Management of grasslands by mowing versus grazing – impacts on soil organic matter quality and microbial functioning

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Keywords

Grassland, grazing, mowing, non-cellulosic polysaccharides, lignin, microbial activity

Highlights

- Grazing and mowing have contrasting effects on soil biogeochemical properties
- Grazing promoted more efficient microbial functioning
- Mowing leads to more degraded lignin than grazing
- Only microbial properties were sensitive to treatment effects in subsurface soil

Abstract

Although 30% of the European surface area is covered with grasslands, little is known about the effect of their management on soil quality and biogeochemical cycling. Here, we analysed soil from an experimental site in Western France, which had been under either grazing or mowing regime for 13 years. We aimed to assess the effect of the two management practices on the biogeochemical functioning of soil system. To this end we compared soil organic matter (SOM) composition and microbial properties at two soil depths. We analysed for elemental, lignin and non-cellulosic polysaccharide content and composition and for microbial biomass, soil microbial respiration and enzyme activities. Our results showed higher soil organic carbon (SOC) and nitrogen contents in the surface soil under grazing as compared to mowing. Soil biogeochemical properties differed between grazing and mowing treatments. In particular, soil under grazing showed lower lignin and higher microbial biomass. Despite the similar non-cellulosic polysaccharide content under both treatments, microbial community under mowing was characterised by higher enzyme production per microbial biomass, leading to more degraded SOM in the mowing system as compared to grazing. We conclude that grazing and mowing regimes impact differently the biogeochemical soil functioning. Higher and more diverse carbon input under grazing compared to mowing may lead to enhanced substrate availability and thus more efficient microbial functioning, which could favour SOC sequestration through formation of microbial products.

1. Introduction

Dangerous climate change can only be avoided if we succeed to remove CO_2 from the atmosphere with negative emission technologies (IPCC, 2018). Soil organic carbon (SOC) sequestration is a nature-based negative emission technology, which may be achievable at scale through the introduction of sustainable management practices with permanent soil cover (Rumpel et al., 2018). Permanent grasslands, which in Europe, occupy about 30% of the agricultural area (Ec.europa.eu, 2018), are responsible for many ecosystem services including forage for animal production and SOC storage (Havstad et al., 2007; Rumpel et al., 2015). Biogeochemical cycling in grassland soils can be influenced by a variety of management practices (Rumpel et al., 2015). The impact of these management practices on processes impacting soil biogeochemical cycling via soil-plant interactions are poorly understood (Dignac et al., 2017). These interactions result in contrasting effects of grassland management on SOC storage potential (Post and Kwon, 2000; Rumpel et al., 2015; Smith et al., 2008).

Grazing and mowing are the most frequently used grassland management practices. Both practices lead to defoliation (removal of plant aboveground tissue). Defoliation alters root exudation and C allocation in plants but the direction of these changes was found to be contrasting (Bazot et al., 2005; Gavrichkova et al., 2010; Medina-Roldán and Bardgett, 2011), related to different climatic and pedological conditions (Pineiro *et al.*, 2010).

Defoliation under grazing management is caused by herbivores during several days (Senapati et al., 2014). This process plays an important role in terms of carbon and nutrient return (Soussana et al., 2006), because about 50-70% of the ingested biomass is returned to soil in the form of excreta. In mowing systems, plant biomass is removed in a day with up to 20% of all cut biomass remaining as green litter in form of harvesting losses (Sanaullah et al.,

71 2010). In order to compensate for nutrient exportation during defoliation events, mineral
72 fertilisers are applied in mowing systems.

73 Due to the different types of biomass returned in the two systems, the quality of
74 biomass input also varies. Mowing systems receive only plant residues while input in grazing
75 systems comprises additionally animal depositions. Dung and urine inputs are characterised
76 by lower C:N ratio, higher amount of easily available compounds (Dungait et al., 2009) and
77 relatively stable compounds, such as crude proteins and fats (Dungait et al., 2005; Ngo et al.,
78 2011). Moreover, in grazing systems, there is a return of senescent brown litter, which
79 contains less N and less soluble compounds compared to the green litter returned as
80 harvesting losses in mowing systems (Sanaullah et al., 2010).

81 These differences may affect belowground processes (Wilson et al., 2018), SOC
82 formation and storage (Cotrufo et al., 2015; Rumpel et al., 2015) through their effect on the
83 soil microbial biomass and its activity (Liang et al., 2017). We therefore hypothesised that the
84 two management systems may lead to contrasting soil microbial functioning and affect
85 differently biogeochemical cycling. The effect of management has been analysed up to now
86 mainly in the first few centimetres of soil, although it has been shown that management can
87 affect SOC stored down to 2 m depth (Tautges et al., 2019). We thus hypothesised that
88 grassland management affects SOC below the first centimetres.

89 We focused on an experimental site with grazing and mowing as two contrasting
90 management practices under similar soil and climatic conditions. We aimed to evaluate the
91 differences in biogeochemical cycling in soil under the two different management practices at
92 two depths. To this end we analysed C and N contents, molecular signatures of
93 polysaccharide and lignin monomers. These variables were compared to the functioning of
94 the soil microbial communities, assessed by the analyses of soil microbial respiration, growth
95 kinetic parameters and activity of 9 enzymes as well as microbial biomass C and N.

2. Materials and methods

2.1. Site description and soil sampling

The field experiment is located in Lusignan (southwest of France, 46°25'12,91"N; 0°07'29,35"E) at the national long-term experimental observatory SOERE ACBB (Agroecosystems, Biogeochemical Cycles and Biodiversity). The mean annual temperature and precipitation for the period 2006–2010 were 11.2°C and 773 mm, (Senapati et al., 2014). The landscape is flat. The soil is classified as a Dystric Cambisol with loamy texture (Chabbi et al., 2009).

The current study is focused on two permanent sown grasslands (each of about 3 ha in size), which were established in 2005 by sowing a mixture of three plant species (*Lolium perenne*, *Festuca arundinacea, Dactylis glomerata* L.) in both treatments. In the grazing system, legume *Trifolium repens* was included in the species mixture but covered only 5% of grazed paddock in 2017. The mown grassland was cut four times per year with biomass exported. To replace the exported nutrients, nitrogen (N) fertilizer was applied at rates between 170 and 380 kg N ha^{-1} $year^{-1}$ (Puche et al., 2019). Grazing in the grazed paddock took place from March to December with 50 days per year using 15 to 20 livestock units per hectare. Grazed grasslands received less nitrogen fertilization (60-150 kg N ha^{-1} $year^{-1}$, Puche *et al.*, 2019) because nitrogen losses were additionally returned by dung and urine and through the presence of the leguminous species. In order to compare the treatments at similar N status, fertilizer application rates were adjusted to maintain the Nitrogen Nutrition Index between 0.9 and 1.0 for both treatments, close to non-limiting nitrogen nutrition to near maximum plant production (Senapati et al., 2016). Moreover, both sites were limed regularly in order to neutralize acid pH.

