# *Fagus sylvatica* in temperate deciduous forests differing in tree species diversity - effects on the soil microbial community and complementary resource use

## Dissertation

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In memory of my mom …

Jena, März 2013 Carolin Thoms

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# Chapter 1

# Introduction

## 1.1 Understanding the role of tree diversity in beech forest ecosystems

Hardly any other tree species in Central Europe is as competitive as the European beech (*Fagus sylvatica*) (Leuschner et al., 2006). According to the potential natural vegetation in Germany, *Fagus sylvatica* would be the most common forest tree of Germany, and mixed beech forests would grow on three-quarters to two-thirds of the area. When *Fagus* spread to Central Europe after the last ice age, the forests were dominated by fast migrating pioneer species like *Pinus* and *Betula* species. *Fagus* is characterized by good rooting and beech seedlings can grow well in the shade of other trees. These properties allow *Fagus* to infiltrate existing forest communities, grow over, and suppress them in many places (Meister and Offenberger, 2004).

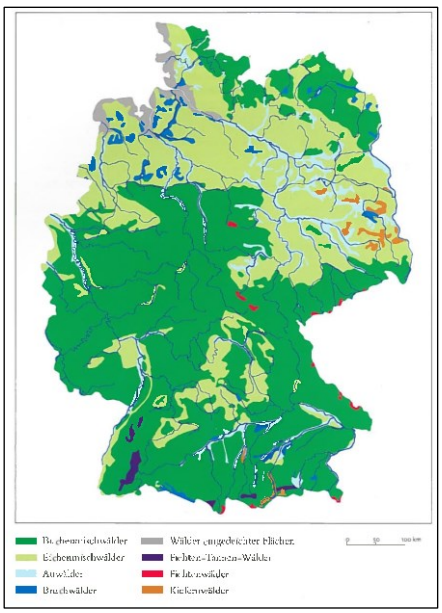

**Fig. 1.1:** Primary type of natural forest vegetationin Germany. (Source: Meister and Offenberger, 2004)

External disturbances, especially human use (timber harvesting, silvicultural replanting, land clearing for agricultural use) resulted in the reduction of the proportion of natural beech forests to less than 10% of the remaining forest area in Central Europe. However, the past conversion of forests to lucrative monocultures of *Picea* and *Pinus*species quickly showed consequences. In the second part of the 19th and during the 20th Century, storms and pests demonstrated that these monocultures are not sustainable and very vulnerable to external disturbances. In the period following this it was difficult to enforce the planting of deciduous trees due to the hunting lobby. The paradigm shifted only at the end of the 20th Century, such that forests are now seen as a cultural asset: not only as a supplier of wood and deer, but also as valuable for recreation and nature conservation (Meister and

Offenberger, 2004). Therefore, some still-existing beech forests in Germany were put under protection, for example the Hainich National Park in Thuringia and the Müritz and Jasmund National Parks in Mecklenburg-Vorpommern. In addition, many states in Germany have created silviculture programs in recent years to increase the proportion of mixed forests. An example of such a program is the LÖWE program in Niedersachsen. With the increase in the proportion of deciduous mixed forests, soon followed the long-term aim to make the forests more stable in the face of external influences and to improve their ecological function (www.landesforsten.de/LOEWE-Langfristige-OEkologische-Waldentwicklung.20.0.html).

The impending danger of climate change and its implications for the composition and abundance of life on earth triggered this rethinking. Consequences like the loss of biodiversity and a shift in the occurrence of species in many regions have been already recorded and resulted in a steadily growing field of biodiversity research (Loreau et al., 2001; McCollin et al., 2000). Recently the loss of biodiversity has been revealed as a major driver of ecosystem change (Hooper et al., 2012). Changes in ecosystems could be relevant for humanity if they negatively affect the functioning of ecosystems and therefore the provision of goods and services. In the current ecological research the link between biodiversity and ecosystem functioning is widely discussed (Loreau et al., 2001; Wardle et al., 2004; Scherber et al., 2010). Recent meta-analyses showed that biodiversity and ecosystem functioning are predominantly positively linked, but also concluded that this link still poses many unresolved questions (Balvanera et al. 2006; Cardinale et al. 2007, 2011). A better understanding of the link could help to better and more directly protect species and ecosystems in the future.

In recent years many attempts have been made to analyze the link by applying different approaches in biodiversity experiments. One approach is experimental-manipulative with artificially planted forest stands, which has been previously done in different ways for grasslands (Tilman et al., 1996; Roscher et al., 2004). But for forests this is a very lengthy process, coupled with intensive care. Nevertheless, one attempt is underway since 2003 with the BIOTREE experiment in western Thuringia (Scherer-Lorenzen et al., 2007). However, these man-made ecosystems have an entirely different dynamic than long-established communities. Hence, the results can only be applied with caution to natural ecosystems. A different approach offers comparative observational studies. The areas with

the highest protection level of National Parks present a unique opportunity for this kind of biodiversity research. The advantage is a better correlation between biodiversity and ecosystem functioning. However, there is a risk that observed correlations are site-specific and cannot be generalized. Above all, both research approaches are necessary, especially for German forestry where large areas that are currently monocultures will be converted into mixed stands. It is expected that increased tree diversity will affect biotic interactions and biogeochemical cycles with consequences for ecosystem functioning in temperate forests. This assumption was the basis for the establishment of the Graduate School 1086 to compare defined forest stands (phase 1) or tree clusters (phase 2) differing in tree species diversity in the Hainich National Park. In the first phase, three tree diversity levels were distinguished, differing in tree species richness and maximum eveness. Higher plant diversity was found to be more reliable (Naeem et al., 1997), and to include more species complementarity (Cardinale et al., 2007) with effects for aboveground-belowground interactions (Eisenhauer, 2012). This work aims to contribute to whether some conclusions on the role of biodiversity are also applicable to beech forest ecosystems differing in tree species diversity.

**Complementary resource use in forest ecosystems**

Loreau and Hector (2001) contributed to a mechanistic understanding of biodiversity effects by partitioning selection and complementarity. The 'complementarity effect' occurs when "resource partitioning or positive interaction lead to increased resource use", while "in the 'selection effect', dominance by species with particular traits affects ecosystem processes" (Loreau and Hector, 2001). Increased resource use due to complementarity can occur through different forms of niche partitioning that allow species to capture resources in ways that are complementary in space, time or in chemical terms (Tilman, 1999; McKane et al., 2002).

