Land management and soil property effects on soil microbial communities and carbon storage in temperate forest and grassland systems

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Chapter 1

Introduction

The world´s soils constitute the largest pool of terrestrial organic carbon (OC) containing about 1550 Gt (Lal, 2004a). Soil OC (SOC) plays an important role in regulating soil fertility and is a strong determinant of numerous ecosystem services. For example, an increase in the SOC pool improves water quality and soil structure as well as increases soil biodiversity (Lal, 2004c). In addition, there is a strong interest to sequester SOC to mitigate the current anthropogenic enrichment of atmospheric carbon dioxide (Lal, 2004b).

The amount of SOC is determined by the balance between carbon (C) inputs by plant production and the release of C during decomposition (Fig. 1). Different land use and management practices affect the quantity and quality of aboveground and belowground litter input into the soil, and thus the spatial distribution of microbial substrates.

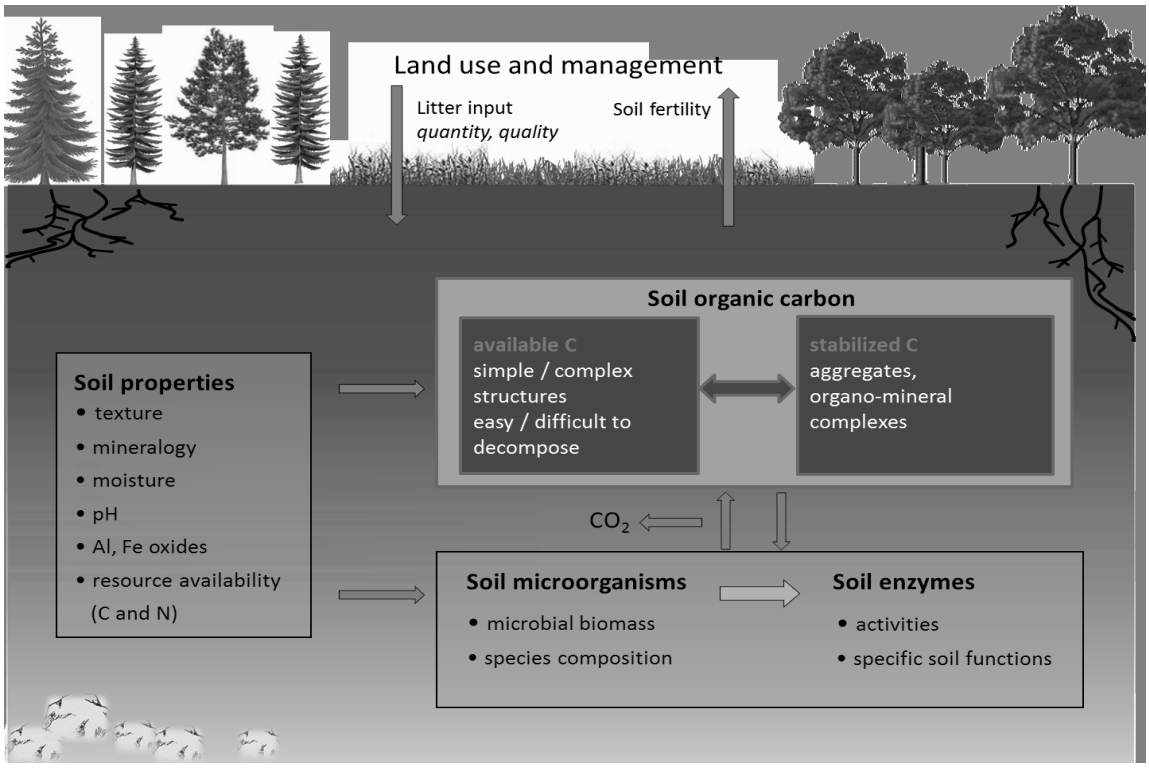

Fig. 1.1 Simplified outline of OC storage in the soil and its controlling factors.

Soil microorganisms and their released enzymes play a key role in the decomposition of soil organic matter (SOM) and break down large polymeric organic compounds such as cellulose, hemicellulose, lignin, and proteins. The decomposition of OM, in turn, provides energy for microbial growth and supplies C for the formation of new cells; however, most of the C in the decomposition process is released as carbon dioxide. The availability of OC for microorganisms is related to the OM fraction in which it resides. Soil OC is part of the SOM, a complex mixture of organic compounds at various stages of decomposition. Beyond the potential impact of land use and management practices, soil properties are likely to influence the microbial community, enzyme activities, storage and turnover of different OM fractions. To realize the potential of soils for C sequestration in the long term, we need to improve our understanding of the interactions between environmental factors, soil microbiological properties, storage and turnover of different OM fractions, that impact OC cycling in soils (Singh et al., 2010).

Microbiological properties are considered to be more sensitive to changes in management and environmental conditions than bulk chemical or physical soil properties (Bandick and Dick, 1999; Acosta-Martínez et al., 2004). Thus, it should be possible to detect changes in microbial community composition and activity before changes in OC. There are several studies that investigate the influence of different management practices on soil microbial biomass, community composition and enzyme activities in forest and grassland systems (Bardgett et al., 1999a; Bardgett et al., 2001; Andersson et al., 2004; Hassett and Zak, 2005; Grayston and Rennenberg, 2006; Maassen et al., 2006). Management effects on soil microbiological properties have largely been studied in field manipulations, especially in grassland systems. Although these experiments help to understand the effects of single management practices, in practice, two or even three grassland management practices are commonly applied simultaneously. The combination of different management practices can lead to different effects on soil microbial communities and enzyme activities compared with single management practices, but studies to analyse combined effects are rare (for instance, (Grayston et al., 2001)).

Forest management practices such as tree species selection, harvesting and thinning have been shown to directly and indirectly affect soil enzyme activities (Hassett and Zak, 2005; Weand et al., 2010). Many studies only considered short-term effects (<10 yr) of various forest management practices on enzyme activities, while much less is known about long-term effects (>20 yr). Further, microbial biomass and enzyme activities decrease with soil depth in association with a decrease in C concentrations (Taylor et al., 2002). Thus,

most studies on microbial communities and enzyme activities affected by forest management have been conducted with samples from the A horizon or the topsoil horizon, whereas studies of subsoil horizons are rare (Taylor et al., 2002; Zhang et al., 2004; Sotomayor-Ramírez et al., 2009). However, a considerable amount of the OC within the soil profile is stored in subsoil horizons despite low C concentrations (Batjes, 1996; Wang et al., 2010). Previous studies showed that roots and rhizodeposition account for substantial belowground litter input into soils (Kögel-Knabner, 2002; Schmidt et al., 2011). In addition, high amounts of aboveground litter input favour the production of dissolved organic carbon (DOC) that can be transferred to deeper soil horizons and thus contribute to new OC in SOM in subsoil horizons (Lorenz and Lal, 2005). Thus, the impact of different forest management practices on microbial functions needs to be investigated with a particular focus on subsoil horizons, which may have significant implications for the global scale cycling of C.

Soil OM can be separated by density fractionation into functional fractions that differ in their sensitivity to land use and management changes. The light fraction of uncomplexed particulate OM is composed mainly of plant and animal tissues with short turnover times, and it was found to vary the most in relation to management (Bremer et al., 1994; Gregorich and Janzen, 1996). In contrast, the mineral-associated OM fraction remains relatively stable in response to management effects with turnover times of decades to centuries (Baisden et al., 2002; Meyer et al., 2012). Although density fractionation is frequently applied to grassland and forest soils, only a few studies have focused on the radiocarbon (^{14}C) content and turnover of OC in different density fractions (John et al., 2005; Swanston et al., 2005; Schulze et al., 2009; Schrumpf et al., 2013). Most of the existing literature determining OC turnover of density fractions under different land management practices has focused on the conversion of forest to pasture or agriculture (Trumbore et al., 1995; Poirier et al., 2006). Even fewer studies have examined different management practices (Leifeld and Fuhrer, 2009; Meyer et al., 2012). To date, there is a lack of knowledge of the effects of different management practices in natural temperate forests and grasslands on OC storage and turnover in different density fractions.

Early research showed that different density fractions react differently to changes in land management and soil properties. It has been shown that OC storage and turnover in the mineral-associated OM fraction is more affected by soil properties than by forest management (Schöning et al., 2013). However, studies that quantify the impact of land use,

management, and soil properties on OC storage and ^{14}C signatures for different density fractions across regional scales are not available.

Analyses of management effects on soil microbiological properties are also influenced by differences in abiotic soil properties between sites; thus it is important to consider soil properties when studying management effects. Differences in climate, topography and geology between sites lead to different soil types and properties (e.g. texture, nutrient availability and pH), which also influence microbial community biomass, composition and enzyme activities (Bossio et al., 1998; Wardle, 1998; Grayston et al., 2001; Sinsabaugh et al., 2008). Zeller et al. (2001) studied the relative importance of different study sites and management practices on the variability of soil microbial biomass and community structure in subalpine grasslands of the European Alps. They concluded that the effect of site had a greater impact on soil microbial biomass compared with management abandonment. This result highlights the need to study management effects on soil microbiological properties in relation to soil properties. Furthermore, it has been shown that results obtained at one site with specific soil characteristics cannot be generalized and transferred to other sites/regions with different soil properties (Gianfreda et al., 2005). There is a need for studies on larger spatial scales to derive general relationships between soil properties and soil microbiological properties (Birkhofer et al., 2012).

One way to study the community composition of soil microorganisms is by phospholipid fatty acid (PLFA) analysis (Vestal and White, 1989). Phospholipid fatty acids are components of cellular membranes of all living cells, and certain PLFAs can be used as biomarkers for specific microbial groups (Vestal and White, 1989). The link between soil microbes and their functions can be made by studying the activity of extracellular enzymes in the soil (Caldwell, 2005). Extracellular enzymes catalyse the degradation of plant, animal and microbial macromolecules and their activities can provide information on biochemical processes important in soil functioning such as the degradation potential (Trasar-Cepeda et al., 2000), and have been used as potential sensitive indicators of sustainability and management changes (Nannipieri, 1994; Beyer et al., 1999). Enzymes can exist freely in the soil solution, but a greater amount is associated with active cells (plants, animals, microbes), dead cells and cell debris as well as complexed with clay minerals and humic colloids (Burns, 1982). While free enzymes in the soil solution are short-lived, they can be stabilized via sorption to clay minerals and humic substances (Sarkar et al., 1989). This sorption to clay particles can lead to shifts in the catalytic potential of enzymes, though they are still active in the clay fraction (Marx et al., 2005).

In the past, numerous chemical and physical fractionation schemes have been developed to separate OM fractions with different chemistries and stabilities. Different chemical fractionation methods have been shown to separate OM pools that have similar turnover rates (Balesdent, 1996). Thus, physical fractionation of OM that is based either on density or particle size fractionation has been suggested to determine OC dynamics and stabilization in soils (Christensen, 1992). Further, ^{14}C analysis is increasingly applied to soil samples to determine the ^{14}C age and turnover of OC in density fractions (Trumbore and Zheng, 1996; Trumbore, 2009).

Considering the literature review and the gaps in our understanding mentioned above, this thesis intends to improve our knowledge of the relationships between environmental factors and soil microbial communities, OC distribution and turnover in OM fractions; in this way, this work should contribute to a better understanding of SOC storage in forest and grassland systems. While previous research has provided important insights, there is a series of research needs that require further attention:

- Studying and comparing the effects of land use and management on soil microbial communities and OC storage and turnover in several regions and natural ecosystem conditions to draw general conclusions about relationships across geographic regions.
- Assessing long-term effects of land use and management and their interactions with microbial communities and ecosystem functioning at regional scales.
- Further exploring the potential of using enzyme activities to study shifts in nutrient and energy supply and demand in the whole soil profile to predict how C and nutrient cycling will respond to environmental changes in the future.
- Combining novel approaches like PLFA, enzyme activity analysis, soil density fractionation, ^{14}C analysis, and various statistical analyses to further enhance our knowledge of the environmental impact on soil microbial communities as well as the spatial variation and turnover of OC in density fractions, which in turn has high implications for OC cycling and storage in soils.

1.1 Objectives

Considering the research needs mentioned in the previous section, the overall aim of this thesis is to improve our understanding of the factors that affect microbial communities and OC storage in temperate forest and grassland systems. The specific focus is on soil

microbial biomass and community composition, enzyme activities, OC distribution and turnover in different density fractions in relation to land use, management and soil properties at the regional scale. This thesis aims to accomplish the following objectives:

(1) To determine the impact of long-term grassland management and soil properties on total PLFA biomass, microbial community composition, and enzyme activities across the regional scale.
(2) To study enzyme activities and nutrient supply and demand in whole soil profiles, and to quantify the relative influence of long-term forest management and soil properties on enzyme activities in different soil horizons across a latitudinal gradient.
(3) To evaluate the storage and radiocarbon signatures of OC in three different density fractions of forest and grassland sites under diverse management practices and soil properties in different regions.

The research presented here is part of a joint initiative for functional biodiversity research called "the Biodiversity Exploratories". Overall, it investigates important questions on the feedback between land use, biodiversity and ecosystem processes in real-world ecosystems (Fischer et al., 2010). The Biodiversity Exploratories were established in three regions in Germany, the Schorfheide-Chorin in northern Germany, the Hainich-Dün in central Germany and the Schwäbische Alb in southern Germany. Within each region, 18 very intensive field plots (VIPs) were set up: half of them in forests and the other half in grasslands, with different management types and intensities. The plots in each region covered three forest management types (spruce/pine and beech forest under age-class management, unmanaged beech forest) and three grassland management types (fertilized mown meadows, fertilized mown pastures, unfertilized pastures). Each management type was studied with three replicates. In the Hainich-Dün, three additional VIPs were established in forests in order to consider the forest under selection cutting as an important management type in these forests. The three regions were characterised by distinct parent materials and climates. In the Schorfheide-Chorin, glacial till was the dominant parent material. The glacial till was often covered by aeolian and fluvial sand or by organic material in depressions. In the Hainich-Dün, the parent material was loess over Triassic shell lime-stone. Soils in the Schwäbische Alb were developed on Jurassic lime-stone. Mean annual temperatures ranged from 8-8.5 °C in the Schorfheide-Chorin, 6.5-8 °C in the Hainich-Dün and 6-7 °C in the Schwäbische Alb. Mean annual precipitation increased

from the Schorfheide-Chorin to the Hainich-Dün to the Schwäbische Alb, ranging from 500 to 1000 mm.

1.2 Thesis organization

The thesis is organized into three results chapters that are thematically aligned with the three main research objectives described above. An evaluation of the impact of long-term grassland management and soil properties on soil microbiological properties in grassland sites across three regions in Germany is provided in Chapter 2. The analysis focuses on total microbial biomass and community composition, enzyme activities, and different soil properties in the topsoil horizon of grassland soils. The relationships between soil properties and soil microbiological properties, after accounting for large-scale effects and management practices, allowed the identification of general relationships between soil properties and biotic responses.

Chapter 3 provides an assessment of soil horizon related variations of enzyme activities in relation to long-term forest management and soil properties across a latitudinal gradient in Germany. Soil enzyme activity ratios were calculated to assess shifts in resource allocation among different regions and soil horizons. Redundancy analysis in combination with variance partitioning is a useful tool to study complex relationships between enzyme activities and management practices when soil properties vary. This offered the possibility of clarifying how much of the total variation in the enzyme activities is attributable to study region, forest management and soil physico-chemical properties encompassing horizon thickness, clay content, OC and total nitrogen (TN) concentrations, C:N ratio, pH, as well as aluminium and iron oxides.

In Chapter 4, OC contents and ^{14}C signatures of three density fractions were measured in order to deepen our understanding of the impact of the study region, land use, management practices, and soil properties on OC dynamics and turnover among density fractions in forest and grassland soils of low mountain ranges. Correlations between ^{14}C values of OC in all three density fractions allowed further insight into how the different fractions are related to each other, which have significant implications for modelling turnover times in density fractions.

Chapter 5 presents a synthesis of the studies on microbial communities, enzyme activities and OC storage in forest and grassland systems emphasizing the impact of land management and soil properties across regional scales. With the implementation of the

findings of this thesis into the current knowledge on effects the of land management and soil properties on soil microbial communities and OC storage, implications towards the use of soil microbiological properties and the light fraction OC as sensitive indicators of soil quality are elaborated. Finally, the conclusions for the main results are presented along with future research perspectives.

Chapter 2

Soil property and management effects on grassland microbial communities across a latitudinal gradient in Germany

Chapter source: Nadine Herold et al., 2013. Soil property and management effects on grassland microbial communities across a latitudinal gradient in Germany. Applied Soil Ecology. (revised version of the submitted manuscript)

Abstract

There is much interest in the identification of the main drivers that control changes in the microbial community that may be related to sustainable land use. We examined the influence of soil properties and land-use intensity (N fertilization, mowing, grazing) on total phospholipid fatty acid (PLFA) biomass, microbial community composition (PLFA profiles) and activities of enzymes involved in the C, N, and P cycle. These relationships were examined in the topsoil of grasslands from three German regions (Schorfheide-Chorin (SCH), Hainich-Dün (HAI), Schwäbische Alb (ALB)) with different parent material.

Soils of the SCH formed on degraded peatlands in lowland areas and are still periodically flooded and are rich in organic carbon (OC) and sand, while soils of the HAI and ALB are in low mountain ranges and are finer textured, drier, and have smaller OC concentrations. Differences in soil properties explained 60 % of variation in PLFA data and 81 % of variation in enzyme activities across regions and land-use intensities. Although soils in the SCH contained lower PLFA biomass, lower concentrations of bacterial, fungal, and arbuscular mycorrhizal PLFAs, they showed greater enzyme activities, and specific enzyme activities (per unit microbial biomass) than soils in the other two regions. After extraction of variation that originated from large-scale differences among regions and differences in land-use intensities between plots, soil properties still explained a significant amount of variation in PLFA data (34 %) and enzyme activities (60 %). Total PLFA biomass and all enzyme activities were mainly related to OC concentration, while microbial community

composition was mainly related to soil moisture. Land-use intensity (LUI) significantly decreased the soil C:N ratio. There was no effect of LUI on total PLFA biomass, microbial community composition, N and P cycling enzyme activities. In contrast, the activities and specific activities of enzymes involved in the C cycle increased significantly with LUI independent of study region and soil properties, which can have impact on soil organic matter decomposition and nutrient cycling.

Our findings demonstrate that microbial biomass and community composition as well as enzyme activities are more controlled by soil properties than by grassland management at the regional scale.

Keywords: Temperate grasslands, Degraded peat soils, Land-use intensity, Phospholipid fatty acid (PLFA), Specific enzyme activities

2.1 Introduction

Soil microorganisms play a significant role in many ecosystem processes such as soil organic matter decomposition, nutrient cycling, and organic carbon (OC) sequestration. At the same time, they are important drivers of plant diversity and productivity in terrestrial ecosystems and thus of sustainable land use (Van der Heijden et al., 2008). There are various factors regulating the abundance, composition and activities of soil microorganisms such as edaphic factors (soil type, texture, moisture, pH, nutrient availability) or land management practices. In the light of the impact of agricultural systems on ecosystem functioning we need to improve our understanding of the main factors that determine the composition and functions of soil microorganisms in such ecosystems.

In Europe, grasslands cover about 13 % of the land area (Eurostat, 2011), and have great potential to sequester carbon by improving grassland management practices (Conant, 2010). Grasslands in Germany exist in different geographical regions such as in lowland areas in northern Germany or in low mountain ranges (upland areas) in central and southern Germany (Fischer et al., 2010). Differences in climate, topography, and parent material among regions result in different soil types and properties. Therefore, the range of OC concentrations in German grassland soils is wide, with the highest OC concentrations and high soil moisture occurring in lowland areas where former peat soils were frequently drained and subsequently managed as grasslands. Soils in upland areas in Germany, in

contrast, often contain much less OC and soil moisture. Variations in soil properties between regions such as texture, soil temperature, soil moisture, nutrient availability, and pH, in turn, have been shown to impact microbial community composition and related enzyme activities in mineral soils (Grayston et al., 2001; Brodie et al., 2002; Lauber et al., 2008; Sinsabaugh et al., 2008). In peat soils, waterlogged conditions are a major driver of microbial community composition and enzyme activities (Kang and Freeman, 1999). At present, however little is known about degraded peat soils. In addition, the aforementioned studies have mainly focused on the relation between soil properties and microbes in only one region under local conditions. It has been shown that results obtained at one site with specific soil characteristics cannot be generalized and transferred to other sites/regions with different soil properties (Gianfreda et al., 2005). So far, little is known about relationships between soil properties and biota shared among different regions with their specific abiotic conditions (Birkhofer et al., 2012). Thus, there is a need for studies on larger spatial scales to derive general patterns.

Grasslands in lowland and upland areas in Germany are all subject to similar management practices such as nitrogen (N) fertilization, mowing, and grazing. The effect of different management practices on soil microbiological properties has usually been studied using field manipulations. Such experiments have shown that N addition and high grazing intensities favor the growth of bacteria as indicated by lower fungi to bacteria ratios (Bardgett et al., 1999a; Bardgett et al., 2001; Grayston et al., 2001), whereas decreasing the overall abundance of microorganisms (Lovell et al., 1995; Bardgett et al., 1999a; Bardgett et al., 2001). In contrast, mowing has been found to increase soil microbial biomass (Uhlířová et al., 2005). In comparison to microbial community composition or growth, enzyme activities show no clear pattern in response of inorganic N fertilization, mowing, or grazing. Some authors reported, that inorganic N fertilization stimulated activities of C, N and phosphorus (P) acquiring enzymes (Stursova et al., 2006; Keeler et al., 2009), while others found that N fertilization inhibited activities of enzymes involved in the N cycle (Dick, 1992; Olander and Vitousek, 2000). Mowing and grazing has been reported to increase (Le Roux et al., 2003) or decrease (Holt, 1997) the activities of soil enzymes that are fundamental to N cycling. Although experiments help to understand the effects of single management practices, farmers commonly apply a number of different management practices simultaneously. Such field studies to analyze these combined effects are rare (for instance, Grayston et al. (2001)). In addition, many experiments run for only a few years, and therefore long-term effects of grassland management are not well known.

In this study, we analyze total phospholipid fatty acid (PLFA) biomass, microbial community composition, and enzyme activities in the topsoil of grasslands from three German regions that have experienced a long history of management and differ in their soil characteristics (degraded peat soils in lowland areas, mineral soils in upland areas). Grassland management in each study region included N fertilization, mowing, and grazing. With this study we aimed to determine 1) general relationships between soil properties and total PLFA biomass, microbial community composition, and enzyme activities, and 2) the impact of long-term grassland management on total PLFA biomass, microbial community composition, and enzyme activities. We used PLFA profiles as a measure for microbial biomass and community composition (Vestal and White, 1989). The link between soil microbes and their function can be made by studying the activity of extracellular enzymes involved in C, N and P cycling (Caldwell, 2005). Extracellular enzymes are responsible for the breakdown of large polymeric compounds and thus control many metabolic pathways in soils.

2.2 Materials and methods

2.2.1 Study sites

We studied 27 continuously managed grassland plots located in the Biodiversity Exploratories comprising Schorfheide-Chorin (SCH) in northern Germany, Hainich-Dün (HAI) in central Germany and Schwäbische Alb (ALB) in southern Germany (Fischer et al., 2010). These three study regions differed in climatic conditions and parent materials (Table 2.1) and constitute a latitudinal gradient of 800 km. The predominant soil groups in the three study regions were Histosols in the SCH, Stagnosols in the HAI, and Leptosols in the ALB (IUSS Working Group WRB, 2006). In each region, nine grassland plots with different land-use intensities were established. For each plot *i*, the land-use intensity (LUI*i*) (Table 2.2) was calculated according to Blüthgen et al. (2012), and is defined as

$$LUI_i = \frac{F_i}{F_R} + \frac{M_i}{M_R} + \frac{G_i}{G_R}$$

where F_i is the intensity of N fertilization (kg N ha^{-1} yr^{-1}), M_i the mowing frequency and G_i the grazing intensity (livestock units days of grazing ha^{-1} yr^{-1}) for the year 2007, and F_R, M_R, G_R their respective mean within its region *R* for that year.

Table 2.1 General characteristics of the study regions.

Study region	MAT[a] °C	MAP[b] mm	Parent material	FAO-Soil group[c]
Schorfheide-Chorin	8.0-8.5	500-600	Glacio-fluvial sand and glacial till	Histosol, Gleysol
Hainich-Dün	6.5-8.0	500-800	Loess and Triassic shell limestone	Stagnosol, Cambisol
Schwäbische Alb	6.0-7.0	700-1000	Jurassic limestone	Leptosol, Vertisol

[a]MAT = Mean Annual Temperature
[b]MAP = Mean Annual Precipitation
[c]IUSS Working Group WRB (2006)

2.2.2 Soil sampling

In spring 2008, five soil samples were taken at each grassland plot (20 x 20 m), one at each corner and one in the plot centre, and mixed to obtain a composite sample for each plot. Prior to the sampling, the aboveground vegetation was cut and removed. The soils in the HAI and ALB were sampled down to the bedrock (HAI 45±1.7 cm, ALB 20±1.4 cm) using a motor driven auger (8.3 cm diameter). In the SCH a split tube sampler (4.8 cm diameter, 40 cm length) was used to sample Histosols and Gleysols. The uppermost soil horizon, the Ah horizon (mean horizon depth in HAI 10.4±3.0 cm, in ALB 12.6±0.8 cm), and the Ha-horizon of the Histosols (here only the 0 to 10 cm depth increment), was separated from the soil core, stored in ice boxes and transported to the field lab facility. After removal of coarse roots and stones one sub-sample of the soil sample was air-dried, a second was stored at -20°C and another one was stored at -80°C.

2.2.3 General soil properties

Air-dried sub-samples were sieved to < 2 mm and used to determine soil texture, pH, OC, total nitrogen (TN), labile inorganic P (P_i), and labile organic P (P_o) concentrations. Soil texture was determined according to Schlichting and Blume (1966). Soil pH was measured in the supernatant of a 1:2.5 mixture of soil and 0.01 M $CaCl_2$ using a glass electrode. Sub-samples for elemental analysis were ground in a ball mill. Total C and N concentrations were determined by dry combustion (Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany). After removal of OC by ignition at 450°C for

Table 2.2 Management of all grassland plots in 2007.

Region	Plot ID	Location latitude, longitude	N fertilization [kg ha^{-1}]	Mowing [times yr^{-1}]	Grazing	Grazing intensity [LU d ha^{-1}]	Land-use intensity
Schorfheide-Chorin	SEG1	53°5'N, 13°58'E	26	2	-	0	3.0
	SEG2	53°5'N, 13°58'E	90	2	-	0	2.6
	SEG3	53°6'N, 13°59'E	120	2	cattle	55.7	3.0
	SEG4	53°6'N, 14°0'E	0	1	cattle	28	1.1
	SEG5	53°6'N, 14°0'E	0	1	cattle	28	1.1
	SEG6	53°6'N, 13°37'E	0	2	-	0	1.5
	SEG7	53°5'N, 13°58'E	0	0	cattle	122.3	0.8
	SEG8	53°6'N, 14°1'E	0	0	cattle	28	0.4
	SEG9	53°5'N, 13°36'E	0	0	cattle	141.5	0.9
Hainich-Dün	HEG1	50°58'N, 10°24'E	135	3	cattle	44.9	3.0
	HEG2	51°0'N, 10°24'E	140	3	cattle	34.8	3.0
	HEG3	50°59'N, 10°25'E	140	3	cattle	34.8	3.0
	HEG4	51°6'N, 10°26'E	27	1	cattle	113.8	1.8
	HEG5	51°12'N, 10°19'E	80	2	cattle	93.6	2.5
	HEG6	51°12'N, 10°23'E	80	1	cattle	9.3	2.1
	HEG7	51°16'N, 10°24'E	0	0	cattle, horses	452.5	2.0
	HEG8	51°16'N, 10°25'E	0	0	cattle, horses	452.5	2.0
	HEG9	51°13'N, 10°22'E	0	0	cattle	71.4	0.8
Schwäbische Alb	AEG1	48°23'N, 9°20'E	35	2	-	0	1.8
	AEG2	48°22'N, 9°28'E	100	3	-	0	2.6
	AEG3	48°24'N, 9°31'E	64	3	-	0	2.3
	AEG4	48°22'N, 9°25'E	35	1	cattle	106.6	1.8
	AEG5	48°23'N, 9°26'E	50	1	cattle, horses	123.5	2.0
	AEG6	48°24'N, 9°26'E	50	1	cattle, horses	687.2	3.0
	AEG7	48°23'N, 9°22'E	0	0	sheep, goats	30.8	0.5
	AEG8	48°25'N, 9°29'E	0	1	sheep, goats	103.7	1.3
	AEG9	48°23'N, 9°30'E	0	0	sheep, goats	38.7	0.6

16 h, inorganic C was quantified with the same elemental analyzer. OC concentration was calculated as the difference between total C and inorganic C. Labile P_i and P_o fractions were extracted with 0.5 M $NaHCO_3$ (adjusted to pH 8.5) following the method of Olsen et al. (1954). P concentrations in the extracts were determined with a continuous flow analyzer (CFA, Seal, Norderstedt, Germany) using the phosphomolybdate blue method (Murphy and Riley, 1962). P_o concentrations were calculated as the difference of total P and P_i concentrations in the $NaHCO_3$ extracts.