Due to the large land requirements (3 ha for plots with cows), it was not possible to establish and maintain a completely replicated field experiment including grazing treatment for several decades. Limitations to generalization of the treatment effects due to the absence of replication of the experiments were limited by choosing homogenous flat areas in close proximity with similar land use history, climate, and soil type. Moreover, we carried out baseline measurements, in form of geostatistical evaluation of the soils SOC and N contents and included initial SOC stocks as a co-variate. These data showed that both plots were significantly different in initial SOC and N contents (n=28). The SOC contents on mowing plots varied between 9.9 and 13.7 mg g^{-1} (average 12.0 ± 1.0 mg g^{-1}), while under grazing it was between 11.9 and 19.1 mg g^{-1} (average 14.8 ± 1.5 mg g^{-1}). N contents varied between 1.0 and 1.4 mg g^{-1} (average 1.2 ± 0.1 mg g^{-1}) under mowing, while under grazing the values ranged between 1.2 and 1.9 mg g^{-1} (average 1.5 ± 0.1 mg g^{-1}). These previous analyses indicated on average non-significant differences in SOC stock changes between grazing and mowing after nine years of treatment (Crème et al., 2020). The study also showed partitioning of the field into different zones with SOC gain and loss (Crème et al., 2020; Fig S1, Supplementary materials).

Five replicated soil samples were taken from each of the two zones, giving a total of 10 replicated field samples per plot. Sampling took place in November 2017, 2 weeks and 5 months after the last grazing and mowing events, accordingly. The shortest distance between samples was 25 m. Soil samples were collected with a mechanical auger (5cm Ø, 30cm) at two depths: 0-10 cm (surface soil) and 20-30 cm (subsurface soil) giving in total 40 samples. All samples were sieved through a 2-mm mesh. Thereafter, half of the samples was air-dried and ground for measurements of physicochemical analysis and the other half was stored at 4°C before microbial analyses. Because of dry field conditions prior to measurements of

145 microbiological analysis, soil samples were moistened by distilled water to adjust 50% of
146 WHC and pre-incubated at 22 °C for 7 days.

147 *2.2. Soil general properties*

148 Soil pH (H_2O) was measured in a soil:water suspension (1:2.5 weight/volume). Soil
149 organic carbon (SOC), nitrogen (N) and stable isotopes (^{13}C and ^{15}N) contents were measured
150 using a CHN auto-analyser (Flash EA, Thermo Electron Corporation, Bremen, Germany)
151 coupled with an isotope ratio mass spectrometer. The isotopic ratios were calculated relative
152 to the Pee Dee Belemnite Standard (PDB) for C and relative to atmospheric N_2 for nitrogen.
153

154 *2.3. Soil chemical properties*

155 Lignin was analysed by the alkaline cupric oxide (CuO) oxidation method (Hedges and
156 Ertel, 1982; Kögel and Bochter, 1985). Briefly, oxidation was carried out under alkaline
157 conditions (2M NaOH) at 172 °C for 4 hours using 500 mg of air-dried soil, 250 mg of CuO,
158 50 mg of ammonium ferrous hexahydrate and 50 mg of glucose. After cooling, samples were
159 acidified with 5 M HCl and left overnight for humic acid precipitation. Removal of humic
160 acids was conducted through centrifugation (10 min at 10000 rpm) and followed by
161 extraction of phenolic oxidation products with C18 reversed phase columns. The phenols
162 were derivatized with BSTFA and quantified as trimethylsilyl derivatives by gas
163 chromatography with a HP gas chromatograph (HP GC 6890) equipped with a flame
164 ionization detector and a SGE BPX-5 column (50 m length, 0.25 mm inner diameter, 0.32 μm
165 coating). Samples were injected in split mode (1:10). The GC oven temperature was
166 programmed at 100 °C for 2 min, then increased from 100 to 172 °C at a heating rate of 8 °C
167 min^{-1}, from 172 to 184 °C at 4 °C min^{-1}, and from 184 to 300 °C at a rate of 10°C min^{-1}.

168 The internal standard ethylvanillin was added before the purification step to quantify
169 lignin recovery and the quantification standard phenylacetic acid was added before GC
170 analyses.
171 The total lignin content (mg g^{-1} dry soil) of the sample was determined as the sum of
172 phenolic oxidation products: vanillyl (V), syringyl (S) and p-coumaryl (C) in their acid (Ac),
173 aldehyde (Al) and ketone forms. Lignin content was also expressed as lignin content per SOC
174 (mg g^{-1} SOC). Lignin decomposition was assessed by the ratios of S, C to V and (Ac/Al)
175 ratios of V and S, which generally indicate decomposition state (Thevenot et al., 2010).
176 Non-cellulosic polysaccharides of plant and microbial origin (Kögel-Knabner, 2002)
177 were determined by gas chromatography after trifluoroacetic acid (TFA) hydrolysis and
178 reduction-acetylation using a method introduced by Rumpel and Dignac (2006) and modified
179 by Eder et al. (2010). The analysis was performed using 700 mg of soil samples. Briefly,
180 hydrolysis of non-cellulose polysaccharides was carried out at 105°C for 4 h with 10 ml of 4
181 M TFA. Thereafter, Myo-inositol was added as quantification standard to account for the
182 losses during the purification procedure. Removal of soil was performed by filtration through
183 glass fibre filters (Whatman GF/C 0.45 μm). Afterwards, TFA was evaporated using a
184 centrifugal Evaporator EZ-2 ENVI at 35°C for 4 hours and dry samples were left overnight in
185 the freezer. Thereafter, dry samples were dissolved in 0.5 ml of H_2O followed by the addition
186 of 0.9 EDTA in order to avoid co-precipitation of organic material with metal oxides and
187 hydroxides (Eder et al., 2010). One mL sodium borohydride (NaBH4) in dimethylsulfoxide
188 (20 g L−1) was added for reduction of polysaccharide monomers into alditol forms and kept
189 at 40°C for 1.5 hours. Then, acetylation was conducted by addition of 0.2 mL acetic acid, 2
190 mL of acetic anhydride and 0.2 mL Methylimidazole. Acetylated alditols were extracted by 1
191 ml of dichloromethane and quantified with a HP GC 6890 gas chromatograph equipped with
192 a flame ionization detector. Separation was achieved with a 60 m fused silica capillary

