

The Effects of Land Use and Management on Belowground Carbon and Nutrient Interactions

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Thesis Outline

Chapter 1 This introduction covers the state of the art of the relevant research topics, identifies research gaps and outlines the main objectives of this thesis.

Chapters 2 – 4 are the thematically aligned result chapters for the above-mentioned three main research objectives. In **Chapter 2**, the mineralization potentials, main drivers and interactions of C, N, P and S across three study regions under differently managed forests and grasslands in Germany across regional scale is quantified.

Chapter 3 how different amounts of glucose addition, and associated variation in the degree to which C limitation is overcome, shape the immediate and short-term microbial respiration response and priming effects for soils under different land use.

Chapter 4 focuses on the intensity of soil priming under different land use and management as well as the mechanisms of soil priming due to nutrient mining. The same amount of ^{13}C labelled glucose is added as labile C substrate to all samples to quantify differences in response among different soils.

Chapter 5 presents a synthesis of the three main findings of this thesis and some implications of the main findings on land use and management practices especially pertaining soil's function as C sequester and reservoir for the bioavailability of nutrients. Finally, future research perspectives that arise from the current findings are presented.

Chapter 1

Introduction

1.1 Soil organic matter mineralization as a function of microbial energy and nutrient requirements

The decomposition and mineralization of soil organic matter (SOM) is driven by microbial processes. Most plant- or microbial-derived organic compounds in soil provide heterotrophic microorganisms with energy and nutrients through complex metabolic processes, leading to the formation and maintenance of SOM (Malik et al. 2015, Kallenbach et al. 2016). In a general term, soil mineralization is defined as a process where the organic forms of soil carbon (C) and other macronutrients such as nitrogen (N), phosphorus (P) and sulfur (S) are converted to inorganic forms due to the breakdown of litter and SOM (Chapin et al. 2011). Mechanistically, gross mineralization refers to the total amount of nutrients release via mineralization regardless of microbial or plant immobilization (uptake), while net mineralization refers to the net accumulation of inorganic nutrients after accounting for immobilization by microbes and or plant over a given time interval (Chapin et al. 2011). Soil mineralization is regulated by a large group of soil microorganisms through a wide range of metabolic processes particularly enzymatic reactions based on their stoichiometric nutrient requirements (Nannipieri et al. 2002). Factors affecting the rates of SOM mineralization including the amount and quality of organic substances (Gunina and Kuzyakov 2015, Stewart et al. 2015), various environmental factors such as the chemical and mineralogical properties of soil and SOM (Sollins et al. 1996), climatic factors and seasonal variations has been well studied (Couteaux et al. 1995, Wang et al. 2000).

As an example, the mineralization of sulfur in soil from ester sulfate via sulfohydrolases is controlled by the need for S (Edwards 1998). Ester sulfate is one of the most commonly occurring S-containing compounds in soil, alongside sulfamates and other C-bonded S compounds (McGill and Cole 1981). When the amount of inorganic S from acquired from gross mineralization exceeds microbial demand (immobilization and assimilation), net mineralization of S will occur and become available for plant uptake. Consequently, most soil

S is assimilated into microbial biomass and lower concentration of available S will be accumulated in soil for plant uptake in S-limited ecosystems (Edwards 1998). Thus, from this point of view, the decomposition of SOM can be considered a response function between energy and nutrient availability (supply) and microbial demand (Billings and Ballantyne 2012). The same theory has been discussed for other soil nutrients such as N (Schimel and Bennett 2004). Therefore, soil mineralization is a key process for the decomposition of SOM as well as the release of plant-available nutrients. Recent research has emphasized that the physicochemical and biological factors in the soil environment play an important role for the persistence of SOM (Schmidt et al. 2011) and therefore the rate of the release of these nutrients via soil mineralization. This development has shifted the view that the persistence of SOM and its decomposition rates depend not only on the characteristics of organic matter itself but the environment in which it interacts, such as soil and land use effects (Fontaine et al. 2003b) which alters the microbial activities in soil and therefore controls the decomposition process of SOM. This highlights the importance of studying various environmental factors such as soil abiotic factors and land use effects on the rates of soil mineralization.

1.2 Coupling of carbon to nutrients in soil organic matter and its mineralization

The coupling between microbial processes and nutrient stoichiometry in the terrestrial environment has been proposed by a number of ecological theories (Zechmeister-Boltenstern et al. 2015) namely Ecological Stoichiometry (EST) (Sturner et al. 2002), nutrient use efficiency (Bridgham et al. 1995), threshold elemental ratio (TER) (Frost et al. 2006), resource allocation theory (Allison and Vitousek 2005) and overflow metabolism (Russell and Cook 1995). These concepts originate mainly from the study of Redfield (1934), who was among the first to propose that carbon:nitrogen:phosphorus (C:N:P) ratios in planktonic biomass is rather well-constrained in relation to substrate concentrations in the marine environment. This was later found to be similar for the case of soil biomass at the global level (Cleveland and Liptzin 2007). The establishment of these theories and findings has enabled more soil ecologists to link substrate concentration and microbial ecology in understanding how nutrient availability constrains microbial activities in soil.

Soil organic matter decomposition and mineralization based on microbial energy to nutrient requirements can therefore be observed by analyzing the patterns of C decomposition in parallel to the mineralization of N, P and S. Following the idea of stoichiometric controls, SOM decomposition is enhanced by higher organic carbon (OC) to nutrient ratios as more C is respired to acquire N, P and S which are more limiting than OC to maintain cellular elemental homeostasis (Manzoni et al. 2010, Mooshammer et al. 2012, Tipping et al. 2016). The biological fluxes of these elements are mainly mediated through redox reactions by microorganisms via catalytic reactions in the presence of oxygen (O₂) and organic C (Falkowski et al. 2008). The elemental homeostasis of microbial biomass has been proposed to occur through (i) the rate of elemental acquisition for limiting elements and (ii) through the turnover times of elements in relative to the more abundant elements (Spohn 2016).

1.3 Effects of labile C input on native soil C

The main C input to soil originates mostly from the input of plant litter such as leaf and root litter. In addition, plant root exudation which contains C-rich compounds is released in the rhizosphere and these are used as labile energy source by soil microbes and mycorrhizae to mineralize other limiting soil nutrients which in turn benefit the plant. The input of these labile C may also trigger additional mineralization of C from SOM, a process known as 'priming effect'. Experimental studies have shown that following the addition of labile C sources such as glucose, additional decomposition of autochthonous or native SOM is triggered. In nature, the priming effect has been suggested to occur in microbial hotspots with high C input (Kuzyakov and Blagodatskaya 2015) and has been observed in the rhizosphere or the soil regions surrounding plant roots with high microbial activities (Dijkstra and Cheng 2007, Bengtson et al. 2012, de Graaff et al. 2014), the detritosphere or immediate regions under plant litter comprising of semi decomposed organic materials (de Neergaard and Magid 2015), the drilosphere or earthworm burrows (Bityutskii et al. 2012, Eck et al. 2015) and microaggregates (Tian et al. 2015) or compound soil structures smaller (<250 μm) constructed from diverse materials bound together during soil formation processes (Totsche et al. 2017).

The priming effect is formally defined either as an increase (positive priming) or decrease (negative priming) of native SOM decomposition in response to the input of labile C (Kuzyakov et al. 2000a) (Figure 1.1). It can also be defined depending on whether C or N represents the main focus of a study (Kuzyakov et al. 2000b). For C, the priming effect is defined as an extra decomposition of native organic C following addition of easily-decomposable organic substances to the soil (Dalenberg and Jager 1989). For N, priming is defined as extra soil N which is taken up by plants after addition of mineral N fertilizer in comparison to non-N treated plants (Jenkinson et al. 1985, Leon et al. 1995, Kuzyakov et al. 1997b).

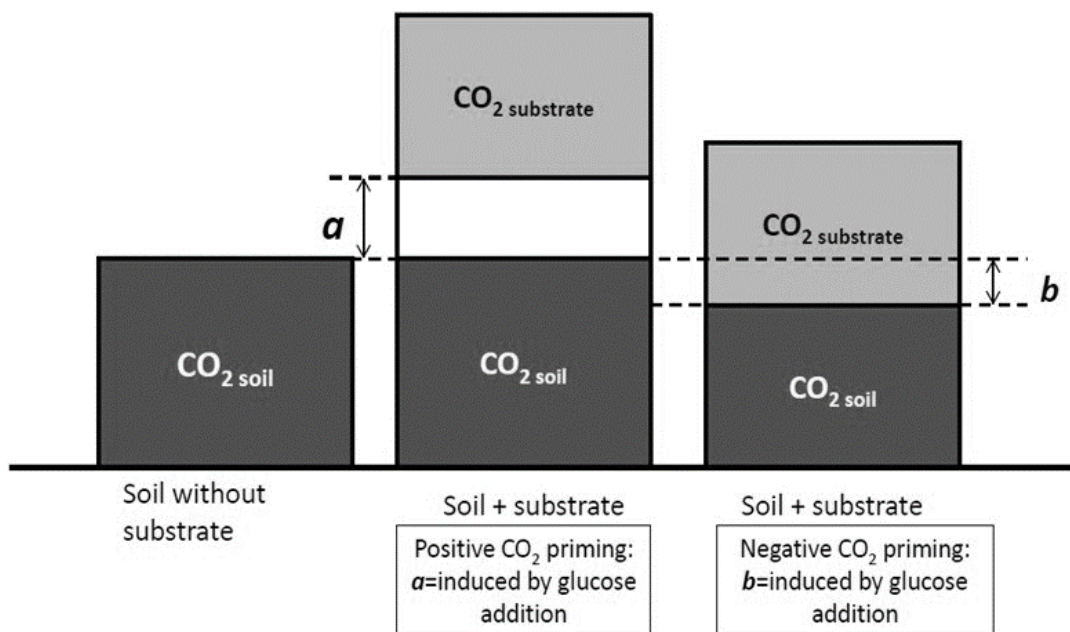


Figure 1.1 Adapted diagrams on the mechanisms of positive and negative priming effects (Kuzyakov et al. 2000a).

1.4 Mechanisms proposed to cause soil priming

The mechanisms behind priming and their controlling factors are still not fully understood. One theory suggests that the addition of an easily available substrate first activates fast growing microorganisms (r-strategists) and enhances their turnover (“apparent” priming), which is then facilitating the growth and activity of slow-growing k-strategists (Fontaine et al. 2003a). These are assumed to be responsible for the decomposition of native SOM even after substrate exhaustion, resulting in “real” priming (Blagodatskaya and Kuzyakov 2008). Other

potential mechanisms for real priming that have been suggested include co-metabolism of SOM and mineralization of SOM to access and mineralize N (Fontaine et al. 2011).

Based on the so-called nutrient mining theory, when energy is not limiting in soil, microorganisms will still mineralize C from SOM in order to access limiting nutrients (Nottingham et al. 2012, Chen et al. 2014). Strong coupling between the addition of root exudates, gross N mineralization and priming suggests that plant C allocated belowground as exudates, is used by microorganisms as labile energy source to produce extracellular enzymes that mineralize N from SOM (Bengtson et al. 2012, Zhu et al. 2014). This is supported by studies that showed reduced priming when mineral N is added, while the effect of adding mineral P alone was less clear (Bradford et al. 2008, Chen et al. 2014, Nottingham et al. 2015). This could be due to catabolic differences between N and P acquisition of soil microorganisms. Microbial assimilation of organic N (i.e. amino sugars or proteins) releases CO₂ through the oxidation of the C skeleton from N-containing compounds, whereas mineral phosphorus (P) could be acquired through enzymatic hydrolysis of organic P esters extracellularly without liberating CO₂ (Dijkstra et al. 2013, Nottingham et al. 2015).

Supporting evidence for priming due to nutrient mining is provided by an increase in the synthesis of extracellular enzymes to acquire nutrients from SOM (Blagodatskaya and Kuzyakov 2008). The addition of labile C has been shown to increase the activities of β -glucosidase (Chen et al. 2014) and phenol oxidase (Nottingham et al. 2012) which indicate the decomposition of more recalcitrant SOM, whereas the increase in phosphomonoesterase (Nottingham et al. 2012) suggests P as the nutrient that limits microbial growth when energy is in abundance. The combination of priming experiments with the analyses of changes in extracellular enzymes responsible for C and nutrient mineralization in soils is therefore a useful approach to get further insights in priming mechanisms and to identify potentially limiting nutrients.

1.5 The effects of land use, management and soil properties

Land use types, management and plant species diversity influence the energy and nutrient limitations of SOM decomposition (Guo and Gifford 2002). For instance, in the temperate climate, different plant species and functional types such as beech (*Fagus sylvatica*), birch (*Betula pendula*), maple (*Acer sp.*), oak (*Quercus sp.*), spruce (*Picea abies*), pines (*Pinus pinea*) and other shrubs in the temperate forest in comparison to different grass species such as purple needlegrass (*Nassella pulchra*), common wild oats (*Avena fatua*), foxtail or spear grass (*Hordeum murinum*), perennial ryegrass (*Lolium perenne*), and buffalo grass (*Buchloe dactyloides*) in the temperate grasslands may affect the soil microclimate, the quality and quantity of litter input, rhizodeposition and nutrient availability (Augusto et al. 2002, Hagen-Thorn et al. 2004). This in return alters the amount of exchangeable base cations, soil acidity and the composition of the decomposer community (Vesterdal 1999, Hansson et al. 2011).

In this thesis, the effects of differences in energy and nutrient supply on mechanisms and stoichiometry of soil organic matter decomposition are evaluated using multiple sites with different land cover and land management. Two land cover types are compared, forest and grassland, in three different regions in Germany. Within each land cover type, sites that differ in land management are compared. For forests, management involves, different tree species i.e. coniferous versus deciduous species produce different quality and quantity of litter, root morphology and exudation which alter soil microbial community (Purahong et al. 2016) and subsequently the rate of SOM decomposition and nutrient availabilities (Berg and Meentemeyer 2002, Hansson et al. 2013). Grassland effects are also influenced by species composition, which in turn reflects management practices. Socher et al. (2013) suggest that fertilization results in the largest and most negative effect on plant species diversity and composition in the grasslands as compared to other land management types and measures of land use intensity such the frequency of as mowing and grazing. It has been shown from previous studies that higher plant diversity increases soil carbon inputs that in turn result in higher microbial carbon accumulation and microbial activities in the rhizosphere (Lange et al. 2015). Therefore, fertilization could affect not only the plant species richness in grasslands but also the input of and storage of SOM.

Even though many studies of SOM decomposition exist, the relationship between stoichiometric constraints under the differing energy and nutrient conditions found with different land use and management types and its importance for the mineralization rates of SOM is still unclear. For example, soils with higher C-to-nutrient (C:N, C:P, C:S) ratios for example forest soils compared to grassland soils, soil microorganisms have to mineralize (or remove) relatively more OC to acquire limiting nutrients. Thus, a positive correlation between C mineralization and C-to-nutrient ratios is expected (Manzoni et al. 2012, Mooshammer et al. 2014a, Spohn and Chodak 2015) (Figure 1.1).

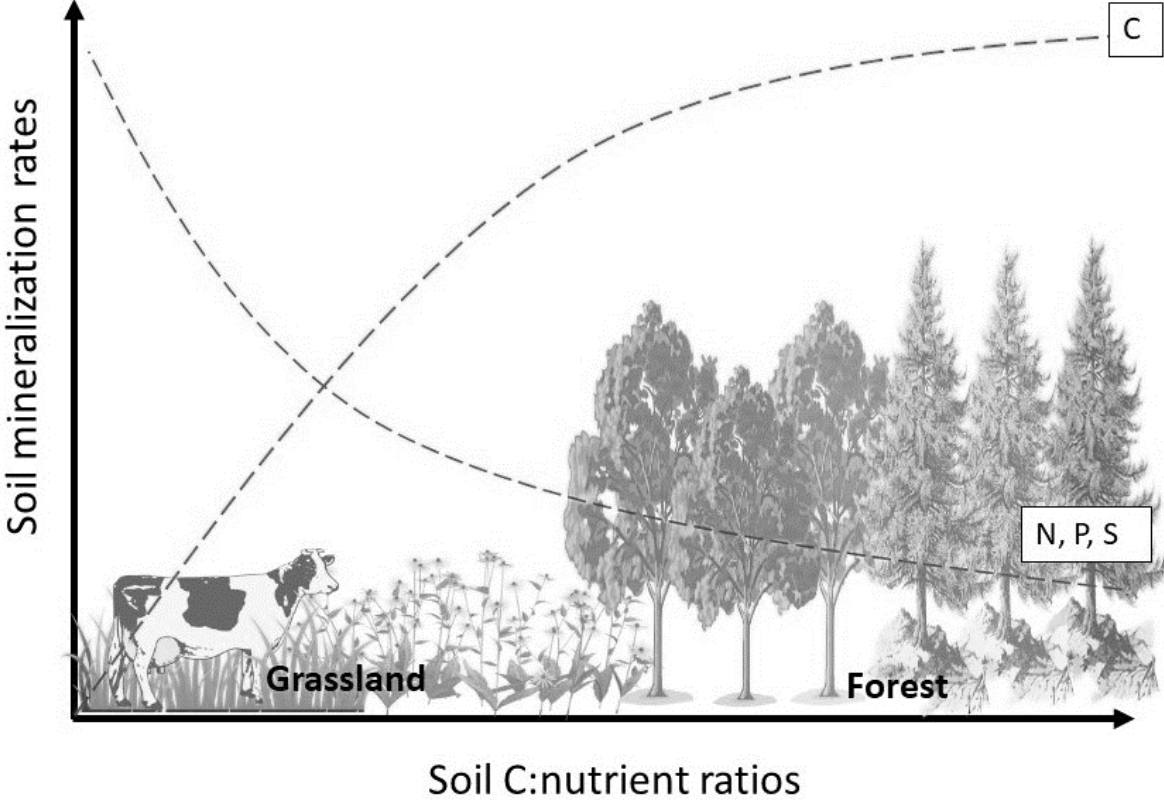


Figure 1.2 Simplified diagram of interactions between carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) mineralization with increasing soil C:nutrient ratios under different land uses.

Further, most previous studies on the effect of land use and management intensity on SOM decomposition were also restricted by smaller study scopes such as a single variable of land use effect or a single study region. Therefore, a more comprehensive study is required to address the multiple key drivers that drive SOM decomposition through the implementation of multiple land use types and intensity across a large study region with different landscape types in a single study.

The magnitude of the priming effect has been shown to depend on both the amount of labile energy sources and soil nutrient availability. As important controls for belowground soil organic carbon (SOC) and nutrient availabilities (Guo and Gifford 2002), land use and management intensity is therefore thought to affect soil priming (Figure 1.3). Priming effects due to co-metabolism are possibly varying with the litter quality and thus tree species composition, as more complex litter constituents, like lignin, require more energy for decomposition (Klotzbücher et al. 2011). In grasslands, fertilization practices may affect the amount of soil priming since the rates of nutrient mineralization has been closely linked to nutrient availability where mineral N and labile C are preferred by soil microorganisms (Nottingham et al. 2012, Chen et al. 2014).

Across large spatial scales, differences in soil biotic properties are shaped by abiotic soil properties, climate and topography (Birkhofer et al. 2012). Differences in soil properties can further affect the portion of SOM directly available for microbial decomposition. The sorption of SOM to mineral surfaces is for example assumed to reduce its accessibility by microbes and is related to clay mineralogy, the amount of pedogenic oxides and soil pH. Accordingly, type and magnitude of priming effects could also vary regionally between soil types and underlying geology. Even though many priming experiments were already conducted, it is unclear how priming varies under different land use and management intensities. Specifically, it is still unknown whether CO₂ respiration and CO₂ respired from priming were significantly different between temperate forests and grasslands due to land use and management effects, and among different regions due to soil abiotic properties, underlying geology and climatic factors.

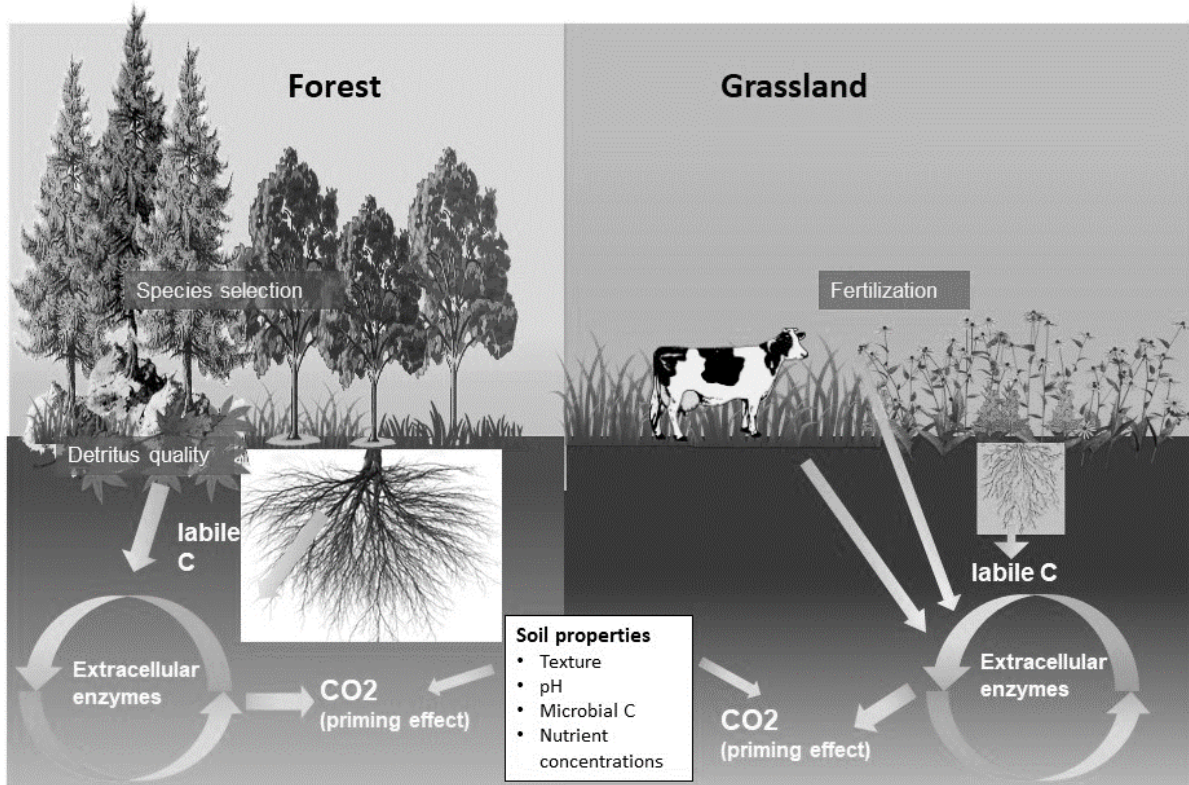


Figure 1.3 Effects of land uses and management practices (tree species in forests and fertilization in grasslands) on soil priming effect, along with the effects of soil properties.

1.6 Objectives

This thesis aims to improve our understanding on the interactions between belowground C and nutrients particularly in relation to regional, land use and management effects. Emphasis is given on understanding the comprehensive interactions between C, N, P and S mineralization rates in relation to different soil properties, C-to-nutrient stoichiometric effects, effects of labile C on SOM decomposition via priming effects and how these processes are controlled by land uses and management effects i.e. tree species effects in forests and fertilization in grasslands. The specific objectives of this thesis are:

- i. To determine how potential mineralization rates are driven by interactions between C, N, P and S under differently managed forests and grasslands in three study regions in Germany.
- ii. To determine how much of the variations in C, N, P and S mineralization across sites are related to land use, management practices and regional differences.
- iii. To analyze the amount of C released from SOM following labile C input in soils due to priming effects.
- iv. To study the relationship of soil priming to nutrient mining and to quantify the magnitude of CO₂ released from priming for soils under different land use types and management.

The research presented in this thesis is part of the Biodiversity Exploratories project, a large-scale joint research comprised of more than 40 projects aimed at studying multiple drivers of biodiversity as a function of land use and management intensities (Fischer et al. 2010). The study sites are distributed across 3 regions in Germany: the (i) UNESCO Biosphere Reserve “Shorfheide-Chorin” (SCH) in the lowlands of North-eastern Germany; (ii) the “Hainich National Park” and its surroundings (HAI) in the hilly landscape of central Germany and (iii) the UNESCO Biosphere Reserve “Schwäbische Alb” (ALB) in the low mountain range of South-western Germany. The distribution of study regions and types of land uses, and management are shown in Figure 1.4. Soils in the ALB region developed mainly on Jurassic limestone and were classified as clay-rich Cambisols or Leptosols (WRB 2014). Soils in

forests and grasslands of the HAI region had loamy or clayey texture due to the dominant geological substrate of loess over limestone. The main HAI soil types were Luvisols, Stagnosols, Vertisols or Cambisols. In the SCH forests, soils were classified as Cambisols and had a texture of sandy loam to pure sand as the geological substrate was glacial till covered by glacio-fluvial or aeolian sand. The grassland soils in the SCH were drained Histosols characterized by high OC contents and a loamy texture. These regions differ in climate and soil parent materials, and accordingly have different dominant soil types and properties (Table 1.1) (Fischer et al. 2010).

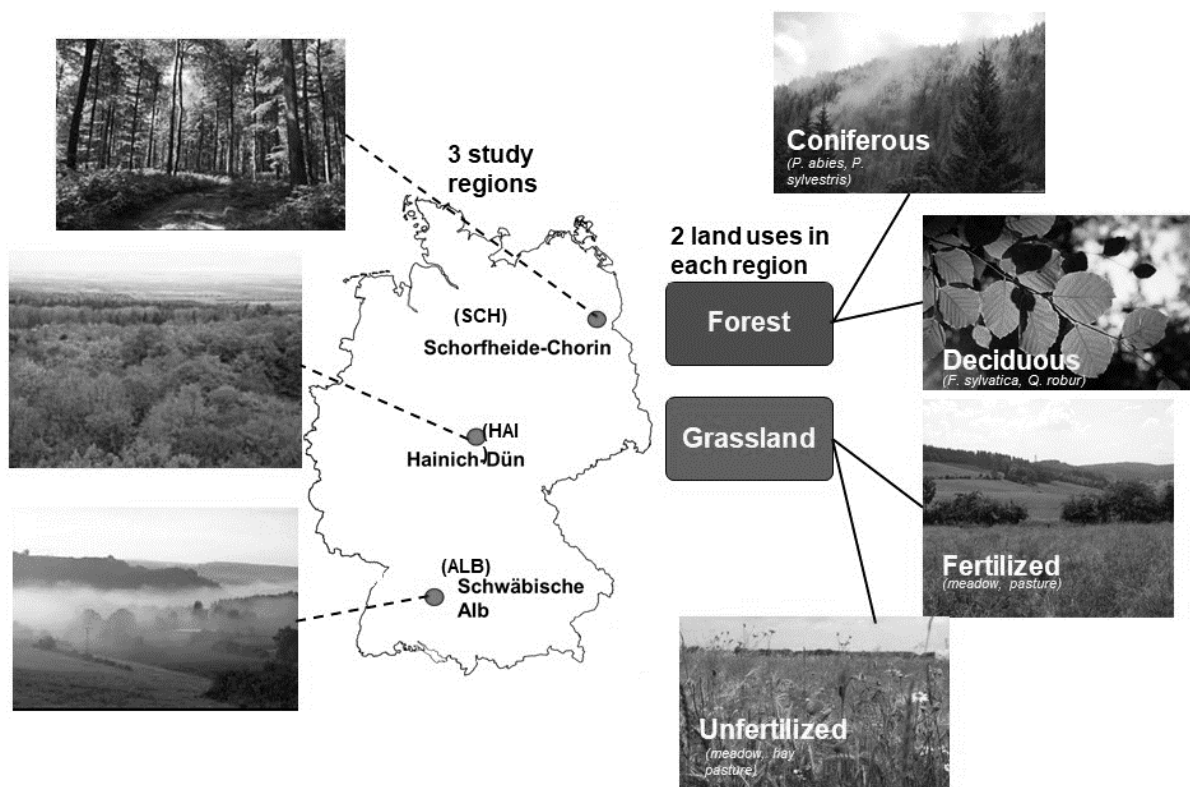


Figure 1.4 Distribution of study regions and types of land use types and management.

In Germany, forests and grasslands are, together with agricultural lands the most important ecosystems with strong anthropogenic influences (Fischer et al. 2010). One hundred plots were selected from each region and equally from each land use type, 50 forest and 50 grassland sites, with a total of 300 study sites in the 3 regions. In forests, the dominant tree species were European beech or common beech (*Fagus sylvatica*), common oak (*Quercus robur*), Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). The forest sites were managed as age class forest, selection forests, and unmanaged forests. Land management for

grasslands in the study sites were classified under four categories (along a gradient of decreasing land use intensity): meadows, pastures, mown pastures and fallows and were either fertilized or unfertilized except for fallows. In fertilized grasslands, livestock manure or slurry was used as fertilizers. An average amount of 283.8 ± 105.2 , 281.9 ± 111.3 and 391.2 ± 100.4 kg N ha⁻¹ had been applied from 2006-2014 in ALB, HAI and SCH respectively. The number as well as each type of land use and management intensities of the study regions is shown in Table 1.2.

Table 1.1 Climatic conditions and geological information of study regions (adapted from Fischer et al. (2010)).

	Schwäbische Alb	Hainich-Dün	Schorfheide-Chorin
Location	South-West Germany	Central Germany	North-East Germany
Coordinates	N 48° 26' E 9° 23'	N 51° 9' E 10° 28'	N 53° 0' E 13° 46'
Size	~422 km ²	~1300 km ²	~1300 km ²
Geology	Calcareous bedrock with karsts phenomena	Calcareous bedrock covered by loess	Young glacial landscape
Soil type			
Grassland	Cambisol, Leptosol	Cambisol, Stagnosol, Vertisol	Histosol, Cambisol, Gleysol, Luvisol, Albeluvisol
Forest	Cambisol, Leptosol	Luvisol, Stagnosol	Cambisol, Albeluvisol, Regosol
Human population density	258 km ⁻¹	116 km ⁻¹	23 km ⁻¹
Altitude (a.s.l.)	460–860m	285–550m	3–140m
Mean annual temperature	6–7 °C	6.5–8 °C	8–8.5 °C
Mean annual precipitation	700–1000mm	500–800mm	500–600mm

Table 1.2 Number of sampling sites according to land use and management intensity in the study regions.