2.2.4 Vegetation sampling

In early summer 2008 we recorded the vegetation of all plots, in a 4 x 4 m area close to the soil sampling area. We identified all vascular plant species and estimated their percentage cover. Based on this we calculated for each plot the mean Ellenberg indicator value for moisture as a reliable measure of soil moisture (Ellenberg et al., 2001).

2.2.5 Microbial biomass and community composition

We performed PLFA analysis on the soil samples which were kept frozen at -80°C after sampling and freeze-dried prior to PLFA extractions. PLFA extractions were performed using a modified Bligh and Dyer (1959) method. Briefly, 2 g freeze-dried sample were extracted twice in a chloroform-methanol-citrate buffer (1:2:0.8), followed by overnight phase separation. Fatty acids in the organic phase were then separated using a silica-bonded solid phase column (SPE-SI; Bond Elut 3CC, 500 mg, Varian Inc.) to remove glyco lipids and neutral lipids. The polar lipids were then converted to fatty acid methyl esters by mild alkaline methanolysis. After this, methyl-esterfied fatty acids were analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with a DB-5ms arylene phase column (0.25 μm internal diameter by 0.25 μm film thickness by 60 m length, Agilent Technologies), and interfaced to an Agilent 5973 mass selective detector. Peak areas were converted to nmol lipid g^{-1} dry weight (dw) using an internal standard (19:0 nonadecanoic methyl ester). The total nmol lipid g^{-1} dw (sum of all lipids present, 20 or less C atoms-long chains) was used as an index of total PLFA biomass (Vestal and White, 1989; Zelles et al., 1992; Frostegård and Bååth, 1996). Individual PLFAs were used to indicate broad groups of the microbial community: 16:1ω5c for arbuscular mycorrhizal fungi (AMF) (Balser et al., 2005); 18:2ω6,9c for saprotrophic fungi (fungi) (Balser et al., 2005); 16:1ω7c for Gram-negative bacteria, 15:0 iso for Gram-positive bacteria (Wilkinson et al., 2002); and 16:0 10 methyl as an indicator of actinomycetes (Frostegård et al., 1993). The ratio of

fungal lipids to bacterial lipids was used to indicate the fungal to bacterial ratio (Frostegård and Bååth, 1996). Relative abundance of individual PLFAs was expressed as mole percentage (mol%) of total PLFA.

2.2.6 Enzyme assays

Sub-samples that were stored frozen at -20°C were thawed to 4°C just prior to enzyme activity analysis. Freezing of samples prior to enzyme analysis is a common practice, which has been shown before in other studies (Kandeler and Eder, 1993; Allison and Vitousek, 2004; Keeler et al., 2009), and is used as logistical constraints and the amount of taken samples prevented assaying fresh samples. Previous work determined no consistent shift in enzyme activities as a result of freezing samples (DeForest, 2009; Wallenius et al., 2010; Abellan et al., 2011), and freezing should not differentially affect enzyme activities in different land-use intensities, which was one of the primary focus of this study. The activities of ß-glucosidase, ß-xylosidase, α-glucosidase, N-acetyl-glucosaminidase, L-aminopeptidase and phosphatase were measured according to the method of Marx et al. (2001). Briefly, a mixture of 1 g field-moist soil was dispersed in 50 ml of sterile deionised water using an ultrasonic disaggregator with a low energy input (60 J ml^{-1}). The soil suspension was continuously stirred while an aliquot of 50 µl was transferred into a black micro-titer plate. Then 50 µl of autoclaved buffer (0.1 M MES-buffer or 0.05 M Trizma-buffer) and 100 µl of the respective 1 mM substrate containing the fluorescent compounds 4-methylumbelliferone (4-MUF, for ß-glucosidase, ß-xylosidase, α-glucosidase, N-acetyl-glucosaminidase and phosphatase) or 7-amino-4-methylcoumarin (7-AMC, for L-leucine aminopeptidase) were added to the soil suspension. Further, 10 µM standards (4-MUF standard, 7-AMC standard) were added to the soil suspension with buffer to obtain final concentrations of 0, 100, 200, 500, 800 and 1200 pmol $well^{-1}$. Wells without soil suspension were used as a control for autocleavage of substrates. Micro-titer plates were incubated in the dark for 210 min at 30°C while they were shaken on a micro-titer plate shaker at 300 rpm. Fluorescence was measured after 30, 60, 90, 150 and 210 min with 360 nm excitation and 460 nm emission using a microplate reader (Infinite 200, Tecan, Crailsheim, Germany). The first 30 min served as preincubation of the micro-titer plates. Enzyme activities were linearly related to the intensity of fluorescence. Results of enzyme activities are expressed as nmol MUF/AMC g^{-1} dw h^{-1}. Specific enzyme activities were determined by dividing total extracellular enzyme activities by total microbial lipid biomass and expressed as nmol MUF/AMC g^{-1} dw h^{-1} nmol lipid $biomass^{-1}$.

2.2.7 Statistical analyses

Redundancy analysis (RDA) is a multivariate technique for linear relations between two sets of variables (Ter Braak, 1986), and it was applied in this study to relate PLFA and enzyme activity data as the dependent variables and soil properties as the independent variables. A preliminary detrended correspondence analysis (DCA) showed that the gradient length was always less than 4 standard deviations. Thus linear responses are expected (Ter Braak and Šmilauer, 2002). Prior to RDA, relative abundance of individual PLFAs (mol%) were arcsine transformed. The variance inflation factor (VIF) was used to exclude collinear soil variables (of those listed in Table 2.3) from the analysis. An independent variable with a VIF > 20 indicates high collinearity (Ter Braak and Šmilauer, 2002). First, RDAs with soil properties (OC, labile P_i and P_o concentrations, C:N ratio, pH, clay content and Ellenberg indicator value for soil moisture) as independent variables were run to obtain the total amount of variance explained in PLFA data (total PLFA biomass, fungal to bacterial ratio, relative abundance of different PLFA groups (mol%), those shown in Table 2.4) and enzyme activities (of those shown in Fig. 2.1 a) across regions and land-use intensities. Second, all models were fitted for study region identity (binary coded variables (0, 1) reflecting one of three study regions) and management (continuous variable using the LUI index), thereby extracting variation that is derived from large-scale differences between regions (e.g. soil types and properties) and differences in land-use intensities between plots. This approach indicates if soil properties were related to soil microbiological properties in a general way, after accounting for study region and land-use intensity. Monte Carlo permutation tests were performed to test the significance level for each model at the 5 % significance level with 999 unrestricted permutations. Further, we used forward selection to select those explanatory variables (soil properties) that explain most of the variance in PLFA and enzyme activity data. Monte Carlo permutation tests were conducted to assess if the selected explanatory variable was statistically significant with 999 unrestricted permutations (Ter Braak and Šmilauer, 2002). We used one-way analysis of variance (ANOVA) to test the effect of study region on soil properties, PLFA data, and enzyme activities. This was followed by Tukey-Kramer HSD test for all pair-wise comparisons of the means ($P < 0.05$). The following parameters were log-transformed to meet the assumptions of ANOVA: OC concentration, TN concentration, clay content, α-glucosidase activity, specific ß-glucosidase activity, and concentration of AMF. Sand content was square-root transformed. Linear regression was used to relate enzyme activities to total PLFA biomass. Analysis of covariance (ANCOVA) was used to analyse the effect

of land-use intensity (LUI index) on PLFA (of those shown in Table 2.4) and enzyme activity data (of those shown in Fig. 2.1 a), while controlling for the effects of study region and differences in soil properties among the plots. In these ANCOVAs study region (categorical variable indicating the three study regions) and the soil property (continuous variable) that explained the most variance in the RDA were used as covariates. Thus, OC concentration was used as the covariate when we tested the PLFA and enzyme activity data, and labile P_i concentration in analyses with specific enzyme activity data. We further used ANCOVA to assess the effect of land-use intensity on soil properties with study region as a covariate. RDAs were performed using CANOCO 4.5 (Ter Braak and Šmilauer, 2002). RDA plots were generated with CanoDraw 4.0 graphics software (Ter Braak and Šmilauer, 2002). All other statistical analyses were conducted in R version 2.9.0 (R Development Core Team, 2008).

Table 2.3 Means (n=9) and standard errors of soil properties in the three study regions. Significant differences among regions ($P < 0.05$) are indicated by different letters.

	Schorfheide-Chorin	Hainich-Dün	Schwäbische Alb
Sand (g kg^{-1})	318 (62.2)^{a}	70 (3.1)^{b}	79 (28.2)^{b}
Silt (g kg^{-1})	426 (46.2)	468 (29.9)	544 (36.3)
Clay (g kg^{-1})	255 (21.3)^{a}	462 (30.2)^{b}	377 (49.5)^{ab}
OC (g kg^{-1})	150 (23.6)^{a}	51 (7.3)^{b}	67 (4.9)^{b}
TN (g kg^{-1})	14.0 (1.8)^{a}	4.8 (0.6)^{b}	6.3 (0.5)^{b}
CN ratio	10.5 (0.3)	10.4 (0.3)	10.6 (0.2)
Labile P_i (mg kg^{-1})	28.3 (5.5)	28.9 (8.2)	34.5 (10.0)
Labile P_o (mg kg^{-1})	18.6 (1.8)	16.2 (2.6)	11.8 (1.2)
pH	6.1 (0.3)	6.6 (0.1)	5.9 (0.2)
EIVmoisture*	6.5 (0.2)^{a}	5.3 (0.2)^{b}	5.1 (0.4)^{b}

*EIVmoisture=Ellenberg indicator value for soil moisture

2.3 Results

2.3.1 Soil properties

The study regions varied widely in soil characteristics (Table 2.3). Soils in the SCH had significantly higher OC and TN concentrations (two to three times), and sand contents

(four times) than soils in the HAI and ALB. Soils in the HAI exhibited significantly higher clay contents than soils in the SCH. The Ellenberg indicator values for soil moisture suggested that soils in the SCH had significantly higher soil moisture conditions than soils in the other two regions.

Table 2.4 Means (n=9) and standard errors of total PLFA biomass, fungal to bacterial ratio, and concentrations of different PLFA groups of the three study regions. Significant differences among regions ($P < 0.05$) are indicated by different letters.

	Schorfheide-Chorin	Hainich-Dün	Schwäbische Alb
Total PLFA biomass (nmol g^{-1} dw)	65.6 (10.0)	88.8 (14.2)	94.2 (6.7)
Fungal to bacterial ratio	2.8 (0.2)^{a}	3.5 (0.1)^{b}	2.9 (0.1)^{a}
Gram-positive bacteria (nmol g^{-1} dw)	2.2 (0.6)^{a}	4.4 (0.8)^{ab}	4.6 (0.7)^{b}
Gram-negative bacteria (nmol g^{-1} dw)	4.3 (0.7)	5.8 (1.0)	4.9 (0.4)
Fungi (nmol g^{-1} dw)	1.5 (0.2)^{a}	3.3 (0.6)^{b}	3.3 (0.3)^{b}
AMF (nmol g^{-1} dw)	2.4 (0.3)	4.5 (0.8)	3.9 (0.3)
Actinomycetes (nmol g^{-1} dw)	4.5 (0.7)	3.4 (0.5)	4.7 (0.2)

2.3.2 Microbial biomass and community composition at each region

Total PLFA biomass was smallest in the SCH and largest in the ALB, but differences were not significant (Table 2.4). The fungal to bacterial ratio was significantly higher in the HAI ($F_{2,24} = 7.2$, $P < 0.01$) than in the ALB and SCH. Soils in the SCH contained lower concentrations of Gram-positive ($F_{2,24} = 3.6$, $P = 0.04$) and Gram-negative bacteria ($F_{2,24} = 1.1$, $P = 0.4$), fungi ($F_{2,24} = 6.0$, $P < 0.01$) and AMF ($F_{2,24} = 3.4$, $P = 0.052$) than soils in the other two regions.

2.3.3 Enzyme activities and specific enzyme activities at each region

All enzyme activities increased in the order ALB < HAI < SCH, except for phosphatase activity that exhibited the lowest values in HAI (Fig. 2.1 a). Specific enzyme activities (per unit microbial biomass) were highest in soils of the SCH (Fig. 2.1 b). Generally, all enzyme activities were positively related to total PLFA biomass, but relationships differed for individual enzymes and study regions (Fig. 2.2). Although differences in the slope for

individual enzyme activities per total PLFA biomass were small between study regions, soils in the SCH contained consistently higher enzyme activities per se (higher intercept) than soils in the HAI and the ALB.

2.3.4 Influence of soil properties on PLFA data and enzyme activities

RDAs were used to determine the influence of soil properties on the total variation in PLFA data and enzyme activities (Fig. 2.3 a-d). Soil properties explained 60 % of variance in the PLFA data ($P < 0.01$) across study regions and land-use intensities.

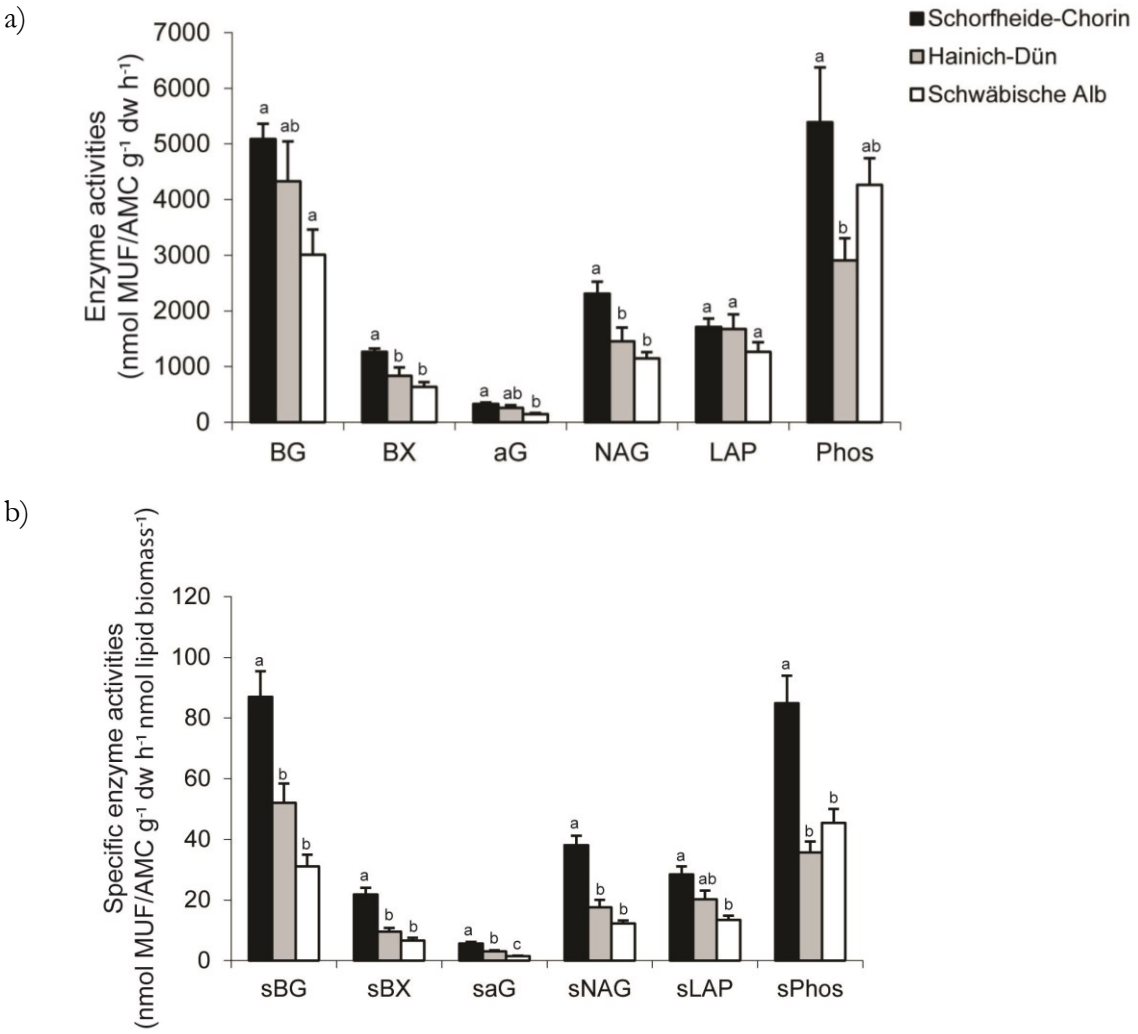

Fig. 2.1 Means (n=9) and standard errors of a) enzyme activities and b) specific enzyme activities (per unit of microbial biomass) of the three study regions. Significant differences between regions ($P < 0.05$) are indicated by different letters.

Abbreviations: BG=ß-glucosidase activity, BX=ß-xylosidase activity, aG=α-glucosidase activity, NAG=N-acetyl-glucosaminidase activity, LAP=L-leucine aminopeptidase activity, Phos=phosphatase activity

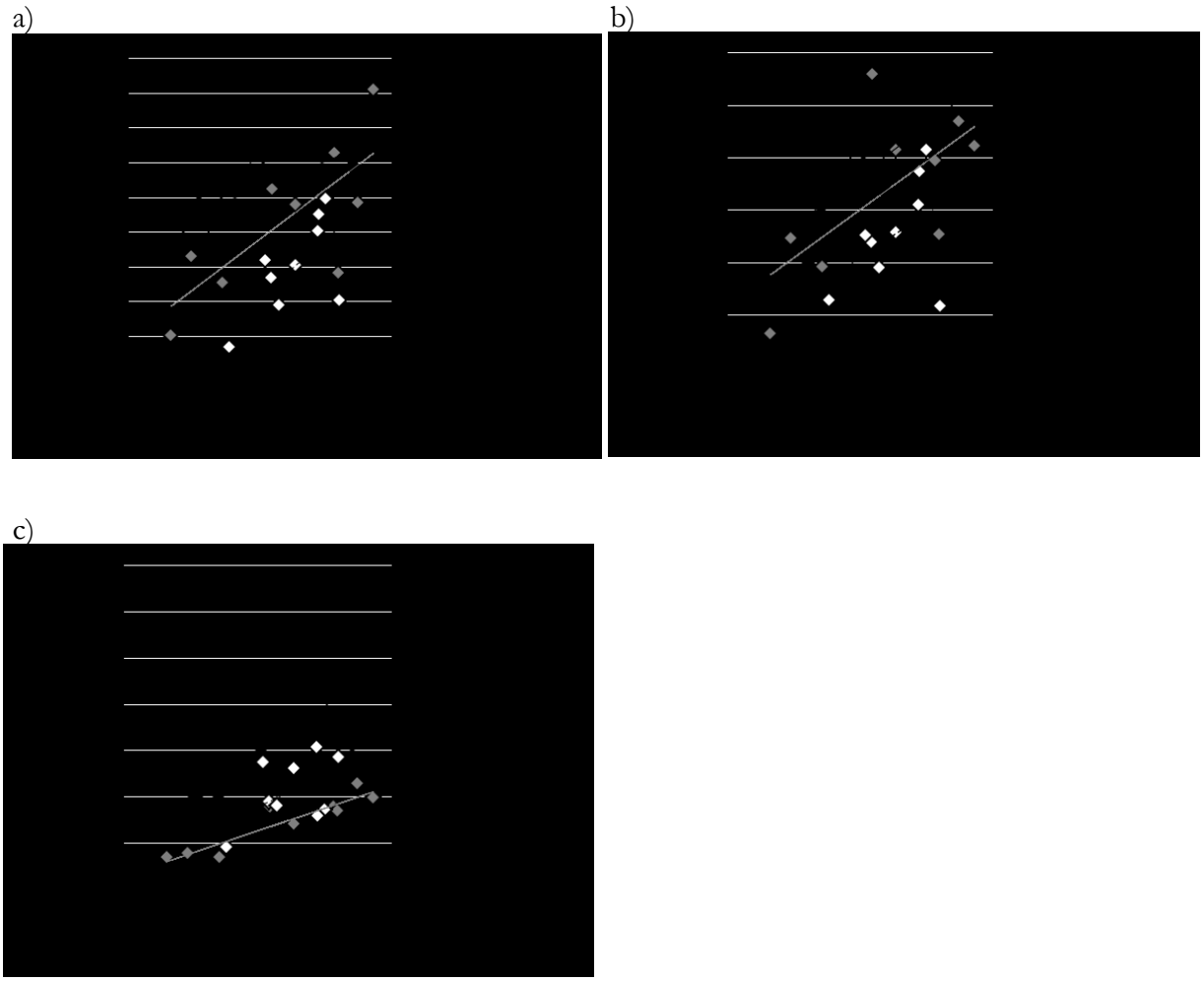

Fig. 2.2 Regressions of three enzyme activities (BG=ß-glucosidase activity, LAP=L-leucine aminopeptidase activity, Phos=phosphatase activity) on total PLFA biomass. Soils in Schorfheide-Chorin: black squares and black line, soils in Hainich-Dün: grey squares and grey line, soils in Schwäbische Alb: white squares and black dashed line. Asterisks (*$P < 0.05$, **$P < 0.01$) indicate significant relationships.

Relationships between soil properties and PLFA data were highly influenced by large-scale variation of soil properties among regions (Fig. 2.3 a). After accounting for variation among the regions and differences in land-use intensities between plots, soil properties still explained 34 % of the total variation in PLFA data ($P < 0.01$). While there was no relationship between total PLFA biomass and OC concentration across study regions and land-use intensities, total PLFA biomass increased with higher OC concentrations after accounting for study region and land-use intensities. The relative abundance of fungi and fungal to bacterial ratio decreased with higher Ellenberg indicator values for soil moisture (Fig. 2.3 b).

Soil properties alone explained 81 % of the variation in enzyme activities ($P < 0.01$) across study regions and land-use intensities with significant contributions of OC concentration, clay content, pH, Ellenberg indicator value for soil moisture, and C:N ratio (Fig. 2.3 c). After accounting for the study region and differences in land-use intensities between plots, soil properties still explained 60 % of variation in enzyme activities ($P < 0.01$). All enzyme activities were positively related to OC concentrations and soil moisture (Fig. 2.3 c, d). The effect of pH on enzyme activities was restricted to phosphatase activity that showed a decline with increasing pH.

2.3.5 Effects of LUI on soil properties and soil microbiological properties

N fertilization ($y = 0.01x + 1.2$, $R^2 = 0.69$, $P < 0.01$) and mowing ($y = 0.6x + 1.0$, $R^2 = 0.64$, $P < 0.01$) resulted in high LUI values (Table 2.2). We tested the influence of LUI on soil properties using ANCOVA with study region as a covariate. Organic carbon ($F_{1,21} = 0.01$, $P = 1.0$), TN ($F_{1,21} = 0.3$, $P = 0.6$), labile P_i ($F_{1,21} = 2.2$, $P = 0.1$), labile P_o concentrations ($F_{1,21} = 0.7$, $P = 0.4$), and pH ($F_{1,21} = 0.1$, $P = 0.8$) were not affected by LUI. Land-use intensity however significantly decreased the soil C:N ratio ($F_{1,21} = 7.4$, $P = 0.01$).

Taking into account that study region and soil properties had a significant impact on soil microbiological parameters, study region identity and the most influential site property(derived from the RDA analysis) were used as covariates when testing the effect of LUI on PLFA data, enzyme activities, and specific enzyme activities. No significant effects of LUI on PLFA data were observed (Table 2.5 a), while activities of enzymes involved in the C-cycle (ß-glucosidase activity, ß-xylosidase activity, α-glucosidase activity) (Table 2.5 b) as well as specific activity of ß-glucosidase and ß-xylosidase were significantly related to LUI (Table 2.5 c), and increased with greater LUI.

2.4 Discussion

General relationships between abiotic soil properties and soil microbiological parameters

We found that all three aspects of the microbial community: biomass, composition, and activity were strongly influenced by soil properties (Fig. 2.3 a, c). After removal of variance specific to study regions and land-use intensities, total PLFA biomass was mainly determined by OC concentrations (Fig. 2.3 b). This is in line with the study of Zeller et al. (2001), where they reported that variations in total PLFA biomass were due to variations in OC contents, and were positively related. Microbial community composition was, in

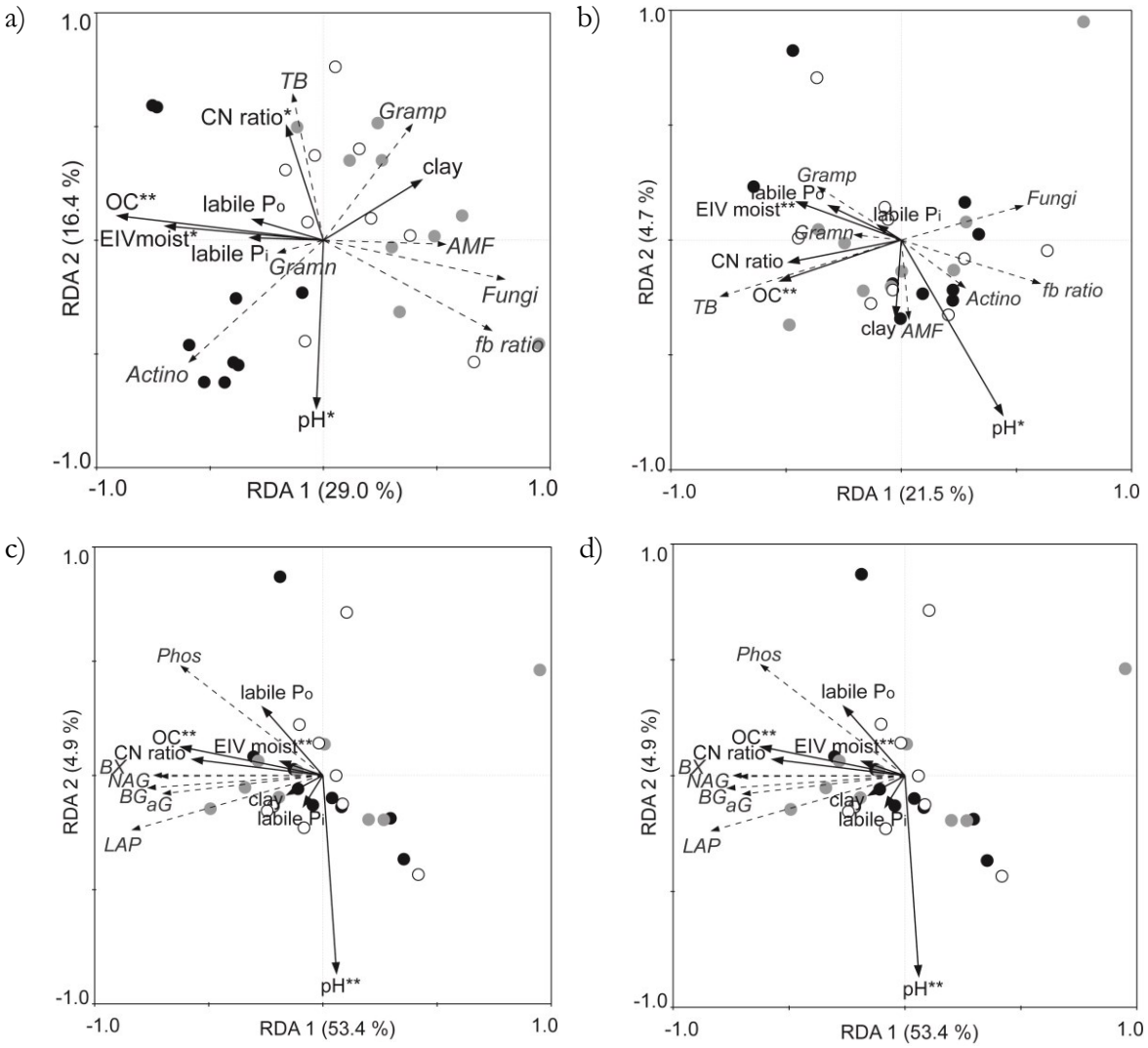

Fig. 2.3 RDA analyses of the effects of soil properties on a) PLFA data and c) enzyme activities across study regions and land-use intensities, and the effects of soil properties constrained by study region and grassland management (LUI) to extract variation that derived from large-scale differences among regions and differences in land-use intensities between plots on b) PLFA data, and d) enzyme activities in all grassland sites. Samples are classified by study region: Schorfheide-Chorin = black points, Hainich-Dün = grey points, Schwäbische Alb = white points. Asterisks (*$P < 0.05$, **$P < 0.01$) indicate significant explanatory variables on the total variation of unrestricted Monte Carlo permutation tests.