193 column (SGE BPX 70, 0.32 mm internal diameter, 0.25 mm film thickness) under the
194 following temperature program: 170 to 250 °C at 8 °C.min^{-1}, followed by 12 min at 250 °C
195 (isothermal). Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The injector
196 was kept at 250 °C and the detector at 260 °C. The non-cellulosic polysaccharides content of
197 soil samples was determined as the sum of monosaccharides: C5 (pentoses: xylose, ribose
198 and arabinose), C6 (hexoses: glucose, galactose and mannose), and desoxyC6
199 (desoxyhexoses: fucose and rhamnose) (Kögel-Knabner, 2002). A higher C6/C5 ratio
200 generally indicates higher contribution of microbial sugars.

201 *2.4. Soil microbial properties*

202 Microbial biomass C (MBC) and nitrogen (MBN) were determined by the chloroform
203 fumigation-extraction method (Vance et al., 1987). Dissolved organic C and N in fumigated
204 and non-fumigated soil samples were extracted in 0.05 M K_2SO_4 and were measured using a
205 multi C/N analyzer (multi C/N analyser 2100S, Analytic Jena). MBC and MBN were
206 calculated with a conversion factor of 0.45 (Jenkinson et al., 2004). For measuring soil
207 microbial respiration (SMR) a half gram of soil sample was placed in 2 ml Eppendorf tubes.
208 The CO_2 efflux was trapped in 3 ml of 0.1 M NaOH and determined by conductometry. The
209 metabolic quotient (qCO_2), reflecting decomposition activity (Anderson, 2003; Anderson and
210 Domsch, 1993), was calculated as soil microbial respiration expressed per gram of microbial
211 biomass carbon: qCO_2 = SMR/MBC (μg CO_2-C g^{-1} MBC h^{-1}).

212 We used microbial growth kinetics technique as an approach to estimate microbial
213 biomass activity state (Blagodatskaya and Kuzyakov, 2013). This approach is based on soil
214 respiratory response to unlimited nutrient amendments (Panikov and Sizova, 1996). For this
215 purpose, soil samples were treated with a solution (0.1 ml per g of dw soil) containing per g
216 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
217 mg KH_2PO_4 for surface soil samples and 10 mg glucose, 1.9 mg $(NH_4)_2SO_4$, 3.8 mg

218 $MgSO_4*7H_2O$, 0.53 mg K_2HPO_4 and 1.35 mg KH_2PO_4 for subsurface soil samples. The
219 amount of mineral salts was preliminary selected in order to avoid soil pH change of more
220 than 0.1 units after addition. For active microbial biomass (AMB) and specific growth rate
221 calculation, the results of substrate induced respiration rate were fitted with a model proposed
222 by Panikov and Sizova (Panikov and Sizova, 1996; Wutzler et al., 2012):

223 $$CO_2(t) = A + B * \exp(\mu * t) \quad (1)$$

224 In order to estimate catabolic (decomposition) activity in regards to specific substrates
225 in soil, we measured extracellular enzyme activity using the fluorometric technique (Koch et
226 al., 2007; Marx et al., 2005; Razavi et al., 2015). Nine types of fluorogenic substrates based
227 on 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) were used: (1)
228 MUF-α-D-glucopyranoside for α-glucosidase, (2) MUF-β-D-glucopyranoside for β-
229 glucosidase, (3) MUF-β-D-galactopyranoside for β-galactosidase, (4) MUF-β-D-
230 xylopyranoside for β-xylosidase, (5) MUF-β-D-cellobioside for β-cellobiohydrolase, (6)
231 MUF-N-acetyl-β-D-glucosamide for chitinase, (7) Leucine-AMC for leucine aminopeptidase,
232 (8) MUF-heptanoate for lipase and (9) MUF-phosphate for phosphatase. Saturation
233 concentrations of fluorogenic substrates were determined in preliminary experiments and
234 comprised 20 μmol g^{-1} soil for all enzymes except lipase with 60 μmol g^{-1} soil. Briefly, a
235 water extract of soil (1:10) was homogenised by low-energy sonication (40 J s^{-1} output
236 energy) for 60 s. Thereafter 50 ml of the soil suspension were added to 150 ml of each
237 substrate solution in a 96-well microplate. Fluorescence was measured at an excitation
238 wavelength of 355 nm and an emission wavelength of 460 nm (Victor3 1420-050 Multilabel
239 Counter, PerkinElmer, USA).
240

2.5. Statistical analysis

All results are presented as arithmetic means with standard error. The statistical analyses were conducted by using R (Studio Version 1.1.447). We used analyses of covariance (ANCOVA) to test treatment effect, depth effect and their interactions using chemical and microbial variables with initial SOC stock as a covariate. The initial SOC stocks data was obtained from exactly the same sampling points based on the geostatistical evaluation before the beginning of the experiment. This procedure allowed us to account for the lack of field replication by taking into account the original difference between the grazed and mowed plots. In order to obtain better understanding of treatment and depth effects, non-transformed data (except C and N contents) were subjected to Principal Component Analysis (PCA) and the results were also tested by ANCOVA with initial SOC stock as a covariate. The equations were fitted by non-linear regression, using Model Maker-3 software (SB technology Ltd.).

3. Results

3.1. Soil properties

Soil physicochemical properties are presented in Table 1. The pH was not controlled by initial SOC stock ($P=0.70$). Lower pH was found for both treatments in surface soil compared to subsurface soil, although, the lowest pH value was recorded in surface soils under mowing treatment. SOC and N contents were nearly twice as high in the surface soil compared to the subsurface soil under both treatments. Even if SOC and N contents were dependant on initial SOC stock ($P=0.03$ and 0.02, respectively), there were still significant effects of depth ($P<0.001$) and treatment ($P<0.001$) after correction by using it as covariate. C:N ratio differed only between soil depths ($P<0.001$) showing slightly higher C:N ratios in surface soils as

compared to subsurface soils. $\delta^{13}C$ followed the same pattern as SOC content and the highest enrichment was recorded for the surface soil of the grazing treatment (depth effect $P<0.001$ and treatment effect $P=0.002$). The $\delta^{15}N$ did not differ between the treatments and was enriched in surface soils compared to subsurface soils.

