sampled at each plot replicate at 3 points (about 10 m apart) resulting in six replicate samples per treatment except for bare soil. For the bare fallow treatment, we sampled soil only at 2 points (n=4) because the plot size was not enough large and the soil is more homogeneous due to plant removal. The plot effects and the unbalanced design were accordingly considered during statistical analysis. Soil samples were collected with a mechanical auger (8cm Ø, 10 cm) at 0-10 cm. In the laboratory, fresh soil samples were sieved at 2 mm and split into two fine soil subsamples: i) a subsample for physico-chemical analysis (air-dried), and ii) a subsample for microbial analyses (stored at 4°C). Prior to microbial analysis, soil samples were pre-incubated at 22 °C for 7 days.

2.3. Soil general properties

Soil pH (H_2O) was measured in a soil:water suspension (1:2.5 weight/volume). SOC, nitrogen (N) and stable isotope (^{13}C and ^{15}N) contents were measured with a CHN auto-analyzer (Flash EA, Thermo Electron Corporation, Bremen, Germany) coupled with an isotope ratio mass spectrometer. The isotopic ratios were calculated relative to the Pee Dee Belemnite Standard (PDB) for C and relative to atmospheric N_2 for N.

2.4. Amino sugar signature

Amino sugars were extracted from soil following a method proposed by Zhang and Amelung (1996). Soil samples were hydrolized with 6 M HCL at 105 °C for 8 h. After the acid was evaporated, samples were purified by 1 M KOH addition and centrifugation. The supernatant was freeze-dried and afterwards amino sugars were extracted by anhydrous methanol. Derivatisation to aldononitrile acetates was performed by a derivatisation reagent consisting of 32 mg ml^{-1} hydroxylamine hydrochloride and 40 mg ml^{-1} 4-(dimethylamino) pyridine in pyridine-methanol (4:1 v/v) for 30 min at 75–80 ˚C. Samples were then reheated for 30 min after adding 1 ml of acetic anhydride. Remaining derivatization reagents were removed by three washing steps with dichloromethane, 6 M HCl and deionised water. The organic phase was then dried under N_2 and dissolved in ethyl acetate-hexane (1:1). Thereafter, 15 µg of the IS 2 tridecanoic acid methyl ester (1 µg $µl^{-1}$) in ethyl acetate-hexane (1:1) were added.

Aminosugars were analysed gaschromatographically with a gaschromatograph (Agilent 7890 A) coupled to an Agilent 7000 A triple quadrupole mass spectrometer (Agilent, Waldbronn, Germany). The compounds were separated on a 30 m OPTIMA® 17 column (phenylmethyl polysiloxane, 50% phenyl, 0.25 mm I.D., 0.50 µm film thickness; Macherey-Nagel, Dueren, Germany). Helium was used as the carrier gas with a flow rate of 1.1 ml min^{-1}. The temperature of the GC oven was programmed at 120°C (isothermal) held for 1 min, then increased to 250°C at the rate 5°C min^{-1} and held for 2 min, then increased to the final temperature 280°C at the rate 10°C min^{-1} and held for 10 min (Banfield et al., 2017).

2.5. Biological properties

Soil microbial biomass C (MBC) and N (MBN) were determined by the chloroform fumigation extraction method (Vance et al., 1987). Dissolved organic C and N in fumigated and non-fumigated soil samples was extracted in 0.05 M K_2SO_4 and were measured using a multi C/N analyzer (multi C/N analyser 2100S, Analytic Jena). MBC and MBN were calculated with a conversion factor of 0.45 (Jenkinson et al., 2004).

Microbial growth kinetic parameters were estimated by using soil respiratory response to unlimited nutrient amendments (Panikov and Sizova, 1996). For this purpose, soil samples were treated with a solution (0.1 ml per g of dw soil) containing per g soil: 10 mg glucose, 1.9 mg $(NH_4)_2SO_4$, 3.8 mg $MgSO_4*7H_2O$, 0.11 mg K_2HPO_4 and 1.68 mg KH_2PO_4. The proportions of K_2HPO_4 and KH_2PO_4 were adjusted in order to avoid soil pH change of more than 0.1 units after addition. The calculation of active microbial biomass (AMB) and specific growth rate was based on the results of the substrate induced respiration rate fitted with a model proposed by Panikov and Sizova 1996; Wutzler et al., 2012:

$$CO_2(t) = A + B * \exp(\mu * t) \quad (1)$$

Extracellular enzyme activity was measured using the fluorometric technique (Koch et al., 2007; Marx et al., 2005; Razavi et al., 2015). Seven types of fluorogenic substrates based on 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) were used: (1) MUF-α-D-glucopyranoside for α-glucosidase, (2) MUF-β-D-glucopyranoside for β-glucosidase, (3) MUF-β-D-xylopyranoside for β-xylosidase, (4) MUF-β-D-cellobioside for β-cellobiohydrolase, (5) MUF-N-acetyl-β-D-glucosamide for chitinase, (6) Leucine-AMC for leucine aminopeptidase and (7) MUF-phosphate for phosphatase. Saturation concentrations of fluorogenic substrates were determined in preliminary experiments and comprised 20 μmol g^{-1} soil for all enzymes except lipase with 60 μmol g^{-1} soil. Briefly, a water extract of soil (1:10) was homogenised by low-energy sonication (40 J s^{-1} output energy) for 60 s. Thereafter, 50 μl of the soil suspension were added to 150 μl of each substrate solution in a 96-well microplate. Fluorescence was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm (Victor3 1420-050 Multilabel Counter, PerkinElmer, USA).