Abbreviations: EIVmoist=Ellenberg indicator value for soil moisture, TB=total PLFA biomass, fb ratio=fungal to bacterial ratio, Gramp=Gram-positive bacteria, Gramp=Gram-negative, Actino=Actinomycetes, BG=ß-glucosidase activity, BX=ß-xylosidase activity, aG=α-glucosidase activity, NAG=N-acetyl-glucosaminidase activity, LAP=L-leucine aminopeptidase activity, Phos=phosphatase activity

contrast to microbial biomass, mainly related to soil moisture. It has been shown that under drier soil conditions fungi are more abundant than bacteria (Guenet et al., 2012). This can further be supported in our study through decreased relative abundance of fungi and fungal to bacterial ratio under higher Ellenberg indicator values for soil moisture (Fig. 2.3 b).

Similar to microbial biomass, enzyme activities were mainly affected by OC concentrations which was already described in earlier studies (Dick et al., 1988). Soil organic matter is the main resource for microorganisms and microbial activities increase with increasing resource availability. Enzyme activities also increased with higher Ellenberg indicator values for soil moisture (Fig. 2.3 d). Guenet et al. (2012) studied a grassland site in Germany. The presence of a gentle slope within the experimental site resulted in a slight moisture gradient. They found that enzyme activities were generally lowest at drier sites and explained this by a reduction in substrate availability. It is not very likely that lower soil moisture per se was responsible for lower enzyme activities in our study. Smaller OC concentrations accompanied by lower soil moisture and less available C likely resulted in decreased enzyme activities (German et al. 2012). Further, our results revealed that phosphatase activity increased with decreased pH (Fig. 2.3 d). Phosphatase activity at the study sites was primarily attributable to microbe- and plant-derived acid phosphatases (Wasaki et al., 2005; Wasaki et al., 2008), which has been shown to decrease with increasing soil pH (Acosta-Martínez and Tabatabai, 2000). This is supported by the negative relationship between pH and phosphatase activity in our study. However, there could still be significant levels of alkaline phosphatases which dominate alkaline soils (Eivazi and Tabatabai, 1977). Overall, our study demonstrates that in grassland soils general relationships between soil properties and microbial community composition and enzyme activities persist over larger spatial scales and land-use intensities.

Differences between study regions

Differences in microbial community composition and enzyme activities among regions were primarily a result of soil moisture conditions. The positive relationship between total PLFA biomass and OC concentration did not hold across regions. This was mainly attributable to Histosols and Gleysols in the SCH with their high OC concentrations, but smaller total PLFA biomass compared to the soils in the HAI and ALB. A fluctuating ground water table in degraded peat soils in the SCH also indicated by high Ellenberg indicator values for soil moisture, led to anaerobic conditions in spring and our samples

Table 2.5 ANCOVA results with a) PLFA data b) enzyme activities, and c) specific enzyme activities as response variables. Explanatory variables (SR=study region identity, OC=OC concentration, labile P_i=labile P_i concentration, LUI=land-use intensity) are given in rows in the order of entering the analysis. Degrees of freedom (df), mean squares (MS) and F-values are presented (*$P < 0.05$, **$P < 0.01$).

a)		TB		fb ratio		Gramp		Gramn		Fungi		AMF		Actinomycetes	
	df	MS	F	MS	F	MF	F	MS	F	MS	F	MS	F	MS	F
Study region	2	0.08	12.1**	0.03	14.2**	0.01	8.8**	0.00	10.0**	0.00	10.2**	0.00	14.4*	0.01	30.8**
OC	1	0.74	116.9**	0.03	15.6**	0.00	0.9	0.00	0.4	0.00	6.6*	0.00	0.1	0.00	1.1
LUI	1	0.02	3.8	0.00	2.1	0.00	0.6	0.00	1.2	0.00	4.1	0.00	4.0	0.00	0.7
SR:OC	2	0.00	0.7	0.00	2.0	0.00	0.8	0.00	0.1	0.00	1.3	0.00	3.3	0.00	0.1
SR:LUI	2	0.02	3.1	0.01	3.4	0.00	1.9	0.00	0.1	0.00	3.6*	0.00	0.4	0.00	2.9
OC:LUI	1	0.03	4.7*	0.01	6.4*	0.00	0.5	0.00	0.5	0.00	3.5	0.00	5.8*	0.00	0.0
Residuals	17	0.01		0.00		0.00		0.00		0.00		0.00		0.00	

b)		BG		BX		aG		NAG		LAP		Phos	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Study region	2	0.18	34.1**	0.29	93.4**	0.36	33.0**	0.24	29.1**	0.05	5.1*	0.17	12.0**
OC	1	0.67	130.0**	0.81	259.1**	0.80	74.4**	0.64	78.4**	0.70	69.1**	0.57	41.2**
LUI	1	0.10	20.1**	0.09	28.5**	0.07	6.7*	0.01	0.7	0.04	3.8	0.00	0.0
SR:OC	2	0.12	23.0**	0.14	44.2**	0.11	10.3**	0.03	3.7*	0.06	6.2**	0.00	0.1
SR:LUI	2	0.03	4.8*	0.06	20.0**	0.03	2.3	0.01	0.7	0.01	1.1	0.03	2.1
OC:LUI	1	0.01	2.6	0.02	6.3*	0.03	2.6	0.05	6.0*	0.00	0.1	0.01	0.5
Residuals	17	0.01		0.00		0.01		0.01		0.01		0.01	

c)		sBG		sBX		saG		sNAG		sLAP		sPhos	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Study region	2	0.50	27.1**	0.66	39.5**	0.76	33.7**	0.58	32.9**	0.25	11.2**	0.33	19.1**
Labile P_i	1	0.25	14.2**	0.24	14.3**	0.14	6.2**	0.05	2.8	0.11	4.9*	0.02	1.2
LUI	1	0.09	4.8*	0.07	4.5*	0.09	3.8	0.02	1.3	0.05	2.4	0.01	0.7
SR: labile P_i	2	0.00	0.2	0.01	0.8	0.00	0.0	0.02	1.2	0.00	0.1	0.03	1.7
SR:LUI	2	0.01	0.7	0.04	2.2	0.02	1.0	0.01	0.6	0.02	0.8	0.01	0.8
Labile P_i:LUI	1	0.00	0.2	0.01	0.7*	0.04	1.7	0.00	0.0	0.01	0.4	0.00	0.0
Residuals	17	0.02		0.02		0.02		0.02		0.02		0.02	

Abbreviations: TB=total PLFA biomass, fb ratio=fungal to bacterial ratio, Gramp=Gram-positive bacteria, Gramp=Gram-negative, BG=ß-glucosidase activity, BX=ß-xylosidase activity, aG=α-glucosidase activity, NAG=N-acetyl-glucosaminidase activity, LAP=L-leucine aminopeptidase activity, Phos=phosphatase activity

were taken only shortly after the water table fell. This type of stagnant flooded conditions has been shown to lead to lower microbial biomass (Unger et al., 2009), likely caused by a decrease in aerobic organisms and an increase in slowly growing anaerobic organisms. This was also shown in three floodplain soils (Gleysol, Fluvisols) in the surrounding of the Elbe River (Germany), where increased flooding duration resulted in a decreased total PLFA biomass (Rinklebe and Langer, 2006). We assume that anaerobic conditions during winter flooding caused a temporary decrease of total PLFA biomass in degraded peat soils of the SCH, and that total PLFA biomass recovers under aerobic conditions in summer. Due to the sampling time, our study may have shown the biggest possible seasonal difference in total PLFA biomass between soils in the SCH and soils in the other two regions. Anaerobic conditions in the SCH also favour the accumulation of refractory organic matter (Canfield, 1994) that is more resistant to decomposition, and thus less available for microbes (Jastrow et al., 2007). Although abiotic conditions provide a less favourable habitat for microorganisms in the SCH, measured enzyme activities and specific enzyme activities were higher than in the HAI and the ALB. While the increase in enzyme activities per unit microbial biomass was similar in all study regions, the intercept of the regression between individual enzyme activities per total PLFA biomass was higher for soils from SCH than HAI and ALB (Fig. 2.2 a-c). This suggests that microorganisms in degraded peat soils are not more productive in terms of the enzymes produced per microorganism, but that these enzymes have longer turnover times. These can be weeks to months, and possibly much longer (Burns, 1982), because of stronger immobilization of enzymes by adsorption and generally slower decomposition under anaerobic conditions.

Land-use intensity effects on PLFA data and enzyme activities

All of our study sites were managed with combinations of N fertilization, mowing, and grazing. To test the effects of grassland management on soil microbiological parameters, either categorical factors like management types or continuous variables that describe the different management practices, can be used. The limitation of using categories is that quantitative changes are not included within one management type, and different management types do not necessarily correspond to different intensities. To overcome this problem we used a land-use intensity index proposed by Blüthgen et al. (2012) that integrates different management practices applied in combination. The advantage of using such an index is to get a general picture of specific relationships under real-world conditions, while saving degrees of freedom in a small number of samples ($n < 50$).

ANCOVA revealed no significant effect of LUI on total PLFA biomass (Table 2.5 a). Other studies however reported negative effects of high intensities of N fertilization or grazing on total microbial biomass (Bardgett et al., 1999a; Bardgett et al., 2001). Both, long-term N fertilization and grazing are believed to reduce root biomass and therefore the amount of root C input, which can result in lower microbial biomass. Hassink (1992) showed that the decline in the amounts of root C input into the soil can have much larger effects than any increase in aboveground biomass returned to the soil as the result of higher fertilization. In our study, LUI mainly depended on N fertilization and mowing, and plots with high LUI received high N additions and were mown two to three times per year. In addition, our fertilized plots received a maximum of 140 kg N ha^{-1} yr^{-1}, while in other studies plots had much higher N additions with 280 kg N ha^{-1} yr^{-1} (Bardgett et al., 1999a) or 200 kg N ha^{-1} yr^{-1} (Lovell et al., 1995). Recent results showed that long-term intensification of land use with high N addition and high mowing intensity have not resulted in reduced root biomass at our sampling plots (Solly et al., 2013). Thus, the release of carbon from roots was sufficient to sustain the soil microbial biomass.

Concurrent with shifts in the soil microbial biomass, several studies have reported changes in microbial community composition with N fertilization and grazing (Bardgett et al., 1999a; Bardgett et al., 2001). The fungal to bacterial ratio was highest in unfertilized and less intensively grazed sites. Thus, highly fertilized and grazed sites are dominated by bacteria. In our study, we did not find any effects of LUI on microbial community composition (Table 2.5 a). Similarly to recent studies (Bardgett et al., 2001), shifts in microbial community composition that we observed were related, in part, to soil N concentrations, but across our study sites N concentrations were not correlated with LUI. Thus, this could be one reason why we did not find changes in microbial community composition with changing LUI.

In contrast to the microbial community, ß-glucosidase activity, ß-xylosidase activity, α-glucosidase activity as well as specific ß-glucosidase activity, and specific ß-xylosidase activity increased with higher LUI. Land-use intensity did not affect nutrient concentrations or pH, but soil C:N ratios increased with lower LUI. Organic matter with high C:N ratio is only slowly degraded by soil microbes (Taylor et al., 1989). This is in line with high activities and specific activities of enzymes involved in the C cycle under high LUI with low C:N ratios. Further, it has been reported that N addition stimulates the degradation of labile, cellulosic organic material (Fog, 1988), and increases the activities of

cellulases, glycosidases, or phosphatases (Stursova et al., 2006; Keeler et al., 2009). The latter authors suggested that N addition stimulates plant productivity and related microbial activity. Because N is essential for enzyme synthesis, N additions also increase the demand for C and P. This could also possibly explain why activities of enzymes involved in the C cycle increased at higher LUI. However, phosphatase activities did not show higher values at high LUI. There was a trend that under high fertilization rates labile P_i concentrations were higher. It is known that high phosphate concentration in the soil solution acts as an inhibitor of enzyme production and expression (Spiers and McGill, 1979), and thus possibly restricted phosphatase activity. In addition, plots with high LUI had also higher mowing frequencies. It has been reported that declining carbon supply to soil microorganisms is likely to arise from long-term defoliation-induced reductions in photosynthetic capacity of plants, plant growth and accumulation of litter (Bardgett et al., 1998; Johnson and Matchett, 2001). Although microorganisms did not respond to LUI in terms of composition or total biomass, microbial enzyme activities increased with higher LUI in order to acquire energy and C.

2.5 Conclusions

Our study demonstrates that differences in soil properties among regions had a high impact on soil microbiological properties. OC concentration could not be used as a good predictor for total PLFA biomass and specific enzyme activities across regions when organic soils are included. A fluctuating water table in degraded peat soils reduced microbial growth, and likely resulted in differences in stabilization and turnover times of enzymes among regions. It is possible that because of the chosen sampling time, directly after water table drawdown, our study may have shown the biggest possible seasonal differences in microbiological parameters between degraded peat soils in the SCH and upland soils in the HAI and the ALB. Further, we found that all three aspects of the microbial community: biomass, composition, and activity, were related to soil properties in a general way. After accounting for large-scale differences among regions and differences in land-use intensities between plots, soil properties still explained a significant proportion of variation in total PLFA biomass, community composition and enzyme activities. Our results further revealed that grassland management in our study regions did not affect total PLFA biomass or microbial community composition, while activities and specific activities of enzymes involved in the C cycle increased with higher land-use intensity and lower soil C:N ratio. We argue that changes in soil microbiological properties in response to grassland

management can only be detected when soil properties were considered as covariables in the analysis to account for differences in soil properties between study plots. Together our results suggest that over larger spatial scales shifts in microbial communities and enzyme activities are more controlled by differences in soil properties than by grassland management practices.

Chapter 3

Vertical and latitudinal gradients of potential enzyme activities in soil profiles of differently managed forest sites

Chapter source: Nadine Herold et al., 2013. Vertical and latitudinal gradients of potential enzyme activities in soil profiles of differently managed forest sites. (to be submitted)

Abstract

Management of forest sites has the potential to modulate soil organic matter decomposition by changing the catalytic properties of soil microorganisms within a soil profile. In this study we examined the impact of forest management intensity and physico-chemical properties on variation in enzyme activities in the A, B1 and B2 horizon in three German regions (Schorfheide-Chorin (SCH), Hainich-Dün (HAI), Schwäbische Alb (ALB)).

The more sandy Arenosols in the Schorfheide-Chorin were characterized by lower organic carbon (OC) and total nitrogen (TN) concentrations, higher C:N ratios, and lower soil pH than the finer textured soils in the Hainich-Dün and Schwäbische Alb. All enzyme activities (ß-glucosidase, ß-xylosidase, α-glucosidase, phenol oxidase, N-acetyl-glucosaminidase, L-leucine aminopeptidase, phosphatase) across all soil horizons were highest in the ALB, followed by the HAI and the SCH. Soils in the SCH had lower ratios of activity of C acquiring enzymes (ß-glucosidase) relative to N acquiring enzymes (N-acetyl-glucosaminidase + L-leucine aminopeptidase), and activity of C acquiring enzymes relative to phosphorous (P) acquiring enzymes (phosphatase) than soils in the other two regions, indicating a shift in investment to N and P acquisition in the SCH. All enzyme activities, except phenol oxidase activity, decreased in deeper soil horizons as concentrations of OC and TN did, while the decrease was much stronger from the A to the B1 horizon than from the B1 to the B2 horizon. In contrast, phenol oxidase activity

showed no significant decrease towards deeper soil horizons. Additionally, the production of more enzymes responsible for the degradation of more recalcitrant C relative to labile C compounds increased in the two subsoil horizons. Subsoil horizons in all regions also indicate a shift to higher N acquisition, while the strength of the shift depended on the soil type. Further, our results clearly showed that most of the total variance of enzyme activities can be explained by soil properties in all soil horizons, followed by study regions and forest management intensity. Partial RDA revealed that clay concentration explained most of the variation in enzyme activities in all soil horizons and it was positively related to all enzyme activities.

Our results highlight the need for large scale studies including different regions and their environmental conditions in order to draw general conclusions on the impact of forest management and soil properties on enzyme activities in the whole soil profile, which should help to better predict how C and nutrient cycling will respond to environmental change in the future.

Keywords: Silvicultural management intensity, Enzyme activity ratios, specific enzyme activities, Redundancy analysis

3.1 Introduction

A considerable amount of the soil carbon (C) within the soil profile is stored in subsoil horizons despite low C concentrations (Batjes, 1996; Wang et al., 2010). Extracellular enzymes mediate the decomposition of soil organic matter (OM) and mineralize soil organic carbon (OC), nitrogen (N) and phosphorus (P) (Bandick and Dick, 1999; Finzi et al., 2006), thereby affecting global C and nutrient cycles (Sinsabaugh et al., 2008).

Surface soils are rich in fresh-C inputs via leaf litter, root litter and root exudates. These organic compounds serve as an energy source for microorganisms and enable the production of extracellular enzymes. In subsoil horizons, where fresh-C inputs are smaller in comparison to surface horizons and a large proportion of soil OC is stabilized by interaction with mineral surfaces only small amounts of OC are easily available for microorganisms (Fontaine et al., 2007; Kögel-Knabner et al., 2008). At the same time temporal variation in soil temperature and moisture are smaller in subsoil horizons, and nutrient availability decrease down the soil profile. Microbial biomass typically declines

with soil depth (Taylor et al., 2002). As microorganisms are the main producers of extracellular enzymes in soils, also enzyme activities should be smaller in subsoil horizon than in surface horizons. Taylor et al. (2002) studied enzyme activities in a loamy and sandy soil profile in three different depths, and explained generally lower rates of enzymatic activity in subsoil samples with lower numbers of microbes and the decrease in OM content. However, most studies on enzyme activities have been conducted with surface soils (Kandeler et al., 1999; Andersson et al., 2004; DeForest et al., 2004), while respective studies in subsoils are rare (Taylor et al., 2002; Zhang et al., 2005; Sotomayor-Ramírez et al., 2009).

The ratios between energy and nutrient acquiring activities of extracellular enzymes (e.g. ratio of ß-glucosidase activity : phosphatase activity, an indicator of potential C:P acquisition activity) can be used to follow shifts in nutrient or energy supply and demand (Sinsabaugh et al., 2008; Sinsabaugh et al., 2009; McDaniel et al., 2013). They help to determine if soil microbial communities assign more effort to acquire one nutrient relative to another, or to acquire energy. In this context, enzyme activity ratios have been used to study climate change effects in a disturbed forest soil (McDaniel et al., 2013), ecoenzymatic stoichiometry of terrestrial soils and freshwater sediments (Sinsabaugh et al., 2009; Sinsabaugh et al., 2012), the importance of OM recalcitrance in 28 ecosystems (Sinsabaugh and Follstad Shah, 2011), and effects of climate and soil properties across 40 ecosystems (Sinsabaugh et al., 2008). To our knowledge no prior study examined enzyme activity ratios in different soil horizons within the soil profile separately to learn more about shifts in energy and nutrient supply along the soil profile.

Forest management practices including the selection of tree species as well as harvesting and thinning can directly and indirectly affect enzyme activities in soils by changing the amount and quality of plant and root litter input to the soil. For example, Wean et al. (2010) showed different enzyme activity patterns of soils under different tree species due to varying litter quality. Hasset and Zak (2005) observed that harvesting of aspen trees resulted in a decline of enzyme activities due to reduced microbial biomass as a result of smaller litter input and changes in soil microclimate. Thinning of a 62-year-old pine stand, however, had no notable effect on enzyme activities (Maassen et al., 2006). While the aforementioned studies determined short-term responses (<10 yr) of different harvesting intensities on enzyme activities, the question remains, whether forest management practices also have long-term (>20 yr) effects on enzyme activities at the stand level.

When forest management is studied over larger regions, inherent climatic and edaphic differences between study regions need to be considered. Usually, study regions vary in climate, soil type and properties, and it has been concluded that the limitation of enzyme activity assessments is that results obtained within one soil type cannot be generalized and transferred to other soil types (Gianfreda et al., 2005). Thus, it is necessary to perform studies in several regions with their specific soil types and properties to identify patterns shared among different regions.

In this study we determined soil enzyme activities mediating the degradation of cellulose, hemicellulose, starch, lignin, chitin, proteins and organic phosphorous in differently managed forests in three German regions. Enzyme activity ratios were calculated to identify shifts in supply and demand for soil C, N and P within soil profiles. We applied redundancy analysis and variance partitioning to separate the effects of region, forest management, and soil properties on enzyme activities in different soil horizons.

3.2 Materials and methods

3.2.1 Study sites

This study was carried out at 25 forest plots located in the three study regions Schorfheide-Chorin (SCH) in northern Germany, Hainich-Dün (HAI) in central Germany, and Schwäbische Alb (ALB) in southern Germany (Fischer et al., 2010), and constitute a latitudinal gradient of 800 km (Table 3.1). The three study regions were characterised by distinct regional climate. Mean annual temperature decreased from the SCH (8.0-8.5 °C) to the HAI (6.5-8.0 °C) to the ALB (6-7 °C). Mean annual precipitation increased in the order SCH, HAI and ALB from 500 to 1000 mm. In the SCH glacial till was the dominant parent material which was often covered by aeolian and fluvial sand. The parent material of the HAI was loess over Triassic shell lime stone. In the ALB soils were developed on Jurassic lime stone. Soils in the SCH were Arenosols, Luvisols in the HAI, and Cambisols in the ALB (IUSS Working Group WRB, 2006). The plots in each region covered three forest management types namely coniferous and deciduous forest under age-class management, and unmanaged broadleaf forest (Table 3.1). In the HAI beech forest under selection cutting was studied as an additional forest management type. Replications per management type differed (Table 3.1). Plots of the managed and unmanaged deciduous forest are dominated by European beech (*Fagus sylvatica* L.) in all regions while in the managed coniferous forest plots the dominant tree species is Scots pine *(Pinus sylvestris* L.) in the

SCH, and Norway spruce (*Picea abies* L.) in the HAI and ALB. Each forest plot can be assigned a specific management intensity. The silvicultural management intensity indicator (SMI) proposed by Schall and Ammer (2013) was used to quantify silvicultural management intensity of stands that differ in species composition, age, silvicultural system (forest under age-class management (even-aged) versus unmanaged forest (uneven-aged)), and thinning grade. The SMI is calculated as the average of two components, the risk of stand loss and the stand density. The risk component of the SMI reflects the species-related survival probabilities and rotation periods, while the stand density component quantifies removal effects and the regeneration method using biomass related to a reference.

Table 3.1 Stand characteristics of all forest plots in three study regions.

	Plot ID	Location latitude, longitude	Age [years]	Basal area [m^2 ha^{-1}]	SMI
Schorfheide-Chorin					
Pine age-class forest	SEW1	52°54'N, 13°50'E	20-29	53.3	0.299
	SEW2	52°57'N, 13°46'E	30-39	53.3	0.265
	SEW3	52°55'N, 13°38'E	50-59	53.3	0.282
Beech age-class forest	SEW4	52°55'N, 13°50'E	90-99	49.5	0.177
	SEW5	53°3'N, 13°53'E	170-179	42.0	0.165
	SEW6	52°54'N, 13°50'E	140-149	42.9	0.322
Unmanaged beech forest	SEW9	53°2'N, 13°48'E	130-139	44.7	0.028
Hainich-Dün					
Spruce age-class forest	HEW2	51°12'N, 10°22'E	50-59	41.9	0.387
	HEW3	51°16'N, 10°18'E	60-69	31.8	0.523
Beech age-class forest	HEW6	51°16'N, 10°14'E	110-119	41.4	0.073
Beech selection forest	HEW7	51°7'N, 10°23'E	150-159	18.7	0.238
	HEW8	51°21'N, 10°31'E	170-179	32.0	0.143
	HEW9	51°7'N, 10°22'E	150-159	27.9	0.178
Unmanaged beech forest	HEW10	51°5'N, 10°27'E	160-169	31.2	0.099
	HEW11	51°6'N, 10°24'E	160-169	37.6	0.004
	HEW12	51°6'N, 10°27'E	180-189	32.3	0.085
Schwäbische Alb					
Spruce age-class forest	AEW1	48°28'N, 9°20'E	40-49	63.7	0.547
	AEW2	48°22'N, 9°21'E	60-69	28.7	0.525
	AEW3	48°24'N, 9°21'E	50-59	42.5	0.514
Beech age-class forest	AEW4	48°23'N, 9°14'E	40-49	18.3	0.204
	AEW5	48°25'N, 9°24'E	140-149	34.5	0.315
	AEW6	48°23'N, 9°26'E	80-89	34.5	0.256
Unmanaged beech forest	AEW7	48°23'N, 9°15'E	130-139	23.8	0.088
	AEW8	48°22'N, 9°22'E	150-159	34.1	0.011
	AEW9	48°22'N, 9°24'E	150-159	18.5	0.151

3.2.2 Soil sampling

In spring 2008 just before foliation, five soil samples were taken at each forest plot (20 x 20 m), one at each corner and one in the plot centre. At each individual plot the aeromorphic organic layer was removed with a metal frame (20 x 20 cm in SCH, ALB and 40 x 40 cm in HAI). Subsequently, the mineral soil was sampled down to the bedrock using a motor driven auger (diameter of 8.3 cm). Arenosols in the SCH and Luvisols in the HAI comprised three mineral horizons, while the Cambisols in the ALB had two mineral horizons. The first mineral horizon, AEh in the SCH, Ah in the HAI and the ALB were termed as A horizon. The second horizon, ABhw in the SCH, BEw/BEg in the HAI and Btw in the ALB were designated as B1 horizon. The third horizon, Bw in the SCH and Btg in the HAI were termed as B2 horizon. Samples were taken from each horizon, stored in ice boxes and transported to the field lab facility. After removal of coarse roots with a diameter of >2 mm and stones, mineral soil samples were split up into two sub-samples. One sub-sample was air-dried, and another was stored at -20 °C for further analyses. The samples from the organic layers were dried in an oven at 70 °C. All soil analyses were performed on five soil sub-samples per plot and horizon, except for soil texture. Here, the five sub-samples per plot and horizon were mixed to obtain a composite sample before the texture analysis.

3.2.3 Physico-chemical soil analyses

For determination of soil texture, OC and TN concentrations, pH, aluminium (Al) and iron (Fe) oxides the air-dried sub-samples were used. Soil texture was the determined according to the method of Schlichting and Blume (1964). Soil pH was measured with a glass electrode in the supernatant of a 1:2.5 mixture of air-dried bulk soil and 0.01 M $CaCl_2$. Oxalate-extractable Al and Fe (Al_o, Fe_o) were extracted with 0.2 M oxalate solution (pH 3) (Schwertmann, 1964). The extraction of dithionite-extractable Fe (Fe_d) was done with Na-dithionite and tri-Na-citrate-dihydrate for 16 h after the method of Holmgren (1967). Al and Fe concentrations were measured with Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, Optima 3300 DV, Perkin-Elmer, Norwalk, CT, USA). Sub-samples of the mineral soil and the litter were ground in a ball mill for elemental analysis. Total C (TC) and TN concentrations were determined by dry combustion (Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany). After removal of OC by ignition at 450°C for 16 h, inorganic C was quantified with the same elemental analyser. OC concentrations of the mineral soil were calculated as the difference between TC and

inorganic C. C stocks (kg m^{-2}) of the organic layer were calculated as the product of OC concentration, the dry weight of the organic layer (70 °C) and its volume.