Study region	Land use	Management type	n
Schwäbische Alb	Forest	deciduous beech (<i>Fagus sylvatica</i> L.)	5
		coniferous spruce (<i>Picea abies</i> L.)	5
	Grassland	fertilized pasture	1
		fertilized meadow	5
		unfertilized pasture	2
		unfertilized meadow	2
Hainich-Dün	Forest	deciduous beech (<i>Fagus sylvatica</i> L.)	6
		coniferous spruce (<i>Picea abies</i> L.)	4
	Grassland	fertilized mown pasture	2
		fertilized meadow	3
		unfertilized pasture	3
		unfertilized mown pasture	2
Schorfheide-Chorin	Forest	deciduous beech (<i>Fagus sylvatica</i> L.)	5
		coniferous pine (<i>Pinus sylvestris</i> L.)	2
		mixed forest (<i>Fagus sylvatica</i> L. and <i>Pinus sylvestris</i> L.)	3
	Grassland	fertilized mown pasture	1
		fertilized meadow	4
		unfertilized meadow	5
		Total	60

Chapter 2

Interactions of soil C, N, P and S mineralization under differently managed forest and grassland soils

Chapter source: Gan et al., 2019. Effects of nutrient stoichiometry on soil organic matter decomposition under different land use and management (manuscript under preparation)

Abstract

Soil organic matter (SOM) mineralization couples the mineralization of carbon with other nutrients, but the overall effect of stoichiometry on soil organic carbon (SOC) mineralization and the release of plant-available nutrients is still unresolved. Variations in study region, land use and management are further important controls of soil mineralization potentials, but few studies address the interacting effects of these factors in one single investigation. Therefore, this chapter determined potential mineralization rates of carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) simultaneously for topsoil samples (0-10 cm) from 300 differently managed forest and grassland plots (deciduous and coniferous forests, fertilized and unfertilized grasslands) of three regions in Germany under controlled conditions in the laboratory. Samples were incubated for two weeks in microlysimeters and CO₂ evolution as well as leachable dissolved organic carbon (DOC), NO₃⁻, NH₄⁺, SO₄²⁻ and PO₄³⁻ were determined as indicators for net nutrient mineralization. It is hypothesized that (1) SOM mineralization for nutrient acquisition would promote higher CO₂ and DOC production relative to nutrient release at sites with higher soil carbon-to-nutrient ratios, and therefore predicted to be higher in (i) forests relative to grasslands, (ii) coniferous relative to deciduous forests, and (iii) unfertilized relative to fertilized grasslands, and (2) that higher C mineralization at sites with higher soil carbon-to-nutrient ratios is related to microbial mining for nutrients by removing excess C via CO₂ evolution and DOC leaching.

Soil respiration and microbial biomass carbon were related to total OC contents but declined with higher soil C:N and C:S ratios. In contrast to hypothesis, CO₂ evolution was significantly higher in grasslands than forests in all regions. No consistent effect of forest management

could be detected on soil respiration and microbial biomass in all regions, and this might be due to the differences in soil properties such as pH and soil texture among regions. Further in contrast to expectations, CO₂-to-N- and CO₂-to-S-leaching ratios were significantly higher in grasslands than in forests in all regions, even though soil C:N and C:S ratios were higher in grasslands than in forests. This was mostly caused by significantly higher CO₂ and significantly lower N and S leaching in grasslands than in forests which might be due to a higher microbial biomass at the grassland sites (thus higher N and S assimilation). According to expectation however, CO₂-to-N- and CO₂-to-S-leaching ratios were significantly higher in coniferous than deciduous forests in two out of the three study regions. This was mostly caused by significantly higher N leaching in deciduous than coniferous forests (2 out of 3 regions) as compared to higher CO₂ evolution (1 out of 3 regions). Additionally, DOC release was significantly higher in forests than grasslands and in coniferous than deciduous forests in two out of three regions which might support the first hypothesis. However, DOC-to-nutrient mineralization ratios were not discussed in this study. In grasslands, no fertilization effect was observed on soil OC and microbial biomass C concentrations, CO₂ and DOC released as well as CO₂-to-N- and CO₂-to-S-leaching ratios.

When normalized to microbial biomass C, soil C:N is positively correlated to C mineralization and DOC leaching. This correlation was much stronger in forests (CO₂: $r = 0.7$, $P < 0.001$, DOC: $r = 0.8$, $P < 0.001$) as compared to grasslands (CO₂: $r = 0.2$, $P < 0.05$, DOC: $r = 0.2$, $P < 0.05$) due to higher range of soil C:N at the forest sites. These findings suggest that low soil N contents increase C losses per microbial biomass as indicated by the nutrient mining theory, supporting the second hypothesis. However, while one might expect higher N immobilization by microbes with increasing soil C:N ratio, N leaching per microbial biomass was observed to increase with higher soil C:N both in forests ($r = 0.7$, $P < 0.001$) and in grasslands ($r = 0.2$, $P < 0.05$).

The findings from this chapter provides evidence that land use types affect the rates of C mineralization (CO₂ production and DOC leaching) relative to nutrient release. The first hypothesis that SOM mineralization for nutrient acquisition would promote higher CO₂ and DOC production relative to nutrient release at sites with higher soil carbon-to-nutrient ratios was partly supported. Higher C-to-nutrients mineralization in grasslands than in forests observed is suggested to be caused by higher microbial CO₂ respiration, microbial biomass C and nutrient immobilization in grasslands than forests. Strong correlations between C

mineralization and soil C:N ratios when normalized to microbial biomass supported the second hypothesis that higher C mineralization is related to microbial mining for nutrients by removing excess C. These results thus supported the paradigm of reduced microbial carbon use efficiency at nutrient poor forest soils, but high N leaching per microbial biomass in N poor soils which questions the concept that this is caused by microbial N deficiency, when higher N assimilation (thus lower N mineralization observed) would be expected.

Keywords: nutrient mineralization, stoichiometry effect, carbon cycling, land use effects, forest management, grassland management, tree species, fertilization

2.1 Introduction

Soil organic matter (SOM) mineralization is an important process in which carbon and nutrients in SOM are mineralized to CO₂ and the mineral forms of N (mainly NH₄⁺ and NO₃⁻), PO₄³⁻ and SO₄²⁻ for plant and microbial uptake (Kowalenko and Lowe 1975, Schimel and Bennett 2004, Chapin et al. 2011). Carbon mineralization couples to N, P and S mineralization due to the co-limitation of these nutrients on microbial processes to maintain their biomass stoichiometry (Jonasson et al. 1999, LeBauer and Treseder 2008, Vitousek et al. 2010). Nutrient co-limitation is the limitation of multiple resources simultaneously on microbial processes or plants productivity (Bracken et al. 2015). For example, Elsar et al. (2007) has shown that addition of both N and P simultaneously results in higher plants productivity in three different ecosystems compared to when only one of those nutrients is added.

In soils with C limitation, net mineral N and S are also released during microbial decomposition of SOM when organic matter is mostly used as energy source, resulting in positive correlations between C and N as well as C and S mineralization (McGill and Cole 1981, Chapin et al. 2011). Previous studies have shown that C mineralization correlates positively to N and S mineralization (Winsor and Pollard 1956, Quan et al. 2014), N mineralization correlates with S mineralization (Kowalenko and Lowe 1975, Tabatabai and Al-Khafaji 1980) as well as with P mineralization (Guesewell and Gessner 2009, Heuck and Spohn 2016, Marklein et al. 2016), suggesting that the availability of these nutrients may co-limit microbial processes. Under nutrient limitation however, microbial mineralization of organic S from sulfate esters and organic P may occur independent of OC mineralization, thus

decoupling C from P and S mineralization (McGill and Cole 1981). Decoupling of C and N could also be observed in N-limited soils where N-containing monomers such as amino acids, amino sugars and nucleic acids are depolymerized through enzymatic reactions and directly taken up by microbes without prior conversion to inorganic nutrients (Schimel and Bennett 2004).

Yet, SOM and plant litter often have C-to-nutrient ratios highly exceeding that of microbial biomass stoichiometry (McGroddy et al. 2004). Even though excess C and N sources are required by soil microbes for the production of energy and extracellular enzymes, microbes may still have to mineralize relative more SOC while decreasing the mineralization of limiting nutrients at sites with large carbon-to-nutrient ratios of SOM, resulting in reduced carbon use (CUE) and increased nitrogen use efficiency (NUE) (Mooshammer et al. 2014b, Spohn 2016). Accordingly, OC-to-nutrient ratios of SOM were positively related to C mineralization and negatively to N mineralization (Janssen and Soil 1996, Bengtsson et al. 2003). Alternatively, excess OC can also be left behind and released to the soil solution as dissolved organic carbon (DOC) after breakdown of large macromolecules when nutrients rather than carbon are limiting growth (Hessen and Anderson 2008). However, to the best knowledge of this thesis, no study has explored the ratios of C-to-nutrient mineralized so far, where higher C-to-nutrient mineralized ratios would then be expected at sites with higher C-to-nutrient ratios of SOM. Although extensive studies have investigated the effects of soil properties and litter stoichiometry on SOM mineralization, few addressed the interactions between different nutrient mineralization rates simultaneously. Consequently, the effects of nutrient stoichiometry on SOM mineralization and potential release of plant available nutrients is still lacking.

Even less clear is how the interaction effects from land use, management and study regions/soil type affect SOM stoichiometry and thus SOM mineralization. Across different study regions, differences in soil biotic properties are shaped by abiotic soil properties, climate and topography (Birkhofer et al. 2012), and this is postulated to affect soil mineralization potentials. Land use and management practices are important controls of soil nutrient contents and availabilities (McGrath et al. 2001, Dupouey et al. 2002). At grassland sites, SOC and total N (TN) stocks in topsoils were reported to be higher than in forests (Hassink 1994a, Ferreira et al. 2016), and this higher soil nutrient content is postulated to result in lower C to N mineralization in grasslands as compared to the forest sites. Forest

management can affect soil organic matter (OM) quality via tree species selection with typically higher C:nutrients ratios in coniferous than deciduous stands, which supposedly lead to higher C compared to N mineralization (Cote et al. 2000, Hansson et al. 2011) and may stimulate DOC production. Nitrogen fertilization of grasslands has been shown to decrease C mineralization (Spohn et al. 2016), but increasing C-degrading enzyme activities following N addition have also been reported (Keeler et al. 2009). Under low soil N sources, C mineralization during litter decomposition has been suggested to be partly due to N mining (Craine et al. 2007a).

The aim of the present study was to examine the potential mineralization rates of C, N, P and S for differently managed forests and grassland systems across three study regions in Germany by performing a multi-factorial soil incubation experiment across 300 sites for 14 days. The first scientific questions asked was: are the variations in C, N, P and S mineralization related to land use and management practices, or due to regional differences (e.g. parent materials)? Based on previous studies, CO₂ and DOC production relative to nutrient release (ratios of C-to-nutrient mineralization) during SOM mineralization have been shown to be higher at sites with higher soil carbon-to-nutrient ratios, and therefore hypothesized to be higher in (i) forests relative to grasslands, (ii) coniferous relative to deciduous forests, and (iii) unfertilized relative to fertilized grasslands. The second question asked was: does reduced microbial carbon use efficiency in nutrient poor soils result in the removal of “excess” C in order to mine for limiting nutrients, which is observable via higher C mineralization? It is thus hypothesized that (iv) CO₂ and DOC production would be higher while net N mineralization would be lower per microbial biomass at sites with lower soil carbon-to-nutrient ratios (forest relative to grasslands, coniferous relative to deciduous forests and unfertilized relative to fertilized grasslands).

2.2 Methods

2.2.1 Study region and soil sampling

The study regions are part of the Biodiversity Exploratories project, a large-scale joint research aimed at studying multiple drivers of biodiversity as a function of land use and management intensities (Fischer et al. 2010). The study sites are distributed across 3 regions in Germany: the (i) Shorfheide-Chorin (SCH) in the lowlands of North-eastern Germany; (ii) the Hainich-Dün (HAI) in the hilly landscape of central Germany and (iii) Schwäbische Alb (ALB) in the low mountain range of South-western Germany. Soils in the ALB region developed mainly on Jurassic limestone and were clay-rich Cambisols or Leptosols (IUSS Working Group WRB, 2014). Soils in forests and grasslands of the HAI region had loamy or clayey texture due to the dominant geological substrate of loess over limestone. The main HAI soil types were Luvisols, Stagnosols, Vertisols or Cambisols. In the SCH forests, soils were classified as Cambisols and had a texture of sandy loam to pure sand as the geological substrate was glacial till covered by glacio-fluvial or aeolian sand. The grassland soils in the SCH were drained Histosols characterized by high OC contents and a loamy texture. These regions differ in climate and soil parent materials, and accordingly have different dominant soil types and properties (Table 1-2). One hundred plots were selected from each region which comprised of 50 forest and 50 grasslands with a total of 300 study sites. The forests were further divided into deciduous and coniferous forests, and grasslands were divided into fertilized and unfertilized sites. More detailed number of plots of each land use and management were shown in Table 1-2. Topsoil samples (0-10cm) were collected in May 2014. At each site, a total of 14 soil cores were sampled along two 40 m transects in forests and two 20 m transects in grasslands and mixed to form composite samples.

2.2.2 Soil incubation with microlysimeters

For each sample, 40 g fresh soil sample was incubated in 250 ml reusable two-chamber filter holders with receiver (Nalgene 300-4000 Polysulfone Graduated Filter Holder) modified into microlysimeters according to the method of Nadelhoffer (1990). The microlysimeters enabled the simultaneous and repeated sampling of CO₂ efflux and nutrient analysis (DOC, NH₄⁺, NO₂⁻, NO₃⁻, SO₄²⁻) with large sample number. To construct the microlysimeters, high-purity

and plasticizer-free tubing with different diameters (Saint-Gobain™ Tygon S3™ E-3603 Flexible Tubings) were used to facilitate the leaching process and the suction of soil filtrate from the lower chamber. Non-biodegradable glass fiber filters with a diameter of 42 mm and particle retention size of 1.2 μm (Sartorius Glass Microfiber Filters) were placed on the filter unit of the microlysimeter. A completed microlysimeter unit was pressure-tested for leakage using vacuum pump. To avoid clogging of soil samples on the filter for long term incubation, 40 g of acid-washed sand was placed on top of the glass fiber filters, followed by the soil samples. To calculate the volume of microlysimeters with soil samples, vacuum pump was again used along with a known volume of standard jar to calculate the volume of the pump. Microlysimeter volume was then calculated using the following equation:

$$V_S = \frac{P_S \times V_P}{(P_{atm} - P_S)}$$

where V_S is the volume of the microlysimeter, P_{atm} is the atmospheric pressure, V_P is the volume of the pump and P_S is the pressure of the microlysimeter. All samples were adjusted to 60% of water holding capacity (WHC) and pre-incubated at 20°C in the dark for 4 days.

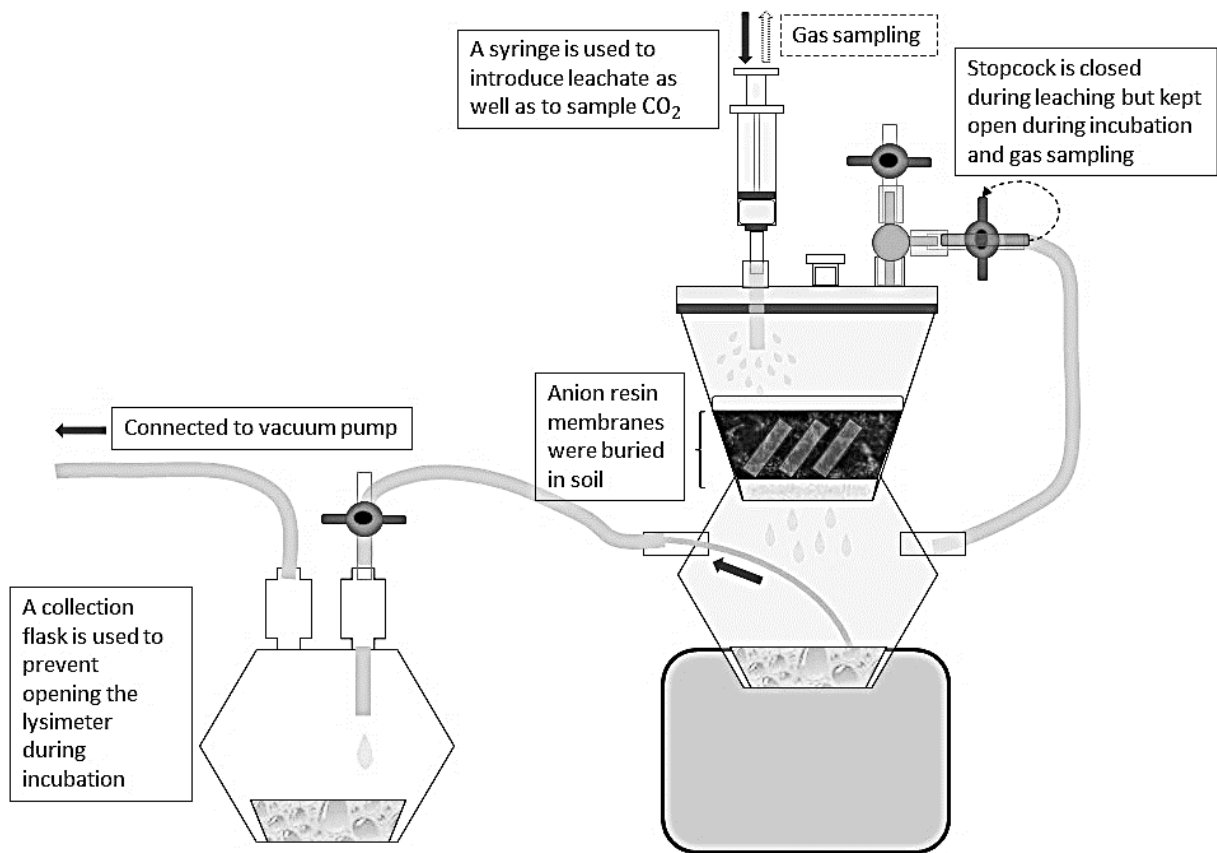


Figure 2.1 Soil incubation set up using lysimeters (a reusable two-chamber filter holders with receiver, left) with an attached soil leachate collector (right) modified according to the method of Nadelhoffer (1990).

2.2.3 Nutrient leaching and phosphate measurement

After 4 days of pre-incubation, samples were leached with 80 ml of 0.01 M calcium chloride (CaCl_2) to remove nutrients accumulated during the pre-incubation. To leach the sample, instead of using a filter funnel in one of the lid outlets of the microlysimeter as demonstrated by Nadelhoffer (1990), a short tube was attached to the outlet where the tube's end in the upper chamber was clammed shut and small holes were formed on the tube, creating a shower effect. This is to avoid strong disturbance of the soil aggregates from the leaching solution, which could cause blockage. The soil samples were leached again after the first and second week of incubation, directly after CO_2 efflux measurement. The filtrate from leaching was

collected through vacuum suction using mason jars with two gas outlets; one attached to the vacuum pump and one attached to the lower chamber of the microlysimeters. After leaching, the microlysimeters were flushed with CO₂-free synthetic gas (20.5 Vol.-% O₂, rest N₂, Westfalen AG, Germany) for at least 2 minutes (psi), sealed and incubated for the next measurement.

Due to the low solubility of mineral P, anion exchange resin membranes were used to absorb PO₄³⁻ in soil solution and to limit the resorption back to soil solution, as modified from standard method (SSSA 1996) and Kouno et al. (1995). For each microlysimeter, a total of 6 x 2 cm resin membranes cut into six 2 x 1 cm stripes were buried for two weeks in soil samples, before the pre-incubation. To elude the mineralized PO₄³⁻, the resin stripes were removed from soil, rinsed with deionized water thoroughly to remove soil aggregates that attached on the membrane surface, and shaken for 2 hours (150 rpm) in 20 ml of 0.2 M nitric acid (HNO₃). PO₄³⁻ retained by the acid solution was quantified colourimetrically (Murphy and Riley 1986). The used resin membranes were regenerated by shaking for one hour with 0.5 M HNO₃ to remove the remaining PO₄³⁻ on the membrane stripes, followed by shaking with 0.5 M NaHCO₃ for an hour, rinsed with deionized water and repeated shaking with 0.5 M NaHCO₃ for another hour. The regenerated membranes were kept moist in deionized water until further use.

2.2.4 CO₂ efflux measurement and microbial C

CO₂ efflux was measured weekly before nutrient leaching using a differential, non-disperse infrared (NDIR) CO₂-analyzer (LI-COR 6262) based on the difference between the adsorption of infrared (IR) through the reference cell with a known gas concentration, and the sample cell. To calculate CO₂ concentration, firstly a regression line was computed from the area of the IR absorbance curve vs. the injected CO₂ with known concentration. CO₂ concentrations (mg CO₂ g⁻¹ DW soil) from samples were then estimated based on this regression line and were corrected to a known amount of soil samples (dried weight, DW) and microlysimeter volume. Filtrate collected from leaching was kept in sample bottles and freeze immediately at -20°C until analysis. For control, the same microlysimeter was set up and sampled without soil samples. Microbial C was quantified using chloroform fumigation extraction (CFE) at the beginning of the incubation to study the relations between microbial biomass and nutrient

mineralization. The measurement of CO₂ efflux and microbial C enabled the calculation of metabolic quotient (qCO₂) to more closely link soil respiration to microbial activities. qCO₂ was calculated by dividing CO₂ concentration with microbial C (mg CO₂ g⁻¹ DW soil g⁻¹ microbial C).

2.2.5 Statistical analysis

Statistical analysis was conducted using R version 3.3.2 (Team 2016). Results for C, N, P and S mineralization and soil properties were presented as means±standard error ($P<0.05$) unless stated otherwise. To see if the mineralization rates were statistically different among land use types and management, Analysis of Variance (ANOVA) with the function “aov” was used. Prior to ANOVA analysis, normality and homogeneity of residual variances were checked using the (i) Residuals versus Fitted plot, (ii) normal Q-Q plot, (iii) Scale-Location plot and the (iv) Residuals versus Leverage plot. Data that did not fulfil the conditions for normality and homogeneity of residual variances were log transformed. Redundancy analysis (RDA) was performed with the function “rda” to identify the correlations between response variables (the mineralization rates of C, N, P and S) and predictor variables (soil clay content, SOC, soil TN, TP, TS, microbial C, soil pH, soil C:N, C:P and C:S ratios). From RDA biplot, factors that showed important correlations with soil mineralization were fitted using analysis of covariance (ANCOVA) using the “aov” function in R.

2.3 Results

2.3.1 Soil properties and C-to-nutrient ratios

Soil properties varied between study regions most probably due to different parent materials and climatic conditions. Soil pH values were significantly higher ($P<0.05$) in grasslands (6.4 ± 0.1) than forests (4.5 ± 0.1) (Table 1). The soil pH in forest soils strongly increased in the order of SCH (3.54 ± 0.09), HAI (4.77 ± 0.09) and ALB (5.26 ± 0.09), whereas the soil pH in the grasslands were in the range between 6 and 7. The soil pH in HAI and ALB was significantly different in coniferous and deciduous forest with lower pH values in coniferous than deciduous forest in the ALB but higher pH values in coniferous than deciduous forest in the HAI. Similar with soil pH, clay contents were significantly higher in grasslands than in

forests. In both land use types, SCH soils contained low amount of clay, while the clay content was intermediate in HAI and was high in the ALB soils. However, there were no significant differences in soil texture between coniferous and deciduous forest in all study regions. Elemental concentrations of C, microbial C, N, P and S also varied between land use types and study regions (Table 1, Table 2). They showed the same overall pattern as clay contents. Organic C, microbial C, and total N, P and S (TN, TP, TS) concentrations were strongly correlated to clay contents in each land use and study region.

Under different forest management, total N concentrations were significantly higher at the deciduous than coniferous forest sites in SCH, while total P concentrations were higher at the coniferous than deciduous forest sites in ALB and HAI. Mixed results were observed for total S where higher concentrations were observed at the coniferous than deciduous forest sites in HAI, while the opposite was observed in SCH forests. The differences in soil nutrient concentrations produced varied C-to-nutrient ratios across sites. In all study regions, soil C:N, C:P and C:S values were significantly higher at all forest than grassland sites, which was consistent with the lower total N, P and S concentrations at all forests compared to grassland sites. Under different managements, C:N values were higher at all coniferous compared to deciduous forest sites and were higher at unfertilized than fertilized grassland sites in ALB and HAI. Similar results were observed for C:P and C:S, where significantly higher ($P < 0.05$) values were observed at unfertilized compared to fertilized grassland sites in ALB. C:S ratios were further higher at coniferous than deciduous forest sites in ALB and SCH. The correlations between soil clay content and SOC, microbial C, TN, TS and TP were shown in Table 3. Overall, the effect of soil clay content was more important on these soil properties in forests compared to grasslands. The effect of soil clay content was especially important for SOC and microbial C concentrations, followed by soil TN, TS and TP.

Table 2.1 Soil pH, clay, silt and sand contents, organic carbon (C) and microbial C concentrations under different study regions, land use types and managements (mean \pm standard errors). Significant differences between study regions and land use types are indicated by capital letters, and between study regions and management types (tree species in forests and fertilization in grasslands) by lowercase letters according to Tukey's test ($P < 0.05$).

		pH	Clay (g kg ⁻¹)	Silt	Sand (mg g ⁻¹)	Organic C (μ g g ⁻¹)	Microbial C
Schwäbische-Alb							
Forest	(n = 50)	5.26 \pm 0.09 ^C	496 \pm 14.4 ^E	444 \pm 15.7 ^C	59.6 \pm 9.84 ^A	62.4 \pm 1.95 ^D	325 \pm 19.0 ^D
Deciduous forest	(n = 38)	5.41 \pm 0.10 ^{cd}	493 \pm 16.5 ^{cd}	439 \pm 18.1 ^{cd}	67.5 \pm 11.2 ^a	61.1 \pm 2.24 ^d	340 \pm 34.0 ^c
Coniferous forest	(n = 12)	4.79 \pm 0.18 ^{bc}	506 \pm 29.4 ^{cd}	460 \pm 32.3 ^{cd}	34.3 \pm 20.2 ^a	65.1 \pm 3.98 ^d	276 \pm 39.8 ^{bc}
Grassland	(n = 49)	6.27 \pm 0.09 ^D	535 \pm 19.3 ^E	407 \pm 15.9 ^C	57.9 \pm 9.94 ^A	65.1 \pm 1.97 ^D	608 \pm 18.8 ^E
Unfertilized grassland	(n = 21)	6.38 \pm 0.14 ^{ef}	510 \pm 22.2 ^d	428 \pm 24.4 ^{cd}	61.9 \pm 15.1 ^a	64.1 \pm 3.01 ^d	623 \pm 28.8 ^d
Fertilized grassland	(n = 28)	6.20 \pm 0.12 ^e	553 \pm 19.2 ^d	392 \pm 21.1 ^{bcd}	54.9 \pm 13.1 ^a	65.8 \pm 2.61 ^d	598 \pm 24.9 ^d
Hainich-Dün							
Forest	(n = 50)	4.77 \pm 0.09 ^B	301 \pm 14.0 ^C	642 \pm 15.7 ^E	58.0 \pm 9.84 ^B	35.6 \pm 1.95 ^B	192 \pm 20.3 ^B
Deciduous forest	(n = 46)	4.67 \pm 0.09 ^b	296 \pm 15.0 ^b	647 \pm 16.5 ^f	57.5 \pm 10.2 ^a	34.4 \pm 2.03 ^b	184 \pm 21.1 ^b
Coniferous forest	(n = 4)	5.96 \pm 0.31 ^{cdef}	355 \pm 50.9 ^{bc}	583 \pm 55.9 ^{def}	63.5 \pm 34.6 ^a	49.8 \pm 6.90 ^{bcd}	286 \pm 76.2 ^{abc}
Grassland	(n = 50)	6.81 \pm 0.09 ^E	423 \pm 18.4 ^D	520 \pm 15.7 ^D	57.6 \pm 9.84 ^A	45.6 \pm 1.95 ^C	307 \pm 18.8 ^{CD}
Unfertilized grassland	(n = 26)	6.83 \pm 0.12 ^f	431 \pm 24.0 ^c	509 \pm 21.9 ^e	59.9 \pm 13.6 ^a	46.9 \pm 2.71 ^c	307 \pm 21.5 ^c
Fertilized grassland	(n = 24)	6.78 \pm 0.12 ^f	413 \pm 28.6 ^c	532 \pm 22.8 ^e	55.1 \pm 14.1 ^a	44.3 \pm 2.82 ^{bc}	307 \pm 25.1 ^c
Schorfheide-Chorin							
Forest	(n = 50)	3.54 \pm 0.09 ^A	44.8 \pm 14.4 ^A	84.8 \pm 15.7 ^A	871 \pm 9.84 ^C	20.8 \pm 1.95 ^A	45.3 \pm 19.8 ^A
Deciduous forest	(n = 28)	3.56 \pm 0.12 ^a	53.7 \pm 19.2 ^a	98.7 \pm 21.1 ^a	848 \pm 13.1 ^c	20.9 \pm 2.61 ^a	55.8 \pm 26.9 ^a
Coniferous forest	(n = 22)	3.53 \pm 0.13 ^a	33.5 \pm 21.7 ^a	67.2 \pm 23.8 ^a	900 \pm 14.7 ^c	20.7 \pm 2.94 ^a	32.6 \pm 29.5 ^a
Grassland	(n = 28)	6.07 \pm 0.12 ^C	126 \pm 19.2 ^B	292 \pm 21.0 ^B	582 \pm 13.1 ^B	32.8 \pm 2.61 ^B	232 \pm 24.9 ^{BC}
Unfertilized grassland	(n = 21)	6.06 \pm 0.14 ^e	128 \pm 38.5 ^a	291 \pm 24.4 ^b	581 \pm 15.1 ^b	33.0 \pm 3.01 ^{ab}	226 \pm 28.8 ^{bc}
Fertilized grassland	(n = 7)	6.08 \pm 0.24 ^{def}	117 \pm 22.2 ^a	297 \pm 42.2 ^{bc}	586 \pm 26.1 ^b	32.1 \pm 5.21 ^{abc}	249 \pm 49.9 ^{bc}

Table 2.2 Mean values of soil total N, P and S concentrations and C:N, C:P and C:S ratios under different study regions, land use types and managements (mean \pm standard errors). Significant differences between study regions and land use types are indicated by capital letters, and between study regions and management types (tree species in forests and fertilization in grasslands) by lowercase letters according to Tukey's test ($P < 0.05$).