2.6. Statistical analysis

All results are presented as arithmetic means with standard error. The statistical analyses were conducted by using R software (Studio Version 1.1.447). We identified significant differences ($P<0.05$) of studied parameters between samples using ANOVA based on Type II sums of squares with Tukey test due to unbalanced experimental design. Treatments and plots were placed as fixed

effects in the ANOVA model. The equation (1) was fitted by non-linear regression, using Model Maker-3 software (SB technology Ltd.). To reveal the treatment effects, non-transformed data were subjected to Principal Component Analysis (PCA).

3. Results

3.1. Soil general properties and amino sugars

Root biomass ranged between three to nine t ha^{-1}. It was lowest under low intensity grazing (LGraz) and highest under mowing (MOW) (Table 3). Soil pH ranged between 5.2 and 5.9 and it was the highest at the unmanaged (UM) and decreased in the order: high intensity grazing (HGraz)=LGraz> (Mow)>Bare fallow (Bare). SOC content was similar under all treatments even though, it tended to be the highest under LGraz. Absence of vegetation (Bare) resulted in 15-46% lower SOC content compared to the other treatments. The C:N ratio was about 11 under UM, LGraz and Mow, whereas it was slightly lower under HGraz and Bare. All managed sites together with unmanaged site showed lower ^{13}C enrichment compared to Bare. Across the managed treatments, ^{15}N was less enriched under LGraz and Mow compared to UM. Bare soil was also enriched in ^{15}N.

Amino sugars contents were the highest under UM followed by lower values under managed sites. The lowest amino sugars contents were observed for bare soil. Amino sugar content per SOC did not differ significantly among all treatments. The ratio of glucosamine to galactosamine (GlcN/GalN) was lowest under UM but there was no difference among other managed treatments and bare soil.

3.2. Microbial functioning and degradation processes

MBC varied between 304 and 1314 μg g^{-1}. It was the highest under UM, followed by similarly lower values for HGraz≥LGraz=Mow (Fig. A). MBC per SOC (MBC mg g^{-1} SOC) and basal respiration (mg CO_2-C g^{-1}) followed a similar pattern as MBC (Fig. 2, 3A). Microbial C:N ratio did not differ between UM and grazing treatments but it increased under Mow and Bare (Fig. 2C). Basal respiration per SOC was the highest under UM (Fig. 3B). The metabolic quotient (qCO_2) did not differ among managed and unmanaged treatments but the highest value was recorded under

bare soil (Fig. 3C). The AMB represented 0.5-1.1% of MBC. It decreased in the order Mow=HGraz≥LGraz≥UM=Bare (Fig. 4A). Specific growth rate μ ranged between 0.15 and 0.24 h^{-1} and was highest under Bare and UM (Fig. 4B).

C-cycle enzymes followed a similar pattern as the SOC contents but showed significant differences between treatments (Fig. 5A). The lowest enzyme activities among managed practices were observed for Mow. Only xylosidase activity was not sensitive to any treatment. Leucine aminopeptidase activity was not affected by any grassland management. The highest phosphatase activity was observed under grazing treatments. The highest enzyme activity per MBC was under LGraz and Bare for all enzymes except leucine aminopeptidase (Fig. 5B). Leucine aminopeptidase activity per MBC was similar among all treatments.

3.3. Response to biomass input

Our results indicated that the biomass input gradient resulted in 5 different kinds of response form (Fig. 6): negative or positive linear, bell-like or reverse bell-like, ripple-like, and specific response based only on the presence/absence of disturbance.

Only amino sugars content and microbial C:N ratio followed a *linear pattern*, which was negative for amino sugars and positive for microbial C:N, i.e. amino sugars decreased with increasing plant biomass input, while microbial biomass C:N increased. Total C and N content, specific growth rate, relative AMB and absolute enzyme activity followed *bell-like form* (or the reverse bell-like form) with highest values for LGraz. pH along with microbial parameters such as MBC, basal respiration and specific enzyme activity had *a ripple-like* form with two peaks for UM and HGraz treatments. Additionally, root biomass response also demonstrated ripple-like form but with peaks at LGraz and Mow. The GluN/GalN ratio was significantly differentiated from other treatments only for UM, whereas the metabolic coefficient (qCO_2) showed significant differences from other treatments only for bare fallow soil.

3.4. Principal component analysis

Principal component analysis enabled separation into three groups: UM, Bare and managed sites all together (HGraz, LGraz and Mow) (Fig. 7A). The separation of control sites from grazed

287 and mowed sites was related to enrichment of ^{13}C and ^{15}N isotopes, higher specific growth rate and
288 lower percentage of AMB. UM and Bare were differentiated by SOC and N concentrations, MBC
289 and C-cycle enzyme activities, which showed higher values under UM. To eliminate the dominance
290 of the PCA by control treatments and thus to check if there is a differentiation among managed
291 sites, we applied PCA with exclusion of UM and Bare (Fig. 7B). There was a clear separation of
292 grazed and mowed sites. Mow was separated from LGraz and HGraz treatments by lower pH, SOC
293 and N contents and lower enzyme activities. Grazing treatments were also separated: HGraz was
294 characterised by higher basal respiration and MBC compared to LGraz.
295
296 **4. Discussion**
297 The study sites were chosen because we hypothesized changes in soil biogeochemical and
298 microbial properties due to management activities. Indeed, PCA analyses showed the sites could be
299 differentiated according to the management effects on biogeochemical and microbial parameters. In
300 particular, differences between mowing and grazing treatments in agreement with another study
301 were driven by contrasting pH, enzyme activities and microbial C:N ratios similar to finding
302 (Gilmullina et al., 2020). We further hypothesized gradual changes of soil biogeochemical and
303 microbial properties due to reduction of plant biomass input because it may be directly related to
304 microbial response and SOM dynamics (Bardgett et al., 1998; Lal, 2002). According to our
305 hypothesis, the biomass input gradient based on the APBL should increase linearly in the order:
306 Bare<Mowing<HGraz<LGraz<UM. However, our result indicated five different response curves:

307 *4.1. Properties linearly depending on plant biomass input*

308 Amino sugar content and microbial C:N ratio showed linear increase with increasing plant
309 biomass input. The site without plant biomass input (bare soil) showed lowest amino sugar content,
310 indicating lowest amounts of microbial residues in soil (Joergensen, 2018). This may most probably
311 be explained by the absence of organic substrates triggering the microbial community to use another
312 source of energy and nutrients (Ding et al., 2017). The amino sugar contents in soils may thus be
313 dependent on plant biomass input, which may provide N as well as C substrates for microbial

activity. High plant-derived C input at the UM site may thus have led to increased microbial biomass resulting in intense microbial residue formation while absence of plant input in bare plots led to their degradation. Despite the gradual increase of amino sugars along the input gradient, the differences between managed grasslands were insignificant, indicating that contrasting management had little impact on this parameter. This might be related to the short time (13 years) of the experiment and to the fact that management effects on soil under similar land use are small. Even after land use change, 6 years were necessary to detect the accumulation of microbial residues (Ding et al., 2011). Similar results in other managed grassland soils indicate that neither the nature of input (plant or animal) (Liang et al., 2007) nor plant diversity (Liang et al., 2016) had a strong effect on amino sugar content.

The positive linear relation of microbial C:N ratio along the disturbance gradient indicated that the decrease of plant input into soil resulted in the starving status of microorganisms or in the selection of microorganisms with slow growth strategies. The absence of differences between unmanaged and grazing treatments indicated that this parameter was only affected by the input quantity but not by its nature. UM and grazing treatments showed similar microbial C:N ratio due to sufficient organic matter input into soil, whereas N was lacking in soil under bare and under Mow despite mineral fertilizer input. This could favor fungal communities, which may be more sensitive to management than bacterial communities (Praeg et al., 2020).

4.2. A bell-like or reverse bell-like form of response along the disturbance gradient

A bell-like response curve along the disturbance gradient was observed for SOC and N content, absolute enzyme activity and the relative proportion of active microbial biomass and the specific growth rate, which followed a reverse bell-like form. It was interesting to note that positive and negative controls presented by UM and Bare did not differ. Both treatments present quite stable systems characterised by either continuous presence or absence of plant litter input.

As the input gradient was established based on differences of aboveground plant biomass input, the bell-like form is most likely explained by belowground biomass input diversity. The top of the “bell” under LGraz may indicate that different input types such as: dung input, root activity

(Shen et al., 2020), slog-off cells and decaying root debris (Berhongaray et al., 2019) could be the reason of higher SOC content and SOC-dependent parameters (Bazot et al., 2005; Shen et al., 2020). Our results are in agreement with studies on grazing exclusion, which was shown to shift to lower belowground C allocation, consequently, decreasing total SOC (Sokol and Bradford, 2019; Wilson et al., 2018). In bare soil, the absence of input coupled with ongoing decomposition will result in continuous loss of SOC (Barré et al., 2010). We therefore suggest that all these bell-like response parameters, were not dependent on the (aboveground) plant input but could reflect total input diversity.

4.3. A ripple-like form of response to disturbance gradient

The pH, MBC, basal respiration and specific enzyme activity followed the ripple-like form, whereas root biomass showed the opposite pattern, indicating that these soil properties were more related to root biomass rather than to APBL. However, the relationship of pH, MBC, basal respiration and specific enzyme activity with root biomass was negative. It was surprising to find higher root biomass negatively influenced the specific enzyme activity supporting the idea that high exudation provides easily-available substrates for the selected groups of microbial community (Esperschütz et al., 2009; López-Guerrero et al., 2013). Probably, this selection could be also an explanation of MBC decrease under high root biomass.

The presence of animals and fertilisation, which were not considered in our framework could also influence pH (Aciego Pietri and Brookes, 2008; Steffens et al., 2008), which in turn affects MBC and basal respiration. Even if it is quite complicated to estimate the amount of total input in the UM and HGraz treatments, probably, high dung input under HGraz could compensate aboveground biomass removal and maintain MBC and basal respiration at the same level as in UM. High dung input activates microbial activity increasing decomposition processes (Bol et al., 2003), however, substrate degradation processes might be directed to labile dung compounds rather than SOM. Whereas, low pH under mowing and bare soil might be an explanation for the lower MBC (Aciego Pietri and Brookes, 2008; Weigand et al., 1995).

Specific enzyme activity (enzyme activity per MBC) also showed a ripple-like response with highest values for LGraz and bare soil. It is not surprising to observe high specific enzyme activity in bare soils, in response to lack of available nutrient for soil microorganisms (Guenet et al., 2010). It is more interesting that specific enzyme activity was also high under low intensity grazing. This might be explained by small amounts of dung and urine input, which stimulated only few microorganisms and was not enough to maintain the large fraction of microbial population at active state. It's still surprising that specific enzyme activity remained high because it could have indicated SOM degradation and consequently SOC content decline (Gilmullina et al., 2020), but SOC content was highest under LGraz. Probably, in this case high specific enzyme activity only demonstrated the microbial strategy investing energy in enzyme production rather than in biomass production (Sauvadet et al., 2018). Thus, the metabolic activity of the microbial community was not following the input gradient, which we established based on aboveground biomass and may not be directly related to management intensity measured by APBL.