3.2. Specific SOM compounds

Non-cellulosic polysaccharide (NCP) content was not affected by initial SOC stock ($P=0.52$) and there was treatment × depth interaction (Table 2, $P<0.001$). Grazing resulted in higher NCP content in both depths compared to mowing. The NCP content per SOC (mg g^{-1} soil C) was affected only by depth ($P=0.002$). Concerning the NCP monomers ratio, C6/C5 and Man/Xyl ratios were controlled by initial SOC stock ($P=0.03$ and 0.04, respectively), consequently, after ANCOVA application the treatment effect was varnished while depth effect remained significant (Table 2, $P<0.001$). All NCP monomers ratios were higher in subsurface soil compared to surface soil under both treatments.

Lignin content was not affected by initial SOC stock correction ($P=0.82$), so the effects of depth ($P<0.001$), treatment ($P<0.001$) and their interactions ($P=0.04$) remained significant (Table 3). Lignin content was higher in surface soils than in subsurface soils and was higher under grazing compared to mowing as well. Correcting for initial SOC stock caused the elimination of all effects on lignin content per SOC content. The C/V ratio was affected only by depth ($P=0.006$) showing higher values in surface soils than in subsurface soils. The S/V ratio was greater under grazing treatment than under mowing treatment at both depths even after correction by initial SOC stock (Table 3, $P=0.01$). Based on the presence of treatment × depth interaction $(Ac/Al)_V$ and $(Ac/Al)_S$ ratios were lower in the surface soil of grazing treatment as compared to mowing treatment ($P<0.001$). In contrast to surface soils, treatments did not show any effects on these lignin ratios in subsurface soils.

290

291 *3.3. Soil microbial properties*

292 The soil microbial respiration (SMR) ranged between 0.2 and 0.7 μg CO_2 –C g^{-1} h^{-1}
293 with highest values in the surface soil under grazing treatment (Fig. 1A). After correcting for
294 initial SOC stock, treatment × depth interaction effect on SMR was significant (Table S1,
295 Supplementary materials, P<0.001). Soil microbial respiration per SOC was around 33%
296 higher in the surface soil under grazing as compared to mowing (Fig. 1B). In contrast, it was
297 greater in the subsurface soil under mowing than under grazing treatment. Including initial
298 SOC stock as covariate resulted only in significant effect of treatment × depth interaction on
299 soil microbial respiration per SOC (Table S1, Supplementary materials, P=0.004).

300 MBC per SOC was highest in the surface soil under grazing (20 μg C mg^{-1} SOC, Fig.
301 1C). Mowing treatment resulted in two times lower MBC per SOC in the surface soil
302 compared to grazing treatment. After correction for initial SOC stock, treatment (P<0.001)
303 and their interaction (P<0.001) showed significant effects on qCO_2. Mowing treatment
304 resulted in higher qCO_2 at both depths as compared to grazing treatment (Fig. 1D, P=0.02).

305 Microbial C:N ratio ranged between 4.9 and 6.4. It was affected by treatments in all
306 depths showing higher values under mowing (Fig. 2A). After taking into account initial SOC
307 stock, the treatment effect was still significant (Table S1, Supplementary materials, P<0.001).
308 Active microbial biomass was also higher under mowing at both depths compared to grazing
309 treatment (Fig. 2B, P=0.02). The highest specific microbial growth rate (Fig. 2C) was
310 recorded in subsurface soils without difference between treatments. But in surface soils, the
311 specific microbial growth rate was higher under grazing than under mowing (Fig. 2C).
312 However, ANCOVA with initial SOC stock as covariate decreased the significance treatment
313 effects (P=0.05) on specific microbial growth rate but increased the depth effect Table S1,
314 Supplementary materials, P<0.001).

315 Treatment effect on absolute enzyme activities is presented only for leucine
316 aminopeptidase in surface soil and chitinase and phosphatase in subsurface soil (Fig S2,
317 Supplementary materials). When the initial SOC stock was used as covariate, treatment
318 differences between enzyme activities per MBC were observed for all enzymes (except
319 leucine aminopeptidase) in surface soil. Soil under mowing treatment showed 2-2.5 times
320 higher enzyme activity per MBC under mowing compared to soil under grazing (Fig. 3). The
321 differences between treatments were more pronounced in surface soil for activities of
322 chitinase, β-galactosidase, β-glucosidase and phosphatase (Table S1, Supplementary
323 materials).
324

325 *3.4. Principal component analysis*

326 Principal component analysis based on SOC normalised data of all soil properties
327 showed that the first two factors explained 54.4% of the variation (Fig. 4). The first
328 component (Dim1) was related to microbial functioning, as it was strongly associated with
329 the soil microbial properties MBC and MBN per SOC in negative direction. The positive
330 direction was related to the lipase activity per MBC. The second component (Dim2) was
331 explained by variables related to polysaccharides. It was positively correlated with enzymes
332 participating in polysaccharide degradation and negatively with polysaccharide ratios. The
333 clustering of samples allowed to separate surface soil and subsurface soil samples along both
334 axes, while surface soil samples were additionally separated by treatments along the first axes
335 (Fig. 4). Subsurface soil samples were differentiated from surface soil by high neutral
336 polysaccharide monomer ratio, low enzymes activities per MBC, MBC and MBN per SOC.
337 Treatments in surface soil were separated by C- and N-cycle enzyme activity and MBC and
338 MBN per SOC. We also applied ANCOVA with initial SOC stock as a covariate on new
339 PCA coordinates which resulted in significant effects of treatment, depth and their

interaction. Treatment effect was more pronounced on Dim1, while Dim2 was more affected by depth.

4. Discussion

4.1. Effect of grazing and mowing on chemical properties of surface soil

Since the primary factor of SOM formation is organic matter input (Fujisaki et al., 2018; Kögel-Knabner, 2002), higher SOC and N contents in the surface soil under grazing system might be explained by greater C input compared to mowing systems. This was shown through ecosystem flux measurements at these plots (Senapati et al., 2014). Moreover, dung return comprising about 50-80% of plant biomass could also favour higher SOC and N content under grazing (Soussana et al., 2006). Even if mowing leads to some biomass input in the form of plant material lost during grass removal (Sanaullah et al., 2010), the amount is not enough to reach a similar input level than under grazing. Additionally, the lower pH under mowing could contribute to indirect losses of SOC via changing C cycle and microbial functioning (Kemmitt et al., 2006). Consequently, our results suggest that temperate loamy soil under grazing is more prone to higher SOC contents when compared to mowing.