		Total N	Total P (g kg ⁻¹)	Total S	C:N	C:P	C:S
Schwäbische-Alb							
Forest	(n = 50)	4.77 \pm 0.18 ^C	0.93 \pm 0.04 ^C	0.56 \pm 0.03 ^C	13.0 \pm 0.20 ^B	69.9 \pm 3.08 ^B	111 \pm 1.77 ^C
Deciduous forest	(n = 38)	4.78 \pm 0.21 ^e	0.94 \pm 0.04 ^{cde}	0.56 \pm 0.03 ^d	12.8 \pm 0.17 ^b	68.4 \pm 3.49 ^{cd}	109 \pm 1.91 ^d
Coniferous forest	(n = 12)	4.73 \pm 0.37 ^{ef}	0.88 \pm 0.08 ^{cde}	0.56 \pm 0.06 ^d	13.8 \pm 0.31 ^{bc}	74.4 \pm 6.21 ^{cde}	117 \pm 3.41 ^d
Grassland	(n = 49)	6.35 \pm 0.18 ^D	1.42 \pm 0.04 ^D	0.86 \pm 0.03 ^D	10.3 \pm 0.20 ^A	48.9 \pm 3.11 ^A	75.0 \pm 1.79 ^A
Unfertilized grassland	(n = 21)	5.99 \pm 0.28 ^{fg}	1.13 \pm 0.06 ^e	0.81 \pm 0.04 ^e	10.8 \pm 0.23 ^a	59.5 \pm 4.69 ^{bcd}	77.6 \pm 2.58 ^{bc}
Fertilized grassland	(n = 28)	6.61 \pm 0.24 ^g	1.64 \pm 0.05 ^f	0.90 \pm 0.04 ^e	9.96 \pm 0.20 ^a	40.9 \pm 4.06 ^{ab}	73.0 \pm 2.23 ^{abc}
Hainich-Dün							
Forest	(n = 50)	2.72 \pm 0.18 ^B	0.36 \pm 0.04 ^A	0.32 \pm 0.03 ^B	13.2 \pm 0.20 ^B	105 \pm 3.08 ^C	114 \pm 1.77 ^C
Deciduous forest	(n = 46)	2.66 \pm 0.19 ^c	0.34 \pm 0.04 ^{ab}	0.31 \pm 0.03 ^c	13.1 \pm 0.16 ^{bc}	106 \pm 3.17 ^f	114 \pm 1.74 ^d
Coniferous forest	(n = 4)	3.41 \pm 0.65 ^{bcde}	0.55 \pm 0.14 ^{abcd}	0.46 \pm 0.10 ^{abcd}	14.7 \pm 0.53 ^c	95.8 \pm 10.7 ^{def}	114 \pm 5.90 ^d
Grassland	(n = 50)	4.47 \pm 0.18 ^C	0.99 \pm 0.04 ^C	0.57 \pm 0.03 ^C	10.3 \pm 0.20 ^A	41.2 \pm 3.08 ^A	66.1 \pm 1.77 ^A
Unfertilized grassland	(n = 26)	4.53 \pm 0.25 ^e	0.95 \pm 0.05 ^{cde}	0.59 \pm 0.04 ^d	10.4 \pm 0.21 ^a	44.6 \pm 4.22 ^{ab}	67.2 \pm 2.31 ^{ab}
Fertilized grassland	(n = 24)	4.40 \pm 0.26 ^{de}	1.03 \pm 0.06 ^{de}	0.55 \pm 0.04 ^d	10.1 \pm 0.22 ^a	37.4 \pm 4.39 ^a	64.8 \pm 2.41 ^a
Schorfheide-Chorin							
Forest	(n = 50)	1.13 \pm 0.18 ^A	0.22 \pm 0.04 ^A	0.14 \pm 0.03 ^A	18.8 ^C \pm 0.20	97.3 ^C \pm 3.08	147 \pm 1.77 ^D
Deciduous forest	(n = 28)	1.23 \pm 0.24 ^{ab}	0.24 \pm 0.05 ^a	0.15 \pm 0.04 ^{ab}	17.0 ^d \pm 0.20	95.3 ^{ef} \pm 4.06	138 \pm 2.23 ^e
Coniferous forest	(n = 22)	0.99 \pm 0.27 ^a	0.21 \pm 0.06 ^a	0.12 \pm 0.04 ^a	21.1 ^e \pm 0.23	100 ^f \pm 4.58	158 \pm 2.52 ^f
Grassland	(n = 28)	3.17 \pm 0.24 ^B	0.72 \pm 0.04 ^B	0.47 \pm 0.04 ^C	10.4 ^A \pm 0.26	42.9 ^A \pm 4.11	79.5 \pm 2.37 ^B 78.6
Unfertilized grassland	(n = 21)	3.18 \pm 0.28 ^{cd}	0.72 \pm 0.06 ^c	0.48 \pm 0.04 ^d	10.5 ^a \pm 0.23	43.0 ^{ab} \pm 4.69	\pm 2.58 ^c
Fertilized grassland	(n = 7)	3.13 \pm 0.49 ^{cde}	0.69 \pm 0.10 ^{bcd}	0.42 \pm 0.07 ^{bcd}	10.4 ^a \pm 0.40	42.7 ^{abc} \pm 8.13	82.2 \pm 4.45 ^{bc}

Table 2.3 Pearson's correlation coefficient of the relationships between soil clay content with soil organic carbon (SOC), microbial C, total N, total S and total P.

	Soil clay content	
	Forest	Grassland
Schwäbische-Alb		
SOC	0.51***	0.20
Microbial C	0.30*	0.02
Total N	0.45***	0.16
Total S	0.32*	0.13
Total P	0.07	0.21
Hainich-Dün		
SOC	0.62***	0.04
Microbial C	0.45**	0.12
Total N	0.63***	0.04
Total S	0.53***	-0.1
Total P	0.57***	-0.1
Schorfheide-Chorin		
SOC	-0.01	-0.12
Microbial C	0.65***	-0.17
Total N	0.24	-0.13
Total S	0.35*	-0.16
Total P	0.12	-0.05

2.3.2 Net C, N, P and S mineralization rates across sites under different regions, land use and management

The rates of C, N, P and S mineralization per g dry weight soil showed considerable variations between study regions, land use and under different forest types (Figure 1). Overall, average C mineralization rates at all grassland sites were more than 2-fold higher compared to the forest sites and increased in the following order: SCH<HAI<ALB. Contrary, nitrogen mineralization rates were on average 23% and 40% higher at the forest sites in ALB and HAI than at grassland sites, while no significant difference was observed between forests and grasslands in SCH. Similar to N mineralization, S mineralization rates were 2-fold higher at the forest sites in ALB and HAI compared to the grassland sites, while no difference was observed in SCH between land use types. On average, phosphorus mineralization rates were almost 3-fold higher at the forest than grassland sites in HAI, while no differences were observed at other regions. Under different forest types, C mineralization rates were on average higher in coniferous forests in HAI but were higher in deciduous forests in SCH ($P<0.001$). Nitrogen mineralization rates were on average 55% and 15% higher at deciduous compared to coniferous forest sites in HAI and SCH. In SCH, average P mineralization rates were more than 2-fold higher at the deciduous compared to coniferous forest sites. The rates of DOC leached were significantly higher in forests than grasslands at ALB and SCH and were significantly higher in coniferous than deciduous forests in HAI and SCH (Figure 3).

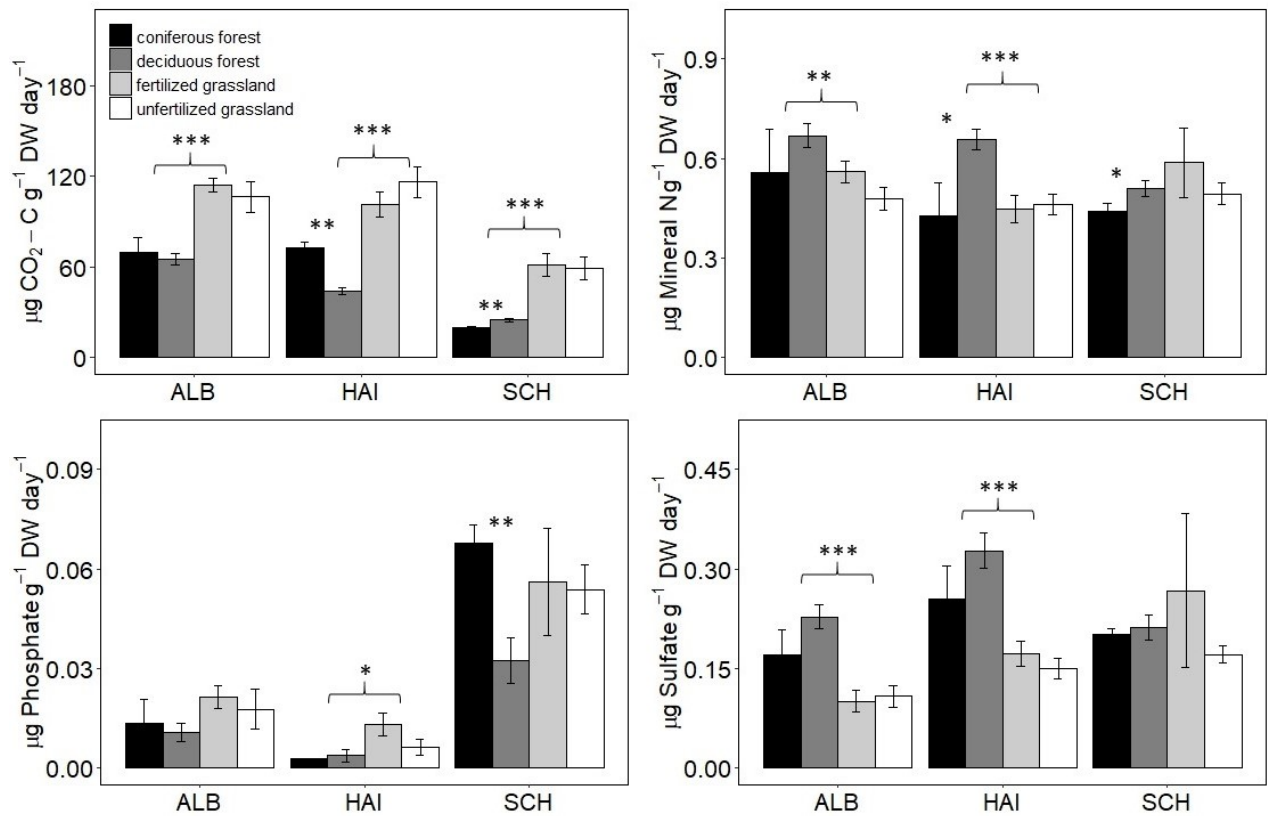


Figure 2.2 Comparison of average mineralization rates of C, N, P and S under different study regions, land use types and managements. Error bars represent SE of means. Number of study sites (n) are shown in Table 1. Significant differences of variance between land use types (shown by brackets) and management are denoted by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

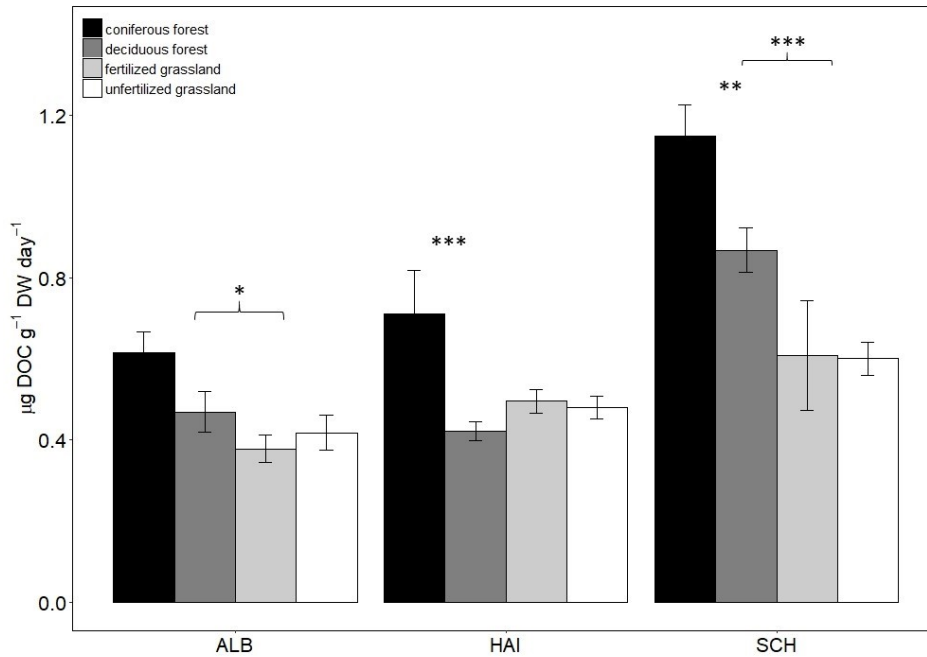


Figure 2.3 Dissolved organic carbon (DOC) leached under different study regions, land use types and managements. Error bars represent SE of means. Number of study sites (n) are shown in Table 1. Significant differences of variance between land use types and management are denoted by * ($P<0.05$), ** ($P<0.01$) and *** ($P<0.001$).

2.3.3 Relations of net mineralization rates and soil properties

Redundancy analysis biplots showed the relations between soil properties (organic C, microbial C, soil pH, nutrient concentrations and soil C-to-nutrient ratios) and net mineralization rates of C, N, P and S mineralization (Figure 3a-b). Data points were clustered according to study regions but not tree species nor fertilization, highlighting the importance of study regions compared to management effects on soil mineralization. Interactions between predictor variables showed that organic C, microbial C, elemental concentrations and soil pH were positively correlated, and this was mainly linked to the ALB and HAI regions (both forest and grassland sites) which have higher soil fertility while the SCH region was linked to lower soil fertility. Carbon mineralization rates in both forests and grasslands were clearly separated from N, P and S mineralization. Instead, C mineralization was positively correlated with sites that associated with higher soil fertility, clay and silt content (Figure 3, Table 1). At the forest sites, N mineralization rates were positively correlated to S mineralization rates and

negatively correlated to P mineralization. In grasslands, N mineralization rates were positively related to P mineralization. The results from RDA biplot were tested and supported using linear model, where the effects of study regions and microbial C concentrations showed significant correlations with soil mineralization (Table 3). Results showed that at both forest and grassland sites, study region explained most of the explained variance that drives C, N, P and S mineralization ($P < 0.001$). Significant positive correlations were observed between microbial C concentration and C mineralization at both forest and grassland sites. Significant positive effects from microbial C were also observed on N and S mineralization at the forest sites, while in both forests and grasslands, microbial C concentrations were negatively correlated to P mineralization

2.3.4 Ratios of C-to-nutrient mineralization and relations to soil nutrient stoichiometry

Ratios of C-to-N mineralization rates were overall more than 2-fold higher at all grassland sites compared to forest sites. The ratios of C-to-N mineralization differed significantly ($P < 0.05$) between study regions at the forest sites and increased in the order SCH (50 ± 18) < HAI (85 ± 17) < ALB (231 ± 16). At the grassland sites, C-to-N mineralization ratios were the lowest in SCH (115 ± 22 , $P < 0.05$) and were not significantly different between HAI (288 ± 16) and ALB (231 ± 16). Average ratios of C-to-S mineralization at the forest sites amounted only to 23% of the average ratios at the grassland sites and were not significantly different between study regions. For grassland sites however, ratios of C-to-S mineralization varied significantly between regions and increased in the order: SCH (367 ± 133) < HAI (893 ± 98) < ALB (1723 ± 109). The ratios of C-to-P mineralization were significantly higher at the forest compared to the grassland sites in ALB ($P < 0.01$), while no differences were observed between land use types in HAI and SCH. Significantly higher C-to-N and C-to-S mineralization ratios were observed at the coniferous forest sites in ALB and HAI as compared to deciduous forests, while C-to-P mineralization ratios were higher at the deciduous than coniferous forest sites in SCH. Higher ratios of C-to-P mineralization ($P < 0.01$) were also observed at unfertilized grasslands compared to fertilized grassland sites in HAI.

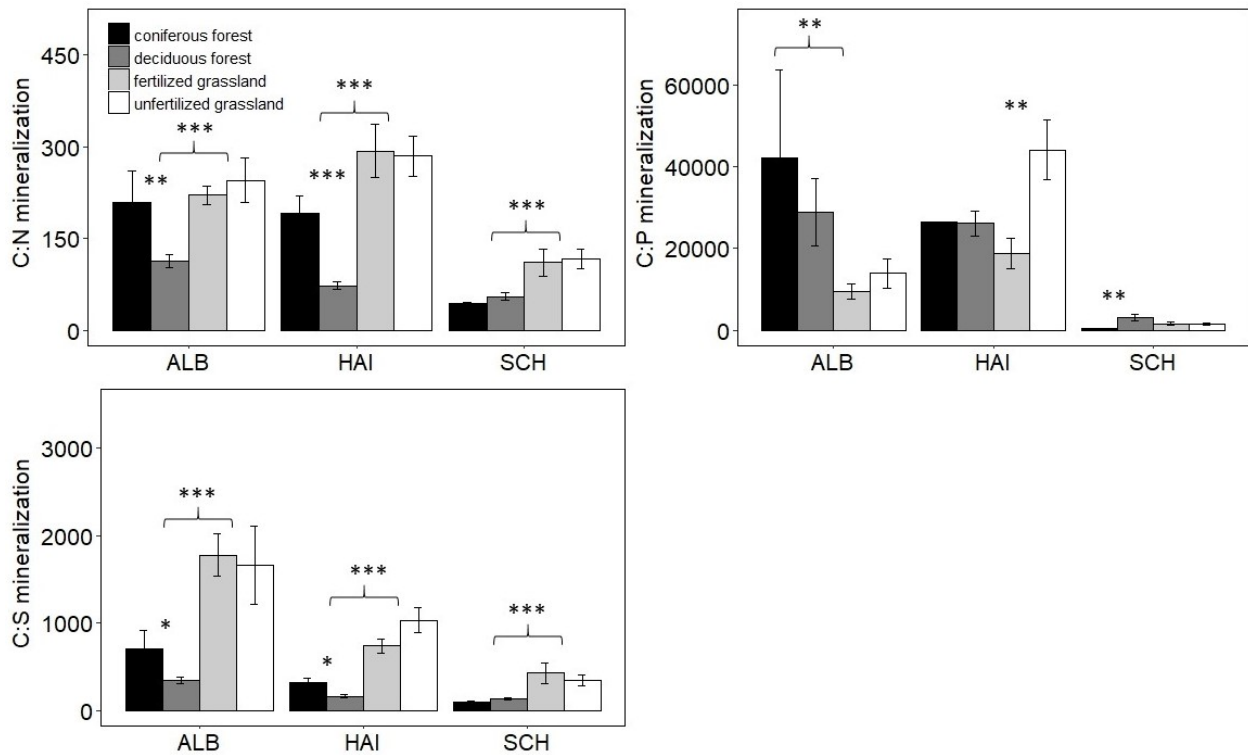


Figure 2.4 Comparison of average mineralization rates of C-to-nutrients under different study regions, land use types and managements. Error bars represent SE of means. Number of study sites (n) are shown in Table 1. Significant differences of variance between land use types and management are denoted by * ($P<0.05$), ** ($P<0.01$) and *** ($P<0.001$).

Redundancy analysis showed that at the forest sites, soil C:N, C:S and C:P were negatively correlated to N, S and P mineralization (Figure 3a). At the grassland sites, soil C:N and C:P were negatively correlated to N and P mineralization, while soil C:S was positively correlated with S mineralization (Figure 3b). Results from linear model showed that after considering the effects of study region and microbial C concentration, soil C:P has significant negative effects on P mineralization at both forest and grassland sites, but no effects of soil C-to-nutrient ratios were observed on C, N and S mineralization (Table 3). Carbon mineralization, DOC leaching and N mineralization per mg microbial C were positively correlated to soil C:N (Figure 4a-c), while microbial C concentrations were negatively correlated to soil C:N (Figure 4d). The correlation strengths were higher in forests than grasslands probably because of higher soil C:N range covered by the studied forest sites.

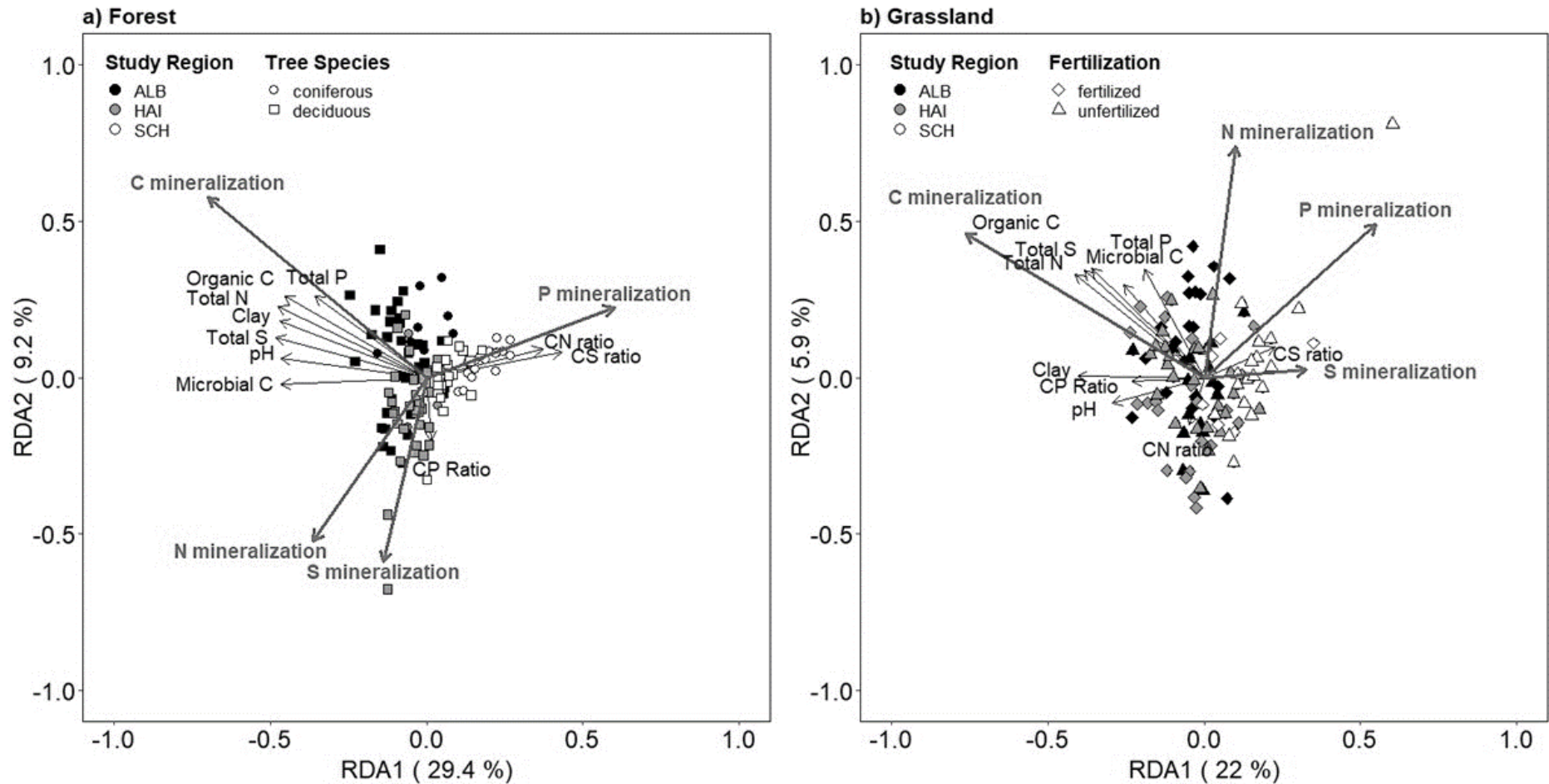


Figure 2.5 Biplots of redundancy analysis (RDA) showing net C, N, P and S mineralization rates ($\mu\text{g DW}^{-1} \text{ day}^{-1}$) as responses variables and soil properties (organic C, microbial C, soil pH, total N, P and S concentrations, and soil C:N, C:P and C:S ratios) as predictor variables. Study regions are denoted by ALB: Schwäbische-Alb, HAI: Hainich-Dün and SCH: Schorfheide-Chorin.

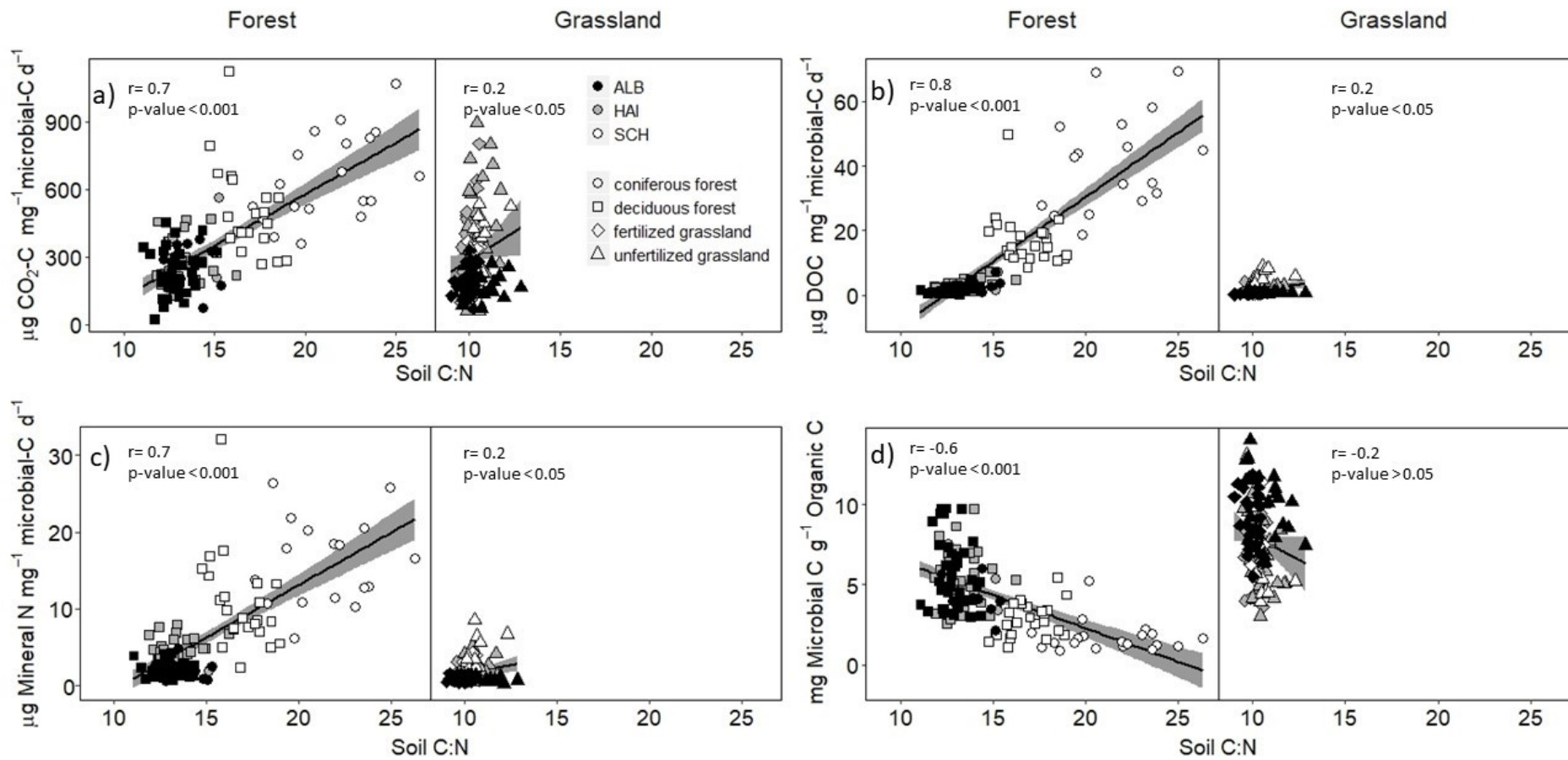


Figure 2.6 Correlations between a) C mineralization per microbial C, b) N mineralization per microbial C, c) DOC leached per microbial C and d) microbial C per organic C and soil C:N ratio. Study regions are denoted by ALB: Schwäbische-Alb, HAI: Hainich-Dün and SCH: Schorfheide-Chorin.

Table 2.4 Linear models with net soil mineralization rates (C, N, P and S) for a) forest sites and b) grassland sites as response variables for all plots. Explanatory variables (SR = study region, Cmic = microbial C and C:nutrient = C:N for C and N mineralization, C:P for P mineralization and C:S for S mineralization) were stated as the order of analysis with all interactions included. Degrees of freedom (df), mean squares (MS) and F-values (F) are presented (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

	C mineralization			N mineralization			P mineralization			S mineralization		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F
a) Forest												
Study region (SR)	2	12.6	110.0 ***	2	0.8	6.7 **	2	58.0	71.5 ***	2	2.8	13.9 ***
Cmic	1	0.9	7.5 **	1	2.6	20.7 ***	1	9.2	11.4 **	1	0.8	4.1 *
C:nutrient	1	0.0	0.3	1	0.1	1.2	1	3.5	4.3 *	1	0.1	0.4
SR:Cmic	2	1.4	12.0 ***	2	0.1	0.8	2	16.6	20.4 ***	2	0.0	0.1
SR:C:nutrient	2	0.0	0.3	2	0.2	1.6	2	0.8	1.0	2	0.1	0.6
Cmic:C:nutrient	1	0.3	2.9	1	1.1	8.6 **	1	2.5	3.1	1	0.2	1.1
SR:Cmic:C:nutrient	2	0.0	0.1	2	0.1	0.6	2	0.1	0.1	2	0.4	2.1
Residuals	117	0.1		11	0.1		7	0.8		11	0.2	
				4			6			7		
b) Grassland												
Study region (SR)	2	4.7	26.0 ***	2	0.4	2.9	2	38.6	58.9 ***	2	4.7	16.1 ***
Cmic	1	4.5	24.8 ***	1	0.6	3.7	1	3.0	4.6 *	1	0.1	0.2
C:nutrient	1	0.2	1.3	1	0.2	1.7	1	15.5	23.7 ***	1	0.2	0.5
SR:Cmic	2	0.3	1.4	2	0.0	0.3	2	0.8	1.2	2	0.2	0.8
SR:C:nutrient	2	0.4	2.1	2	0.2	1.3	2	0.3	0.4	2	0.3	0.9
Cmic:C:nutrient	1	1.3	7.3 **	1	0.0	0.2	1	0.3	0.5	1	0.0	0.1
SR:Cmic:C:nutrient	2	0.1	0.6	2	0.2	1.5	2	0.6	1.0	2	1.1	3.9 *
Residuals	112	0.2		10	0.1		9	0.6		10	0.3	
				9			3			3		

2.4 Discussion

2.4.1 The effects of study region on soil mineralization

Soil organic matter mineralization potentials for C, N, P and S differed significantly across three different study regions (Figure 1a-d). This was confirmed by the linear model which showed that study region explained the highest proportion of variance of SOM mineralization for all elements studied (Table 3). Study regions are related to differences in climatic factors as well as in soil properties (Fischer et al. 2010). However, since soil incubations in this study were performed in a climate chamber with fixed temperature and humidity with similar moisture treatments prior to pre-incubation, only differences in soil properties across regions were considered as possible drivers for the variations of soil mineralization observed.

As C mineralization rates were observed to correlate positively with clay content, soil pH, OC, total N, total P, total S, and microbial C concentrations (Figure 3), the variations of these parameters among study regions could partly be attributed from the effects of study region on C mineralization. Compared to SCH, soils in the ALB and HAI regions were characterized by higher clay and silt content, soil pH, OC, microbial C, total N, total P and total S concentrations (Table 1-2). This thus explained the overall higher C mineralization rates in ALB and HAI, while the low C mineralization in SCH could probably be attributed to its soil texture with low clay, high sand content and low microbial C concentrations (Figure 1a, Table 1). Positive relationships between clay content, SOM content and microbial activities have been previously reported (Hassink 1994b, Wang et al. 2003, Kemmitt et al. 2006). Further, a previous study from the same regions has shown that OC storage in different fractions of SOM is mainly influenced by soil texture and mineralogy (Herold et al. 2014c). The importance of clay particles on OC retention is due to their cation exchange properties and in reacting with organic matter to form stabilized SOM complexes (Dixon 1991).

Overall, higher P mineralization rates were observed in SCH compared to ALB and HAI (Figure 1). As compared to N mineralization rates which are mainly affected by biological factors such as microbial activities and SOM content, soil P mineralization has been shown to be more strongly controlled by P availability in soil (Chapin et al. 2011). Low P availability in soil has been shown to increase microbial P acquisition from organic P sources mainly via phosphatase activities and thus increase P mineralization (Olander and Vitousek 2000). High P mineralization in SCH which also contained the lowest total P concentrations compared to

ALB and HAI (Table 2) is therefore suggested to be induced by low amount of P in this region. Similar with the impact of clay content on C mineralization, low concentrations of total P in SCH could probably be explained by its soil texture with low clay and high sand content (Table 1). This is supported by a recent study conducted on 147 sites across the German forest landscape, where the concentrations of different solubility forms of soil P in sandy soils were found to be significantly lower compare to other soil texture types (Niederberger et al. 2018).