Several studies including meta-analyzes revealed that the magnitude of complementarity increases as biodiversity experiments run longer than five years (Cardinale et al., 2007; Fargione et al., 2007; Reich et al., 2012). Eisenhauer (2012) highlighted the multiplicity and relevance of aboveground-belowground interactions as a source of complementarity between plants. However, analysis of complementarity effects refers mainly to grassland biodiversity experiments. If marked differences in the species complementarity can be

identified in grassland biodiversity experiments after a few years, it is likely that in highly diverse and established forest communities complementarity effects and complementary resource use in the narrow sense might also be pronounced and even more complex. The results of different forests in eastern Canada showed that complementarity effects are different across biomes (Paquette and Messier, 2011). It was concluded that in the more environmentally stressed boreal climates, beneficial complementary interactions between tree species may become more important than in temperate forests where competitive exclusion is the most probable outcome of species interactions. However, the results of Zhang et al. (2012), the only meta-analysis of forest ecosystems, showed a more similar distribution of complementarity effects across biomes. They found a positive relationship between productivity and species richness in forest ecosystems, which was attributed to an improved resource partitioning and/or interspecific facilitation, especially as these played out over the multiple years of a study. In forest communities with high tree diversity the better availability of resources might be crucial in times of shortage.

**The effect of tree diversity on the soil microbial community**

In forest ecosystems tree species have a strong influence on soil properties in the topsoil (Augusto et al., 2003). Just a few years are enough to significantly reduce the soil pH after arable land has been reforested (Fornaçon et al., 2005). The soil pH is strongly influenced by the quality of litter and significantly affects all trophic levels of the decomposer community like earthworms (Vahder and Irmler, 2012), nematodes (Bjornlund et al., 2002) and soil microorganisms (Anderson and Joergensen, 1997; Blagodatskaya and Anderson, 1998; Högberg et al., 2007). There is strong evidence that individual plant traits that affect resource quality are more important than plant diversity *per se* for the decomposer community (De Deyn et al., 2004; Milcu et al., 2006).

Soil microorganisms decompose and mineralize organic substances left by plants and animals and therefore drive major ecosystem processes, such as organic matter turnover and nutrient cycling (Bardgett, 2005).Van der Heijden et al. (2008) concluded that soil microorganisms must be considered as important drivers of plant diversity and productivity in terrestrial ecosystems. Increasing tree species diversity in beech forest ecosystems accompanied by an increase in litter quality should have an influence on the structure and abundance of the microbial community, directly or indirectly. Recent long-term studies

show significant positive relationships between plant diversity and the soil biota in grasslands (Scherber et al., 2010; Eisenhauer et al., 2011a). In a temperate forest, a positive relationship was found between the herbaceous layer and the soil biota (Eisenhauer et al., 2011b). Therefore it can be assumed that more diverse tree communities promote a greater abundance of soil microorganisms. The underlying mechanisms include a provision of different habitat structures for the soil biota affecting all trophic levels. This comprises differences in the shrub, herb and litter layer as well as in the density and structure of roots. Decomposers can contribute to a positive plant diversity-productivity relationship through increasing resource availability for plants (Eisenhauer et al., 2012). Increased complementarity in highly diverse plant communities may increase organic matter turnover and therefore could contribute to a higher activity of the soil microbial community.

It is expected that decomposition of leaf litter affect primarily the uppermost soil layer. Microbial biomass generally decreases rapidly with increasing soil depth (Snaidr et al., 2008). In deeper soil layers, it was shown that the distribution of the microbial community is explained by the variation in soil recources (root exudates, soil C and N, exchangeable K) (Marilley and Aragno, 1999; Steenwerth et al., 2008, Fierer et al., 2003). It is likely that differences in tree species diversity contribute to changes of edaphic factors in deeper soil layers affecting the microbial communities.

However, it should be noted that the relationship between plants and soil food webs operates over seasonal timescales (Bardgett et al., 2005). Seasonal differences in the soil microbial community in a grassland diversity experiment were distinctly greater than differences between plant diversity levels (Habekost et al., 2008). Temperate forest ecosystems are also heavily influenced by the seasonal succession of spring - summer - autumn - winter. It is likely that the seasonal dynamics of forests has a great impact on the abundance and composition of soil microorganisms and that sampling in only one season can hide diversity effects on the microbial community.

## 1.2 Main objectives and hypotheses of this study

In this thesis the following hypotheses should be addressed:

- Complementary resource use plays a crucial role in near-natural mixed beech forests with high tree species diversity.
- Increased tree species diversity in beech forest ecosystems associated with increased litter quality and complementarity effects promotes high microbial abundances (Fig. 1.2).
- In deeper soil layers mainly edaphic factors influence the microbial communities.
- The impact of tree species diversity on the soil microbial community varies throughout the year, as direct effects of species-specific tree characteristics changes with the season.

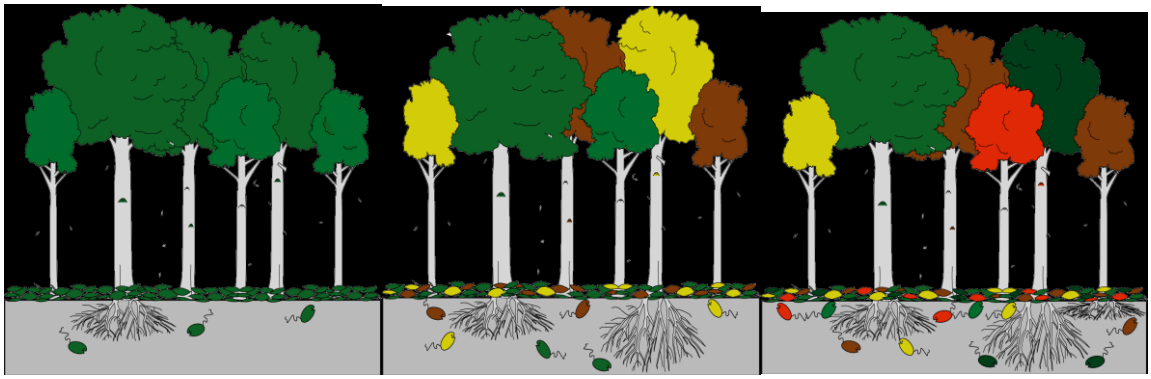

**Fig. 1.2:** Does increased tree species diversity in beech forest ecosystems promotes higher soil microbial abundances and complementary resource use?