With regards to the biogeochemical composition of SOC, we did not find any differences in non-cellulosic polysaccharide concentrations. These results are in agreement with other studies showing that the soils' polysaccharide content is more or less stable and even plant removal does not have a strong effect on the total polysaccharide concentrations (Marchus et al., 2018). Soil lignin content, in contrast, was lower under grazing than mowing. As lignin is a biomarker for plant-derived organic matter and more difficult to decompose, because it requires a specific enzyme system (Buswell et al., 1987; Thevenot et al., 2010), lower exportation of plant biomass and lignin input via dung deposition in soil under grazing would suggest the opposite trend. However, dung contains only small amounts of lignin

(Dungait et al., 2005), which is relatively instable being degraded during one year (Dungait et al., 2008). All lignin parameters (except the C/V ratio) suggested that lignin was less degraded in the grazing than the mowing system. More acid pH in fertilised mowing systems could have favoured the activity of lignin-degrading fungi (Couto et al., 2006). In mowing systems microbial activity is fuelled exclusively by plant litter, whereas in grazing systems organic matter input is supplied also by animal depositions. We hypothesise that this could lead to contrasting quantitative lignin inputs, but could also impact its decomposition. Our data show that lignin degradation in the mowing system is slower and less complete than in the grazing system, leading to accumulation of partially degraded lignin molecules (Filley et al., 2006). Therefore, lignin in the mowing system was characterised by a higher state of degradation and at the same time its contribution to SOC was higher as compared to the grazing system.

4.2. Effect of grazing treatment on biological properties of surface soil

Higher maturity and sustainability of the grazing system was shown by higher MBC per SOC together with a lower qCO_2 (Anderson and Domsch, 2010). Higher qCO_2 in the mowing system indicates that the microbial communities were less efficient and respired more C to maintain metabolic activity as compared to those under grazing (Anderson, 2003). Microorganisms are the main SOM decomposers leading to release of greenhouse gases and nutrients in natural as well as in managed soils (Bardgett et al., 2008; Gougoulias et al., 2014). This is particularly relevant for grazed pastures. Higher soil microbial respiration and microbial CO_2 –C per unit SOC (soil microbial respiration per SOC) in the grazing system was probably related to dung input with a huge amount of easily available compounds (Chu et al., 2007; Marinari et al., 2000).

Contrary to our expectations, absolute enzyme activity did not differ among the treatments, even after normalisation by SOC. A treatment effect was only observed after

390 normalisation by MBC, which expresses microbial activity in terms of enzyme production.
391 The enzymatic activities per MBC were higher in the mowing system as compared to the
392 grazing one, indicating that microorganisms in mowed soil produced enzymes more actively
393 than those under grazing. Microbial communities in the mowing system stayed active and
394 were investing in enzyme production probably to adapt to less decomposable organic
395 materials with higher lignin contents (see above). This maintenance of active state requires a
396 lot of energy, consequently, it could change C-cycling rates and decomposition of SOM
397 (Schimel and Schaeffer, 2012; Wang et al., 2014).

398 Microbial communities in the mowed soil are probably characterised by a higher
399 contribution of fungi than those of the grazed soil because we recorded a higher C:N ratio of
400 the microbial biomass (Joergensen and Emmerling, 2006) and more acid pH. Lower specific
401 growth rates in the mowing system may indicate relative domination of K-strategists in the
402 microbial community, which are more adapted to nutrient poor conditions (Strickland and
403 Rousk, 2010; Xu et al., 2017) and the decomposition of specific substances, such as plant
404 material containing high amounts of biopolymers (Fontaine et al., 2003). As illustrated by
405 lower enzyme activity per MBC, microorganisms in the grazing system invested less energy
406 for the degradation of complex compounds than those of the mowing system, most probably
407 because of higher availability of easily decomposable substrates. These conditions favour r-
408 strategists (Fierer et al., 2007; Xu et al., 2017) and thus stimulate microbial activity, as shown
409 by higher MBC per SOC and higher soil microbial respiration under the grazing as compared
410 to mowing system. As a consequence, the biogeochemical soil functioning under the two
411 management practices is quite different. This may affect significantly SOM formation, which
412 is favoured in systems with intensive microbial processing of C input (Kallenbach et al.,
413 2016; Liang et al., 2017) thus corroborating the high SOC contents observed under grazing.

4.3. Less pronounced treatment effects in subsurface soil

Treatment effects on soil properties were less pronounced in subsurface soil compared to surface soil. Enhanced leaching and activity of soil fauna (Bohlen et al., 2004; Rumpel and Kögel-Knabner, 2011) promote nutrient transport to subsurface soil under grazing which resulted in higher SOC and N contents in subsurface soil under grazing than the one under mowing. Treatment effects in the subsurface soil were neither observed for non-cellulosic polysaccharide content and origin nor for lignin content or its degradation status. Since lignins are typical indicators of plant input (Kögel-Knabner, 2002), this could indicate that grazing and mowing have only small effects on plant rooting behaviour at lower depths.

On the other hand, the treatment effects on MBC and MBN was also observable in subsurface soil. Soil microbial respiration did not differ between the treatments but microbial CO_2 –C per SOC and qCO_2 were higher in the subsurface soil under mowing indicating that the microbial communities used C inefficiently, similarly to surface soil. Higher galactosidase activity in the subsurface soil of the mowing treatment is related to higher contribution of galactose monomers in grass roots compared to grass leaves (Schädel et al., 2010). As lipase is hydrolysing triglycerides, higher lipase activity in the subsurface soil indicates accumulation of lipid compounds at depth, which probably serve as C source for microorganisms under C-limiting conditions (Heitkötter et al., 2017).

The absence of treatment separation for the subsurface soils on the PCA plot might indicate that in deeper soil probably more time is required to make treatment effects observable. It was interesting to note that chemical properties related to SOM composition were not sensitive to treatment effects in the subsurface soil, whereas microbial properties were. This is in agreement with other studies, which showed that microbial properties are most sensitive to changes introduced by management activities (Allison and Martiny, 2008; Bending et al., 2004).