2.4.2 Land use and management effects

Overall, C mineralization rates were consistently higher in grasslands than forests across all study regions (Figure 1a). This may be attributed to microbial C concentrations in grasslands which is twice as high as the concentrations in forests (Table 1). Results from linear model showed that after considering the effects of study region, microbial C concentrations still showed significant positive effects on C mineralization in both forests and grasslands (Table 3a-b). Land use has been shown to be an important driver of microbial biomass and activities. For example, previous studies on the same sites have shown that higher microbial activities in grasslands compared to forests led to faster fine root decomposition (Solly et al. 2014) and faster litter-derived C turnover (Herold et al. 2014c). This could further be explained by higher litter quality (lignin content, lignin-to-N ratios and C-to-N ratios) in grasslands than in forests (Solly et al. 2014) which is more labile and susceptible to microbial decomposition and thus results in higher microbial activities in grasslands than in forests (Melillo et al. 1982, Taylor et al. 1989). Positive correlations between microbial C concentrations and soil C mineralization have been widely reported by previous studies (Anderson and Domsch 1990, Brookes and Soils 1995, Bastida et al. 2008, Whitaker et al. 2014). Further, as C mineralization rates significantly correlate with microbial C concentration at all sites in this study ($r = 0.6$, $P < 0.001$), microbial C concentration is suggested to represent an active proportion of microbial biomass and served as a reliable parameter of microbial activities (Anderson and Domsch 1990, Haney 2008).

Overall, DOC concentrations were higher in forests compared to grasslands, and in coniferous compared to deciduous forests (Figure 3). In this study, higher DOC concentrations in forests compared to grassland may be attributed to higher N mineralization (Figure 1b) and soil C:N (Table 2) in forests than grasslands, which has been identified as important positive predictors

of DOC (Camino-Serrano et al. 2014), or that DOC production increased the availability of N-containing substrates which led to higher N mineralization. Further, higher DOC concentrations in coniferous compared to deciduous forests was probably linked to slower decomposition rates of coniferous litter as well as thicker organic layer under coniferous stands (Camino-Serrano et al. 2014). This may also explain higher average SOC content in the mineral soils from coniferous compared to deciduous forests in some regions (Table 2.1), even though mineral soils were incubated without litter layer in this study.

Mineralization rates of N and S were significantly higher in forests compared to grasslands in ALB and HAI (Figure 1b, Figure 1d). Similar patterns observed for N and S mineralization suggests that S mineralization rates are coupled to N mineralization. In unpolluted ecosystems, S cycling is tightly coupled to N and P cycling due to their important roles as the essential components of protein (Chapin et al. 2011), and the coupling of N and S mineralization rates has been demonstrated in previous studies (Tabatabai and Al-Khafaji 1980, McGill and Cole 1981). Higher N and S sources at the grassland than forest sites did not result in higher mineralization potentials because much of the nutrient sources may be resistant to decomposition (Schimel 1986). Most soil N is in organic forms and contributes to complex polymers such as proteins, nucleic acids and chitin which requires enzymatic reactions for depolymerisation, mineralization, and immobilization (Jørgensen 2009) while a small proportion of this N source is immobilized as microbial biomass (Paul and Juma 1981, Azam et al. 1986, Marumoto and Azad 1991).

Higher net N and S mineralization observed in forests than grasslands could also be explained by the differences between microbial C concentrations between the two land use types (Table 1). In forests, higher net N and S mineralization is probably due to lower microbial biomass and activities compared to grasslands, which resulted in lower N and S immobilization in microbial biomass and therefore higher net mineralization (Vitousek and Matson 1984, Schimel 1986, Barrett and Burke 2000). However, as compared to ALB and HAI, N mineralization is not significantly different between forests and grasslands in SCH even though higher microbial C concentrations were also observed in grasslands compared to forests in this region. This may be explained by the significantly lower clay content and soil pH in SCH grasslands compared to other grassland sites which resulted in overall lower microbial activities and lower N immobilization (Schimel 1986, Hassink 1994b, Pal and Marschner 2016). Another explanation may be that for most SCH grasslands in the peaty

soils, the ratio of microbial biomass to SOC is smaller compared to grasslands from the other two regions which may led to relatively smaller N immobilization.

Higher rates of N and S mineralization in deciduous than coniferous forests were further observed in HAI and SCH (Figure 1b, Figure 1d). Different mineralization rates are suggested to be due to differences in litter quality, where litter composition from different tree species has been reported to vary widely and may potentially affect soil N mineralization (Gower and Son 1992, Hobbie 1992, Reich et al. 1997). This finding supported previous studies which observed higher N mineralization in the mineral soil (Côté et al. 2000) as well as in the forest floor under deciduous compared to coniferous stands in boreal forests (Côté et al. 2000, Jerabkova et al. 2006). However, the effects of tree species on N mineralization should be interpreted with caution due to the other forest management practices at the study sites, which were not considered in this study. Most deciduous and coniferous forest sites in HAI as well SCH were managed as commercial forest under different age class, selective harvesting and as mixed forests, while some deciduous forest sites were unmanaged (Fischer et al. 2010). While some evidence supported the finding that variations in soil mineralization was attributable to tree species effect since forest types were compared within the same study region with similar soil properties and structure (Table 1), it could not be ruled out that higher N mineralization rates under deciduous forest type could be due to other management effects such as stand age and age class management which has been shown in previous studies (Côté et al. 2000, Mueller et al. 2012, Lucas-Borja et al. 2016).

As for C and P mineralization, significant differences too were observed between different forest types (Figure 1c). However, these variations could be partly due to the influence of study regions since no consistent trends were observed in other regions. This is supported by the result of linear model which showed that the interaction effects between study region and microbial C concentrations were significant for C and P mineralization in forests (Table 3a). Overall, it could be concluded based that land use types and management related practices in forest such as tree species selection are important controls of soil mineralization potentials due to differences in the amount and quality of organic substrates input to soil which affects microbial activities. Some studies have shown that C mineralization declined with N fertilization (Craine et al. 2007b, Riggs et al. 2015, Spohn et al. 2016). However, significant differences in SOM mineralization between fertilized and unfertilized grassland sites were not observed. This may be due to the effects of other grassland management practices not

considered in this study such as mowing and grazing which have been shown to affect plant species richness (Socher et al. 2012).

2.4.3 Stoichiometry effects on soil mineralization

Using RDA, the interactions among C, N, P and S mineralization along with the effects of soil properties under different regions and land uses were observed (Figure 3a-b). In forests, P mineralization negatively correlated to C, N and S mineralization. In other words, increasing P availability in forests was related to reduced leachable N, S and C respired, and this could be due to lower mineralization or higher immobilization. Potential P limitation as a result of soil P saturation in temperate forests has been reported by previous studies (Gallardo and Schlesinger 1994, Gress et al. 2007, Shaw and DeForest 2013). Although temperate forests are generally thought to be more N than P-limited, low bioavailability of inorganic P may still occur (DeForest et al. 2012, Shaw and DeForest 2013). As compared to ALB and HAI, higher P mineralization in SCH is expected to reduce P limitation at this site and increase microbial N immobilization, or that higher P mineralization may be related to low soil clay content in this region. However, since N mineralization rates did not differ greatly between study regions (Figure 1b), this may be an indicator that other factors may have constrained N and P immobilization in SCH compared to ALB and HAI, which was not measured in this study. Although previous study showed that P mineralization may be driven by microbial C limitation (Spohn and Kuzyakov 2013), this is unclear given that higher C removal per microbial C was also observed in SCH (Figure 4a-b). An alternative explanation is that distinctive soil properties such as low clay content, soil pH, microbial C concentrations and overall lower microbial activities in SCH compared to ALB and HAI (Table 1) resulted in lower N and P immobilization (Hart et al. 1994, Côté et al. 2000). In this case, the increase in C mineralization and DOC leached per microbial C especially in SCH forests may indicate microbial metabolic stress rather than overflow C removal to meet nutrient requirement (Wardle and Ghani 1995, Aciego Pietri and Brookes 2008). In contrast, P mineralization positively correlated to N and S mineralization in the grasslands (Figure 3b), suggesting that soil P availability probably did not limit microbial N and S immobilization as compared to the forests. Previous study at the same sites has reported lower bioavailable soil P concentrations in forest than grasslands, and that grassland management such as lime-containing fertilizer may increase overall P bioavailability in grasslands than forests (Alt et al. 2011b).

Based on previous studies, soils with high C:N ratios should have low N mineralization as more N is immobilized while excess C is removed via overflow respiration or reduced C use efficiency (CUE) (Manzoni et al. 2012, Mooshammer et al. 2014a, Spohn and Chodak 2015). Therefore at sites with higher soil C:N, higher C and lower N mineralization are expected to result in higher ratios of C:N mineralized as compared to sites with lower soil C:N (Barrett and Burke 2000, Côté et al. 2000). This was partly observed in this study where C mineralization and DOC leached per microbial C increased with increasing soil C:N especially in forests (Figure 4a-b). However, instead of increased N immobilization as predicted, increasing N mineralization per microbial C with increasing soil C:N was observed (Figure 4c). Increasing N mineralization per g SOC with decreasing soil N sources may be explained by microbial C mining for N acquisition, where excess C from a C-rich source is mineralized to decompose nitrogenous SOM (Mooshammer et al. 2014a). This however raised an important question: if microbes are N limited, most of the N sources would be expected to be assimilated quickly (in the form of depolymerized organic N) rather than being available for ammonification and nitrification processes, as total net NH_4^+ and NO_3^- were measured as net N mineralized in this study (Schimel and Bennett 2004, Chapin et al. 2011).

Higher ratios of mineralized C:N and C:S were further observed at sites with lower soil C:N ratios i.e. in grasslands compared to forests and coniferous compared to deciduous forests (Figure 2b, Figure 2c), even though soil C:N and C:S ratios were significantly lower in grasslands than forests at all sites (Table 2.2). This contrasts to the findings of Côté et al (2000) which showed higher C:N mineralized ratios in coniferous compared to deciduous forests in ALB and HAI are suggested to be due to lower SOM quality under coniferous stands. These findings suggest that SOM stoichiometry was probably not responsible for the differences in the ratios of C:N and C:S mineralized observed in grasslands than forests in this study. Other results discussed above show that C mineralization was lower while N and S mineralization was higher in forests compared to grasslands (Figure 1a, Figure 1b, Figure 1d). This thus explained lower C:N and C:S mineralized ratios in forests compared to grasslands (Figure 2a, Figure 2c).

The results also showed that microbial C concentration had significantly positive effects on C, N and S mineralization (Table 3). Taken together, these findings suggest that lower C:N and C:S ratios of SOM mineralization in forests than grasslands are likely due to lower microbial biomass and activities in forest soils which resulted in overall lower C mineralization and

lower N and S immobilization, leading to the observed higher N and S leaching rates. This is supported by the result which shows decreasing microbial C concentration with increasing soil C:N in forests, even after normalization with OC concentration per site (Figure 4d). Lower microbial biomass-C concentrations and thus overall low N immobilization as biomass particularly in the acidic and sandy forest soils in SCH (Figure 4c) may probably explained high inorganic N retention with increasing soil C:N rather than N mining (Schimel 1986, Booth et al. 2005).

2.5 Conclusion

Findings showed that land use, management practices and regional differences are important controls for C turnover and the mineralization of N, P and S in soil organic matter. Soil respiration and microbial biomass carbon were showed to be related to total OC contents and declined with higher soil C:N and C:S ratios, which were higher in grassland than forest soils. In contrast to the first hypothesis, CO₂-to-N- and CO₂-to-S-leaching ratios were significantly higher in grasslands than forests in all regions, even though soil C:N and C:S were lower in grassland than forest soils. This was explained by significantly higher CO₂ and significantly lower N and S leaching in grasslands than forests which might be due to higher microbial biomass at the grassland sites, indicating higher N and S immobilization. Supporting the second hypothesis however, CO₂-to-N- and CO₂-to-S-leaching ratios were observed to be significantly higher in coniferous than deciduous forests in two out of the three study regions, while no important differences were observed between fertilized and unfertilized grasslands. Finally, increasing CO₂ and DOC production per g microbial biomass-C with soil C:N particularly in the forest soils (and in coniferous relative to deciduous forest soils) supports the hypothesis that reduced microbial carbon use efficiency in nutrient poor soils did result in the removal of “excess” C for nutrient acquisition. However, increasing N mineralization with soil C:N showed the uncertainties of whether high N leaching per g microbial biomass-C in N poor soils is a result of microbial N deficiency, or simply due to higher retention of soil inorganic N due to low microbial immobilization.

Chapter 3

Effects of substrate concentrations on soil respiration and soil C priming under different land use

Chapter source: Gan et al., 2018. Effects of substrate concentrations on soil respiration and soil C priming under different land use. (Manuscript ready for submission)

Abstract

The addition of labile C substrates to soil can accelerate or inhibit the decomposition of soil organic matter (SOM). Future changes in vegetation productivity or climate are expected to alter the amount, location and quality of labile C added to soils, with unknown consequences for terrestrial carbon cycling. In this chapter, the effects of labile C input on the mobilization of native C across a range of sites, land use and land management were determined. The main objectives were to (i) quantify the magnitude of C released with different concentrations of labile C input and (ii) the effects of labile C concentrations in relation to the microbial biomass in each soil with short incubation approach for 48 hours. Using labelled ^{13}C glucose as labile C substrate, different fractions of CO_2 respired according to their sources were able to be fractionated (glucose, basal respiration and extra CO_2 respired due to priming effects).

Positive priming was observed in all glucose additions, which were in amounts equivalent to 30-920% of microbial C. This range is much higher compared to previous studies which concluded that the addition of more than 50% microbial C would result in the inhibition of SOM decomposition and hence negative priming. Our findings highlight that the amount of C added affects the amount of soil priming (extra native C released due to labile C addition) observed. Further, this chapter showed that using the same incubation time when different amounts of labile C added may not be adequate to observe the full effects of priming.

Keywords Soil priming effects, Soil microbial carbon, glucose concentrations, stable isotope, soil carbon cycling

3.1 Introduction

The input of labile carbon (C) in soil accelerates or retards microbial activities and the decomposition of soil organic matter (SOM) through priming effect (Blagodatskaya and Kuzyakov 2008). Many studies have been conducted to quantify the amount of native C released from priming triggered by the addition of chemically different C substrates, from different concentrations of the same C substrate and in different soils and ecosystems (Dilly 2004, Guenet et al. 2010, Nottingham et al. 2012). To a lesser degree, studies attempted to identify the mechanisms underlying priming effects. These include the co-metabolism of SOM with the added substrate, mining of SOM to release additional nutrients (Nottingham et al. 2015) and due to succession in microbial groups (Bernard et al. 2007, Morrissey et al. 2017). However, comparing the amount of CO₂ respired from priming among published studies can be problematic as they add different amounts of labile C to soils especially when compared to the size of the existing microbial C pool in soil.

Adding different amounts of labile C relative to microbial C pool size has been shown to significantly affect the amount of CO₂ respired due to priming. Blagodatskaya & Kuzyakov (2008) reported that the additions of C up to 15% of microbial C induces a linear increase of CO₂ respired from priming, while C addition up to and exceeding 50% of microbial C resulted in exponential decrease or negative priming. When the amount of labile C added relative to microbial C is very small ($\mu\text{g C g}^{-1}$), the release of CO₂ or SOM-C uptake as biomass via priming has been suggested to reflect a “triggering effect”, where microbial metabolism and respiration activity increase without an increase in microbial biomass (De Nobili et al. 2001). This process has been suggested as a response strategy of soil microbes to stay metabolically alert to survive in a C-poor environment (De Nobili et al. 2001). Dungait et. al (2013) reported that adding low amount of labile C ($15 \mu\text{g C g}^{-1}$) results in an overall increase in PLFA, and therefore biomass, which was on average greater than the amount of C added and therefore must be derived from or “primed” from soil organic matter (SOM). Similar findings have also been reported by De Nobili et al. (2001) that the addition of low amounts of labile C ($143.6 \mu\text{g organic C ml}^{-1}$) results in higher total CO₂ respired than the amount of C added.

In contrast, adding labile C in amounts greater than soil microbial biomass-C has been reported to result in microbial growth and microbial C turnover (Blagodatskaya and Kuzyakov 2008). Similarly, previous substrate-induced respiration studies have shown that

the addition of labile C exceeding the minimum amount required for microbial metabolism (similar or slightly above soil microbial C) resulted in biomass growth (Anderson and Domsch 1985). Lower CO₂ released via priming with higher fresh organic input has been suggested to be due to “preferential substrate utilization”, a process where soil microbes switch from decomposing SOM to more easily available C substrates and resulted in a net increase of C balance observed at the end of soil incubation (Blagodatskaya et al. 2011).

Most previous studies measured priming responses on a more extended time scale (>1 month), and less attention has been given on the dynamics of C respiration immediately after substrate addition and how that may inform about underlying processes. The aims of this study were (1) to evaluate total soil CO₂ respiration and CO₂ respired from priming in response to adding different amounts of glucose relative to microbial biomass-C in two land use types (2 forest and 2 grassland soils), and (2) to follow the dynamics of these processes in the hours following addition using an online method to monitor headspace stable isotope and CO₂ concentrations.

3.2 Materials and methods

3.2.1 Study sites and soil sampling

Soil samples were collected in May 2014 from two forests and two grasslands in Hainich-Dün (HAI). All soils were characterized by loamy or clayey texture due to the dominant geological substrate of loess over limestone and were classified as Cambisols (IUSS Working Group WRB, 2014). Detailed information climatic conditions and soil parent materials of the study region has been published previously (Fischer et al. 2010). The first forest site (Forest 1) was a commercial forest dominated by *Picea abies*, and the second (Forest 2) was a naturally managed forest dominated by *Fagus sylvatica*. The first grassland site (Grassland 1) was an unfertilized cattle-grazed pasture, while the second (Grassland 2) was an ungrazed, fertilized meadow and was mowed once a year. The top 10 cm of mineral soil was sampled in May 2014 using split tubes, and 14 soil cores with a diameter of 4.8 cm were sampled in each site and mixed to form composite samples that were stored at 4 °C prior to incubations.

3.2.2 Microbial C, Total C and Total N

Microbial C content was determined using chloroform-fumigated extraction (Jenkinson 1987, Vance et al. 1987). Total C (TC) and nitrogen (TN) (g kg^{-1} soil) were determined by dry combustion (VarioMax, Elementar Analysensysteme GmbH, Hanau, Germany). Inorganic C was quantified after removing OC by ignition at 450°C for 16 hours. Organic C was calculated as the difference between TC and inorganic C. Soil pH was determined with a pH electrode in a 1:2.5 supernatant of soil and 0.01 M CaCl_2 . Data on soil clay content was adopted from Solly et al (2014) and determined by wet sieving and sedimentation (pipette method).

3.2.3 Soil incubation with ^{13}C labelled glucose

Prior to incubation, soils were sieved ($2 < \text{mm}$) and pre-incubated for 3 days in dark at 20°C at field moisture. One g of soil was incubated in 12 ml glass vials with PTFE/silicon septa for 48 hours with different glucose concentrations (200, 400, 800 and 1600 μg glucose-C, $\delta^{13}\text{C} = 100\%$) dissolved in 100 μl deionized water. Controls were prepared using the same volume of glucose-free deionized water. After glucose or water addition, the vials were flushed with CO_2 -free synthetic gas (20.5 % O_2 , 79.5 % N_2 , Westfalen AG, Germany). The samples were incubated in the ‘gas bench’ autosampler coupled to isotope ratio mass spectrometer (GC-IRMS) (MS, Finnigan Delta S) via a variable open-split interface (Conflo II, all from Finnigan Mat, Bremen). Isotope ratios of CO_2 evolution was reported in the conventional δ -notation below:

$$\delta^{13}\text{C} = \left[\left(\frac{^{13}\text{Rsa}}{^{13}\text{Rref}} \right) - 1 \right] \times 1000$$

where Rsa and Rref were the ratios of ^{13}C to ^{12}C in samples and reference, respectively. The vials were flushed again with CO_2 -free synthetic gas after 24 hours to avoid CO_2 saturation. The ^{13}C labelled glucose solution was obtained by diluting purchased D-Glucose- $^{13}\text{C}_6$ (99 atom % ^{13}C , Sigma-Aldrich GmbH Munich, Germany) with unlabelled glucose using the isotope balance equation below (Brand and Coplen 2012):

$$\delta^{13}\text{C}_{\text{glucose}} = \delta^{13}\text{C}_{\text{unlabelled}} \times x_{\text{unlabelled}} + \delta^{13}\text{C}_{\text{labelled}} \times x_{\text{labelled}}$$

where x is the mole fraction of elements in different glucose pools. $\delta^{13}\text{C}_{\text{glucose}}$ refers to the target ^{13}C abundance in the glucose substrate added to incubation vials (100 %). CO_2 respired from glucose was quantified using the equation below (Nottingham et al. 2012):

$$\% \text{CO}_2_{\text{glucose}} = \frac{\delta_c - \delta_T}{\delta_c - \delta_{\text{glucose}}} \times 100$$

where δ_c and δ_T are the $\delta^{13}\text{C}$ values of respired CO_2 from control soils and soils with glucose addition, respectively. Priming effect was calculated using the equation below (Bastida et al. 2013):

$$\text{Priming effect} = \text{Total CO}_2(\text{labeled soil with glucose}) - \text{CO}_2(\text{glucose label}) - \text{Total CO}_2(\text{control})$$

3.2.4 Statistical analysis

All statistical analyses and plots were performed and produced in R version 3.3.2 (Team 2016). Results for CO_2 efflux and soil abiotic properties were presented as means \pm standard error ($P < 0.05$) unless stated otherwise. CO_2 measurements and $\delta^{13}\text{C}$ values of the time-series data were linearly interpolated to one standardized measurement time series using the “approxfun” function for a uniformed comparison.

3.3 Results

3.3.1 Soil abiotic properties

Organic C (OC) concentrations differed between Forest 1 and 2 but were similar in the two grassland. Soil C:N was higher in Forest 1 than the rest of the study sites; this site also had the highest OC concentrations. Soil pH was on average more acidic in forests (pH 4.8 ± 0.1) than grasslands (pH 6.6 ± 0.1). Clay content was similar for all four sites.

Table 3.1 Soil abiotic properties for the samples used.

Sample	Organic C		Clay content	
	(mg g ⁻¹)	C:N	Soil pH	(mg g ⁻¹)
Forest 1	53.742	16.333	4.9	409
Forest 2	35.143	11.795	4.7	447
Grassland 1	34.823	11.109	6.8	387
Grassland 2	33.924	10.788	6.5	436

3.3.2 CO₂ respiration following glucose addition

Total amount of CO₂ respired from all samples with glucose addition was higher compared to control soils with only water added after 48 hours of incubation (Table 2). Higher CO₂ respiration was also observed with increases in the glucose concentration added for all soils (Table 2; Figure 3.1). In soils from both forests sites, the addition of 200 µg glucose-C resulted in a constant rate of CO₂ respiration followed by gradual decrease with time, while adding glucose in higher concentrations resulted an increasing delay until the maximum rates of CO₂ respiration are attained. With 1600 µg glucose-C addition, respiration rates remained high (Forest 1) or continued increasing (Forest 2) for the duration of the experiment. In grassland soils, CO₂ respiration rates increased before the first measurement and remained high, with a small maximum at highest levels of glucose addition, before declining. The time delay from glucose addition to decline was overall faster in grasslands compared to forests. Another interesting observation was that in both grassland soils, the initial respiration rate right after glucose addition increased with glucose levels, while in both forests, the initial respiration rate was similar for all levels of glucose addition.

Table 3.2 Microbial C, the absolute amount of C added and per microbial C and soil CO₂ respiration derived from different fractions. For control soils, means±standard deviation of total CO₂ was reported (n = 4). Significant differences between samples are indicated by lowercase letters.

Sample	Microbial C (mg g ⁻¹ soil)	Glucose added (mg C g ⁻¹ soil)	Glucose added (% microbial C)	Total CO ₂ (mg C g ⁻¹ soil)	Glucose-derived CO ₂ (%)	Primed CO ₂ (mg C g ⁻¹ soil)
Forest 1	0.17	Control	-	0.07±0.00 ^b	-	-
		0.2	115	0.18	24	0.01
		0.4	230	0.34	43	0.03
		0.8	460	0.59	71	0.07
		1.6	920	1.00	92	0.04
Forest 2	0.67	Control	-	0.04±0.00 ^a	-	-
		0.2	30	0.13	30	0.01
		0.4	60	0.25	51	0.02
		0.8	119	0.49	81	0.06
		1.6	238	0.86	93	0.05
Grassland 1	0.47	Control	-	0.08±0.00 ^b	-	-
		0.2	42	0.14	24	0.01
		0.4	85	0.26	39	0.04
		0.8	169	0.42	54	0.29
		1.6	338	0.74	80	0.11
Grassland 2	0.55	Control	-	0.10±0.00 ^c	-	-
		0.2	36	0.17	23	0.01
		0.4	73	0.31	40	0.03
		0.8	146	0.56	53	0.15
		1.6	291	1.11	81	0.13

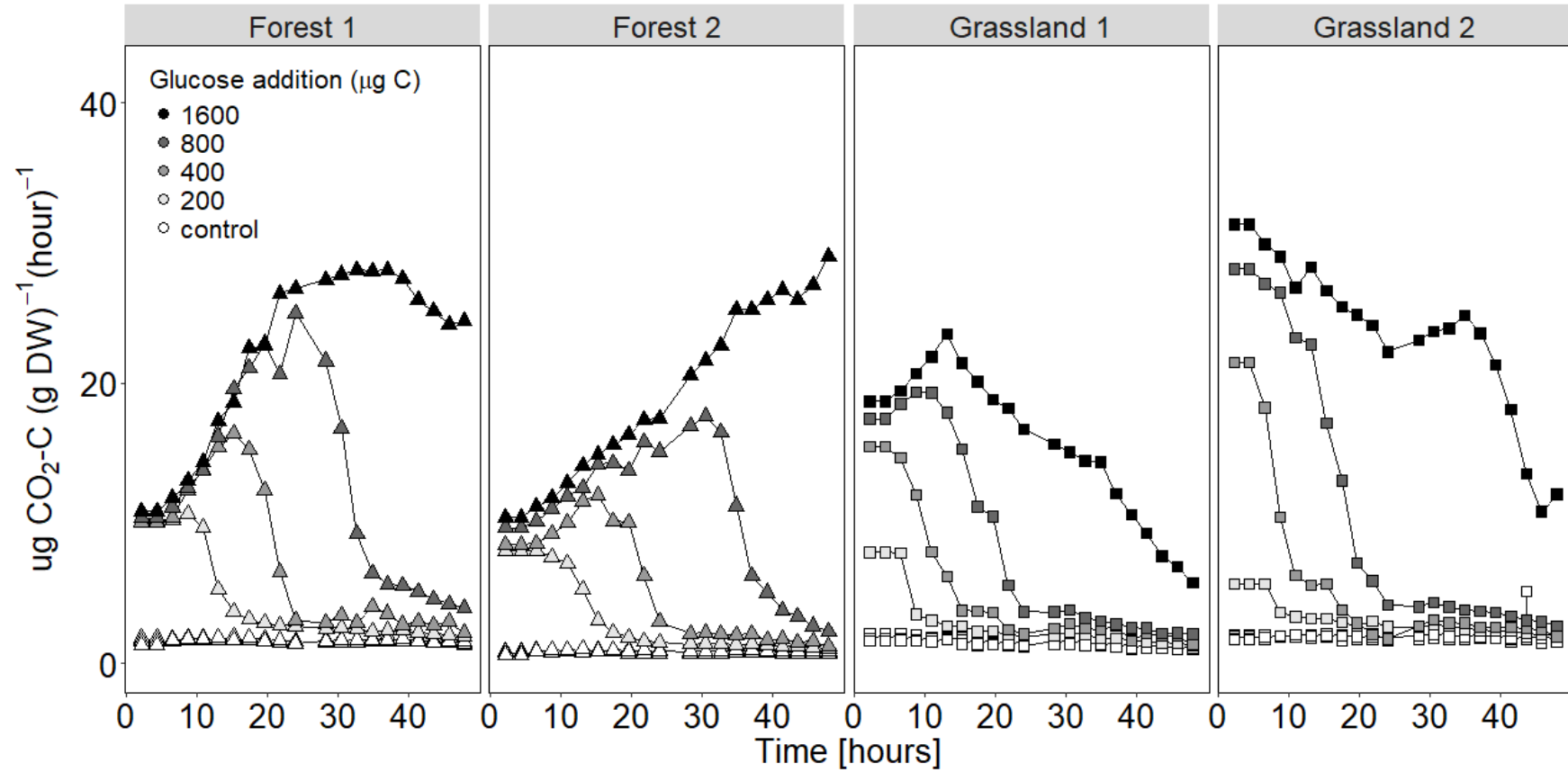


Figure 3.1 Hourly CO₂ efflux during 48 hours of incubation with different glucose concentration addition in 2 forest and 2 grassland plots in Hainich-Dün (HAI).

3.3.3 The amounts of soil C priming with different glucose concentration

In all soil samples, the addition of all different glucose concentrations resulted in positive priming (Figure 3.2, Table 2). In this study, the amount of added glucose ranged from 30-920% of the microbial C pools (115-920% for Forest 1, 30-238% for Forest 2, 42-338% for Grassland 1 and 36-291% for Grassland 2). These are within the range where previous studies indicated that negative priming should be expected. However, in all cases, there was positive priming (i.e. addition of glucose stimulated the release of non-glucose soil carbon by respiration). While both total CO₂ respiration and glucose-derived CO₂ increased with glucose addition, the amount of CO₂ released from priming increased with glucose concentration only up to the addition of 800 µg C (Figure 3.3a, Table 2). Interestingly, the total priming for all samples was highest with addition of 800 µg glucose-C and declined when glucose addition was increased to 1600 µg C. The additions of 800 µg glucose-C were equivalent to 460% of microbial-C in Forest 1 soils, 119% in Forest 2, 169% in Grassland 1 and 146% in Grassland 2, respectively. (Figure 3.3).

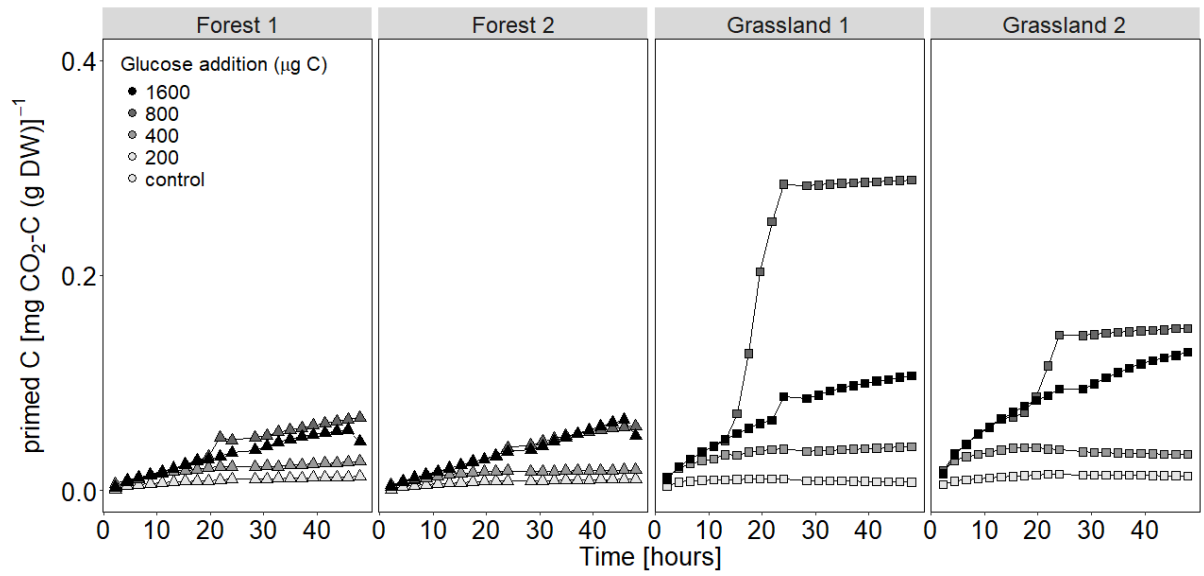


Figure 3.2 Cumulative primed CO₂ during 48 hours of incubation with different glucose concentration addition in 2 forest and 2 grassland plots in Hainich-Dün (HAI).