3.2.4 Enzyme assays

The activities of ß-glucosidase (BG), ß-xylosidase (BX), α-glucosidase (aG), N-acetyl-glucosaminidase (NAG), L-leucine aminopeptidase (LAP) and phosphatase (Phos) were measured according to the method of Marx et al. (2001). The following solutions were prepared for enzyme assays: (1) 0.1 M MES buffer (2-[N-Morpholino]ethanesulfonic acid) (pH 6.1), and (2) 0.05 M Trizma buffer (a mixture of α-α-α-Tris-(hydroxymethyl)-methylamin and Tris-(hydroxymethyl)-aminomethane hydrochloride) (pH 7.8). Substrates containing the fluorescent compounds 4-methylumbelliferone (4-MUF) or 7-amino-4-methylcoumarin (7-AMC) were dissolved in 300 µl dimethyl sulfoxide (DMSO) and brought to a final volume of 10 ml with sterile deionised water for a 10 mM stock solution. An aliquot of 5 ml of the stock solution was mixed with 45 ml of autoclaved buffer (MES-buffer for 4-MUF-substrates, Trizma-buffer for 7-AMC-substrates) to obtain a working solution of 1 mM. Only the activity of L-leucine aminopeptidase was analysed with the 7-AMC-substrate. Standards were dissolved in methanol and sterile deionised water to obtain final concentrations of 10 mM 4-MUF and 5 mM 7-AMC stock solutions. Subsequently, these stock solutions were diluted in MES-buffer or Trizma-buffer to 10 µM. A mixture of 1 g field-moist soil and 50 ml of sterile deionised water was dispersed using an ultrasonic disaggregator with a low energy input (60 J ml^{-1}). The soil suspension was continuously stirred while an aliquot of 50 µl was transferred into a black micro-titer plate. 50 µl of autoclaved buffer and 100 µl of the respective substrate were added. Standards were mixed with soil suspension and buffer to obtain final concentrations of 0, 100, 200, 500, 800 and 1200 pmol $well^{-1}$. Wells without soil suspension were used as a control for autocleavage of substrates. Micro-titer plates were incubated in the dark for 210 min at 30°C while the micro-titer plate was shaken at 300 rpm min^{-1}. Fluorescence was measured after 30, 60, 90, 150 and 210 min with 360 nm excitation and 460 nm emission using a microplate reader (Infinite 200, Tecan, Crailsheim, Germany). The first 30 min served as preincubation of the micro-titer plates. Enzyme activities were linearly related to the intensity of fluorescence. Results of enzyme activities are expressed as nmol MUF/AMC g^{-1} dry weight soil (dw) h^{-1}.

A new method was developed to estimate phenol oxidase activity (PhOx) in small soil samples. This method is based on a recent paper of Johnsen and Jacobsen (2008), who

used tetramethylbenzidine (TMB) to measure peroxidase activities of organic surface soil from a forest. We used the same substrate, buffer and temperature of incubation as Johnsen and Jacobsen (2008), but we adapted the method to estimate phenol oxidase in micro-titer plates and improved the precision of the method by following the linear development of colour during the 60 min incubation. In detail, the substrate (TMB) (Sigma-Aldrich) and the buffers sodium acetate and sodium citrate dehydrate (Merck) were used for the assays. The 50 mM sodium acetate buffer was adjusted to pH 5.0 using a 12 % acetic acid. The 205 mM sodium citrate buffer was adjusted to pH 4.0 with citric acid (1 M). TMB was dissolved in 2.5 ml DMSO and then brought to a final volume of 5 ml with sterile deionised water for a stock solution of 60 mM. TMB working solution (12 mM) was prepared with autoclaved sodium citrate buffer. 0.4 g of field-moist soil was weighed and added to 50 ml acetate buffer and dispersed by an ultrasonic disaggregator with low energy input (60 J ml^{-1}). Each sample well of the white micro-titer plates received an aliquot of 200 µl of the soil suspension while stirring this suspension on a magnetic stir plate and 50 µl of the TMB working solution. The negative control wells contained 200 µl acetate buffer and 50 µl TMB working solution, and the blank well received 200 µl of the soil suspension and 50 µl acetate buffer. Micro-titer plates were incubated in the dark for 1 h at 25°C. Enzyme activities were determined spectrophotometrically by measuring absorbance at 630 nm immediately after adding the TMB to the wells of one micro-titer plate and after an additional 15, 30, 45 and 60 min using the same microplate reader as for fluorescence measurements. Results of enzyme activities are expressed as excitation 630 nm ($E_{630\ nm}$) g^{-1} dw h^{-1}. All enzyme assays were performed with six replicates for each soil sample.

We calculated three ratios in extracellular enzyme activities to determine changes in resource allocation with respect to different soil horizons. For all activity ratios, specific enzyme activities were calculated in units of µmol g^{-1} OC h^{-1} or E_{630nm} g^{-1} OC h^{-1}. The first activity ratio ln BG : ln (NAG+LAP) was used as an indicator of potential C:N acquisition activity and designated as E_C:E_N. The second activity ratio ln BG : ln Phos was used as an indicator of potential C:P acquisition activity and designated as E_C:E_P. The third activity ratio ln BG : ln (10+PhOx) was used as an indicator of potential labile C : recalcitrant C acquisition activity and termed as E_L:E_R.

3.2.5 Statistical analyses

Statistical analyses were conducted in R version 2.15.1 (R Development Core Team, 2012). Two-way analysis of variance (ANOVA) was used to study differences between soil horizons and study regions on soil properties, enzyme activities, and enzyme activity ratios. This was followed by Tukey-Kramer HSD test for all pair-wise comparisons of the means ($P < 0.05$). Prior to ANOVA, we tested if the normality and homogeneity of the variances of the residuals was fulfilled, otherwise the data was log-transformed. Direct linear relationships between the environmental parameters and enzyme activities were analysed by redundancy analysis (RDA) using CANOCO 4.5 (Ter Braak and Šmilauer, 2002). A variance partitioning procedure was used to evaluate the proportion of the total variance of the enzyme activities that can be attributed to (i) study region (binary coded variables), (ii) forest management (SMI, C:N ratio of the organic layer, C stocks of the organic layer), and (iii) soil physico-chemical properties (of those listed in Table 3.2). First, a full RDA with all three groups of explanatory variables was run to obtain the total amount of variance explained in enzyme activities. Second, partial RDAs were run using one of the three environmental variable groups as constrained explanatory variable and the other two groups together as covariables to gain the variance explained by each environmental group. Monte Carlo permutation tests were performed to test the statistical significance of the fractions of variance, with 999 permutations under the reduced model. Prior to RDA, the variance inflation factor (VIF) was used to exclude collinear soil variables from the analysis. A step-by-step forward procedure with a Monte Carlo permutation test under the full model was performed with 999 unrestricted permutations. An independent variable with a VIF > 20 indicates high collinearity (Ter Braak and Šmilauer, 2002). The variable with the highest VIF was excluded from the model and the remaining variables were tested again for collinearity. This procedure was repeated until all collinear variables (VIF > 20) were removed from analysis. In addition, soil properties whose conditional effects were not significant were also excluded from the analysis when the effect of soil properties on the variance of enzyme activities was tested. This procedure was done for each horizon.

3.3 Results

3.3.1 Soil properties

Table 3.2 shows differences in soil properties among study regions and soil horizons. The depth of the soil profile was smaller in the Cambisols of the ALB than in the Arenosols of

Table 3.2 Means and standard errors of soil properties in different soil horizons in three study regions. Two-way ANOVA results are presented with Tukey HSD test ($P < 0.05$). Significant differences between study regions across all soil horizons are indicated by *capital letters*, and between soil horizons for all study regions by *lowercase letters*.

	Schorfheide-Chorin			**Hainich-Dün**			**Schwäbische Alb**	
	A horizon	B1 horizon	B2 horizon	A horizon	B1 horizon	B2 horizon	A horizon	B1 horizon
n	7	7	6	9	9	9	9	8
Horizon thickness [cm]	9.2 ± 1.3^{Aa}	18.3 ± 3.4^{Ab}	21.4 ± 1.9^{Ab}	8.7 ± 1.0^{Aa}	17.6 ± 1.8^{Ab}	18.1 ± 1.7^{Ab}	9.0 ± 1.5^{Ba}	13.4 ± 1.2^{Bb}
Sand concentration [g kg^{-1}]	914 ± 14^{Aa}	939 ± 12^{Aa}	943 ± 13^{Aa}	45 ± 7^{Ba}	45 ± 9^{Ba}	28 ± 3^{Ba}	61 ± 10^{Ca}	80 ± 14^{Ca}
Silt concentration [g kg^{-1}]	69 ± 11^{Aa}	55 ± 11^{Aab}	49 ± 9^{Ab}	660 ± 32^{Ba}	656 ± 31^{Ba}	407 ± 35^{Bb}	491 ± 38^{Ca}	519 ± 50^{Ca}
Clay concentration [g kg^{-1}]	17 ± 4^{Aa}	6 ± 3^{Aa}	9 ± 6^{Aa}	295 ± 34^{Ba}	299 ± 32^{Ba}	565 ± 35^{Bb}	448 ± 39^{Ba}	402 ± 50^{Ba}
OC concentration [g kg^{-1}]	24.6 ± 2.4^{Aa}	6.6 ± 1.0^{Ab}	2.9 ± 0.5^{Ac}	48.3 ± 3.8^{Ba}	14.2 ± 1.9^{Bb}	9.7 ± 1.2^{Bb}	69.1 ± 6.1^{Ca}	30.2 ± 2.8^{Cb}
TN concentration [g kg^{-1}]	1.4 ± 0.1^{Aa}	0.5 ± 0.1^{Ab}	0.2 ± 0.04^{Ac}	3.3 ± 0.3^{Ba}	1.2 ± 0.2^{Bb}	1.0 ± 0.1^{Bb}	4.9 ± 0.4^{Ca}	2.5 ± 0.2^{Cb}
C:N ratio	18.2 ± 0.5^{Aa}	14.5 ± 1.1^{Ab}	12.6 ± 0.6^{Ac}	14.8 ± 0.4^{Ba}	11.7 ± 0.4^{Bb}	9.3 ± 0.3^{Bc}	14.1 ± 0.4^{Ca}	12.2 ± 0.5^{Cb}
pH	3.2 ± 0.1^{Aa}	3.7 ± 0.1^{Ab}	3.9 ± 0.1^{Ab}	4.3 ± 0.2^{Ba}	4.2 ± 0.1^{Ba}	5.3 ± 0.2^{Bb}	4.2 ± 0.3^{Ba}	4.6 ± 0.4^{Ba}
Alo concentration [g kg^{-1}]	1.7 ± 0.2^{Aa}	1.7 ± 0.1^{Aa}	2.0 ± 0.2^{Aa}	2.4 ± 0.3^{Ba}	2.5 ± 0.3^{Ba}	2.8 ± 0.2^{Ba}	4.0 ± 0.4^{Ca}	3.8 ± 0.3^{Ca}
Feo concentration [g kg^{-1}]	1.8 ± 0.2^{Aa}	1.6 ± 0.1^{Aa}	1.5 ± 0.2^{Aa}	4.0 ± 0.3^{Ba}	4.0 ± 0.4^{Ba}	3.2 ± 0.2^{Ba}	3.6 ± 0.5^{Ba}	3.3 ± 0.3^{Ba}
Fed concentration [g kg^{-1}]	2.9 ± 0.3^{Aa}	2.6 ± 0.3^{Aab}	2.5 ± 0.4^{Ab}	11.0 ± 0.9^{Ba}	13.6 ± 1.1^{Ba}	21.9 ± 1.0^{Bb}	25.3 ± 1.7^{Ca}	28.2 ± 2.2^{Ca}

the SCH and the Luvisols of the HAI. Soil pH ranged between 3.2 and 4.3 in the A horizons, and between 3.7 and 5.3 in the subsoil horizons. The SCH had a significantly lower pH than the other two regions. The soil pH differed between the A and B1 horizon in the SCH, and was significantly higher in the B2 than in the other two horizons in the HAI. Arenosols of the SCH significantly differed in their soil texture with higher sand and lower silt and clay concentrations from the Luvisols of the HAI and Cambisols of the ALB. Luvisols of the HAI had significantly higher clay concentrations in the B2 horizon than in the A and B1 horizon. Oxalate-extractable Al and dithionite-extractable Fe oxides were highest in the ALB followed by the HAI and the SCH across all horizons. Pedogenic Al and Fe oxides revealed no significant differences between the three soil horizons in all three regions, only dithionite-extractable Fe oxides in the B2 horizon of the SCH was significantly lower than in the A horizon. In in the HAI dithionite-extractable Fe oxides were significantly higher in the B2 than in the other two horizons. OC and TN concentrations differed significantly between the study regions for all soil horizons with the highest values in the ALB followed by the HAI and the SCH. Organic C and TN concentrations decreased from the A to the B1 and B2 horizon in all study regions, whereas the decrease was much stronger from the A to the B1 horizon than from the B1 to the B2 horizon. The ALB showed a lower decrease in both concentrations from the A to the B1 horizon than the SCH and HAI. The C:N ratios across all horizons significantly differed between all regions with the highest ratios in the SCH followed by the HAI and the ALB. The C:N ratio in all study regions were widest in the A horizon and decreased in deeper soil horizons.

3.3.2 Enzyme activities

Enzyme activities were highest in the ALB for all soil horizons, followed by the HAI and the SCH (Table 3.3). BG, NAG and Phos had the highest activities compared to the other enzymes across all regions and soil horizons. Differences in enzyme activities between soil horizons were enzyme specific. Overall, enzyme activities decreased from the A to the B1 and B2 horizon. The BG, BX, aG, NAG, and LAP activities were significantly higher in the A horizon than in the B1 and B2 horizon in all study regions. Phos activity also significantly differed between the B1 and B2 horizon with higher values in the B1 horizon. In contrast, PhOx activity was the only enzyme activity which showed no significant decrease in deeper soil horizons.

Table 3.3 Means and standard errors of enzyme activities in different soil horizons in three study regions. Two-way ANOVA results are presented with Tukey HSD test ($P < 0.05$). Significant differences between study regions across all soil horizons are indicated by *capital letters*, and between soil horizons for all study regions by *lowercase letters*.

	Schorfheide-Chorin			**Hainich-Dün**			**Schwäbische Alb**	
	A horizon	B1 horizon	B2 horizon	A horizon	B1 horizon	B2 horizon	A horizon	B1 horizon
BG [nmol MUF g^{-1} dw h^{-1}]	205 ± 30^{Aa}	32 ± 6^{Ab}	15 ± 4^{Ab}	1244 ± 249^{Ba}	183 ± 41^{Bb}	149 ± 34^{Bb}	1388 ± 251^{Ca}	436 ± 70^{Cb}
BX [nmol MUF g^{-1} dw h^{-1}]	147 ± 25^{Aa}	14 ± 3^{Ab}	6 ± 1^{Ab}	295 ± 42^{Ba}	75 ± 14^{Bb}	46 ± 10^{Bb}	311 ± 27^{Ca}	122 ± 12^{Cb}
aG [nmol MUF g^{-1} dw h^{-1}]	23 ± 3^{Aa}	4 ± 1^{Ab}	NA	65 ± 9^{Ba}	18 ± 3^{Bb}	15 ± 3^{Bb}	101 ± 11^{Ca}	39 ± 6^{Cb}
PhOx [E_{630nm} g^{-1} dw h^{-1}]	0.5 ± 0.1^{Aa}	0.3 ± 0.04^{Aa}	0.3 ± 0.04^{Aa}	1.3 ± 0.2^{Ba}	1.1 ± 0.1^{Ba}	1.0 ± 0.1^{Ba}	1.8 ± 0.2^{Ca}	1.6 ± 0.2^{Ca}
NAG [nmol MUF g^{-1} dw h^{-1}]	298 ± 63^{Aa}	53 ± 10^{Ab}	28 ± 9^{Ab}	752 ± 76^{Ba}	163 ± 28^{Bb}	137 ± 33^{Bb}	1265 ± 280^{Ca}	339 ± 69^{Cb}
LAP [nmol AMC g^{-1} dw h^{-1}]	120 ± 20^{Aa}	27 ± 4^{Ab}	13 ± 2^{Ab}	412 ± 76^{Ba}	114 ± 29^{Bb}	137 ± 32^{Bb}	921 ± 128^{Ca}	409 ± 71^{Cb}
Phos [nmol MUF g^{-1} dw h^{-1}]	2146 ± 339^{Aa}	281 ± 57^{Ab}	122 ± 27^{Ac}	4735 ± 607^{Ba}	1092 ± 204^{Bb}	510 ± 50^{Bc}	6026 ± 660^{Ca}	1557 ± 272^{Cb}

Abbreviations: BG=ß-glucosidase activity, BX=ß-xylosidase activity, aG=α-glucosidase activity, PhOx=phenol oxidase activity, NAG=N-acetyl-glucosaminidase activity, LAP=L-leucine aminopeptidase activity, Phos=phosphatase activity

All specific enzyme activities (per unit of C) were lowest in the SCH for all soil horizons (examples Fig. 3.1). Differences in specific enzyme activities between soil horizons were also enzyme specific. Overall, specific enzyme activities were highest in the A horizon and lower in the B1 and B2 horizon in all study regions, with exceptions of specific LAP and PhOx activities. The specific BG, BX, NAG, and Phos activities were significantly higher in the A than in the B1 horizon in all study regions, but not always higher than the B2 horizon. In contrast, specific LAP activity significantly differed between the B1 and B2 horizon with the highest values in the B2 horizon. Specific PhOx activity was the only enzyme activity which showed a significant increase from the A to the B1 to the B2 horizon.

The SCH revealed significantly lower enzyme activity ratios across all soil horizons than the HAI and ALB (Fig. 3.2). The E_C:E_N ratio and the E_L:E_R ratio were significantly greater in the A horizon than in the two subsoil horizons in all regions (Fig. 3.2 a, c). In contrast, the E_C:E_P ratio showed no significant decrease in deeper soil horizons in all regions.

3.3.3 Effects of region, forest management, and soil properties on enzyme activities

Prior to RDA collinear soil properties (of those listed in Table 3.2) were removed to keep only the best soil variables explaining the variance in enzyme activities. In all three soil horizons the clay content of the soil were kept in the RDA model as a significantcontributor to the variance explained. In addition to this factor, pH and oxalate-extractable Fe oxides were significant contributors in the A horizon, oxalate-extractable Al and Fe oxides and horizon thickness in the B1 horizon, and oxalate-extractable Al oxides in the B2 horizon. The total variance of enzyme activities explained by all groups of environmental variables together was similar for each horizon, ranging from 86.8 % to 90.9 % (Table 3.4). Forest management did not significantly affect the variance of enzyme activities all soil horizons. Study region explained more variance in enzyme activities in the B1 horizon with 7.6 %compared to the others horizons (4.8 in B2 horizon, 1.8 in A horizon). The proportion of the total variance in enzyme activities explained by soil properties was highest in the A horizon with 15.2 %, followed by the B1 horizon with 11.5 %, and the B2 horizon with 8.5 %. Variance partitioning further showed that the shared variance fraction was very high in all soil horizons and ranged between 64.5 % and 70.3 %. Figure 3.3 a-c show the relationships between all environmental variables and enzyme activities in the three different horizons. When all environmental variable groups were considered together, clay concentrations were highly positively related to all enzyme

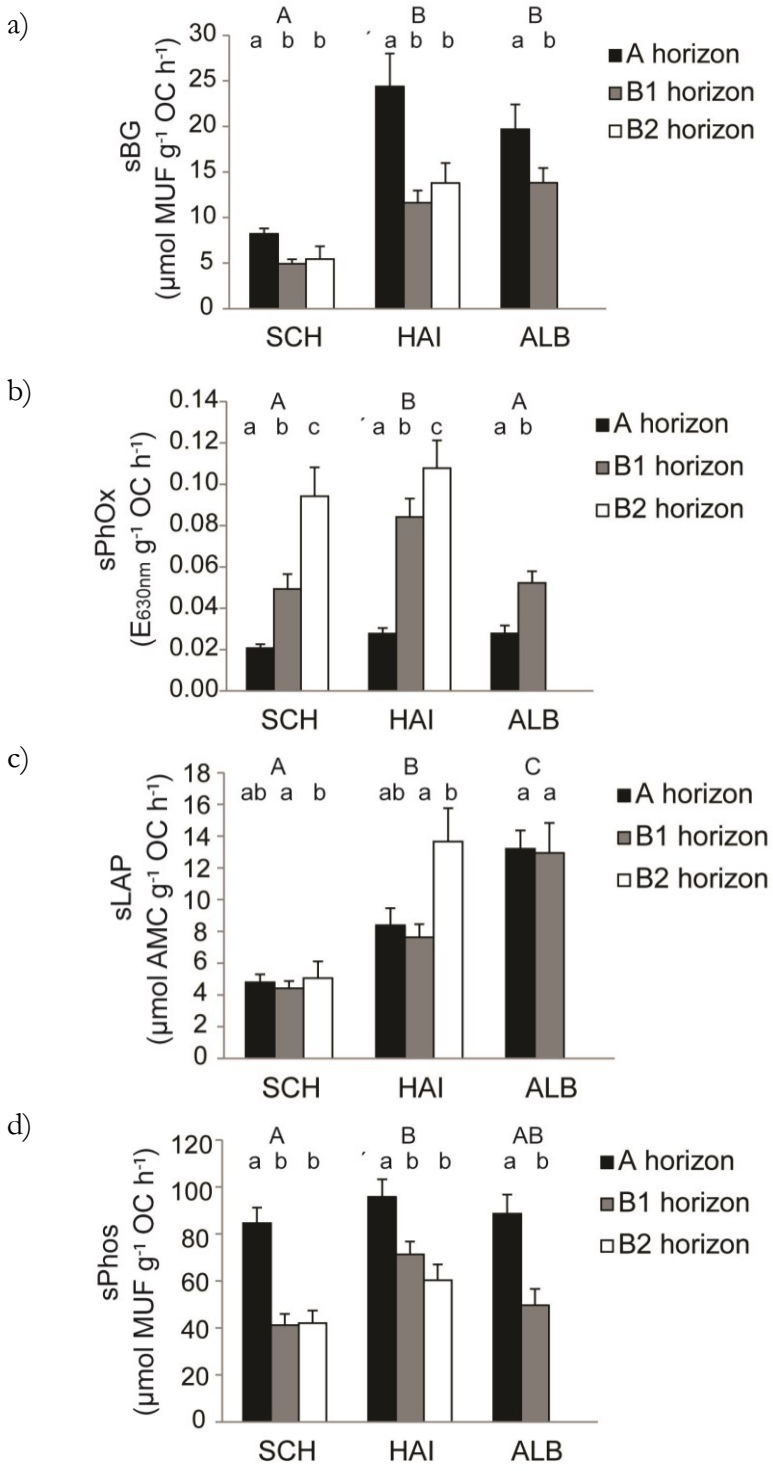

Fig. 3.1 Specific activities of a) ß-glucosidase b) peroxidase, c) L-leucine aminopeptidase, and d) phosphatase of three soil horizons in three study regions (SCH=Schorfheide-Chorin, HAI=Hainich-Dün, ALB=Schwäbische Alb). Bars represent means (n = 6-9) and standard errors.

activities in all soil horizons. In the A horizon, Phos activity was positively related to oxalate-extractable Fe oxides, whereas PhOx activity was positively related to the soil pH (Fig. 3.3 a). C stocks of the organic layer negatively impacted enzyme activities. In the B1

and B2 horizon (Fig. 3.3 b, c), the SCH was negatively related to all enzyme activities with lower values in the SCH than in the other two regions. In addition, enzyme activities decreased with higher horizon thickness in the B1, while the C:N ratio of the organic layer positively influenced Phos activity in the B2 horizon.

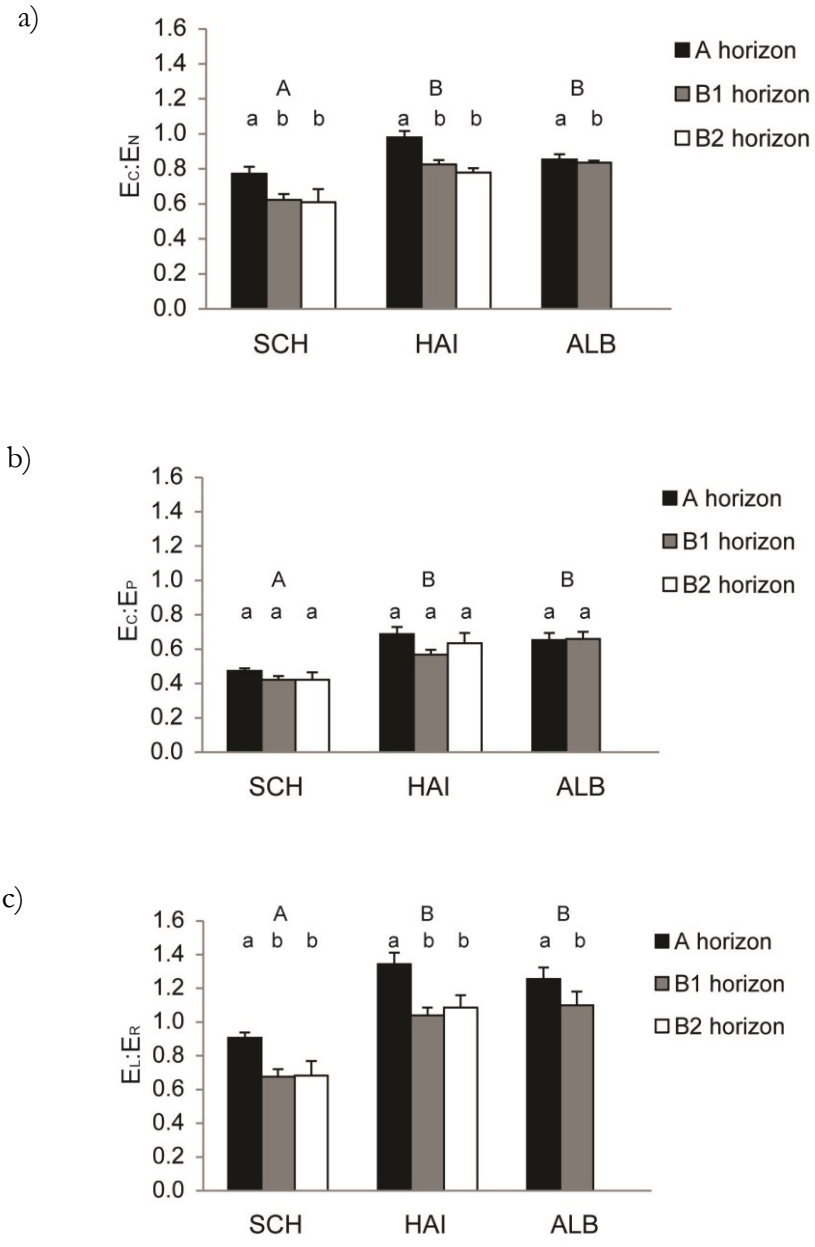

Fig. 3.2 Ratios of a) E_C:E_N is ln BG : ln (NAG+LAP) b) E_C:E_P is ln BG : ln Phos and c) E_L:E_R is ln BG : ln (10+PhOx) of three soil horizons in three study regions (SCH=Schorfheide-Chorin, HAI=Hainich-Dün, ALB=Schwäbische Alb). Bars represent means (n = 6-9) and standard errors.

Table 3.4 Variance partitioning results showing the fractions of explained variance of enzyme activities in three soil horizons that can be attributed to study region, forest management, and soil properties. The fraction of the variance explained and its significance revealed by unrestricted Monte Carlo permutation tests (*$P < 0.05$, **$P < 0.01$) by each of the environmental groups is given separately, after eliminating the variance due to the respective other two environmental groups, which are used as covariables.