## 1.3 Thesis organisation

The main part of the thesis (Chapter 3 and Chapter 4) focuses on the impact of tree species diversity in beech forests on the soil microbial community. First, the abundance and composition of the microbial community in the soils of the almost pure beech forest stands (DL 1) in comparison with the mixed beech forest stands (DL2-3) directly after litter fall were determined (Chapter 3). The microorganisms should be analyzed in the most active part of the soil. We used the first 20 cm of the uppermost soil layer at three depth intervals (0-5, 5-10, 10-20 cm) of the main rooting zone. In this chapter we discuss results that are related to the second and third main hypothesis.

Another soil sampling was carried out in late spring/early summer in the topsoil and should give more insight into the seasonal dynamic of the microbial community (Chapter 4). We expected differences due to advanced decomposition processes of the litter layer and due to a higher activity in the rhizosphere strengthening the tree diversity effect on the soil microbial community. Therefore, Chapter 4 mainly addresses the fourth main hypothesis. For our analysis we took advantage of a lot of useful data that have been explored by colleagues from the Graduate School.

The third research approach (Chapter 5) involves the competition between tree species in more diverse forest stands with implications for the complementary use of resources, and therefore refers to the first main hypothesis. Here, the water use strategy of *Fagus sylvatica* during a severe summer drought was investigated. During this year, continuous measurements of precipitation, throughfall, and soil water were carried out which were used for isotope measurements providing information for the source water of metabolic products in *Fagus sylvatica*.

# Chapter 2

# Material and methods

## 2.1 The study site

The study was conducted in the Hainich National Park, a deciduous forest in Thuringia, in central Germany. The National Park was established in 1997 and is one of the largest connected semi-natural forests in Europe, covering a total area of 7500 ha. The presence of extended close-to-nature beech forests on limestone at medium altitude makes the Hainich National Park unique in the world and led to its inclusion in the UNESCO World Heritage Site "Beech Forests of the Carpathians and the Ancient Beech Forests of Germany" in 2011.

Different forest management strategies in the past resulted in a small-scale forest mosaic with high tree species diversity. A detailed description of available historical data from Mund (2004) showed that the region of the Hainich National Park was forested with deciduous forests since the early 1500s. In the first half of the 20th century the southern part of Hainich, known as “Weberstedter Holz”, was mainly used for selective cutting. In the last decades before the establishment of the National Park, the region was a military training area, and only occasional selective cutting took place.

In the north-east section of the National Park, 12 study plots (50 m x 50 m each) within a radius of approximately 4 km were established, representing three tree diversity levels (DL 1-3) with four replicates per level (Fig. 2.1). Selection criteria for the study plots were (i) comparable pedological conditions (Triassic limestone covered by loess), (ii) similar climate conditions (precipitation 600–670 mm $yr^{-1}$, annual mean temperature 7.5–8.0 °C), (iii) comparable stand structure in terms of basal area and the diameter at breast height (DBH), and (iv) tree diversity and abundance patterns according to pre-defined diversity classes ranging from low (0.2) to high values (1.5) of the Shannon diversity index (Leuschner et al., 2009).

