Effect of mowing and grazing on soil organic matter quality and microbial functioning

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THESE

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The dictum of Leo Tolstoy from « Anna Karenina » saying “All happy families are all alike; each unhappy family is unhappy in its own way” was widely adopted in science : physics, zoology and microbiology (Zaneveld et al., 2017). I would like to adopt it to PhD studies and state that “Every finished PhD is happy and finished PhD” which is also applicable for my situation. But it would not be possible without supervision, help and support of many people around me during these three years.

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RESUME

Les prairies peuvent contribuer à l'atténuation du changement climatique par la séquestration du carbone organique dans le sol (COS). Cependant, l'ampleur de cette séquestration dépend des pratiques de gestion et des conditions pédoclimatiques. Le pâturage et le fauchage sont tous deux des techniques de récolte, mais leur effet sur le système plante-sol peut être différent. Dans ce contexte, l'objectif général de la thèse était de déterminer l'effet du pâturage et du fauchage sur la qualité de la matière organique du sol (MOS) et les processus biogéochimiques du sol dans des conditions pédoclimatiques contrastées. Pour cela, j'ai analysé les paramètres du sol et des végétaux en pâturage et en fauchage sur deux sites expérimentaux de SOERE ACBB à Lusignan et Clermont-Ferrand.

Mes résultats indiquent que les pratiques de gestion des prairies ont modifié la chimie des plantes, en particulier sa teneur en lignine, ce qui se traduit par une meilleure qualité de la litière végétale sous pâturage par rapport au fauchage. Cependant, la composition de la lignine du sol n'était pas liée à la composition de la lignine des parties aériennes et des racines, ce qui suggère que la lignine du sol est contrôlée par la décomposition microbienne. De plus, la gestion des prairies a influencé la quantité de la biomasse racinaire, qui contrôlait par conséquent le fonctionnement microbien. Les conditions pédoclimatiques ont déterminé les effets de la gestion des prairies sur le COS et l'azote: le pâturage a entraîné une teneur plus élevée en COS par rapport au fauchage sous un climat océanique tempéré, tandis que sous un climat semi-continental, les deux ont produit des teneurs en COS similaires à celles des prairies sans gestion particulier. Cependant, quelles que soient les conditions pédoclimatiques, le fauchage a conduit à une SOM plus dégradée et un fonctionnement microbien moins efficace par rapport au pâturage.

Pour conclure, le pâturage et le fauchage ont le potentiel d'augmenter la séquestration du COS, bien que le pâturage ait un plus grand potentiel dans les sols pauvres en C, ce qui peut s'expliquer par les effets contrastés sur les processus biogéochimiques du sol.

Mots clefs : prairie, pâturage, fauche, matière organique du sol, fonctionnement microbien

ABSTRACT

Grasslands can contribute to climate change mitigation through soil organic carbon (SOC) sequestration, however, the magnitude of SOC sequestration is dependent on the management practices and pedoclimatic conditions. Grazing and mowing are both harvesting techniques, but their effect on the plant-soil system may be different. In this context the general aim of the PhD was to determine the effect of grazing and mowing on soil organic matter (SOM) quality and soil biogeochemical processes under contrasting pedoclimatic conditions. To this end, I analysed soil and plant parameters in grazing and mowing at two experimental sites of SOERE ACBB in Lusignan and Clermont-Ferrand.

My results indicate that grassland management practices altered plant chemistry, in particular its lignin content, resulting in higher plant litter quality under grazing compared to mowing. However, the soil lignin composition was not related to shoot and root lignin composition suggesting that soil lignin is controlled by microbial decomposition. Moreover, grassland management influenced the root biomass, which consequently controlled microbial functioning. Pedoclimatic conditions determined the grassland management effects on SOC and N: grazing resulted in higher SOC content compared to mowing under temperate oceanic climate whereas under semi-continental climate both resulted in similar SOC contents as in unmanaged grassland. However, regardless of the pedoclimatic conditions, mowing led to more degraded SOM and less efficient microbial functioning as compared to grazing.

To conclude, both grazing and mowing have the potential to increase SOC sequestration albeit grazing has bigger potential in temperate oceanic climate, which may be explained by contrasting effects of grazing and mowing on soil biogeochemical processes.

Keywords: grassland, grazing, mowing, soil organic matter, microbial functioning

ABBREVIATIONS

(Ac/Al)s	= Syringyl acid/ syringyl aldehyde lignin monomers ratio
(Ac/Al)v	= Vanillyl acid/ vanillyl aldehyde lignin monomers ratio
aglu	= α-glucosidase
AMB	= Active microbial biomass
ANPP	= Aboveground net primary production
APBL	= Aboveground plant biomass leftover
Bare	= Bare fallow
bgala	= β-galactosidase
bglu	= β-glucosidase
C	= Carbon
C/V	= Coumaril/vanillyl lignin monomers ratio
C6/C5	= (galactose+mannose)/(arabinose+xylose) ratio
cello	= cellobiosidase
chit	= chitinase
Cler	= Clermont-Ferrand experimental site
DesoxyC6/C5	= (rhamnose+fucose)/(arabinose+xylose) ratio
DM	= Dry matter
GHG	= Greenhouse gas
GlcN/GalN	= Glucosamine/Galactosamine ratio
GlcN/MurN	= Glucosamine/muramic acid ratio
HGraz	= High intensity grazing
leu	= leucine aminopeptidase
LGraz	= Low intensity grazing
lip	= lipase
Lus	= Lusignan experimental site
Man/Xyl	= mannose/xylose ratio
MBC	= Microbial biomass carbon
MBN	= Microbial biomass nitrogen
MCP	= Microbial carbon pump
Mow	= Mowing treatment
N	= Nitrogen

NCP	= Non-cellulosic polysaccharides
OM	= Organic matter
P	= Phosphorus
PCA	= Principal Component Analysis
phosph	= phosphatase
qCO2	= metabolic quotient
S/V	= Syringyl/vanillyl lignin monomers ratio
SMR	= Soil microbial respiration
SOC	= Soil Organic Carbon
SOERE ACBB	= Observational and experimental centre for long-term research in environment – agroecosystems, biogeochemical cycles and biodiversity
SOM	= Soil organic matter
UM	= Unmanaged treatment
WoS	= Web of Science database
xyl	= xylosidase

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CHAPTER 1. INTRODUCTION

1.1. General introduction

Sustainable agriculture requires to keep balance between social needs and preservation of the environment. The main aim of sustainable agriculture is to meet the needs of growing population simultaneously considering the economic viability and environmental benefits of the agricultural strategies. The growing meat consumption will require increasing forage production through intensification of grasslands use, thereby, creating environmental risk in the form of greenhouse gasses (GHG) emission and soil degradation. Grasslands are in the main focus of soil scientists, because in addition to their socio-economic benefits, grasslands serve as a C sinks via their capacity to store stable soil organic carbon (SOC). However, the magnitude of SOC storage will be highly dependent on pedoclimatic conditions, the regime and intensity of grassland management practices in terms of harvesting through grazing or mowing and fertilizer use. Therefore, the study of different grassland management practices impact on soil processes should be carried out under similar pedoclimatic conditions. However, to account for ecosystem complexity similar grassland management practices should be studied under contrasting pedoclimatic conditions.

Most grasslands in Europe are managed by the practices aimed to feed domestic livestock either directly via grazing in the field or producing forage (mowing) (Conant et al., 2001; Rumpel et al., 2015). Grasslands covering about 40% of Earth's land surface are a significant contributor to the global carbon cycle. Although several meta-analyses have been conducted and identified the importance of climate, soil properties and grassland type on the grassland management intensity effect (Abdalla et al., 2018; McSherry and Ritchie, 2013), the information about biogeochemical processes is still scarce.

Here we focus on the two grassland harvesting practices: grazing and mowing. Both systems are considered in frames of permanent grasslands and are not separated in EU statistical reports. However, grazing is more common grassland management and covers bigger surfaces compared to mowing. Additionally, the importance of mowing lays in silage production. Both serve the same agricultural function of livestock feeding, but may have very different effects on the plant-soil system. The effect of grazing management on the plant-soil system is well-studied, but is known to give contrasting results depending on climate, soil texture and grassland type. Mowing effects on the plant-soil system received less attention. In consequence, there are

only few comparative studies of grazing versus mowing mainly addressing plant community composition (Tälle et al., 2016) and many knowledge gaps in terms of their effect on biogeochemical C cycling are remaining (see below).

In this PhD study, I focused on grasslands at two experimental sites with contrasting pedoclimatic properties located in France. I analysed plant chemistry and soil chemical and biological properties in order to determine the grazing and mowing impact on microbial processes involved in soil organic carbon dynamics.

Firstly, I carried out a literature review and determined the knowledge gaps concerning grassland management effect on SOM composition and turnover. I also focused on the microbial functioning due to growing interest in their contribution to SOM formation (Chapter 1). In the second Chapter I show to what extend grazing and mowing change the plant aboveground and belowground biomass chemistry and if these plant-related changes are reflected by SOM composition (Chapter 2). Chapters 3 and 4 investigated microbial functioning under grazing and mowing in two different environmental contexts: (1) natural grassland on sandy loam and C-rich Eutric Cambisol under semi-continental climate (Chapter 3) and (2) sown grasslands on loamy clay and C-low Dystric Cambisol under temperate oceanic climate (Chapter 4). In the general discussion, I identified general responses of SOM quality and microbial functioning to grazing and mowing regardless of the pedoclimatic conditions (Chapter 5). In this chapter, I also identified research perspectives and proposed how this PhD study results can be applied in different agricultural contexts.

1.2. Agriculture and climate change mitigation

By the end of the current century, it is predicted that the global mean temperature will increase by 2-7°C if the anthropogenic activity remains unchanged (IPCC, 2014; Wu et al., 2011). Agricultural activities release significant amounts of greenhouse gases (GHG) into the atmosphere, including CO_2, CH_4 and N_2O. Grassland practices such as nutrient management, grazing intensity and species biodiversity influence greenhouse gas emission and could potentially have mitigation effect on GHG emissions in particular through increasing carbon sequestration in soils (Smith et al., 2008).

1.2.1. Soil organic matter under grasslands

Organic carbon accumulates in soil in form of soil organic matter (SOM) composed of plant litter, animal residues at different stages of decomposition, soil organisms' cells, tissues and metabolites (Fig. 1). The largest part of SOM is C, however, other important constituting nutrients are N, P and S (Dungait et al., 2012).

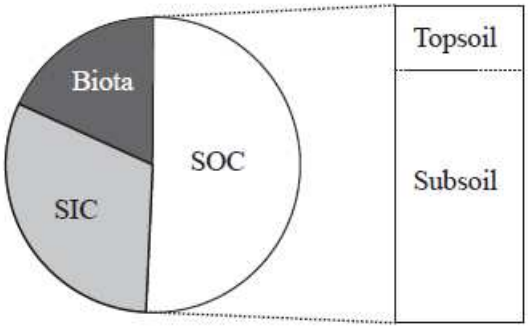


Figure 1. The global distribution of C between soil alive (soil biota) and dead part (soil inorganic C (SIC) and SOC). Source: Dungait et al 2012

Total SOC content is dependent on the pedoclimatic conditions: it increases with precipitation and clay content and decreasing temperature (Jobbágy and Jackson, 2000). Comparing SOC content among biomes differing by plant diversity does not necessarily change SOC, however, SOC content increases under higher plant diversity when it is compared within one biome (De Deyn et al., 2008).

The potential of C sequestration is linked to SOC and its saturation. Soil C saturation is defined by soil limits to C stabilization in three different SOC pools (biochemical, physical and mineral protection) (Six et al., 2002; Stewart et al., 2007). The dependency of SOC and C input

level has asymptotic nature: C storing capabilities of soil decrease by the increase of C input (Fig. 2).

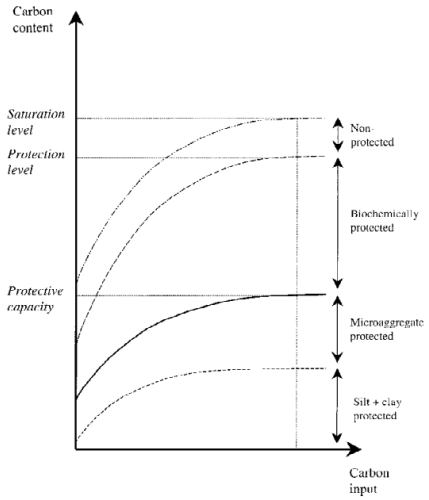


Figure 2. The protection and saturation capacity of soil. Source: Six et al 2002.

Due to varying SOC saturation levels in different soil types, the effect of grassland managements on SOC changes will differ as well and will probably be linked to grassland type, soil texture and microbial activity (Chenu et al., 2019; Frasier et al., 2019).

1.2.2. C sequestration under grasslands

Globally, the SOC stocks in grasslands is about 50-120 t C ha^{-1} in the first meter (Leifeld et al., 2005), whereas SOC storage in temperate grasslands is estimated about 170 Gt C in 0-3 m depths (Jobbágy and Jackson, 2000). C sequestration (C sink capacity) is the transfer of atmosphere C to stable SOC fractions, storing it securely and protecting it from release back into the atmosphere. The OC sequestration potential of world soils under temperate grasslands varies from 0.4 to 1.2 Gt C $year^{-1}$ depending on the climatic conditions and land management (Lal, 2004; Stockmann et al., 2013).

Soil C sequestration is related to SOC persistence and vulnerability. Primarily, recalcitrant plant-derived aromatic components (e.g. lignin-like) were considered as dominant contributors to SOC (Lützow et al., 2006; Thevenot et al., 2010), whereas, nowadays the components incorporated into microbial biomass are expected to result in more efficient SOM formation (Cotrufo et al., 2015, 2013). The latest proposition of SOC persistence is derived from the concept of functional complexity which is which is the interplay between spatial and temporal variation of molecular diversity and composition (Lehmann et al., 2020; Schmidt et

al., 2011). However, it seems both of lignin and necromass are important because SOM composition is linked to environmental and geochemical variables (Hall et al., 2020b).

In this PhD I focus on SOC content, biogeochemical and microbial properties of the soil systems, which can be directly or indirectly influenced by management practices and may be related to the SOC sequestration potential of grasslands.

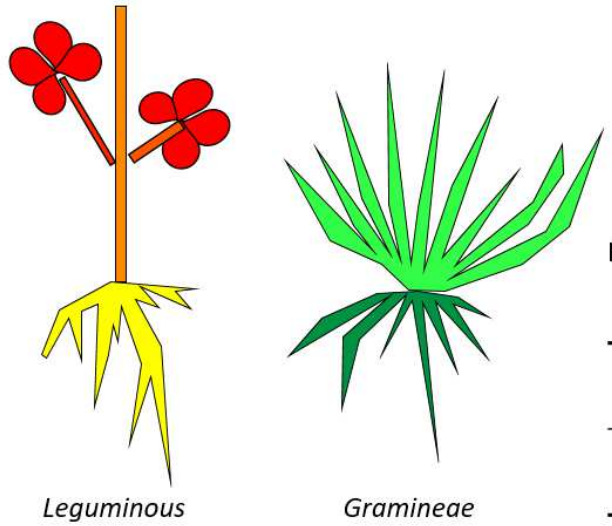
1.3. Biogeochemical composition and quality of organic C input in grasslands

Organic C input in grassland is derived from aboveground and belowground biomass input. However, in managed grasslands aboveground biomass is consumed or exported, whereas, belowground biomass contribution to OC input may increase due to defoliation effect on the increase of root biomass (Reeder et al., 2004) and root exudation (Hamilton et al., 2008). Consequently, belowground input comprises about 50-90% of grassland annual net primary productivity (Piñeiro et al., 2010; Ziter and MacDougall, 2013). Additionally, under grazed grasslands, 20-40% of aboveground intake is returned as dung (Soussana et al., 2006).

1.3.1. Physiological traits of plants

Plant traits (green leaf traits or chemistry) may be related to soil properties and ecosystem functions. Plant traits such as leaf/litter/root quality and chemistry were stronger controlling factor of decomposition rates than pedoclimatic conditions (Cornwell et al., 2008; Orwin et al., 2010). Based on a global database of plant traits (TRY) more than 2000 plant traits characterising plant life cycle were recently determined (Kattge et al., 2011). However, for soil scientist the most important traits are structure-related traits (lignin, C, dry matter) and nutrient-related traits (N, P, pH, phenols), which mainly influence tissue decomposability (Freschet et al., 2012). Plant tissue quality is mainly expressed by C:N and lignin-to-N ratio: higher values of the ratios indicate low tissue quality and low decomposability whereas lower values of the ratios indicate high tissue quality and high decomposability (De Deyn et al., 2008).

The factors likely causing changes in litter quality are the plant growth strategy, the development stage and stress adaptation to disturbance, for example due to grassland management activities. Fast growing plants tend to have higher tissue quality and produce more exudates, whereas slow-growing plants contribute to C input with nutrient-poor litter (De Deyn et al., 2008). Lignin concentration increases with increasing plant maturity, and may also be higher in senescent plant litter (Abiven et al., 2011; Jung and Casler, 2006; Sanaullah et al., 2010). Moreover, the tissue quality differs within the plant organs (Fig. 3). Irrespective of the plant species, roots have often higher C:N ratio compared to the leaves (Yang et al., 2018). Based on the tissue quality, the decomposability of plant organs decreases in the order of leaves>fine stems>fine roots>coarse stem (Freschet et al., 2012).



C:N ratio

High quality Low quality

Plant family	C:N ratio	
	Shoot	Root
Leguminous	10-14	17-26
Gramineae	26-45	33-46

Figure 3. The plant tissue quality based on the C:N ratio differing within plant organs in Leguminous and Gramineae. Source: Crème et al., 2016; Whitehead et al., 1979

1.3.2. Aboveground plant input

Aboveground plant input consists of leaves, stems, and reproductive organs. Under managed grasslands it may occur due to trampling or tillage application during some grassland management practices.

The major components of aboveground plant tissue are polysaccharides (45-57%), lignin (10-25%) and 2-15% raw proteins (Kögel-Knabner, 2002). The soluble components in plant biomass is about 29-52% depending on the plant species (Garland, 1992). The main polysaccharides are cellulose, hemicellulose and pectin. The non-cellulosic polysaccharides of plant cell walls are mainly presented by hemicelluloses. In dicotyledonous plants the main hemicelluloses are xyloglucans, being principally composed of glucose and xylose. In monocotyledons and in leguminous leaves and stems, the main hemicelluloses are arabinoxylans.

The litter chemistry of aboveground input differs based on the species. Leguminous plants have higher quality of litter compared to graminoids due to their possibility to symbiotic association with N_2-fixing bacteria which allows to overcome N limitation in soil (Dovrat et al., 2020).

1.3.3. Belowground plant input

Belowground plant input is composed of belowground plant organs (roots, rhizomes) and components released by living roots (exudates). 80-90% of roots are located in the first 30 cm

in temperate grasslands and belowground primary production accounts for 60-80% of total primary production (Jackson et al., 1996). During one vegetation period, grass plants allocate the C into belowground in total at about 1500-2200 kg C ha^{-1} (roots and root exudates) (Kuzyakov and Domanski, 2000). Root exudates present about 10-20% of the total belowground C allocation (Grayston et al., 1996; Jones et al., 2004). The composition of root exudates is characterised by low molecular weight compounds such as amino acids, organic acids, sugars, phenolics, and by high-molecular weight compounds, such as mucilage (polysaccharides) and proteins (Bais et al., 2006).

Roots are less decomposable due to higher chemical recalcitrance (Rasse et al., 2005), because root organs have more lignin with higher contribution of cynnamyl moieties compared to other organs (Abiven et al., 2011).

1.3.4. Animal-produced C inputs

Dung is animal-produced input in grasslands. However, dung is plant-originated but transformed by ruminant digestion. Dung contains 80% of soluble carbohydrates in the form of free sugars, oligopolysaccharides and aminosugars; 7% of lignin, 12 % crude protein and 3-5% of fats (Dungait et al., 2010, 2005). The C decomposability in dung depends on the herbivore diet (Ajwa and Tabatabai, 1994). The 75% of dung in the grassland is incorporated/mineralised between one month to one year (Dungait et al., 2005).

Another particular animal-produced input in grasslands is urine. The N concentration in cattle urine is about 8.0 g l^{-1}. Urea is a dominant constituent of urine and contributes to total N at rate more than 50% (Dijkstra et al., 2013).

1.4. OC decomposition, transformation and stabilisation in grasslands

The understanding of SOC cycling is possible if the soil processes are investigated on all three levels: input, transformation and stabilization. Based on the concept of functional complexity concept the grassland managements should be designed by introduction of various practices (plant mixtures, amendments, perennial vegetation) which in turn should be adjusted depending on the pedoclimatic conditions (Hall et al., 2020b; Lehmann et al., 2020).

1.4.1. Degradation processes driven by microorganisms

The soil microbial community is presented by diverse groups of archaea, bacteria, fungi and protozoa (Martiny et al., 2006). However, the magnitude of participation in soil biogeochemical cycles will depend on their activity, biomass and community structure (Joergensen and Wichern, 2018; Strickland and Rousk, 2010).

The widely used categorization of soil microbial community is the division into fungi and bacteria. The importance of this categorisation lay in the differences of these groups in the decomposition pathway and physiology (Six et al., 2006; Strickland and Rousk, 2010). Fungi are characterised by filamentous growth resulting in higher biomass increasing the contact surface in the environment and, consequently, are more adapted to nutrient-poor ecosystems than bacteria (Rousk and Bååth, 2007). The fungal communities differ in their physiology as well: saprotrophic fungi predominate in litter-rich environment contributing better to C cycle, whereas ectomycorrhizal fungi predominate in deeper layers of soil and mobilise N (Hobbie and Horton, 2007). Fungi and bacteria also differ by their stoichiometry, fungi have higher biomass C:N ratio than bacteria. Because of this, fungi have lower nutrient requirements (Strickland and Rousk, 2010).

Four activity (physiological) states of microorganisms were determined lately: active, potentially active, dormant and dead (Blagodatskaya and Kuzyakov, 2013). Active microorganisms contribute only 0.1-2% of the total microbial biomass. They are involved in the nutrient transformation processes (Hobbie and Hobbie, 2013). Microorganisms in potentially active state are maintaining their reduced metabolism to be able to switch into active state within very short time period. When the substrate availability is limited and under unfavourable environmental conditions the microorganisms switch to dormant state

Activity of microorganisms can be measured indirectly via several parameters, such as basal respiration (CO_2 efflux), enzyme activities, and growth kinetic parameters. Basal

respiration provides evidence about the total catabolic use of substrates whilst basal respiration per microbial biomass (metabolic quotient qCO_2) demonstrates the age and the stress of microbial populations (Anderson, 2003; Joergensen and Emmerling, 2006). Extracellular enzyme activities reflect more specifically the processes related to C, N, P and S cycling (Nannipieri et al., 2003). Extracellular enzymes depolymerize organic compound to soluble forms and transform them into metabolizable compounds. The production of extracellular enzymes by active microorganisms is energy consuming. It seems that extracellular enzyme activity is controlled by abiotic (pH, soil moisture, temperature) (Burns et al., 2013) and biotic factors (substrate quality, plant community composition) (Chuan et al., 2020; Hewins et al., 2015), which may be affected by grassland management practices. The effect of grassland management itself can be contrasting and unpredicted. This is why extracellular enzyme activities respond to grassland management practices (e.g. to grazing) differently depending on the plant community composition and the intensity of grassland management (Cui and Holden, 2015; Hewins et al., 2015; Stark et al., 2015; Xu et al., 2017).