	A horizon	B1 horizon	B2 horizon
Variance fractions			
Explained variance	87.1**	86.8**	90.9**
Study region	1.8	7.6*	4.8*
Forest management	5.2	3.2	7.3
Soil properties	15.2**	11.5*	8.5*
Shared	64.9	64.5	70.3
Residuals	12.9	13.2	9.1

Partial RDA (biplots not shown) revealed significant correlations between study region and enzyme activities in the B1 and B2 horizon, with forest management and soil properties as covariables. Enzyme activities were separated by study region with high correlations of all enzyme activities to the ALB in the B1 horizon, and negative correlations of all enzyme activities to the SCH in the B2 horizon. Further partial RDA showed significant correlations between soil properties and enzyme activities in all soil horizons, with study regions and forest management as covariables. The clay content was the most important contributor to explain variation in enzyme activities in all horizons, and was positively related to them. No significant correlations were found between forest management and enzyme activities in all horizons. There was a trend that all enzyme activities, except PhOx activity, were negatively related to C stocks of the organic layer in the A horizon.

3.4 Discussion

Enzyme activities among regions

Enzyme activities in the whole soil profile were region specific. There was a strong pattern that the enzyme activities across all soil horizons were highest in the ALB, followed by the HAI and the SCH (Table 3.3). The more sandy Arenosols in the SCH were characterized by lower OC and TN concentrations, higher C:N ratios, and lower soil pH than the finer textured soils in the HAI and ALB (Table 3.2). These differences in soil type and properties

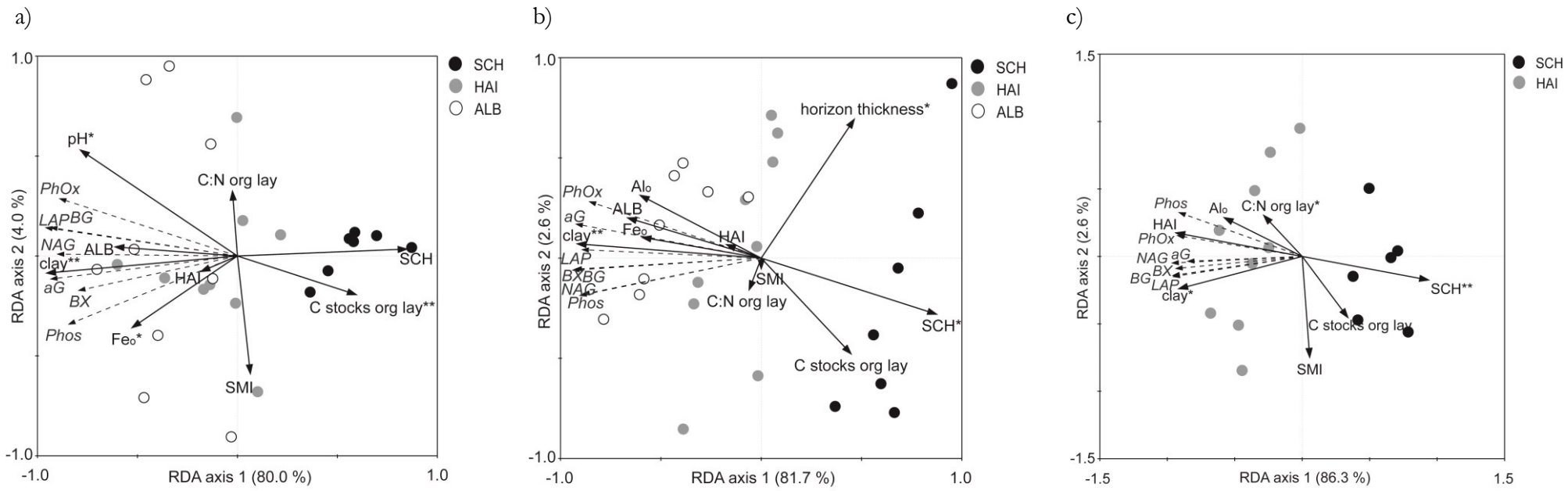

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Fig. 3.3 Biplots of the RDAs for enzyme activities (nmol MUF/AMC g^{-1} dw h^{-1}) and environmental variables (study region, forest management, and soil properties) of the a) A horizon, b) B1 horizon, and c) B2 horizon. Asterisks (*$P < 0.05$, **$P < 0.01$) indicate significant explanatory variables on the total variance of enzyme activities revealed by unrestricted Monte Carlo permutation tests.

Abbreviations: SCH=Schorfheide-Chorin, HAI=Hainich-Dün, ALB=Schwäbische Alb, BG=ß-glucosidase activity, BX=ß-xylosidase activity, aG=α-glucosidase activity, PhOx=phenol oxidase activity, NAG=N-acetyl-glucosaminidase activity, LAP=L-leucine aminopeptidase activity, Phos=phosphatase activity, SMI=Silvicultural management intensity indicator, Al_o=oxalate-extractable Al oxides, Fe_o=oxalate-extractable Fe oxides, C:N org lay=C:N ratio of the organic layer, C stocks org lay=C stocks of the organic layer

were responsible for the lower enzyme activities in the SCH, and support earlier findings where enzyme activities were lower with smaller OC and TN concentrations and pH (Dick et al., 1988; Sinsabaugh et al., 2008). Variations in substrate supply can be expected from different climate and soil types between the study regions, thus leading to shifts in C and N acquisition strategies. The higher C:N ratios and the lower TN concentrations in the Arenosols of the SCH indicate that these soils are more depleted in N than the Luvisols in the HAI and the Cambisols in the ALB. Thus, soils in the SCH have a higher demand for N, which is in line with the lower E_C:E_N ratios in the SCH than in the other two regions (Fig. 3.2 a), indicating that soil microbial communities in Arenosols direct more effort to acquire N relative to process C. Sinsabaugh et al. (2008) showed that mean enzymatic C:P acquisition ratio for 40 ecosystems declined with mean annual temperature (MAT) and precipitation (MAP). The lower E_C:E_P ratios in the SCH than in the other two regions (Fig. 3.2 b) only partly support the relations found in the aforementioned study. This is because MAP was lowest in the SCH, whereas MAT was highest compared to the other two regions. One reason for different trends in the E_C:E_P ratio with climatic variables in our study could be due to the studied range of climatic gradients by Sinsabaugh et al. (2008), suggesting that P availability declines relative to C as soil-weathering intensity increases. This suggests that in our study the parent material more strongly determines the availability of nutrients than climatic conditions. However, the lower E_C:E_P ratios in the SCH indicates lower P availability and a shift in investment to P acquisition. The range in E_L:E_R ratio (0.45-1.61) in our study was slightly wider than that reported by Sinsabaugh and Follstad Shah (2011) with 0.75-1.4. They proposed that the BG:PhOx activity is negatively related to the relative abundance of recalcitrant carbon, and that with increasing recalcitrance of decomposing OM, C and nutrient availability decreases. The lower nutrient availability in Arenosols of the SCH would suggest that the OM is more degraded, thus the labile substrate pool is more depleted than in the other two regions. This can be supported by the lower E_L:E_R ratio in the SCH (Fig. 3.2 c) with larger production of enzymes responsible for the degradation of more recalcitrant C compounds.

Enzyme activities in the soil profile

All enzyme activities, except PhOx activities, declined more from the A to the B1 horizon than from the B1 to the B2 horizon in all study regions (Table 3.3). Differences in enzyme activities among soil horizons were also due to variations in soil properties, such as OC and TN concentrations, the C:N ratio, or the microbial biomass. The decreased soil C:N ratios

in deeper soil horizons indicate that the soil OM is more degraded and humified (Dick, 1983). Together with lower fresh-C inputs by plants into deeper soil horizons (Fontaine et al., 2007) microbes and their released enzymes cannot sustain their catalytic capacity in deeper soil horizons relative to that of the surface horizon. This can be supported by some of the specific enzyme activities (BG, BX, aG, NAG, Phos), which were highest in the A horizon and lower in the B1 and B2 horizon in all study regions (Fig. 3.1); indicating lower enzyme activity per unit of C in subsoil horizons. Further, enzyme activities are to a large extend derived from soil microorganisms (Ladd, 1978), and the microbial biomass has been shown to decrease with increasing soil depth as a consequence of altered resource availability in form of decreasing nutrient concentrations in deeper soil horizons (Fritze et al., 2000; Fierer et al., 2003). Recent results of our study plots showed that total microbial biomass was also lower in deeper soil horizons than in the A horizon (personal communication Jessica Gutknecht).

In contrast to hydrolytic enzyme activities, PhOx activity showed a different depth distribution, with no significant decrease from the A to the subsoil horizons (Table 3.3). Our findings are in accordance with Kramer et al. (2013) who found similar results in an arable soil. They explained differences in depth distribution between hydrolytic and oxidative enzyme activities by substrate distribution within the soil profile and enzyme stability (binding to different particle size fractions). The increasing specific PhOx activities towards deeper soil horizons (Fig. 3.1 b), indicating higher PhOx activity per unit of C in subsoil horizons, further support the findings of different depth distributions between hydrolytic and oxidative enzyme activities. Further the E_L:E_R ratio revealed higher values in the A horizon than in the B1 and B2 horizon (Fig. 3.2 c). This indicates a relative increase in enzyme activities degrading more recalcitrant C relative to labile C compounds in the subsoil horizons. The study of Fontaine et al. (2007) showed that the higher stability of OC and smaller OC decomposition in deep soil layers reflect the lack of fresh C inputs for microorganisms, which probably suppressed the C acquiring enzyme BG in deeper soil horizons. In addition, it has been shown that root-derived C is the main litter input into deeper soil horizons (Rasse et al., 2005), which could be chemically more recalcitrant with higher concentrations of lignin (Lorenz and Lal, 2005).

The relatively larger decline in the activity of BG than of NAG and LAP in deeper soil horizons (thus a decrease in E_C:E_N ratio (Fig. 3.2a)) for all regions suggests a shift to higher N acquisition in subsoils. The higher E_C:E_N ratios in the A horizon could be due to higher

N availability in topsoils which was found to suppress the activity of N acquiring enzymes NAG and LAP (Olander and Vitousek, 2000; Saiya-Cork et al., 2002). Further, the strength of the shift towards higher N acquisition in subsoil horizons depended on the soil type. Cambisols in the ALB showed a smaller decrease in the E_C:E_N ratio from the A to the B1 horizon than Arenosols in the SCH and Luvisols in the HAI. This suggests that the N demand in the B1 horizon of Cambisols is lower than that of the B1 in the other two soil types and is in line with a lower decrease in OC and TN concentrations from the A to the B1 horizon in the Cambisols than in the Arenosols and Luvisols. In contrast to the E_C:E_N ratio and the E_L:E_R ratio, the E_C:E_P ratio showed no differences between soil horizons in all study region (Fig. 3.2 b), indicating no shift in the investment to P acquisition relative to C along the soil profile. Achat et al. (2013) studied the phosphorus status of Siberian forest soils, and showed that total organic P fractions represent high proportions of the total P in the surface soil layers and it decrease with increasing soil depth. They also observed that the potential contribution of the microbiological processes to P availability decreases in deeper soil horizons consistent with the decreases in total C and N. This suggests that P relative to C availability does not change from surface soils to subsoils in these forests, which could also be the case in our studied forest sites.

Impact of region, forest management and soil properties on enzyme activities

The high overall explained variance in enzyme activities by all environmental groups together (Table 3.4) showed that we were able to include the important factors in the variance partitioning analysis. Our results clearly show that from the three tested environmental groups most of the total variance of enzyme activities can be explained by soil properties in all soil horizons (Table 3.4). This seems not only to be the case in forest ecosystems. Another study by Floch et al. (2009) also showed that variances in enzyme activities were more attributable to soil properties than management strategy in 0-20 cm depth in an apple orchard agroecosystem.

Partial RDA revealed significant correlations between soil properties and enzyme activities constrained by study region and management. Soil texture is an important control in stabilizing soil enzymes, especially the interactions with clay minerals and humic colloids affects the stability of the enzymes (Burns, 1982). Thus, in our study, among all included soil properties, clay concentration explained most of the variation in enzyme activities in all horizons and it was positively related to all enzyme activities. It has to be pointed out, that clay concentrations were positively correlated to OC concentrations and Al and Fe oxides

(excluded from RDA, because of high collinearity), which are also known as a substrate and/or sorbent for enzyme activities. Next to the soil properties, study regions was the second most important factor in explaining variation in enzyme activities which is mostly the effect of the different soil types between study regions with their intrinsic soil properties. Other studies such as Bossio et al. (1998) already showed the importance of soil type on microbiological properties. They studied the relative importance of various environmental variables in governing the microbial community composition in farming systems and found that soil type was the most important control on microbial community composition next to time, specific farming operation, management system, and spatial variation in the field.

Partial RDA revealed no significant impact of long-term forest management intensity on enzyme activities constrained by study region and soil properties. There was a trend that enzyme activities were lower in the A horizon with higher C stocks of the organic layer. We would expect a positive correlation between C stocks of the organic layer and enzyme activities in the A horizon, because higher C stocks of the organic layer following higher leaf litter input lead to higher C storage in mineral horizons through higher leaching of dissolved OC from the organic layer (Fröberg et al., 2005). Thus, higher C concentrations in the mineral horizon can enhance enzyme activities. We assume that this negative trend is an effect of the study region, because forests in the SCH had higher C stocks of the organic layer than the other two study regions, but lower enzyme activities.

However, it needs to be pointed out that a major part of the variance in enzyme activities was explained by the shared variance fraction which represents the shared variation between all three environmental groups. Therefore, forest management intensity possibly affected enzyme activities indirect via the effect of management intensity on soil properties.

3.5 Conclusions

Our study showed that soil type and soil properties have significant effects on enzyme activities and acquisition of energy and nutrients in the whole soil profile of forest stands. There was a strong pattern that enzyme activities across all soil horizons were highest in the ALB, followed by the HAI and the SCH, which was a result of different soil types between regions and their intrinsic soil properties such as OC and TN concentrations, C:N ratios, and soil pH. Calculated enzyme activity ratios indicated a shift in investment to N and P acquisition in the SCH compared to the other two study regions. The decrease in all

enzyme activities, except PhOx activity, from the A to the B1 to the B2 horizon followed the decrease in OC and TN concentrations, while the decrease was much stronger from the A to the B1 horizon than from the B1 to the B2 horizon. Subsoil horizons showed higher enzyme activities degrading more recalcitrant C relative to labile C compounds than the A horizon, which is in line with no significant decrease of PhOx activity towards deeper soil horizons compared to hydrolytic enzyme activities. Further, subsoil horizons in all regions indicate a shift to higher N acquisition, while the strength of the shift depended on the soil type. Forest management intensity seems to have no major impact on enzyme activities on the long-term. These results highlight the need for studying different regions and their environmental conditions in order to draw general conclusions on the impact of forest management and soil properties on enzyme activities in the whole soil profile, which should help to better predict changes in C and nutrient cycling in response to changes in environmental conditions in the future.

Chapter 4

Soil and land-use types influence soil organic carbon storage and radiocarbon signatures in temperate soils more than land management

Chapter source: Nadine Herold et al., 2013. Soil and land-use types influence soil organic carbon storage and radiocarbon signatures in temperate soils more than land management. Soil Biology and Biochemistry. (submitted)

Abstract

Land use and management practices are considered as important controls for soil organic carbon (OC) storage and turnover. We compared the impact of land use (forest, grassland), forest management (spruce and beech forest under age-class management, unmanaged beech forest), and grassland management (meadow, mown pasture, pasture) on OC stocks and radiocarbon signatures ($\Delta^{14}C$) in A horizons. Soils samples were taken from 36 plots in the Hainich-Dün (HAI) and the Schwäbische Alb (ALB) in Germany. Bulk samples were separated in two light fractions and the mineral-associated organic matter (MOM) fraction using polytungstate with a density of 1.6 g cm^{-3}.

An important part (27±1.8 %) of the total OC was located in the light fractions of A horizons in forests and grasslands of both study regions, while forest soils stored significantly higher proportions of total OC (33±1.9 %) than grassland soils (20±2.3 %). The $\Delta^{14}C$ values of the density fractions indicated that the two light fractions had shorter turnover times in grasslands than in forests. For the free light fraction we found a high variation of $\Delta^{14}C$ in grasslands with higher $\Delta^{14}C$ values under pasture than under mown pasture, whereas differences in OM input between management types in forests and grasslands were probably not large enough to affect OC storage in density fractions. Large OC storage in the MOM fraction was associated with higher stability indicated by depleted $\Delta^{14}C$ values and correlations of the MOM fraction-OC to pedogenic Al and Fe oxides as

well as clay concentrations. Further, $\Delta^{14}C$ signatures of all density fractions were correlated to each other, but the strength of the relationship depended on the soil and land-use type.

We conclude that land-use type is more important for OC storage and turnover of the light fractions, while amount and turnover of OC in the MOM fraction were rather affected by soil and site conditions than by vegetation. In contrast, different forest and grassland management practices only had minor impact on OC storage and turnover of density fractions.

Keywords: Physical fractionation, Radiocarbon (^{14}C) measurements, A horizon, Turnover, Temperate Forest, Temperate Grassland

4.1 Introduction

Temperate forest and grassland soils store significant quantities of the total terrestrial soil organic carbon (OC) (Jobbágy and Jackson, 2000). Soil OC storage is determined by the balance between C inputs by leaf and root litter and the release of C during decomposition. The management practices applied in forest and grassland systems are known to modify soil OC storage through biomass removal, plant species selection, fertilization, or grazing, thereby affecting the amount, quality, and distribution of new OC entering the soil (Conant et al., 2001; Guo and Gifford, 2002; Carter et al., 2003; Jandl et al., 2007). Tree species selection influences soil OC storage in forests for example because of slower decay of coniferous tree litter compared to deciduous tree litter (Vesterdal and Raulund-Rasmussen, 1998; Fischer et al., 2002). Forest harvesting seems to have little- or no net effect on soil OC storage (Johnson and Curtis, 2001), but results might depend on soil types (Nave et al., 2010). To date, however, the effect of harvesting has predominantly been studied in pine forests and little is known about temperate broadleaf forests (Nave et al., 2010). Published studies in temperate grasslands mainly refer to effects of single management practices on soil OC storage (Soussana et al., 2004; Piñeiro et al., 2010), but commonly a number of different management practices are applied simultaneously, which has been studied much less (Ammann et al., 2007).

Changes in soil OC as a result of different land use and management are difficult to measure in the bulk soil because of the high spatial variability of soil OC (Conen et al., 2004; Grüneberg et al., 2010; Schrumpf et al., 2011). Soil organic matter (OM) in bulk soils

is a heterogeneous mixture of organic substances in various stages of decomposition with different chemical composition and turnover. The isolation of more sensitive OM fractions enhances the probability of determining soil OC stock changes in relation to management.

Density fractionation has been used to isolate light fractions of uncomplexed particulate OM from mineral-associated OM (Christensen, 2001). The light fraction consists mainly of weakly decomposed plant and animal tissues with short turnover times (annual to decadal), whereas the mineral-associated OM fraction is a more stable fraction with turnover times of decades to centuries (Baisden et al., 2002; John et al., 2005; Crow et al., 2007). The light fraction is often be separated in one fraction that is free or exterior to stable aggregates (free light fraction), and another fraction that is occluded within stable aggregates (occluded light fraction) (Golchin et al., 1994). It has been concluded that these two fractions are functionally specific fractions (Meyer et al., 2012), and they apparently differ in turnover times (^{14}C) and chemical composition (Golchin et al., 1994; Wagai et al., 2009).

Land use has been reported to influence the distribution of carbon to functionally different OM fractions (John et al., 2005). The light fraction was found to be sensitive to soil management (Bremer et al., 1994; Gregorich and Janzen, 1996; Chan, 2001), which was mostly studied in agricultural systems, but rarely in forest and grassland systems (Leifeld and Fuhrer, 2009; Meyer et al., 2012; Schrumpf et al., 2013). In contrast, OC storage in the mineral-associated OM fraction is mainly controlled by soil properties, particularly the amount of clay and associated iron and aluminium oxides (Kaiser et al., 2002; Mikutta et al., 2006; Schöning et al., 2013; Schrumpf et al., 2013). To our knowledge no study has compared effects of different management practices in natural forests and grasslands from the same region on OC storage in the three density fractions.

Radiocarbon (^{14}C) produced by aboveground testing of nuclear bombs during the 1950s and 1960s can be used to study OC turnover in soil density fractions (Trumbore and Zheng, 1996; Six and Jastrow, 2002). Only few studies are available, that determined the impact of different land-use and management types on ^{14}C in density fractions of temperate ecosystems. Meyer et al. (2012) studied OC turnover in the upper mineral soil (0-10 cm) of Cambisols in three different mountainous grassland systems (hay meadow, pasture, abandoned grassland) in the European Alps. They found, that the OC turnover of the free light fraction varied most in relation to management, while the occluded light fraction and mineral-associated OM fraction were not affected by grassland abandonment. Schöning et al. (2013) studied the decadal effect of forest management on the ^{14}C signatures of the

mineral-associated OM fraction and related turnover times in Ah horizons, but found no significant differences between the forest management types. Instead, ^{14}C signatures of the mineral-associated OM fraction and related turnover times were controlled by clay contents and associated OC concentrations. This shows that soil properties are important determinants of OC turnover in the mineral-associated OM fraction which could be confirmed by other studies (Don et al., 2009; Leifeld and Fuhrer, 2009). However, there is overall little information about the impact of land use and management practices on ^{14}C signatures of density fractions in forest and grassland systems.

Here, we used density fractionation and radiocarbon analyses to study variations of OC in A horizons of forest and grassland sites in two German regions (Hainich-Dün, Schwäbische Alb), that differ in climatic conditions and parent material. The forest sites covered a range of three different management types including spruce and beech forest under age-class management and unmanaged beech forest. The grassland sites were managed as meadow, mown pasture, and pasture. The large number of samples and replications allowed an evaluation of the results using statistical methods such as analysis of covariance. Our objective was to examine the impact of land use, land management, and soil properties on OC storage and ^{14}C signatures in density fractions at the regional scale.

4.2 Materials and methods

4.2.1 Study sites

We studied 18 grassland and 18 forest plots in Germany, located in the Hainich-Dün (HAI) and Schwäbische Alb (ALB) (Fischer et al., 2010). Soils in the HAI were Luvisols in the forests and Stagnosols in the grasslands (Table 4.1). In the ALB, Cambisols occurred as the soil group in the forests and Leptosols in the grasslands (IUSS Working Group WRB, 2006). Three different forest management types were studied including Norway spruce (*Picea abies* L.) and European beech (*Fagus sylvatica* L.) dominated forests (Table 4.2). The unmanaged forests are uneven-aged forests with trees of different sizes and ages. Plots of the unmanaged beech forest in the HAI belong to a National park, in which no trees at all have been harvested since 1997. From 1965-1997, this area was used as a military training ground and even in this time only small amounts of wood were removed. Plots of the unmanaged beech forest in the ALB experienced restricted timber harvesting under nature conservation aspects (e.g. removal of spruce trees to protect old beech trees), or were not managed at all for the last 80 years. Forests under age-class management were even-aged

regenerations after a clear cut (harvested at 80-120 year intervals). Forests under age-class management were separated in different growth stages according to diameter at breast height (DBH): thicket and pole wood (DBH 7-15 cm), young timber (DBH = 15-30 cm), and old timber (DBH > 30cm). In addition to the forest sites, grassland sites were studied, which were managed as meadow (fertilized and mown), mown pasture (fertilized, mown and grazed), and pasture (unfertilized and grazed) (Table 4.3). The three grassland management types differ in N-fertilization (kg N per ha^{-1} yr^{-1}), mowing (times mowed yr^{-1}) and grazing intensity (livestock units days of grazing ha^{-1} yr^{-1}). All grassland plots experienced the 2008 management practice for at least four years prior to sampling.

Table 4.1 General characteristics of the study regions.

Study region	MAT[a] [°C]	MAP[b] [mm]	Parent material	FAO-Soil group[c]
Hainich-Dün	6.5-8.0	500-800	Loess and Triassic shell limestone	Luvisol (forest) Stagnosol (grassland)
Schwäbische Alb	6.0-7.0	700-1000	Jurassic limestone	Cambisol (forest) Leptosol (grassland)

[a]MAT = Mean Annual Temperature
[b]MAP = Mean Annual Precipitation
[c]IUSS Working Group WRB (2006)

4.2.2 Soil sampling

In spring 2008, five soil samples were taken at each plot (20 x 20 m), one at each corner and in the plot centre. Prior to sampling, the organic layer and the above ground vegetation were removed. Subsequently, the mineral soil was sampled down to the bedrock using a motor driven auger (8.3 cm diameter). Soil horizons were designated according to the guidelines for soil description (FAO, 2006). The uppermost soil horizon (A horizon) was separated from the soil core (Table 4.4). The five soil samples per plot were mixed to obtain a composite sample for each plot. Samples were stored in ice boxes and transported to the field lab facility. After removal of coarse roots and stones, soil samples were air-dried and sieved to < 2 mm to isolate the fine earth.

Table 4.2 Management of the forest plots in 2007.

Region	Management type	Plot ID	Location latitude, longitude	Stage of development	Stand age [yr]	Basal area [m^2 ha^{-1}]
Hainich-Dün	Spruce age-class forest	HEW1	51°11'N, 10°19'E	Old timber	70-79	42.2
		HEW2	51°12'N, 10°22'E	Old timber	50-59	41.9
		HEW3	51°16'N, 10°18'E	Old timber	60-69	31.8
	Beech age-class forest	HEW4	51°22'N, 10°31'E	Thicket	20-29	42.9
		HEW5	51°15'N, 10°14'E	Old timber	80-89	23.6
		HEW6	51°16'N, 10°14'E	Old timber	110-119	41.4
	Unmanaged beech forest	HEW10	51°5'N, 10°27'E	Old timber	160-168	31.2
		HEW11	51°6'N, 10°24'E	Old timber	160-169	37.6
		HEW12	51°6'N, 10°27'E	Old timber	180-189	32.3
Schwäbische Alb	Spruce age-class forest	AEW1	48°28'N, 9°20'E	Young timber	40-49	63.7
		AEW2	48°22'N, 9°21'E	Old timber	60-69	28.7
		AEW3	48°24'N, 9°21'E	Old timber	50-59	42.5
	Beech age-class forest	AEW4	48°23'N, 9°14'E	Pole wood	40-49	18.3
		AEW5	48°25'N, 9°24'E	Old timber	140-149	34.5
		AEW6	48°23'N, 9°26'E	Old timber	80-89	34.5
	Unmanaged beech forest	AEW7	48°23'N, 9°15'E	Old timber	130-139	23.8
		AEW8	48°22'N, 9°22'E	Old timber	150-159	34.1
		AEW9	48°22'N, 9°24'E	Old timber	150-159	18.5

Table 4.3 Management of the grassland plots in 2007.

Region	Management type	Plot ID	Location latitude, longitude	N-fertilization [kg ha^{-1}]	Mowing [times yr^{-1}]	Grazing	Grazing intensity [LU d ha^{-1}]	Management since
Hainich-Dün	Meadow	HEG1	50°58'N, 10°24'E	135	3	cattle	44.9	2005
		HEG2	51°0'N, 10°24'E	140	3	cattle	34.8	1995
		HEG3	50°59'N, 10°25'E	140	3	cattle	34.8	2003
	Mown pasture	HEG4	51°6'N, 10°26'E	27	1	cattle	113.8	1980
		HEG5	51°12'N, 10°19'E	80	2	cattle	93.6	1993
		HEG6	51°12'N, 10°23'E	80	1	cattle	9.3	2004
	Pasture	HEG7	51°16'N, 10°24'E	0	0	cattle, horses	452.5	2005
		HEG8	51°16'N, 10°25'E	0	0	cattle, horses	452.5	2005
		HEG9	51°13'N, 10°22'E	0	0	cattle	71.4	1991
Schwäbische Alb	Meadow	AEG1	48°23'N, 9°20'E	35	2	-	0	1950
		AEG2	48°22'N, 9°28'E	100	3	-	0	1977
		AEG3	48°24'N, 9°31'E	64	3	-	0	1987
	Mown pasture	AEG4	48°22'N, 9°25'E	35	1	cattle	106.6	1950
		AEG5	48°23'N, 9°26'E	50	1	cattle, horses	123.5	1950
		AEG6	48°24'N, 9°26'E	50	1	cattle, horses	687.2	1950
	Pasture	AEG7	48°23'N, 9°22'E	0	0	sheep, goats	30.8	1995
		AEG8	48°25'N, 9°29'E	0	1	sheep, goats	103.7	1977
		AEG9	48°23'N, 9°30'E	0	0	sheep, goats	38.7	1907

4.2.3 Basic soil analyses

Soil texture was determined according to Schlichting and Blume (1964). Soil pH was measured in the supernatant of a 1:2.5 mixture of air-dried soil and 0.01 M $CaCl_2$ using a glass electrode. Sub-samples of the air-dried soil were ground in a ball mill for elemental analysis. Total C (TC) and total N (TN) concentrations were determined by dry combustion (Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany). After removal of OC by ignition at 450°C for 16 h, inorganic C was quantified with the same elemental analyzer. OC concentrations were calculated as the difference between TC and inorganic C. Soil OC stocks (kg m^{-2}) were calculated as the product of OC concentration, the weight of air-dried fine earth and its volume. Oxalate-extractable Al and Fe (Al_o, Fe_o) were extracted with 0.2 M oxalate solution (pH 3) (Schwertmann, 1964). The extraction of dithionite-extractable Fe (Fe_d) was done with Na-dithionite and tri-Na-citrate-dihydrate for 16 h after the method of Holmgren (1967). Al and Fe concentrations were measured with an Inductively-Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, Optima 3300 DV, Perkin-Elmer, Norwalk, CT, USA).