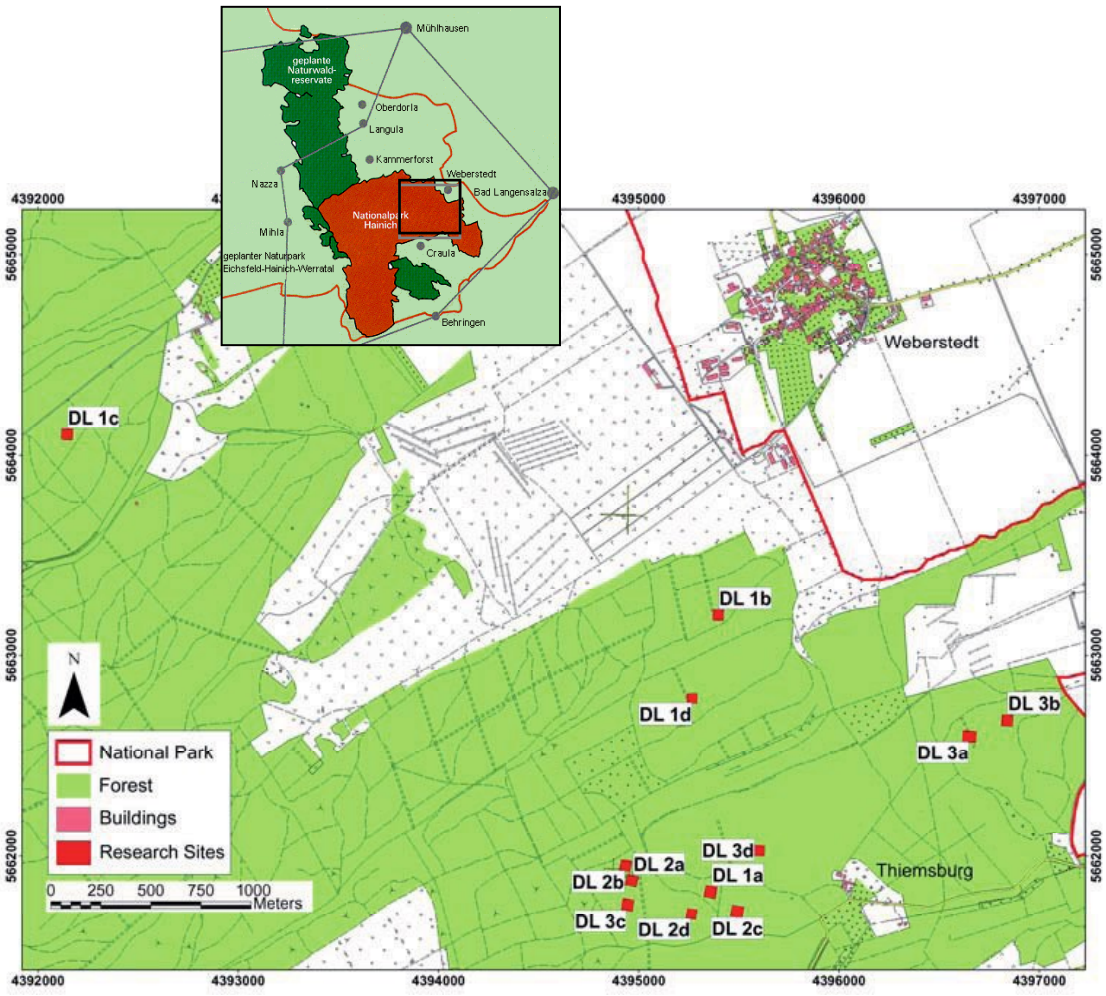


**Fig.2.1:**Topographic map with the location of the study sites of the three diversity levels (DL 1-3).The ensuing letters represent different replicates.The small map on the top shows the National Park Hainichand the black square shows the cutout of the topographic map.Topographic mapmade by M. Daenner,map data from the Thüringer Kataster- und Vermessungsverwaltung.

At DL 1, *Fagus sylvatica* L. is the predominant tree species. Plots of DL 2 contain three dominating tree genera: *Fagus*, *Tilia* species (*Tilia cordata* Mill. and *Tilia platyphyllos* Scop.) and *Fraxinus excelsior* L. Plots assigned to DL 3 are characterized by five dominating tree genera: *Fagus*, *Tilia*, *Fraxinus*, *Carpinus betulus* L. and *Acer* species (*Acer pseudoplatanus* L. and *Acer platanoides* L.). The investigated plots also contained higher percentages of *Quercus* litter (*Quercus robur*, *Quercus petraea*). In general other tree species like *Prunus avium*, *Ulmus glabra,* and *Populus* species were also found in low numbers, especially in the mixed stands.

All soils in the study area developed from loess underlain by limestone and were classified as Luvisols. There are variations in the clay content between the study plots with much lower clay contents in the topsoils of the pure beech stands. The strong accumulation of

clay between 30 and 40% begins at soil depths of 30-40 cm and can lead to stagnic properties during winter and spring, but the soils were typically dry during summer. Some important tree and site characteristics are summarized in Table A1 (Appendix).
One plot per diversity level (DL 1a, DL 2c, DL 3a) was chosen to carry out time consuming and complex measurements for the different projects of the Graduate School. Within each of the study plots three transects of 30 m length were chosen randomly without overlap. Along transects several subplots were established where the main studies of the different projects were carried out. For maps with the distribution of trees and subplots of all study plots see Figs. A1-A12 and for pictures of the study plots see Figs. A13-18 (Appendix).

## 2.2 Methods used to analyse biodiversity effects

Molecular biomarkers and their isotopic contents are useful tools to explore key organisms and processes in biogeochemical cycles, and therefore can give novel insights to the link between biodiversity and ecosystem functioning. In the following, two molecular biomarkers used in this study are briefly introduced. More details including the description of the extraction methods and the measurements as well as further references will be found in the given sections.

**Phospholipid fatty acids (PLFA)** as microbial biomarkers were used to characterize the soil microbial community. The extraction of PLFAs represents a phenotypic analysis and an important quantitative assessment of the microbial community. This method is applied in broad scientific fields and is independent from the culturability of microbial cells. Phospholipids exist in the membranes of all living cells but different subsets of the microbial community differ in their fatty acid composition. For some of these subsets or groups (fungi, arbuscular mycorrhizal fungi, protozoa, Gram-negative bacteria, Actinobacteria and Gram-positive bacteria in general) certain indicator PLFAs could be detected (Frostegård and Bååth, 1996; Olsson, 1999; Zelles, 1999). Importantly, the method measures only the living part of the microbes. After cell death of the microorganisms, phospholipids are immediately metabolized. Interesting information about the development and application of microbial PLFA as biomarkers can be read in Frostegård et al. (2011). The popularity of this method in soil microbial research increased

during the 1990s and continues to the present. This is due to several advantages, PLFA-based methods are rapid, sensitive, and reproducible, but more importantly the changes of the microbial community can be recorded, and in $^{13}C$-labeling experiments the active groups can even be identified (Frostegård, 2011).For more details refer to section 3.2.3. and 4.2.3.

**Compound-specific hydrogen isotope ratios (δD) of *n*-alkanes** as constituents of the leaf-wax layer were used to determine the water use strategy of *Fagus sylvatica* in the different diversity levels during a drought. The use of *n*-alkanes as molecular biomarker preserved in lake sediments is widely used as palaeoclimate proxy (Sachse et al., 2006; Mügler et al., 2008; Günther et al., 2011). The informational content of this biomarker is based on the fact that the organic hydrogen of *n*-alkanes records the isotopic composition of the ambient water (environmental water) used during photosynthesis (Sternberg, 1988). In plants, it can reflect the δD of xylem water, which may correspond in some cases to the δD of the soil water. However, the compound-specific hydrogen isotope ratio (δD) of newly synthesized *n*-alkanes can be also influenced by environmental conditions over the growing season (Smith and Freeman, 2006). For example, leaf transpiration is controlled by relative humidity and, together with stomata closing, leads to an enrichment of deuterium in the leaf water (Farquhar et al., 2007). This is a dominant process during periods of drought, and *n*-alkanes being synthesized during that time may reflect these processes in their δD values. The renewal rate of *n*-alkanes in the individual leaves over the growing season is still largely unknown. It probably depends on the plant species and environmental conditions, which varies due to the intensity of abrasion by wind, water, animals, or radiation (Lockheart et al, 1997). A two-week survey classified the renewal rate of *n*-alkanes in the shade leaves of deciduous trees as being on the order of weeks (Sachse et al., 2009) and provided the precondition that a prolonged drought can be reflected in the isotopic signature of leaf-wax lipids. For more details refer to section 5.2.2 and 5.2.3.

# Chapter 3

## Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest

Chapter source:
Thoms, C., Gattinger, A., Jacob, M., Thomas, F.M., Gleixner, G., 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. *Soil Biology & Biochemistry* 42, 1558-1565.