5. Conclusions

In this study we investigated the effect of grazing and mowing treatments on soil biogeochemical and microbial properties. Our data indicated significant differences in the soil organic matter composition as well as microbial functioning of the two treatments. Both plots were also characterized by contrasting SOC contents and pH values. The grazing system was characterized by (1) more efficient microbial community and (2) less decomposed organic matter as compared to the mowing system. We conclude that the harvesting regime by grazing or mowing affects the biogeochemical functioning of grassland soils. Even though both systems are favorable to SOC storage, grazing might be preferable to mowing because it leads to better substrate quality and more efficient microbial functioning. Although SOM changes were only evident in surface soil, microbial properties suggest that these processes are also occurring in subsurface soil.

6. Conflicts of interests

We state that there is no conflict of interests.

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Tables

Table 1. General soil properties under two grassland management practices (grazing and mowing) at in surface soil (0-10 cm) and subsurface soil (20-30 cm).

	Treatment	pH	SOC content	N	$\delta^{13}C$	$\delta^{15}N$	C:N ratio
			mg g^{-1}	mg g^{-1}	‰	‰	
Surface soil	**Grazing**	5.95±0.09	21.4±0.81	2.2±0.09	-27.4±0.06	4.9±0.13	9.6±0.05
	Mowing	5.51±0.08	14.6±0.51	1.5±0.05	-27.0±0.05	5.0±0.09	9.6±0.07
Subsurface soil	**Grazing**	5.99±0.12	11.8±0.62	1.3±0.06	-26.7±0.06	6.2±0.08	9.2±0.06
	Mowing	6.01±0.13	8.6±0.44	0.9±0.05	-26.3±0.10	6.4±0.10	9.1±0.07
ANCOVA, F value (P values)							
SOC stocks in 2005		0.15 (0.70)	5.31 (0.03)	6.26 (0.02)	6.86 (0.01)	1.90 (0.18)	1.33 (0.26)
Treatment		3.37 (0.08)	30.3 (<0.001)	28.7 (<0.001)	17.2 (0.002)	0.35 (0.56)	1.19 (0.28)
Depth		5.89 (0.02)	181.8 (<0.001)	153.8 (<0.001)	132.5 (<0.001)	157.9 (<0.001)	52.5 (<0.001)
Treatment×Depth		4.27 (0.04)	0.68 (0.41)	0.86 (0.36)	0.28 (0.06)	0.25 (0.62)	0.37 (0.55)

Values are shown as the average of ten replicates and ±SE. Significant differences between the treatments are indicated by capital case letters. Lower case letters show significant differences with depth ($P < 0.05$).

Table 2. Non-cellulosic polysaccharides (NCP) signature in soil under two grassland management practices (grazing and mowing) at two depths (0-10 cm and 20-30 cm).

	Treatment	NCP content	NCP content per SOC	NCP monomers ratios		
				C6/C5[1]	DesoxyC6/C5[2]	Man/Xyl[3]
		mg g^{-1}	mg g^{-1} SOC			
Surface soil	**Grazing**	6.61±0.23	308.98±6.3	0.80±0.02	0.35±0.01	0.54±0.02
	Mowing	4.45±0.18	306.63±11.5	0.84±0.02	0.34±0.01	0.61±0.02
Subsurface soil	**Grazing**	3.09±0.15	263.39±6.4	1.03±0.02	0.43±0.01	0.87±0.03
	Mowing	2.50±0.11	292.41±10.5	1.01±0.02	0.46±0.01	0.91±0.03
ANCOVA, F value (P values)						
SOC stocks in 2005		0.43 (0.52)				
Treatment		36.6 (<0.001)	2.50 (0.12)	5.42 (0.03)	3.81 (0.06)	4.74 (0.04)
Depth		241.1 (<0.001)	0.11 (0.74)	0.87 (0.36)	0.01 (0.91)	0.64 (0.43)
Treatment×Depth		19.7 (<0.001)	11.5 (0.002)	122.2 (<0.001)	102.8 (<0.001)	166.3 (<0.001)
			3.17 (0.08)	3.14 (0.09)	3.18 (0.08)	0.52 (0.48)

[1]C6/C5 – the ratio of C6- to C5- sugar monomers, [2]DesoxyC6/C5 – the ratio of desoxy C6- to desoxy C5- sugar monomers, [3]Man/Xyl - the ratio of mannose to xylose. These ratios indicate the origin of non-cellulosic polysaccharides (microbial or plant).

709 **Table 3.** Lignin signature in soil under two grassland management practices (grazing and
710 mowing) in surface soil (0-10 cm) and subsurface soil (20-30 cm).

	Treatment	Lignin content	Lignin content per SOC	Lignin monomers ratios			
				C/V	S/V	$(Ac/Al)_V$	$(Ac/Al)_S$
		mg g^{-1}	mg g^{-1} SOC				
Surface soil	**Grazing**	0.35±0.01	16.31±0.64	0.45±0.03	1.34±0.02	0.53±0.02	0.46±0.01
	Mowing	0.26±0.01	17.86±0.43	0.45±0.03	1.24±0.02	0.65±0.02	0.57±0.02
Subsurface soil	**Grazing**	0.19±0.02	16.22±0.57	0.37±0.02	1.33±0.03	0.66±0.01	0.54±0.02
	Mowing	0.16±0.01	18.86±0.89	0.37±0.03	1.30±0.02	0.63±0.03	0.56±0.02
ANCOVA, F value (P values)							18.6 (<0.001)
SOC stocks in 2005		0.05 (0.82)	10.9 (0.002)	1.59 (0.22)	1.99 (0.17)	0.08 (0.78)	2.82 (0.10)
Treatment		15.3 (<0.001)	1.14 (0.29)	0.64 (0.43)	7.91 (0.01)	2.83 (0.10)	5.95 (0.02)
Depth		96.3 (<0.001)	0.62 (0.44)	8.58 (0.006)	0.95 (0.34)	9.13 (0.005)	13.1 (<0.001)
Treatment×Depth		4.83 (0.04)	0.88 (0.36)	0.015 (0.90)	2.19 (0.15)	16.3 (<0.001)	