4.4. Properties, which were responding only to control treatments

The GlcN/GalN ratio indicating bacteria and fungal contribution (Joergensen, 2018) responded to the presence of harvesting activities but was not influenced by biomass removal intensity. Our results were supported by a study showing that arable land restoration by pasture installation resulted in decrease of galactosamine (Lauer et al., 2011). Predominantly fungi-derived galactosamine was demonstrated to be more resistant to degradation compared to bacteria-derived components (Dippold et al., 2019; Gunina et al., 2017), thus, lower GlcN/GalN under UM could also be explained by higher fungal residue contribution in the unmanaged system. In addition, it seems that any long-term management activities decreases GlcN/GalN: higher GlcN/GalN was found under undisturbed soil used as control compared to treatments receiving N addition or climate change simulation (Liang et al., 2015). However, it was earlier proposed that this ratio could represent amino sugars accumulation (Joergensen, 2018; Liang et al., 2015). In our case it is tricky to claim the same. We would expect that galactosamine would increase under grazing decreasing

GlcN/GalN because cow dung contains much more galactosamine compared to different plant materials (Jost et al., 2011).

In contrast, the metabolic quotient qCO_2 was sensitive only to the absence of any kind of input: highest value under bare soil treatment indicated low efficient metabolism of fast growing microorganisms and was mainly driven by belowground C allocation. However, the metabolic quotient qCO_2 is known to be a representative and sensitive indicator of soil health (Okolo et al., 2020), in our case this property was not sensitive and did not reflect the differences between grassland management practices. This could be explained by the fact that grassland management practices did not have a very strong effect on qCO_2 as compared to more destructive agricultural management practices e.g. overgrazing or tillage systems (Kooch et al., 2020; Pabst et al., 2016) and could maintain their soil health due to less destructive management.

5. Conclusions

We analyzed the effect of management intensity related to different harvesting regimes of grassland soil on physicochemical, biogeochemical and microbial soil parameters. Our results indicated that pH, microbial C:N ratio and enzyme activities could differentiate different soil management practices. The response forms of the different parameters along a plant aboveground biomass input gradient allowed us to identify their controls. Only amino sugar content and microbial C:N ratio showed a linear pattern and were thus directly dependent on the plant biomass input indicating its relationship to microbial necromass formation and microbial community composition. The bell-like form group reflected the influence of other inputs and their quality (belowground and animal input). Ripple-like form indicated that microbial activity was sensitive to the change of soil physiochemical conditions and root biomass, which were probably in turn altered by grassland management. The bacteria and fungal contributions and the metabolic quotient were not influenced by management intensity.

We conclude that a gradient based on the evaluation of aboveground biomass input is suited to evaluate management impacts on belowground functioning of grassland soils. Moreover, we

suggest that (aboveground) plant biomass input is a poor control of belowground microbial functioning determining SOC dynamics, which might be more related to root activity.

6. Conflicts of interests

We state that there is no conflict of interests.

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687 **Tables**

688 Table 1. Initial soil general parameters before beginning of the experiment in 2005.

Treatments	pH	SOC content	N content	C:N ratio
		mg g^{-1}	mg g^{-1}	
UM	6.04±0.02	43.5±1.2	4.04±0.15	10.8±0.1
LGraz	5.78±0.04	41.9±1.3	3.82±0.14	11.0±0.1
HGraz	5.87±0.05	43.7±1.3	4.00±0.11	10.9±0.1
Mow	5.88±0.07	36.1±1.6	3.29±0.10	10.9±0.2
Bare	5.86±0.14	38.5±2.5	3.55±0.26	10.8±0.1

689

690

691 Table 3. Soil general parameters. Values are shown as the average of six (four for Bare fallow) replicates and ±SE. Significant differences

692 between the treatments are indicated by lower case letters ($P < 0.05$).

Treatment	Root biomass	pH	SOC content	C:N ratio	N content	$\delta^{13}C$	$\delta^{15}N$
	t ha^{-1}		mg g^{-1}		mg g^{-1}	‰	‰
UM	5.58±0.95a	5.9±0.07a	82.4±4.8a	11.1±0.1ab	7.4±0.4a	-27.4±0.07b	5.6±0.1b
LGraz	7.74±2.07a	5.7±0.05b	84.9±5.2a	11.1±0.1ab	7.6±0.5a	-27.7±0.06bc	4.9±0.1c
HGraz	3.69±1.1b	5.8±0.05ab	79.2±3.3a	10.8±0.1bc	7.3±0.3a	-27.8±0.08c	5.3±0.1bc
Mow	8.51±1.2a	5.3±0.05c	73.0±1.6a	11.2±0.1a	6.5±0.1a	-27.6±0.04bc	4.8±0.1c
Bare	-	5.2±0.06c	49.3±2.3b	10.7±0.0c	4.6±0.2b	-26.8±0.03a	6.3±0.1a

693

694 Table 4. Amino sugar signatures. Values are shown as the average of six (four for Bare
695 fallow) replicates and ±SE. Significant differences between the treatments are indicated by
696 capital case letters ($P < 0.05$).