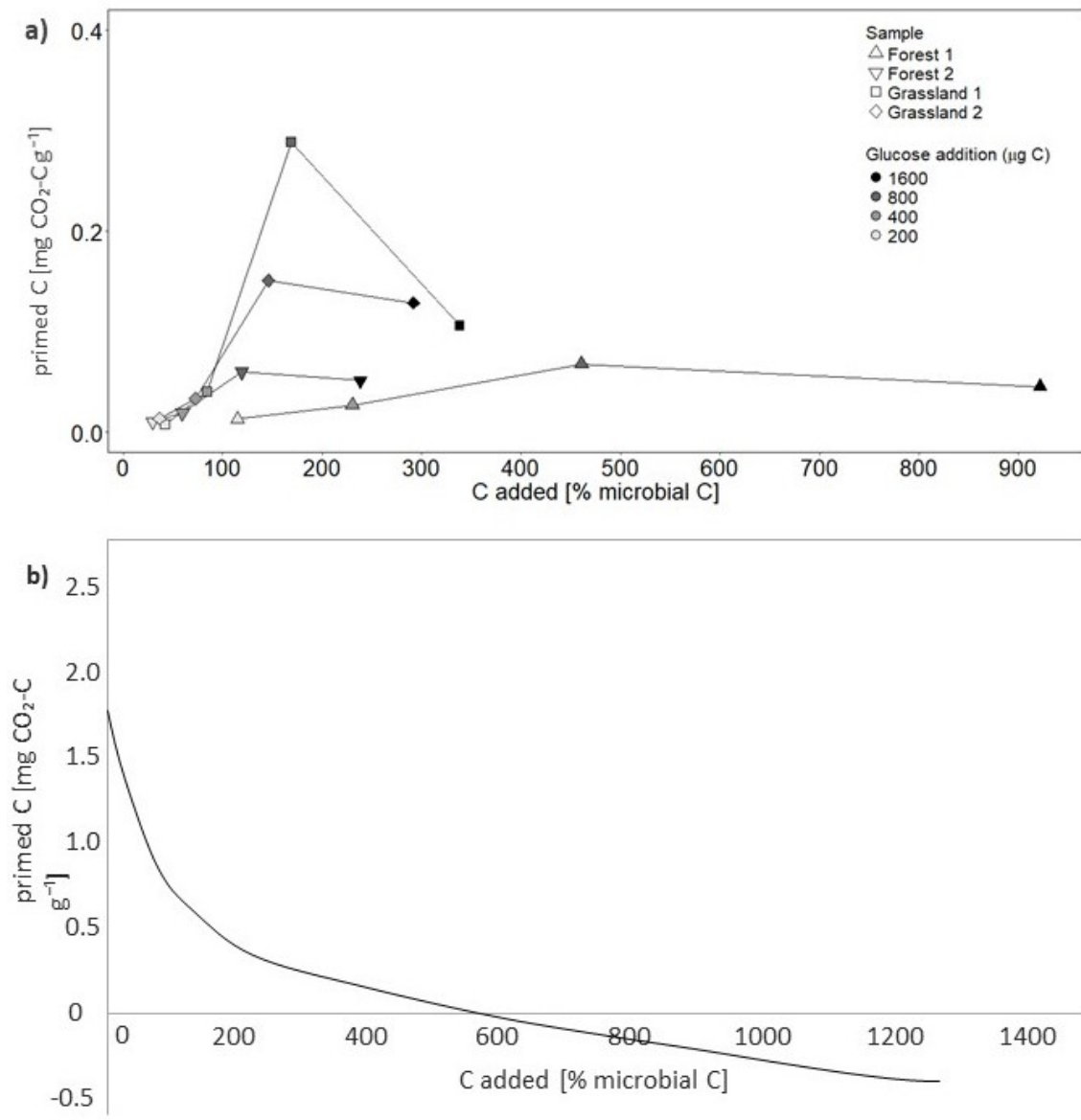


Figure 3.3 a) Cumulative primed CO₂ during 48 hours of incubation with different glucose concentration addition in 2 forest and 2 grassland plots versus C concentrations added per microbial C in Hainich-Dün (HAI) and b) primed CO₂ with different glucose concentration added as % microbial C as reported in a comprehensive review by Blagodatskaya and Kuzyakov (2008) (plot reproduced).

3.4 Discussion

3.4.1 Different CO₂ respiration rates between grasslands and forests following glucose additions

From all soils, total CO₂ respiration with glucose addition was higher as compared to control soils with only water addition after 48 hours of incubation, but the rates of response differed markedly between forests and grasslands (Table 3.2; Figure 3.1). Higher total CO₂ respiration due to C-substrates has been observed by Bastida et al. (2013) and Brant et al. (2006), while higher CO₂ respiration with increasing glucose concentrations in all samples has been reported by Guenet et al. (2010) and Lai et al. (2013). As fresh organic matter input supports rapid growth of microbial population, this explains the positive relationship between CO₂ respiration and the concentration of C input (Stotzky and Norman 1961). In forest soils, initially similar rates of CO₂ respiration following addition of different amounts of glucose-C were also reported by Stotzky and Norman (1961) and Anderson and Domsch (1985). With lag times that increased with the amount of added glucose, distinct maxima in forest soil respiration rates developed. In contrast, the first measurements of respiration rates after glucose addition in grasslands already differed with the amount of added glucose.

The explanation for these different temporal responses could be due to different amount of time for different total microbial biomass-C pool size or existing microbial activities to respond. In this study, the evidence of overall lower microbial activity in forests than grasslands were shown from the much lower respiration rates in control incubations in forests compared to grasslands as shown in Table 4.4 (Chapter 4). Even though higher microbial biomass-C concentration was observed in Forest 2 compared to grassland soils (Table 1), previous studies on the same regions have reported lower average concentrations of microbial biomass-C in forests compared to grasslands (Solly et al. 2014). In forest soils, where microbial biomass-C was lower than in grasslands, approximately ten hours were required in this study to increase microbial population to utilize glucose added (Stotzky and Norman 1961). In grassland soils, the absence of this lag phase may indicate higher existing microbial biomass and activities, and that the increase in different initial respiration rates showed C limitation on microbial respiration (Stotzky and Norman 1961). Higher existing microbial activities in grasslands than in forests may also be due to higher soil pH compared to the

forest soils (grasslands soil pH = 6.8 and 6.5, forest soil pH = 4.9 and 4.7, Table 1) which may retard microbial functions such as extracellular enzyme activities as shown by lower extracellular enzyme activities in Chapter 4 (Figure 4.3) and previous study (Herold et al. 2014b), or that the differences in CO₂ respiration kinetics reflected different microbial community composition between forests and grasslands related to differences in soil pH values (Nacke et al. 2011).

3.4.2 The intensity of soil priming due to different glucose concentrations

In all soil samples, the addition of all different glucose concentrations resulted in positive priming (Figure 2, Table 2). This result did not agree with the meta-analysis by Blagodatskaya and Kuzyakov (2008) which reported that the addition of SOM exceeding 50% of microbial C results in a decrease of priming activities, or even in negative priming. In this study, glucose concentrations added per microbial C all showed positive priming although they mostly exceeded this 50% limit. One explanation could be that the meta-analysis conducted by Blagodatskaya and Kuzyakov (2008) was based on studies that measured respiration responses over a wide range of time scales, ranging from hourly measurements to field conditions monitored over 19 months.

In this study, the amount of CO₂ released from priming increased with glucose concentration only up to the addition of 800 µg C (Figure 3, Table 2), then declined, though still indicating positive priming at 1600 µg C addition. In addition, the absolute amount of primed C as a function of glucose addition depended on observation time, i.e. more than C concentration per microbial C is needed to predict the amount of priming. One reason why adding 1600 µg C resulted in lower total priming compared to 800 µg may be that the C saturation was reached and microbes switched to mainly metabolizing the more recent and labile C, a process previously termed as “preferential substrate utilization” (Blagodatskaya et al. 2011), and that the observation time was insufficient to capture the same priming intensity compared to the addition of lower glucose concentration. Another explanation may be that the higher glucose concentration resulted in microbes do not utilize SOC to outcompete microbial groups specializing in SOC decomposition (Fontaine et al. 2004, Bradford et al. 2008), even though this was not tested in this study.

3.5 Conclusion

In this study, positive priming was observed during 48 hours of incubation following the addition of various amounts of glucose, equivalent to 30-920% of the soil microbial C. Although previous studies suggested that the addition of fresh organic matter exceeding 50% of microbial C results in a decrease of priming activities or in negative priming, we observed positive priming in all treatments and soils. The highest cumulative priming in both forest and grassland soils was observed after 48 hours with the addition of 800 ug glucose-C; this amounted to 119 - 460% of soil microbial C. This study showed that the absolute amount of C input affects timing of response and the total amount of priming observed, and that priming was not a function of fresh organic matter input per microbial C alone. A major issue was due to differences in timing of response for different soils and amounts of C substrate addition: a certain incubation time that describes CO₂ released from priming for one amount of input of C substrate may not be sufficient to capture the same amount of priming, which might take less time with the addition of higher substrate concentrations. While it is agreed that substrate concentration used to study soil priming should always be considered and based on the size of microbial C pool to predict the type of outcome (microbial turnover or soil organic matter decomposition), it is also suggested that the relationship between the amount of labile C added per microbial C and total CO₂ respired from priming should be interpreted with caution regarding the length of observation time.

Chapter 4

Factors controlling substrate respiration and soil priming effect under different land use and management

Chapter source: Gan et al., 2018. Factors controlling substrate respiration and soil priming effect under different land use and management. (manuscript ready for resubmission)

Abstract

Soil priming effect has been widely studied, but the effects of land use and management across large spatial scale has not been investigated in a single study. Under different land use and management, mineral soils receiving different qualities of aboveground litter, with different acidity level and nutrient availability affect belowground energy limitation is hypothesized to have different effects on the respiration rates of labile C and soil priming. While most published studies on soil priming was performed through longer incubation periods to quantify priming intensity, this study focused on short term incubation to study the dynamics of labile C respiration, and the effects of land use and soil abiotic properties on soil priming at the ecosystem level.

Topsoils from 60 sites under different land use and management across three regions in Germany were studied: Schwäbische-Alb (ALB), Hainich-Dün (HAI) and Schorfheide-Chorin (SCH). Short laboratory incubations (48 hours) with ^{13}C labelled glucose coupled with the analyses of β -glucosidase, N-acetyl-glucosaminidase, sulfatase, and phosphatase activities were carried out to study if soil priming is driven by nutrient mining.

Across different study regions, respiration rates of the added glucose after normalization with organic C content were observed to be significantly higher ($P < 0.05$) in forests than grasslands. This was linked to significant increase in phosphatase activities in all forest soils, suggesting that glucose addition stimulated higher microbial growth and P acquisition in forests than grasslands. Higher soil pH and C:N enhanced microbial activities and priming

effect across different regions and land use types. This shows that factors that constrain microbial activities in soil were also the most important factors that control priming.

Keywords Soil C priming, microbial nutrient mining, land use, extracellular enzyme activities, stable isotope, tree species effect, fertilization effect

4.1 Introduction

Soil priming is an increase (positive priming) or decrease (negative priming) of soil organic matter (SOM) decomposition in response to the input of labile C sources (Kuzyakov et al. 2000a). This process occurs in microbial hotspots with high C input. It has been described in the rhizosphere (Dijkstra and Cheng 2007, Bengtson et al. 2012, de Graaff et al. 2014), the detritosphere (de Neergaard and Magid 2015), and the drilosphere (Bityutskii et al. 2012, Eck et al. 2015). Identifying factors that control priming is crucial to predict the response of large soil C stocks to future vegetation or climate change.

Even though soil priming has been widely studied, only small number of studies focused on the effect of land use and management (Bell et al. 2003, Bassem et al. 2014, Mazzilli et al. 2014, Diochon et al. 2016) and different soil abiotic properties on soil priming (Hamer and Marschner 2005, Frøseth and Bleken 2015, Tian et al. 2016). To the best knowledge of this thesis, the effects of land use and management across large spatial scale with different soil abiotic properties on soil priming has not been investigated in a single study. Previous studies on soil priming usually focused only on a few selected sites that were insufficient to use advanced statistical analysis to try and tease apart complex relationships between soil properties and site history or management.

Land use is an important driver of belowground organic C (OC) and nutrient availabilities. In managed forests, the selection of tree species affects the understory cover species, quality and quantity of litter, as well as root morphology and exudation (Augusto et al. 2003). Forest management practices in Central Europe such as age management and selective logging as well as unmanaged forests has been shown to significantly affect microbial community successions via its effect on leaf litter quality, soil macronutrients and soil pH (Purahong et al. 2015). A meta-analysis by Berg and Meentemeyer (2002) showed that while climatic factor played an important role in foliar decomposition in the initial stage, foliar decomposition was

strongly affected by foliar N concentration and C:N ratio at the later stage of decomposition, and this was controlled by tree species. In grasslands, the addition of N from fertilization has been linked to an increase in SOM and β -glucosidase activities due to increased plant productivity (Cenini et al. 2016). The importance of land use and management as an important control of soil nutrient status on soil priming was confirmed by previous studies which showed that soil priming occurred due to co-metabolism of SOM to access limiting nutrients (Fontaine et al. 2011). Increases in β -glucosidase and phenol oxidase activities followed by an increase in enzymes that catalyze phosphorus (P) and nitrogen (N), such as phosphomonoesterase and leucine amino peptidase, can provide a proxy for microbial nutrient mining (Nottingham et al. 2012). Across different study regions, soil abiotic properties such soil pH and clay mineralogy affect the sorption of dissolved organic matter and reduce its accessibility to soil microbes (Nyborg and Hoyt 1978, Hinsinger 2001, Herold et al. 2014a), and thus potentially influence soil priming. Most published results focused on quantifying long term priming intensity and the change in soil C stock ranged from days to annual scale (Chen et al. 2014, Chowdhury et al. 2014, Fontaine et al. 2011, Nottingham et al. 2012, Zhang et al. 2017), but none has focused on the short-term dynamics of labile C respiration.

The aim of this study was to determine the short-term dynamics of labile C respiration and soil priming under different land use type (forest versus grassland) and management (tree species in forest and fertilization in grassland). At the same time, this study considered the potential effects of soil properties on priming. It is hypothesized that priming of SOM would be related to the need for nutrient mining and therefore greatest in soils with lower plant litter quality and nutrient status. Therefore, priming is predicted to be (1) higher in forest compared to grassland soils, (2) higher under coniferous forest than under deciduous forest (3) higher in unfertilized than fertilized grasslands. To test these hypotheses, C priming of SOM in topsoil (0-10 cm) from 60 sites under different land use and management in three regions in Germany was studied and important soil properties that could be relevant for priming were considered. To assess priming, ^{13}C labelled glucose was added to mineral soil (0-10 cm) and compared respired $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ in short term laboratory incubations to that of unamended soils. By analysing soil extracellular enzyme activities at the end of incubations, the degree to which priming occurred due to microbial nutrient mining when C is not limiting was assessed.

4.2 Method

4.2.1 Study site and soil sampling

The study sites belong to the Biodiversity Exploratories project (www.biodiversity-exploratories.de), a large interdisciplinary study of the effect of grassland and forest management on biodiversity and ecosystem functions. This study is conducted in three regions in Germany: (i) UNESCO Biosphere Reserve “Schwäbische Alb” (ALB), (ii) “Hainich-Dün National Park” and its surroundings (HAI), and (iii) UNESCO Biosphere Reserve “Schorfheide-Chorin” (SCH). These regions differ in climate and soil parent materials, and accordingly have different dominant soil types and properties (Fischer et al. 2010). Soils in the ALB region developed mainly on Jurassic limestone and were clay-rich Cambisols or Leptosols (WRB 2014). Soils in forests and grasslands of the HAI region had loamy or clayey texture due to the dominant geological substrate of loess over limestone. The main HAI soil types were Luvisols, Stagnosols, Vertisols or Cambisols. In the SCH forests, soils were classified as Cambisols and had a texture of sandy loam to pure sand as the geological substrate was glacial till covered by glacio-fluvial or aeolian sand. The grassland soils in the SCH were drained Histosols characterized by high OC contents and a loamy texture. Twenty plots were selected from each region, 10 forest and 10 grassland sites, with a total of 60 study sites. In each region, half of the forest sites were dominated by deciduous stands (ALB = 5 plots *Fagus sylvatica*, HAI = 6 plots *Fagus sylvatica*, SCH = 3 *Fagus sylvatica*, 2 *Quercus robur*) and another half were dominated by coniferous stands (ALB = 5 plots *Picea abies*, HAI = 4 plots *Picea abies*, SCH = 5 *Pinus sylvestris*). Of the studied grassland sites, five were hay pasture or meadow fertilized with livestock manure or slurry, while the other five were unfertilized hay pasture, pasture or meadow. On fertilized plots, an average amount of 283.8 ± 105.2 , 281.9 ± 111.3 and 391.2 ± 100.4 kg N ha⁻¹ had been applied from 2006-2014 in ALB, HAI and SCH respectively.

Soil samples were collected in May 2014. A total of 14 soil cores were sampled along two 40 m transects in forests and two 20 m transects in grasslands and mixed to form composite samples. Before sampling the mineral soil at forest sites, organic layers were removed. In grasslands, aboveground plants were cleared by cutting. Split tubes with a diameter of 4.8 cm were then driven manually into the ground to sample the upper mineral soil (0-10 cm).

Samples from all 14 soil cores were subsequently mixed to form one representative composite sample per plot. Composite samples were split up into two sub-samples. Samples for physico-chemical analyses were air dried at 40 °C, while samples used for incubation and enzyme analysis were stored at 4 °C.

4.2.2 Soil abiotic properties

Gravimetric water content (dry basis of soil) was determined by drying 20 g soil at 105 °C until the weight of samples remained constant. Soil pH was determined with a pH electrode in a 1:2.5 supernatant of soil and 0.01 M CaCl₂. Total C (TC) and nitrogen (TN) (g kg⁻¹ soil) were determined by dry combustion (VarioMax, Elementar Analysensysteme GmbH, Hanau, Germany). Inorganic C was quantified after removing OC by ignition at 450 °C for 16 hours. Organic C was calculated as the difference between TC and inorganic C. Total sulfur (TS) (g kg⁻¹ soil) was determined after digestion of soil with aqua regia in a closed vessel. Aliquots of 1.5 g were extracted with 3.5 ml HNO₃ and 10.5 ml HCl and boiled for 3 hours at 96 °C. Inorganic S was extracted with 0.016 M KH₂PO₄ (Ensminger 1954) and 0.5 M NH₄F (Prietzl and Hirsch 2000) for more acidic soil in the SCH forests (pH = 3.5±0.1) and determined using Dionex IC-System DX-500 (ThermoFisher, Dreieich). Organic and inorganic P were extracted with 0.5 M NaHCO₃ (adjusted to pH 8.5) following the method of Olsen et al. (1954). Data on soil texture was adopted from Solly et al. (2014) and determined by wet sieving and sedimentation (pipette method).

4.2.3 Soil incubation

Soil samples were incubated for 48 hours at 20 °C in dark to examine the effect of glucose addition on soil respiration and C priming. One g of each soil sample was filled into 12 ml glass vials with PTFE/silicon septa and 800 µg glucose-C ($\delta^{13}\text{C} = 100\text{‰}$) dissolved in 100 µl deionized water was added. Pre-experiments showed that the addition of 800 µg glucose-C resulted in positive priming in both forests and grasslands, and the peak CO₂ efflux was observed in 48 hours. The amount of glucose-C addition was similar in relation to microbial C in grasslands and was about two times higher in forests. Additionally, control samples were prepared using the same volume of glucose-free deionized water. After glucose or water addition, the vials were flushed with CO₂-free synthetic gas (20.5 % O₂, 79.5 % N₂, Westfalen

AG, Germany) for 2 minutes at 10 psi. Subsequently, the samples were incubated on top of an elemental analyzer coupled to a gas chromatography isotope ratio mass spectrometer (GC-IRMS) (MS, Finnigan Delta S) via a variable open-split interface (Conflo II, all from Finnigan Mat, Bremen). Soil CO₂ efflux and δ¹³C values of each sample were determined every 2.2 hours for a total of 48 hours. Isotope ratios of CO₂ evolution were reported in the conventional δ-notation below:

$$\delta^{13}\text{C} = \left[\left(\frac{^{13}\text{Rsa}}{^{13}\text{Rref}} \right) - 1 \right] \times 1000$$

where Rsa and Rref were the ratios of ¹³C to ¹²C in samples and reference respectively. The δ values were expressed as per mill (‰) based on the Vienna Pee Dee Belemnite (VPDB) scale for ¹³C. To avoid CO₂ saturation during the incubation period, the vials were flushed again with CO₂-free synthetic gas after 24 hours as described above.

The abundance of ¹³C (100‰) in glucose solution was obtained by diluting labelled (D-Glucose-¹³C₆, 99 atom % ¹³C, Sigma-Aldrich GmbH Munich, Germany) and unlabelled glucose using the isotope balance equation below (Brand and Coplen 2012):

$$\delta^{13}\text{C}_{\text{glucose}} = \delta^{13}\text{C}_{\text{unlabelled}} \times x_{\text{unlabelled}} + \delta^{13}\text{C}_{\text{labelled}} \times x_{\text{labelled}}$$

where x is the mole fraction of elements in different glucose pools. δ¹³C_{glucose} refers to the target ¹³C abundance in the glucose substrate (100‰). δ¹³C_{unlabelled} and δ¹³C_{labelled} are the δ values of unlabelled glucose and labelled glucose respectively. The sum of x in all pools equals to 1.

CO₂ respired from glucose was quantified using the equation below (Nottingham et al. 2012):

$$\% \text{CO}_{2\text{glucose}} = \frac{\delta_c - \delta_T}{\delta_c - \delta_{\text{glucose}}} \times 100$$

where δ_c and δ_T are the δ¹³C values of respired CO₂ from control soils and soils with glucose addition respectively. Priming effect was calculated as the difference between CO₂ evolved from native soil OC after glucose addition (total CO₂ evolution of soil samples after glucose amendment minus glucose-derived CO₂) and CO₂ evolved from control samples without glucose addition using the equation below (Bastida et al. 2013):

$$\text{Priming effect} = \text{Total CO}_2(\text{soil with glucose}) - \text{CO}_2(\text{glucose}) - \text{Total CO}_2(\text{control})$$

Results of CO₂ efflux rates and priming are presented per g OC in the samples to account for the large differences in OC contents of samples, which strongly influence mineralization rates.

4.2.4 Enzyme analysis

Enzyme assays based on conjugates of the fluorescent compound 4-methylumbelliferone (MUF) were performed at the end of incubations according to the method of Marx et al. (2001). Four potential extracellular enzyme activities involved in different nutrient cycles were determined: (i) β -glucosidase (C cycle), (ii) N-acetyl-glucosaminidase (N cycle), (iii) sulfatase (S cycle) and (iv) acid phosphatase (P cycle). Three different solutions were prepared prior to analysis: (i) 0.1 M 2-[N-Morpholino] ethanesulfonic acid (MES) buffer solution (pH 6.1) for method standardization and stabilization of the fluorescence intensity of MUF, (ii) 10 μ M MUF working solution and (iii) 0.01 M working solutions of each MUF-substrate. Soil aggregates were broken down by dispersing 1 g of incubated soil in 50 ml of sterile deionized water using an ultrasonic disaggregator with low energy input (60 Jml⁻¹). Supernatants were continuously stirred while 50 μ l aliquots were transferred to a 96-well micro titer plate using a multi-channel pipette. Subsequently, 50 μ l of autoclaved MES buffer and 100 μ l of substrate working solution were transferred to each well. For the standard plate without substrate addition, different volumes of MES buffer and MUF working solution were added in addition to 50 μ l of soil suspension to achieve MUF concentrations of 0, 100, 200, 500, 800 and 1200 pmol/well for calibration. All solutions except the soil suspensions were transferred using a pipetting robot equipped with software for automated pipetting (Freedom EVO 75, Tecan Trading AG, Switzerland). Micro titer plates were incubated in dark at 30 °C while shaken at 300 rpm using an orbital shaker. Fluorescence intensity was measured after 30, 60, 120 and 180 minutes with 360 nm excitation and 460 nm emissions using a micro plate reader (Infinite 200, Tecan Trading AG, Switzerland). The first 30 minute served as pre-incubation of the micro titer plates. Potential soil enzyme activities were quantified through comparison with the standard concentration of MUF substrates. Enzyme activities were linearly related to the intensity of fluorescence and were expressed as

nmol MUF g⁻¹ dry weight (DW) soil h⁻¹. Specific enzyme activities were calculated by normalization to OC contents (nmol MUF g⁻¹ OC h⁻¹).

4.2.5 Statistics

All statistical analyses were conducted in R version 3.3.2 (Team 2016). Results for CO₂ efflux, enzyme activities and soil abiotic properties were presented as means ± standard error ($P < 0.05$) unless stated otherwise. To see if the magnitudes of priming and extracellular enzyme analysis were statistically different among land use types and management, Analysis of Variance (ANOVA) with the function “aov” was used. Prior to ANOVA analysis, normality and homogeneity of residual variances were checked using the (i) Residuals versus Fitted plot, (ii) normal Q-Q plot, (iii) Scale-Location plot and the (iv) Residuals versus Leverage plot. Data that did not fulfill the conditions for normality and homogeneity of residual variances were log transformed. Principle component analysis (PCA) was performed with the function “prcomp” to identify the correlations between C priming (mg CO₂-C g⁻¹ OC) and soil abiotic properties, and differences in extracellular enzyme activities between control and glucose treated samples. From PCA biplot, factors that showed important correlations with priming magnitude were selected for three analysis of covariance (ANCOVA) using the “aov” function in R: a) the effect of both forest and grassland land use types were tested, b) tree species effect in forests and c) fertilization effect in grasslands.

4.3 Results

4.3.1 Soil abiotic properties and nutrient concentrations

Soil abiotic properties differed between forests and grasslands as well as among ALB, HAI and SCH study region (Table 1, Table 2). Soil type and texture at the study sites were shown in Table 1. Of all three study sites, mineral soils in SCH had the highest sand content and was significantly higher in the Cambisols (forest sites) than Histosols (grassland sites). Mineral soils in ALB and HAI on the other hand contained similar concentrations of sand. Silt and clay concentrations were similar in ALB, HAI and in the SCH Histosols, and was the lowest in SCH Cambisols. Soil pH was on average more acidic in forests (pH 4.4 ± 0.1) than grasslands (pH 6.6 ± 0.1). There was no consistent trend for soil acidity for forests with

different tree species, or between fertilized and unfertilized grasslands except in SCH. Grassland sites in SCH which were established on degraded peatlands contained high OC, total N and inorganic S concentration compared to other sites.

In all study regions, organic carbon-to-total nitrogen ratios (C:N ratios) were on average higher in forests than grasslands and were higher in coniferous than deciduous forests. Inorganic P concentration was on average higher in all grassland than forest sites, while organic P concentration was higher in forests. There was no clear trend for S concentration according to land use types. Phosphorus and S concentration were similar among all forest sites with different tree species except for ALB, where P concentrations were higher in coniferous than deciduous forests. Contrary to expectation, fertilization of grassland soils did not result in higher OC and total N concentrations in any of the three study regions. Only P concentrations (both organic and inorganic) were on average higher in fertilized than in unfertilized grassland sites.

Table 4.1 Soil type and texture at forest and grassland sites in the study regions. All values are shown as means±standard error. Two-way ANOVA results are presented with Tukey HSD test ($P<0.05$). Significant differences for soil properties between study regions are indicated by capital letters while differences between land uses within the same region are indicated by lowercase letters.

Study region	Land use	Management	Soil types (WRB)	Sand [g kg ⁻¹]	Silt [g kg ⁻¹]	Clay [g kg ⁻¹]
Schwäbische Alb	Forest	Coniferous	Cambisols/Leptosols	36.2±5.1 ^{Aa}	539.0±60.1 ^{BCa}	424.8±59.4 ^{BCa}
		Deciduous	Cambisols	40.2±13.5 ^{Aa}	473.6±50.0 ^{BCa}	486.4±53.9 ^{Ca}
	Grassland	Fertilized	Cambisols	93.0±28.9 ^{Aa}	338.2±45.9 ^{ABa}	568.8±39.8 ^{Ca}
		Unfertilized	Cambisols/Leptosols	58.0±15.0 ^{Aa}	419.2±83.8 ^{BCa}	522.8±98.0 ^{Ca}
Hainich-Dün	Forest	Coniferous	Luvisols/ Stagnosols	63.5±11.3 ^{Aa}	582.7±61.1 ^{BCa}	355.5±67.5 ^{BCa}
		Deciduous	Luvisols/ Stagnosols	65.0±6.7 ^{Aa}	585.3±29.6 ^{BCa}	351.0±34.8 ^{BCa}
	Grassland	Fertilized	Cambisols/Vertisols	57.0±13.1 ^{Aa}	630.8±94.2 ^{Ca}	312.4±104.1 ^{ABCa}
		Unfertilized	Cambisols/Stagnosols	55.6±7.2 ^{Aa}	564.6±73.1 ^{BCa}	381.2±77.8 ^{BCa}
Schorfheide-Chorin	Forest	Coniferous	Cambisols	858.6±28.7 ^{Cb}	102.8±24.0 ^{Aa}	38.6±5.2 ^{Aa}
		Deciduous	Cambisols	883.2±24.5 ^{Cb}	75.4±19.9 ^{Aa}	42.2±7.4 ^{Aa}
	Grassland	Fertilized	Histosols	300.4±6.9 ^{Ba}	531.4±21.8 ^{BCc}	168.2±25.0 ^{ABb}
		Unfertilized	Histosols	276.8±86.0 ^{Ba}	388.2±51.9 ^{BCb}	335.0±47.1 ^{BCc}

WRB= World Reference Base

Table 4.2 Soil management, pH and nutrient concentrations at forest and grassland sites under different managements in the study regions. All values are shown as means±standard error. Two-way ANOVA results are presented with Tukey HSD test ($P<0.05$). Significant differences between study regions are indicated by capital letters while differences between land uses within the same region are indicated by lowercase letters.