1.4.2. Microbe-derived compounds in soil

Microbes promote SOM accumulation by producing more stable and diverse components (Kallenbach et al., 2016). The higher C use efficiency and increased turnover of microbial biomass can result in the accumulation of microbial residues in soil (Cotrufo et al., 2013). Because plants do not produce amino sugars and amino sugars in soil are not easily degraded, amino sugars can be used as the indicator of bacterial and fungal residues. About 26 microbial amino sugars were identified, allowing to determine the ones specific to bacteria and fungi. Muramic acid (MurN) is an exclusive bacterial component whereas glucosamine (GlcN) is a fungal component. Galactosamine (GalN) and mannosamine (ManN) are amino sugars, which can be produced by both groups and their origins are still debated. Based on this, the ratios GlcN/MurN and GlcN/GalN may indicate the origin of microbial residues. However, GlcN/GalN is still indefinite to interpret, but due to the highest recalcitrance of GalN among other amino sugars this ratio may indicate the accumulation of amino sugars in soil (Liang et al., 2015).

The microbial residues were lately identified as a source for SOM formation (Kallenbach et al., 2016; Kögel-Knabner, 2017; Miltner et al., 2012). The conception of “microbial carbon pump” proposed by Liang et al 2017 elucidates the microbial processing of plant-derived OC, which leads to SOC stabilization (Fig. 4). There are two pathways of these processes: *ex vivo*

modification and *in vivo* turnover based on the microbial anabolism/catabolism. Once the organic matter passed through this microbial C pump it is less disposed to degradation because of their chemical structure and ability to be sorbed to mineral surfaces as compared to unchanged plant originated material. This mechanistic understanding of microbe-driven SOM stabilization may be used to explain the effects of human disturbance in soil systems.

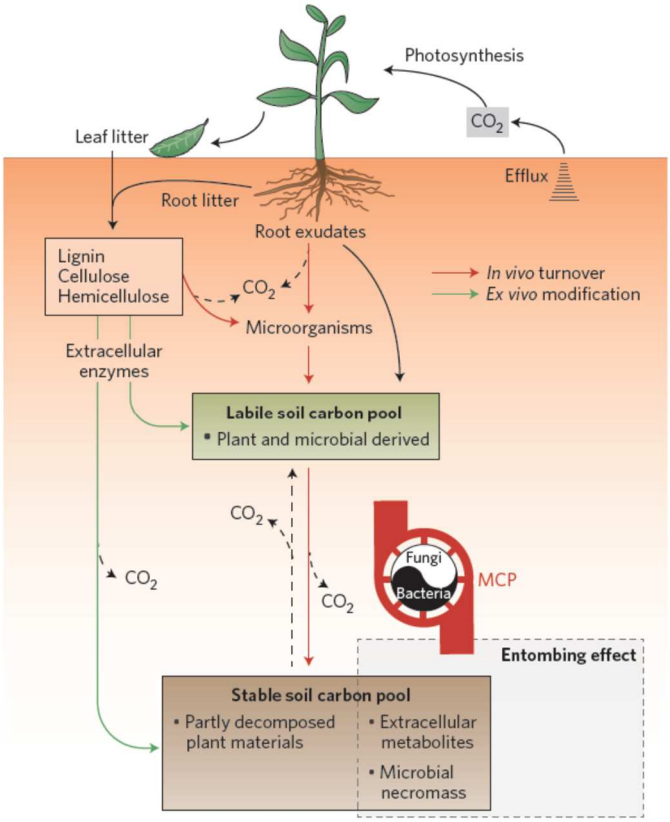


Figure 4. The microbial metabolic processes in plant-soil system involving microbial C pump (MCP). Source: Liang et al., 2017

Because microbial necromass is recalcitrant, the investigation of how grassland management impacts the microbial necromass could reinforce the understanding of SOC accumulation. It was already shown that under grasslands the amino sugars contribution to SOC increases when the contribution of lignin decreases (Ma et al., 2018) because the pedoclimatic properties influence on amino sugars and lignin contribution to SOM differently (Hall et al., 2020b).

1.5. Effects of grassland management on processes affecting soil organic carbon

Forage harvesting in grassland occurs through grazing or mowing. These two grassland management practices serve the same function, however, the organisation is different. Grazing involves livestock presence in the grassland whereas under mowing the grass is mowed and transported to cowsheds. Mowing differs from grazing mainly by the more homogeneous impact on the plant-soil system and absence of return of animal dejections. In other words, under mowing the defoliation is full plant removal and occurs in a short time (1 day); the nutrient input is homogeneous through inorganic fertilisation. Whereas under grazing the defoliation is selective and occurs during long period of time (several days or weeks); the nutrient input is heterogeneous because of urine and dung patches. These differences between grazing and mowing may alter plant-soil system in different ways.

Grazing and mowing may influence on soil biogeochemical processes directly and indirectly. Direct impact of grazing and mowing may occur via soil compaction and fertilisation, whereas the indirect effect is via plant physiology alteration.

1.5.1. Mowing

Mowing is a grassland management practice, which removes the plant aboveground biomass at a height of about 5-6 cm at once. This treatment can be also called “hayed”, “defoliation”, “meadow” and “silage production”. When mowing treatment is simulated, it can be called “defoliation” or “plant clipping”. Less often simulated grazing can be called “mowing” (Ziter and MacDougall, 2013). The effect of mowing studied under laboratory conditions is usually called as clipping. Hereafter, in this study we use “mowing” to indicate the grassland management, whereas we use “defoliation” to indicate the plant removal process (which also happen under grazing).

The grassland practices applied under mowing treatment include fertilisation, lime application and irrigation. Mowed grasslands can be natural and sown. The impact of mowing management can be divided into three components: defoliation, fertilisation and soil compaction.

Mowing impact on plant community and physiology

Plant defoliation under mowing induces the increase of plant exudation in short-term (Bazot et al., 2005; Hamilton et al., 2008). However, the root production and quality response to mowing is not always the same. Root biomass can increase but with no changes in quality (Ziter and MacDougall, 2013) and it can also stay stable in response to mowing with increasing quality (Bazot et al., 2005; Medina-Roldán and Bardgett, 2011). It seems that the different responses are related to different plant species: graminoids respond by decreasing or not responding whereas leguminous plants tend to increase the root biomass (Schmitt et al., 2013). At the same time, mowing together with fertilisation decreases the plant community biodiversity by selecting the species with wider ecological niche (graminoids) (Zechmeister et al., 2003). The abandonment (cessation) of mowing treatment can also results in the biodiversity decrease due to replacement of grass vegetation with shrubs and trees (Louault et al., 2005; Sienkiewicz–Paderewska et al., 2020).

Mowing impact on soil microbial functioning

Mowing impact on soil microbial functioning is mainly based on plant clipping effects, which increases microbial biomass and C use efficiency due to rapid root exudates release (Gavrichkova et al., 2008; Uhlířová et al., 2005). Plant clipping decreases soil C mineralization in short-term and favours bacterial communities (Shahzad et al., 2012; C. J. Zhang et al., 2018). There is little information about mowing impact on the microbial activity, probably because mowing impact is mainly affecting the microbial activity indirectly through plant responses.

1.5.2. Grazing

In contrary to mowing, grazing impact is generally more complex. This is because additionally to removing intact components (defoliation), herbivores release patchy dung and urine input into soil and altering soil compaction by trampling (Bardgett and Wardle, 2003). Grazing effects vary by intensity, animal type, and regime. Grazing occurs in a longer time and its impact is less homogeneous compared to mowing.

The changes in SOC storage by grazing are driven by changes in net primary production, N storage and changes in decomposition processes (Piñeiro et al., 2010). Previous studies found that the effect of grazing intensity is dependent on climate and grassland type. Regardless of the intensity, SOC storage under grazing increases in moist warm climate and in moist cold climate. Moreover, C4-dominated grasslands has bigger potential to increase SOC storage

compared to C3-dominated grasslands because C4-plants compensate grazing impact by having many rhizomes and other storage organs (Abdalla et al., 2018). However, climate impact is also dependent on soil physicochemical parameters: at high precipitation, clay soils face negative effect of grazing whereas on coarse textured soils grazing may have positive effect on SOC (McSherry and Ritchie, 2013).

Grazing impact on plant community and physiology

Plants respond to grazing at two levels: at individual level by changing the physiology and at community-level by changing the diversity because of defoliation-resistant plants selection (Bardgett et al., 1998) (Fig. 5). On the one hand, physiological changes may increase the plant biomass C:N ratio (Semmartin et al., 2008). On the other hand, animals alter the plants community composition in two ways: (1) consumption of dominant plant species and, thus, increasing the plant biodiversity (Olff and Ritchie, 1998) and (2) preferential consumption of palatable plants, i.e. with low C:N ratio, consequently, plants with high C:N ratio are expected to dominate and further contribute to input (Bardgett et al., 1998). Based on a meta-analysis, grazing in general induces the increase of aboveground plant quality (He et al., 2020; Heyburn et al., 2017). However, the belowground plant parts responded to grazing differently and resulted in either higher (Heyburn et al., 2017) or lower quality (He et al., 2020). Nutrient input presented by dung and urine and grazing impact of plant stoichiometry changes might explain SOC storage alterations (Heyburn et al., 2017; Poeplau et al., 2018).

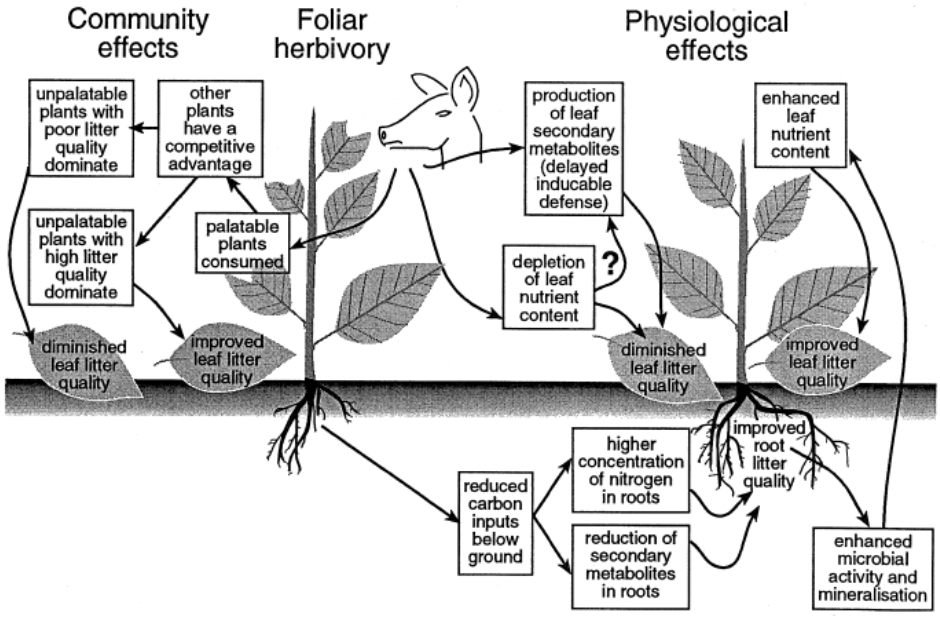


Figure 5. Schematic image of plant-microbial response to grazing. Source: Bardgett et al 1998

Physiologically plants respond to moderate grazing by increasing the root biomass and consequently resulting in the increase of belowground deposition (Wilson et al., 2018; Zhan et al., 2020). However, root biomass may also decline in short term after grazing treatment application (Klumpp et al., 2009) as well as in a long-term experiment regardless of the intensity (Li et al., 2018).

Grazing impact on soil microbial functioning

First of all, soil microbial parameters are more sensitive than plant traits and respond to grazing faster, which may be explained by direct effect of dung and urine inputs (Attard et al., 2008). In a longer-term, plant changes driven by grazing affect soil microbial activity.

Bardgett et al., 1998 proposed a model of grazing effects on decomposition pathways. The model indicates that heavy grazing will promote fast cycles with labile substrates and bacteria-dominated community whereas light grazing or the cessation of grazing will favour slow cycles because of more resistant substrates and fungi. However, investigations of this conceptual model were contradictory, as they either supported (Oates et al., 2012; Xu et al., 2017) or rejected (Bagchi et al., 2017; Ingram et al., 2008) this model.

Thus, while effects on plants are well studied, it is not entirely clear how grazing affects soil biogeochemical processes. These effects are, however, important, as microbes are fast-reacting to environmental changes, and their effect on global C balance could be crucial.

1.5.3. Grazing versus mowing

In order to find the articles focusing on the comparison of grazing versus mowing I used the database Web of Science (WoS) by searching "grazing mowing". The search results consist of 1084 articles in total (Fig. 6). The distribution between the research areas showed that comparison of grazing versus mowing is mainly plant-focused. Based on a meta-analysis of plant biodiversity affected by grazing versus mowing, the study concluded that grazing has higher conservation value compared to mowing but the effect size is also dependent on the grassland type (Tälle et al., 2016). Studies, which focused on soil biogeochemical functioning of grazing and mowing in different pedoclimatic environments are scarce.

In order to examine only in soil parameters, I, thereafter, chose the recordings referring to "Soil science "and "Environmental sciences" giving in total 282 research articles.

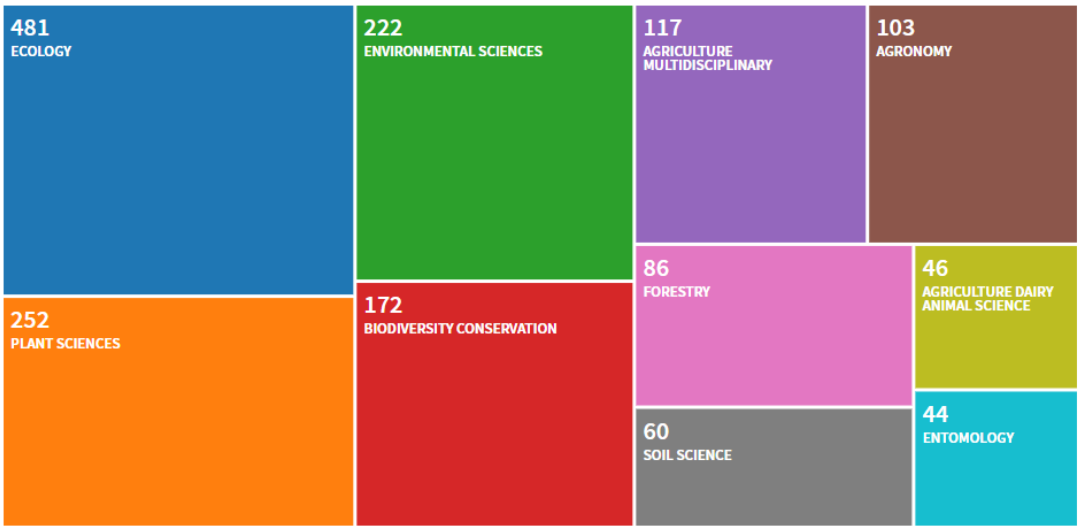


Figure 6. The tree map of search results in WoS by the keywords "grazing mowing", in total 1084 articles. Source: webofknowledge.com

As plant-focused studies may also refer to "Soil science" and "Environmental sciences", I added the criterion "microbial" in order to narrow the search. After application of these criteria, there were only 14 articles published during 2004-2020 presented in the Table 1 (three were excluded because of review article on grassland management impact (Rumpel et al., 2015); litter degradation experiment not related to grazing and mowing (Sanaullah et al., 2010); and the article from this thesis). Finally, there were only six articles truly investigating grazing versus mowing effect on soil biogeochemical properties and the rest of articles was excluded due to simulation of grazing/mowing. Because I was interested only in the experiments carried out under the same pedoclimatic conditions, only three articles met this requirement (Gong et al., 2014; LIU et al., 2017; Wang et al., 2020). Albeit these studies were all located in one geographical zone (China), grazing animals only included sheep or yaks. Based on this, no dairy cows or beef were presented in these grazing versus mowing studies. However, dairy cows and beef are dominating grazing animals under temperate climate in most other parts of the world.

Table 1. The search results on the articles matching the criteria in WoS: "grazing mowing" in "Soil science" and "Environmental science" areas and refined by "microbial".

N	*Authors*	*Country*	*Grazing treatment*	*Mowing treatment*	*Measured parameters*	*Pedoclimatic conditions*
1	*Tian et al 2004*	USA	simulated grazing	simulated mowing	MBC; denitrifier and nitrifier communities	different
2	*Kohler et al 2005*	Switzerland	simulated grazing	simulated mowing	Plants, CLPP	different
3	*Robson et al 2007*	France	light grazing (cattle and sheep)	mowing (+/- fertilisation)	Available soil N, N-cycle enzymes	different
4	*Gavrichkova et 2008*	Italy	grazing mixed with mowing	mowing once a year	MBC, soil C mineralization potential	Haplic Phaezem
5	*Olofsson 2009*	Sweden	-	simulated mowing	Plants, N mineralization, soil t°	different

6	*Shahzad et al 2012*	France	-	simulated mowing	Soil respiration, PLFA	Cambisol
7	*Morris et al 2013*	Germany	cattle/sheep , different intensities	mowing 2-3 per year	AMF, plants	different
8	*Gong et al 2014*	China	sheep light grazing	mowing once year	Soil, root respiration, litter quality, soil t°	sandy loamy chestnut soil
9	*Herold et al 2014*	Germany	cattle/sheep , different intensity	mowing 2-3 per year	Soil N and P, PLFA, plants	different
10	*Shengjie et al 2017*	China	yak seasonal grazing	mowing once a year	Soil fauna, MBC, plants	subalpine meadow soil
11	*Jozefowska et al 2018*	Poland	-	mowing (+/- fertilisation)	Soil fauna, MBC, dehydrogenase activity, microbial community	Eutric Cambisol
12	*Zhang et al 2018*	China	-	mowing (+/- fertilisation)	N-cycle enzymes, ammonia oxidifier and denitrifier communities	Chestnut soil
13	*Zaitsev et al 2018*	Germany	extensive cattle grazing	mowing (several times per year)	Soil fauna and soil respiration	different
14	*Wang et al 2020*	China	sheep grazing at 2 rates	mowing once a year	Soil N and P, plants, phosphatase and urease activities, microbial community	Sandy loam chestnut soil

The studies from China with yak or sheep indicated that grazing versus once-per-year mowing resulted in lower microbial biomass and less diverse soil fauna (LIU et al., 2017). Contrastingly, moderate grazing compared to mowing and to cessation of grazing led to higher plant and microbial biodiversity (Wang et al., 2020). The study of Gong et al. 2014 indicated that the effect of grazing versus mowing on soil respiration was dependent on precipitation.

From this literature survey it became clear that there were a lot of published articles, which were focused separately on grazing and on mowing, but there were only few comparative studies focusing on grazing and mowing simultaneously. However, the comparison of these two management practices using dominant grazing animals in the temperate climate is important, because their impact to SOC storage and soil biogeochemical processes may be contrasting.

1.6. Objectives and Scientific questions

This study focuses on the investigation of the grassland management effects on soil organic matter (SOM) quality and microbial functioning under contrasting pedoclimatic conditions. Particularly I studied (1) aboveground and belowground plant stoichiometry and chemical composition as potential OC input quality indicators under grassland and (2) biogeochemical soil and microbial parameters as indicators of biogeochemical processes.

My general objective was to determine the effect of grazing and mowing on SOM composition and soil biogeochemical processes under two pedoclimatic conditions being characterised by OC-poor soil under temperate oceanic climate and OC–rich soil under semi-continental climate.

The first part focused on plant input quality impacted by management under natural grassland. The specific objective was to investigate the management impact on the quality of aboveground and belowground plant organic matter under 4 grassland management practices.

I addressed the following questions:

(1) how does aboveground and belowground plant quality and chemistry respond to abandonment of grassland management?

(2) does aboveground and belowground plant quality and chemistry respond similarly to grassland management practices?

(3) do the differences in plant chemistry reflect the differences in soil chemistry among differently managed grasslands?

The second chapter is focused on the comparison of light intensity grazing versus mowing effects on soil organic matter and microbial parameters under sown grassland. I aimed to evaluate the differences in soil biogeochemical cycling under grazing and mowing at two depths (0-10 and 20-30 cm).

To distinguish the differences, I addressed the following questions:

(1) does SOM content and biogeochemical composition differ between grazing versus mowing?

(2) if it differs, is it related to microbial-driven processes (changes in microbial activity and physiology)?

(3) does grassland management affect soil properties below the surface horizon?

The third part is focused on the study of microbial functioning influenced by grazing and mowing under natural grassland. The grassland managements present a gradient of aboveground plant biomass input in grassland: unmanaged < light grazing < high grazing < mowing < bare fallow. In this system, I aimed to determine the response of SOM quality, degradation processes and microbial functioning using the gradient of aboveground plant biomass input resulting from contrasting grassland managements. Particularly, the following questions were investigated:

(1) does aboveground plant biomass input quantity controls SOC content?
(2) how does microbial contribution to SOC respond to grassland management and is it related to SOC content and aboveground plant biomass input?
(3) is microbial functioning dependent on aboveground plant biomass input or driven by other factors?

The last chapter synthesizes all data in order to unravel the differences of soil functioning and potential SOC sequestration capabilities between grazed and mowed soils. Moreover, I aim to determine which soil chemical or microbial properties are responding similarly to grassland managements regardless of pedoclimatic conditions. This chapter also contains some future research perspectives.

MATERIALS AND METHODS

1.7. Study sites

1.7.1. Experimental site SOERE ACBB in Theix (Clermont-Ferrand)

Site description

The study was conducted at the national long-term experimental observatory SOERE ACBB (Agroecosystems, Biogeochemical Cycles and Biodiversity), which was setup in 2005 on permanent grassland area, in the Massif-central region in France. Climate at the site is semi-continental 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 was managed by a mixed regime of fertilized mowing and grazing. The soil type at the site is an Cambisol which developed on granitic bedrock. Each grassland management practice has four independent field replicates representing two blocks under slightly different soil characteristics (Eutric Cambisol and Colluvic Cambisol). Because we wanted to avoid the effect of edaphic conditions, we focused only in one block with two field replicates of each grassland management practice of Eutric Cambisol. We have chosen this block due to its higher homogeneity and location on a flat landscape. The initial soil general parameters before the beginning of experiment are presented in the table 2.

Table 2. Initial soil general parameters before beginning of the experiment in 2005.

Treatments	pH	SOC content mg g^{-1}	N content mg g^{-1}	C:N ratio
Aband	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
Mowing	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

We focused on the grassland management practices: three grassland management practices (low and high intensity cattle grazing, mowing), positive (abandoned) and negative (bare) control plots. At the unmanaged site, all plant biomass is returned to soil and this treatment may thus be considered as a positive control. In grazing and mowing systems, plant biomass is exported at increasing level. As a negative control we consider bare soil.

The study focused on four treatments corresponding to different utilization of the grasslands, with mowing fertilized, grazing by cattle at high or low intensities and abandon. In plots under mowing, there are three cutting events per year and NPK fertilization to allow high nutritional status to plant biomass (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, plots were rotationally grazed during the same times five 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 same duration of grazing. Plots under abandon were not used at all (cessation of management since 2005). In addition, a bare soil 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 abandoned and mowing treatments) and 30 m^2 for bare soil.