Treatment	Aminosugars		
	mg g^{-1} dry soil	mg g^{-1} SOC	GlcN/GalN
UM	2.54±0.23a	30.5±1.6a	1.49±0.05b
LGraz	2.29±0.15ab	27.2±1.0a	1.71±0.04ab
HGraz	2.46±0.16ab	28.4±1.7a	1.77±0.05a
Mow	1.98±0.14ab	27.2±1.7a	1.78±0.03a
Bare	1.69±0.26b	34.8±6.3a	1.79±0.13a

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Figure captions

Figure. 1. Conceptual framework showing the management practices on the disturbance gradient based on aboveground plant biomass removal.

Figure 2. Microbial biomass C concentration (MBC) and content (MBC per SOC), microbial C:N ratio under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Values are shown as the average of six (four for Bare soil) replicates and ±SE. Significant differences between the treatments are indicated by lower case letters ($P < 0.05$).

Figure 3. Basal respiration (A), Basal respiration (CO2 per SOC) (B) and metabolic quotient qCO2 (C) under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Values are shown as the average of six (four for Bare fallow) replicates and ±SE. Significant differences between the treatments are indicated by lower case letters ($P < 0.05$).

Figure 4. Active microbial biomass (AMB) (A) and specific growth rate μ (B) under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Values are shown as the average of six (four for Bare fallow) replicates and ±SE. Significant differences between the treatments are indicated by lower case letters ($P < 0.05$).

Figure 5. Absolute enzyme activity (A) and enzyme activity per MBC for the 7 enzymes under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Aglu, bglu, xyl, cello, chit, leu, phosph represent α-glucoside, β-glucoside, xylosidase, cellobiosidase, chitinase, leucin aminopeptidase and phosphatase, accordingly. Values are shown as the average of six (four for Bare soil) replicates and ±SE. Significant differences between the treatments are indicated by lower case letters ($P < 0.05$).

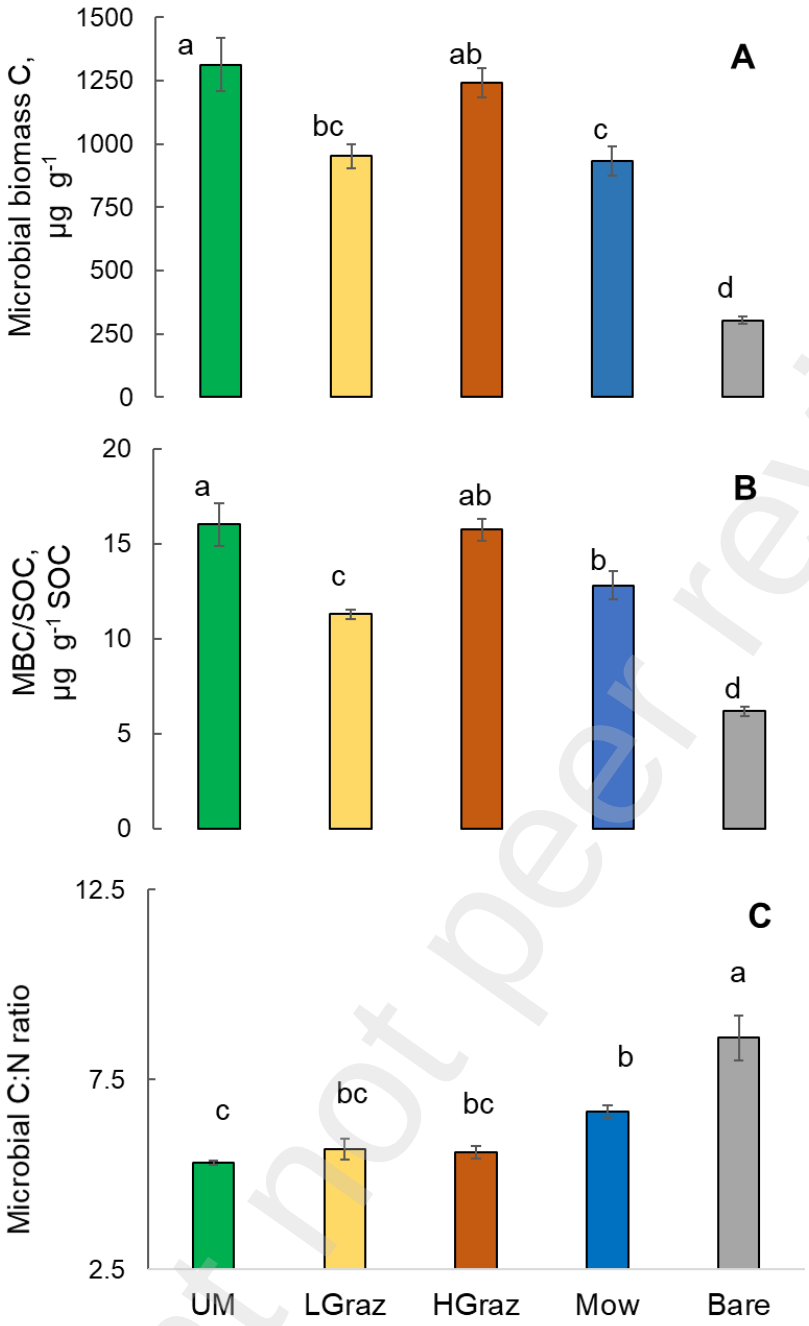
724 **Figure 6**. The response patterns of measured soil biogeochemical parameters to disturbance
725 gradient based on aboveground plant biomass leftover level.
726 **Figure 7**. Principal component analysis (PCA) of all measured soil variables under (A) three
727 grassland management practices (low intensity grazing (LGraz), high intensity grazing
728 (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). PCA score plot (B)
729 represents only three grassland management practices (low intensity grazing, high intensity
730 grazing and mowing). Only variables with quality of representation (cos^2) higher than 0.6
731 were shown on PCA plots.
732