**Abstract**

We investigated the link between aboveground and belowground diversity in temperate deciduous forest ecosystems. To this end, we determined the effects of the tree species composition on the biomass and composition of the soil microbial community using phospholipid fatty acid (PLFA) profiles in the Hainich National Park, a deciduous mixed forest on loess over limestone in Central-Germany. We investigated the effects of the leaf litter composition on the microbial community, hypothesizing that distinctive leaf litter compositions increase signature PLFAs. In addition, we studied the impact of clay content, pH and nutrient status of the soil on the microbial community in different surface soil layers. Consequently, soil was sampled from depths of 0-5 cm, 5-10 cm and 10-20 cm. Plots with highest leaf litter diversity had the largest total amounts of fatty acids, but only PLFA 16:ω15, which is a common marker for arbuscular mycorrhizal fungi, was significantly increased. In the uppermost soil layer, the pH explained most of the variance in microbial composition. In the deeper surface soil layers, nutrients such as carbon, nitrogen and phosphorus determined the microbial abundances and composition. Our results suggest that the soil microbial community is mainly indirectly influenced by aboveground diversity. Changes in soil pH or the soil nutrient status that are driven by specific plant traits like leaf litter quality drive these indirect changes. Specific direct interactions are mostly reasonable for mycorrhizal fungi.

**Keywords:** microorganisms, biodiversity, PLFA, leaf litter, soil resources, beech forest, Hainich National Park

## 3.1 Introduction

Little is known about the link between the aboveground tree species diversity and the soil microbial diversity in mixed-species forests (Gleixner et al., 2005). In general, the distribution and abundance of the soil microbial communities is a function of abiotic (physical and chemical) conditions and biotic factors (interactions among species/food supply) (Scherer-Lorenzen et al., 2005). In forest soils, the vertical and horizontal distribution of soil microorganisms can differ extremely but microbes are generally most abundant in the upper surface soil layer - in particular, within the first few centimeters of the topsoil (Fierer et al., 2003; Steenwerth et al., 2008). The large food supply through decomposition of plant litter and plant residues supports a high microbial abundance at the topsoil's surface. Evidently, the decomposer community is affected by plant litter characteristics (Wardle et al., 2006) such as the provision of microhabitats (Ettema, 1998; Hansen, 2000) and the chemical composition of the foliage (e.g. Ricklefs and Matthew, 1982; Hättenschwiler et al., 2008). The C/N ratio, which is often used as an indicator of tissue quality, correlates negatively with litter decomposition (Taylor et al., 1989; Jacob et al., in press) and influences the composition of the decomposer community.

Under aerobic conditions, complex compounds such as lignin or the lignocelluloses complex are broken down by fungi (de Boer et al., 2005). Using PLFA 18:2ω6,9 as an indicator, Snajdr et al. (2008) found that the fungal biomass was largest in the L-layer, where it was 4.5 times larger than in the H-layer and continued to rapidly decrease with increasing soil depth. Currently, it is largely unknown whether different tree litter types in forest ecosystems promote specific groups of fungi or bacteria. But earlier investigations have shown that distinct soil microbial communities can be found in ecosystems that differ in plant community composition (Bauhus et al., 1998; Myers et al., 2001; Hackl et al., 2005; Habekost et al., 2008). Generally, the abundances of fungi and protozoa decline with increasing soil depth (Ekelund et al., 2001; Taylor et al., 2002; Fierer et al., 2003), and those of actinomycetes increase (Federle et al., 1986; Fritze et al., 2000). Furthermore, the vertical distribution of Gram-negative bacteria is linked to the root distribution (Söderberg et al., 2004). Their biomass increased when rapidly decomposable C compounds such as sugars, organic and amino acids were available, which leaked out of roots and fungal

mycelia (Marilley and Aragno, 1999). Consequently, in a soil profile, bacteria populations changed from a higher Gram-negative abundance at the soil surface to a higher Gram-positive abundance at deeper soil layers (Franzmann et al., 1998; Blume et al., 2002; Fierer et al., 2003). There, additional factors such as root C inputs, detritus, species interactions and abiotic conditions determine the microbial composition (Brant et al., 2006; de Boer et al., 2005; Bardgett, 2005). Steenwerth et al. (2008) showed that the variation in soil resources (i. e., soil C and N and exchangeable K) explained the distribution of soil microbial communities in greater depths, and the results suggest that lower pH values restrict the growth of Gram-negative bacteria. The influence of the pH on the soil microbial composition is widely accepted (Anderson and Joergensen, 1997; Blagodatskaya and Anderson, 1998; Högberg et al., 2007). Bååth and Anderson (2003) found that the concentrations of PLFAs 16:1ω5 and 16:1ω7 increased with increasing pH, while those of i16:0 and cy19:0 decreased; in contrast, the concentration of 18:2ω6,9, which is indicative of fungi, was affected to a small extent only. Dominant tree species with a low litter quality such as beech may decrease the soil pH (Guckland et al., 2009) and an influence of the tree species composition on the microbial community is very likely. However, more information on biotic and abiotic factors controlling the composition of microbial PLFAs is needed to understand the regulation and functional significance of biodiversity (Wardle et al., 2006). Therefore, we studied the effects of increasing leaf litter diversity and specified the dominant environmental factors in vertical soil profiles on the microbial community in near-natural deciduous forest stands with increasing tree species diversity. We hypothesize that a) an increase in tree diversity is correlated by an increasing diversity and abundance of PLFAs in the uppermost soil layer; whereas b) edaphic factors such as pH, C:N ratio and clay content drive the composition of the microbial community in deeper soil layers.

## 3.2 Material and methods

### 3.2.1 Study site

The study was conducted in the Hainich National Park, a deciduous forest in Thuringia, Central-Germany. The National Park is one of the largest connected semi-natural forests in Europe and covers a total area of 7500 ha. Different forest management strategies in the

past resulted in a small-scale forest mosaic with high tree species diversity. In the north-east of the National Park, nine fenced study plots (50 m x 50 m each) were established representing three tree diversity levels (DL 1-3) with three replicates per level (Leuschner et al., 2009). At DL 1, *Fagus sylvatica* L. is the predominant tree species. Plots of DL 2 contain three dominating tree genera: *Fagus*, *Tilia* species (*Tilia cordata* Mill. and *Tilia platyphyllos* Scop.) and *Fraxinus excelsior* L. Plots assigned to DL 3 are characterized by five dominating tree genera: *Fagus*, *Tilia*, *Fraxinus*, *Carpinus betulus* L. and *Acer* species (*Acer pseudoplatanus* L. and *Acer platanoides* L.). The investigated plots contained also higher percentages of *Quercus* litter. In general other tree species, like *Quercus robur*, *Quercus petraea*, *Prunus avium*, *Ulmus glabra* and *Populus* species, were found in low numbers especially in the mixed stands. Site characteristics were comparable regarding climate, stand structure and physical soil properties (Guckland et al., 2009). All stands are near-natural without distinct anthropogenic impact on their structure in the past several decades. The main stand characteristics (basal area, stem density, tree height, tree age) are published in Jacob et al. (2010) and Guckland et al. (2009).