The grassland management practices may be placed along two disturbance gradients based on: (1) aboveground plant biomass input (APBL) and the gradient of the belowground input quality. The level of APBL presents plant material (i.e. shoots, stubble, litter), which is left after disturbance the events (grazing and mowing). APBL present the difference between the aboveground net primary production (ANPP) and the used biomass by grazing or mowing (Table 3). However, under mowing there is loss of plant biomass during mechanical removal comprising 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.

Table 3. Aboveground net primary production and aboveground plant biomass leftover

Treatments	Aboveground net primary production (ANPP)	Used biomass	Used	Aboveground plant biomass leftover (ARBL)
	t ha^{-1} $year^{-1}$	t ha^{-1} $year^{-1}$	%	t ha^{-1} $year^{-1}$
Aband	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
Mowing	9.01±0.23	9.01±0.23	100	0
Bare	0	0	0	0

The ANPP was measured for mowing and grazing managements 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 in each replicate plot. 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, fence was placed to avoid animal defoliation. ANPP, in g DM m^{-2} $year^{-1}$, is the sum of the successive biomass accumulation along the year. The harvested biomass was estimated in grazed plot based on the daily animal intake, which was calculated accordingly to animal live weight and the number of animal grazing days per year per plot. In mowed plots, harvested biomass was based on the harvested forage yield. For unmanaged and bare soil treatments, the harvested biomass was set to zero (0). According to APBR we conceptualized the first gradient in the order of **Abandoned < low intensity grazing < high intensity grazing < mowing < bare** (Fig. 7, 8.).

Figure 7. Conceptual picture of treatment placement based on the disturbance gradients of aboveground plant biomass leftover.

Figure 8. Photographs of grassland management practices at experimental site of Clermont-Ferrand. Gilmullina, 2018

Soil and plant sampling

The sampling was conducted only once before the last grazing event in order to avoid the short-term effect of grazing. The sampling days was chosen to have similar weather and field

conditions as it was during the first sampling in Lusignan. In late October 2018, soil was sampled at each field replicate at 3 points (about 10 m apart) resulting in 2 replicate samples per treatment except bare soil. For bare treatment we sampled soil only at 2 points (n=1) because due to plant removal the soil is more homogeneous and the plot size was not enough large. Each pseudo-replicate was analysed separately and then the mean of pseudo-replicates was used as a real replicates. The differences in replicate numbers between the treatments 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 plant aboveground (shoot and litter) and belowground biomass (roots and rhizomes) and two fine soil subsamples: i) subsample for physico-chemical analysis (air-dried), and ii) subsample for microbial analyses (stored at 4°C during two months). Plant belowground biomass materials (roots and rhizomes) were dried a 60°C. Prior to microbial analysis, soil samples were pre-incubated at 22 °C for 7 days. The particularity of field sampling is the impossibility of direct measurement: experimental sites are located enough far, the samples need to be transported and stored during the transportation time. Another difficulty is related to the fact that biogeochemical analysis requires homogeneous soil samples, this is why soil samples are ground and sieved. These limitations can cause some artefacts but it should not influence to the differences between treatments because all soil samples are treated similarly.

Plant aboveground materials were removed by scissors from the soil cores. Dead material was separated and presented the aboveground litter. Additionally, during root washing the particular organic matter with the size bigger than 5 mm was also referred to litter. Dung was collected from the grazing plots and they were characterized by different stage of degradation.

1.7.2. Experimental site SOERE ACBB in Lusignan

Site description

The field experiment is located in Lusignan (southwest of France, 46°25'12,91"N; 0°07'29,35"E, Fig. 9) 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).

Figure 9. Photograph of experimental site in Lusiganan. Gilmullina, 2017

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 show 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 system, 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. The study also showed partitioning of the field into different zones with SOC gain and loss (A. Crème, personal communication; Fig. 28, Supplementary materials in Annexes.).

Soil sampling

The sampling was conducted only once before the last grazing event in order to avoid the short-term effect of grazing.. 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. The choice of depths was related to the fact that surface soil has direct contact with grazing and mowing whereas subsurface soil would show indirect effect of grassland management. 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 microbiological analysis, soil samples were moistened by distilled water to adjust 50% of WHC and pre-incubated at 22 °C for 7 days.

1.8. Methods

1.8.1. General soil parameters

pH, C and N content

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) content of soil and plant samples was 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.

1.8.2. Chemical parameters

Lignin concentration and composition

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

The total lignin content (mg g^{-1} dry soil) in the sample was determined as the sum of phenolic oxidation products: vanillyl (V), syringyl (S) and p-coumaryl (C) in their acid (Ac), aldehyde (Al) and ketone forms. Lignin content was also expressed as lignin content per SOC

(mg g^{-1} SOC). Lignin decomposition was assessed by the ratios of S, C to V and (Ac/Al) ratios of V and S, which generally indicate decomposition state (Thevenot et al., 2010).

Non-cellulosic polysaccharides

Non-cellulosic polysaccharides of plant and microbial origin (Kögel-Knabner, 2002) were determined by gas chromatography after trifluoroacetic acid (TFA) hydrolysis and reduction-acetylation using a method introduced by Rumpel and Dignac (2006) and modified by Eder et al. (2010). The analysis was performed using 700 mg of soil samples. Briefly, hydrolysis of non-cellulose polysaccharides was carried out at 105°C for 4 h with 10 ml of 4 M TFA. Thereafter, Myo-inositol was added as quantification standard to account for the losses during the purification procedure. Removal of soil was performed by filtration through glass fibre filters (Whatman GF/C 0.45 µm). Then TFA was evaporated using centrifugal Evaporator EZ-2 ENVI at 35°C for 4 hours and dry samples were left overnight in the freezer. Thereafter, dry samples were dissolved in 0.5 ml of H_2O followed by the addition of 0.9 EDTA in order to avoid co-precipitation of organic material with metal oxides and hydroxides (Eder et al., 2010). One mL sodium borohydride (NaBH4) in dimethylsulfoxide (20 g L−1) was added for reduction of polysaccharide monomers into alditol forms and kept at 40°C for 1.5 hours. Then, acetylation was conducted by addition of 0.2 mL acetic acid, 2 mL of acetic anhydride and 0.2 mL Methylimidazole. Acetylated alditols were extracted by 1 ml of dichloromethane and quantified with a HP GC 6890 gas chromatograph equipped with a flame ionization detector. Separation was achieved with a 60 m fused silica capillary column (SGE BPX 70, 0.32 mm internal diameter, 0.25 mm film thickness) under the following temperature program: 170 to 250 °C at 8 °C.min^{-1}, followed by 12 min at 250 °C (isothermal). Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The injector was kept at 250 °C and the detector at 260 °C. The non-cellulosic polysaccharides content of soil samples was determined as the sum of monosaccharides: C5 (pentoses: xylose, ribose and arabinose), C6 (hexoses: glucose, galactose and mannose), and desoxyC6 (desoxyhexoses: fucose and rhamnose) (Kögel-Knabner, 2002). A higher C6/C5 ratio generally indicates higher contribution of microbial sugars.

Amino sugars

Amino sugars were extracted from soil following (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

lyophilized and then 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), and 15 µg of the IS 2 tridecanoic acid methyl ester (1 µg $µl^{-1}$) in ethyl acetate-hexane (1:1) were added. Compounds were separated gas chromatographically 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) followed by flame ionisation detection (GC-FID system Agilent GC7820A, Waldbronn, Germany).

1.8.3. Biological parameters

Microbial biomass C and N

Microbial biomass C (MBC) and nitrogen (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 were 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).

Basal respiration

For measuring soil microbial respiration (SMR) a half gram of soil sample was placed in 2 ml Eppendorf tubes. The CO_2 efflux was trapped in 3 ml of 0.1 M NaOH and determined by conductometry. The metabolic quotient (qCO_2), reflecting decomposition activity (Anderson, 2003; Anderson and Domsch, 1993), was calculated as soil microbial respiration expressed per gram of microbial biomass carbon: qCO_2 = SMR/MBC (µg CO_2-C g^{-1} MBC h^{-1}).

Enzyme activities

The extracellular enzyme activity was measured by using the fluorometric technique (Koch et al., 2007; Marx et al., 2005; Razavi et al., 2015). Nine 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-galactopyranoside for β-galactosidase, (4) MUF-β-D-xylopyranoside for β-xylosidase, (5) MUF-β-D-cellobioside for β-cellobiohydrolase, (6) MUF-N-acetyl-β-D-glucosamide for chitinase, (7) Leucine-AMC for leucine aminopeptidase, (8) MUF-heptanoate for lipase and (9) 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 ml of the soil suspension were added to 150 ml 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).

Microbial growth kinetics

We used microbial growth kinetics technique as an approach to estimate microbial biomass activity state (Blagodatskaya and Kuzyakov, 2013). This approach is based on 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 for surface soil samples and 10 mg glucose, 1.9 mg $(NH_4)_2SO_4$, 3.8 mg $MgSO_4*7H_2O$, 0.53 mg K_2HPO_4 and 1.35 mg KH_2PO_4 for subsurface soil samples. The amount of mineral salts was preliminary selected in order to avoid soil pH change of more than 0.1 units after addition. For active microbial biomass (AMB) and specific growth rate calculation, the results of substrate induced respiration rate were fitted with a model proposed by Panikov and Sizova (Panikov and Sizova, 1996; Wutzler et al., 2012):

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

1.8.4. 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).

In the Chapter 2 we used two-way ANOVA in order to test sample type effect, treatment effect and their interaction on elemental properties, lignin concentration and composition of plant samples. We also used Principal Component Analysis (PCA) in order to investigate whether and how the plant sample types will separate.

In the Chapter 3 we identified significant differences ($P<0.05$) of studied parameters between samples using ANOVA based on Type III sums of squares with Tukey test due to unbalanced experimental design. When normality was not passed successfully, Kruskall-Wallis test was used. The equations (1) were fitted by non-linear regression, using Model Maker-3 software (SB technology Ltd.). To reveal the treatment effects, non-transformed data (except C and N content) were subjected to Principal Component Analysis (PCA).

In the Chapter 4 we used analyses of covariance (ANCOVA) to test treatment effect, depth effect and their interactions on measured 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 and to control 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 (1) were fitted by non-linear regression, using Model Maker-3 software (SB technology Ltd.).

CHAPTER 2. DO GRASSLAND MANAGEMENT PRACTICES AFFECT SOIL LIGNIN CHEMISTRY BY CHANGING THE COMPOSITION OF ORGANIC MATTER INPUT?

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2.1. Abstract

Grassland management practices alter plant tissue quantity and quality via defoliation, fertilisation and trampling. These alterations may impact litter decomposition and soil organic carbon (SOC) accumulation. Here, we aimed to investigate the effect of four grassland management practices (unmanaged, low and high intensity of grazing, and mowing) on organic matter (OM) input quality and its relation with soil organic matter composition. The OM types were represented by intact aboveground and belowground material, partly degraded aboveground litter and dung material. We assessed the quality of OM types based on their elemental (C and N) contents and lignin composition.

The results showed that C:N ratios differed among plant sample types but did not differ among treatments. In contrast, lignin biogeochemistry and lignin:N ratios of plant tissues were affected by both, sample types and treatment. High grazing intensity resulted in the highest plant shoot and litter quality, reflected by low C:N and lignin:N ratios. Lignin chemistry of aboveground and belowground OM under all grazing treatments indicated that plants were characterised by low maturity stage. The absence of management and mowing effects resulted in similar lignin chemistry of all plant sample types. Despite the similar lignin contribution to SOC under all grassland management practices, soil lignin was more degraded under mowing compared to other practices. However, in all other treatments the plant lignin chemistry was not linked to soil lignin chemistry. We conclude that plant tissue quality is impacted by the presence or absence of grazing animals. These impacts affected the aboveground plant OM molecular composition rather than its stoichiometry which consequently altered the soil lignin composition as well.

2.2. Resume

Les pratiques de gestion des prairies modifient la quantité et la qualité des tissus végétaux par défoliation, fertilisation et piétinement. Ces altérations peuvent avoir un impact sur la décomposition de la litière et l'accumulation de carbone organique (COS) dans le sol. Ici, nous avons cherché à étudier l'effet de quatre pratiques de gestion des prairies (non gérées, de faible et haute intensité de pâturage et de tonte) sur la qualité des intrants de matière organique (MO) et sa relation avec la composition de la matière organique du sol. Les types de MO ont été présentés par des matériaux intacts au-dessus du sol et souterrains, de la litière aérienne partiellement dégradée et des déjections. Nous avons évalué la qualité des types de MO en fonction de leur teneur en éléments (C et N) et de leur composition en lignine.

Les résultats ont montré que les rapports C:N différaient selon les types d'échantillons de plantes, mais ne différaient pas entre les traitements. En revanche, la biogéochimie de la lignine et les rapports lignine:N des tissus végétaux ont été affectés par les deux types d'échantillons et le traitement. Une intensité de pâturage élevée a donné la meilleure qualité de pousses et de litière, reflétée par de faibles rapports C:N et lignine: N. La chimie de la lignine de la MO aérienne et souterraine sous tous les traitements de pâturage a indiqué que les plantes étaient caractérisées par un faible stade de maturité. L'absence de gestion et de tonte a entraîné une chimie de la lignine similaire pour tous les types d'échantillons de plantes. Malgré la contribution similaire de la lignine au COS dans toutes les pratiques de gestion des prairies, la lignine du sol était plus dégradée lors du fauchage que d'autres pratiques. Cependant, dans tous les autres traitements, la chimie de la lignine végétale n'était pas liée à la chimie de la lignine du sol. Nous concluons que la qualité des tissus végétaux est affectée par la présence ou l'absence d'animaux au pâturage. Ces impacts ont affecté la composition moléculaire de MO de la plante plutôt que sa stœchiométrie et ont été reflétés d'une manière ou d'une autre par la composition de la lignine du sol.

2.3. Introduction

Carbon sequestration in soils is controlled by organic matter (OM) input (De Deyn et al., 2008; Lal, 2004; Orwin et al., 2010), which in grasslands occurs via root activity, belowground and aboveground OM input (Bardgett et al., 1998). Belowground input is composed of root exudation, dead cells slog-off and root litter (Kuzyakov and Domanski, 2000), while aboveground input consists of green and/or senescent plant material (leaves and stems). Root activity is sensitive to stress caused by nutrient limitation or defoliation (Hamilton et al., 2008; Medina-Roldán and Bardgett, 2011; Schmitt et al., 2013), whereas aboveground input occurs due to senescence processes or trampling (Bardgett et al., 1998). These inputs strongly control soil organic carbon (SOC) dynamics via their effect on soil microbial processes (Fontaine et al., 2003). Indeed, addition of fresh OM may lead to priming of native soil organic matter (SOM) (Kuzyakov et al., 2000). It is important to note that not the microbial status but the input quality influences these processes (Aerts, 1997; Fanin and Bertrand, 2016). Thus, input quality will select the microbial community altering the whole decomposition process (Grayston et al., 1996; Sauvadet et al., 2019). Thereby, in natural ecosystems the input quality can be important factor driving the soil processes and any human activity or climate change may influence this relationship.

Grassland management practices directly alter the quantity and quality of OM inputs via defoliation, dung input and plant physiological response. The impact of grassland management on input quality is interdependent with the plant response to management activities (Bardgett and Wardle, 2003). In general, defoliation induces root exudation (Bazot et al., 2005; Hamilton et al., 2008). Complete defoliation by mowing followed by a long period of regrowth either does not change or decreases aboveground plant quality (Bazot et al., 2005; Medina-Roldán and Bardgett, 2011). However, when plants are only browsed (i.e. partially removed) under light grazing, they will remain actively growing, resulting in higher litter quality (He et al., 2020; Heyburn et al., 2017). Consequently, similar grassland management with different grazing intensity will alter the development stage of plant communities (Bardgett et al., 1998). Another factor influencing the input quality is the alteration of the plant community composition because the quality of plant tissue chemistry varies widely depending on the plant species (De Long et al., 2019; Dovrat et al., 2020). While defoliation itself may not change plant richness, fertilisation decreases the plant diversity (Clark and Tilman, 2008; Lezama and Paruelo, 2016). Moreover, under grazing defoliation is selective due to the preference of

livestock to consume OM with higher quality, consequently this selective defoliation will also influence the species diversity (Bardgett et al., 1998) resulting in less diverse OM input (Bakker et al., 2006).

OM quality is mainly defined by C:N and lignin:N ratios. Low OM quality presenting high C:N and lignin:N ratios slows down the degradation rates whereas high OM quality with high N contribution results in the opposite. While the C:N ratio is a more global indicator of plant tissue chemistry, lignin and it's monomers ratios depend not only on the quality but also on the plant's development stage and differ according to the plant species and plant organs (Abiven et al., 2011; Zhang et al., 2016). Additionally, for soil samples, the lignin monomer ratios may indicate the lignin's degradation state and lignin's origin (Thevenot et al., 2010). Consequently, the plant tissue chemistry together with the soil lignin composition can be used to evaluate soil processes (De Long et al., 2019). Generally, estimation of grassland management impact on the plant traits was based on C:N ratio, lignin:N and some other physiological properties (leaf size, root length, etc) but the information how grassland management could alter the lignin composition in shoots and roots separately is still missing.

We hypothesised that understanding the relationships between OM input and its fate in soil is crucial for developing sustainable agricultural practices. Mowing impacts the soil-plant system through complete defoliation and the need for mineral fertiliser input. The effects of grazing include trampling, selective defoliation, dung and urine input depending on grazing intensity. The consequences of management practices will further depend on grassland type, climatic and edaphic conditions. We hypothesised that the comparison of different harvesting regimes (grazing, mowing and unmanaged) under similar pedoclimatic conditions could allow to understand the key plant responses with regards to management impacts on plant-derived OM input.

Our study aimed to assess the elemental and isotopic composition, and lignin chemistry of OM input under 4 grassland management practices. We have distinguished 4 types of OM input in soil: intact material presented by (1) aboveground (shoots) and (2) belowground (roots) plants material, dead material presented by (3) senescent aboveground plant litter which was partly degraded and (4) animal produced dung. We addressed the following questions: (1) how does grassland management practices affect aboveground and belowground plant OM chemistry? (2) how does plant OM chemistry influence SOM chemistry, in particular its lignin composition?

2.4. Results

2.4.1. Organic matter input quality

The C and N contents of the different sample types (shoot, root, aboveground litter and dung) are presented in Table 4. Root samples showed the lowest values compared to shoot and aboveground litter. However, C and N contents of all OM types were similar among treatments (Table 5). The C:N ratio was the highest in roots and the lowest in dung but it did not differ between the treatments in any sample type (Table 4).

Table 4. C and N contents, C:N ratio and natural abundance $\delta^{13}C$ of living (shoot, root) and non-living (aboveground litter) plant and dung material under different grassland management practices. Values are shown as the average of 4 replicates and ±SE.

Sample type		C content %	N content %	C:N ratio %	Natural abundance $\delta^{13}C$ ‰
Shoot	UM	41.3±0.8	2.3±0.2	18.3±2.1	-28.4±0.2
	LGraz	42.8±0.6	2.3±0.2	18.8±1.8	-27.9±0.2
	HGraz	42.1±0.3	2.4±0.2	17.9±1.6	-27.6±0.3
	Mowing	41.6±0.7	2.2±0.1	19.2±0.8	-28.4±0.3
Root	UM	40.2±0.3	1.6±0.1	26.0±1.3	-27.5±0.2
	LGraz	40.4±0.5	1.5±0.1	26.6±1.4	-27.5±0.5
	HGraz	40.6±0.1	1.6±0.1	25.9±1.5	-28.4±0.2
	Mowing	40.8±0.1	1.4±0.1	28.9±2.0	-28.1±0.1
Litter	UM	41.4±0.4	1.9±0.03	21.5±0.4	-27.7±0.2
	LGraz	41.6±1.0	2.3±0.2	18.6±1.5	-28.3±0.1
	HGraz	40.7±1.2	2.2±0.1	18.7±0.6	-29.6±0.6
	Mowing	42.4±0.2	2.1±0.1	20.4±1.1	-28.9±0.5
Dung	UM	-	-	-	-
	LGraz	39.1±0.4	2.6±0.1	15.2±0.9	-29.3±0.3
	HGraz	40.3±1.1	2.7±0.1	15.3±1.0	-29.4±0.3
	Mowing	-	-	-	-

In contrast, the natural abundance of ^{13}C was differentiated by the treatment as illustrated by changing $\delta^{13}C$ ratios. High grazing (HGraz) and mowing (Mowing) treatments showed low $\delta^{13}C$ ratios in roots and aboveground litter. Aboveground litter and dung were most depleted in $\delta^{13}C$ compared to the plant shoots (Table 4).

Table 5. Summary of two-way ANOVA results for different variables of plant material under different grassland management practices.

ANOVA, F values (P values)	C content %	N content %	C:N ratio	Natural abundance $\delta^{13}C$ ‰
Nature	5.61 (0.007)	31.98 (<0.001)	36.77 (<0.001)	7.71 (0.002)
Treatment	0.75 (0.52)	0.83 (0.49)	1.02 (0.36)	4.83 (0.006)
Nature x Treatment	0.83 (0.56)	0.42 (0.86)	0.46 (0.83)	3.98 (0.004)

ANOVA, F values (P values)	Lignin content mg g^{-1}	Lignin:N	$(Ac/Al)_v$	$(Ac/Al)_s$	C/V	S/V
Nature	32.38 (<0.001)	36.77 (<0.001)	11.4 (<0.001)	26.11 (<0.001)	12.14 (<0.001)	7.23 (0.002)
Treatment	5.78 (0.003)	5.83 (0.002)	1.72 (0.18)	0.67 (0.57)	5.31 (0.004)	5.69 (0.003)
Nature x Treatment	7.78 (<0.001)	7.4 (<0.001)	5.88 (<0.001)	0.85 (0.54)	1.33 (0.27)	0.97 (0.46)

The highest lignin content was recorded for aboveground litter (Fig. 10A). Treatments did not influence lignin contents of roots but they affected lignin contents of shoots and aboveground litter (Table 5). The highest lignin content in plant shoots was recorded for the unmanaged treatment (UM) followed by low intensity grazing (LGraz) > Mow > HGraz. Grazing with both intensities resulted in lower lignin content of aboveground litter compared to Mow and UM sites. Lignin:N followed a similar pattern as lignin content (Fig. 10B).

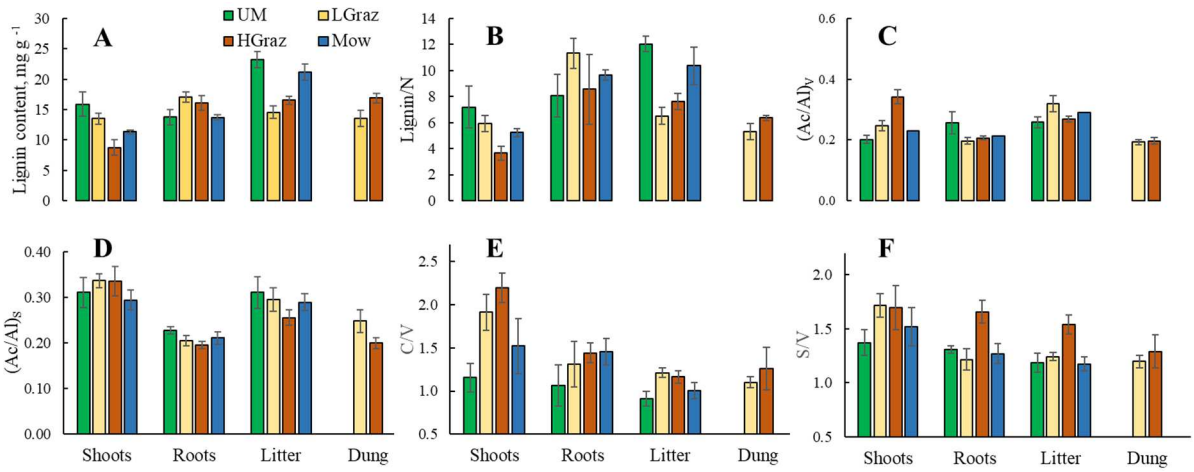

Figure 10. Lignin content and its monomer ratios of living (shoot, root) and non-living (litter) plant and dung material under different grassland management practices. Values are shown as the average of 4 replicates and ±SE.