711 C/V – the ratio of cinnamyl phenols to syringyl phenols; S/V - the ratio of syringyl phenols to vanillyl
712 phenols; (Ac/Al)V – acid to aldehyde ratio of vanillyl phenols; (Ac/Al)s – acid to aldehyde ratio of syringyl
713 phenols. These ratios are indicators of lignin degradation state in soil.
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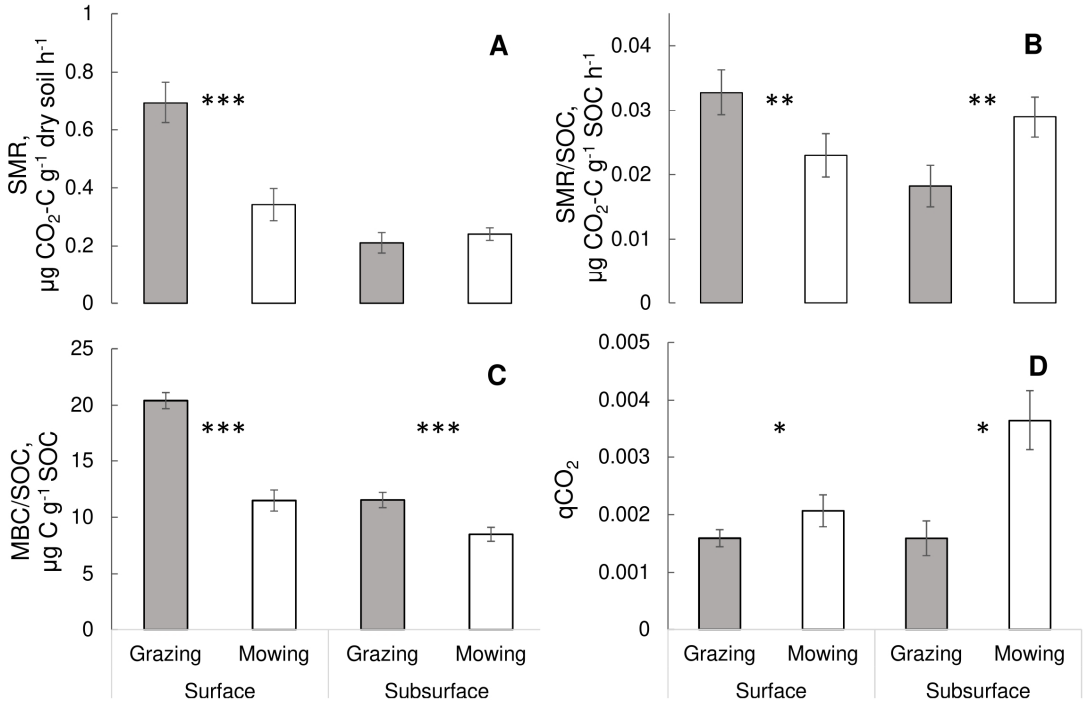
717 **Figure captions**



718

719 **Figure 1.** (A) Soil microbial respiration (SMR), (B) soil microbial respiration (SMR)
720 per soil organic carbon (SOC), (C) microbial biomass carbon (MBC) per soil organic carbon
721 (SOC) and (D) metabolic quotient (qCO_2) in soil under two grassland management practices
722 (grazing and mowing) in surface soil (0-10 cm) and subsurface soil (20-30 cm). Significant
723 differences between the treatments are indicated by *, ** and ***, representing probability at
724 the 5, 1, and 0.1% levels, respectively.

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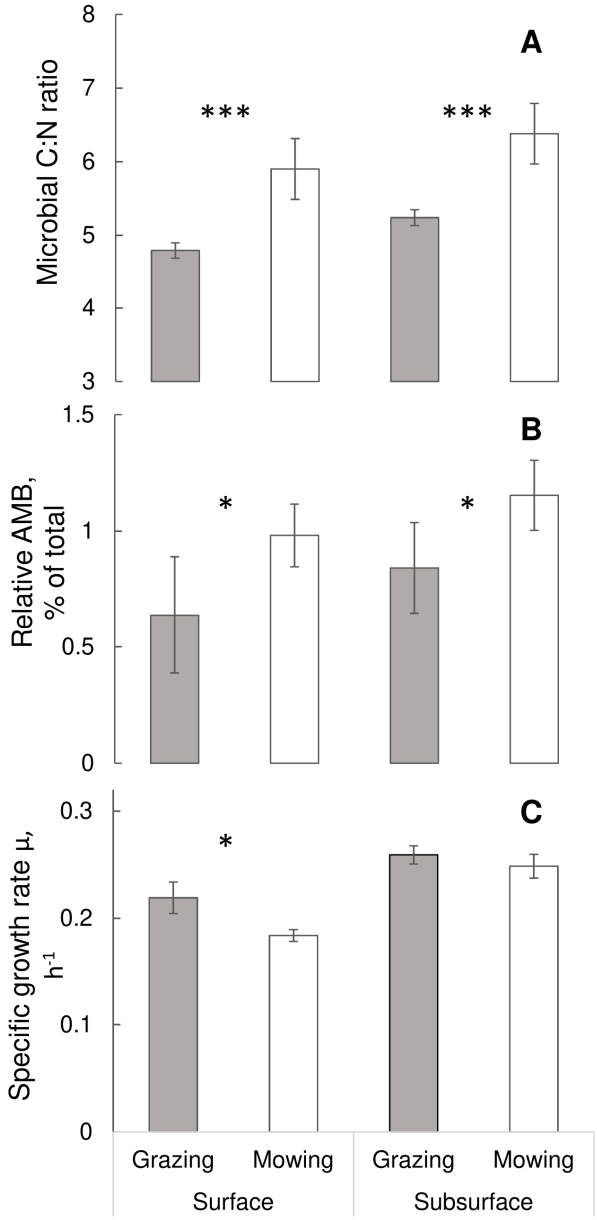

726

727 **Figure 2**. (A) Microbial C:N ratio, (B) the percentage of active microbial biomass
728 (AMB) and (C) specific microbial growth rate (μ) in soil under two grassland management
729 practices (grazing and mowing) in surface soil (0-10 cm) and subsurface soil (20-30 cm).
730 Significant differences between the treatments are indicated by *, ** and ***, representing
731 probability at the 5, 1, and 0.1% levels, respectively.