### 3.2.2 Soil sampling

In November 2005, soil samples were taken from all nine plots at three randomly chosen transects of 30 m x 1 m within the plots. We took twelve samples per plot from depths of 0-5 cm, 5-10 cm and 10-20 cm using a split tube (Eijkelkamp, Giesbeek, Netherlands) with 5.3 cm diameter. The samples were randomly taken at a minimum distance of 4 m, and were mixed to obtain one composite sample per depth and plot. All samples were stored at -20°C and sieved <2 mm to remove visible stones, animals, roots and plant material prior to lipid extraction. The water content was quantified using a sub sample dried to 105°C. For C and N analyses another sub sample was air dried, ball milled and analyzed in an automated element analyzer (Elementaranalysator varioMAX, Elementar Analysensysteme GmbH, Hanau, Germany). Soil pH was measured in 1 M KCl solution.

### 3.2.3 PLFA analysis

100 g of fresh soil were extracted according to the method of Bligh and Dyer (1959) and Zelles and Bai (1993). As described previously (Kramer and Gleixner, 2006), soil lipids were extracted using a mixture of chloroform, methanol and phosphate buffer (1:2:0.8 v/v/v). Phospholipids were isolated on silica columns and hydrolyzed and methylated

using a methanolic KOH solution. Fatty acid methyl esters (FAME) were separated into saturated, polyunsaturated and monounsaturated fatty acids using aminopropyl-modified and silver-impregnated SPE columns. After addition of methyl nonadeconoate n19:0 as an internal standard, the samples were quantified with a GC-AED System (GC: HP 6890 Series, AED: G 2350 A, Agilent Technologies, United States) using a BPX 70 column (50 m x 0.32 mm I.D., 0.25 mm film thickness) in the split mode (10:1). Helium was used as a carrier gas at a flow rate of 1.3 ml $min^{-1}$. The temperature program started at 100°C (for 1 min). Thereafter, the temperature was raised to 135°C at a rate of 4°C $min^{-1}$, then to 230°C at 2°C $min^{-1}$. The final temperature of 260°C was reached after further raising the temperature at 30°C $min^{-1}$, and was kept constant for 1 min. PLFAs were identified by a comparison with a standard mixture (Supelco) of saturated fatty acids and unsaturated fatty acids and by using mass spectral data from a database obtained from analyses of archaeal, bacterial and eukaryotic monocultures (Gattinger et al., 2003).

The sum of the PLFAs i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 was chosen to describe Gram-positive bacteria excluding the actinomycetes, and saturated fatty acids with a methyl group on the 10th C atom were used to quantify the Gram-positive actinomycetes (Lechevalier, 1977; Kroppenstedt, 1985). The sum of the PLFAs i14:0, i15:0, a15:0, 15:0, i16:0, 10Me17:0, 11Me17:0, i17:0, a17:0, cy17:0, 10Me19:0, cy19:0, 16:1ω7 and 18:1ω7 represented the total bacterial biomass. The biomass of Gram-negative bacteria was calculated subtracting the biomass of actinomycetes and other Gram-positive bacteria from the total bacterial biomass. Cyclopropyl fatty acids such as cy17:0 and cy19:0 were used as indicators of environmental stress (Kaur et al., 2005), and the fatty acids 20:2, 20:3, 20:4 were used for indicating soil protozoa (Shaw, 1966; White et al., 1996). The fatty acid 18:2ω6,9 was used as a general fungal marker (Federle, 1986; Tornberg et al., 2003), and the fatty acid 16:1ω5, for arbuscular mycorrhizal fungi (Nordby et al., 1981; Olsson, 1999). The following ratios were also calculated: cyclopropyl fatty acids:monoenoic precursors (cy17:0, cy19:0/16:1ω7, 18:1ω7) and total saturated:total monounsaturated fatty acids (15:0, 16:0, 18:0, 20:0, 22:0/15:1ω11, 16:1ω7, 16:1ω5, 17:1ω8, 18:1ω9, 18:1ω7, 20:1ω11). These two ratios were used as indicators of nutritional stress in bacterial communities (Knivett and Cullen, 1965; Kieft et al., 1997; Bossio et al., 1998; Feng and Simpson, 2009).

### 3.2.4 Statistical analysis

Statistic calculations were performed using R software (version 2.6.2, R Development Core Team, 2008). To test differences between the three diversity levels and the three soil depths, we performed an analysis of variance (ANOVA) followed by Tukey-Kramer HSD test for all pairwise comparisons of the means ($p < 0.05$ in all cases).

PLFA profiles were analyzed using CANOCO software (version 4.5, Microcomputer Power, Inc., Ithaca, NY). The results of the amounts of individual PLFA were subjected to principal component analysis (PCA) and redundancy analysis (RDA) based on a covariance matrix, where mol% of fatty acids was centered. PCA as an indirect gradient analysis was used to extract the major patterns from the variation in PLFA data, and to interpret their relevance with the available environmental data in regression analyses. In RDA as a direct gradient analysis, the ordination axes represented aggregates of the environmental data that best explain the PLFA data. All environmental data were tested for significant contribution ($p < 0.05$) to the explanation of the variation in the PLFA data using the Monte Carlo permutation test.

## 3.3 Results

### 3.3.1 Soil characteristics

We found significantly increased pH values and significantly decreased C:N ratios with increasing diversity level in the upper 5 cm of soil (Table 3.1). In 5-10 and 10-20 cm soil depth both parameter differed only between the first diversity level and the other two. The effect was mostly driven by the lower presence of *Fagus* species at higher diversity levels. From DL 1 to DL 3 increasing litter diversity could be observed (Fig. 3.1a).