All lignin monomer ratios were sensitive to the sample type (Table 5). $(Ac/Al)_v$ in shoots was the highest under HGraz and did not differ among other treatments and sample types (Fig.10C). $(Ac/Al)_s$ ratio was sensitive only to sample type (Table 5, Fig. 10D), showing higher values in aboveground shoot and litter than in roots. C/V ratios of shoots were also affected by grazing treatments (Fig. 10E). HGraz resulted in increased S/V ratios of roots and aboveground

litter but was not affecting shoots (Fig. 10F). Lignin content was similar in aboveground litter and dung of grazing treatments, while $(Ac/Al)_s$ and $(Ac/Al)_v$ were higher in aboveground litter.

2.4.2. Soil lignin content and composition

Lignin concentrations (mg g^{-1} dry soil) and contents (mg g^{-1} SOC) in soil did not differ between the treatments (Fig. 11A, B). $(Ac/Al)_v$ was highest under Mow as compared to the other treatments, which showed no differences (Fig. 11C). The C/V ratio was highest under UM and lowest under Mow (Fig. 11E). Mow was characterized by lower C/V ratio compared to grazing treatments. The S/V ratio was highest in soil under HGraz followed in the order of <LGraz=Mow<UM (Fig. 11F).

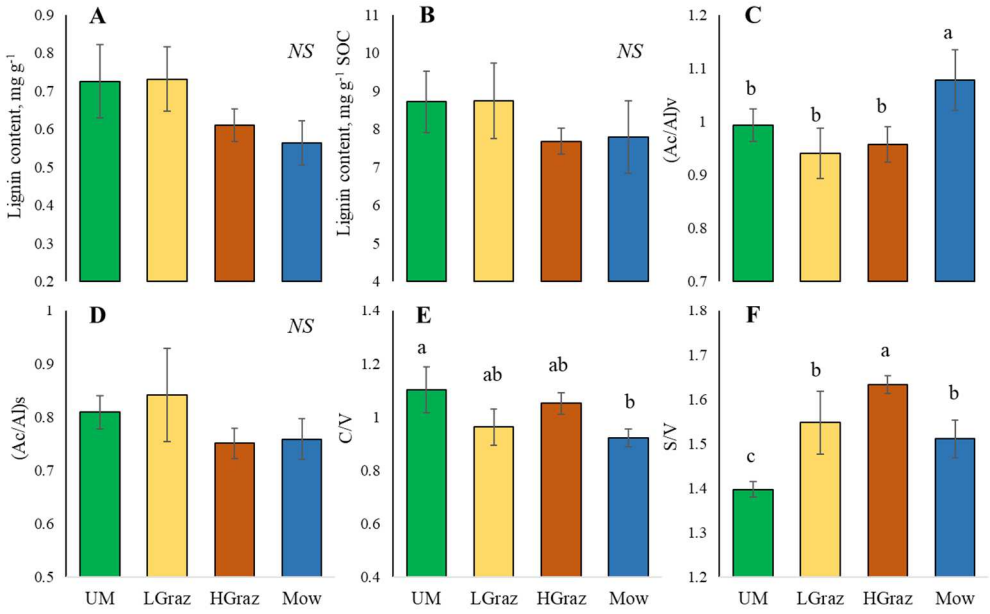

Figure 11. Soil lignin content and its monomer ratios under different grassland management practices. Values are shown as the average of 6 replicates and ±SE.

2.4.3. Correlations and PCA

Among all parameters only one significant linear dependency was found: natural abundance $\delta^{13}C$ with S/V (Fig. 12). The $\delta^{13}C$ ratio did not correlate with lignin contents but was correlated with the S/V ratios. However, a significant negative correlation was observed only for root samples but not for all treatments.

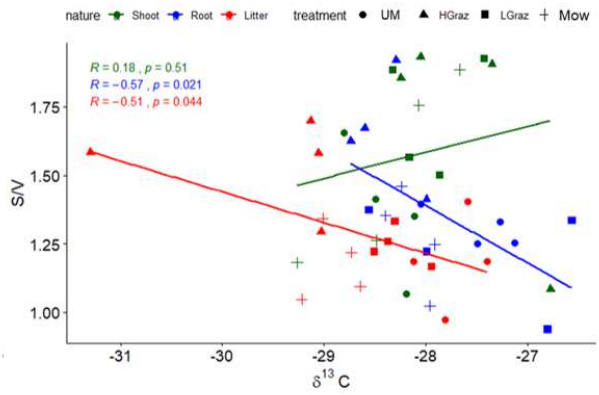

Figure 12. Correlation between lignin S/V ratio and natural abundance $\delta^{13}C$.

Principal component analysis (PCA) was carried out with all plant samples. The first two dimensions explained 60.4% of the total variability (Fig. 13). The treatments were not separated, though there was a clear separation of sample types. Aboveground litter was separated from shoot and root OM because of its higher lignin content and lower C/V ratio. Plant shoot and its litter differed from roots because of higher N and, consequently, lower C:N ratio.

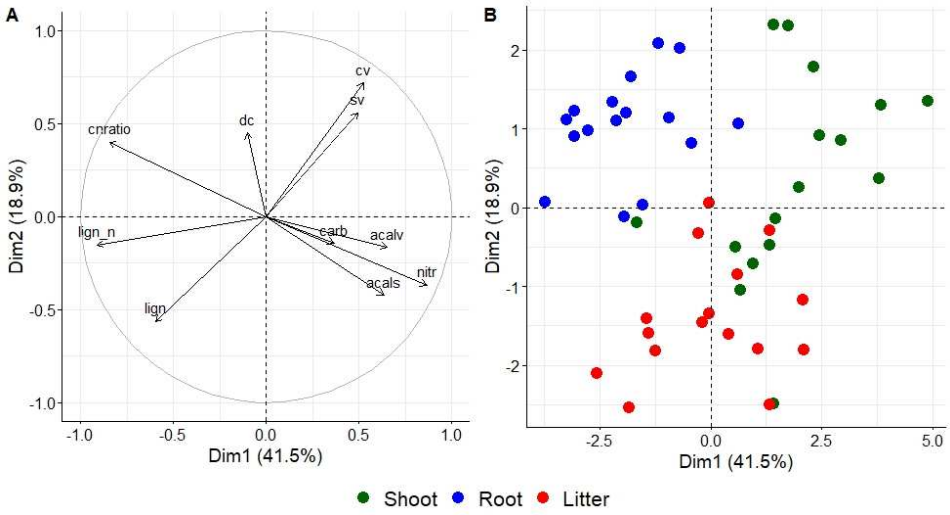

Figure 13. Principal component analysis (PCA) for living (shoot, root) and non-living (litter) plant material under different grassland management practices.

When the soil samples were included into PCA analysis, 80.2% of the variability could be explained by the first two dimensions, but the separation of plant samples varnished (Fig. 14). Only soil samples were well-separated from plant samples which grouped all together. Soil samples differed from aboveground litter and intact plant material by lower C and N contents and higher Ac/Al ratios.

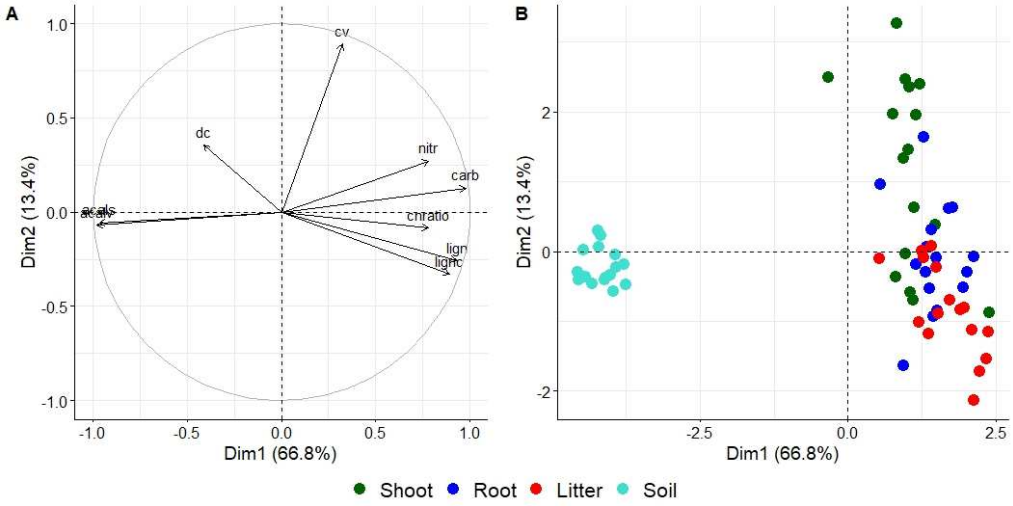

Figure 14. Principal component analysis (PCA) for living (shoot, root) and non-living (litter) plant material and soil under different grassland management practices.

2.5. Discussion

2.5.1. Lignin chemistry of aboveground OM input (shoot and litter) is altered by grassland management

We analyzed the elemental and lignin composition of aboveground (shoot, litter, dung) and belowground (root) OM input. Grassland treatment did not alter the elemental composition of shoot and aboveground litter but altered lignin parameters. Lowest lignin content in shoots under HGraz may indicate that plants affected by grazing had a low maturity, whereas the highest lignin contents in shoot material under unmanaged treatment indicated that the plants were developed and mature. Low lignin content in aboveground litter samples under both grazing treatments is probably related to trampling, consequently, resulting in fresh green plant material input, which has low lignin content as compared to the senescent plant material (Sanaullah et al., 2010). This suggests that aboveground plant-derived OM input under these four grassland management interventions has contrasting chemical composition (Ziter and MacDougall, 2013), while showing a similar C and N stoichiometry.

Lignin:N is another widely used indicator to estimate OM quality and may be a better predictor of litter decomposability than stoichiometric traits (Freschet et al., 2012). Consequently C:N and lignin:N ratios may indicate different processes, for example, as low C:N can be related to the other factors, i.e the C:N ratio change during plant development (Zhang et al., 2020). Moreover, plants transport N to the organs with active photosynthesis, which are not always coupled with lignin content of plant tissues (J. Zhang et al., 2018). Lignin synthesis in plants may be related to plant's defense system and thus can be altered by defoliation or biotic stress (Bennett et al., 2015; Zhang et al., 2016). In the case of aboveground litter, which is partly degraded, the strong positive relationship might indicate that the C:N ratio increases together with Lignin:N ratio during degradation (He et al., 2019; Thomas and Asakawa, 1993).

The shoot quality based on the Lignin:N was lowest under UM and LGraz, whereas higher values were recorded under HGraz and Mow. The higher quality under HGraz as compared to LGraz could be related to either lower maturity of the plants due to constant removal or differences in plant community composition. Grazing increases the plant community diversity as compared to unmanaged and the intensity increases the presence of leguminous species (Herrero-Juregui and Oesterheld, 2018; Louault et al., 2005; Proulx and Mazumder, 1998). The increase of leguminous species, consequently, results in higher plant input quality (Faust et al.,

2018). However, LGraz also faces defoliation but because of less destructive browsing, plants may be more capable to recover and partial removal allows to keep plants at high maturity stage, which results in contrasting elemental and lignin composition as compared to HGraz (Alber et al., 2014).

Lignin composition of shoots and aboveground litter was also impacted by grassland treatments. However, $(Ac/Al)_v$ and $(Ac/Al)_s$ ratios of shoot and aboveground litter were not different among treatments, except for $(Ac/Al)_v$ of shoots under HGraz. Grazing treatments regardless of the intensity increased C/V ratios of shoot and aboveground litter, with higher magnitude for shoot OM. These differences are probably related to the grazing effect on plant maturity (see above). Because of partial or selective defoliation under grazing treatments, plants are always on growing process producing fresh mature material which has higher C/V ratio (Abiven et al., 2011). Aboveground litter, which is partly degraded OM originating from shoots, followed a similar pattern, but showed fewer changes. The S/V ratio of aboveground litter was also higher under HGraz compared to all other treatments. S units are preferentially degraded compared to V units (Bahri et al., 2006) and it was surprising to find them undecomposed in aboveground litter in HGraz. This is probably due to dung input, which might be degraded preferentially, compared to plant-derived OM (Dungait et al., 2009).

The changes in lignin molecular composition could have resulted from plant community changes (Heim and Schmidt, 2007a; Whitehead et al., 1979). Few investigations exist in this regards as forage quality of plants is usually assessed only based on the lignin content but not based on the lignin molecular composition. Otto and Simpson (2006) analysed grass shoot, root and decomposed litter and support the molecular differences between sample types we found here (see below 3.3.). But they did not provide the information about the community composition of the grass community, so the question whether there are differences in molecular composition between different species of grassland plants remains unclear.

2.5.2. Belowground input (root) quality is not sensitive to grassland management

Similarly to aboveground plant samples, elemental properties (C, N, C:N ratio) of root OM were similar in all treatments. Similarly, lignin content and lignin:N of roots did not differ among grassland treatments, although root biomass was reported to be affected by grassland management (Bardgett et al., 1998; Klumpp et al., 2009; Medina-Roldán and Bardgett, 2011). Our results may be related to the fact that even if the root biomass is altered by grassland management, the lignin composition remains the same. In contrast to aboveground plant organs,

lignin composition of roots is not altered during plant development (Zhang et al., 2020). Roots quality is strongly correlated with soil properties but it is not correlated with the plant physiological changes (Orwin et al., 2010). Our results may thus suggest that root lignin composition is not affected by grassland management although this may change root growth and physiology (biomass, exudation) (Alber et al., 2014; Klumpp et al., 2009; Shen et al., 2020).

Concerning lignin molecular composition of roots, only the S/V ratio was affected by the treatment. The highest S/V of roots was recorded for HGraz, which can be explained by plant community changes. Graminous species contain more S units as compared to leguminous species (Heim and Schmidt, 2007b). HGraz generally increases the abundance of some leguminous species (red and white clover) (Kruess and Tscharntke, 2002; Louault et al., 2005; Sternberg et al., 2000), so we would expect the opposite result: the reduction of S/V under HGraz. Graminoids produce higher root biomass as compared to some grazing-tolerant leguminous species (Bolinder et al., 2002). Even though the leguminous abundance increased under HGraz, probably due to less root biomass it could not contribute to the increased S/V ratio of plant roots.

2.5.3. More degraded soil lignin under mowing

Soil lignin content did not differ between grassland managements, however, the lignin composition was sensitive to grassland managements. Mow treatment resulted in higher degraded soil lignin state as compared to UM and grazing treatments regardless of intensity. Based on the same soil lignin content per SOC and similar lignin content of aboveground and belowground OM input, we suggest that more degraded lignin state is not related to initial OM input quality but to microbial degradation processes in soil. Additionally, the dung input under grazing systems may serve as supplementary lignin source whereas under Mow the inorganic fertilisation do not contribute to lignin input but still might enhance total organic C (Poeplau et al., 2018; Yu et al., 2012).

Although, there was still a link between lignin chemistry of OM input and soil which was related to S/V ratio under HGraz. S/V ratios is still interpreted in different ways: it may indicate (1) the plant source if there is a shift between angiosperms and gymnosperms (Otto and Simpson, 2006) or (2) the source of plant organ (Abiven et al., 2011) and the degradation status (Bahri et al., 2006). In our case, under HGraz, high soil S/V ratio together with high root S/V ratio may indicate that the source of soil lignin under HGraz might be more root-derived compared to the other treatments.

2.5.4. The differentiation of plant sample types

In the PCA plan showing only plant materials, the sample types were separated. Roots were separated from shoots and aboveground litter because of their higher N content $(Ac/Al)_s$, $(Ac/Al)_v$ and lower C:N ratio. Intact plant materials and aboveground litter were separated by lignin content, lignin:N ratio and C/V ratio. When soil samples were added to the PCA sample type, except for soil or treatment could no longer be distinguished. Soil separation was related to high $(Ac/Al)_v$ and $(Ac/Al)_s$ ratios and low C and N contents. We would expect that partially degraded aboveground litter is located between soil and plant parts. However, when lignin is incorporated into soil, it may undergo further degradation and stabilization mechanisms by the interaction with clay minerals (Rasse et al., 2006; Thevenot et al., 2010).

The $(Ac/Al)_v$ ratio is widely used as an indicator of SOM degradation degree, whereas it does not seem to be a representative degradation indicator for the grass tissue degradation. We gathered the results of lignin monomer ratios for each sample type (shoot, root, litter, soil) without considering the treatment effect (Fig. 15).

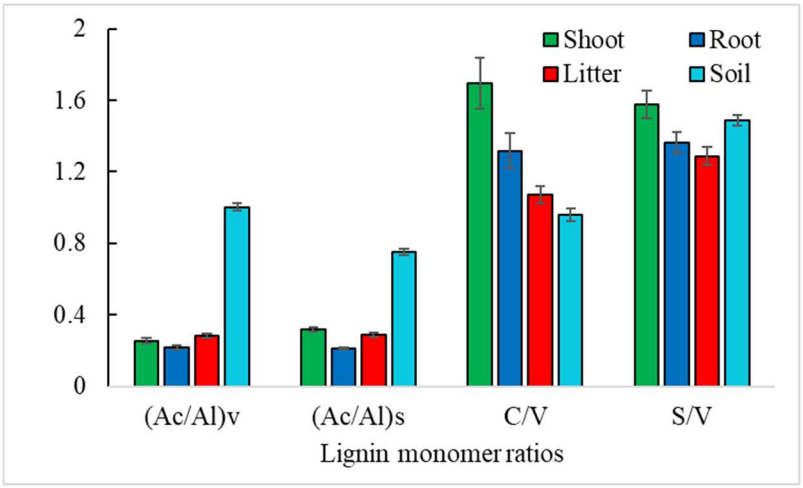

Figure 15. Lignin monomer ratios in different sample types (shoot, root, litter and soil) summarized within grassland treatments.

There was no difference between intact aboveground material and dead partly degraded aboveground litter in $(Ac/Al)_v$, however, it drastically increased in soil. Probably, this ratio may indicate the degradation degree of lignin only in soil and in woody tissue (Otto and Simpson, 2006), but not in non-woody plants (grasses, herbs) as in our case. The C/V ratio was smoothly decreasing from shoot>litter>soil and root>soil (Fig. 15). This supports the earlier findings that C/V could be a good indicator of plant litter degradation degree (Baumann et al., 2013; Thevenot et al., 2010).

The only lignin monomer ratio showing the link between soil and plant aboveground material was S/V ratio (R^2 = 0.85, p value <0.05). Generally, S/V ratio indicates the lignin source in soil (Heim and Schmidt, 2007a). It was surprising that the link was observed only between soil and aboveground material indicating that lignin also enters from aboveground part and may impact the lignin composition in soil. Grazing treatments increase S/V ratio in aboveground plant biomass which consequently increase the soil S/V ratio. However, such S/V increase under grazing treatments is still unclear and needs further investigation of lignin in different wild plant species material.

2.6. Conclusions

This study investigated the plant OM quality based on the elemental composition and lignin chemistry under four grassland management systems. Lignin composition was found to be more sensitive to treatments compared to C, N stoichiometry. Our results demonstrated the lignin molecular differences in plant tissue chemistry in response to grassland management. Intact aboveground material was more affected by grassland management than intact root material, which showed only few changes. High intensity grazing had the greatest impact on lignin composition in all sample types. However, grazing regardless of the intensity altered the lignin composition as compared to all other treatments most probably because of partial and selective defoliation altering the plant maturity stage. Unexpectedly, we did not find any links between the input lignin chemistry and soil lignin chemistry suggesting that the latter is the result of the abiotic and biotic transformation processes after plant litter is returned to soil.

CHAPTER 3. DOES ABOVEGROUND PLANT INPUT QUANTITY DRIVE MICROBIAL FUNCTIONING OF GRASSLAND SOILS? -TOWARDS THE EVALUATION OF MANAGEMENT INTENSITY ON SOIL PROCESSES.

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3.1. Abstract

Grassland management practices vary in intensity (stocking rates, fertilization) and plant removal strategies (grazing versus mowing). The combination of these factors can have multidirectional effect on microbial activity and thus influence soil organic matter (SOM) degradation. We performed analysis of soil samples from a thirteen-year experiment in Central France with different grassland management practices (unmanaged, light and high grazing, mowing, bare fallow), which we conceptualised as disturbance gradient based on the plant biomass leftover level. We aimed to determine how disturbance through grassland management alters microbial necromass accumulation, microbial functioning and soil degradation processes. In order to investigate the mechanisms involved we characterised (1) amino sugars as microbial necromass indicator and (2) microbial biomass C and N, basal respiration, seven enzyme activities and microbial growth kinetics as microbial functioning and degradation processes indicators.

Our results demonstrated that plant input quantity influenced the amino sugar content and microbial C:N ratio. While microbial parameters related to growth kinetics and enzyme activities did not follow the linear dependency and were probably related to input quality. Microbial biomass C, basal respiration and specific enzyme activities showed contrasting pattern along the disturbance gradient, which was explained by soil pH and the amount of root biomass. Aboveground input quantity depending on grassland harvesting intensity does not necessary control the soil organic C content and soil degradation processes.

3.2. Resume

Les pratiques de gestion des prairies varient en intensité (taux de chargement, fertilisation) et en stratégies d'élimination des plantes (pâturage par rapport à la tonte). La combinaison de ces facteurs peut avoir un effet multidirectionnel sur l'activité microbienne et ainsi influencer la dégradation de la matière organique du sol (MOS). Nous avons effectué une analyse d'échantillons de sol d'une expérience de treize ans dans le centre de la France avec différentes pratiques de gestion des prairies (non géré, pâturage léger et élevé, fauchage, jachère nue), que nous avons conceptualisé comme un gradient de perturbation basé sur le niveau de restes de biomasse végétale. Nous visions à déterminer comment la perturbation due à la gestion des prairies modifie l'accumulation de nécromasse microbienne, le fonctionnement microbien et les processus de dégradation des sols. Afin d'étudier les mécanismes impliqués, nous avons caractérisé (1) les sucres aminés comme indicateur de nécromasse microbienne et (2) la biomasse microbienne C et N, la respiration basale, sept activités enzymatiques et la cinétique de croissance microbienne en tant qu'indicateurs du fonctionnement microbien et des processus de dégradation.

Nos résultats ont démontré que la quantité d'intrants végétaux influençait la teneur en sucre aminé et le rapport microbien C:N. Alors que les paramètres microbiens liés à la cinétique de croissance et aux activités enzymatiques ne suivaient pas la dépendance linéaire et étaient probablement liés à la qualité des intrants. La biomasse microbienne C, la respiration basale et les activités enzymatiques spécifiques ont montré un schéma contrasté le long du gradient de perturbation, ce qui était expliqué par le pH du sol et la quantité de biomasse racinaire. La quantité d'intrants hors sol dépendant de l'intensité de récolte des prairies ne contrôle pas nécessairement la teneur en C organique du sol et les processus de dégradation du sol.