4.2.4 Density fractionation

Three density fractions including a light fraction (LF1) that contains the free particulate OC and OC occluded in macroaggregates, a light fraction that contains OC occluded in microaggregates (LF2), and the mineral-associated OM (MOM fraction) were separated according to the method of Don et al. (2009). An amount of 10 g air-dried and sieved soil (< 2 mm) from the A horizons was suspended in 100 ml sodium polytungstate of a density of 1.6 g cm^{-3}. A gentle ultrasonic treatment with an energy of 60 J ml^{-1} was applied. Subsequently, the suspension was centrifuged for 30 min with a relative centrifugal force of 2889 g. Using a pipette, the floating organic particles (LF1) were transferred with a pipette to a glass-fiber filter on a vacuum bottle. The remaining soil sample was again mixed with 100 ml sodium polytungstate solution of the same density and treated with ultrasonic sound (energy of 450 J ml^{-1}) to break micro-aggregates. Subsequently, the suspension was centrifuged and treated as described above to separate the floating particles (LF2) and the MOM. All fractions were washed with distilled water to remove the sodium polytungstate until the conductivity was < 10 μSm^{-1}. The light fractions were dried at 40°C, and the MOM fraction was freeze-dried. Organic C and TN concentrations of the density fractions were determined with the same method as described above for bulk soil samples. A mass

balance showed that overall 99±0.5 % of the dry bulk soil mass and 88±1.1 % of the total OC were recovered in the density fractions. The recovery of total OC in density fractions did not differ between different regions and land-use types (see results, Table 4.5).

4.2.5 Radiocarbon analyses

Radiocarbon concentrations (^{14}C) in the density fractions were analysed by accelerator mass spectrometry (AMS) in Jena, Germany (Steinhof et al., 2004). After dry combustion of the sample, a small part of the evolving CO_2 was used to determine $\delta^{13}C$, while the remaining part of the CO_2 was reduced to graphite by heating a mixture of H_2 and CO_2 with Fe powder at 550°C. The resulting graphite-coated iron was pressed into targets and measured with the AMS facility for ^{14}C. Radiocarbon activity is expressed as $\Delta^{14}C$, the difference in parts per thousand (‰) between the $^{14}C/^{12}C$ ratio in the sample compared to that of the standard oxalic acid (Stuiver and Polach, 1977). All ^{14}C values were corrected for mass-dependent fractionation of ^{14}C using the measured $\delta^{13}C$. The average measurement precision of the $\Delta^{14}C$ values was 2.7 ‰.

4.2.6 Statistical analyses

All statistical analyses were conducted in R version 2.15.1 (R Development Core Team, 2012). Results are presented as means ± standard error of the mean. We used two-way analysis of variance (ANOVA) to test the effect of study region and land use on soil properties. For this analyses, the A horizon thickness, sand contents, oxalate-extractable Al concentrations, C:N ratios of the MOM fraction, and OC stocks of the LF1 were log-transformed. Analysis of covariance (ANCOVA) was performed to test the effect of study region, land-use and management types on C:N ratios and OC stocks of the bulk soil as well as C:N ratios, proportions of total OC concentration, OC stocks, and $\Delta^{14}C$ values in the three density fractions. The clay concentration was used as a covariate to account for inter-group variation. OC stocks of the LF1 and $\Delta^{14}C$ values of the LF2 were log-transformed. Linear regression was used to analyse relations between $\Delta^{14}C$ values in different density fractions, and to assess the effect of soil properties on OC storage and ^{14}C signatures in density fractions.

4.3 Results

4.3.1 Soil properties

Table 4.4 shows soil properties of forest and grassland plots in the two study regions. Leptosols in the ALB had significantly thicker A horizons than Cambisols in the ALB. Forest soils in the HAI contained more silt and less clay than HAI grassland soils, but in the ALB soil texture did not differ between land-use types. In both study regions soil pH was significantly lower in forest soils than in grassland soils. The oxalate-extractable Al and dithionite-extractable Fe concentrations were about two times higher in the ALB than in the HAI.

Table 4.4 The A horizon thickness, sand, silt and clay concentrations, pH, oxalate-extractable Al and Fe (Al_o, Fe_o) concentrations, and dithionite-extractable Fe (Fe_d) concentrations in forest and grassland soils in Hainich-Dün and Schwäbische Alb. Two-way ANOVA results are presented with Tukey HSD test ($P < 0.05$). Significant differences between study regions are indicated by *capital letters*, and between land-use types by *lowercase letters*.

	Hainich-Dün		Schwäbische Alb	
	Forest	Grassland	Forest	Grassland
A horizon thickness [cm]	9.5 ± 0.8^{ab}	10.4 ± 3.0^{ab}	9.0 ± 1.5^{a}	12.6 ± 0.8^{b}
Sand [g kg^{-1}]	41±6	70±3	61±10	79±28
Silt [g kg^{-1}]	631 ± 34^{a}	468 ± 30^{b}	491 ± 38^{b}	544 ± 36^{ab}
Clay [g kg^{-1}]	329 ± 37^{a}	462 ± 30^{b}	448 ± 39^{ab}	377 ± 50^{ab}
pH	4.4 ± 0.3^{a}	6.6 ± 0.1^{b}	4.2 ± 0.3^{a}	5.9 ± 0.2^{b}
Al_o [g kg^{-1}]	2.8 ± 0.3^{A}	2.1 ± 0.1^{A}	4.0 ± 0.4^{B}	3.7 ± 0.2^{B}
Fe_o [g kg^{-1}]	3.7±0.3	3.2±0.2	3.5±0.5	3.3±0.4
Fe_d [g kg^{-1}]	14.2 ± 1.8^{A}	13.4 ± 0.7^{A}	25.3 ± 1.7^{B}	23.3 ± 1.4^{B}

4.3.2 Organic C in the bulk soil and density fractions

Averaged OC concentrations across all land-use types and regions were highest in the LF2 (377±8 g kg^{-1}), followed by the LF1 (364±5 g kg^{-1}), and the MOM fraction (40±2 g kg^{-1}).

Soils in the ALB had significantly higher OC concentrations in the bulk soil and the MOM fraction than soils in the HAI, while the two light fractions showed significantly higher OC concentrations in forest soils than in grassland soils (Table 4.5).

Table 4.5 Organic carbon (OC) concentrations, C:N ratios, proportions of total OC concentration, and OC stocks of the three density fractions, and OC concentrations, C:N ratios, and OC stocks of the bulk soil in forest and grassland soils in Hainich-Dün and Schwäbische Alb. Two-way ANOVA results are presented with Tukey HSD test ($P < 0.05$). Significant differences between study regions are indicated by *capital letters*, and between land-use types by *lowercase letters*.

	Hainich-Dün		Schwäbische Alb	
	Forest	Grassland	Forest	Grassland
OC concentration [g kg^{-1}]				
LF1	380.7±7.2^{a}	357.3±3.1^{b}	383.9±4.9^{a}	334.2±11.8^{b}
LF2	394.8±9.0^{a}	361.1±21.4^{b}	395.6±13.2^{a}	357.3±16.5^{b}
MOM	33.2±3.6^{A}	33.3±4.1^{A}	44.1±3.3^{B}	47.9±3.5^{B}
Bulk soil	56.0±5.4^{A}	50.2±7.2^{A}	68.8±6.0^{B}	67.0±4.7^{B}
C:N ratio				
LF1	26.0±0.6^{Aa}	15.5±0.5^{Ab}	27.2±0.7^{Ba}	18.7±1.4^{Bb}
LF2	23.7±0.9^{Aa}	14.5±0.4^{Ab}	25.9±0.7^{Ba}	16.5±1.1^{Bb}
MOM	10.9±0.4^{a}	8.8±0.3^{b}	10.6±0.5^{a}	9.0±0.2^{b}
Bulk soil	14.2±0.5^{a}	10.4±0.3^{b}	14.1±0.4^{a}	10.6±0.2^{b}
Proportion of total OC concentration [%]				
LF1	26.8±1^{Aa}	18.7±2.0^{Ab}	20.4±1.0^{Ba}	8.4±0.8^{Bb}
LF2	9.1±0.7^{a}	6.6±1.0^{b}	9.3±1.1^{a}	6.6±0.9^{b}
MOM	64.1±2.1^{Aa}	74.7±2.5^{Ab}	70.3±1.7^{Ba}	85.0±1.3^{Bb}
OC stock [kg m^{-2}]				
LF1	1.0±0.1^{a}	0.6±0.2^{b}	0.7±0.1^{a}	0.4±0.02^{b}
LF2	0.3±0.1^{a}	0.2±0.02^{b}	0.3±0.04^{ab}	0.3±0.1^{a}
MOM	2.4±0.3^{Aa}	2.5±0.5^{Aa}	2.5±0.4^{Ba}	4.4±0.4^{Bb}
Bulk soil	4.3±0.5^{ab}	3.6±0.7^{a}	4.0±0.5^{a}	6.1±0.4^{b}
Recovery of total OC in fractions [%]	87.1±1.6	91.3±2.6	88.0±1.9	83.8±2.1

Across all samples, the highest C:N ratios (21.8±1.3) were found in the LF1, followed by the C:N ratios of the LF2 (20.2±1.3), and the MOM fraction (9.8±0.3). The C:N ratios of the two light fractions were significantly higher in the ALB than in the HAI. Forest soils had significantly higher C:N ratios in the bulk soil and in all density fractions than grassland soils.

Overall, the highest proportions of total OC were stored in the MOM fraction (73±1.6 %), followed by the LF1 with 19±1.3 %, and the LF2 with 8±0.5 %. HAI soils stored higher proportions of the total OC in the LF1 than ALB soils. Both light fractions showed significantly higher proportions of total OC in forest soils than in grassland soils.

Averaged OC stocks across all plots were highest in the MOM fraction (2.9±0.2 kg m^{-2}), followed by the LF1 (0.7±0.1 kg m^{-2}), and the LF2 (0.3±0.02 kg m^{-2}). OC stocks of the MOM fraction were significantly higher in soils of the ALB than in soils of the HAI. MOM OC stocks were significantly higher in grassland soils than in forest soils in the ALB, but not in the HAI.

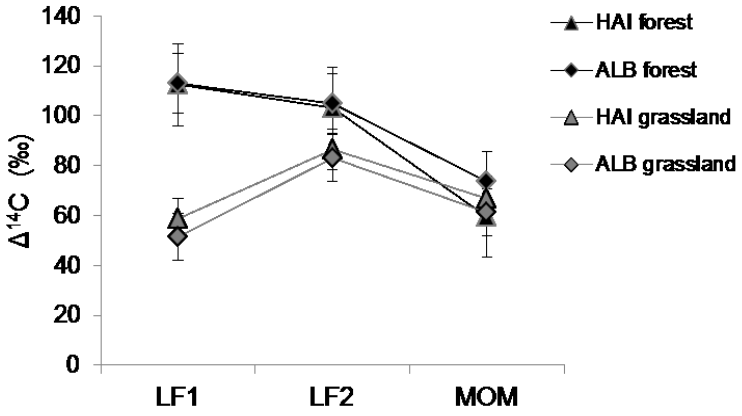

Fig. 4.1 Mean $\Delta^{14}C$ values and standard errors of the LF1, LF2 and MOM fraction in forest and grassland soils in the Hainich-Dün (HAI) and Schwäbische Alb (ALB). HEG6 (mown pasture plot in the Hainich-Dün) was excluded from the analyses due to negative $\Delta^{14}C$ values in the LF1 and LF2.

4.3.3 Radiocarbon signatures

Radiocarbon signatures (measured in 2008) for the LF1 ranged from -9 ‰ to 176 ‰, for the LF2 from -8 ‰ to 156 ‰, and for the MOM fraction from 21 ‰ to 133 ‰ (Fig. 4.2 a-c). Soils in the ALB had slightly higher $\Delta^{14}C$ values in all three density fractions compared to the HAI, but this was not significant (Fig. 4.1, Table 4.6). In both regions, forest soils showed the highest mean $\Delta^{14}C$ values in the LF1 (113±6 ‰), followed by the LF2 (104±7 ‰), and the MOM fraction (67±7 ‰) (Fig. 4.1). In contrast, mean $\Delta^{14}C$ values in grassland soils were highest in the LF2 (85±5 ‰), followed by the MOM fraction (64±6 ‰) and the LF1 (55±6 ‰).

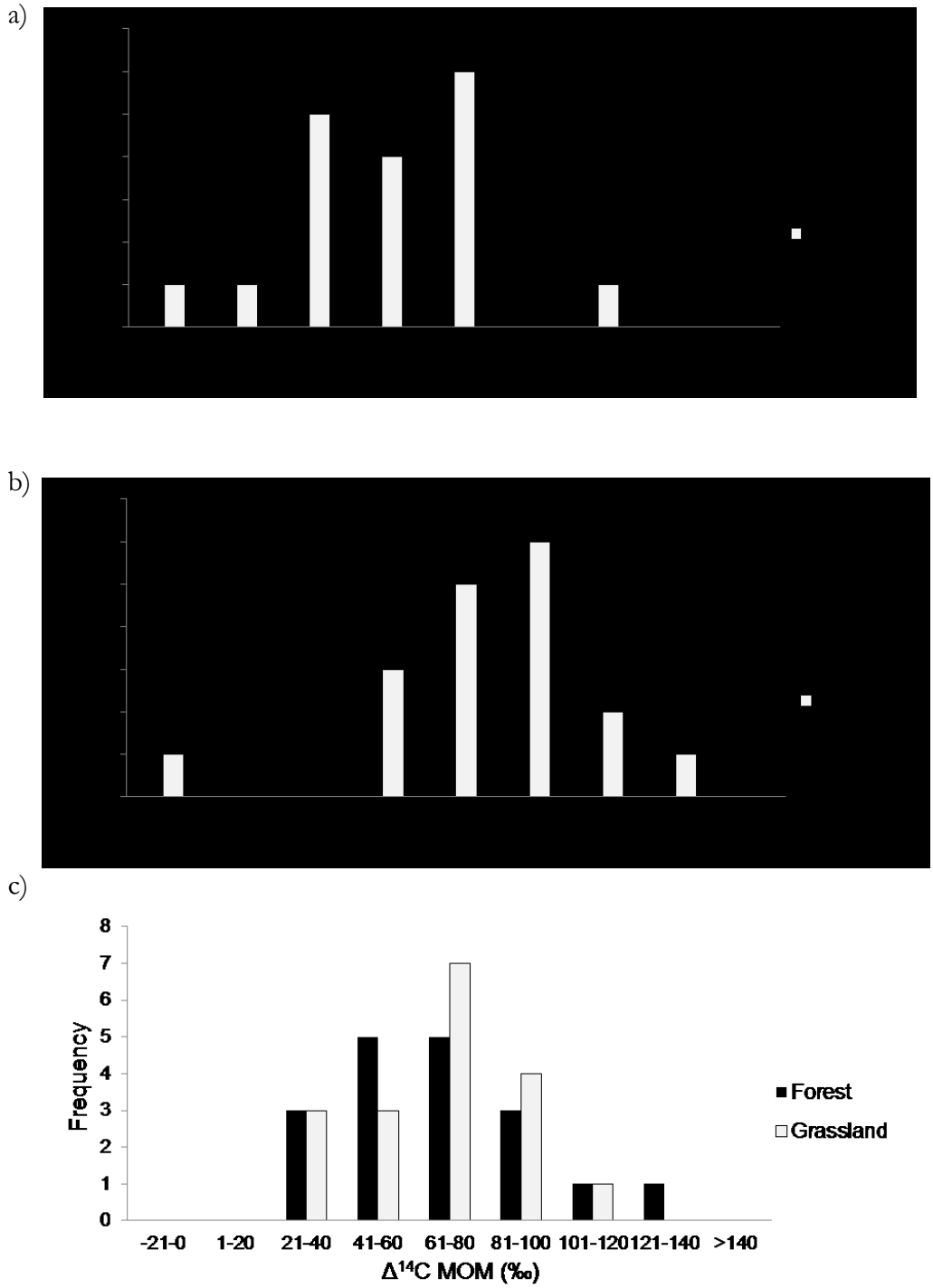

Fig. 4.2 Histograms of $\Delta^{14}C$ values of the a) LF1, b) LF2, and c) MOM fraction in forest and grassland soils in Hainich-Dün and Schwäbische Alb.

Across the two regions, the two light fractions revealed a higher range of $\Delta^{14}C$ values in grassland soils (in LF1 -9 ‰ to 117 ‰, LF2 -8 ‰ to 137 ‰) than in forest soils (LF1 69 ‰ to 176 ‰, LF2 36 ‰ to 156 ‰) (Fig. 4.2 a, b). The two light fractions from grasslands had significantly lower $\Delta^{14}C$ values than forest soils (Fig. 4.1, Table 4.6). The

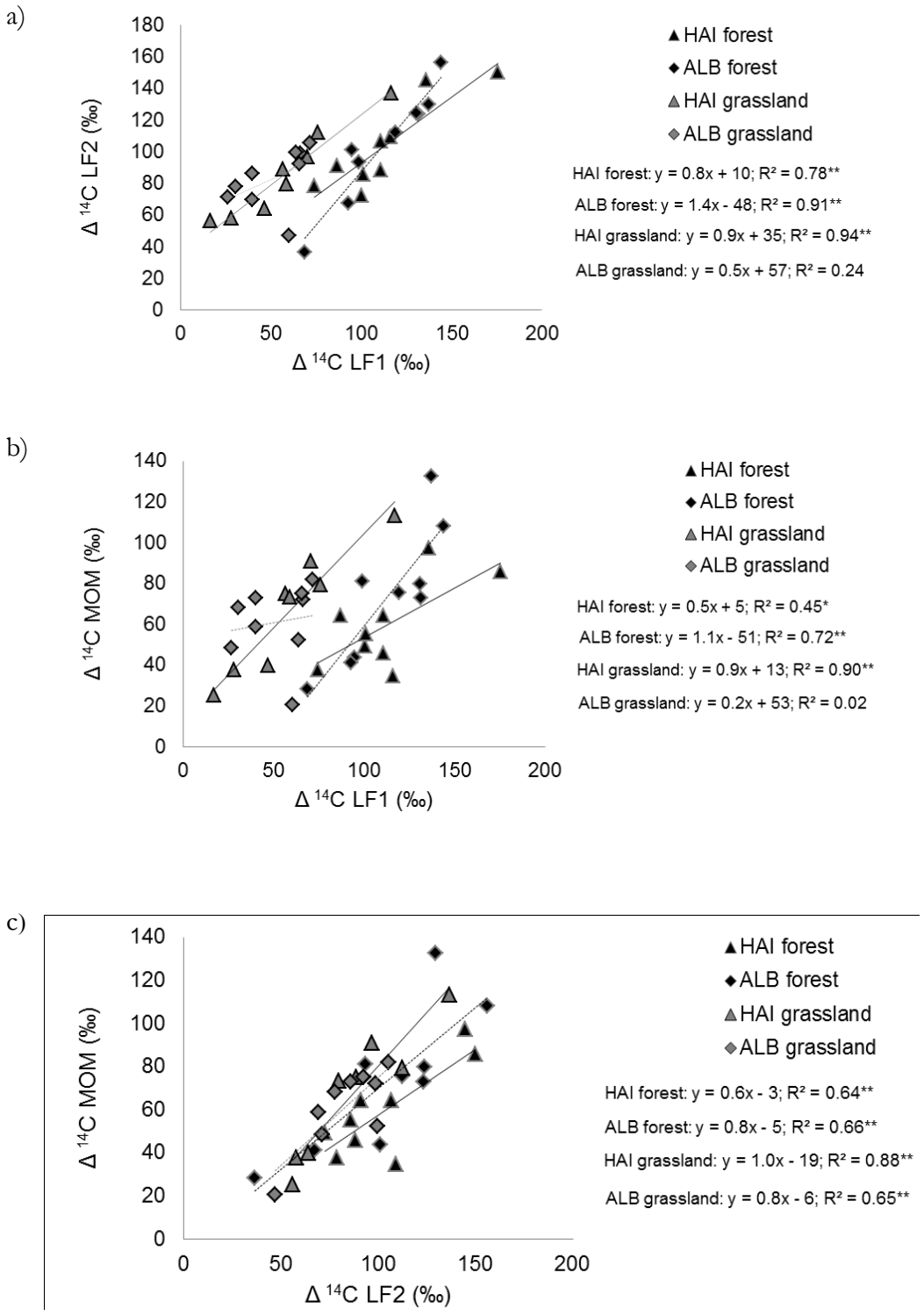

Fig. 4.3 Relations between $\Delta^{14}C$ values of the three density fractions (LF1, LF2, MOM fraction) separated for forest and grassland soils and regions (HAI=Hainich-Dün, ALB=Schwäbische Alb). R-values are presented (*$P < 0.05$, **$P < 0.01$). HEG6 (mown pasture plot in the Hainich-Dün) was excluded from the analyses due to negative $\Delta^{14}C$ values in the LF1 and LF2. HAI forest: black line; ALB forest: grey line; HAI grassland: black dotted line; ALB grassland: grey dotted line.

range of $\Delta^{14}C$ values in the MOM fraction was slightly smaller in grassland (21 ‰ to 113 ‰) than in forest soils (28 ‰ to 133 ‰) (Fig. 4.2 c). The $\Delta^{14}C$ values of the MOM fraction did not significantly differ between the two land-use types (Table 4.6).

Correlations between $\Delta^{14}C$ values of density fractions indicate a link between turnover times of those fractions (Fig. 4.3 a-c). The $\Delta^{14}C$ values of the two light fractions were correlated with each other in the HAI and ALB forest soils, and in the HAI grassland soils, but not in the ALB grassland soils. The same applies to the relation between the $\Delta^{14}C$ values of LF1 and the MOM fraction. The $\Delta^{14}C$ values of the LF2 were highly correlated to the $\Delta^{14}C$ values of the MOM fraction independent of study region and land-use type.

4.3.4 Impact of management on OC storage and $\Delta^{14}C$ values

The C:N ratios of the bulk soil and MOM fraction were significantly higher in soils of spruce forest under age-class management than in soils of beech forest under age-class management in both regions, and C:N ratios of the two light fractions were significantly higher in the pasture soils than in meadow soils in the ALB. Management type had no significant effect on OC storage in density fractions, but on $\Delta^{14}C$ values (Table 4.6). Pasture soils showed significantly higher $\Delta^{14}C$ values in the LF1 than mown pasture soils in both regions, and the $\Delta^{14}C$ values in the LF2 were also significantly higher in pasture than in mown pasture soils, but only in the HAI.

4.3.5 Impact of soil properties on OC storage and $\Delta^{14}C$ values

The amounts of OC per kg bulk soil in the LF2 and MOM fraction significantly increased with higher oxalate-extractable Al and dithionite-extractable Fe oxide concentrations (Fig. 4.4 a-c). In addition, a significant linear relation was found between the clay concentration and the amount of OC per kg bulk soil in the MOM fraction (Fig. 4.4 d). No significant relations existed between the amount of OC per kg bulk soil in the LF1, $\Delta^{14}C$ values of the density fractions and soil properties.

Table 4.6 ANCOVA results with C:N ratios, proportions of total OC concentration, OC stocks, and $\Delta^{14}C$ values of the three density fractions, and C:N ratios and OC stocks of the bulk soil as response variable. Explanatory variables are given in rows in the order of entering the analysis. Degrees of freedom (df), mean squares (MS) and F-values are presented (*$P < 0.05$, **$P < 0.01$). HEG6 (mown pasture plot in the Hainich-Dün) was excluded from the ANCOVA analyses when tested for significant effects on $\Delta^{14}C$ values of the different density fractions due to negative $\Delta^{14}C$ values in the LF1 and LF2.