Study region	Land use	Management	pH	Organic C [g kg ⁻¹]	Total N [g kg ⁻¹]	C:N ratio	Inorganic P [g kg ⁻¹]	Organic P [g kg ⁻¹]	Total S [g kg ⁻¹]	Inorganic S [mg kg ⁻¹]
Schwäbische Alb	Forest	Coniferous	4.2±0.4 ^{Aa}	61.3±4.4 ^{BCb}	4.3±0.3 ^{ABCb}	14.2±0.5 ^{Ca}	9.0±2.7 ^{ABb}	50.8±19.2 ^{Bb}	0.5±0.0 ^{ABCa}	39.6±4.8 ^{Ab}
		Deciduous	5.6±0.3 ^{Bb}	52.7±4.6 ^{ABCa}	3.9±0.2 ^{ABCa}	13.3±0.4 ^{Ca}	3.7±0.5 ^{Aa}	13.4±2.2 ^{Aa}	0.5±0.0 ^{ABCa}	31.3±6.4 ^{Aa}
	Grassland	Fertilized	6.0±0.2 ^{BCa}	56.5±4.3 ^{ABCa}	5.5±0.4 ^{BCa}	10.2±0.2 ^{ABa}	26.9±12.0 ^{ABb}	14.2±3.1 ^{Aa}	0.7±0.0 ^{BCa}	34.1±7.6 ^{Aa}
		Unfertilized	6.0±0.3 ^{BCa}	64.2±4.2 ^{Cb}	6.5±0.5 ^{Cb}	10.0±0.3 ^{Aa}	13.9±3.1 ^{ABa}	16.6±4.0 ^{Ab}	0.9±0.1 ^{Cb}	40.1±3 ^{Ab}
Hainich-Dün	Forest	Coniferous	5.7±0.4 ^{Bb}	51.9±2.4 ^{ABCb}	3.6±0.3 ^{ABCb}	14.6±0.9 ^{CDb}	6.0±0.6 ^{ABa}	12.4±3.7 ^{Aa}	0.5±0.0 ^{ABCb}	36.0±2.8 ^{Aa}
		Deciduous	4.3±0.1 ^{Aa}	33.9±1.9 ^{ABCa}	2.7±0.2 ^{ABa}	12.8±0.4 ^{Ca}	5.7±0.4 ^{Aa}	15.7±2.0 ^{Ab}	0.3±0.0 ^{ABa}	34.8±2.4 ^{Aa}
	Grassland	Fertilized	7.0±0.1 ^{CDa}	34.8±4.9 ^{ABCa}	3.5±0.5 ^{ABCa}	9.9±0.2 ^{Aa}	9.0±1.7 ^{ABb}	7.6±1.1 ^{Aa}	0.5±0.1 ^{ABCb}	12.4±2.2 ^{Aa}
		Unfertilized	7.0±0.1 ^{CDa}	44.9±7 ^{ABCb}	4.4±0.7 ^{BCb}	10.2±0.2 ^{ABa}	6.6±0.9 ^{Aa}	6.1±1.2 ^{Aa}	0.3±0.1 ^{ABa}	16.4±2.4 ^{Ab}
Schorfheide-Chorin	Forest	Coniferous	3.5±0.1 ^{Aa}	23.1±2 ^{ABb}	1.2±0.1 ^{Aa}	19.3±0.8 ^{Eb}	22.1±8.9 ^{ABa}	32.7±3.6 ^{ABa}	0.1±0.0 ^{Aa}	15.6±0.9 ^{Ab}
		Deciduous	3.6±.01 ^{Aa}	20.0±2 ^{Aa}	1.2±0.1 ^{Aa}	17.0±0.7 ^{DEa}	23.1±4.9 ^{ABa}	33.7±3.6 ^{ABa}	0.1±0.0 ^{Aa}	13.6±2.1 ^{Aa}
	Grassland	Fertilized	7.2±0.0 ^{Db}	134.9±18.4 ^{Da}	13.1±1.7 ^{Da}	10.3±0.1 ^{ABa}	32.8±5.6 ^{Bb}	19.2±2.5 ^{Ab}	1.8±0.2 ^{Da}	305.9±70.9 ^{ABa}
		Unfertilized	6.4±0.3 ^{BCDa}	244.7±17.6 ^{Eb}	19.5±0.9 ^{Eb}	12.6±0.8 ^{BCb}	23.6±9.6 ^{ABa}	13.8±4.6 ^{Aa}	3.4±0.3 ^{Eb}	614.5±371.2 ^{Bb}

4.3.2 Glucose respiration, primed CO₂ and C balance

Total CO₂ respired per g OC was significantly higher ($P<0.05$) for soils treated with glucose compared to controls and was on average higher in forests than in grasslands in all regions (Table 3). For control soils without glucose addition, total CO₂ respired per g OC was not significantly different between land use types. Compared to other sites, SCH forests showed the highest total CO₂-C per g OC after 48 hours of incubation (15.5 ± 1.2 mg) while SCH grasslands showed the lowest CO₂-C per g OC (3.7 ± 0.4 mg). The maximum CO₂ efflux per hour occurred earlier in grassland soils after 2, 9 and 13 hours and only after 24, 28 and 30 hours for the forest soils in ALB, HAI and SCH respectively (Figure 1). Glucose respired was indicated by the more positive values of $\delta^{13}\text{C}$ in the respired CO₂ after glucose addition (Table 3). For soils without glucose addition, mean $\delta^{13}\text{C}$ values did not differ between grassland ($-23\pm 0.6\text{‰}$) and forest ($-25\pm 0.4\text{‰}$) sites across regions. With glucose addition, the highest mean $\delta^{13}\text{C}$ values in forests observed was $87.4\pm 0.7\text{‰}$, and $45.1\pm 1.6\text{‰}$ in grasslands.

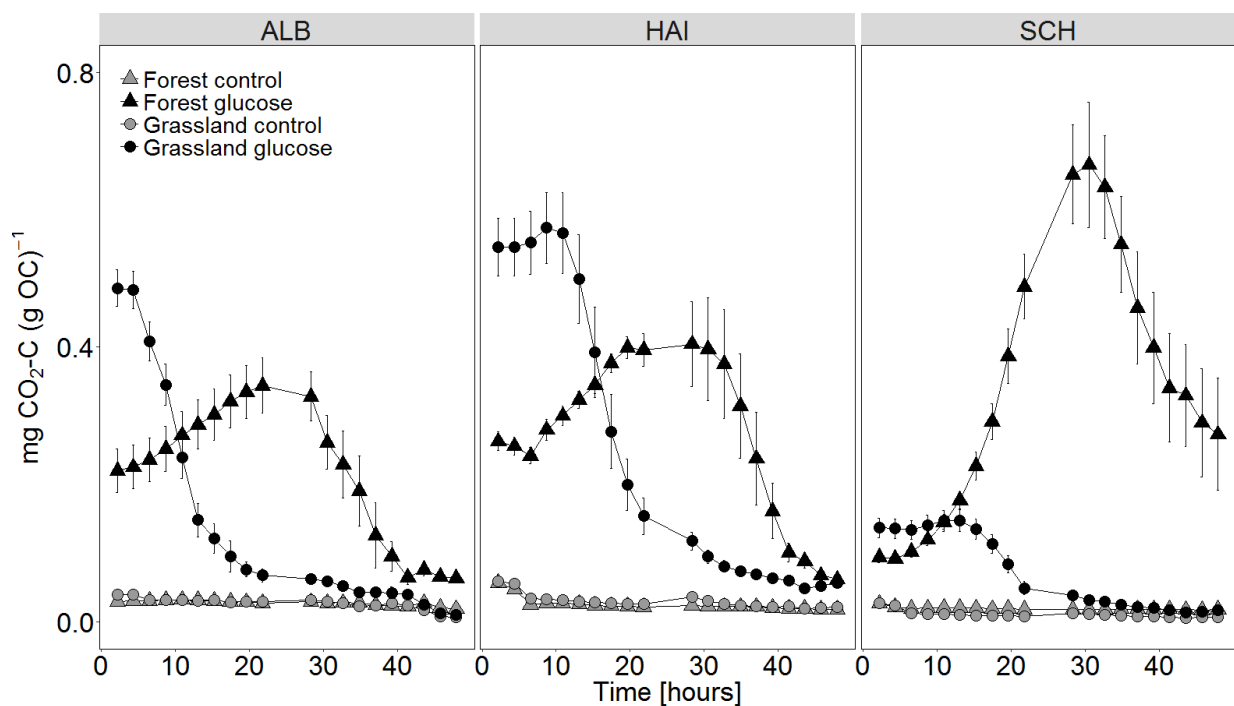


Figure 4.1 CO₂ efflux over time during 48 hours of incubation following glucose addition in grassland and forest plots in i) Schwäbische-Alb (ALB), ii) Hainich-Dün (HAI) and iii) Schorfheide-Chorin (SCH). Data is presented as mean \pm SE. Each data point represents 10 replicates.

In all study regions, the proportion of added glucose-C respired in 48 hours was higher in forests compared to grasslands. Positive priming was observed in both forests and grasslands in all regions (Figure 2a). In ALB and SCH, total C priming normalized to OC was not significantly different between land use types. In HAI, however, priming was significantly higher ($P<0.05$) in grasslands (2.2 ± 0.3 mg CO₂-C g⁻¹ OC) than in forests (1.4 ± 0.1 mg CO₂-C g⁻¹ OC) (Table 4). Within forest sites, priming was significantly higher ($P<0.05$) in deciduous than in coniferous forest in ALB (Table 3). Within grasslands, priming was observed to be higher in fertilized sites in all regions, which was statistically significant in HAI and SCH ($P<0.05$). Carbon balance for all sites after 48 hours of incubation is shown in Table 4. Total native OC loss through priming was in the same order of magnitude or even higher than CO₂ flux of the control samples. On average, losses of native soil OC due to priming were only 2-30% of the amount of CO₂ respired from added glucose, so that there was a net C gain after 48 hours in 48 hours of incubation.

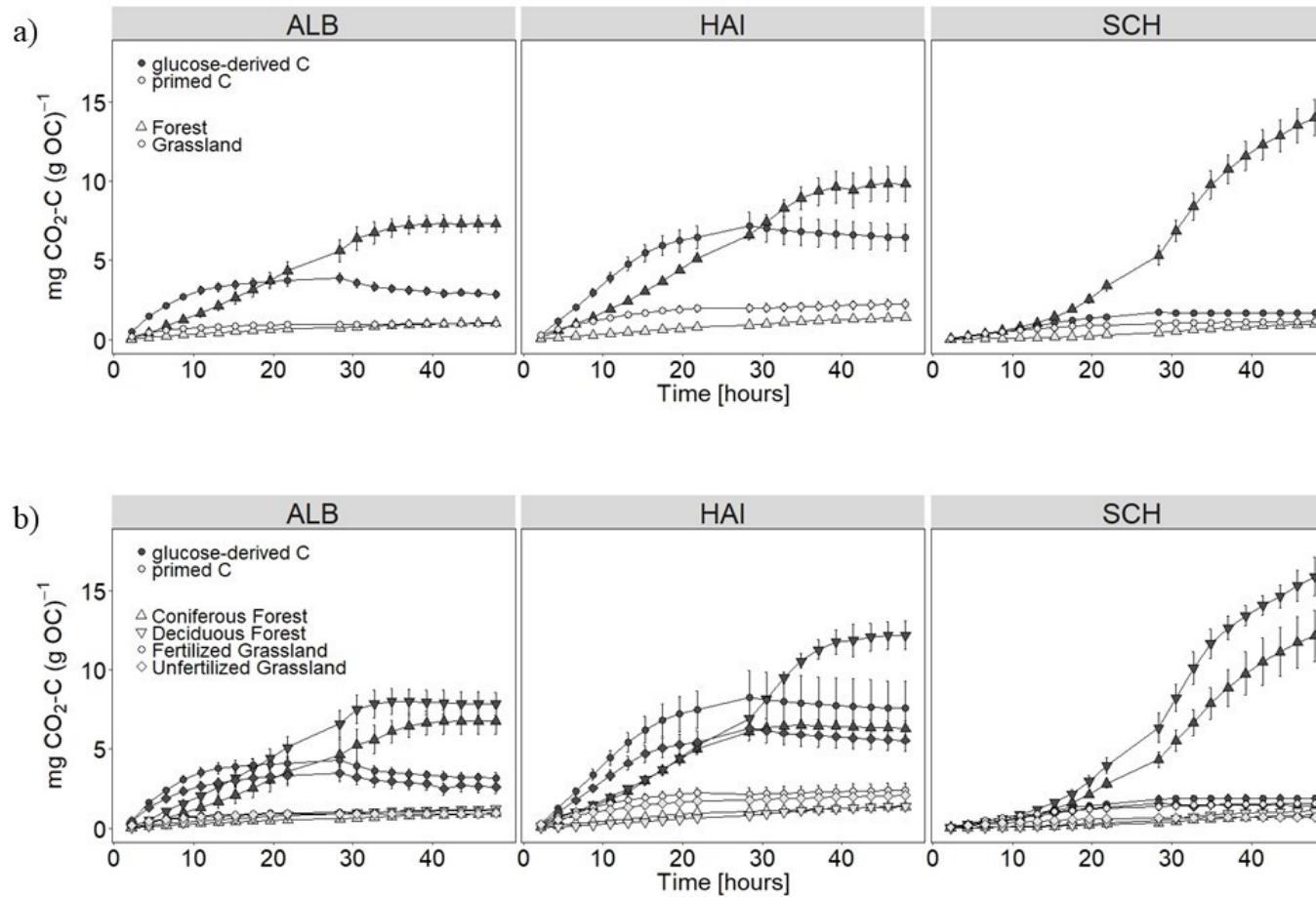


Figure 4.2 CO₂ efflux over time during 48 hours of incubation following glucose addition in a) grassland and forest plots and b) under different management in i) Schwäbische-Alb (ALB), ii) Hainich-Dün (HAI) and iii) Schorfheide-Chorin (SCH). Data is presented as mean±SE, each data point represents 10 replicates.

Table 4.3 Means±standard error of total CO₂, δ¹³C of CO₂ efflux, glucose-derived CO₂ and primed CO₂ in forest and grassland in the study regions. Two-way ANOVA results are presented with Tukey HSD test (*P*<0.05). Significant differences between study regions are indicated by capital letters while differences between land uses within the same region are indicated by lowercase letters.

Study region	Land use	Treatment	n	Total CO ₂ (mg CO ₂ -C g ⁻¹ OC)	δ ¹³ C (‰)	Glucose-derived CO ₂ (%)	Primed CO ₂ (mg CO ₂ -C g ⁻¹ OC)
Schwäbische-	Forest	Glucose	10	10.1±0.7 ^{Db}	65.3±3.8 ^{Db}	72.3±3.1 ^{Cb}	1.1±0.1 ^{Aa}
		Control	10	1.3±0.1 ^{ABa}	-25.6±0.6 ^{Aa}	-	-
Alb	Grassland	Glucose	10	6.6±1.4 ^{Cb}	26.3±1.5 ^{Bb}	42.1±1.0 ^{Aa}	1.0±0.1 ^{Aa}
		Control	10	1.4±0.3 ^{ABa}	-26.9±0.6 ^{Aa}	-	-
Hainich-	Forest	Glucose	10	12.6±0.9 ^{Db}	69.8±4.7 ^{Db}	75.7±3.9 ^{Cb}	1.4±0.1 ^{Aa}
		Control	10	1.1±0.1 ^{ABa}	-25.2±0.8 ^{Aa}	-	-
Dün	Grassland	Glucose	10	11.5±1.2 ^{Db}	45.1±1.6 ^{Cb}	55.2±1.2 ^{Ba}	2.3±0.3 ^{Bb}
		Control	10	1.7±0.2 ^{ABa}	-22.5±0.9 ^{Aa}	-	-
Schorfheide-	Forest	Glucose	10	15.5±1.2 ^{Eb}	87.4±0.7 ^{Eb}	89.9±0.5 ^{Db}	1.0±0.1 ^{Aa}
		Control	10	0.8±0.1 ^{ABa}	-24.9±0.5 ^{Aa}	-	-
Chorin	Grassland	Glucose	10	3.7±0.4 ^{Bb}	33.1±1.4 ^{Bb}	45.2±1.5 ^{Aa}	1.1±0.2 ^{Aa}
		Control	10	0.7±0.1 ^{Aa}	-22.2±1.0 ^{Aa}	-	-

Table 4.4 Means±standard error of C balance in forest and grassland in the study regions. C-balance is calculated by subtracting the sum of C in each sample (sum of organic C and glucose-C) with CO₂-C control, CO₂-C glucose and primed CO₂-C. Significant differences between study regions are indicated by capital letters while differences between land uses within the same region are indicated by lowercase letters.

Study region	Land use	n	Organic C (mg g ⁻¹ DW soil)	Glucose-C addition	CO ₂ -C total	CO ₂ -C control	CO ₂ -C glucose	Primed CO ₂ -C	C-balance
				(μg g ⁻¹ DW soil)					
Schwäbische-Alb	Forest	10	57.0±3.3 ^{Aa}	800	556.6±11.5 ^{DEb}	72.5±5.3 ^{Ba}	401.3±15.2 ^{Cb}	82.8±14.7 ^{Ba}	57.2±3.3 ^{Aa}
	Grassland	10	60.3±3.1 ^{Aa}	800	392.2±26.9 ^{ABa}	84.5±7.2 ^{Ba}	166.4±13.8 ^{Aa}	142.5±11.6 ^{Cb}	60.2±3.4 ^{Aa}
Hainich-Dün	Forest	10	41.1±3.3 ^{Aa}	800	494.8±8.6 ^{CDb}	49.0±7.4 ^{ABa}	372.8±16.2 ^{Cb}	73.0±14.4 ^{Ba}	41.4±3.3 ^{Aa}
	Grassland	10	39.8±4.3 ^{Aa}	800	420.4±4.3 ^{BCa}	66.3±8.1 ^{Ba}	231.8±6.3 ^{ABa}	124.2±6.0 ^{BCb}	40.9±4.8 ^{Aa}
Schorfheide-Chorin	Forest	10	21.5±1.4 ^{Aa}	800	322.0±16.0 ^{Aa}	18.5±1.9 ^{Aa}	289.4±14.0 ^{Ba}	14.1±1.4 ^{Aa}	22.0±1.4 ^{Aa}
	Grassland	10	189.8±21.9 ^{Bb}	800	636.6±41.3 ^{Eb}	129.3±17.5 ^{Cb}	287.3±20.3 ^{Ba}	220.1±17.5 ^{Db}	190.0±21.9 ^{Bb}

4.3.3 The effect of glucose on extracellular enzyme activities

Potential extracellular enzyme activities (normalized to OC) differed among ALB, HAI and SCH as well as under different land uses (Figure 3). Following glucose addition, only phosphatase activities in forest sites showed significant increases ($P < 0.05$), and this observation was consistent in all study regions (Figure 3). The largest increase was found in HAI forest ($142.7 \pm 36.5 \text{ nmol MUF g}^{-1} \text{ OC h}^{-1}$) followed by SCH ($52.2 \pm 19.9 \text{ nmol MUF g}^{-1} \text{ OC h}^{-1}$) and ALB ($27.3 \pm 5.9 \text{ nmol MUF g}^{-1} \text{ OC h}^{-1}$). Within forests, phosphatase activity increase was significantly higher ($P < 0.01$) in deciduous forests in HAI than in coniferous forests, while the opposite trend, though not significant, was observed for ALB and SCH. The increases of phosphatase activities in grasslands were not significant ($P < 0.05$) in all regions, and there was also no effect of fertilization observed. The activities of β -glucosidase, N-acetyl-glucosaminidase and sulfatase all increased slightly after glucose addition, but these were not statistically significant.

4.3.4 Effect of study region, land use, and soil properties on C priming

Principal component analysis showed the correlations between C priming (both per g OC and per DW soil), shifts in extracellular enzyme activities after glucose addition, and main soil abiotic properties (soil pH, C:N ratio, clay, inorganic P and inorganic S content) in both forest and grassland sites in all regions (Figure 4). The first two axes of PCA explained a total of 68.2% of the variation in the dataset. The PCA biplot showed a clear separation of samples specific to study regions and land use types. From PCA loadings, OC-normalized priming was positively correlated with soil clay content and pH, negatively correlated to soil C:N ratio, inorganic P and inorganic S concentration. Non-OC-normalized priming showed positive correlations with soil pH, OC, total N, inorganic P and S concentration.

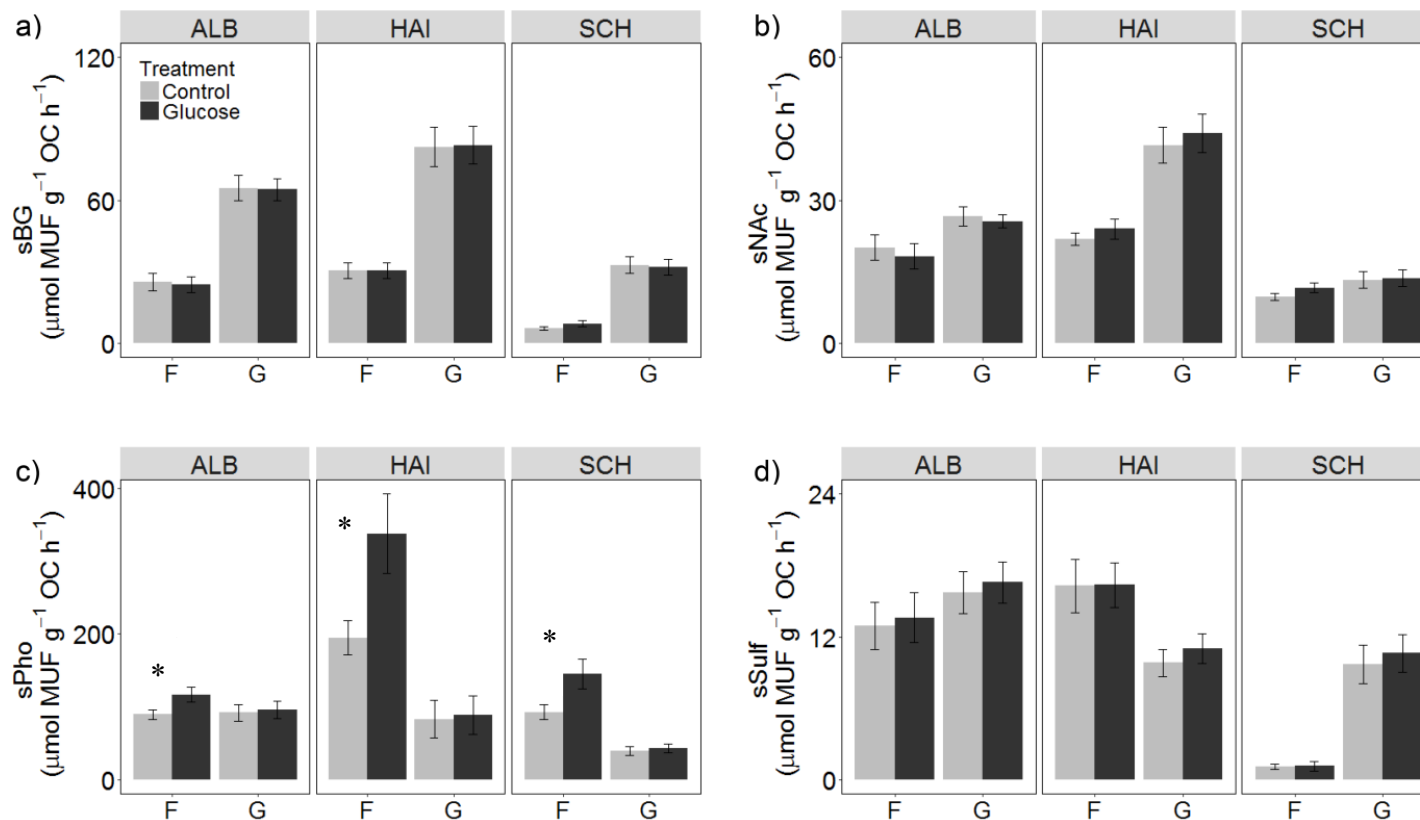


Figure 4.3 Specific extracellular enzyme activities with and without glucose addition: (a) β -glucosidase (sBG) (b) N-acetylglucosaminidase (sNAC) (c) phosphatase (sPho) and (d) sulfatase (sSulf) in different land use types i) Schwäbische-Alb (ALB), ii) Hainich-Dün (HAI) and iii) Schorfheide-Chorin (SCH). Bars represent means ($n=10$) and standard errors. Statistical differences are presented as: * ($P<0.05$), ** ($P<0.01$) and *** ($P<0.001$) according to Student's t-test performed on each subset of land use in each study region. Abbreviations: F= forest, G= grassland.

Analyses of covariance showed significant effects of study region, pH, clay content, C:N ratio, inorganic P, inorganic S and land use on C priming (OC normalized) (Table 5a-c). When both forest and grassland sites were included in one single model, significant proportion of variance of priming was explained by study region ($F(2,48) = 11.41, P < 0.001$) and soil pH ($F(1,48) = 8.97, P < 0.01$), while clay content, C:N ratio, inorganic P, inorganic S and land use were not statistically significant. In forests where tree species effect was tested, significant proportion of variance was explained by study region ($F(2,21) = 31.34, P < 0.001$), soil pH ($F(1,21) = 26.82, P < 0.001$), C:N ratio ($F(1,21) = 62.29, P < 0.001$), and inorganic S ($F(1,21) = 24.74, P < 0.001$). In grasslands, where fertilization effect was tested, study region ($F(2,18) = 25.28, P < 0.001$), soil pH ($F(1,18) = 25.28, P < 0.01$), clay content ($F(1,18) = 10.0, P < 0.01$), inorganic S content ($F(1,18) = 6.0, P < 0.05$) and fertilization ($F(1,18) = 6.23, P < 0.05$) explained a significant proportion of variance. We additionally examined the indirect effect of coniferous vs. deciduous forests on soil pH, C:N ratio and inorganic S with a t-test but could not find any significant effect ($P < 0.05$).

Table 4.5 Analysis of covariance for (a) different land uses (b) different tree species and c) fertilization effect on priming. Explanatory variables: Degrees of freedom (df), mean squares (MS) and F-values are presented as: * ($P<0.05$), ** ($P<0.01$), *** ($P<0.001$). Priming effects were normalized to organic C content.

Coefficients	Df	Mean square	F value	Variance partitioning (%)
a) Land use effect (forest vs. grassland)				
Study region	2	3.76	11.41 ***	26.6 15.7
pH	1	2.96	8.97 **	
Clay	1	0.01	0.04	
C:N ratio	1	0.23	0.71	
Inorganic P	1	0.03	0.10	
Inorganic S	1	1.20	3.64	
Land use	1	0.47	1.43	1.7
Residuals	48	0.33		56.0
b) Tree species (coniferous vs. deciduous)				
Study region	2	0.96	31.34 ***	27.3 50.9
pH	1	0.82	26.82 ***	
Clay	1	0.01	0.43	
C:N ratio	1	1.90	62.29 ***	
Inorganic P	1	0.08	2.72	
Inorganic S	1	0.75	24.74 ***	
Tree species	1	0.01	0.37	0.5
Residuals	21	0.03		21.3
c) Fertilization (fertilized vs. unfertilized)				
Study region	2	8.95	25.28 ***	38.7 22.0
pH	1	3.18	8.98 **	
Clay	1	3.54	10.0 **	
C:N ratio	1	1.29	3.65	
Inorganic P	1	0.00	0.01	
Inorganic S	1	2.13	6.0 *	
Fertilization	1	2.21	6.23 *	4.8
Residuals	18	0.44		34.5

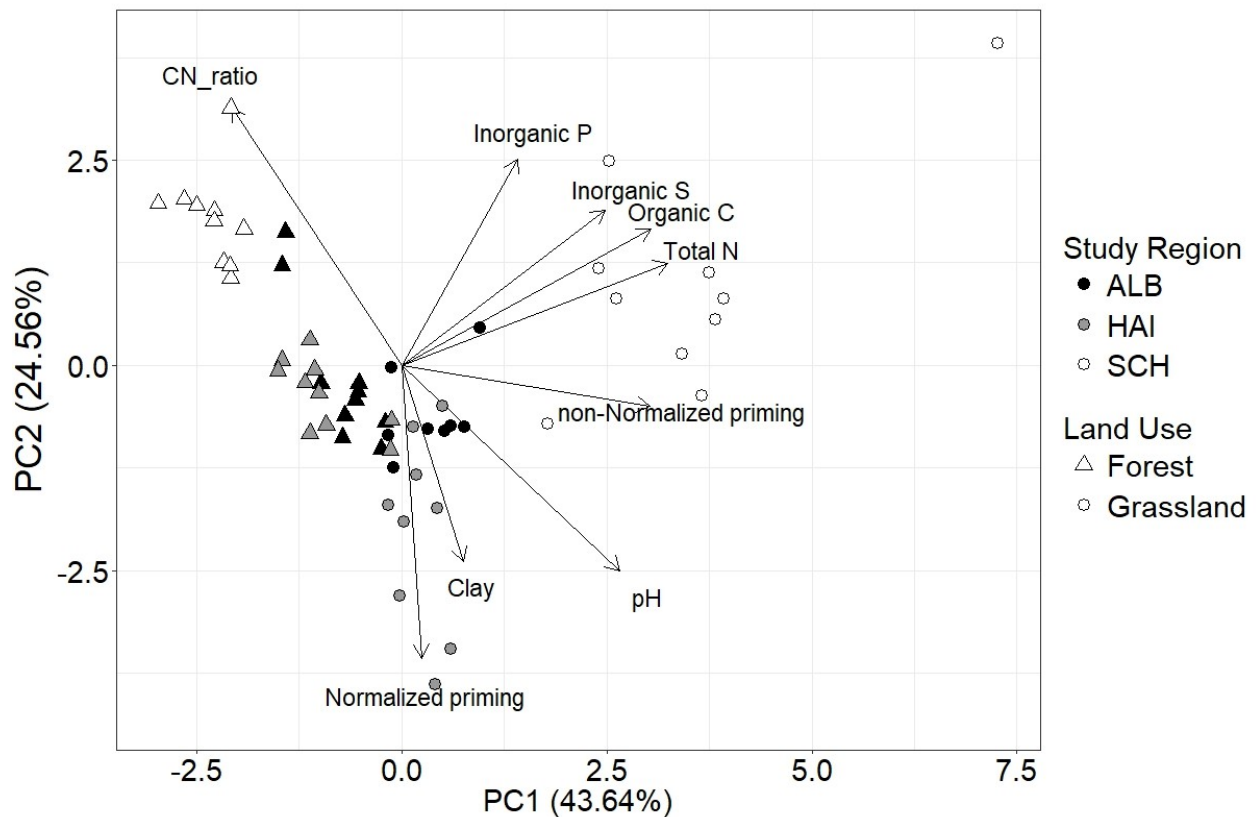


Figure 4.4 Distance biplots of principal component analysis (PCA) for OC-normalized and non-normalized priming, soil abiotic properties and shift in extracellular enzyme activities. Samples are shown in different colours and shapes according to study regions and land use respectively. Abbreviations: ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin, normalized priming = total priming (after 48 hours of incubation) normalized with organic C ($\text{mg CO}_2\text{-C g}^{-1}\text{ OC}$) and non-normalized priming = total priming not normalized to organic C ($\text{mg CO}_2\text{-C g}^{-1}\text{ DW}$). All enzyme activities were calculated as a difference in enzyme activities between control and glucose treated samples after 48 hours of incubation: sBG = β -glucosidase, sNAc = N-acetylglucosaminidase, sPho = phosphatase and sSulf = sulfatase.

4.4 Discussion

4.4.1 Glucose respiration pattern under different land uses

The addition of glucose resulted in an increased respiration producing CO₂ respiration curves that were unique to forests and grasslands respectively (Figure 1). Based on previous studies at the same sites, microbial C and PLFA biomass was reported to be higher in grasslands than forests (Herold et al. 2014b, Solly et al. 2014). Since the same amount of glucose was added to all sites, this meant more glucose compared to the amount of microbial C was added in forests than grasslands. This suggests that the glucose added to forest soils at the study sites might have exceeded the energy required to maintain microbial metabolism, and resulted in greater microbial growth in forests compared to grasslands (Anderson and Domsch 1985, 2010).