3.3. 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). However, their actual role in climate change mitigation may be related to 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 disturbance (van Andel and van den Bergh, 1987) of soil processes and biogeochemical cycling, through their impact on abiotic (e.g. soil compaction) and biotic processes (e.g. plant activity). While for plant ecologists disturbance is defined by natural processes (e.g. fire, grazing, etc) affecting plants *directly* (Hobbs and Huenneke, 1992), its impact on the soil microbial community may be *indirect* by altering habitat and substrate availability. Grazing and mowing are two grassland harvesting strategies, which were widely considered as disturbance for the studies of plant communities changes (Olff and Ritchie, 1998; van Andel and van den Bergh, 1987) but an attempt to adapt this idea to soil microbial community is still missing. In this study, we thus investigated different grassland management practices (mowing and two different grazing intensities), which we conceptualized as disturbance gradients in terms of plant litter input.

Differences between grazing and mowing are related to the complex relationships between quality and quantity of aboveground and belowground litter and their effects on SOM dynamics (Liu et al., 2014). 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) and plant physiological traits (Niu et al., 2016). 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). These changes may have direct or indirect effects on degradation processes in soil via their influence on microbial activity (Chuan et al., 2020; Millard and Singh, 2010; Oates et al., 2012; Sayer et al., 2013). During plant litter degradation, microorganisms not only produce CO_2 but they also convert plant-derived C into microbial biomass, which can be further stabilized in soil (Liang et al.,

2017; Ma et al., 2018). These processes can be addressed by combining microbial and biogeochemical analysis, enabling to identify simultaneously microbial functioning, plant litter degradation products and microbial residues. Whereas, the effect of land-use management on microbial SOM degradation processes is broadly studied (Ali et al., 2018; Cui and Holden, 2015; Xu et al., 2017), microbial SOM formation has received less attention (Liang et al., 2016).

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 comparing management practices under comparable climatic conditions (Franzluebbers and Stuedemann, 2009; Liu et al., 2014). Recently it was shown that both, light grazing and mowing (with N addition), do promote C sequestration, however, light grazing may lead to more efficient microbial functioning and thus to higher C sequestration due to dung input (Gilmullina et al., 2020).

Here we examine 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 were assumed to represent a disturbance gradient because of contrasting aboveground plant biomass input. We chose this disturbance gradient, because input quantity may regulate SOC accumulation through alteration of microbial activity and functioning. However, the direction of the response of microbial activity and soil organic matter quality along the disturbance gradient and the mechanisms driving their response are poorly known. These important knowledge gaps need to be addressed in order to develop grassland management practices favouring the accumulation of SOC. We aimed to determine the biogeochemical response patterns along the disturbance gradients. To this end, we determined soil parameters, such as SOC content, pH, and amino sugars composition. Microbial functioning was characterised by biomass C and N content, fraction of active microorganisms, specific growth rate, basal respiration, metabolic quotient qCO_2 and specific enzyme activity.

3.4. Results

3.4.1. Soil general properties and amino sugars

Soil pH ranged between 5.2 and 5.9 and it was the highest at the unmanaged (UM) and the site with high intensity grazing (HGraz) (Table 6). For the other treatments, pH decreased in the order low intensity grazing (LGraz)>Mowing (Mow)> Bare fallow (bare).

Table 6. Soil general parameters. Values are shown as the average of six (four for Bare fallow) replicates and ±SE. Significant differences between the treatments are indicated by capital 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±4.8ab	11.1±0.1a	7.4±0.4a	-27.4±0.07ab	5.6±0.1a
LGraz	7.74±2.07a	5.7±0.05b	84.9±5.2a	11.1±0.1a	7.6±0.5a	-27.7±0.06a	4.9±0.1a
HGraz	3.69±1.1b	5.8±0.05a	79.2±3.3ab	10.8±0.1b	7.3±0.3ab	-27.8±0.08ab	5.3±0.1ab
Mow	8.51±1.2a	5.3±0.05c	73.0±1.6b	11.2±0.1a	6.5±0.1bc	-27.6±0.04b	4.8±0.1bc
Bare	-	5.2±0.06c	49.3±2.3c	10.7±0.0b	4.6±0.2c	-26.8±0.03c	6.3±0.1c

Root biomass was lowest under LGraz. The highest root biomass was under Mow, though HGraz and UM treatments resulted at insignificantly lower root biomass compared to Mow. The highest SOC content was observed under LGraz followed by similar values under HGraz and at the UM site. SOC content under Mow was 15% lower compared to LGraz and 46% higher compared to the bare fallow treatment. N content was the highest under UM and LGraz and followed by HGraz<Mow. The C:N ratio were about 11 under UM, LGraz and Mowing, whereas it was slightly lower under HGraz and Bare. All managed sites showed lower ^{13}C enrichment compared to UM and Bare. Across the managed treatments, ^{15}N was less enriched under LGraz and Mow compared to HGraz. Bare soil showed the highest enrichment of both stable isotopes.

Amino sugars contents were the highest under UM followed by lower values under managed sites (Table 7). 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.

Table 7. Amino sugar signatures. Values are shown as the average of six (four for Bare fallow) replicates and ±SE. Significant differences between the treatments are indicated by 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±0.04a
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

3.4.2. Microbial functioning and degradation processes

MBC varied between 304 and 1314 µg g^{-1}. It was the highest under UM and HGraz, followed by similarly lower values for LGraz and Mow (Fig. 16A). MBC per SOC (MBC mg g^{-1} SOC) and basal respiration (mg CO_2-C g^{-1}) followed a similar pattern as MBC (Fig. 16, 17A).

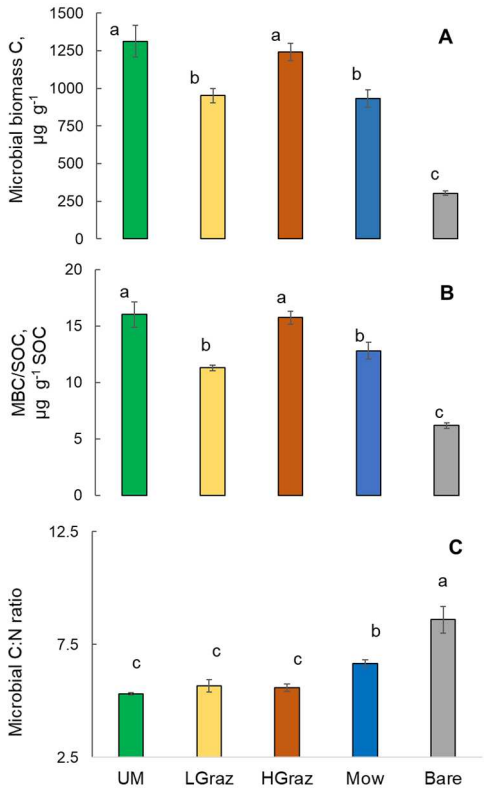


Figure 16. Microbial biomass C concentration (MBC) and content (MBC per SOC), microbial C:N ratio under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Values are shown as the average of six (four for Bare soil) replicates and ±SE. Significant differences between the treatments are indicated by lower case letters ($P < 0.05$).

Microbial C:N ratio did not differ between UM and grazing treatments but it increased under Mow and Bare (Fig. 16C). Basal respiration per SOC was the highest under UM and HGraz (Fig. 17B).

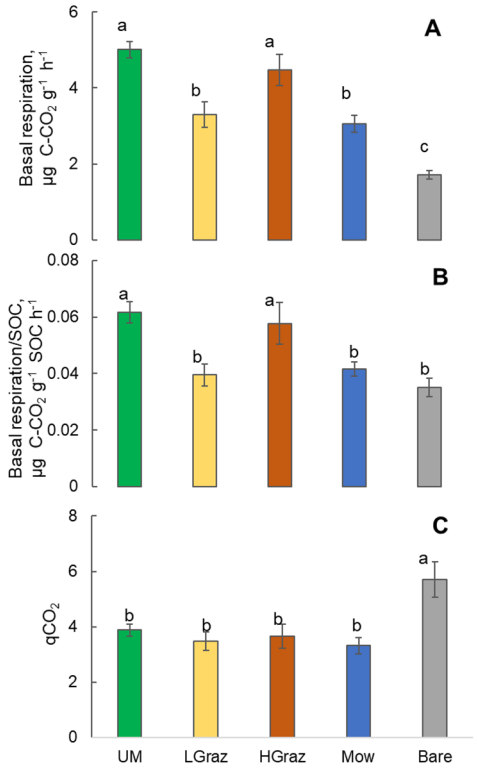


Figure 17. Basal respiration (A), Basal respiration (CO_2 per SOC) (B) and metabolic quotient qCO_2 (C) under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Values are shown as the average of six (four for Bare fallow) replicates and ±SE. Significant differences between the treatments are indicated by lower case letters ($P < 0.05$).

The metabolic quotient (qCO_2) did not differ among managed and unmanaged treatments but the highest values were observed under Bare soil (Fig. 17C). The AMB represented 0.5-1.1% of MBC. It decreased in the order Mow≥HGraz≥LGraz>UM=Bare (Fig. 18A). Specific growth rate µ ranged between 0.15 and 0.24 h^{-1} and was the highest under Bare followed by UM and it was the lowest under grazing treatments and Mow (Fig. 18B).

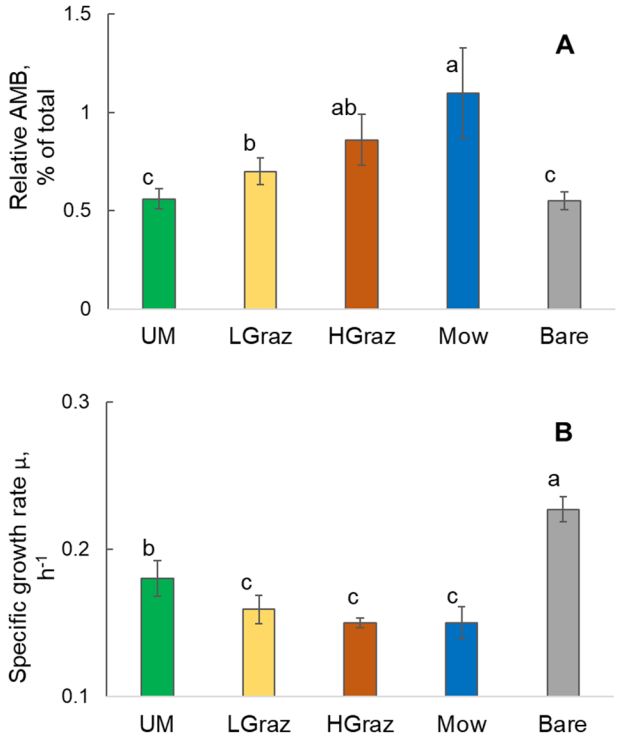


Figure 18. Active microbial biomass (AMB) (A) and specific growth rate μ (B) under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Values are shown as the average of six (four for Bare fallow) replicates and ±SE. Significant differences between the treatments are indicated by lower case letters ($P < 0.05$).

C-cycle enzymes followed a similar pattern as the SOC content resulting in the highest values under LGraz (Fig. 19A). The lowest enzyme activities among managed practices were observed under Mow. Leucine aminopeptidase activity was not affected by grazing management: resulting in highest value under UM and grazing treatments, then followed by decrease in the order of >Mow>Bare. The lowest phosphatase activity was observed under UM and Bare. In managed soils, phosphatase activity increased in the order LGraz<HGraz<Mow. Enzyme activity per MBC was the highest under LGraz and Bare for all enzymes except leucine aminopeptidase (Fig. 19B). Leucine aminopeptidase activity per MBC was similar among all treatments except for Mow, which showed the lowest value.

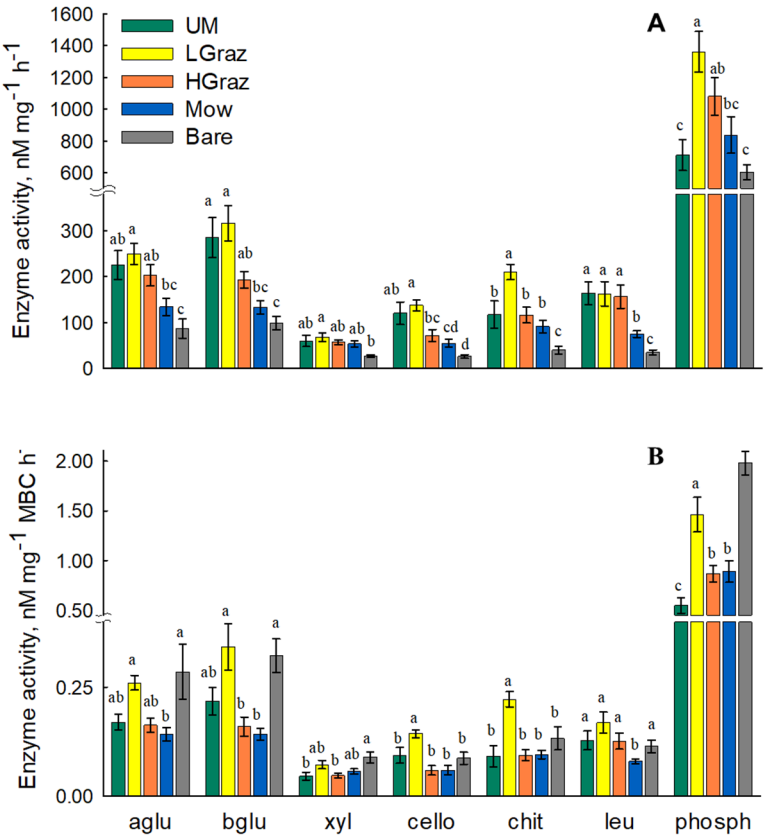


Figure 19. Absolute enzyme activity (A) and enzyme activity per MBC for the 7 enzymes under three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). Aglu, bglu, xyl, cello, chit, leu, phosph represent 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 ($P < 0.05$).

3.4.3. The response to disturbance gradient

Our results indicated that the disturbance gradient resulted in 5 different kinds of response form (Fig. 20): negative or positive linear, bell-like or reverse bell-like, ripple-like, and specific response based only on the presence/absence of disturbance.

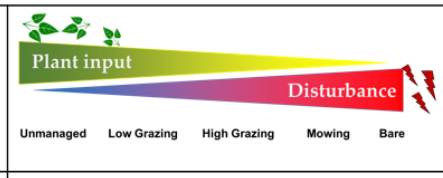
Name of dependence		Properties
Linear - Positive		Microbial C:N ratio
- negative		Amino sugars content
Bell-like form		SOC and N content Active microbial biomass Absolute enzyme activity
Reverse bell-like form		Specific growth rate
Ripple-like form		pH Microbial biomass C Basal respiration Specific enzyme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

Figure 20. The response patterns of measured soil biogeochemical parameters to disturbance gradient based on aboveground plant biomass leftover level.

Only amino sugars content and microbial C:N ratio followed the *linear pattern*: negative and positive, accordingly. C and N content, specific growth rate, relative AMB and absolute enzyme activity followed *bell-like form* (or the reverse bell-like form). pH and root biomass along with microbial parameters such as MBC and basal respiration had *a ripple-like* form with two peaks including UM and HGraz treatments. The GluN/GalN ratio was significantly different only in UM treatment, whereas the metabolic coefficient (qCO_2) was significantly different only in bare fallow soil.

3.4.4. Principal component analysis

Principal component analysis enabled separation into three groups: UM, Bare and managed sites all together (HGraz, LGraz and Mow) (Fig. 21A). The separation of UM and Bare from managed sites was related to enrichment of ^{13}C and ^{15}N isotopes, higher specific growth rate and lower percentage of AMB. UM and Bare were differentiated by SOC and N concentrations, MBC and C-cycle enzyme activities, which showed higher values under UM. To eliminate the dominance of the PCA by the non-managed sited and thus to check if there is a differentiation among managed sites, we applied PCA with exclusion of UM and Bare (Fig. 21B). There was a clear separation of grazed and mowed sites. Mow was separated from LGraz

and HGraz treatments by lower pH, SOC and N contents and lower enzyme activities. Grazing treatments were also separated: HGraz was characterised by higher basal respiration and MBC compared to LGraz.

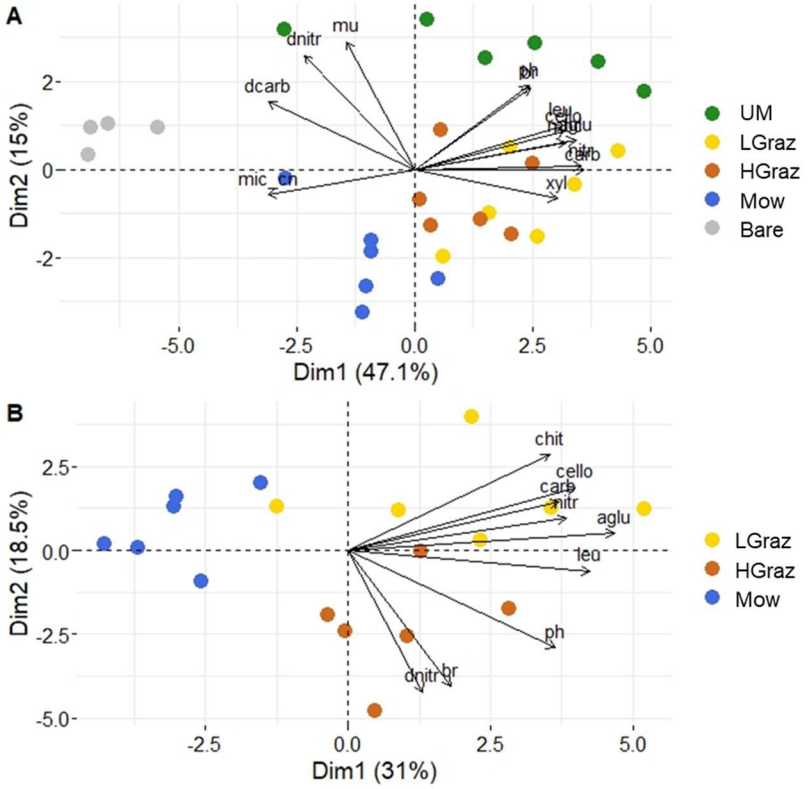


Figure 21. Principal component analysis (PCA) of all measured soil variables under (A) three grassland management practices (low intensity grazing (LGraz), high intensity grazing (HGraz) and mowing (Mow)), unmanaged (UM) and bare fallow (Bare). PCA score plot (B) represents only three grassland management practices (low intensity grazing, high intensity grazing and mowing). Only variables with quality of representation (cos^2) higher than 0.6 were shown on PCA plots.

3.5. Discussion

The study sites were chosen because we hypothesized gradual changes in soil biogeochemical and microbial properties with reduction of plant biomass input due to management activities. We made this hypothesis due to the general observation of plant biomass input being directly related to SOM dynamics (Bardgett et al., 1998; Lal, 2002). According to our hypothesis, the disturbance based on the APBL should increase linearly in the order: UM<LGraz< HGraz<Mowing<Bare. However, our result indicated five different response curves, which will be discussed below.

3.5.1. Properties linearly depending on the disturbance gradient

Linearly dependent properties on the disturbance gradient were amino sugar content and microbial C:N ratio. Amino sugars are the indicators of microbial residues in soil (Joergensen, 2018). The most disturbed site (bare soil) showed decreased amino sugar content most probably due to the absence of biomass input, which may trigger the microbial community to use SOM components instead (Ding et al., 2017). Low C:N ratio of amino sugars make them as a reasonable source of N (Li et al., 2019) and their degradation may thus explain that the contents in soils are depending 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 lead to increased microbial biomass resulting in intense microbial residue formation. Despite the gradual decrease of amino sugars on the disturbance gradient, the differences between managed grasslands were insignificant, indicating that contrasting management had only 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 see the accumulation of microbial residues (Ding et al., 2011). Similar results in other managed grassland soils indicated 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 results in the starving status of microorganisms or in the selection of microorganisms with slow growing 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 results due to sufficient organic matter input into soil, whereas N was lacking in soil under bare and under mow despite mineral fertilizer input, thus favoring fungal community.

3.5.2. 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, which followed a bell-like form, and the specific growth rate, which followed a reverse bell-like form. It was interesting to note that positive and negative controls presented by unmanaged site and bare soil did not differ. Both treatments present quite stable systems characterised by either continuous presence or absence of plant litter input at all. However, based on the other parameters (specific enzyme activity, microbial C:N ratio, MBC:SOC), the microbial functioning and the microbial community status were contrasting under bare soil and unmanaged treatments.

As the disturbance gradient was related to decreasing aboveground plant biomass input, the observed bell-like form is most likely explained by belowground biomass input via root activity (Shen et al., 2020), slog-off cells and decaying root debris (Berhongaray et al., 2019). 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). Higher SOC content and SOC-dependent parameters under low intensity grazing were induced not only by aboveground plant input but also by dung and defoliation-driven belowground plant input (Bazot et al., 2005; Shen et al., 2020). In bare soil, the absence of input coupled with ongoing decomposition will result in continuous loss of C (Barré et al., 2010). All these bell-like response parameters, therefore, were not dependent on the aboveground plant input but dependent on the belowground input and input quality.

3.5.3. 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. These parameters were not related to aboveground biomass input either. It seems that these soil properties were more related to root biomass rather than to APBL. It was surprising to find higher root biomass declined 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 factors such as the presence of animals and fertilisation, which were not considered in the disturbance framework could also influence pH, which in turn had effects on 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 followed ripple-like form with the peaks on LGraz and bare soil. It is not surprising to observe high specific enzyme activity in bare soils, which occurred due to the 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. Therefore, specific enzyme activity remained high and likely this reflects the degradation of the stable components of SOM. Thus, the metabolic activity of microbial community was not following the disturbance gradient and may not be directly related to management intensity measured by aboveground plant biomass input.

3.5.4. Properties, which were responding only to the presence or absence of disturbance

The GlcN/GalN ratio was responding to the presence of disturbance but not influenced by disturbance intensity. This ratio was lower in unmanaged soils due to their high galactosamine content, which was much lower under other grassland management practices. Our results were supported by a study showing that arable land restoration into pasture 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 be also explained by higher fungal residue contribution in the unmanaged system. In addition, it seems that any long-term soil disturbance increases 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 faeces contain 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 loading.

3.6. Conclusions

Even though it is challenging to identify the main driver of the changes occurring in such complex grassland systems we tried to simplify and to focus only on one factor: disturbance gradient based on the level of aboveground biomass leftover which was a simplified indicator of the aboveground substrate input quantity. We have isolated the parameters and arranged them into four groups of response. Only amino sugar content and microbial C:N ratio were dependent on the aboveground plant biomass leftover. The bell-like form group reflected the influence of other inputs (belowground and animal input). Ripple-like form indicated that microbial activity was sensitive to the change of soil physiochemical conditions, which were probably in turn altered by grassland management. Only two properties were sensitive to the control sites only: GluN/GalN was sensitive to any kind of disturbance whereas metabolic quotient qCO_2 was sensitive to the absence of any kind of input. We therefore suggest that in order to evaluate disturbance of belowground systems under agricultural management, aboveground plant biomass input may only be used if the interest is in microbial necromass formation and microbial community composition. Root biomass input and activity might be a better indicator for disturbance of belowground microbial functioning. This disturbance gradient based on microbial activity parameters would follow the order Mow<LGraz<UM<HGraz<Bare.