### 3.3.2 Microbial biomass

Total amount of PLFA in 0-20 cm soil depth increased with increasing tree diversity (Fig. 3.1b, Table 3.2). The increase from DL 1 to DL 3 was observed for all microbial groups, but was only significant for the group of arbuscular mycorrhizal fungi (AM fungi) (Table 3.2).

**Table 3.1:** Soil characteristics at the tree species diversity levels (DL) (means ± 1 SE of three plots per DL). Different letters indicate significant differences among DL in a given soil depth ($p < 0.05$).

| Diversity level | Soil depth (cm) | C (%) | N (%) | C/N | pH (KCl) |
|---|---|---|---|---|---|
| DL 1 | 0 – 5 | 3.3 ± 0.9a | 0.21 ± 0.04a | 15.9 ± 1.7a | 3.4 ± 0.2a |
| DL 2 | 0 – 5 | 3.8 ± 1.0a | 0.30 ± 0.08a | 12.6 ± 0.3b | 4.3 ± 0.1b |
| DL 3 | 0 – 5 | 3.9 ± 0.9a | 0.31 ± 0.08a | 12.6 ± 0.7b | 5.2 ± 0.1c |
| DL 1 | 5 – 10 | 1.1 ± 0.2a | 0.07 ± 0.01 a | 16.6 ± 3.0a | 3.3[a] ± 0.1a |
| DL 2 | 5 – 10 | 2.0 ± 0.5a | 0.18± 0.05ab | 11.2 ± 0.4b | 4.1[a] ± 0.1b |
| DL 3 | 5 – 10 | 2.5 ± 0.9a | 0.21 ± 0.07 b | 11.8 ± 1.0b | 4.3[a] ± 0.3b |
| DL 1 | 10 – 20 | 0.7 ± 0.0 a | 0.05 ± 0.00a | 14.3 ± 1.8a | 3.4[b] ± 0.1a |
| DL 2 | 10 – 20 | 1.3 ± 0.3ab | 0.14 ± 0.03b | 10.0 ± 1.3b | 4.5[b] ± 0.5b |
| DL 3 | 10 – 20 | 1.5± 0.4 b | 0.14 ± 0.04b | 10.7 ± 1.1b | 4.7[b] ± 0.3b |

[a] Calculated from Guckland et al. (2009).
[b] Data from Guckland et al. (2009).

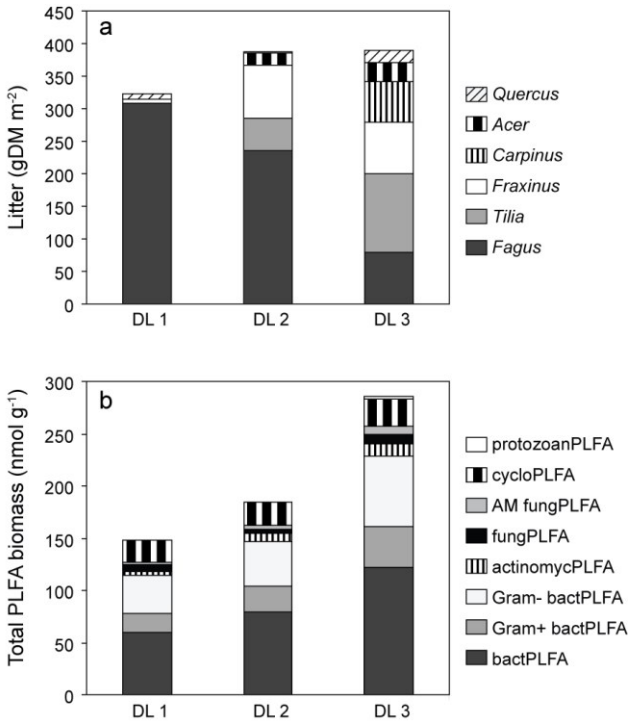


**Fig. 3.1:** Characteristics of the topsoil at the different diversity levels (DL), a) Litter amounts in the year 2005 (g DM $m^{-2}$) (data from Jacob et al., 2010); b) Biomass of fatty acid groups (nmol $g^{-1}$ dw) at the diversity levels (DL, N = 3 per DL) in a soil depth of 0-20 cm.

Total amount of PLFA strongly decreased with soil depth. Correlation between the biomass of the microbial groups and litter composition in the uppermost soil layer demonstrate that only the abundance of AM fungi was significantly correlated to some litter species (Table 3.3). The percentage of *Fagus* litter was negatively correlated with

AM fungi, whereas *Tilia* litter and also the pH were positively correlated. Soil characteristics were not significantly differentamong DL in the uppermost soil layer (data not shown) but exhibited highly significant differences in a soil depth of 10-20 cm. Except for fungi, the microbial biomass of nearly all microbial groups were significantly and positively correlated with the pH, the clay content and the soil nutrients C, N, P.

### 3.3.3 Microbial Composition

Significant differences of the microbial groups in the diversity gradient were found in soil depths of 0-5 cm and 0-20 cm for PLFA 16:1ω5 (AM fungi) and PLFA cy17:0 and cy19:0 (cyclo) (Table 3.2). In these soil layers, the AM fungi increased and the cyclo decreased with increasing tree species diversity. In deeper soil layers, only the bacterial biomass increased with increasing diversity level. The proportion of protozoan was very small, and was hardly detectable below 5 cm of soil depth. In the first diversity level, no significant change in the microbial composition with depth was observed, except for protozoa (Table 3.2). However, in the mixed-species stands (DL 2, 3), the proportions of bacterial biomass, actinomycetes and other Gram-positive bacteria increased with soil depth. The two calculated PLFA ratios, cyclopropyl fatty acids:monoenoic precursors and total saturated:total monounsaturated fatty acids, increased with depth in all diversity levels and were highest at diversity level 1 (Table 3.2).