Higher respiration of glucose derived OC was observed in forests than grasslands, providing evidence that that higher amount of glucose-C added in relative to microbial C pool size in forest than grasslands resulted in higher metabolism of glucose (Table 3). In previous study, high glucose-C added per microbial C may result in the switch of SOM to glucose consumption, a process previously termed as “preferential substrate utilization”, and may result in negative priming (Kuzyakov 2002). In line with those results, a previous study showed that microbes did not decompose native SOM for the first two weeks following the addition of high amounts of labile C relative to microbial C (ratio microbial C to glucose C of 1:8) in Chernozems (Blagodatskaya et al. 2007). It is observed, in contrast, positive priming in the first 48 hours of incubation for all samples (Figure 2). This suggests that with the glucose-C added in this study (ratio microbial C to glucose C of 1:2), the saturating point where microbial C usage was switched completely to glucose metabolism was not reached. Even if the amount of glucose-C added exceeded the amount of microbial C, microbes still decomposed SOM. This indicates the microbial need for further elements than C, H and O to maintain the microbial metabolism which can only be obtained from native SOM.

4.4.2 Extracellular enzyme activities as predictors of microbial nutrient mining

Following glucose addition, significant increase in phosphatase activities in forests was found (Figure 4). This finding was similar with the study by Nottingham et al. (2012) who showed that sucrose-C addition resulted in higher phosphatase activities as compared to controls, providing an evidence that P mining due to P deficiency occurred via increase in microbial phosphomonoesterase production. Contrary to forest sites, significant increase in phosphatase activities was observed after glucose addition in grassland soils (Figure 4). As discussed above, higher concentration of glucose-C added in relative to microbial pool size in forests than in grasslands probably resulted in higher microbial growth and higher microbial P demand for nucleic acid production (Fontaine et al. 2003a). Another reason why significant increase in phosphatase activities was not observed in grasslands was probably because of higher soil P concentration in grasslands than in forests, resulting in lower microbial P limitation. This was shown by lower inorganic P concentration observed in this study (Table 2), and further supported by previous study who reported higher inorganic P concentrations in grasslands than forests at the same study sites (Alt et al. 2011a). However, acid phosphatase was measured in this study, it could also be that alkaline phosphatase may be more important in grasslands soils with high soil pH as indicated by the metabolomics study of Kaiser et al (2016).

In this study, higher phosphatase activities in forest soils following glucose addition were not correlated to soil priming (Figure 4). At the same time, significant effect of inorganic P on priming in forests could not be showed when soil pH and CN ratio were considered as covariates (Table 5). Previous studies used the increase in extracellular enzyme activities that mineralize limiting nutrients from native SOM as a proxy to test whether priming occurred due to nutrient mining (Blagodatskaya and Kuzyakov 2008, Dijkstra et al. 2013). For P, however, it has been shown that an increase in phosphatase activities does not necessarily result in an increase in C priming due to the decoupling between C and P mineralization (Dijkstra et al. 2013). In contrast to N, P can be released from organic P esters by hydrolysis without mineralization of the C-skeleton from SOM (Dijkstra et al. 2013, Nottingham et al. 2015). Accordingly, previous studies showed that priming was often reduced when mineral N

is added, while the effect of adding mineral P alone did not increase priming (Bradford et al. 2008, Chen et al. 2014, Nottingham et al. 2015).

Significant increase in the activities of β -glucosidase, N-acetyl-glucosaminidase and sulfatase was not observed following glucose addition (Figure 4). These findings agreed with Nottingham et al. (2012) but in contrast with Chen et al. (2014), which may be due to different incubation period where up to 210 hours of incubation time was performed by Chen et al. (2014) following sucrose addition, compared to 48 hours performed in this study. As CO₂ released from SOM was observed, priming may be due to microbial C turnover from glucose consumption, or that existing β -glucosidase concentrations were sufficient to decompose cellobiose from SOM into its sugar monomers during the two days of incubation. While the same argument could explain why an increase in sulfatase and N-acetyl-glucosaminidase activities was not observed, it was also possible that N mineralization occurred through other N-degrading enzymes than the N-acetyl-glucosaminidase. For example, an increase in leucine amino peptidase has been observed by Chen et al. (2014) following sucrose addition which decreased again with mineral N addition.

4.4.3 Regional and land use effects on C priming

More than one fourth of variance from factors that control C priming was explained by regional effects alone (Table 5a-c). Within study regions, variations in soil abiotic properties explained a significant proportion of variance in priming. Soil priming has been suggested to occur in soils with high C:N ratios due to microbial N mining (Kuzyakov 2010). In contrast, higher total priming after OC normalization was observed at sites with lower C:N ratio and higher pH and clay content, and at sites with lower inorganic S and P (Table 5a-c, Figure 4), suggesting that factors associated with higher microbial activities supported higher C priming. This was further supported by the lowest C priming (not normalized to OC) at sites with the lowest amounts of total microbial and PLFA biomass as reported previously (Herold et al. 2014b, Solly et al. 2014).

In line with those studies, positive correlations between priming and soil pH (Figure 4) showed that higher pH probably promoted an overall increase in microbial and enzyme activity, leading to higher priming rates (Table 5a-b). Previous studies have shown the importance of soil pH in affecting bacterial community structure, and that high pH favours

extracellular enzyme activities (Herold et al. 2014a, Kaiser et al. 2016). The stronger response of priming to pH in forests than grasslands (Table 5b-c) suggests that the pH effect on microbial activity was stronger in forest than grassland soils (Table 5). This can be explained by much more pronounced pH gradient in forest compared to grassland sites. As compared to forests, soil pH and C:N ratio in grasslands showed less significant effect on C priming (Table 5b). One reason could be that these parameters were rather similar between sites and regions and that their observed range was not large enough to show important effects. Negative correlations were also observed between priming and inorganic S concentrations in forests but not in grasslands (Table 5b-c). This might be a hint that lower S availability increased priming by nutrient mining and more so in forests than in grasslands.

Positive correlation was observed between priming and clay content (Figure 4, Table 5c). At the global level, higher clay content results in higher SOC content due to the stabilizing effect of clay mineral surfaces and reduces OC accessibility for soil microorganisms (Jobbagy and Jackson 2000). However, as positive correlation between clay content and OC-normalized priming was observed, higher stability of OC on minerals was probably not causing results presented in this study. Higher priming (not normalized to OC) was observed in grasslands than in forests in all study regions (Table 4). Together with higher microbial biomass and the activities of β -glucosidase (Figure 3) in grasslands than in forests, higher priming may also be linked to faster OC turnover in grasslands. This was further supported by litter decomposition studies from Solly et al. (Solly et al. 2014). On overall, the results presented here suggest that the nutrient mining theory was only partially supported as a driver of C priming, and that higher priming was typically observed at sites with more favourable conditions for microbial growth. Similar findings were reported by Nottingham et al. (2012) who observed that the highest priming occurred in tropical soils with medium fertility, suggesting that priming is partly constrained in soils with high fertility due to high nutrient availability, and also in soils with low fertility due to low SOM content to support microbial activities.

4.4.4 Tree species and fertilization effects on priming

Because organic C contents and C:N ratios were higher at coniferous than deciduous forests (Table 2), higher priming in coniferous forests is expected but the opposite was observed (Figure 2b). After accounting for the effect of study region and soil abiotic properties, the

effect of tree species alone was not significant in explaining the variance of priming (Table 5b). Overall, the findings from this chapter did not support the hypothesis that different tree species in forests affects priming via changes in litter quality and soil nutrient status. Mean values of soil abiotic properties that could significantly affect priming (soil pH, C:N ratio and inorganic S concentrations) were also not significantly different between coniferous and deciduous forests (Table 5b), thereby excluding the possibility that there may be indirect effect of tree species on priming. One possible explanation could be that soil pH was lower in coniferous than deciduous forests in HAI, whereas soil pH was higher in both deciduous forests in ALB and SCH (Table 2). The coniferous forests at HAI were only recently reforested on calcareous soils due to changes in ownership after the year 1949 (Mund 2004), so that different from the other regions, pH values in the soil were still higher for coniferous forest soils.

Total priming after normalized to OC was consistently higher in fertilized grasslands (Fig 2b). This contrasted with predicted hypothesis that priming would be higher in unfertilized than fertilized grasslands because of lower nutrient concentration and higher microbial nutrient mining. One possible reason could be that fertilization had a smaller effect on soil nutrient status than was expected, as C:N ratios did not differ between fertilized and unfertilized sites (Table 2). Additionally, soil OC, TN and inorganic S concentrations were even smaller at fertilized sites. This could reflect more intensive management of fertilized sites by mowing or grazing, which reduces aboveground biomass and thus the return of OC and nutrients to the soil, compared to unfertilized grassland with grazing and manure input (Naeth et al. 1991). Since there were no significant differences in soil pH between fertilized and unfertilized grasslands, the differences in P concentrations may not be caused by the dissolution equilibrium of P-containing minerals due to calcareous bedrock or by the effect of liming, but directly from fertilization (Alt et al. 2011a, Oelmann et al. 2017). However, significant effect of soil inorganic P concentration was not observed on priming in grasslands, suggesting that microbial P mining was probably not the main driver of priming between fertilized and unfertilized grasslands as discussed above.

4.5 Conclusion

Short-term incubation revealed the dynamics of labile C respiration in soils under different study regions, land use, management and different soil abiotic properties. Using this approach, this study showed that labile C was respired at different rates under different land use and management, supporting the initial hypothesis that land use affects nutrient availability and energy limitation in the mineral soil and may affect priming intensity. This may be overlooked when performing a long-term incubation with a large between-measurement gap. Study region and soil abiotic properties (soil pH, clay content, C:N, inorganic P and inorganic S concentrations) explained a significant proportion of variance in priming. Priming was positively correlated with soil pH and clay content and was negatively correlated with C:N, showing that factors that constrain microbial activities in soil were also the most important factors that control priming. A strong support for the nutrient mining theory being an important driver of priming was not found, but this was probably because of a relatively short incubation compared to previous studies. However, within a short incubation period, it is observed that among all study regions, glucose addition resulted in a consistent and significantly higher increase in the P acquisition capacity in forests than grasslands, which linked to significant increase in phosphatase activities in forest than grasslands.

Chapter 5

Synthesis and conclusions

5.1 Land use and management effects on belowground C and nutrient cycling

Many factors associated with land use and management intensity have been reported to directly affect the decomposition of soil organic matter through the alteration of microbial processes. One of the main objectives of this thesis aims at understanding the effect of land use and management on soil C and nutrient interactions. Specifically, soil samples were collected from 300 study sites ranging from different land use types (forest and grassland) and management intensity (deciduous and coniferous forest, fertilized and unfertilized grasslands), and three main experiments are carried out to determine land use and management effects on: (i) soil mineralization potential for C and other nutrients, its main drivers and interactions between nutrients (Chapter 2), (ii) the relation between native C mobilization due to priming effects following the addition of different amounts of labile C soil microbial biomass (Chapter 3) and (iii) the comparison of native C mobilization due to priming effects at different sites as driven by microbial nutrient mining (Chapter 4).

Overall, this thesis shows that land use effects are important drivers of the interactions of soil C and nutrients (N, P and S) in both soil mineralization potentials (Chapter 2) and of the amount of native C released due to priming effects (Chapters 3 and 4). Chapter 2 shows that C mineralization rates were consistently higher in grasslands than forests across different study regions, and this is suggested to be due to the fact that concentrations of microbial biomass-C in grasslands is on average two times higher than in forests. Higher C and lower N and S mineralization rates in grassland compared to forest soils in turn results in higher C-to-N and C-to-S mineralization/ leaching rates in grassland than forest soils, which contrasts to the hypothesis where higher C-to-nutrients is expected in soils with higher C-to-nutrients. This important finding suggests that SOM mineralization does not depend on soil nutrient stoichiometry (carbon to nutrient availability) alone. The synthesis of these findings is illustrated in Figure 5.1 as shown below:

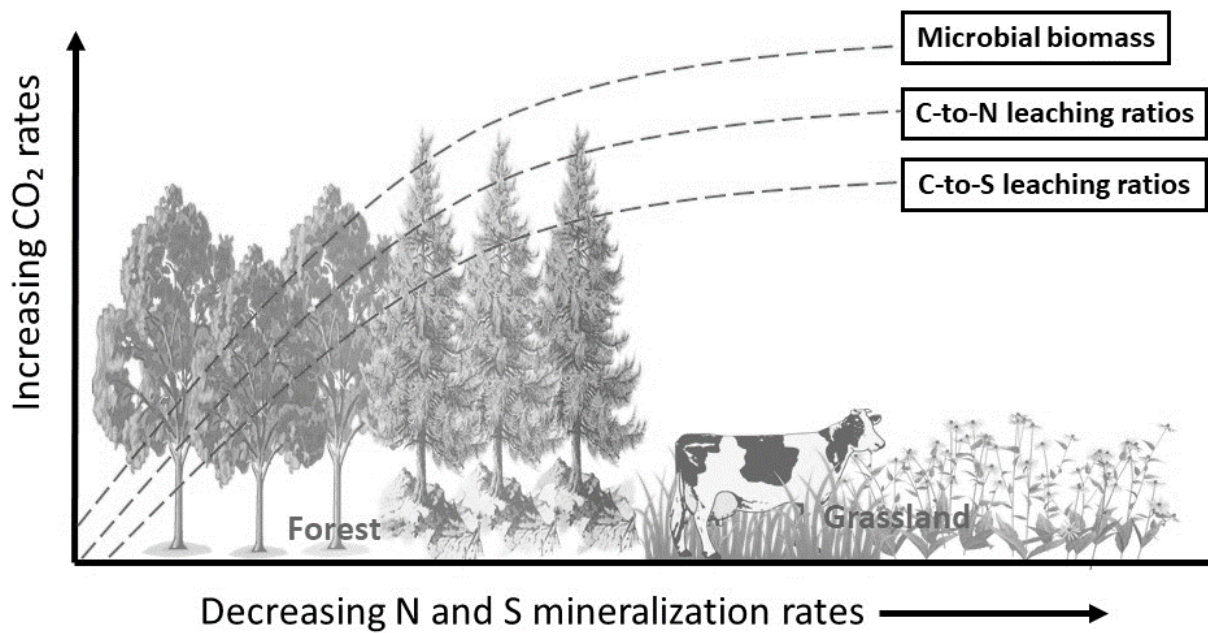


Figure 5.1 Higher CO₂ respiration rates and lower nitrogen (N) and sulfur (S) mineralization rates in grassland compared to forest soils resulted in higher CO₂-to-N and CO₂-to-S leaching rates in grassland compared to forest soils, where microbial biomass-C is also observed to be higher in grassland soils.

In Chapters 3 and 4, the effects of land use on microbial activities is further shown by the different overall responses and temporal patterns in CO₂ respiration rates between forests and grasslands following glucose addition as a labile C source. Chapter 3 shows that respiration rates in grassland soils respond quickly to the different amounts of added C, while forest soils have a delayed response with a time lag that increases with greater C addition, even though native SOM-C released from priming are similar from both land use types. Additionally, forest soils respired higher glucose-derived CO₂ compared to grassland soils, indicating faster turnover of labile C sources. Another interesting observation in Chapter 3 shows that the initial respiration rates right after glucose addition increase with glucose levels in grassland soils, while the initial rates remain the same for forest soils regardless of glucose levels. The synthesis of these findings is illustrated in Figure 5.2 as shown below:

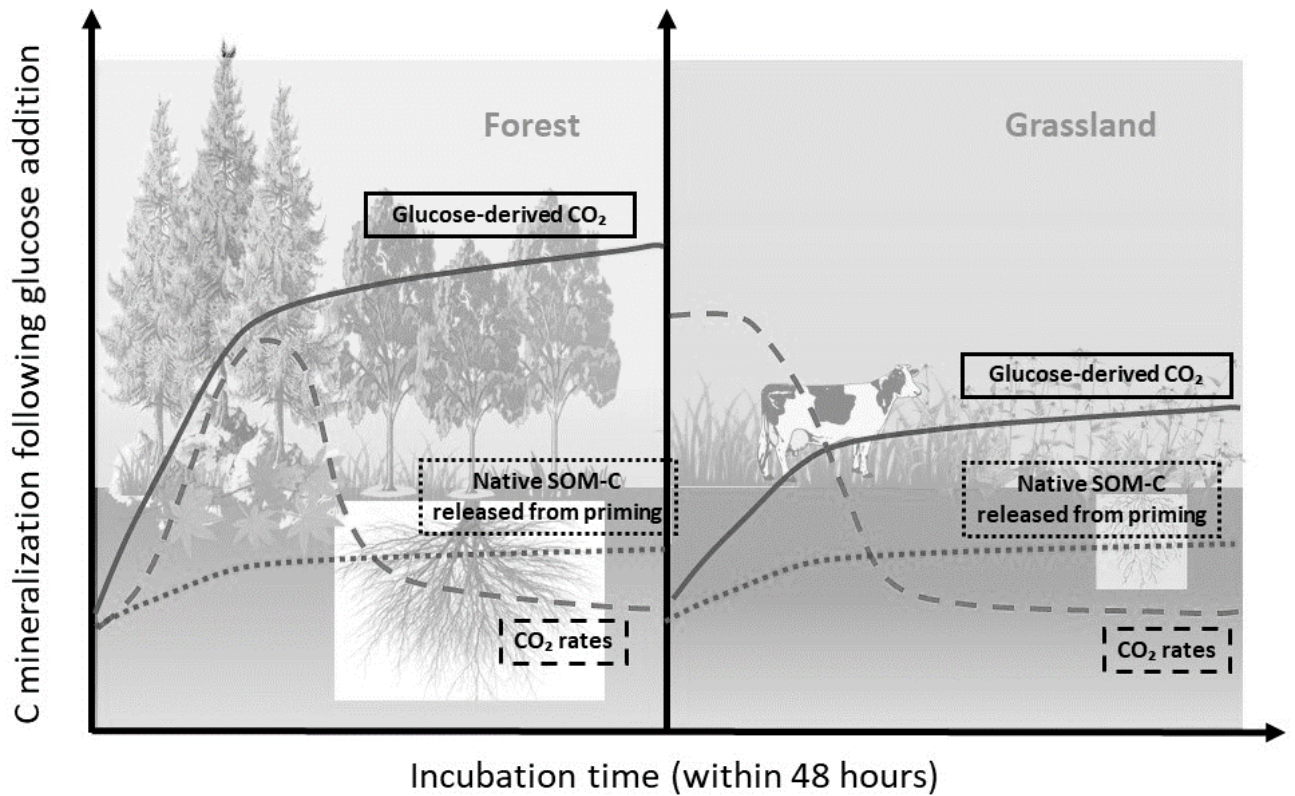


Figure 5.2 Comparison of glucose-derived CO₂ rates, overall CO₂ respiration rates and native SOM-C released from priming between forests and grasslands following glucose addition as a labile C source.

According to previous studies, differences in respiration responses between forests and grasslands is linked to differences in microbial activities between the two land use types (Stotzky and Norman 1961). Forest soils take longer to respond to the addition of the glucose, indicating that the microbial community takes longer to respond than in grasslands (Chapters 3 and 4). Evidence that this is related to overall lower microbial activity in forests comes from the much lower respiration rates in control incubations in forests compared to grasslands (Chapter 4).

In Chapter 2, even though microbial biomass as assessed with fumigation-incubation does not show consistent differences between forest and grassland soils in this study, microbial biomass has been shown by previous studies as well as in Chapter 4 to be on average lower in forest compared to grassland soils at the same study sites. Microbial activities in forest soils such as extracellular enzyme activities may also be retarded by low soil pH compared to the grassland soils as shown in Chapter 4 and previous study (Herold et al. 2014b), or different

microbial community composition between forests and grasslands related to differences in soil pH values (Nacke et al. 2011). This finding also highlights the problems of comparing microbial activities using fumigation/extraction method to assess microbial biomass between soils with very different microbial communities. Higher C mineralization rates and microbial activities in grasslands than forests is further explained by faster fine root decomposition (Solly et al. 2014) and faster litter-derived C turnover (Herold et al. 2014c), which is attributed to higher litter quality in grasslands than in forests and is more susceptible to microbial decomposition and microbial activities (Melillo et al. 1982, Taylor et al. 1989).

This thesis further shows that management practices in forests and grasslands are important drivers of soil mineralization potentials and the intensity of priming effects, even though such effects are not as pronounced as land use types. Chapter 2 shows higher rates of N and S mineralization in deciduous than coniferous forests. This is suggested to be due to differences in litter quality where litter composition from different tree species have been reported to vary widely and may potentially affect soil N mineralization (Gower and Son 1992, Hobbie 1992, Reich et al. 1997). Chapter 3 shows that the intensity of soil priming is significantly higher under fertilized than unfertilized grasslands, while no significant differences are shown between different forest types. Even though some previous studies have also reported tree species and fertilization effects on soil C and nutrient interactions, these findings are interpreted with caution as the observations may also be due or partly due to other forest management practices at the study sites which were not considered in this thesis. From the study sites, most deciduous and coniferous forests are managed as commercial forest under different age class, selective harvesting and as mixed forests, while some deciduous forest sites were unmanaged. Other grassland management practices not considered in this thesis are for example mowing and grazing.

5.2 Soil properties and site effects on belowground C and nutrient cycling

By studying an extensive number of study sites across different regions with variations in different soil properties and soil nutrient concentrations, this thesis provides new insights on the important drivers of soil mineralization, the rate of C mobilization from SOM and which nutrient limits microbial processes when energy limitation is eliminated across regional scale. Soil abiotic properties vary across the large study region due to different soil abiotic properties, climate and topography, and this has been shown to affect microbial processes in soil (Birkhofer et al. 2012). From the first result part of Chapter 2, soil mineralization potentials for C, N, P and S show important variations across the three different study areas, and this is mainly attributed to the differences in soil texture i.e. clay content among different regions in the following ascending order: SCH<HAI<ALB (Chapter 2, Table 2.1). Higher C mineralization rates are linked to higher microbial activities as shown by significant positive relations between these two variables ($r = 0.6$, $P < 0.001$, Figure 5.1 and Figure 5.3). Carbon mineralization potential is shown to be higher in regions with higher clay content; where high clay content also indirectly links to higher concentrations of SOC, microbial biomass and total soil nutrients (Chapter 2, Table 2.3).

Redundancy analysis from Chapter 2 also reveals the confounding effects of soil clay content, SOC, microbial C, total N, P and S as well as soil pH as the main drivers of C mineralization in both forests and grasslands. The effects of soil properties among regions is also apparent on P mineralization. In contrast to C mineralization which is driven by higher SOM concentrations and microbial activities, P mineralization is triggered by the lack of P bioavailability in soil. Chapter 2 shows the importance of soil clay content on soil P mineralization (Figure 2.2 and Figure 2.5). In SCH compared to the other two study regions, the highest P mineralization is observed alongside with the lowest soil clay content at this site, while such distinctive different is not observed for N and S mineralization (Figure 2.2, Figure 5.3). Indeed, the concentrations of differently soluble forms of soil P in sandy soils were found to be significantly lower compared to other soil texture types across the German forest landscape in previous study (Niederberger et al. 2018).

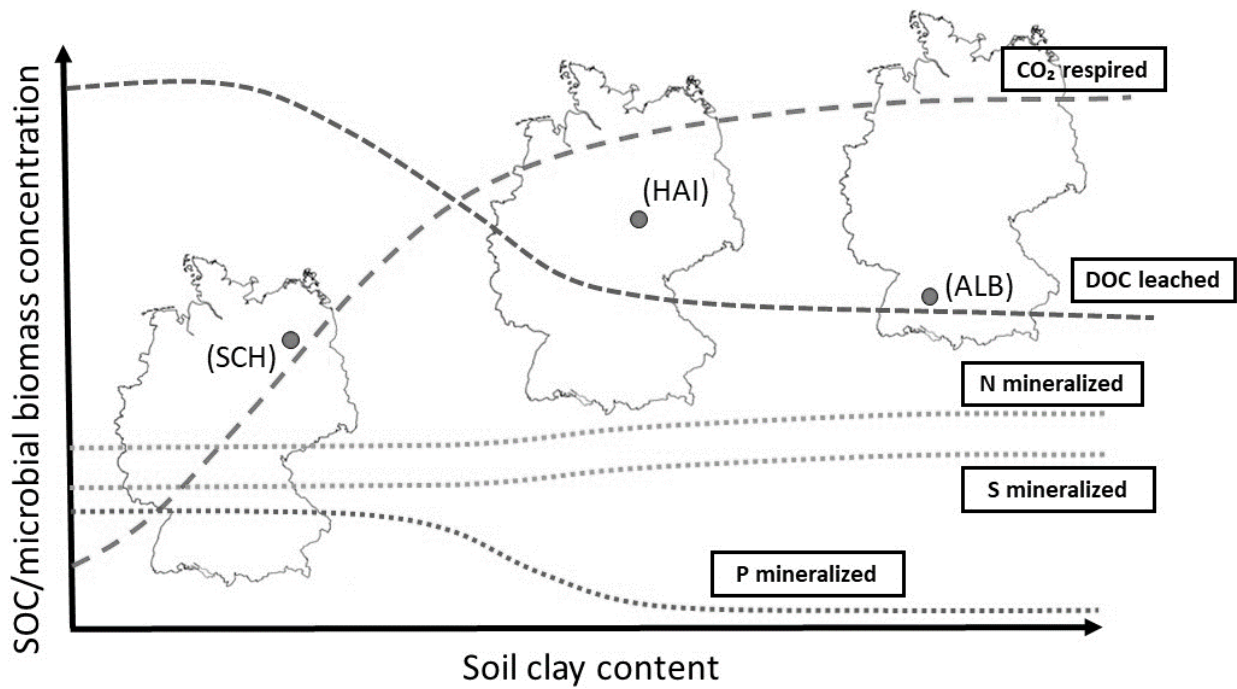


Figure 5.3 Simplified diagram of positive correlations between soil clay content and soil organic C (SOC) and microbial biomass concentrations across three study sites, and its effects on C and nutrients mineralization.

The important effect of soil clay content is further shown in Chapter 3. Even though clay content does not explain a significant proportion of variance on the amount of CO₂ released via priming, this could be due to a normalization effect, as we report priming normalized with OC concentrations for each sample. Chapter 2 shows that SOC concentration is confounded with soil clay content. However, the effects of clay content are apparent from the results of principal component analysis, where it positively correlates with soil priming. Positive relationships between clay content, SOM content and microbial activities have been previously reported (Hassink 1994b, Wang et al. 2003, Kemmitt et al. 2006). The importance of clay particles for the retention of SOM is due to their cation exchange properties and in reacting with organic matter to form stabilized SOM complexes (Dixon 1991), further affecting its accessibility to soil microbes (Nyborg and Hoyt 1978, Hinsinger 2001, Herold et al. 2014a). It can be concluded from the findings in Chapter 2 and 3 that across different regions, the clay content in soil is an important driver of both soil C mineralization and C released via priming effects.

5.3 Interaction between soil C and nutrients (N, P and S)

Carbon mineralization couples to N, P and S mineralization due to the co-limitation of these elements on microbial processes to maintain their biomass stoichiometry (Jonasson et al. 1999, LeBauer and Treseder 2008, Vitousek et al. 2010). This is because in soil, SOM decomposition and mineralization are regulated by heterotrophic microorganisms as nutrient- and energy-driven processes. Soil organic matter decomposition and mineralization based on microbial energy to nutrient requirements can therefore be observed by analyzing the patterns of C decomposition in parallel to the mineralization of N, P and S. Following the idea of stoichiometric controls, SOM decomposition is enhanced by higher OC to nutrient ratios as more C is respired to acquire N, P and S which are more limiting than OC to maintain cellular elemental homeostasis. For example, in soils with C limitation, net mineral N and S are released during microbial decomposition of nitrogenous organic matter and C-bonded S for energy sources, resulting in positive correlations between C and N and between C and S (McGill and Cole 1981, Chapin et al. 2011).

In Chapter 2, increasing C and N mineralization and DOC leached per microbial C with increasing soil C:N shows that indeed more C is being removed while N acquisition increases with decreasing N relative to C source, suggesting that soil microbes need to invest more energy to acquire nutrients in nutrient poor soils which in overall supports the hypothesis of excess C removal due to reduced microbial carbon use efficiency (Figure 5.4). Overall, results also show lower microbial biomass per SOC in nutrient poor soils, and that CO₂ is released per g microbial biomass-C at these sites are also higher compared to nutrient rich soils. However, the increase of N mineralization with increasing soil C:N due to N mining is unclear, as most of the N mineralized would be expected to be assimilated quickly (Schimel and Bennett 2004, Chapin et al. 2011). This uncertainty may be further addressed for example by studying net N immobilization as higher microbial N uptake would be expected despite increasing net N mineralization with decreasing soil N sources. The estimation of net N immobilization is however beyond the scope of this thesis.

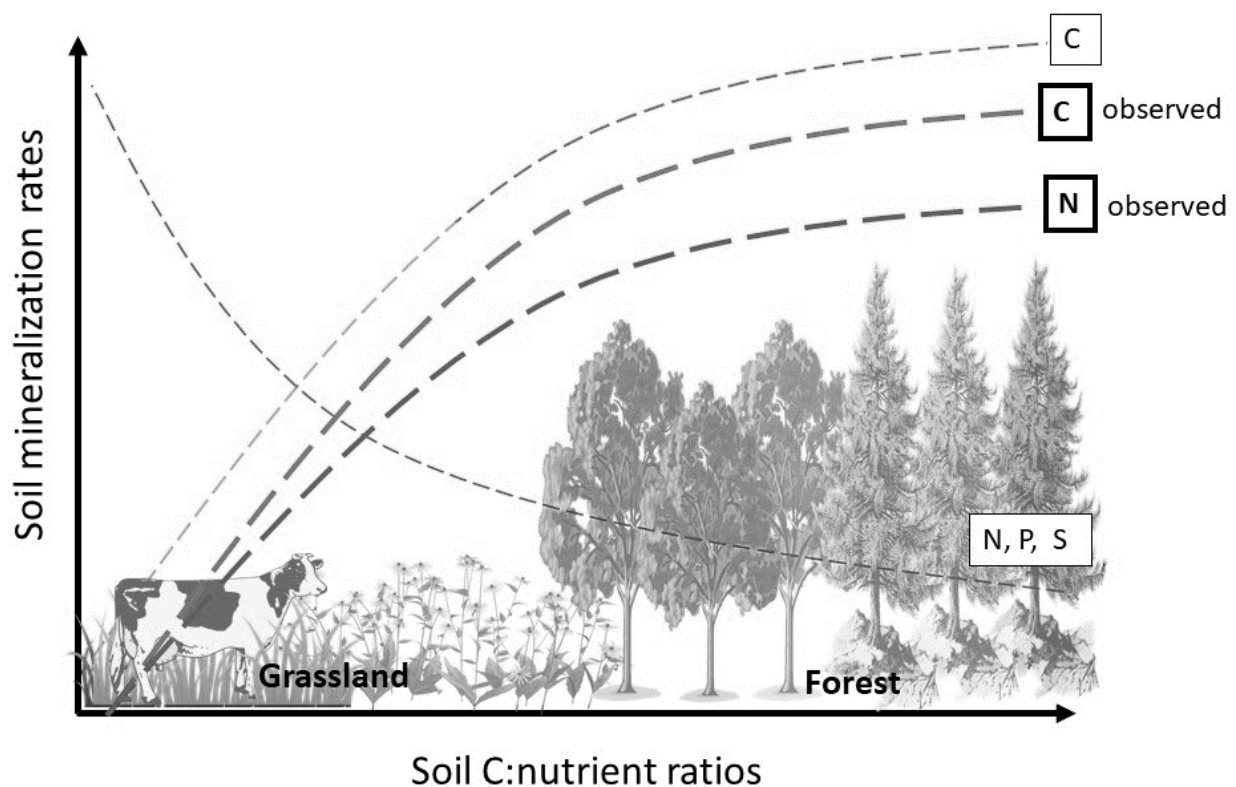


Figure 5.4 Predicted interactions (as shown in Chapter 1) vs observations between carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) mineralization with increasing soil C:nutrient ratios under different land uses.