CHAPTER 4. MANAGEMENT OF GRASSLANDS BY MOWING VERSUS GRAZING – IMPACTS ON SOIL ORGANIC MATTER QUALITY AND MICROBIAL FUNCTIONING

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4.1. 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.

4.2. Resume

Bien que 30% de la surface européenne soit couverte de prairies, on sait peu de choses sur l'effet de leur gestion sur la qualité des sols et le cycle biogéochimique. Ici, nous avons analysé le sol d'un site expérimental de l'ouest de la France, qui était sous régime de pâturage ou de fauche depuis 13 ans. Nous visions à évaluer l'effet des deux pratiques de gestion sur le fonctionnement biogéochimique du système pédologique. À cette fin, nous avons comparé la composition de la matière organique du sol (MOS) et les propriétés microbiennes à deux profondeurs du sol. Nous avons analysé la teneur et la composition des polysaccharides élémentaires, lignines et non cellulosiques, ainsi que la biomasse microbienne, la respiration microbienne du sol et les activités enzymatiques. Nos résultats ont montré des teneurs plus élevées en carbone organique (COS) et en azote dans le sol de surface sous pâturage par rapport à la tonte. Les propriétés biogéochimiques du sol différaient entre les traitements de pâturage et de tonte. En particulier, le sol sous pâturage a montré une lignine plus faible et une biomasse microbienne plus élevée. Malgré la teneur similaire en polysaccharides non cellulosiques sous les deux traitements, la communauté microbienne sous fauchage était caractérisée par une production d'enzymes plus élevée par biomasse microbienne, conduisant à une SOM plus dégradée dans le système de tonte par rapport au pâturage. Nous concluons que les régimes de pâturage et de tonte ont un impact différent sur le fonctionnement biogéochimique du sol. Un apport de carbone plus élevé et plus diversifié sous pâturage par rapport au fauchage peut conduire à une meilleure disponibilité du substrat et donc à un fonctionnement microbien plus efficace, ce qui pourrait favoriser la séquestration du COS par la formation de produits microbiens.

4.3. 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 achieved 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 the 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., 2010). In order to compensate for nutrient exportation during mowing events, mineral fertilisers are applied in mowing systems.

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

and less soluble compounds compared to the green litter returned as harvesting losses in mowing systems (Sanaullah et al., 2010).

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

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

4.4. Results

4.4.1. Soil properties

Soil physicochemical properties are presented in Table 8. 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 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 in the surface soil of 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.

Table 8. 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).

4.4.2. 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 9, 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 9, P<0.001). All NCP monomers ratios were higher in subsurface soil compared to surface soil under both treatments.

Table 9. 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)	2.50 (0.12)	5.42 (0.03)	3.81 (0.06)	4.74 (0.04)
Treatment		36.6 (<0.001)	0.11 (0.74)	0.87 (0.36)	0.01 (0.91)	0.64 (0.43)
Depth		241.1 (<0.001)	11.5 (0.002)	122.2 (<0.001)	102.8 (<0.001)	166.3 (<0.001)
Treatment×Depth		19.7 (<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).

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 10). 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 correction (Table 10, 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.

Table 10. Lignin signature in soil under two grassland management practices (grazing and 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)							
SOC stocks in 2005		0.05 (0.82)	10.9 (0.002)	1.59 (0.22)	1.99 (0.17)	0.08 (0.78)	18.6 (<0.001)
Treatment		15.3 (<0.001)	1.14 (0.29)	0.64 (0.43)	7.91 (0.01)	2.83 (0.10)	2.82 (0.10)
Depth		96.3 (<0.001)	0.62 (0.44)	8.58 (0.006)	0.95 (0.34)	9.13 (0.005)	5.95 (0.02)
Treatment×Depth		4.83 (0.04)	0.88 (0.36)	0.015 (0.90)	2.19 (0.15)	16.3 (<0.001)	13.1 (<0.001)

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

4.4.3. Soil microbial properties

The soil microbial respiration (SMR) ranged between 0.2 and 0.7 μg CO_2 –C g^{-1} h^{-1} with highest values in the surface soil under grazing treatment (Fig. 22A). After correcting for initial SOC stock effect, treatment × depth interaction effect on SMR was significant (Table 11, Supplementary materials in Annexes, P<0.001). Soil microbial respiration per SOC was around 33% higher in the surface soil under grazing as compared to mowing (Fig. 22B). In contrast, it was greater in the subsurface soil under mowing than under grazing treatment. Including initial SOC stock as covariate resulted only in significant effect of treatment × depth interaction on soil microbial respiration per SOC (Table 11, Supplementary materials in Annexes, P=0.004).

MBC per SOC was highest in the surface soil under grazing (20 μg C mg^{-1} SOC, Fig. 22C). Mowing treatment resulted in two times lower MBC per SOC in the surface soil compared to grazing treatment. After correction for initial SOC stock depth, treatment (P<0.001) and their interaction (P<0.001) showed significant effects on qCO_2. Mowing treatment resulted in higher qCO_2 at both depths as compared to grazing treatment (Fig. 22D, P=0.02).

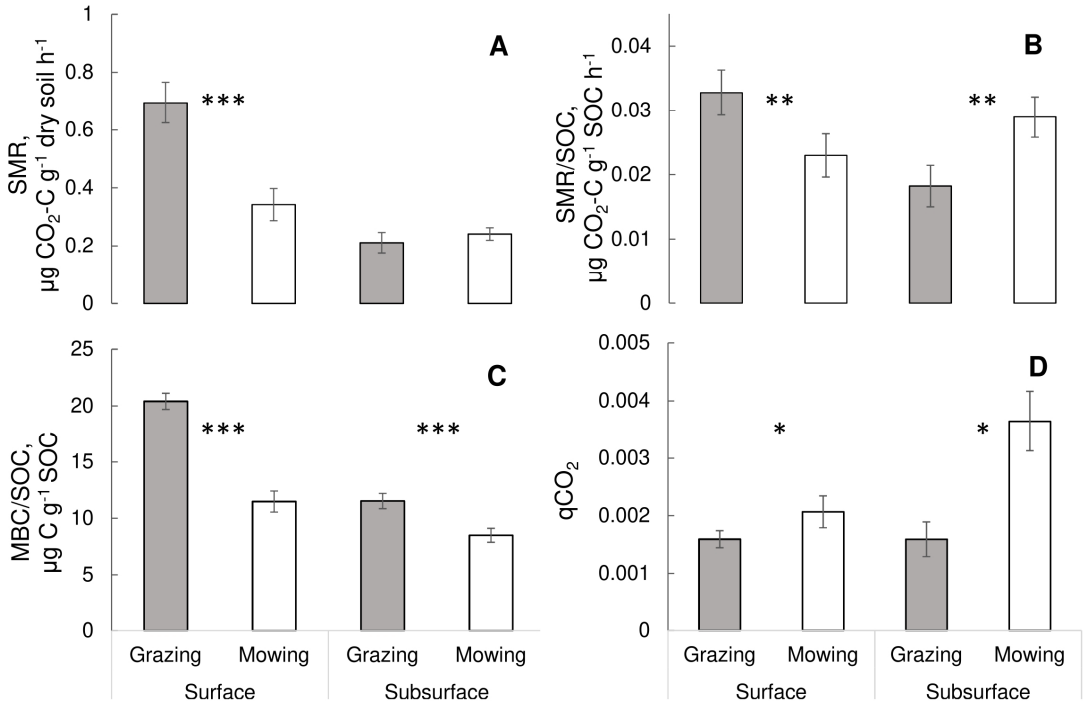

*Figure 22. (A) Soil microbial respiration (SMR), (B) soil microbial respiration (SMR) per soil organic carbon (SOC), (C) microbial biomass carbon (MBC) per soil organic carbon (SOC) and (D) metabolic quotient (qCO_2) in soil under two grassland management practices (grazing and mowing) in surface soil (0-10 cm) and subsurface soil (20-30 cm). Significant differences between the treatments are indicated by *, ** and ***, representing probability at the 5, 1, and 0.1% levels, respectively.*

Microbial C:N ratio ranged between 4.9 and 6.4. It was affected by treatments in all depths showing higher values under mowing (Fig. 23A). After adjustment by initial SOC stock, the treatment effect was still significant (Table 11, Supplementary materials in Annexes, $P<0.001$). After correction by initial SOC, the percentage of active microbial biomass was higher under mowing at both depths compared to grazing treatment (Fig. 23B, $P=0.02$). The highest specific microbial growth rate (Fig. 23C) was recorded in subsurface soils without difference between treatments. But in surface soils, the specific microbial growth rate was higher under grazing than under mowing (Fig. 23C). However, ANCOVA with initial SOC stock correction decreased the significance treatment effects ($P=0.05$) on specific microbial growth rate but increased the depth effect (Table 11, Supplementary materials in Annexes, $P<0.001$).

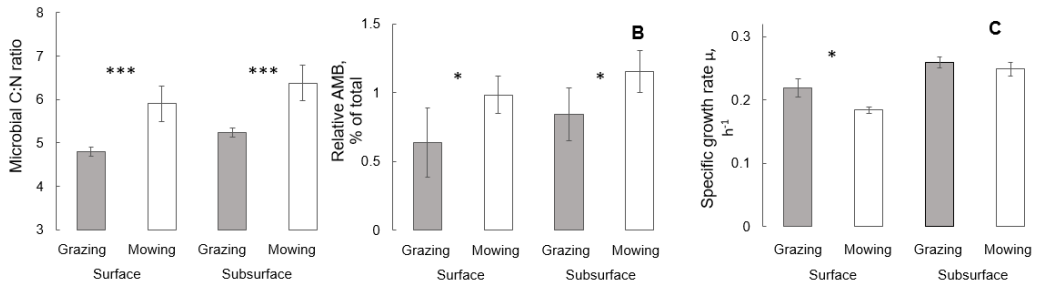

*Figure 23. (A) Microbial C:N ratio, (B) the percentage of active microbial biomass (AMB) and (C) specific microbial growth rate (μ) in soil under two grassland management practices (grazing and mowing) in surface soil (0-10 cm) and subsurface soil (20-30 cm). Significant differences between the treatments are indicated by *, ** and ***, representing probability at the 5, 1, and 0.1% levels, respectively.*

Treatment effects on absolute enzyme activities is presented only for leucine aminopeptidase in surface soil and chitinase and phosphatase in subsurface soil (Fig 31, Supplementary materials in Annexes). When the initial SOC stock was used as covariate, treatment differences between enzyme activities per MBC were observed for all enzymes (except leucine aminopeptidase) in surface soil. Soil under mowing treatment showed 2-2.5 times higher enzyme activity per MBC under mowing compared to soil under grazing (Fig. 24). The differences between treatments were more pronounced in surface soil for activities of chitinase, β-galactosidase, β-glucosidase and phosphatase (Table 11, Supplementary materials in Annexes).

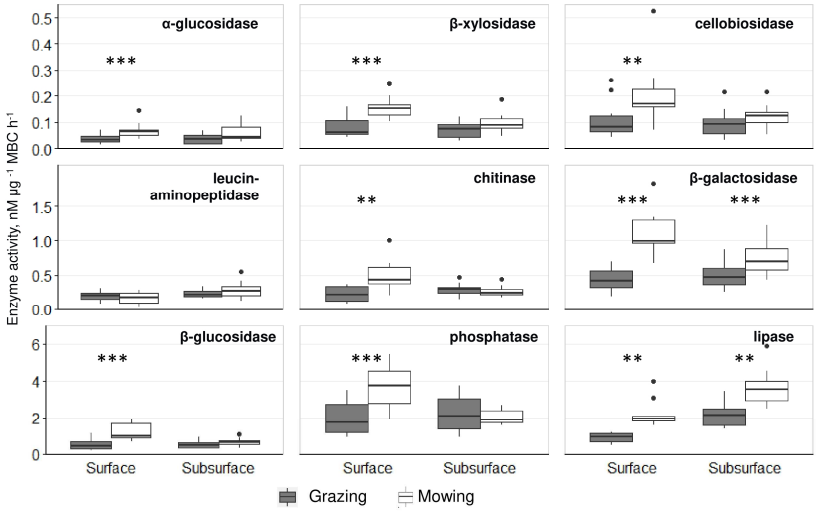

*Figure 24. Boxplot of enzyme activity per unit of microbial biomass C (MBC) for nine enzymes under two grassland management practices (grazing and mowing) in surface soil (0-10 cm) and subsurface soil (20-30 cm). Significant differences between the treatments are indicated by *, ** and ***, representing probability at the 5, 1, and 0.1% levels, respectively.*

4.4.4. Principal component analysis

Principal component analysis based on SOC normalised data of all soil properties showed that the first two factors explained 54.4% of the variation (Fig. 25). The first component (Dim1) was related to microbial functioning, as it was strongly associated with the soil microbial properties MBC and MBN per SOC in negative direction. The positive direction was related to the lipase activity per MBC. The second component (Dim2) was explained by variables related to polysaccharides. It was positively correlated with enzymes participating in polysaccharide degradation and negatively with polysaccharide ratios. The clustering of samples allowed to separate surface soil and subsurface soil samples along both axes, while surface soil samples were additionally separated by treatments the first axes (Fig. 25). Subsurface soil samples were differentiated from surface soil by high neutral polysaccharide monomer ratio, low enzymes activities per MBC, MBC and MBN per SOC. Treatments in surface soil were separated by C- and N-cycle enzyme activity and MBC and MBN per SOC. We also applied ANCOVA with initial SOC stock as a covariate on new 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.

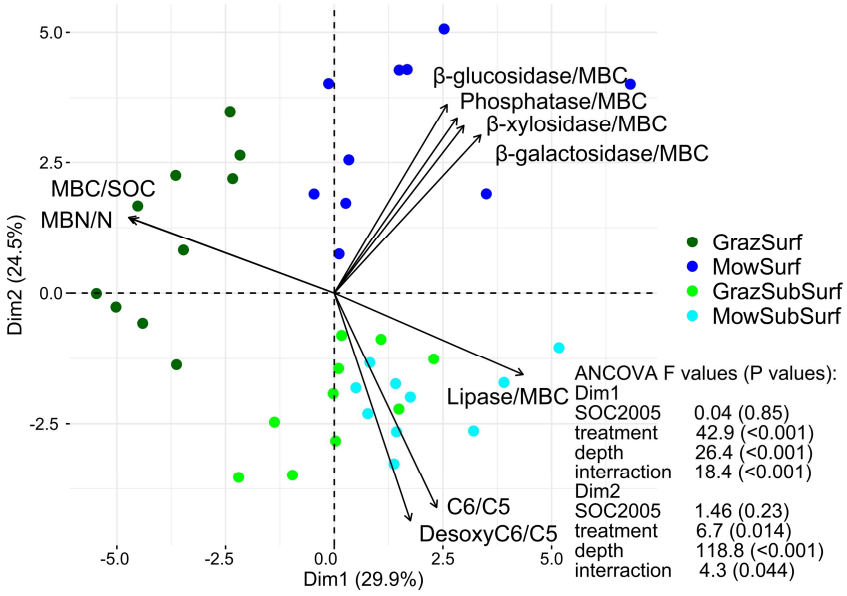

Figure 25. Principal component analysis (PCA) for soil under grazing and mowing in surface soil (0-10 cm) and in subsurface soil (20-30 cm). Only variables with quality of representation (cos^2) higher than 0.75 was shown on PCA plot.

4.5. Discussion

4.5.1. Effect of grazing and mowing on chemical properties in 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, which was shown through ecosystem flux measurements at these plots (Senapati et al., 2014). Besides, 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 can get some biomass input in the form of lost plant material during grass removal (Sanaullah et al., 2010), it is not enough to reach a similar level of input 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 treatment 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, is lower under grazing than mowing. As lignin is a biomarker for plant-derived organic matter and more difficult to decompose, since it requires a specific enzymatic 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 monomer ratios (except C/V ratio) suggested that lignin was less degraded in the grazing than the mowing system. More acidic 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 shows 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.

4.5.2. Effect of grazing treatment on biological properties in surface soil

Higher maturity and sustainability of grazing system was showed by higher MBC per SOC together with a lower qCO_2 (Anderson and Domsch, 2010). Higher qCO_2 in 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 normalisation by MBC, which expresses microbial activity in terms of enzyme production. The enzymatic activities per MBC were higher in the mowing system as compared to the grazing one, indicating that microorganisms in mowed soil produced enzymes more actively than those under grazing. Microbial community in the mowing system stayed active and were investing in enzyme production probably to adapt to less decomposable organic materials with higher lignin contents (see above). This maintenance of active state requires a lot of energy, consequently, it could the change of C-cycling rates and decomposition of SOM (Schimel and Schaeffer, 2012; Wang et al., 2014).

Microbial communities in the mowed soil are probably characterised by a high contribution of fungi because we recorded a higher C:N ratio of the microbial biomass (Joergensen and Emmerling, 2006) and more acid pH. Lower specific growth rates in the mowing system indicated relative domination of K-strategists in the microbial community, which are more adapted to nutrient poor conditions (Strickland and Rousk, 2010; Xu et al., 2017) and the decomposition of specific substances, such as plant material containing high amounts of biopolymers (Fontaine et al., 2003). As illustrated by lower enzyme activity per MBC, microorganisms in the grazing system invested less energy for the degradation of complex compounds than those of the mowing system, most probably because of higher

availability of easily decomposable substrates. These conditions favour r-strategists (Fierer et al., 2007; Xu et al., 2017) and thus stimulate microbial activity, as shown by higher MBC per SOC and higher soil microbial respiration under grazing as compared to mowing system. As a consequence, the biogeochemical soil functioning under the two management practices is quite different. This may affect significantly SOM formation, which is favoured in systems with intensive microbial processing of C input (Kallenbach et al., 2016; Liang et al., 2017) thus corroborating the high SOC contents observed under grazing.

4.5.3. 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 under grazing subsurface soil. Treatment effects in the subsurface soil were not observed for non-cellulosic polysaccharide content and their origin and neither it was 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).

4.6. 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 efficient microbial functioning. Although SOM changes were only evident in surface soil, microbial properties suggest that these processes are also occurring in subsurface soil.

CHAPTER 5. GENERAL CONCLUSIONS AND PERSPECTIVES

Soil organic carbon (SOC) storage is dependent on the plant input and its microbial processing. Primarily, grassland management practices affect on the aboveground plant biomass and the magnitude varies based on the management strategy and intensity. The aboveground plant biomass removal consequently changes the belowground production. The root-originated SOC might be better retained and it may be the main source of SOC in (grassland) soils (Piñeiro et al., 2010; Rasse et al., 2005). High belowground productivity of grasslands potentially leads to high input of plant-derived organic matter (OM), which can be transformed into soil organic matter (SOM). However, the magnitude of its contribution to SOC storage can depend on the grassland management and pedoclimatic conditions characterised by soil texture, precipitation and grassland type (Abdalla et al., 2018; McSherry and Ritchie, 2013).

While numerous studies reported the effect of grazing and its intensity on SOC storage, soil stoichiometry this PhD thesis investigated the processes occurring behind the SOC stock changes driven by different management practices, in particular, grazing versus mowing. Comparative investigations of these two practices are scare and mainly focused on plant physiology. Due to increasing interests of microbial participation in SOM formation we conducted this study in order to understand the differences of grazing and mowing effect on soil biogeochemical properties and microbial functioning under two different pedoclimatic conditions.

Chapter 2 demonstrated that grassland management alters the lignin chemistry of aboveground OM input but not of belowground OM input. Simultaneously, neither of both OM inputs reflected the soil lignin signature, although, there might be a small link between root and soil lignin composition under high intensity of grazing. This finding suggested to test microbial functioning because it most likely led to more degraded SOM under mowing compared to grazing and unmanaged sites. In the Chapter 3, we demonstrated that aboveground input quantity may control microbial necromass accumulation and microbial C:N ratio but not microbial functioning. Microbial functioning is probably driven by belowground input quantity and total input diversity. Also we showed that grazing and mowing in natural grassland under semi-continental climate resulted in contrasting microbial functioning but similar SOC content. Thus, this result led us to investigate further whether grazing and mowing impact on soil biogeochemical properties will be different under contrasting pedoclimatic conditions (Chapter

4). Our results indicated that sown grassland under temperate oceanic climate resulted in more clear differences beween grazing versus mowing related to alterations of microbial functioning as well as SOC content.

Hereby, we provide a synthesis of our work by answering some relevant questions and identify some future research perspectives

How do plants respond to grazing and mowing and does plant chemistry reflect soil chemistry?

This PhD study showed that grassland management alters lignin chemistry of aboveground OM input via the changes of plant maturity and plant community changes. Moreover, he aboveground plant OM input quality reflected the soil lignin composition. However, management-induced alterations of belowground OM input quality were not reflected either by stoichiometry or soil lignin composition.

Belowground input quantity also controlled soil microbial functioning. In this study we did not measure the total OM input quality (including the root exudates) and therefore cannot exclude that microbial functioning was also related to the total OM input diversity. Input diversity may be the reason, why grazing (with urine and dung input) and mowing (with only inorganic fertilisation) resulted in differences in microbial functioning.

However, we investigated plant organs material, which demonstrates only probable future plant OM input. During lifetime plants secrete root exudates, which could have greatly influenced microbial functioning. In order to account for all input sources, the additional investigation of root productivity and root exudates is required. Characterisation of such labile compounds may be important for the understanding of soil biogeochemical cycles, as recent studies focusing on the belowground exudate input support its strong effect on the SOC storage (Shen et al., 2020).

Another aspect, which needs to be elucidated, is the grassland plant community composition and the differences in lignin chemistry among grassland plant species. This is important because some plant species (e.g. leguminous) contain more S units and thus plant composition alterations may further determine the fate of lignin in soil. Future work could be also related to the investigation of labelled plant- and dung-derived OM decomposition and fate under grazing and mowing in field conditions. This could help to understand the effect of grazing and mowing on the transitional step between OM input and its fate in soil.

How do pedoclimatic conditions influence the impact of harvesting regimes on soil biogeochemical properties?

We investigated two soils under grazing and mowing regime at two sites with contrasting pedoclimatic conditions: (1) Clermont-Ferrand with sandy loam Eutric Cambisol (75-78 mg C g-1 soil) under natural grassland (Chapter 3) and (2) Lusignan with loamy-clay Dystric Cambisol (14-20 mg C g-1 soil) under sown grassland (Chapter 4). In order to reveal how the pedoclimatic conditions influence the effect of grassland management on soil properties, we carried out Principal Component Analysis with the data from the Chapters 3 and 4. For site comparison with similar treatments, we selected the high intensity grazing treatment from the Clermont-Ferrand site because the amimal charge was similar to the intensity of grazing in Lusignan (13.9 vs 15-20 LSU ha^{-1}).

PCA with the whole data set, i.e. soil biogeochemical variables and variables related to microbial activity, showed that the first two factors explained 66.3% of variation. The first component (Dim1) was strongly correlated with SOC content and the SOC-dependent parameters and caused the separation of the experimental sites (Fig. 26). The second component (Dim2) was correlated with microbial C:N ratio and some enzyme activities which resulted in the separation of the treatments.