		C:N ratio								*Proportion of total OC concentration [%]*					
		LF1		LF2		MOM		bulk soil		LF1		LF2		MOM	
	DF	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Study region (SR)	1	44.4	26.0**	39.1	16.3**	0.03	0.04	0.02	0.03	630	32.4**	0.1	0.01	617	15.7**
Clay concentration (clay)	1	86.1	50.3**	67.2	28.1**	5.1	6.1*	4.4	5.1*	5.2	0.3	11.6	1.5	1.3	0.03
Land use (LU)	1	747	447**	738	308**	27.1	32.7**	118	136**	905	46.6**	67.9	8.5**	1469	37.4**
Management type (MT)	4	10.9	6.3**	9.3	3.9*	2.6	3.1*	4.5	5.1**	35.3	1.8	4.7	0.6	41.2	1.1
SR:clay	1	4.0	2.3	2.2	0.9	0.9	1.0	1.2	1.3	1.4	0.07	1.1	0.1	4.9	0.1
SR:LU	1	0.1	0.04	8.0	3.3	0.03	0.04	0.03	0.03	45.8	2.4	2.6	0.3	26.4	0.7
Clay:LU	1	3.9	2.3	1.1	0.4	2.0	2.4	0.00	0.00	23.9	1.2	21.5	2.7	0.1	0.0
SR:MT	4	14.9	8.7**	14.1	5.9**	0.4	0.4	0.5	0.5	19.5	1.0	3.8	0.5	38.7	1.0
Clay:MT	4	15.3	9.0**	6.8	2.9	2.0	2.4	1.0	1.2	16.8	0.9	7.4	0.9	26.1	0.7
Residuals	17	1.7		2.4		0.8		0.9		19.4		8.0		39.3	

		OC stock [kg m^{-2}]								*Δ^{14}C value [‰]*					
		LF1		LF2		MOM		bulk soil		LF1		LF2		MOM	
	DF	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Study region (SR)	1	0.7	3.3	0.04	2.2	8.6	6.1*	10.6	3.9	200	0.7	0.07	0.08	177	0.4
Clay concentration (clay)	1	0.2	0.09	0.0	0.04	0.0	0.0	0.3	0.1	392	1.3	2.1	2.3	1397	3.2
Land use (LU)	1	2.8	13.2**	0.04	2.5	8.8	6.2*	4.5	1.7	28819	99.0**	9.5	10.3**	217	0.5
Management type (MT)	4	0.2	1.0	0.01	0.4	3.0	2.1	4.6	1.7	1784	6.1**	2.4	2.6	479	1.1
SR:clay	1	0.07	0.3	0.0	0.02	0.1	0.1	0.01	0.0	874	3.0	5.8	6.3*	1279	2.9
SR:LU	1	0.1	0.6	0.1	8.2*	8.4	5.9*	19.8	7.3*	420	1.4	0.00	0.0	871	2.0
Clay:LU	1	0.2	0.9	0.01	0.3	0.8	0.6	1.8	0.7	2585	8.9**	9.2	10.0**	4952	11.2**
SR:MT	4	0.2	1.1	0.02	1.2	1.0	0.7	2.5	0.9	684	2.4	3.4	3.7*	942	2.1
Clay:MT	4	0.07	0.4	0.01	0.8	1.0	0.7	1.3	0.5	715	2.5	3.1	3.3*	233	0.5
Residuals	17	0.2		0.02		1.4		2.7		291		0.9		441	

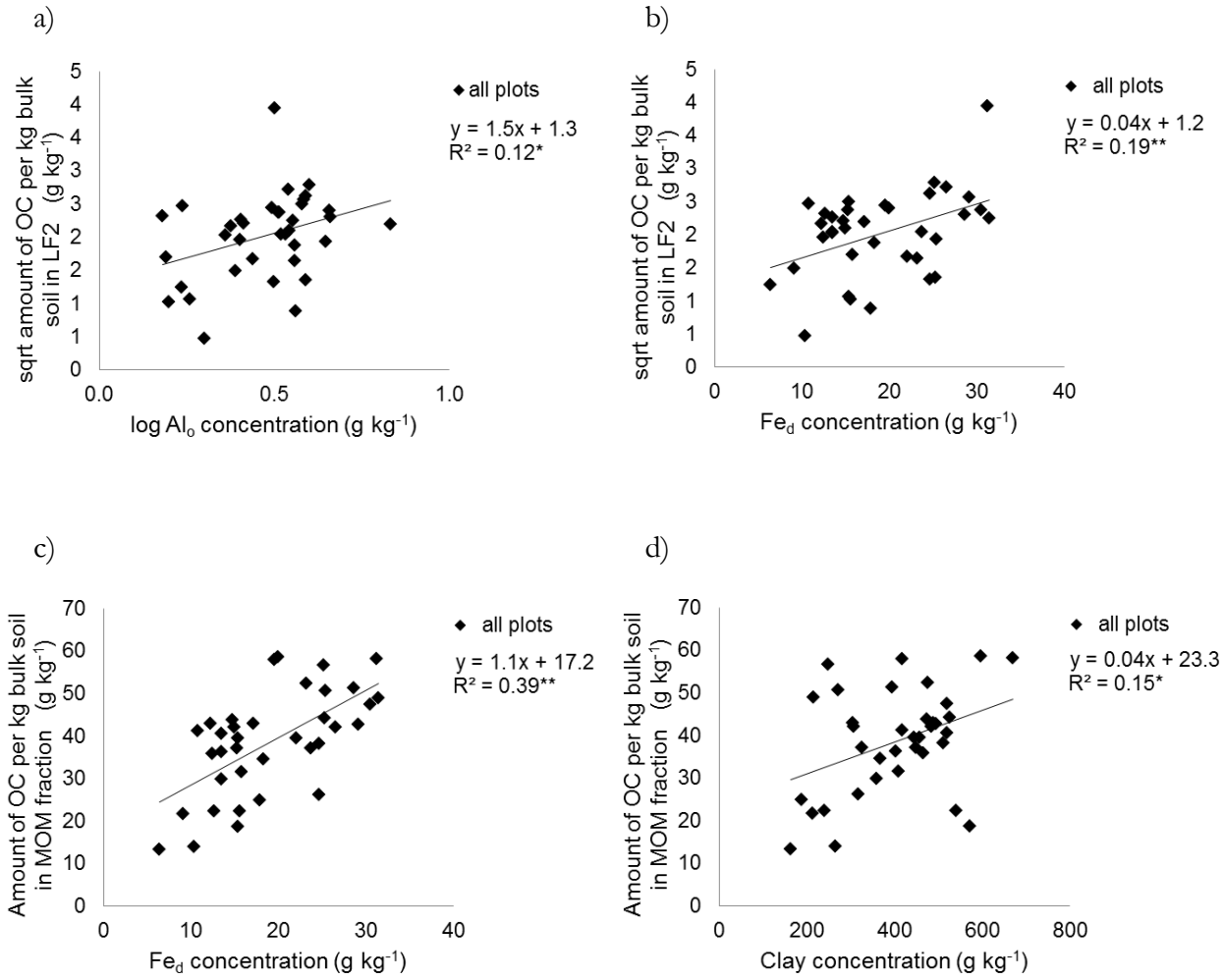

Fig. 4.4 Linear regressions of OC storage of the LF2 and the MOM fraction on soil properties (Al_o=oxalate-extractable Al concentration, Fe_d=dithionite-extractable Fe concentration) across all forest and grassland sites. R-values are presented (*$P < 0.05$, **$P < 0.01$).

4.4 Discussion

Density fractionation separated soil OC into distinct fractions of different C concentrations, C:N ratios and $\Delta^{14}C$ values (Fig. 4.2, Table 4.5). This is in line with other studies in forest and grassland ecosystems (Golchin et al., 1994; Baisden et al., 2002; Swanston et al., 2005; Schrumpf et al., 2013; Schöning et al., 2013).

Land use effects on OC storage and turnover, and relation between density fractions

Forest soils had much higher proportions of total OC in the light fractions, and higher OC stocks in the LF1 than grassland soils (Table 4.5). Differences in the amount and quality of litter, pH and thus in microbial activity between forests and grasslands probably resulted in slower decomposition (alternative: in an accumulation) of OM in the two light fractions of

forest soils. In forests more aboveground litter enters the soil compared to grasslands where the aboveground litter was partly removed by mowing and/or grazing. Further, the quality of the litter, principally its C:N ratio and lignin content, impacts how litter is degraded by microorganisms (Hobbie, 1992). The higher C:N ratios of the two light fractions in forest compared to grassland soils (Table 4.5) suggest that forest litter is less degradable and thus can accumulate to higher OC stocks in the light fractions of forests compared to grasslands. Lower pH values in forest than in grassland soils (Table 4.4) could also have hampered microbial activity (Olsson et al. 1996) and favoured OC accumulation in forests.

The infiltration of the bomb ^{14}C tracer after the early 1960s provides a sensitive indicator of the recent accumulation or loss of C. The ^{14}C signature of atmospheric CO_2 has decreased since the nuclear test ban treaty in 1963 (Riley et al. 2008). Long-term integrated $\Delta^{14}CO_2$ measurements from the monitoring station in the Black Forest (Schauinsland, Germany) showed that the mean summer (May-August) $\Delta^{14}CO_2$ of the atmosphere in 2007 was 50.1±1.0 ‰ (Levin et al., 2013). Radiocarbon values <0 ‰ indicate long turnover times of OC (several centuries), while values higher than the atmospheric $\Delta^{14}C$ value represent either C that turns over on time scales of several years to decades, or a possible mixture of OM that cycles more fast and more slow (Trumbore, 2000). The LF1 of grassland soils had significantly lower $\Delta^{14}C$ values than forest soils in both study regions (Fig. 4.1, Table 4.6). The mean $\Delta^{14}C$ value of the LF1 in grassland soils of the HAI and the ALB (51±7 ‰) was close to the summer mean $\Delta^{14}C$ of the atmospheric CO_2 in 2007 indicating that OC input to the LF1 is young and decomposition fast. In contrast, the LF1 of forest soils had larger contributions of bomb-derived ^{14}C as indicated by a mean $\Delta^{14}C$ value of the LF1 of 113±6 ‰ in the HAI and the ALB. This can be either due to aged OC entering the soil at the forest sites, or to slower decomposition rates of the LF1, or both. Based on ^{14}C measurements, faster turnover of OC in the free light fraction of grassland than forest soils was assumed. For example Baisden et al. (2002) suggest that ≥90 % of the free light fraction of an annual grassland in California turns over in less than 10 yrs while turnover times of 70-710 yrs were modelled by Schulze et al. (2009) for the free light fraction in a Norway spruce forest in Germany. However, Solly et al. (2013) showed for our study sites that the average $\Delta^{14}C$ values of fine roots in forests and grasslands differed on average by 50 ‰ (forest fine roots were older than grassland fine roots), suggesting that at least some of the observed difference in $\Delta^{14}C$ of the LF1 was inherited from root litter input. Accordingly it can be assumed that differences in modelled turnover times of OC in

the LF based on ^{14}C values between forests and grasslands are overestimated if differences in lag-times are not accounted for.

The LF2 is supposed to represent a fraction occluded within stable aggregates (Golchin et al., 1994) and consists of more degraded organic material than the free density fraction. The smaller C:N ratios of the LF2 than the LF1 in our study (Table 4.5) implies that OC of the LF2 was more degraded than OC of the LF1. Larger $\Delta^{14}C$ values of the LF2 than in the LF1 in grassland soils suggest that the LF2 still contains larger portions of bomb-C, and accordingly has older C (C fixed earlier in the post-bomb period) than the LF1, supporting the idea of OC stabilization by occlusion in aggregates. The $\Delta^{14}C$ values of the LF2 in forest soils were however in between those of the LF1 and the MOM fraction, suggesting either that there is limited stabilization in forest aggregates or that the LF2 is comprised of a mixture of younger (LF1) and older (MOM) material. These results support other studies showing that OC occluded in aggregates has slower average turnover than the physically unprotected OC with faster turnover times (Golchin et al., 1994; Swanston et al., 2005). Time series of $\Delta^{14}C$ values would be necessary to better explain why the LF2-material is more bomb-OC-enriched than the LF1 in grassland, but not in forest sites. Larger $\Delta^{14}C$ values of OC in the LF2 of forests as compared to grasslands in both study regions (Fig. 4.1, Table 4.6) probably still reflect $\Delta^{14}C$ differences between land-use types in the LF1.

The $\Delta^{14}C$ values of the MOM fraction were depleted relative to those of the LF2, indicating slower turnover and stabilization against degradation. Many studies already showed that mineral associated OC contained more stabilized (decadal) OM, and on average turned over more slowly than the low density fractions (Baisden et al., 2002; Swanston et al., 2005; Schrumpf et al., 2013). That $\Delta^{14}C$ values of the MOM fraction are nevertheless close to those of the LF1 of grasslands is probably because the MOM fraction is not a homogenous pool but comprises of a mixture of older, more stable, and more recent, fast cycling OC as stated for example by Swanston et al. (2005). The $\Delta^{14}C$ values of the MOM fraction in forest soils in both regions did not differ from those in grassland soils (Fig. 4.1, Table 4.6). Accordingly, it seems that the differences in $\Delta^{14}C$ signatures of the light fractions between the land-use types are not much reflected in the $\Delta^{14}C$ values of the MOM fraction although $\Delta^{14}C$ values of the LF1 and the LF2 were correlated with the $\Delta^{14}C$ values of the MOM fraction (Fig. 4.3 b, c). While forest soils in both study regions displayed significantly higher $\Delta^{14}C$ values in the LF1 and LF2 than grassland soils (Fig. 4.1,

Table 4.6), this was not the case in the MOM fraction. Nevertheless positive correlations between $\Delta^{14}C$ values of all three density fractions suggests either transfer of OC from LF1 to the MOM fraction, transferring the bomb-signal, or related turnover of OC in all fractions (e.g. because of similar environmental constraints). Some studies showed that new, litter derived, OC is transferred from the physically unprotected light fraction to the aggregate protected and mineral-associated fractions (Hassink and Dalenberg, 1996; Sanaullah et al., 2011). It is therefore assumed that decomposed material of the LF1 was transferred to the LF2, thereby partly transferring also the larger $\Delta^{14}C$ values of the LF1 to the LF2 at the forest sites. This could also be the case for the active portion of OC in the MOM fraction, although the diminishing ^{14}C difference between land-use types suggests that also other OC sources could have contributed to MOM formation. If similar environmental constraints would limit turnover times of the fractions like soil pH or C:N ratio, differences in the slopes of the correlations between fractions would be expected for different land-use types, but there was no consistent picture. Possibly besides OC amounts, also OC turnover of the MOM fraction is rather determined by soil properties than by vegetation.

Long-term stabilization

OC accumulation in the MOM fraction is often positively related to hydrous Al and Fe oxides (Kaiser and Guggenberger, 2000; Kaiser et al., 2002). Similarly in our study, OC concentrations in the MOM fraction were linked to oxalate-extractable Al and dithionite-extractable Fe concentrations. As soils of the ALB had overall larger amounts of pedogenic Al and Fe oxides than soils of the HAI (Table 4.4), this is possibly an explanation for the larger OC stocks observed in the MOM fraction of the ALB (Table 4.5). Further our study revealed that complexation of OM by Al and Fe oxides is also an important mechanism for the stabilization of OM in the LF2 and MOM fraction across all sites (Fig. 4.4 a-c). In addition to the metal oxides, clay minerals play an important role for OC stabilization in the MOM fraction (Fig. 4.4 d). Several studies in forest and grassland soils showed that stronger stabilization of the MOM fraction is induced by higher silt and clay contents (Eusterhues et al., 2003; Leifeld and Fuhrer, 2009; Schöning et al., 2013). However, higher OC storage in MOM fraction of ALB soils than in the HAI soils is not reflected in the ^{14}C signatures (Fig. 4.1, Table 4.6). This could be because soil texture, which did not differ between the study regions (Table 4.4), exerts a stronger control on OC turnover in the MOM fraction than pedogenic Al and Fe oxides. Overall, $\Delta^{14}C$ signatures

of the MOM fraction exceeded that of the 2007 atmosphere similar to the LF1 and LF2 (Fig. 4.2 c) and indicate significant accumulation of bomb-produced ^{14}C (Trumbore, 2000) in all density fractions, and thus no long-term stabilization of OM in these soil A horizons.

Management effects

Neither forest management nor grassland management affected OC storage of the three density fractions in our study (Table 4.6). In contrast, Meyer et al. (2012) reported higher OC stocks in the free light fraction in abandoned grasslands compared to meadows in two study regions of the European Alps. This was explained with the higher litter input into the soil with abandonment. In our study, aboveground biomass is removed through mowing and/or grazing in all three grassland management types (Table 4.2). This suggests that differences in aboveground litter input between management types were small compared to the study of Meyer et al. (2012). Recent studies showed that root-derived C rather than C derived from aboveground litter is the main source of OC in the light fractions (Kramer et al., 2010; Schmidt et al., 2011). In grassland ecosystems, long-term N fertilization (Bardgett et al., 1999b) and defoliation by mowing or grazing (Schuman et al., 1999) can reduce root biomass and therefore the amount of root C input. However, Solly et al. (2013) showed that N addition or mowing intensity had no effect on root biomass at our studied grassland sites. Root-derived C did therefore probably not differ significantly between the grassland management types. Together with small variation in the aboveground litter between management types OC storage of the density fractions was not affected by grassland management.

In forests, OC storage of the light fractions also did not vary in relation to management, although we analysed not only different harvesting activities, but also different tree species (spruce and beech) under age-class management. Other studies (Crow et al., 2007; Laganière et al., 2011) reported large OC storage in the LF in coniferous forests, and explained this by low quality of C inputs and environmental constraints to decomposition like small pH values. Also at our study sites, slightly wider C:N ratios of the two light fractions suggest lower litter quality in the spruce forest than in the beech forest under age-class management. Kaiser et al. (2002) found similar proportions of OC in the light fraction in the topsoil of a spruce and a beech dominated site with acidic soil conditions. Also the potential faster decomposition of deciduous than coniferous root litter, as observed by Silver and Miya (2001), could be offset by larger root litter input at deciduous

forest sites due to larger fine root biomass (Finér et al., 2007), leading to similar net OC storage in both forest types.

Our study confirmed previous observations that among density fractions the $\Delta^{14}C$ values and the turnover of the free light fraction varied most in relation to management (Leifeld and Fuhrer, 2009; Meyer et al., 2012). The LF1 revealed significantly higher $\Delta^{14}C$ values in pasture soils than in mown pasture soils in both regions. Leifeld et al. (2009) showed that the free light fraction in pasture soils had slightly longer turnover times and wider C:N ratios than meadow soils. They proposed that the higher $\Delta^{14}C$ values in the free light fraction in pasture soils was caused by a slower degradation of OM in this fraction together with the allocation of slightly older C. The C:N ratios of the LF1 were only higher in pasture soils of the ALB, but not in the HAI, thus in our study area additional factors contribute to the higher $\Delta^{14}C$ of the LF1 in pasture soils. Recent results of our study plots showed that increased N fertilization resulted in decreased mean $\Delta^{14}C$ of fine roots (Solly et al., 2013). Thus, younger fine roots as an important C source could further account for the faster turnover times of the LF1 in mown pasture ($\Delta^{14}C$ values closer to that of the 2007 atmosphere) than in pasture soils.

4.5 Conclusions

The separated density fractions LF1, LF2, and MOM fraction displayed clear differences in OC storage, C:N ratios, and $\Delta^{14}C$ values. Land-use affected OC storage and turnover of the two light fractions. Forest soils stored more OC in both light fractions than grassland soils, possibly as a result of reduced turnover due to lower litter quality and pH values in forest soils. Accordingly, $\Delta^{14}C$ values of both light fractions were significantly higher in forest soils than in grassland soils suggesting slower turnover in forests. However, time lags between CO_2-fixation by plants and the time when OC enters the soil as litter are longer in forest than in grassland soils, and therefore offset some of the observed difference in LF-turnover between forest and grassland sites. Differences in $\Delta^{14}C$ values of the LF1 between grasslands and forests were to some degree transferred to the LF2, while they diminished in the MOM fraction, where amount and turnover were probably rather affected by soil and site conditions than by the vegetation. Nevertheless, positive correlations between $\Delta^{14}C$ of density fractions suggest that decomposed material of the low density fraction can be a source of OM for the higher density fractions which needs to be considered in the modelling of turnover times in density fractions. Despite the fact that $\Delta^{14}C$ values of the LF1 were affected by grassland management, differences in OM input

between management types in forests and grasslands were probably not large enough to affect OC storage in density fractions.

Chapter 5

Synthesis and Conclusions

The soil microbial community and enzyme activities mediate many ecosystem processes, including the decomposition of OM, and thus affect OC storage in soils. In this context, one aim of the research presented here was to further knowledge of driving factors of microbial communities. This study specifically focussed on variations in microbial biomass, community composition, and enzyme activities that are related to land management and soil properties in forest and grassland systems (Chapter 2, 3). In addition, the analysis of C dynamics in density fractions provided a novel set of data for better understanding the OC storage and turnover of density fractions in response to land use, management and soil properties in forest and grassland systems (Chapter 4). Key methods that were applied and combined included PLFA and enzyme activity analyses, density fractionation, ^{14}C measurements and various analyses of soil properties.

5.1 Land management effects

This thesis revealed that long-term land management, independent of study region and soil properties, was a minor control on microbial biomass, community composition, enzyme activities and OC storage in density fractions compared with the soil properties, such as clay concentration, OC and TN concentrations, soil moisture and pH.

We compared total PLFA biomass, microbial community composition and enzyme activities between different land-use intensities (combinations of N fertilization, mowing, and grazing) in grassland soils across three different regions [Chapter 2]. This work highlighted that the microbial biomass and community composition did not change in response to land-use intensity. In contrast, activities and specific activities of enzymes involved in the C cycle increased with higher land-use intensity and lower soil C:N ratios leading to a greater acquisition of energy and C. Enzyme activities proved to be more sensitive indicators of changes in land management in the studied grassland systems across the regional scale than PLFA data. The results obtained here are in contrast to many other studies reporting management effects, for example from N fertilization or grazing, on microbial biomass and community composition (Bardgett et al., 1999a; Bardgett et al., 2001). However, these studies mostly examined short-term management effects on microbial communities in laboratory and field experiments, whereas the results in this study

provided data on long-term land management in natural grassland systems where different management practices like N fertilization and mowing/grazing were combined.

Different forest management practices, including harvesting or the selection of tree species, and associated variations in the quantity and quality of aboveground and belowground litter inputs into the soil have been shown to affect enzyme activities when management practices persist over short time periods (<10 yr) (Hassett and Zak, 2005; Weand et al., 2010). The results in Chapter 3 aimed to clarify whether enzyme activities are sensitive indicators when forest management differences persist over several decades. Different tree species (spruce versus beech), ages, and harvesting systems (forest under age-class management versus unmanaged forest) resulted in different silvicultural management intensities for the forest stands (Schall and Ammer, 2013). Multivariate analysis, such as redundancy analysis combined with a variance partitioning procedure, is a useful tool to determine how biotic properties are affected by different environmental factors (Floch et al., 2009). The results presented in Chapter 3 showed that long-term forest management intensity (>20 yr), constrained by the study region and soil properties, only explained a minor portion of the variance of enzyme activities. Furthermore, the explained variance of enzyme activities by forest management intensity did not change for deeper soil horizons with 5.2 % in the A horizon, 3.2 % in the B1 horizon, and 7.3 % in the B2 horizon. The results indicate two possibilities. First, differences in the studied long-term forest management intensities between forest stands may not have been big enough to affect enzyme activities in the whole soil profile. Second, enzyme activities may not be potential indicators for the assessment of the effect of long-term management on the microbial activity, and thus on soil quality of forest soils.

Soil microbiological properties have been considered as early indicators of environmental changes compared to bulk soil properties, but the separation of OM into physical fractions with different turnover times enhances the detection of land use and management effects on OC storage in soils. The light fraction was found to change with land use and management changes, and thus it is potentially an early indicator of changes in soil C (Bremer et al., 1994). Density fractionation was a useful tool to separate OC into distinct fractions of different C concentrations, C:N ratios and $\Delta^{14}C$ values, which is in line with previous studies in forest and grassland ecosystems (Golchin et al., 1994; Baisden et al., 2002; Swanston et al., 2005; Schrumpf et al., 2013). Results presented in Chapter 4 revealed that site and soil conditions as well as the land-use type (forest, grassland) had a higher

impact on OC storage and turnover in density fractions than land management (spruce and beech forest under age-class management, unmanaged beech forest, meadow, mown pasture, pasture). Forest soils stored more OC in the light fractions than grassland soils due to higher aboveground litter entering the forest soils, less degradable litter (higher C:N ratios of the two light fractions in forest soils), and lower pH values under forests. Additional data on ^{14}C measurements for three density fractions provided new information on OC stabilization and turnover in relation to land use and different management practices across two study regions (Hainich-Dün, Schwäbische Alb). The $\Delta^{14}C$ values of the different density fractions indicated that the two light fractions had shorter turnover times in grassland than in forest soils for the two regions. Faster OC turnover of the free light fraction could be explained by faster decomposition rates of the free light fraction in grassland soils compared with forest soils. Another explanation is the lag-times between carbon dioxide fixation by plants and the time when OC enters the soil as litter, which was shorter in grassland than in forest soils. Larger $\Delta^{14}C$ values of OC in the occluded light fraction of forests as compared to grasslands in both study regions probably still reflects $\Delta^{14}C$ differences between land-use types in the free light fraction. Further, the results revealed that differences in $\Delta^{14}C$ signatures of the light fractions between the land-use types are not very much reflected in the $\Delta^{14}C$ values of the mineral-associated OM fraction. OC storage and turnover of the mineral-associated OM fraction seems to be determined more by soil properties than by vegetation. In accordance with other studies (Leifeld and Fuhrer, 2009; Meyer et al., 2012), the $\Delta^{14}C$ values of the free light fraction varied the most in relation to grassland management. Pasture soils showed higher $\Delta^{14}C$ values in the free light fraction than mown pasture soils. This could be a result of slower degradation of OM in the free light fraction in pasture soils together with the allocation of slightly older C. The study of Meyer et al. (2012) reported higher OC stocks in the free light fraction in abandoned grasslands compared to meadows in two study regions of the European Alps. By contrast, in this study differences in OM input between management types in forests and grasslands were probably not large enough to affect OC storage in density fractions.

Soil biological and chemical properties, such as microbial biomass, enzyme activities, and the light fraction OC, have been shown to be early and sensitive indicators of the effects of management changes on soil quality and may be useful for monitoring trends in a soil over time (Bremer et al., 1994; Ajwa et al., 1999; Bandick and Dick, 1999). The results of this thesis indicate that long-term management, independent of the study region and soil properties, has only minor effects on microbial biomass, microbial community

compositions and OC storage of the light fractions in these forest and grassland soils. These findings reflect the importance of other factors, such as site and soil conditions, and raise doubts about the use of these microbiological and chemical parameters as indicators of the effects of long-term management on soil quality.

5.2 Soil property effects

Many studies have explored distribution patterns of microorganisms and enzyme activities in soils under land use and management change. However, often such studies do not directly link biotic responses with information on edaphic properties, even though these properties have been demonstrated to affect soil microbiological properties (Zeller et al., 2001; Lauber et al., 2008; Sinsabaugh et al., 2008). Redundancy analysis combined with a variance partitioning procedure revealed the proportion of variance for enzyme activities that can be explained by study region, management intensity and soil properties in vertical soil profiles in forests [Chapter 3]. The results clearly showed that soil properties explained a significant proportion of the total variance (8.5 % to 15.2 %) in enzyme activities. Clay concentrations had the largest overall effect on enzyme activity patterns in all of the studied soil horizons, and they were positively related to all enzyme activities. The OC concentration, which is a substrate and sorbent for enzyme activities, was positively correlated with clay concentrations in each region. In other studies, the OC concentration has also been shown to be positively related to enzyme activities (Deng and Tabatabai, 1996, 1997). Further, the study region was a significant factor in explaining the variation in enzyme activities in forests; this is mostly related to the effects of the different soil groups in the study regions with their intrinsic soil properties. Large-scale study designs comprising different regions naturally lead to considerable diversity in the soil groups, because every soil group occurs within a certain geographic region (Simonson, 1959). In Chapter 2, grassland microbial communities across a latitudinal gradient in Germany were studied. The main soil group in the Schorfheide-Chorin is the Histosol, which varied mainly in OC and TN concentrations and in soil texture compared with the Hainich-Dün (Stagnosol) and Schwäbische Alb (Leptosol). Our results indicated that differences in microbial community composition and enzyme activities among regions and management practices were primarily a result of soil moisture conditions due to different soil groups in the regions. A fluctuating water table in degraded peat soils in the Schorfheide-Chorin reduced microbial growth, and likely resulted in differences in stabilization and turnover times of enzymes between the study regions. The PLFA profiles measured in soils of

long-term agriculture farming systems by Bossio and Scow (1998) support the importance of soil groups in governing the composition of microbial communities. It can be concluded that large-scale differences among regions affect forest and grassland microbial activities, and highlight the need for large-scale studies including different regions and environmental conditions.

The results in this thesis further contribute to furthering our knowledge of general patterns between soil properties and microbiological properties for different management practices and regions that differ in climatic conditions and parent materials. Birkhofer et al. (2012) already emphasised that comprehensive field studies, in which soil biological and abiotic soil properties are determined across different regions and land-use types, are important to further our knowledge of unifying principles in soil biology. Soil properties were related to total PLFA biomass, community composition, and enzyme activities using redundancy analysis [Chapter 2]. Soil properties explained 34 % and 60 % of the total variation in PLFA and enzyme activity data, respectively, after accounting for large-scale differences among regions and management practices. Total PLFA biomass was mainly related to OC concentrations and pH, while microbial community composition was mainly related to soil moisture. Similar to microbial biomass, enzyme activities were mainly affected by the OC concentration. The results demonstrated that PLFA data and enzyme activities were related to soil properties in a general way. This has been shown before for the general relationships between soil properties and soil biota (Birkhofer et al., 2012).

Differences in enzyme activities between soil horizons were also due to variations in soil properties, such as OC and TN concentrations and the C:N ratio [Chapter 3]. Phenol oxidase was the only enzyme, which activity did not significantly decline from the topsoil to the subsoil horizons. These findings are in accordance with Kramer et al. (2013), who found similar results in an arable soil. Enzyme activity ratios (e.g. ratio of ß-glucosidase activity : (N-acetyl glucosaminidase activity + L-leucine aminopeptidase activity), an indicator of potential C:N acquisition activity), were used to determine if soil microbial communities assigned more effort to acquire one nutrient relative to another nutrient (e.g. N to C) in vertical soil profiles. Such ratios have been used in other studies to study, for example, climate change effects in a disturbed forest soil (McDaniel et al., 2013), or ecoenzymatic stoichiometry of terrestrial soils and freshwater sediments (Sinsabaugh et al., 2009; Sinsabaugh et al., 2012). In this thesis, forest subsoil horizons across the regional scale indicated a shift to higher N acquisition, while the strength of the shift depended on

the soil type. Further, this study indicated a relative increase in the production of C-acquiring enzymes degrading recalcitrant OM relative to labile C-acquiring enzymes in subsoil horizons of forest soils. Probably, this was due to the higher stability of OC, the lack of fresh C inputs for microorganisms in deeper soil horizons, or differing sorption mechanisms of hydrolytic (e.g. ß-glucosidase) and oxidative (phenol oxidase) enzymes. The decreasing specific ß-glucosidase activity (per unit of C) and increasing specific phenol oxidase activity towards deeper soil horizons further support the findings of different depth distributions between hydrolytic and oxidative enzyme activities. In contrast, an indicator of potential C:P acquisition activity suggested that in the studied forest soils the acquisition of P relative to C did not change along the vertical soil profile.