Chapter 2 also provides new insights that the ratios of C-to-N and C-to-S mineralized is a useful index for microbial activities across the different regions, land uses and management types. In grasslands, high C-to-N and C-to-S mineralized ratios indicates high microbial activities (high respiration, high N and S uptake therefore low net N and S mineralized), while lower C to N mineralized ratios are shown in forests (Figure 5.1). Lower microbial activities in forests compared to grasslands has been shown and discussed in the previous sections. By studying the interactions of C, N, P and S at the same time, this thesis also points out that in forests, the immobilization of N and S may be limited by C and P availability. The limitations of these nutrients may be attributed to land use as well as regional factors, such as low clay content in SCH compared to ALB and HAI regions, and low soil pH in forests compared to grasslands (Hart et al. 1994, Côté et al. 2000).

Chapters 3 and 4 show the effects of eliminating C limitation on C and nutrients interactions through soil priming effects. In soil, microbial activities are mainly constrained by energy limitation (Hobbie and Hobbie 2013). The addition of glucose and thus the elimination of C limitation increases microbial processes until another nutrient becomes limited (Fontaine et al. 2011). Microbes overcome this limitation by decomposing native SOM to ‘mine’ for the specific limiting nutrient and release CO₂ as by products known as the priming effects (Kuzyakov et al. 2000a). One important mechanisms of priming effects is thus due to microbial response to labile energy sources by increasing the production of enzymes to acquire limiting nutrients (Nottingham et al. 2012). This coupling of C to nutrients is also shown in Chapter 2, where the excess C source is invested to mineralize for example N-containing SOM (Mooshammer et al. 2014a). Chapter 4 shows that the addition of labile C increases phosphatase activities only in forests. The increase in extracellular enzyme activities following labile C addition has been used in previous studies as a proxy to study nutrients limiting microbial activities when C is not limiting, and whether soil priming occurred due to microbial nutrient mining (Blagodatskaya and Kuzyakov 2008, Dijkstra et al. 2013). This shows that at the forest sites, the addition of labile C eliminates C limitation on microbial processes and part of this C is invested to produce phosphatase to mine P, which is probably the next limiting nutrient after C. This complements the results from Chapter 2 where RDA shows that in forests, microbial processes (C mineralization, N and S uptake) may be limited by C and P availability.

5.4 Implications for future research

Chapter 2 provides a comprehensive overview of the interactions between C, N, P and S mineralization particularly the relationships between C and other nutrients. While this provides the first insights on how these interactions differ especially under different contexts (study regions, land uses and management practices), more in-depth studies would be necessary to improve our understandings on these interactions and processes. For instance, as this thesis only focuses on the measurements of net nutrient mineralization, it would be interesting to also measure gross nutrient mineralization and net nutrient immobilization to estimate the amount of nutrient partition between microbial uptake and release. More challenging would be the inclusion of plant-microbial-soil interactions in studying soil nutrient interactions. This is because soil mineralization and microbial processes are the most intensive at the regions with the presence of plants (e.g. rhizosphere and detritosphere) due to the input of rhizodeposition and plant litter as food sources (Kuzyakov and Blagodatskaya 2015). Further, the inclusion of plants in studying soil mineralization is especially important, given that the amount of C input is shown from previous studies and from this thesis to affect the mineralization of other nutrients.

Chapter 3 and 4 shows that the input of labile C increases native C release and microbial nutrient mining via increase in extracellular enzyme activities. While previous studies have shown the link between soil priming and microbial N mining (Bradford et al. 2008, Chen et al. 2014, Nottingham et al. 2015), only increase in phosphatase activities is observed but not in N-acetyl glucosaminidase and sulfatase in this thesis. One reason may be that short incubation period (48 hours as conducted by this thesis) and a single substrate addition may not suffice to trigger or observe the mining of other nutrients, or that the study sites may not be N limited as shown by Chapter 2. Previous studies have shown that soil priming observed may also be 'apparent', meaning that the increase in CO₂ released is rather due to higher microbial turnover of labile C e.g. via microbial growth (Kuzyakov 2010). Therefore, a combination of observing microbial C uptake and a longer incubation period until substrate depletion is important in studying soil priming due to microbial nutrient mining.

Another mechanism reported which is responsible for soil priming is the succession between different groups of microorganisms. This theory suggests that the addition of labile C first activates fast growing microorganisms (r-strategists) and enhances their biomass turnover (“apparent” priming), which then facilitates the growth of slow-growing k-strategists which feed on the necromass of r-strategists (Fontaine et al. 2003a). Estimating the increase in specific microbial groups following labile C input and the stages thereafter (e.g. using stable isotope probing, SIP and followed by nucleic acid extraction) would provide important insights on the potential microbial groups responsible for priming and nutrient mining. Other potential future study is to look into are differences between microbial groups in different land use types, and potentially linking the genes or functions related to production of different enzymes to enzyme abundance that related to soil priming as well as nutrient mineralization potentials.

Extensive investigation of other management practices (e.g. age class, selective harvesting, mixed forests, mowing and grazing) on soil C and nutrient interactions is beyond the scope of this thesis, but is highly recommended to further improve our understandings on how different management practices and intensities affect these soil processes. This may be tested using further statistical analysis i.e. by further separating each land use type into different categories of managements and testing the relations of these managements with the responses of soil processes such as soil priming and mineralization rates using simple regressions or simple models (as performed in Chapters 2, 3 and 4) and link it later on with other environmental parameters such as soil properties using multivariate analyses (as performed in Chapter 2 using Redundancy Analysis and in Chapter 4 using Principal Component Analysis) or linear models (as performed in Chapters 2 and 3). Since it is observed in Chapter 2 that C mineralization rates are related to SOC concentrations, it is therefore interesting to specifically test if biomass removal from forests (through harvesting) and grasslands (mowing versus grazing) decreases soil C-to-nutrient ratios and overall rates of C mineralization. Additionally, it would be interesting to test if different grassland managements could explain the significantly higher release of total native SOM-C due to priming from the grassland soils in HAI compared to all other sites as observed in Chapter 4 (Table 4.3), since this observation could not be linked to extracellular enzyme activities (Figure 4.3).

Lastly, as Chapter 2 shows that microbial processes in forests are potentially limited by C and P, it would be interesting to (i) verify this by conducting further experiments such as through C and P additions (ideally with stable isotopes) in soils and by following how these may affect microbial N and S uptake during incubations and (ii) apply these findings on-site i.e. to how forest management could be optimized to eliminate these limitations and increase organic N and S sources (e.g. lower harvest, P fertilization).

5.5 Main conclusions

The main objective of this thesis is to improve our understanding on the interactions between soil C and nutrient cycling, and how these processes are affected by study regions, land use and management practices. To understand the potentials, interactions and main drivers of soil, N, P and S mineralization, a large scale, short term incubation was designed to estimate the mineralization rates of these elements simultaneously. Substrate induced respiration experiments were designed to study the effects of labile C addition on the mobilization of native C via priming effects. To investigate whether soil priming was induced by microbial nutrient mining, extracellular enzyme assays of four different enzymes involved in the mineralization of different elements were performed. The study sites were provided through the framework of the Biodiversity Exploratories project, a large-scale joint research platform aimed at studying multiple drivers of biodiversity as a function of land use and management intensities (Fischer et al. 2010). The design of this project which comprised of a hierarchical set of standardized experimental field plots in three different regions in Germany under different land use and management practices enabled this thesis to evaluate the effects of study regions, land use and management practices on the interactions between soil C and nutrient cycling. The following conclusions based on the main findings of this thesis were drawn:

- I. Soil respiration and microbial biomass carbon were related to total OC contents and declined with higher soil C:N and C:S ratios. As hypothesized, CO₂ and DOC released were significantly higher in grasslands than in forests in all regions. The rates of DOC release were significantly higher in coniferous than deciduous forests in two out of three regions. No consistent effect of forest management could be detected on soil respiration and microbial biomass in all regions, and this might be due to the differences in soil properties such as pH and soil texture among regions. In contrast to expectations, CO₂-to-N- and CO₂-to-S-leaching ratios were significantly higher in in grasslands than forests in all regions. This was mostly caused by significantly higher CO₂ and significantly lower N and S leaching in grasslands than forests which might be due to higher microbial biomass at the grassland sites. According to expectation however, CO₂-to-N- and CO₂-to-S-leaching ratios were significantly higher in coniferous than deciduous forests in two out of the three study

regions. This was mostly caused by significantly higher N leaching in deciduous than coniferous forests. In grasslands, no fertilization effect was observed on soil OC and microbial biomass C concentrations, CO₂ and DOC released as well as CO₂-to-N- and CO₂-to-S-leaching ratios.

- II. When normalized to microbial biomass C, soil C:N positively correlated to C mineralization and DOC leaching. This correlation was much stronger in forests (CO₂: $r = 0.7$, $P < 0.001$, DOC: $r = 0.8$, $P < 0.001$) as compared to grasslands (CO₂: $r = 0.2$, $P < 0.05$, DOC: $r = 0.2$, $P < 0.05$) due to higher range of soil C:N at the forest sites. These findings suggest that low soil N contents increase C losses per microbial biomass as indicated by the nutrient mining theory. However, while one might expect higher N immobilization by microbes with increasing soil C:N ratio, N leaching per microbial biomass was observed to increase with higher soil C:N both in forests ($r = 0.7$, $P < 0.001$) and in grasslands ($r = 0.2$, $P < 0.05$). In the most acidic study region, low CO₂ evolution rates co-occurred with high P release rates in coniferous forests, so that CO₂-to-P-release ratios were significantly smaller in coniferous than deciduous forests.
- III. Various glucose concentrations (equivalent to 30-920% microbial C) addition results in positive soil priming, which is a much higher range compared to previous study which reported that the addition of more than 50% microbial C would result in the inhibition of SOM decomposition and hence negative priming. The addition of glucose results in different respiration curves in forests and grasslands. This has been previously linked to differences in microbial activities between the two land use types (Stotzky and Norman 1961).

IV Across different study regions, cumulative respiration after normalization with organic C following glucose addition is significantly higher in forests than grasslands. Fractionation of CO₂ respired to its sources using isotopic mass balance equation shows that the higher cumulative respiration of forest soils is due to significantly higher amounts of glucose-derived CO₂ and therefore faster turnover of the added labile C in forests than grasslands. This was linked to significant increase in phosphatase activities in forest soils, suggesting that glucose addition stimulated higher microbial growth and P acquisition in forests than grasslands.

Summary

Carbon (C) turnover has been increasingly shown to be linked to the mineralization of other nutrients due to the co-limitation of these elements on microbial processes. Regional factors such as climate and parent materials, land use and management practices are important controls of soil nutrient and availabilities that potentially result in mineral soils with varied carbon-to-nutrient ratios. These factors are thought to have important effects on both soil mineralization potentials and the intensity of priming effect. Still, the overall effects of nutrient stoichiometry on soil organic matter (SOM) mineralization and the release of plant-available nutrients is still unresolved. Additionally, few experimental studies have tried to disentangle the confounding effects of regional effects, land use and management practices in one single study.

The main objectives of this thesis were to study the interactions between C and nutrients in the mineral soils particularly in relation to regional, land use and management effects (i.e. tree species effects in forests and fertilization in grasslands). Emphasis was given on understanding (i) the comprehensive interactions between C, N, P and S mineralization rates in relation to C-to-nutrients stoichiometric effects, (ii) the effects of different concentrations of labile C on native C mobilization via priming effects and (iii) the intensity of priming effects under different land uses and managements via microbial nutrient mining.

To answer research questions pertaining to the interactions and main drivers of soil C and nutrient cycling, the potential mineralization rates of carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) were studied simultaneously under controlled conditions in the laboratory. Samples were incubated for two weeks in microlysimeters and CO₂ evolution as well as leachable dissolved organic carbon (DOC), NO₃⁻, NH₄⁺, SO₄²⁻ and PO₄³⁻ were determined as indicators for net nutrient mineralization. Substrate-induced soil incubations using different concentrations of ¹³C-labelled glucose solutions were designed to study how these different labile C concentrations especially in relative to microbial C concentrations in soils affect the extent of native C mobilization. To study the intensity of soil priming under different land uses and management, again substrate-induced soil incubations were performed but with the same concentration of ¹³C-labelled glucose solution for all soil samples. Extracellular enzyme assays of four different enzymes were performed to investigate which nutrient(s) limit microbial activities when C limitation in soil is eliminated through glucose addition, and

whether soil priming was induced by microbial nutrient mining. The study sites were distributed across 3 regions in Germany: (i) “Shorfheide-Chorin” (SCH), (ii) “Hainich-Dün” (HAI) and (iii) “Schwäbische Alb” (ALB) and comprised of differently managed forests (deciduous and coniferous forests) and grasslands (fertilized and unfertilized). One hundred plots were selected from each region and equally from each land use type, 50 forest and 50 grassland sites, with a total of 300 study sites in 3 regions.

Overall, land use and management types were observed to affect the rates of CO₂ production relative to nutrient release. As hypothesized, this was mainly due to the differences in soil C-to-nutrient ratios between these land use types, where higher ratios were observed in forests than grasslands. The rates of CO₂ and DOC released was significantly higher in grasslands than forests in all regions, while DOC release was significantly higher in coniferous than deciduous forests in two out of three regions. No consistent effect of forest management could be detected on soil respiration and microbial biomass in all regions, and this might be due to the differences in soil properties such as pH and soil texture among regions. In contrast to expectations, CO₂-to-N- and CO₂-to-S-leaching ratios were significantly higher in grasslands than forests in all regions. This was mostly caused by significantly higher CO₂ and significantly lower N and S leaching in grasslands than forests which might be due to higher microbial biomass at the grassland sites. According to expectation however, CO₂-to-N- and CO₂-to-S-leaching ratios were significantly higher in coniferous than deciduous forests in two out of the three study regions. This was mostly caused by significantly higher N leaching in deciduous than coniferous forests. In grasslands, no fertilization effect was observed on soil OC and microbial biomass C concentrations, CO₂ and DOC released as well as CO₂-to-N- and CO₂-to-S-leaching ratios. Overall, higher microbial CO₂ respiration and lower nutrient mineralization is suggested to be due to higher nutrient immobilization due to higher microbial biomass C in grasslands than forests.

When normalized to microbial biomass C, soil C:N positively correlated to C mineralization DOC leaching. This correlation was much stronger in forests (CO₂: $r = 0.7$, $P < 0.001$, DOC: $r = 0.8$, $P < 0.001$) as compared to grasslands (CO₂: $r = 0.2$, $P < 0.05$, DOC: $r = 0.2$, $P < 0.05$) due to higher range of soil C:N at the forest sites. These findings suggest that low soil N contents increase C losses per microbial biomass as indicated by the nutrient mining theory. However, while one might expect higher N immobilization by microbes with increasing soil C:N ratio, N leaching per microbial biomass was observed to increase with higher soil C:N both in

forests ($r = 0.7$, $P < 0.001$) and in grasslands ($r = 0.2$, $P < 0.05$). In the most acidic study region, low CO₂ evolution rates co-occurred with high P release rates in coniferous forests, so that CO₂-to-P-release ratios were significantly smaller in coniferous than deciduous forests. As hypothesized, different from N and S, soil C:P ratios were observed to be uncoupled from C mineralization, which could be due to the extracellular enzymatic cleavage of PO₄³⁻. Overall, the results support the paradigm of reduced microbial carbon use efficiency at nutrient poor forest soils, but high N leaching per microbial biomass in N poor soils questions the concept that this is caused by microbial N deficiency.

Positive priming was observed following the addition of various glucose concentrations which were equivalent to 30-920% microbial C concentrations to the soil samples. This range was much higher compared to previous study which reported that the addition of more than 50% microbial C would result in the inhibition of SOM decomposition and hence negative priming. Additionally, it was observed that glucose input resulted in different respiration curves in forests and grasslands which was influenced by the differences in microbial activities between the two land use types.

Using ¹³C labelling and isotopic mass balance equation, CO₂ respired was fractionated according to its sources. Results showed that the higher cumulative respiration in forests was due to significantly higher amount of glucose-derived CO₂, showing faster microbial turnover of the added labile C in forests than in grasslands. Results from extracellular enzyme assays revealed that when C is not limiting, P may limit microbial activities in forest soils. Increase in phosphatase activities following glucose addition only in forest soils showed that part of the labile C added may be used by soil microbes to produce more extracellular enzyme in order to mine P. Together with positive priming observed, these results confirmed that priming effects at the forest sites was most probably induced by microbial P mining, even though priming due to nutrient mining in grasslands was less clear.

Overall, the findings from this thesis confirmed the importance of nutrient stoichiometry on SOM mineralization, and highlight the important interactions between C, N, P and S mineralization under differently managed soils in forests and grasslands. However, additional data are required to answer questions that are still open as well as new questions that were formed. Regional differences especially in soil clay content were shown to be important drivers of soil mineralization potentials. Therefore, it is important for future studies to take

into consideration the confounding effects of soil clay content with the concentrations of SOC, microbial C, total N, P and S as well as soil pH, which were all observed to be main positive drivers of soil C mineralization. Higher C mineralization rates, higher C:N and C:S mineralization ratios and different C mineralization rates in grasslands than forests were indicated by higher microbial activities and nutrient (N and S) immobilization in grasslands than in forests at the temperate region. The coupling of C to nutrient mineralization as constrained by soil nutrient availability was observed. However, it wasn't clear whether increasing C mineralization, DOC leaching and at the same time N mineralization with increasing soil C:N was due to decreased CUE and increasing NUE, or that lower microbial activities at sites with high C:N ratios and low soil pH resulted in overall lower CO₂ respiration and N immobilization. This uncertainty may be addressed in future studies by studying for example net N immobilization on top of net mineralization, as higher microbial N uptake would be expected despite increasing net N mineralization with decreasing soil N sources. Additionally, both mineralization and priming studies showed that microbial processes in forests are probably be limited by both C and P availability. It would be therefore interesting to verify this by conducting further experiments i.e. C and P addition and the its effects on microbial N and S uptake. Lastly, land use types were shown to be important driver for both soil mineralization potentials and soil priming due to differences in microbial biomass and thus activities at these two sites. However, these effects may be also due to other management practices (e.g. age class, selective harvesting, mixed forests, mowing and grazing) which were not included in this thesis. This poses further challenge in future studies to conduct a more in-depth investigation of other management practices on soil C and nutrient interactions.

Zusammenfassung

Der Umsatz von Kohlenstoff (C) hängt mit der Mineralisierung anderer Nährstoffe zusammen, da diese Elemente gleichzeitig bei mikrobiellen Prozessen begrenzt verfügbar sind. Regionale Faktoren wie Klima- und Ausgangsmaterialien, Landnutzung und Bewirtschaftungstypen sind wichtige Regulatoren von Bodennährstoffen und Nährstoffverfügbarkeit die unterschiedliche Kohlenstoff-Nährstoff-Verhältnissen (C:Nährstoff) hervorufen. Es wird angenommen, dass diese Faktoren wichtige Auswirkungen auf das Bodenmineralisierungspotenzial und die Intensität des Priming Effekts haben. Dennoch ist bislang nur wenig bekannt, wie die Gesamtauswirkungen regionaler Effekte sowie Landnutzung und Bewirtschaftungstypen die Bodennährstoffmineralisierung und den Priming-Effekts beeinflussen.

Das Hauptziel dieser Arbeit bestand darin, die Wechselwirkungen zwischen C und Nährstoffen in den Mineralböden auf regionale Landnutzungs- und Bewirtschaftungseffekte zu untersuchen. Der Schwerpunkt lag auf dem Verständnis (i) der umfassenden Wechselwirkungen zwischen den C- und Nährstoff Mineralisierungsraten in Bezug auf stöchiometrische C-zu-Nährstoff-Effekte, (ii) der Auswirkungen verschiedener Konzentrationen von labilem C auf die native C-Mobilisierung durch Priming-Effekte und (iii) die Intensität der Priming-Effekte bei verschiedener Landnutzung und -managementformen durch mikrobiellen Nährstoffabbau.

Um Forschungsfragen zu den Wechselwirkungen und Haupttreibern des Bodens C und des Nährstoffkreislaufs zu beantworten, wurden die potenziellen Mineralisierungsraten von Kohlenstoff (C), Stickstoff (N), Phosphor (P) und Schwefel (S) gleichzeitig unter kontrollierten Bedingungen im Labor untersucht. Die Proben wurden zwei Wochen lang in Mikrolysimeter inkubiert und die CO₂-Entwicklung sowie auslaugbarer gelöster organischer Kohlenstoff (DOC), NO₃⁻, NH₄⁺, SO₄²⁻ und PO₄³⁻ als Indikatoren für die Netto-Nährstoffmineralisierung bestimmt. Um zu untersuchen, wie unterschiedliche labile C-Konzentrationen das Ausmaß der nativen C-Mobilisierung beeinflussen, wurden substratinduzierte Bodeninkubationen unter Verwendung verschiedener Konzentrationen von ¹³C-markierten Glukoselösungen entworfen. Um die Intensität der Bodengrundierung bei verschiedenen Landnutzungen und -managementformen zu untersuchen, wurden substratinduzierte Bodeninkubationen mit der gleichen Konzentration an ¹³C-markierter

Glukoselösung für alle Bodenproben durchgeführt. Um zu untersuchen, welche Nährstoffe die mikrobielle Aktivität einschränken, wenn die C-Begrenzung im Boden durch Glukosezugabe aufgehoben wird, und ob das Boden-Priming durch mikrobiellen Nährstoffabbau induziert wird, wurden extrazelluläre Enzymtests mit vier verschiedenen Enzymen durchgeführt. Die Untersuchungsflächen waren auf drei Regionen in Deutschland verteilt: (i) „Schorfheide-Chorin“ (SCH), (ii) „Hainich-Dün“ (HAI) und (iii) „Schwäbische Alb“ (ALB) und bestanden aus unterschiedlich bewirtschafteten Wäldern (Laub- und Nadelwälder) und Wiesen (gedüngt und ungedüngt). Insgesamt wurden 300 Untersuchungsgebieten in drei Regionen (je 50 Wald- und 50 Grünlandflächen) ausgewählt.

Insgesamt wurde beobachtet, dass Landnutzungs- und Bewirtschaftungstypen die CO₂-Produktionsrate im Verhältnis zur Nährstofffreisetzung beeinflussen. Wie vermutet, lag dies hauptsächlich an den Unterschieden der Boden-C-Nährstoffverhältnisse zwischen den Landnutzungstypen, bei denen in Wäldern höhere Verhältnisse als bei Grünland beobachtet wurden. Die Freisetzungsraten von CO₂ und DOC waren in allen Regionen in Grünland signifikant höher als in Wäldern, während die DOC-Freisetzung in Nadelwäldern in zwei von drei Regionen signifikant höher war als in Laubwäldern. In allen Regionen konnte kein konsistenter Effekt der Waldbewirtschaftung auf die Bodenatmung und mikrobielle Biomasse festgestellt werden. Dies könnte auf die unterschiedlichen Bodeneigenschaften wie pH-Wert und Bodentextur der Regionen zurückzuführen sein. Entgegen den Erwartungen waren die CO₂:N- und CO₂:S-Auslaugungsquoten im Grünland in allen Regionen deutlich höher als in den Wäldern. Dies wurde hauptsächlich durch signifikant höhere CO₂-Emissionen und eine deutlich geringere N- und S-Auslaugung im Grünland verursacht als in den Wäldern, was möglicherweise auf eine höhere mikrobielle Biomasse an den Grünlandstandorten zurückzuführen ist. Erwartungsgemäß waren die CO₂:N- und CO₂:S-Auslaugungsraten in Nadelwäldern jedoch signifikant höher als in Laubwäldern in zwei der drei Untersuchungsgebiete. Dies wurde hauptsächlich durch eine signifikant höhere N-Auslaugung in Laubwäldern gegenüber den Nadelwäldern verursacht. In Grünland wurde kein Befruchtungseffekt auf die OC- und mikrobiellen Biomasse-C-Konzentrationen im Boden, auf freigesetztes CO₂ und DOC sowie auf die CO₂:N- und die CO₂:S-Auslaugungsrate beobachtet. Insgesamt wird davon ausgegangen, dass eine höhere mikrobielle CO₂-Atmung und eine geringere Nährstoffmineralisierung auf eine höhere Nährstoffimmobilisierung

zurückzuführen sind. Dies ist auf eine höhere mikrobielle Biomasse C im Grünland als in den Wäldern zurückzuführen.

Bei der Normalisierung auf mikrobielle Biomasse C korrelierte der Boden C:N positiv mit der DOC-Auslaugung von C. Diese Korrelation war aufgrund höherer Bodenreichweite C:N an den Waldstandorten deutlich stärker (CO_2 : $r = 0,7$, $P < 0,001$, DOC: $r = 0,8$, $P < 0,001$) als im Vergleich zu Grünland (CO_2 : $r = 0,2$, $P < 0,05$, DOC: $r = 0,2$, $P < 0,05$). Diese Ergebnisse deuten darauf hin, dass niedrige N-Gehalte im Boden die C-Verluste pro mikrobielle Biomasse erhöhen, wie durch die Theorie des Nährstoffabbaus angezeigt wird. Während man mit zunehmendem Boden-C:N-Verhältnis eine höhere N-Immobilisierung durch Mikroben erwarten könnte, wurde jedoch mit zunehmendem Boden-C:N sowohl in Wäldern ($r = 0,7$, $P < 0,001$) als auch in Wiesen ($r = 0,2$, $P < 0,05$) steigende N-Auslaugung beobachtet. In der am stärksten säurehaltigen Untersuchungsregion traten niedrige CO_2 -Entwicklungsraten mit hohen P-Freisetzungsraten in Nadelwäldern auf, sodass das Verhältnis von CO_2 zu P-Freisetzung in Nadelwäldern signifikant geringer war als in Laubwäldern. Zur Unterstützung der zweiten Hypothese, wurde beobachtet, dass die C:P-Verhältnisse im Boden, im Gegensatz N und S, von der C-Mineralisierung entkoppelt wurden, was auf die extrazelluläre enzymatische Spaltung von PO_4^{3-} zurückzuführen sein könnte. Die Ergebnisse unterstützen das Paradigma der reduzierten Effizienz der mikrobiellen Kohlenstoffnutzung in nährstoffarmen Waldböden. Eine hohe N-Auslaugung pro mikrobielle Biomasse in N-armen Böden stellt jedoch die Vorstellung in Frage, dass dies auf mikrobiellen N-Mangel zurückzuführen ist.

Ein positives Priming nach Zugabe von verschiedenen Glukosekonzentrationen (die 30-920% mikrobiellen C-Konzentrationen entsprachen) zu den Bodenproben wurde beobachtet. Dieser Bereich war im Vergleich zu früheren Studien, in denen berichtet wurde, dass die Zugabe von mehr als 50% mikrobiellem C zu einer Hemmung der SOM-Zersetzung führen würde, viel höher. Darüber hinaus wurde beobachtet, dass der Glukoseeintrag zu unterschiedlichen Atmungskurven in Wäldern und Grünland führte, was durch die unterschiedlichen mikrobiellen Aktivitäten der beiden Landnutzungsarten beeinflusst wurde.

Unter Verwendung der ^{13}C -Markierung und isotopischen Massenbilanzgleichung wurde das atmende CO_2 gemäß seinen Quellen fraktioniert. Die Ergebnisse zeigten, dass die höhere kumulative Atmung in Wäldern auf eine wesentlich höhere Menge an Glukose-gewonnenem

CO₂ zurückzuführen war. Ergebnisse von extrazellulären Enzymtests zeigten, dass P mikrobielle Aktivitäten in Waldböden einschränken kann, wenn C kein limitierender Faktor ist. Die Zunahme in Phosphatase Aktivitäten nach der Zugabe von Glukose zeigte sich nur in Waldböden und dass ein Teil des zugesetzten labilen C von Bodenmikroben verwendet werden kann, um extrazelluläres Enzym zu produzieren. Zusammen mit dem beobachteten positiven Priming bestätigten diese Ergebnisse, dass die Priming-Effekte an den Waldstandorten höchstwahrscheinlich durch mikrobiellen P-Abbau ausgelöst wurden.

Insgesamt bestätigten die Ergebnisse dieser Arbeit die Bedeutung der Nährstoffstöchiometrie bei der SOM-Mineralisierung und unterstreichen die wichtigen Wechselwirkungen zwischen der C-, N-, P- und S-Mineralisierung unter unterschiedlich bewirtschafteten Böden in Wäldern und Grünland. Es sind jedoch zusätzliche Daten erforderlich, um noch offene Fragen und neugebildete Fragen zu beantworten. Regionale Unterschiede, insbesondere beim Bodentongehalt, erwiesen sich als wichtige Treiber für Bodenmineralisierungspotenziale. Für zukünftige Studien ist es jedoch wichtig, die miteinander verbundenen Wechselwirkungen des Bodensandgehalts mit den Konzentrationen von SOC, mikrobiellem C, Boden-N, P und S-Konzentration sowie dem Boden-pH-Wert zu berücksichtigen. Unterschiedliche C-, C:N-, C:S-Mineralisierungsraten und C-Mineralisierungsraten im Grünland als in Wäldern wurden durch höhere mikrobielle Aktivitäten und die Immobilisierung von Nährstoffen (N und S) im gemäßigten Klima angedeutet. Die Kopplung von C an die Nährstoffmineralisierung, wie sie durch die Verfügbarkeit von Bodennährstoffen eingeschränkt wird, wurde beobachtet. Es ist jedoch nicht klar, ob die zunehmende C-Mineralisierung, die DOC-Auslaugung und die N-Mineralisierung mit zunehmendem Boden-C:N auf einen verringerten CUE und einen zunehmenden NUE-Wert zurückzuführen ist oder ob die niedrigen mikrobiellen Aktivitäten an Standorten mit hohen C:N-Verhältnissen und niedrigerem pH-Wert in niedrigen Gesamt CO₂ Atmung und N Immobilisation resultieren. Dieser Unsicherheit kann in zukünftigen Studien entgegengewirkt werden, indem die N-Immobilisierung zusätzlich zur Netto-Mineralisierung untersucht wird, wobei trotz einer zunehmenden N-Mineralisierung mit abnehmenden Boden-N-Quellen eine höhere Aufnahme von mikrobiellem N zu erwarten wäre. Es wurde zusätzlich beobachtet, dass mikrobielle Prozesse in Wäldern durch die Begrenzungen von C und P begrenzt sind. Daher wäre es interessant dies durch weitere Experimente beispielweise die Zugabe von C und P und die Auswirkungen auf die N- und S-Aufnahme von Mikroben zu überprüfen.

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Selbständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angefertigt habe.

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Ort, Datum

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Huei Ying Gan