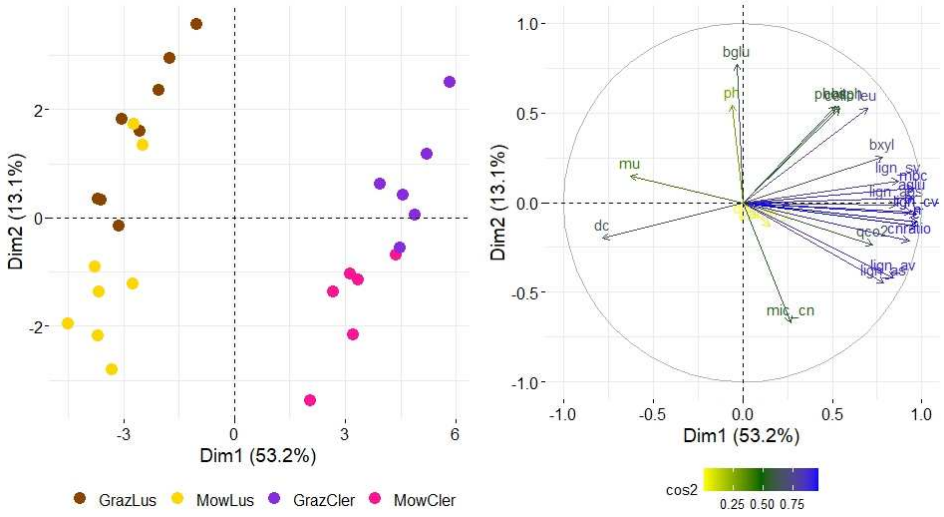

Figure 26. Principal component analysis of soil chemical and biological (microbial) properties under grazing and mowing at Lusignan (Lus) and Clermont-Ferrand (Cler) sites.

It was also interesting to note that the separation of the treatments under different pedoclimatic conditions occurred due to different reasons (Fig. 27). When the PCA was applied separately for chemical and microbial properties, the separation was driven by chemical properties for the Clermont-Ferrand site (Fig. 27 left) whereas at Lusignan site the separation of treatments was driven by microbial properties (Fig. 27 right).

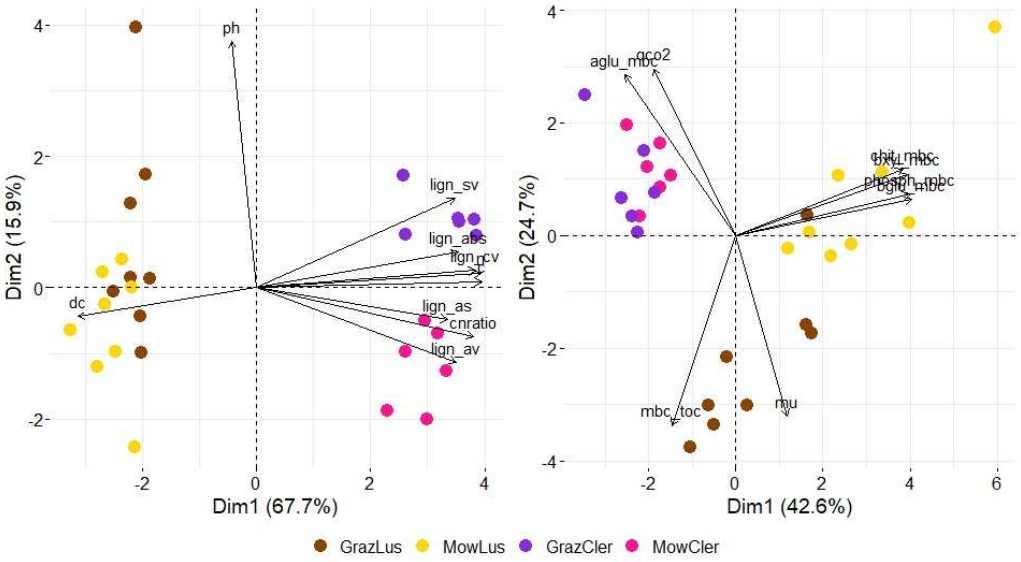

Figure 27. Principal component analysis based on the chemical (left) and biological (microbial) properties (right) separately.

However, the treatments at both site differed chemically as well biologically when each site was compared separately.

The comparison of both sites together allows to determine the key factor determining the differences between treatments. Our results indicate that semi-continental climate with lower clay content but higher SOC content in Clermont-Ferrand results in the separation of grazing and mowing by pH (Fig. 27 left), while temperate oceanic climate with higher clay content and lower SOC content results in separation of grazing and mowing by microbial functioning differences (Fig. 27 right). While for grazing intensity impact depends on grassland type, climate and soil mineralogy (Abdalla et al., 2018; McSherry and Ritchie, 2013), the impact of grassland management strategy (grazing and mowing) in our study was dependent from pedoclimatic conditions such as climate, soil type and grassland type. However, based only on this study we are not able to determine whether this is due to the combined effect of three factors or only one of them.

How does harvesting regime influence the microbial- and plant-derived compounds of SOM?

In order to understand why the SOC content was different between treatments at Lusignan and did not differ in Clermont-Ferrand, we investigated the contribution of plant-derived and microbial-derived C in the two soils. To this end, we used lignin content per SOC as plant-contribution, whereas microbial biomass C per SOC was interpreted as microbial contribution (Fig. 28). However, it would have been more correct to use amino sugars content, but we did

not measure it in soil samples of Lusignan. This is why, we indirectly estimated microbial C contribution via microbial biomass contribution to SOC (MBC/SOC) as the increase of microbial biomass formation is coupled with the contribution of microbial residues (Khan et al., 2016).

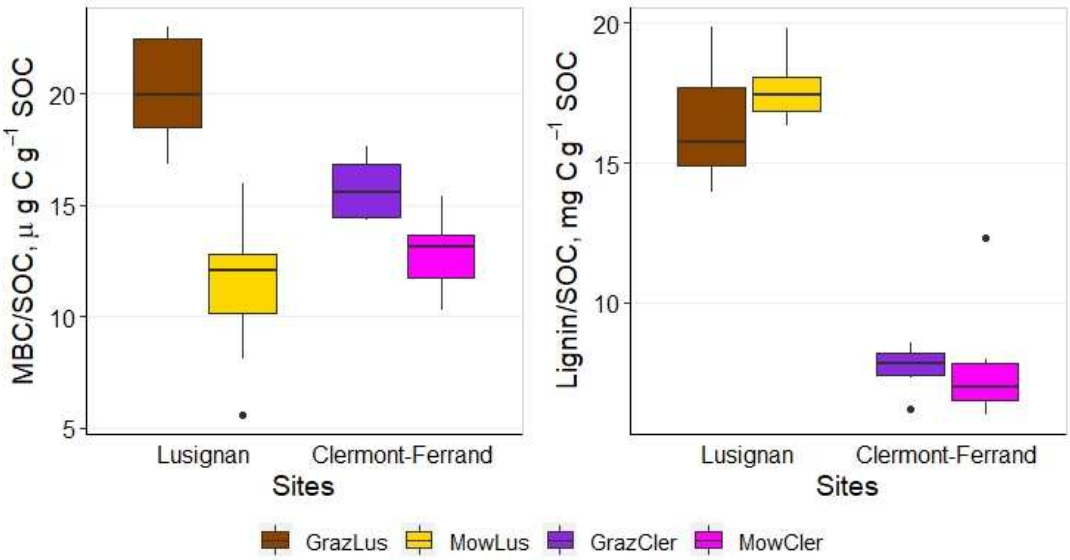

Figure 28. Microbe and plant contribution to SOC

The contribution of plant and microbial-derived compounds to SOC responded differently to both factors: treatments and pedoclimatic conditions. Microbial-derived SOC contribution was higher under grazing at both sites, although the magnitude of increase under grazing was different between the sites (Fig. 28 left). The difference between treatments was stronger at the Lusignan site than at the Clermont-Ferrand site. The pedoclimatic conditions of Lusignan induced higher microbial contribution to SOC under grazing. Probably, pedoclimatic conditions with higher clay content and the absence of winter snow cover at the Lusignan site could have stimulated microbial contribution under grazing. Even if we did not measure amino sugars content at the Lusignan site, microbial biomass contribution can indirectly indicate that grazing at this site has the potential to gain microbial C.

Lignin contribution was similar between the treatments but completely different between the sites (Fig. 28 right). Soils at Lusignan showed higher lignin contribution to SOC compared to soils at Clermont-Ferrand. This could be related to higher clay content and mean annual temperature in Lusignan site, which may have caused the increase of lignin contribution to SOC (Thevenot et al., 2010).

Which parameters are sensitive to treatments regardless of pedoclimatic conditions?

We determined three parameters which responded similarly whatever pedoclimatic condition: pH, lignin degradation state based on the $(Ac/Al)_v$ ratio and microbial C:N ratio (Fig. 29).

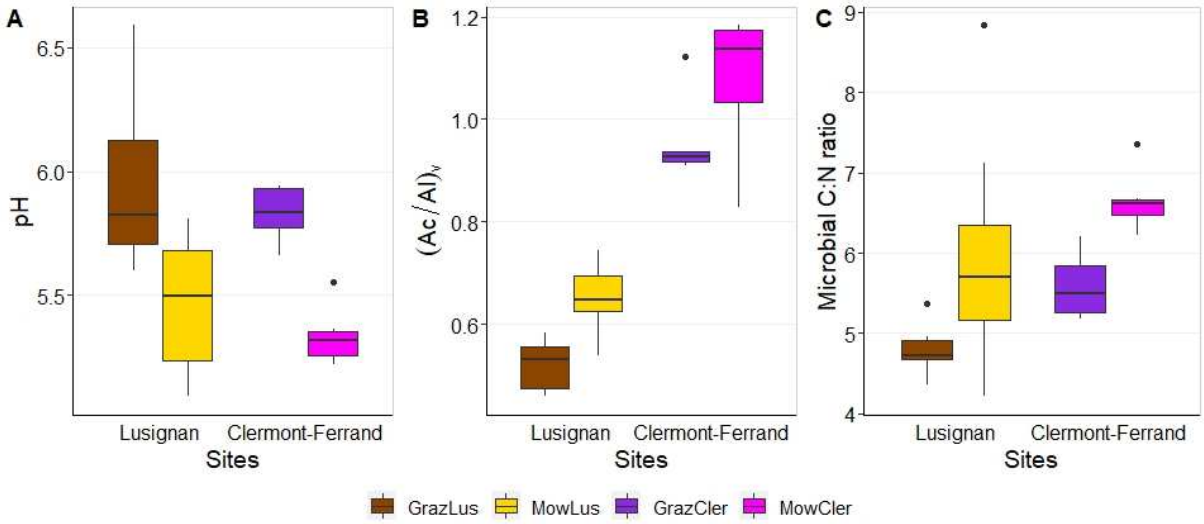

Figure 29. The soil properties sensitive to treatments regardless of pedoclimatic conditions.

The pH (Fig. 29A) was similarly affected by grazing and mowing at both sites. This is not surprising as dung input increases the pH, whereas, the mineral fertilisation decreases pH. At Lusignan, liming probably reduced the pH difference between the treatments, thus resulting in greater difference between grazing and mowing treatments at Clermont-Ferrand site. Because pH is a very important soil property influencing the plant physiology as well as microbial functioning, the differences in pH between grazing and mowing could have cause the grazing and mowing impacts of the other two soil properties sensitive to treatment $(Ac/Al)_v$ and microbial C:N ratio).

Lignin degradation as indicated by the $(Ac/Al)_v$ ratio was the most representative degradation indicator among other lignin ratios and it was higher under mowing than grazing treatment at both sites (Fig. 29B). The reasons of more degraded lignin under mowing may be related to (1) the lack of fresh OM input stimulates starving microorganisms to decompose lignin (Virzo De Santo et al., 2009) (2) pH values favouring microbial communities capable of degrading lignin (Couto et al., 2006). Although, it was interesting to note that lignin degradation state was impacted, lignin contribution to SOC was still similar between the treatments. This means that lignin contribution to SOC did not differ between grazing and mowing, albeit more degraded lignin under mowing. Lignin decomposition is linked to N availability, consequently, more degraded lignin could indicate the lower N availability for microorganisms under mowing

(Hall et al., 2020a). The N input under grazing is higher and more diverse in N forms (dung and urine) compared to mowing fertilised only by NPK which meets only the plants' needs.

However, the soil $(Ac/Al)_v$ ratio was in general higher in Clermont-Ferrand than in Lusignan. There could be two explanations of higher $(Ac/Al)_v$ in Clermont-Ferrand: (1) the presence of natural grassland due to higher plant community diversity could have increased the soil $(Ac/Al)_v$ ratio (Crème et al., 2016); (2) the semi-continental climate driving fungi domination during low temperature conditions (Pietikäinen et al., 2005) might have led to higher lignin degradation state.

Microbial C:N ratio was another property responding similarly to treatments regardless of the pedoclimatic conditions (Fig. 29C). Grazing results in the decrease of the microbial C:N ratio which can be explained by higher pH, more diverse organic input and higher N availability under grazing. Although we did not measure bacterial and fungal biomasses separately, indirectly the microbial C:N ratio may indicate bacterial dominance under grazing, characterised by high pH values (Blagodatskaya and Anderson, 1998). As mineral fertilisation under mowing is dosed in order to meet only plant nutrient needs, most probably this N input does not compensate the N removal from the mowing system. This could have been also a reason for microbial starvation demonstrated by high microbial C:N ratio.

Finally, all these three properties were related to each other. Regardless of pedoclimatic conditions, mowing resulted in low pH, high microbial C:N ratio and more degraded lignin likely indicating fungi dominated and starving microbial community compared to grazing.

We can thus suppose that more diverse input and higher N availability under grazing promotes higher C use efficiency and turnover rates based on lower microbial C:N ratio and higher microbial biomass contribution to SOC will consequently drive the "microbial pump" leading to stabilisation of microbial-derived SOM components formation (Cotrufo et al., 2013; Liang et al., 2017; Sokol and Bradford, 2019).

Here, we have not focused on the microbial community composition but only assumed based on the indirect indicators. Thus, future work should be directed to a more detailed characterisation of microbial community composition in order to investigate differences and similarities of grazing and mowing treatments under different pedoclimatic conditions and how they are related to SOC storage. Because lignin degradation state was the key difference between grazing and mowing treatments, the focus of future studies of grazing and mowing

impact on soil, should be the lignocellulose degrading system (lignin peroxidase, laccase activities coupled with functional gene coding these enzymes).

Management recommendations

My PhD work indicated that grazing could be a better option of grassland management in temperate oceanic climate with loamy clay soil under sown grassland compared to mowing. Whereas under semi-continental climate in sandy loam soil under natural grassland both of grassland treatments may be beneficial in terms of maintaining the SOC content. But still considering the importance of mowing for forage production, other options could be considered are either a combination of grazing with mowing instead of only mowing or replacement of mineral fertilisation by manure addition. Such alternative practices should be addressed in future investigations.

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PUBLICATIONS:

Gilmullina A., Rumpel C., Blagodatskaya E., Chabbi A. (2020), Management of grasslands by mowing versus grazing – impacts on soil organic matter quality and microbial functioning. *Appl. Soil. Ecol*. https://doi.org/10.1016/j.apsoil.2020.103701

Gilmullina A, Rumpel C, Klumpp K., Louault F., Chabbi A. Do grassland management practices affect soil lignin chemistry by changing the composition of organic matter input? *Submitted in Plant and Soil*

Gilmullina A, Rumpel C, Blagodatskaya E., Klumpp K., Louault F., Bertrand I., Dippold M., Chabbi A. Does aboveground plant input quantity drive microbial functioning of grassland soils? -Towards the evaluation of management intensity on soil processes. *Submitted in Soil Biology and Biochemistry*

PRESENTATIONS AT CONFERENCES

EGU General Assembly 2020. Vienna, Austria. 4–8 May 2020. *Oral presentation (Online)*

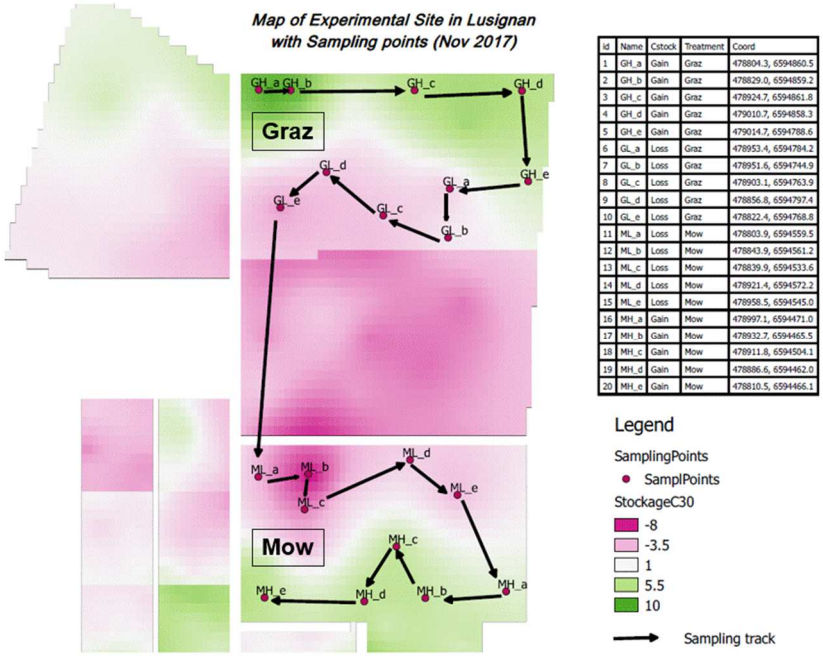
7th International Symposium on Soil Organic Matter. Adelaide, Australia. 6-11 October 2019. *Oral presentation*

International conference: Food security and climate change (4p1000 initiative). Poitiers, France. 18–20 June 2019. *Poster presentation.*

EGU General Assembly 2018. Vienna, Austria. 9–13 April, *2018. Poster presentation.*

ANNEXES

Supplementary materials to Chapter 4



Id	Name	Cstock	Treatment	Coord
1	GH_a	Gain	Graz	478804.3, 6594860.5
2	GH_b	Gain	Graz	478829.0, 6594859.2
3	GH_c	Gain	Graz	478924.7, 6594861.8
4	GH_d	Gain	Graz	479010.7, 6594858.3
5	GH_e	Gain	Graz	479014.7, 6594788.6
6	GL_a	Loss	Graz	478953.4, 6594784.2
7	GL_b	Loss	Graz	478951.6, 6594744.9
8	GL_c	Loss	Graz	478903.1, 6594763.9
9	GL_d	Loss	Graz	478856.8, 6594797.4
10	GL_e	Loss	Graz	478822.4, 6594768.8
11	ML_a	Loss	Mow	478803.9, 6594559.5
12	ML_b	Loss	Mow	478843.9, 6594561.2
13	ML_c	Loss	Mow	478839.9, 6594533.6
14	ML_d	Loss	Mow	478921.4, 6594572.2
15	ML_e	Loss	Mow	478958.5, 6594545.0
16	MH_a	Gain	Mow	478997.1, 6594471.0
17	MH_b	Gain	Mow	478932.7, 6594465.5
18	MH_c	Gain	Mow	478911.8, 6594504.1
19	MH_d	Gain	Mow	478886.6, 6594462.0
20	MH_e	Gain	Mow	478810.5, 6594466.1

Figure 30. Supplementary material 1. Soil sampling map at experimental site in Lusignan. Grazing site is located on the top of map and indicated by "Graz", mowing site is located on the bottom of map and indicated by "Mow". Green colour on the map presents zones with C gain and pink colour presents zones with C loss. Red points show sample points.

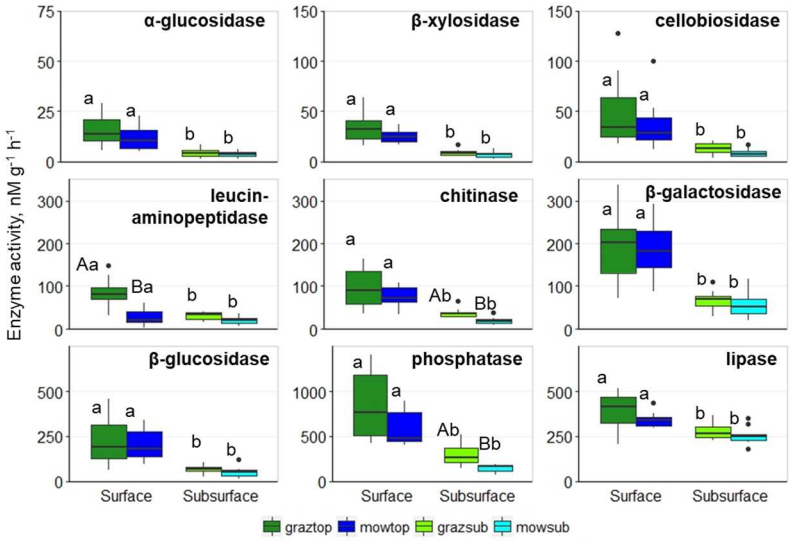


Figure 31. Supplementary material 2. Boxplot of enzyme activity for the nine enzymes. Grazing treatment is presented by green colour and mowing treatment is presented by blue colour. Whereas dark shades show surface soil samples (0-10 cm) and light shades show subsurface soil samples (20-30 cm). Significant differences between the treatments are indicated by capital case letters. Lower case letters show significant differences within depth

($p < 0.05$). Similar letters or the absence of letters indicate that significant differences were not found.

Table 11. Supplementary material 3. Summary of ANCOVA results for microbial variables measured in soil under two grassland management practices (grazing and mowing) at in surface soil (0-10 cm) and subsurface soil (20-30 cm).

Variable	Effect	F value (P values)
Soil microbial respiration	SOC stocks in 2005	0.31 (0.57)
	Treatment	5.45 (0.03)
	Depth	34.4 (<0.001)
	Treatment×Depth	14.7 (<0.001)
Soil microbial respiration per SOC	SOC stocks in 2005	0.32 (0.57)
	Treatment	0.05 (0.83)
	Depth	1.64 (0.21)
	Treatment×Depth	9.39 (0.004)
Microbial biomass C per SOC	SOC stocks in 2005	0.02 (0.87)
	Treatment	41.1 (<0.001)
	Depth	61.6 (<0.001)
	Treatment×Depth	15.2 (<0.001)
Metabolic quotient (qCO_2)	SOC stocks at 2005	0.99 (0.32)
	Treatment	6.25 (0.02)
	Depth	5.54 (0.03)
	Treatment×Depth	5.55 (0.03)
Microbial C :N ratio	SOC stocks in 2005	1.93 (0.19)
	Treatment	15.9 (<0.001)
	Depth	3.23 (0.08)
	Treatment×Depth	0.01 (0.92)
Relative active microbial biomass	SOC stocks in 2005	0.28 (0.61)
	Treatment	6.74 (0.02)
	Depth	2.49 (0.13)
	Treatment×Depth	0.66 (0.43)
Specific growth rate μ	SOC stocks in 2005	0.18 (0.68)
	Treatment	4.08 (0.05)
	Depth	23.9 (<0.001)
	Treatment×Depth	1.33 (0.026)
α-glucosidase	SOC stocks in 2005	1.59 (0.22)
	Treatment	14.0 (<0.001)
	Depth	0.55 (0.45)
	Treatment×Depth	0.27 (0.61)
β-xylosidase	SOC stocks in 2005	1.58 (0.22)
	Treatment	17.3 (<0.001)
	Depth	8.09 (0.007)
	Treatment×Depth	3.97 (0.05)
Cellobiosidase	SOC stocks in 2005	0.38 (0.54)
	Treatment	7.91 (0.008)
	Depth	2.42 (0.13)
	Treatment×Depth	1.27 (0.27)
Leucine-aminopeptidase	SOC stocks in 2005	0.81 (0.37)
	Treatment	0.73 (0.39)
	Depth	6.77 (0.01)
	Treatment×Depth	2.17 (0.15)
Chitinase	SOC stocks in 2005	0.02 (0.89)
	Treatment	4.84 (0.03)
	Depth	0.45 (0.51)
	Treatment×Depth	8.93 (0.005)
β-galactosidase	SOC stocks in 2005	1.13 (0.29)

	Treatment	28.6 (<0.001)
	Depth	3.87 (0.06)
	Treatment×Depth	8.52 (0.006)
β-glucosidase	SOC stocks in 2005	0.15 (0.71)
	Treatment	13.7 (<0.001)
	Depth	3.35 (0.08)
	Treatment×Depth	5.78 (0.02)
Phosphatase	SOC stocks in 2005	2.11 (0.15)
	Treatment	9.29 (0.004)
	Depth	3.07 (0.09)
	Treatment×Depth	7.95 (0.008)
Lipase	SOC stocks in 2005	0.33 (0.57)
	Treatment	43.5 (<0.001)
	Depth	54.3 (<0.001)
	Treatment×Depth	3.64 (0.07)

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

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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 the soil system. To this end we compared soil organic matter (SOM) composition and microbial properties at two depths. We analysed for elemental, lignin and non-cellulosic polysaccharide content and composition, 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 also 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 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 (Piñeiro et al., 2010; Abdalla et al., 2018).