733

734 **Figure. 1.** Conceptual framework showing the management practices on the plant

735 biomass input gradient

736



737

738 **Figure 2.** Microbial biomass C concentration (MBC) and content (MBC per SOC), microbial
739 C:N ratio under three grassland management practices (low intensity grazing (LGraz), high
740 intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare).
741 Values are shown as the average of six (four for Bare soil) replicates and ±SE. Significant
742 differences between the treatments are indicated by lower case letters ($P < 0.05$).

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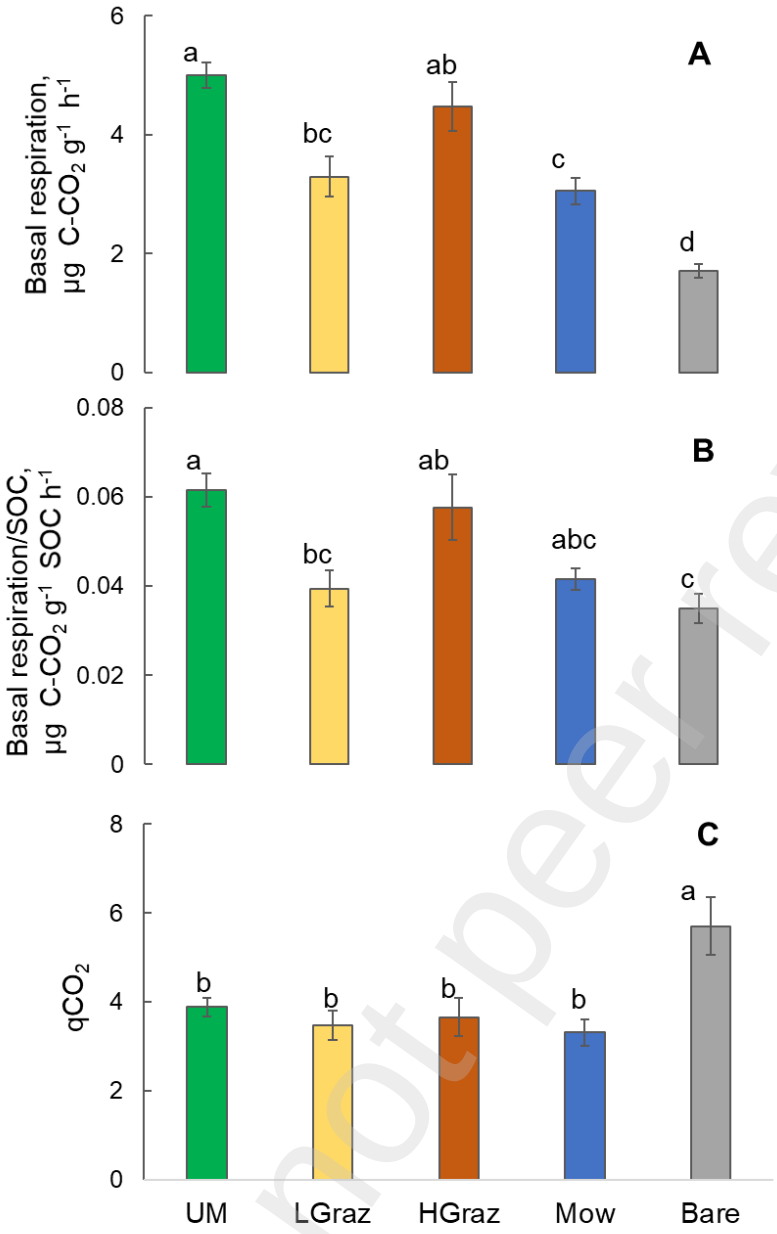

744

745 **Figure 3**. Basal respiration (A), Basal respiration (CO_2 per SOC) (B) and metabolic quotient
746 qCO_2 (C) under three grassland management practices (low intensity grazing (LGraz), high
747 intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare).
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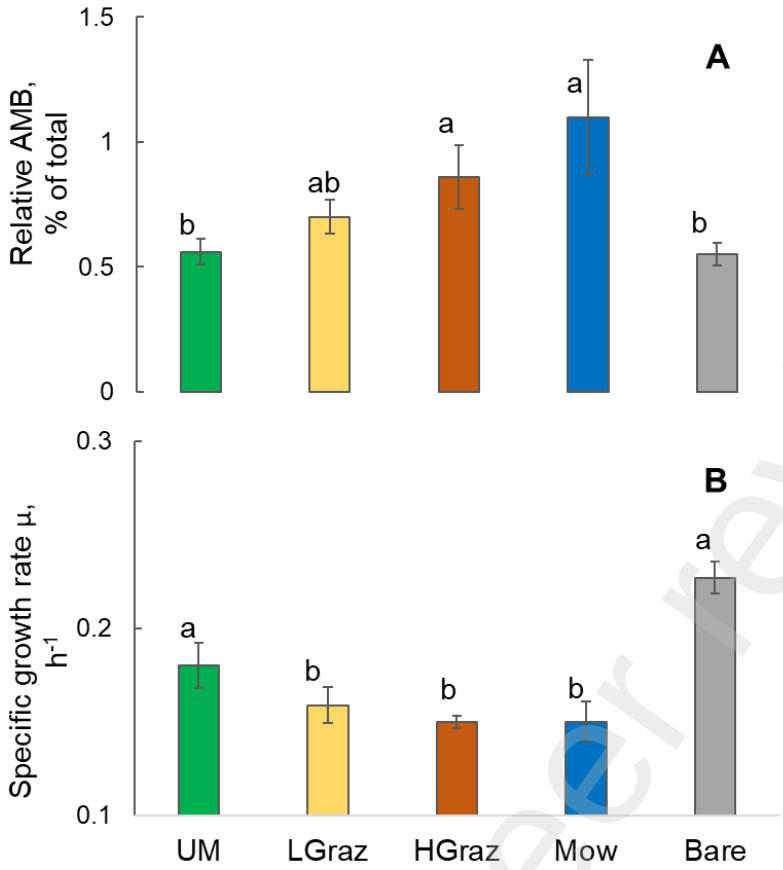