Organic C storage in density fractions, especially in low-density fractions such as the mineral-associated OM fraction, has been shown to be related to aluminium and iron oxides (Kaiser and Guggenberger, 2000; Kaiser et al., 2002). Similarly, the results of Chapter 4 showed that complexation of OM by aluminium and iron oxides is an important mechanism for the stabilization of OM in the occluded light fraction and the mineral-associated OM fraction across forest and grassland sites in the Hainich-Dün and Schwäbische Alb. Larger OC stocks in the mineral-associated OM fraction of the Schwäbische Alb could also be explained by larger amounts of pedogenic aluminium and iron oxides in soils of the Schwäbische Alb compared with soils of the Hainich-Dün. Further, clay minerals play an important role in OC stabilization in the mineral-associated OM fraction. This is comparable with the results of several other studies in forest and grassland soils that revealed stronger stabilization of mineral-associated OC by higher silt and clay contents (Eusterhues et al., 2003; Leifeld and Fuhrer, 2009; Schöning et al., 2013). In contrast, the availability and nature of binding sites at mineral surfaces were neither related to $\Delta^{14}C$ signatures of the occluded light fraction nor to the mineral-associated OM fraction. Together with $\Delta^{14}C$ signatures (measured in 2008) of all three density fractions that exceeded the $\Delta^{14}C$ signatures of the 2007 atmosphere and indicated a significant accumulation of bomb-produced ^{14}C (Trumbore, 2000) in all density fractions, these results suggest no long-term stabilization of OM in the studied soil A horizons.

5.3 Overall conclusions

A new large-scale and long-term project for functional biodiversity, the Biodiversity Exploratories, provided a hierarchical set of standardized field plots in three different regions of Germany encompassing various management types and intensities in forests and

grasslands (Fischer et al., 2010). This study design made it possible to improve our understanding of the key drivers of soil microbial communities and OC storage in natural ecosystems at the regional scale. Overall, the findings of this thesis revealed that the impact of forest and grassland management practices on microbial biomass, community composition, enzyme activities, OC storage and turnover in density fractions was less important than that of soil properties across different regions. Further, this work highlight the need for large-scale studies including different regions and their environmental conditions in order to draw general conclusions on the impact of land management and soil properties on microbial communities and OC storage. This knowledge should be considered in future research and models of SOM as well as applied to effective environmental management.

5.4 Research perspectives

To further knowledge of land management and soil property effects on SOC storage in natural ecosystems, future research should examine the underlying biological and biochemical processes. Different enzymes are produced by specific groups of microorganisms, but their relationship to shifts in community composition in natural systems is still poorly understood (Waldrop et al., 2000). Future studies should specifically focus on a better understanding of the link between enzyme activities and changes in microbial community composition as a result of changing environmental conditions. This could provide parameters that can be related to potential rates of OM decomposition, which in turn has important implications for OC storage in soils.

The results obtained in this study showed that the use of soil microbiological or chemical properties as reliable indicators of management-induced changes in soil quality is highly questionable. Therefore, further efforts to obtain soil quality indicators of general use should be focused on the search for more complex indicators that can more accurately describe the complexity of the soil. This would require testing such indicators in different areas under diverse land use and management practices in order to verify their general validity.

The combination of measured potential enzyme activities with proteomic and metagenomic enzyme analyses would provide further insights into the relationship between enzyme production and activities in soils. The focus should be on an integrated analysis of microbial species diversity (DNA sequencing, proteomics and PLFAs), functional diversity

of soil organisms (proteomic enzyme analyses, identification of enzyme encoding genes and enzyme activities), and abiotic soil properties as affected by land use and management across regional scales. The application of different methods for analysing microbial diversity and function enables the evaluation and improvement of each method. Moreover, it allows more comprehensive and general conclusions to be drawn due to the different strengths of each method.

Modelling of OC turnover in density fractions was beyond the scope of this study and remains a future task. The results of this study showed that the transfer of OC between OM fractions, and lag-times between the carbon dioxide fixation by plants and the time OC enters the soil both need to be considered when modelling OC turnover in density fractions; thus a more complex modelling approach is required. In this context, the influence of different land management practices in forest and grassland systems on organic litter input into the soil is an important model parameter that also needs further exploration.

Summary

Soil microorganisms and enzymes mediate many biochemical processes that are important in soil functioning, such as the decomposition of soil organic matter (OM), which in turn impact carbon (C) cycling of the soils. Still, little is known about the factors that determine soil microbial communities and their functions in temperate forest and grassland systems. The aim of the work presented in this thesis was to improve our understanding of the factors that affect soil microbial communities and OC storage in temperate forest and grassland systems at the regional scale. Soil OM in bulk soils is a heterogeneous mixture of organic substances in various stages of decomposition with different chemical compositions and turnover times. The isolation of more sensitive OM fractions increases the probability of determining changes in soil organic C (OC) stocks related to different land management practices.

This thesis is part of a new large-scale and long-term project for functional biodiversity, called the Biodiversity Exploratories, which includes a hierarchical set of standardized field plots in three different regions of Germany (Schorfheide-Chorin, Hainich-Dün, Schwäbische Alb) encompassing various management types and intensities in forests and grasslands. This study design made it possible to address the following objectives in the present thesis:

- to understand how soil microbial biomass, microbial community composition, and enzyme activities are influenced by grassland management and soil properties across the regional scale,
- to investigate enzyme activities and nutrient supply and demand for whole soil profiles in forests, and to evaluate the proportion of the total variance in enzyme activities that can be attributed to large-scale differences between regions, long-term forest management, and soil properties in different soil horizons, and
- to assess the OC storage and ^{14}C signatures in three density fractions of forest and grassland sites under diverse management practices and soil properties in different regions.

The impact of soil properties and land-use intensity (N fertilization, mowing, grazing) on microbial biomass, community composition (phospholipid fatty acid (PLFA) profiles) and enzyme activities involved in the C, N, and P cycles in the topsoil horizon of grasslands in

three study regions was examined. The study revealed that microbial biomass, community composition and enzyme activities were more affected by soil properties than by land-use intensity, independent of the study region and soil properties. There was no effect of land-use intensity on microbial biomass or microbial community composition, but the activities and specific activities (per unit microbial biomass) of enzymes involved in the C cycle increased significantly with land-use intensity, which can have an impact on soil OM decomposition and nutrient cycling. Further, all three aspects of the microbial community (biomass, composition, and enzyme activities) were related to soil properties in a general way. After accounting for large-scale differences among the regions and land-use intensity, soil properties still explained a significant amount of the variation in the PLFA data (34 %) and enzyme activities (60 %). Microbial biomass and all enzyme activities were mainly related to OC concentration whereas microbial community composition was mainly related to soil moisture.

Enzyme activities in the whole soil profiles of forests under different management intensities (spruce and beech forest under age-class management, unmanaged beech forest) across a latitudinal gradient in Germany were studied and found to be region specific. There was a strong pattern showing that the enzyme activities involved in the C, N, and P cycles across all soil horizons were highest in the Schwäbische Alb, followed by the Hainich-Dün and the Schorfheide-Chorin. The more sandy Arenosols in the Schorfheide-Chorin were characterized by lower OC and TN concentrations, higher C:N ratios, and lower soil pH than the more fine-textured soils in the other two regions. These differences in the soil groups and properties were responsible for the lower enzyme activities in the Schorfheide-Chorin. The calculated enzyme activity ratios to follow shifts in nutrient or energy supply and demand in whole soil profiles indicated a shift in investment to N and P acquisition in the Schorfheide-Chorin, because of the higher N and P demand in these soils. Further, all enzyme activities, except phenol oxidase activity, significantly decreased in deeper soil horizons as the concentrations of OC and TN decreased. The decrease was much greater from the topsoil (A horizon) to the first subsoil horizon (B1 horizon) than from the B1 horizon to the second subsoil horizon (B2 horizon). In contrast, phenol oxidase activity did not significantly decrease from the A to the subsoil horizons. Higher phenol oxidase activity per unit of C, but lower ß-glucosidase activity per unit of C towards deeper soil horizons further supports the findings of different depth distributions between hydrolytic and oxidative enzyme activities. In addition, this study indicated a relative increase in enzyme activities degrading more

recalcitrant C relative to labile C compounds in subsoil horizons of the forest soils, probably due to the higher stability of OC, the lack of fresh C inputs for microorganisms in deeper soil horizons, or differing sorption mechanisms of hydrolytic and oxidative enzymes. The forest subsoil horizons in all regions indicated a shift to higher N acquisition, while the strength depended on the soil type. In contrast, an indicator of potential C:P acquisition activity suggested that in the studied forest soils the acquisition of P relative to C does not change along the vertical soil profile.

Redundancy analysis (RDA) in combination with variance partitioning revealed that the study region was a significant factor in explaining the variation in enzyme activities in forests. This is mostly related to the effects of the different soil groups in the study regions with their intrinsic soil properties. Further, the results clearly showed that a large proportion of the total variance in enzyme activity data could be explained by soil properties for all soil horizons whereas forest management intensity only had minor effects. Partial RDA revealed that the clay concentration explained most of the variation in enzyme activities in all soil horizons and it was positively related to all enzyme activities.

The impact of land use (forest, grassland), management (spruce and beech forest under age-class management, unmanaged beech forest, meadow, mown pasture, pasture), and soil properties on the OC storage and ^{14}C signatures of the free light fraction, occluded light fraction, and mineral-associated OM (MOM) fraction in A horizons of two German regions (Hainich-Dün, Schwäbische Alb) was assessed. Density fractionation separated three OM fractions that clearly differed in terms of OC concentrations, C:N ratios, and radiocarbon signatures ($\Delta^{14}C$). An important part (27±1.8 %) of the total OC was located in the light fractions of the A horizons in forests and grasslands of both study regions. Land use affected OC storage and turnover of the two light fractions. In particular, forest soils stored more OC in both light fractions than grassland soils, possibly as a result of higher aboveground litter entering the forest soils, and slower decomposition of organic material in the two light fractions due to the lower litter quality and lower pH values in forest soils. Accordingly, the $\Delta^{14}C$ values of both light fractions were also significantly higher in forest soils than in grassland soils suggesting slower turnover in forests. Differences in the $\Delta^{14}C$ values of the free light fraction between grasslands and forests were to some degree transferred to the occluded light fraction, while they diminished in the MOM fraction. In the MOM fraction, large OC storage was associated with higher stability indicated by depleted $\Delta^{14}C$ values and correlations of the MOM fraction-OC to pedogenic

Al and Fe oxides as well as clay concentrations. Nevertheless, positive correlations between the $\Delta^{14}C$ values of the density fractions suggested that the decomposed material of the low-density fraction could be a source of OM for the higher density fractions, which should be considered in future modelling of turnover times in density fractions. Among the density fractions, the $\Delta^{14}C$ values of the free light fraction varied the most in relation to grassland management with higher $\Delta^{14}C$ values in pasture soils than in mown pasture soils. Overall, the relatively high $\Delta^{14}C$ signatures of all density fractions indicated the significant accumulation of bomb-produced ^{14}C in all density fractions, and thus no long-term stabilization of OM in these soil A horizons.

Overall, it can be concluded that soil microbial biomass, microbial community composition, enzyme activities, OC storage and turnover in density fractions were more affected by soil properties than by forest and grassland management practices at the regional scale. Further, the results presented here highlight the need for large-scale studies including different regions and their environmental conditions in order to draw general conclusions about the impact of land management and soil properties on soil microbial communities and OC storage. This knowledge should be included in future research and models of soil OM as well as applied to effective environmental management to enhance OC storage in soils.

Kurzfassung

Bodenmikroorganismen und Enzyme steuern viele biochemische Prozesse, die in der Funktionsweise von Böden von großer Bedeutung sind – zum Beispiel der Zersetzung der organischen Bodensubstanz, was wiederum den Kohlenstoff (C)-Kreislauf von Böden beeinflusst. Dennoch ist bislang nur wenig über die Faktoren bekannt, die die mikrobiellen Gemeinschaften und deren Funktionen in temperierten Wald- und Grünlandsystemen beeinflussen. Ziel dieser Promotionsarbeit war es, das Verständnis über die Faktoren, die die mikrobiellen Gemeinschaften und die organische C-Speicherung in temperierten Wald- und Grünlandsystemen auf der regionalen Skala beeinflussen, zu verbessern. Die organische Bodensubstanz im Gesamtboden besteht aus einer heterogenen Mischung von organischen Bestandteilen in verschiedenen Stadien der Zersetzung mit unterschiedlicher chemischer Zusammensetzung und Umsatzzeiten. Isoliert man empfindliche Fraktionen der organischen Bodensubstanz, erhöht dies die Wahrscheinlichkeit, die Veränderungen in Bodenkohlenstoffvorräten unter verschiedener Landbewirtschaftung besser zu beurteilen.

Diese Arbeit ist Teil eines großen und langfristigen Projektes zur funktionellen Biodiversitätsforschung: die Biodiversitäts-Exploratorien. Sie umfassen standardisierte Untersuchungsflächen in drei Regionen Deutschlands (Schorfheide-Chorin, Hainich-Dün, Schwäbische Alb), in denen sich jeweils vielfältige Typen und Intensitäten der Wald- und Grünlandnutzung finden. Im Rahmen dieses Forschungsdesigns bestanden die detaillierten Zielsetzungen der vorliegenden Arbeit im Folgenden:

- Entwicklung eines besseren Verständnisses davon, in welchen Maß die mikrobielle Biomasse, die mikrobielle Artenzusammensetzung und die Enzymaktivitäten im Boden durch die Grünlandbewirtschaftung und die Bodeneigenschaften auf der regionalen Skala beeinflusst werden.
- Untersuchung der Enzymaktivitäten, das Nährstoffangebots und der Nährstoffnachfrage im gesamten Bodenprofil in Wäldern und Bestimmung des Anteils der Gesamtvarianz von den Enzymaktivitäten, der auf Unterschiede zwischen den Regionen, langfristige Waldbewirtschaftung und Bodeneigenschaften in verschiedenen Bodenhorizonten zurückgeführt werden kann.
- Untersuchung der Speicherung sowie der ^{14}C-Gehalte des organischen C in drei Dichtefraktionen in Wald- und Grünlandflächen unter verschiedener

Bewirtschaftung und Bodeneigenschaften in verschiedenen Regionen Deutschlands.

Im Oberboden von Grünländern der drei Untersuchungsregionen wurde der Einfluss von Bodeneigenschaften und Landnutzungsintensität (Stickstoffdüngung, Mahd, Beweidung) auf die mikrobielle Biomasse, Artenzusammensetzung (Phospholipidfettsäuren (PLFA)) und Enzymaktivitäten untersucht, die am C-, Stickstoff (N)- und Phosphor (P)-Kreislauf beteiligt sind. Die Studie ergab, dass die Bodeneigenschaften einen größeren Einfluss auf die mikrobielle Biomasse, die mikrobielle Artenzusammensetzung und die Enzymaktivitäten hatten als die Grünlandbewirtschaftung, unabhängig von der Untersuchungsregion und den Bodeneigenschaften. Es konnte kein Effekt der Landnutzungsintensität auf die mikrobielle Biomasse oder die Zusammensetzung der mikrobiellen Gemeinschaft festgestellt werden. Jedoch stiegen die Enzymaktivitäten und spezifischen Enzymaktivitäten (Aktivität pro Einheit mikrobielle Biomasse), die am C-Zyklus beteiligt sind, signifikant mit der Landnutzungsintensität an. Dies kann sich wiederum auf den Abbau der organischen Bodensubstanz und den Nährstoffkreislauf auswirken. Darüber hinaus konnte festgestellt werden, dass alle drei Aspekte der mikrobiellen Gemeinschaft (Biomasse, Zusammensetzung und Enzymaktivitäten) allgemein zu den Bodeneigenschaften in Beziehung standen. Unter Berücksichtigung von großräumigen Unterschieden zwischen den Regionen und Landnutzungsintensität erklärten die Bodeneigenschaften einen signifikanten Anteil in der Streuung von PLFA Daten (34%) und Enzymaktivitäten (60%). Die mikrobielle Biomasse und alle Enzymaktivitäten wurden hauptsächlich von den organischen C-Konzentrationen beeinflusst, während die Artenzusammensetzung der mikrobiellen Gemeinschaft vor allem mit der Bodenfeuchte in Beziehung stand.

Weiterhin konnte festgestellt werden, dass sich die Enzymaktivitäten im gesamten Bodenprofil unter verschiedenen Intensitäten der Waldnutzung (Altersklassenwald unter Fichte und Buche, unbewirtschafteter Buchenwald), getestet über einen Nord-Südgradienten in Deutschland hinweg, zwischen den Regionen unterschieden. Die Enzymaktivitäten, die am C-, N-, und P-Kreislauf beteiligt sind, waren über alle Bodenhorizonte hinweg in der Schwäbischen Alb am höchsten gefolgt von der Hainich-Dün Region und der Schorfheide-Chorin. Die sandigeren Arenosole in der Schorfheide-Chorin waren durch niedrigere organische C-Konzentrationen und Gesamtstickstoffkonzentrationen, höhere C:N Verhältnisse sowie niedrigere pH-Werte

charakterisiert als die feinkörnigeren Böden in den beiden anderen Regionen. Diese Unterschiede in den Bodengruppen und Bodeneigenschaften waren verantwortlich für die niedrigeren Enzymaktivitäten in der Schorfheide-Chorin. Die Enzymaktivitätsratios wurden berechnet, um Veränderungen in der Nährstoff- oder Energieversorgung und -nachfrage im gesamten Bodenprofil zu verfolgen. Sie ergaben eine höhere Investition hin zur N- und P-Akquisition in der Schorfheide-Chorin aufgrund der höheren N- und P-Nachfrage in diesen Böden. Mit Ausnahme der Phenoloxidase Aktivität, sanken alle Enzymaktivitäten in tieferen Bodenhorizonten signifikant, was mit der Reduktion der organischen C-Konzentrationen und den Gesamtstickstoffkonzentrationen einherging. Die Abnahme dieser Stoffkonzentrationen vom Oberboden (A Horizont) zu dem ersten Unterbodenhorizont (B1 Horizont) war signifikant deutlicher als von dem B1 Horizont zum zweiten Unterbodenhorizont (B2 Horizont). Dagegen war die Phenoloxidase Aktivität in den Unterbodenhorizonten nicht signifikant niedriger als im A Horizont. Die höhere Phenoloxidase Aktivität pro C-Einheit, aber niedrigere ß-Glucosidase Aktivität pro C-Einheit in tieferen Bodenhorizonten unterstützten weiterhin die Ergebnisse unterschiedlicher Tiefenverteilungen zwischen den hydrolytischen und den oxidativen Enzymaktivitäten. Darüber hinaus zeigte die Studie eine Zunahme der Enzymaktivitäten, die schwerer abbaubare C-Verbindungen zersetzen relativ zu denen die labile C-Verbindungen zersetzen, in den Unterbodenhorizonten der Waldböden. Dies kann wahrscheinlich auf die höhere Stabilität des organischen Kohlenstoffs, den Mangel an frischem C-Eintrag für Mikroorganismen in tiefere Bodenschichten oder auf unterschiedliche Sorptionsmechanismen von hydrolytischen und oxidativen Enzymen zurückgeführt werden. Die Unterbodenhorizonte im Wald in allen Untersuchungsregionen deuteten auf eine Verschiebung hin zu höherer N-Akquisition, während die Stärke der Verschiebung abhängig von dem Bodentyp war. Im Gegensatz dazu ließ ein Indikator für potenzielle C:P Akquisitionsaktivität vermuten, dass sich der Erwerb von P relativ zu C in den untersuchten Waldböden nicht entlang des vertikalen Bodenprofils änderte.

Die Redundanzanalyse (RDA) in Verbindung mit der Varianzpartitionierung ergab, dass das Untersuchungsgebiet eine wichtige Rolle bei der Erklärung der Variation in Enzymaktivitäten von Wäldern spielt. Dies ist vor allem auf die Effekte die verschiedenen Bodengruppen in den Untersuchungsgebieten und ihre jeweils spezifischen Bodeneigenschaften zurückzuführen. Ferner haben die Ergebnisse deutlich gezeigt, dass ein großer Teil der Gesamtvarianz von Enzymaktivitäten durch Bodeneigenschaften in allen Bodenhorizonten erklärt werden konnte, während die Intensität der

Waldbewirtschaftung nur einen geringen Einfluss auf die Enzymaktivitäten im gesamten Bodenprofil hatte. Die partielle RDA ergab, dass der Tongehalt, der positiv mit allen Enzymaktivitäten korreliert war, den Großteil der Streuung in Enzymaktivitäten in allen Bodenschichten erklären konnte.

Weiterhin wurden die Auswirkungen der Landnutzung (Wald, Grünland), der Bewirtschaftung (Altersklassenwald unter Fichte und Buche, unbewirtschafteter Buchenwald, Wiese, Mähweide, Weide) und der Bodeneigenschaften auf die Speicherung sowie die ^{14}C-Gehalte des organischen C in drei Dichtefraktionen (freie leichte Fraktion, okkludierte leichte Fraktion, mineral-assoziierte Fraktion der organischen Bodensubstanz) von A Horizonten in zwei Regionen Deutschlands (Hainich Dün, Schwäbische Alb) untersucht. Die Dichtefraktionierung ermöglichte es, drei Fraktionen der organischen Bodensubstanz zu isolieren, die sich deutlich hinsichtlich der C-Konzentrationen, der C:N Verhältnisse und der ^{14}C-Gehalte unterschieden. Ein signifikanter Teil (27 ± 1,8 %) des Gesamt-C war in den beiden leichten Fraktionen des A Horizonts der Wälder und Grünländer der beiden Untersuchungsgebiete gebunden. Die Landnutzung beeinflusste die organische C-Speicherung und -Umsetzung der beiden leichten Fraktionen. Insbesondere Waldböden speicherten mehr C in den beiden leichten Fraktionen als Grünlandböden, möglicherweise als Folge des höheren oberirdischen Streueintrags in die Waldböden und der langsameren Zersetzung des organischen Materials in den beiden leichten Fraktionen bedingt durch die geringere Streuqualität und die niedrigeren pH-Werte in Waldböden. Dementsprechend waren die ^{14}C-Gehalte der beiden leichten Fraktionen in den Waldböden ebenfalls signifikant höher als in den Grünlandböden, was wiederum auf langsamere Verweilzeiten in den Wäldern schließen lässt. Die Unterschiede in den ^{14}C-Gehalten der freien leichten Fraktion zwischen Grünländern und Wäldern wurden bis zu einem gewissen Grad in die okkludierte leichte Fraktion verlagert, während diese Unterschiede in der mineral-assoziierten Fraktion der organischen Bodensubstanz nahezu verloren gingen. In der mineral-assoziierten Fraktion der organischen Bodensubstanz war die hohe C-Speicherung mit höherer Stabilität verbunden, was sich durch die abgereicherten ^{14}C-Signale sowie die Korrelationen des mineral-assoziierten-C zu den pedogenen Aluminium- und Eisenoxiden sowie den Tongehalten äußerte. Trotzdem ließen die positiven Korrelationen zwischen den ^{14}C-Gehalten der einzelnen Dichtefraktionen vermuten, dass das abgebaute organische Material der Fraktionen mit niedrigerer Dichte eine Quelle für Fraktionen mit höherer Dichte sein kann.

Unter den einzelnen Dichtefraktionen, variierten die ^{14}C-Gehalte der freien leichten Fraktion am stärksten unter der Grünlandbewirtschaftung mit höheren ^{14}C-Gehalten in den Böden der Weiden als in den Mähweiden. Insgesamt deuteten die relativ hohen ^{14}C-Signaturen auf eine signifikante Akkumulation des bombenproduzierten ^{14}C in allen Dichtefraktionen hin, was wiederum auf keine langfristige Stabilisierung der organischen Bodensubstanz in diesen A Horizonten hinweist.

Insgesamt kann festgestellt werden, dass die mikrobielle Biomasse, die mikrobielle Artenzusammensetzung, die Enzymaktivitäten, die C-Speicherung und -Umsatz in den Dichtefraktionen mehr von den Bodeneigenschaften beeinflusst wurde als von der Wald- und Grünlandbewirtschaftung auf der regionalen Skala. Darüber hinaus stellen die hier vorgestellten Ergebnisse die Notwendigkeit für großräumige Studien heraus, die verschiedene Regionen und deren Umweltbedingungen berücksichtigen, um allgemeine Schlussfolgerungen über die Auswirkungen der Landbewirtschaftung und Bodeneigenschaften auf die mikrobiellen Gemeinschaften und C-Speicherung aufzuzeigen. Dieses Wissen sollte in Zukunft in die Forschung und in Modelle über die organische Bodensubstanz integriert werden sowie für wirksames Umweltmanagement angewendet werden, um die C-Speicherung in Böden weiterhin zu verbessern.

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Author Contributions to Manuscripts of the Dissertation of Nadine Herold

Manuscript 1: Soil property and management effects on grassland microbial communities across a latitudinal gradient in Germany
Authors: Nadine Herold, Ingo Schöning, Jessica Gutknecht, Fabian Alt, Steffen Boch, Jörg Müller, Yvonne Oelmann, Stephanie A. Socher, Wolfgang Wilcke, Tesfaye Wubet, Marion Schrumpf

- **Nadine Herold** is the first author and responsible for writing this paper. She carried out the soil sampling campaign, performed all enzyme activity and soil property analyses, conducted the final statistical analyses, and submitted the manuscript.
- **Dr. Marion Schrumpf** and **Dr. Ingo Schöning** were responsible for the overall experimental design, reviewed and edited drafts of the manuscript. Ingo Schöning was further involved in the sampling of the soil samples.
- **Dr. Jessica Gutknecht** and **Dr. Tesfaye Wubet** performed the PLFA extraction from the soil samples and determined the PLFA biomass and composition. Jessica Gutknecht further reviewed and made several corrections on manuscript drafts. Tesfaye Wubet was involved in the sampling of the soil samples.
- **Dr. Steffen Boch**, **Dr. Jörg Müller**, and **Dr. Stephanie A. Socher** recorded the vegetation of the plots and provided the Ellenberg indicator value for soil moisture. Steffen Boch and Jörg Müller further commented on a late draft of the manuscript.
- **Prof. Dr. Yvonne Oelmann**, **Prof. Dr. Wolfgang Wilcke**, and **Dr. Fabian Alt** provided the data of the phosphorous analyses for all samples and commented on a late draft of the manuscript. Fabian Alt was involved in the sampling of the soil samples.

Manuscript 2: Vertical and latitudinal gradients of potential enzyme activities in soil profiles of differently managed forest sites.
Authors: Nadine Herold, Ingo Schöning, Doreen Berner, Heike Haslwimmer, Ellen Kandeler, Beate Michalzik, Marion Schrumpf

- **Nadine Herold** is the first author and responsible for writing this paper. She carried out the soil sampling campaign, obtained the data in the laboratory, analyzed and discussed the data.
- **Dr. Marion Schrumpf** and **Dr. Ingo Schöning** were responsible for the overall experimental design, reviewed and edited drafts of the manuscript. Ingo Schöning was further involved in the sampling of the soil samples.
- **Doreen Berner** adapted the enzyme assays using fluorogenic substrates. **Heike Haslwimmer** developed the phenol oxidase assay. **Prof. Dr. Ellen Kandeler** and Doreen Berner revised the manuscript.
- **Prof Dr. Beate Michalzik** carried out a critical review of the manuscript and made some suggestions for improvement.

Manuscript 3: Soil and land-use types influence soil organic carbon storage and radiocarbon signatures in temperate soils more than land management.
Authors: Nadine Herold, Marion Schrumpf, Ingo Schöning, Beate Michalzik, Susan Trumbore

- **Nadine Herold** is the first author and responsible for writing this paper. She carried out the soil sampling campaign, performed soil analyses, analysed and discussed the data, and submitted the manuscript.
- **Dr. Marion Schrumpf** and **Dr. Ingo Schöning** were responsible for the overall experimental design, reviewed and edited drafts of the manuscript. Ingo Schöning was further involved in the sampling of the soil samples.
- **Prof Dr. Beate Michalzik** and **Prof. Dr. Susan Trumbore** carried out a critical review of the manuscript and made a number of suggestions for improvement

Selbständigkeitserklärung

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

…… ……..

Ort, Datum Nadine Herold