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

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

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

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

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, the legume *Trifolium repens* was included in the species mixture but covered only 5% of the 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 achive 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 overcome to some extent 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 at the beginning of the experiment in 2005 and included initial SOC stocks as a co-variate during statistical analyses (see below). The data recorded in 2005 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 at places, where grazing treatment was established, 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}) at mowing plot, while at the grazing plot, the values ranged between 1.2 and 1.9 mg g^{-1} (average 1.5 ± 0.1 mg g^{-1}). The study showed partitioning of the field into different zones with SOC gain and loss (A. Crème, personal communication; 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 (5 cm Ø, 30 cm) 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 were 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 microbiological analysis, soil samples were moistened by distilled water to adjust 50% of WHC and pre-incubated at 22 °C for 7 days.

2.2. Soil general properties

Soil pH (H_2O) was measured in a soil:water suspension (1:2.5 weight/volume). Soil organic carbon (SOC), nitrogen (N) and stable isotopes (^{13}C and ^{15}N) contents were measured using a CHN autoanalyser (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 N2 for nitrogen.

2.3. Soil chemical properties

Lignin was analysed by the alkaline cupric oxide (CuO) oxidation method (Hedges and Ertel, 1982; Kögel and Bochter, 1985). Briefly, oxidation was carried out under alkaline conditions (2 M NaOH) at 172 °C for 4 h using 500 mg of air-dried soil, 250 mg of CuO, 50 mg of ammonium ferrous hexahydrate and 50 mg of glucose. After cooling, samples were acidified with 5 M HCl and left overnight for humic acid precipitation. Removal of humic acids was conducted through centrifugation (10 min at 10,000 rpm) and followed by extraction of phenolic oxidation products with C18 reversed phase columns. The phenols were derivatized with BSTFA and quantified as trimethylsilyl derivatives by gas chromatography with a HP gas chromatograph (HP GC 6890) equipped with a flame ionization detector and a SGE BPX-5 column (50 m length, 0.25 mm inner diameter, 0.32 μm coating). Samples were injected in split mode (1:10). The GC oven temperature was programmed at 100 °C for 2 min, then increased from 100 to 172 °C at a heating rate of 8 °C 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}.

The internal standard ethylvanillin was added before the purification step to quantify lignin recovery and the quantification standard phenylacetic acid was added before GC analyses.

The total lignin content (mg g^{-1} dry soil) of the sample was determined as the sum of phenolic oxidation products: vanillyl (V), syringyl (S) and p-coumaryl (C) in their acid (Ac), aldehyde (Al) and ketone forms. Lignin content was also expressed as lignin content per SOC

(mg g^{-1} SOC). Lignin decomposition was assessed by the ratios of S, C to V and (Ac/Al) ratios of V and S, which generally indicate decomposition state (Thevenot et al., 2010).

Non-cellulosic polysaccharides of plant and microbial origin (Kögel-Knabner, 2002) were determined by gas chromatography after trifluoroacetic acid (TFA) hydrolysis and reduction-acetylation using a method introduced by Rumpel and Dignac (2006) and modified by Eder et al. (2010). The analysis was performed using 700 mg of soil samples. Briefly, hydrolysis of non-cellulose polysaccharides was carried out at 105 °C for 4 h with 10 mL of 4 M TFA. Thereafter, myo-inositol was added as quantification standard to account for the losses during the purification procedure. Removal of soil was performed by filtration through glass fibre filters (Whatman GF/C 0.45 µm). Afterwards, TFA was evaporated using a centrifugal Evaporator EZ-2 ENVI at 35 °C for 4 h and dry samples were left overnight in the freezer. Thereafter, dry samples were dissolved in 0.5 mL of H_2O followed by the addition of 0.9 EDTA in order to avoid co-precipitation of organic material with metal oxides and hydroxides (Eder et al., 2010). One mL sodium borohydride (NaBH4) in dimethylsulfoxide (20 g L^{-1}) was added for reduction of polysaccharide monomers into alditol forms and kept at 40 °C for 1.5 h. Then, acetylation was conducted by addition of 0.2 mL acetic acid, 2 mL of acetic anhydride and 0.2 mL methylimidazole. Acetylated alditols were extracted by 1 mL of dichloromethane and quantified with a HP GC 6890 gas chromatograph equipped with a flame ionization detector. Separation was achieved with a 60 m fused silica capillary column (SGE BPX 70, 0.32 mm internal diameter, 0.25 mm film thickness) under the following temperature program: 170 to 250 °C at 8 °C min^{-1}, followed by 12 min at 250 °C (isothermal). Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The injector was kept at 250 °C and the detector at 260 °C. The non-cellulosic polysaccharides content of soil samples was determined as the sum of monosaccharides: C5 (pentoses: xylose, ribose and arabinose), C6 (hexoses: glucose, galactose and mannose), and desoxyC6 (desoxyhexoses: fucose and rhamnose) (Kögel-Knabner, 2002). A higher C6/C5 ratio generally indicates higher contribution of microbial sugars.

2.4. Soil microbial properties

Microbial biomass C (MBC) and nitrogen (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 were extracted in 0.05 M K_2SO_4 and were measured using a multi C/N analyser (multi C/N analyser 2100S, Analytic Jena). MBC and MBN were calculated with a conversion factor of 0.45 (Jenkinson et al., 2004). For measuring soil microbial respiration (SMR) a half gram of soil sample was placed in 2 mL Eppendorf tubes. The CO_2 efflux was trapped in 3 mL of 0.1 M NaOH and determined by conductometry. The metabolic quotient (qCO_2), reflecting decomposition activity (Anderson, 2003; Anderson and Domsch, 1993), was calculated as soil microbial respiration expressed per gram of microbial biomass carbon: qCO_2 = SMR / MBC (µg CO_2-C g^{-1} MBC h^{-1}).

We used microbial growth kinetics technique as an approach to estimate microbial biomass activity state (Blagodatskaya and Kuzyakov, 2013). This approach is based on 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 for surface soil samples and 10 mg glucose, 1.9 mg $(NH_4)_2SO_4$, 3.8 mg $MgSO_4*7H_2O$, 0.53 mg K_2HPO_4 and 1.35 mg KH_2PO_4 for subsurface soil samples. The amount of mineral salts was preliminary selected in order to avoid soil pH change of more than 0.1 units after addition. For active microbial biomass (AMB) and specific growth rate calculation, the results of substrate induced respiration rate were fitted with a model proposed by Panikov and Sizova (1996) and Wutzler et al. (2012):

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

In order to estimate catabolic (decomposition) activity in regards to specific substrates in soil, we measured extracellular enzyme activity using the fluorometric technique (Koch et al., 2007; Marx et al., 2005; Razavi et al., 2015). Nine 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-galactopyranoside for β-galactosidase, (4) MUF-β-D-xylopyranoside for β-xylosidase, (5) MUF-β-D-cellobioside for β-cellobiohydrolase, (6) MUF-*N*-acetyl-β-D-glucosamide for chitinase, (7) leucine-AMC for leucine aminopeptidase, (8) MUF-heptanoate for lipase and (9) 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 mL of the soil suspension were added to 150 mL 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).

2.5. 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. Equation 1 was 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

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.

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		
		mg g^{-1}	mg g^{-1} SOC	C6/C5[1]	DesoxyC6/C5[2]	Man/Xyl[3]
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)	2.50 (0.12)	5.42 (0.03)	3.81 (0.06)	4.74 (0.04)
Treatment		36.6 (<0.001)	0.11 (0.74)	0.87 (0.36)	0.01 (0.91)	0.64 (0.43)
Depth		241.1 (<0.001)	11.5 (0.002)	122.2 (<0.001)	102.8 (<0.001)	166.3 (<0.001)
Treatment × Depth		19.7 (<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).
Values are shown as avarage of 10 replicates ± SE.

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 monomer ratios, C6/C5 and Man/Xyl 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 monomer 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/

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

	Treatment	Lignin content	Lignin content per SOC	Lignin monomers ratios			
		mg g^{-1}	mg g^{-1} SOC	C/V	S/V	$(Ac/Al)_V$	$(Ac/Al)_S$
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)							
SOC stocks in 2005		0.05 (0.82)	10.9 (0.002)	1.59 (0.22)	1.99 (0.17)	0.08 (0.78)	18.6 (<0.001)
Treatment		15.3 (<0.001)	1.14 (0.29)	0.64 (0.43)	7.91 (0.01)	2.83 (0.10)	2.82 (0.10)
Depth		96.3 (<0.001)	0.62 (0.44)	8.58 (0.006)	0.95 (0.34)	9.13 (0.005)	5.95 (0.02)
Treatment × Depth		4.83 (0.04)	0.88 (0.36)	0.015 (0.90)	2.19 (0.15)	16.3 (<0.001)	13.1 (<0.001)

C/V – the ratio of cinnamyl phenols to syringyl phenols; S/V - the ratio of syringyl phenols to vanillyl phenols; (Ac/Al)V – acid to aldehyde ratio of vanillyl phenols; (Ac/Al)s – acid to aldehyde ratio of syringyl phenols. These ratios are indicators of lignin degradation state in soil.
Values are shown as average of 10 replicates ± SE.

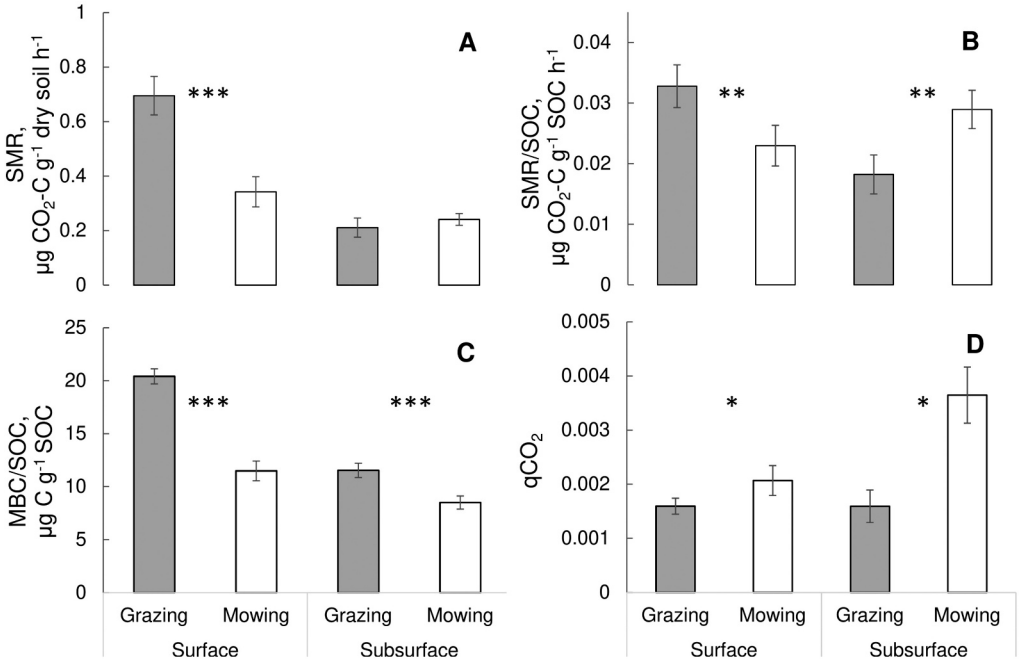

Fig. 1. (A) Soil microbial respiration (SMR), (B) soil microbial respiration (SMR) per soil organic carbon (SOC), (C) microbial biomass carbon (MBC) per soil organic carbon (SOC) and (D) metabolic quotient (qCO_2) in soil under two grassland management practices (grazing and mowing) in surface soil (0–10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

$Al)_V$ 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.

3.3. Soil microbial properties

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

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

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

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

3.4. Principal component analysis

Principal component analysis based on SOC normalised data of all soil properties showed that the first two factors explained 54.4% of the variation (Fig. 4). The first component (Dim1) was related to microbial functioning, as it was strongly associated with the soil microbial properties MBC and MBN per SOC in negative direction. The positive direction was related to the lipase activity per MBC. The second component (Dim2) was explained by variables related to polysaccharides. It was positively correlated with enzymes participating in polysaccharide degradation and negatively with polysaccharide ratios. The clustering of samples allowed separating surface soil and subsurface soil samples along both axes, while surface soil samples were additionally separated by treatments along the first axis (Fig. 4). Subsurface soil samples were differentiated from surface soil by high neutral polysaccharide monomer ratio, low enzymes activities per MBC, MBC and MBN per SOC. Treatments in surface soil were separated by C- and N-cycle enzyme activity and MBC and MBN per SOC. We also applied ANCOVA with initial SOC stock as a covariate on new 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 systems might be explained by greater C input compared to mowing systems. This was shown through

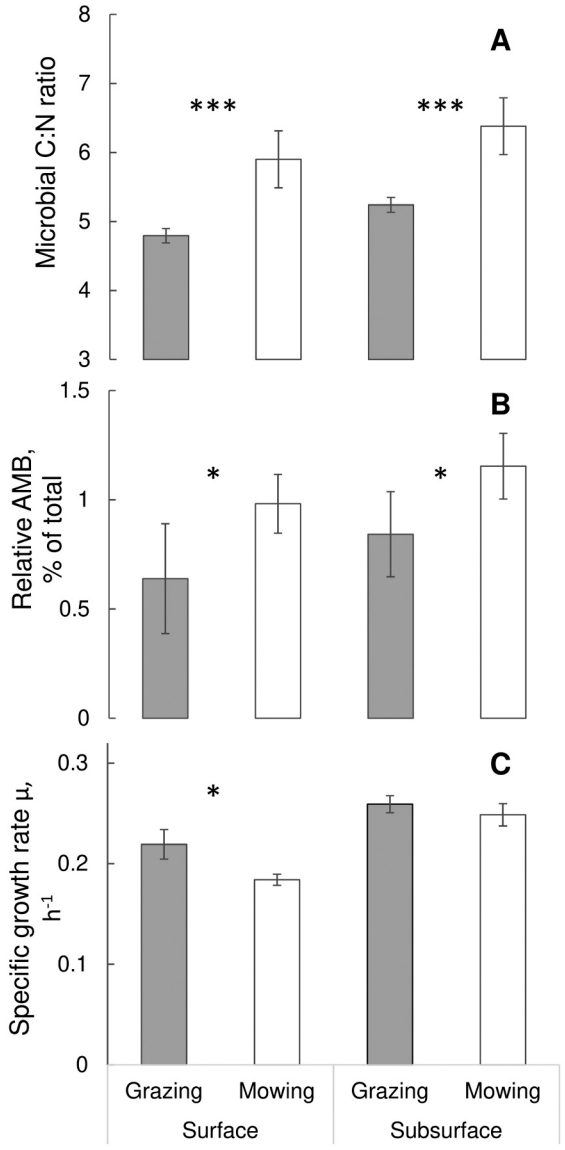

Fig. 2. (A) Microbial C:N ratio, (B) the percentage of active microbial biomass (AMB) and (C) specific microbial growth rate (μ) in soil under two grassland management practices (grazing and mowing) in surface soil (0–10 cm) and subsurface soil (20–30 cm). Significant differences between the treatments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

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

Microbial communities in the mowed soil are probably characterised by a higher contribution of fungi than those of the grazed soil because we recorded a higher C:N ratio of the microbial biomass (Joergensen and Emmerling, 2006) and more acid pH. Lower specific growth rates in the mowing system may indicate relative domination of K-strategists in the microbial community, which are more adapted to nutrient poor conditions (Strickland and Rousk, 2010; Xu et al., 2017) and the decomposition of specific substances, such as plant material containing high amounts of biopolymers (Fontaine et al., 2003). As illustrated by lower enzyme activity per MBC, microorganisms in the grazing system invested less energy for the degradation of complex compounds than those of the mowing system, most probably because of higher availability of easily decomposable substrates. These conditions favour r-strategists (Fierer et al., 2007; Xu et al., 2017) and thus stimulate microbial activity, as shown by higher MBC per SOC and higher soil microbial respiration under the grazing as compared to the mowing system. As a consequence, the biogeochemical soil functioning under the two management practices is quite different. This may affect

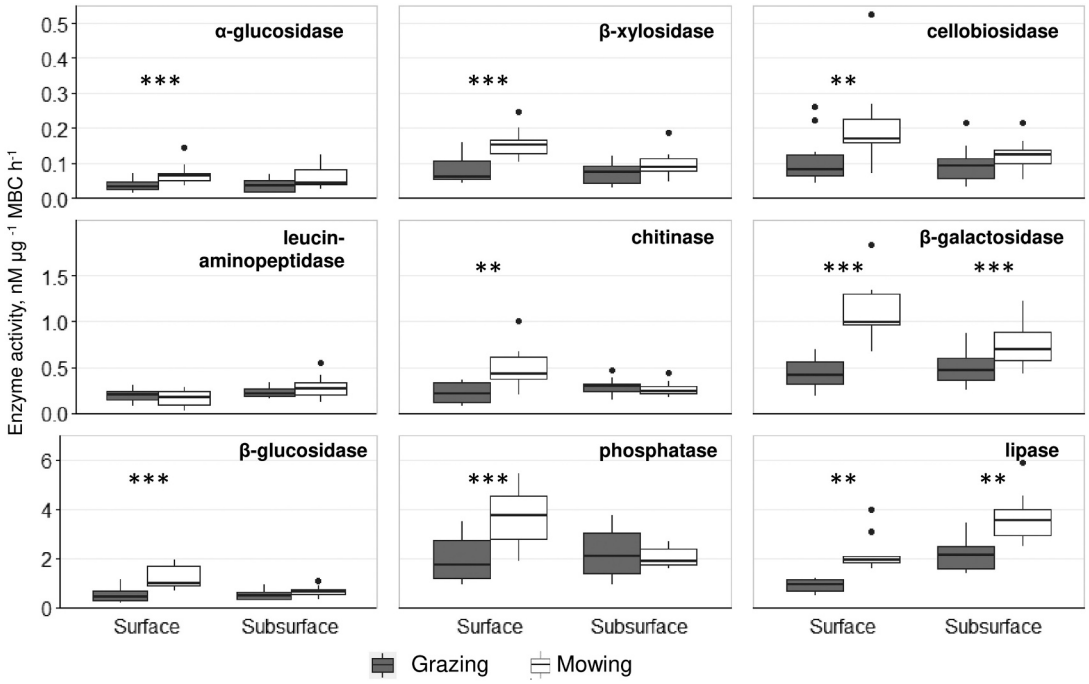

Fig. 3. Boxplot of enzyme activity per unit of microbial biomass C (MBC) for nine enzymes under two grassland management practices (grazing and mowing) in surface soil (0–10 cm) and subsurface soil (20–30 cm). Significant differences between the treatments are indicated by *, ** and ***, representing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

significantly SOM formation, which is favoured in systems with intensive microbial processing of C input (Kallenbach et al., 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

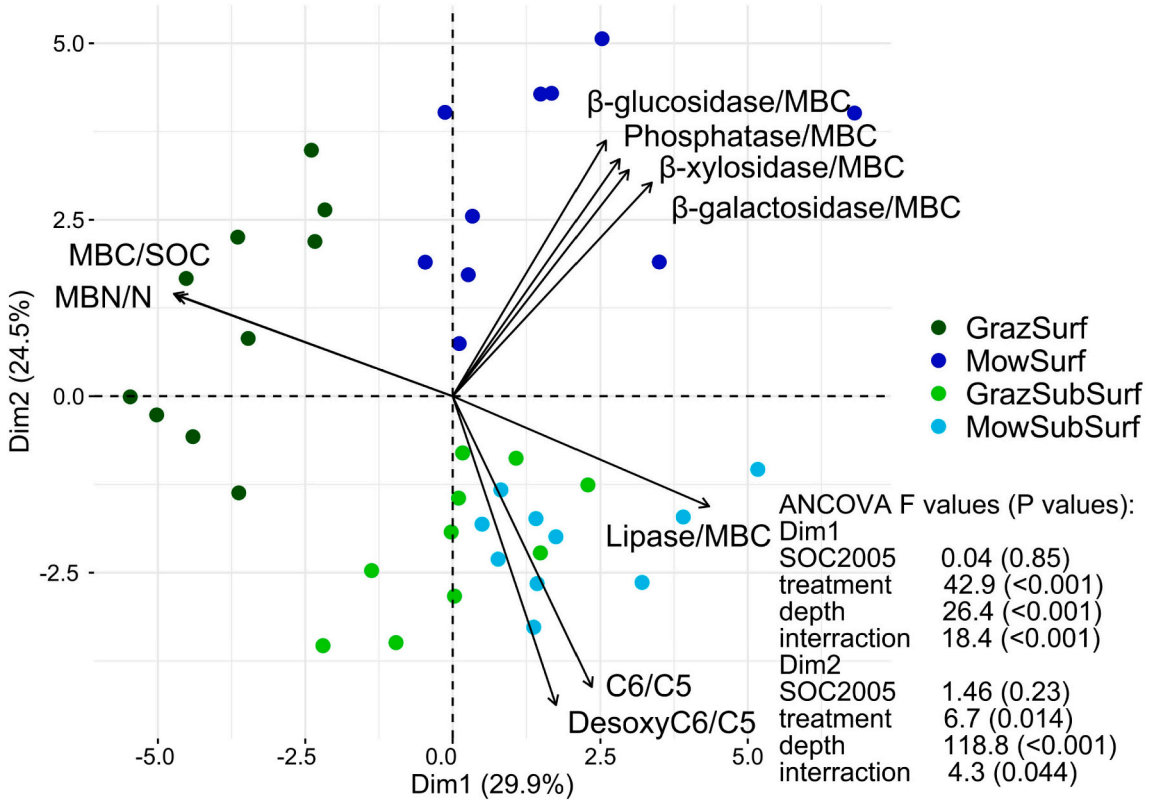

Fig. 4. Principal component analysis (PCA) for soil under grazing and mowing in surface soil (0–10 cm) and in subsurface soil (20–30 cm). Only variables with quality of representation (cos^2) higher than 0.75 was shown on PCA plot.

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 both treatments. Both plots were also characterised by contrasting SOC contents and pH values. The soil under the grazing system was characterised by (1) more efficient microbial communites and (2) less decomposed organic matter as compared to the one under 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 favourable 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.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103701.

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