751

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753 grassland management practices (low intensity grazing (LGraz), high intensity grazing
754 (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Values are shown as
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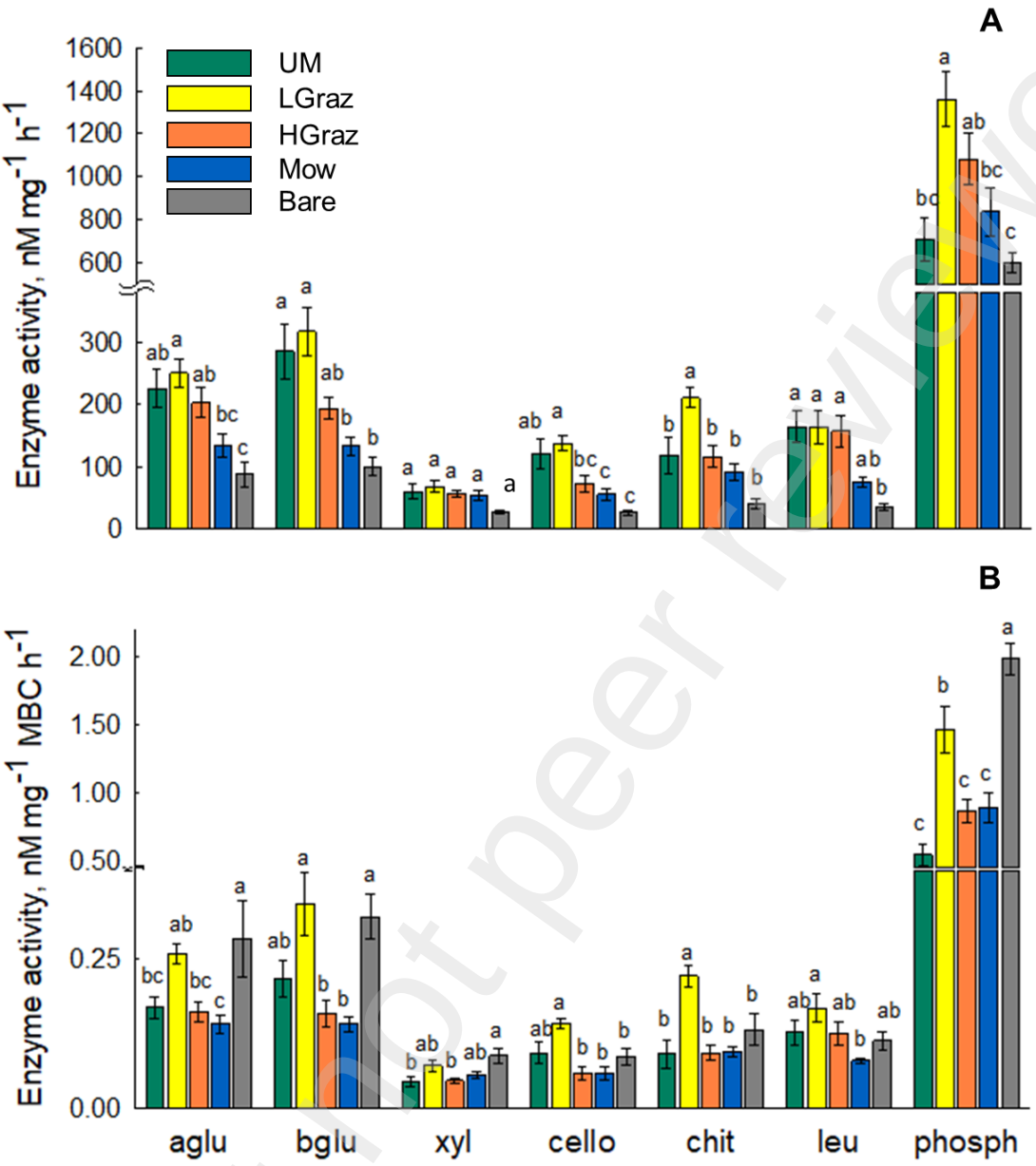
757



758

759 **Figure 5.** Absolute enzyme activity (A) and enzyme activity per MBC for the 7 enzymes
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761 grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Aglu, bglu,
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763 chitinase, leucin aminopeptidase and phosphatase, accordingly. Values are shown as the
764 average of six (four for Bare soil) replicates and ±SE. Significant differences between the
765 treatments are indicated by lower case letters ($P < 0.05$).