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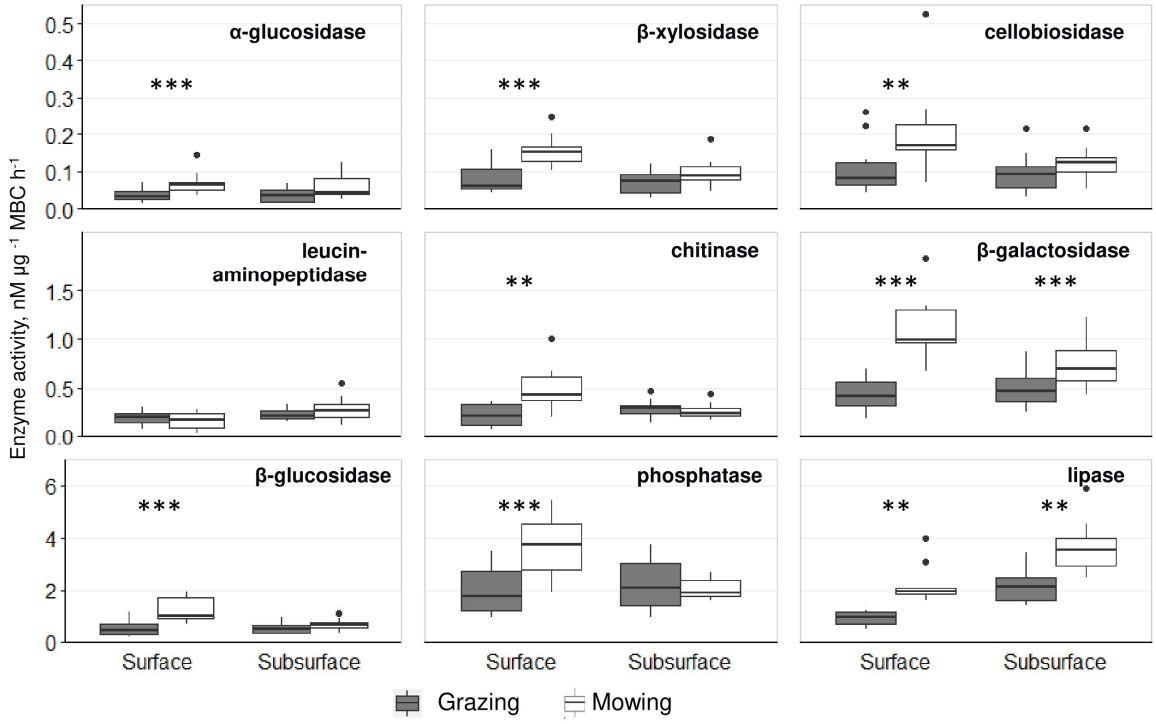

733

734 **Figure 3**. Boxplot of enzyme activity per unit of microbial biomass C (MBC) for nine
735 enzymes under two grassland management practices (grazing and mowing) in surface soil (0-
736 10 cm) and subsurface soil (20-30 cm). Significant differences between the treatments are
737 indicated by *, ** and ***, representing probability at the 5, 1, and 0.1% levels, respectively.

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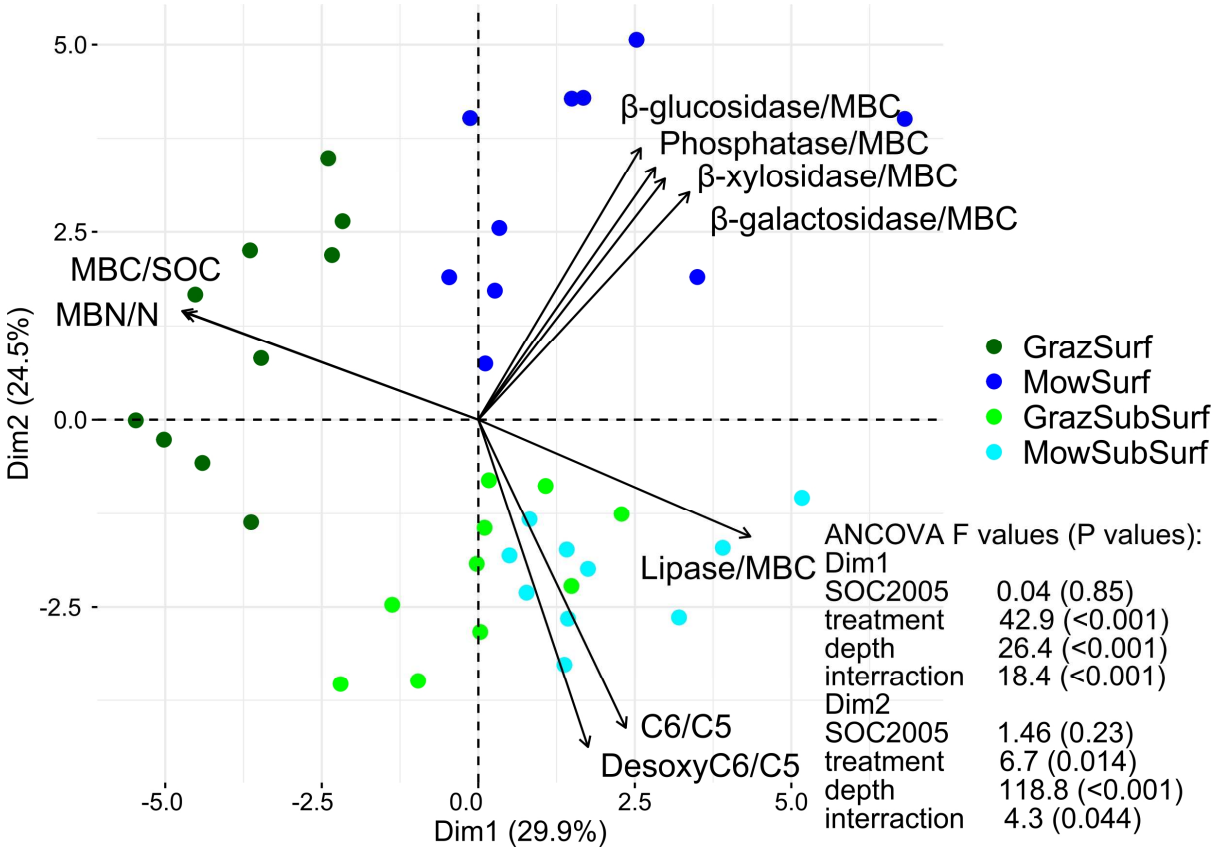

740

741 **Figure 4**. Principal component analysis (PCA) for soil under grazing and mowing in

742 surface soil (0-10 cm) and in subsurface soil (20-30 cm). Only variables with quality of

743 representation (cos^2) higher than 0.75 was shown on PCA plot.