766

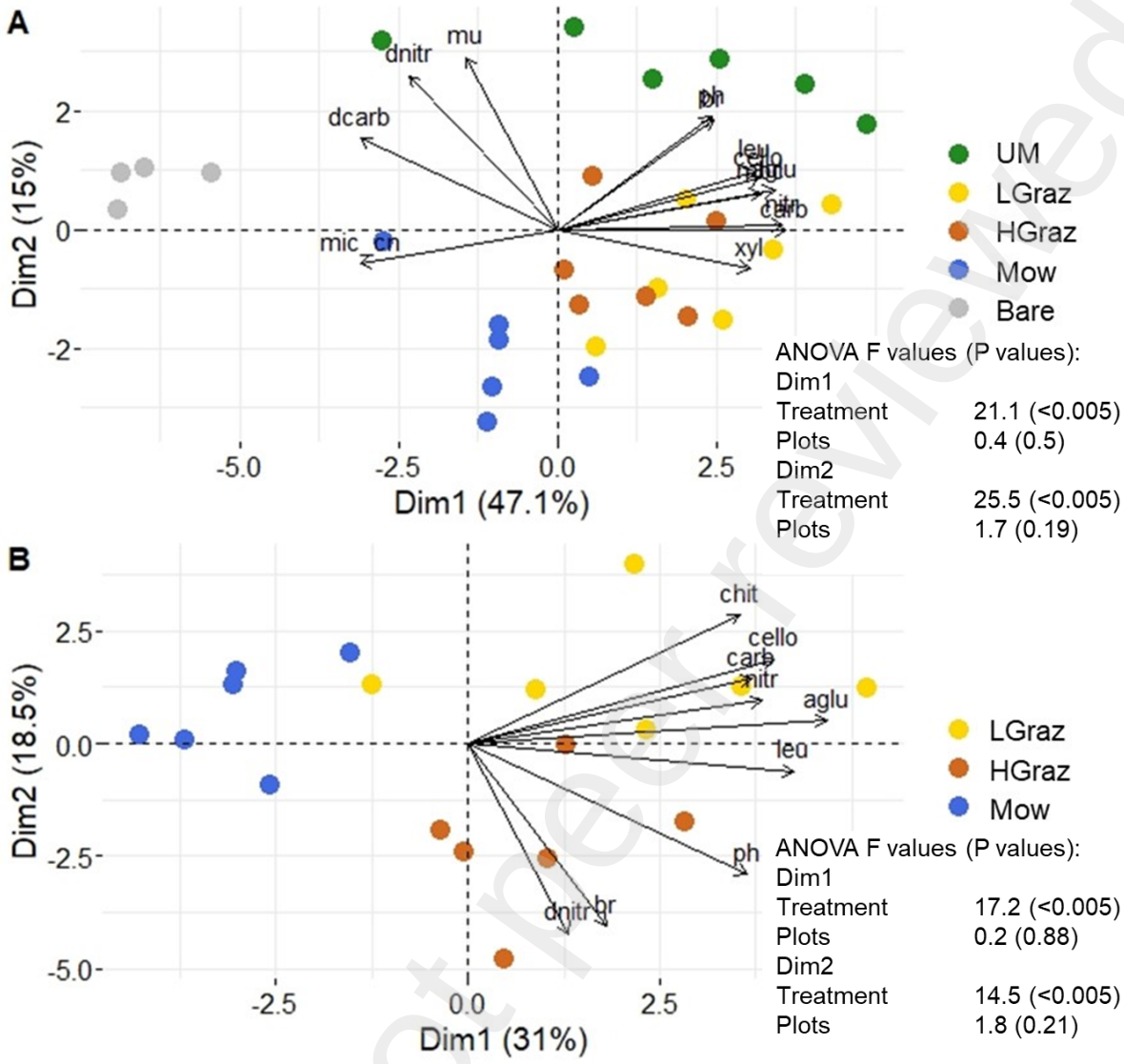
Name of dependence		Properties
Linear - Positive		Microbial C:N ratio
- negative		Amino sugars content
Bell-like form		SOC and N content Active microbial biomass Absolute enzyme activity
Reverse bell-like form		Specific growth rate
Ripple-like form		pH Microbial biomass C Basal respiration Specific enzyme activity
Specific - sensitive to any disturbance		Amino sugars GluN/GalN
- sensitive to absence of any input		Metabolic quotient qCO_2

767

768 **Figure 6**. The response patterns of measured soil biogeochemical parameters to

769 disturbance gradient based on aboveground plant biomass leftover level.

770



771

772 **Figure 7**. Principal component analysis (PCA) of all measured soil variables under (A)
773 three grassland management practices (low intensity grazing (LGraz), high intensity grazing
774 (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). PCA score plot (B)
775 represents only three grassland management practices (low intensity grazing, high intensity
776 grazing and mowing). Only variables with quality of representation (cos^2) higher than 0.6
777 were shown on PCA plots.

778

779

780 Table 2. Aboveground net primary production and aboveground plant biomass leftover under

781 four grassland management practices.

Treatments	Aboveground net primary production(ANPP)	Used biomass	Used	Aboveground plant biomass leftover (APBL)
	t ha^{-1} $year^{-1}$	t ha^{-1} $year^{-1}$	%	t ha^{-1} $year^{-1}$
UM	5.28±0.27	0	0	5.28
LGraz	5.28±0.27	2.88±0.09	50	2.4
HGraz	6.34±0.57	5.71±0.15	90	0.63
Mow	9.01±0.23	9.01±0.23	100	0
Bare	0	0	0	0

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