### 3.3.4 Indirect gradient analysis

Principal component analysis (PCA) with PLFA data including all soil depths showed a good separation between the uppermost soil layer (0-5 cm) and the deeper soil layer (10-20 cm), whereas the intermediate layer strongly overlapped (data not shown). Consequently, only PLFA data from soil depths of 0-5 cm (Figs. 3.2a and 3.3a) and of 10-20 cm (Figs. 3.2b and 3.3b) were further evaluated. The first two principal components explained approximately 80% of the variation in PLFA composition in both soil depths. In the uppermost soil layer, the first principal component explained 55.2% of variance and the second component 22.3%(Fig. 3.2a), the explained variance between the two axes differed only slightly in the deeper soil layer (Fig. 3.2b). In the soil depth of 0-5 cm, the PLFA 18:1ω7, 16:1ω7, 18:1ω9 and 16:1ω5 had strong positive loadings on PC 1, while the PLFA cy19:0, 16:0, i15:0, 22:0, br17:0, cy17:0 and 15:0 had strong negative loadings on PC 1.

**Table 3.2:** Microbial compositions of the diversity levels (DL) (means ± 1 SE of three plots per DL). Different letters indicate significant differences among DL in a given soil depth ($p < 0.05$). *Significant differences among the different soil depths (0-5 cm/5-10 cm/10-20 cm).

| Diversity level | Soil depth (cm) | Bacteria (mol%) | Gram-positive bacteria (mol%) | Gram-negative bacteria (mol%) | Actinomycetes (mol%) | Fungi (mol%) | AM fungi (mol%) | Cyclo (mol%) | Protozoa (mol%) | Cyclo: Precursors | Total sat.: Total mono. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DL 1 | 0 – 5 | 61.1 ± 1.5a | 16.7 ± 1.3a | 39.6 ± 3.6a | 3.0 ± 0.2a | 5.8 ± 4.7a | 2.5 ± 0.8a | 22.3 ± 3.7a | 1.0 ± 0.5a | 1.9 ± 0.6 | 0.8 ± 0.2 |
| DL 2 | 0 – 5 | 61.2 ± 4.2a | 16.2 ± 4.0a | 38.4 ± 6.5a | 4.1 ± 0.4a | 6.1 ± 2.7a | 2.5 ± 0.5a | 17.2 ± 0.9a | 1.0 ± 0.6a | 0.9 ± 0.4 | 0.6 ± 0.2 |
| DL 3 | 0 – 5 | 8.3 ± 7.8a | 16.2 ± 4.8a | 35.9 ± 2.0a | 4.2 ± 2.2a | 6.9 ± 5.2a | 5.1 ± 0.9b | 10.8 ± 2.3b | 1.8 ± 1.4a | 0.5 ± 0.1 | 0.3 ± 0.1 |
| DL 1 | 5 – 10 | 64.1 ± 2.2a | 18.8 ± 3.9a | 37.8 ± 4.0a | 4.9 ± 2.3a | 4.0 ± 1.9a | 1.5 ± 1.4a | 22.8 ± 3.9a | 0.4 ± 0.3a | 2.8 ± 2.0 | 1.7 ± 1.4 |
| DL 2 | 5 – 10 | 71.1 ± 2.2b | 17.3 ± 6.6a | 46.2 ± 10.8a | 5.4 ± 1.8a | 1.2 ± 0.7a | 2.0 ± 1.7a | 27.1 ± 11.7a | 0.0 ± 0.3a | 1.7 ± 1.2 | 0.5 ± 0.2 |
| DL 3 | 5 – 10 | 69.0 ± 1.9b | 21.0 ± 3.6a | 36.8 ± 5.7a | 8.0 ± 0.7a | 2.1 ± 0.6a | 2.0 ± 1.8a | 21.5 ± 7.1a | 0.2 ± 0.2a | 1.5 ± 0.6 | 0.7 ± 0.2 |
| DL 1 | 10 – 20 | 63.8 ± 4.7a | 22.6 ± 5.7a | 32.7 ± 2.2a | 7.2 ± 3.0a | 4.0 ± 1.8a | 0.5 ± 0.9a | 20.9 ± 2.1a | 0.0 ± 0.0a | 3.5 ± 2.6 | 2.4 ± 1.5 |
| DL 2 | 10 – 20 | 74.2 ± 2.3b | 30.0 ± 2.0a | 30.3 ± 1.9a | 10.3 ± 1.7a | 1.4 ± 0.5a | 1.9 ± 0.3a | 18.9 ± 0.8a | 0.2 ± 0.1a | 2.0 ± 0.6 | 1.2 ± 0.3 |
| DL 3 | 10 – 20 | 73.1 ± 1.9b | 27.6 ± 5.0a | 31.3 ± 4.8a | 10.3 ± 2.2a | 2.5 ± 1.1a | 1.0 ± 1.0a | 20.5 ± 3.2a | 0.2 ± 0.2a | 2.4 ± 1.0 | 1.5 ± 0.7 |
| DL 1 | 0 – 20 | 61.9 ± 1.9a | 17.4 ± 0.4a | 38.9 ± 2.3a | 3.8 ± 0.4a | 5.1 ± 2.9a | 2.2 ± 0.6a | 22.1 ± 3.1a | 0.8 ± 0.3a | 1.6 ± 0.5 | 2.8 ± 0.5 |
| DL 2 | 0 – 20 | 67.1 ± 3.6a | 20.9 ± 4.7a | 37.5 ± 6.5a | 6.0 ± 2.1a | 3.9 ± 2.7a | 2.5 ± 0.4a | 19.1 ± 1.5a | 0.7 ± 0.4a | 1.2 ± 0.4 | 2.0 ± 0.7 |
| DL 3 | 0 – 20 | 61.0 ± 6.5a | 18.2 ± 4.6a | 34.9 ± 1.7a | 5.5 ± 2.5a | 5.9 ± 4.1a | 4.3 ± 0.9b | 12.9 ± 2.0b | 1.4 ± 1.0a | 0.6 ± 0.2 | 1.4 ± 0.2 |
| DL 1 | * | a/a/a | a/a/a | a/a/a | a/a/a | a/a/a | a/a/a | a/a/a | a/ab/b | | |
| DL 2 | * | a/b/b | a/a/b | a/a/a | a/a/b | a/b/b | a/a/a | a/a/a | a/b/b | | |
| DL 3 | * | a/b/b | a/ab/b | a/a/a | a/ab/b | a/a/a | a/ab/b | a/a/a | a/a/a | | |

**Table 3.3:** Spearman's rank correlation coefficients (rho) for correlations between the biomass of the PLFA groups and site characteristics (*** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$). Significance is marked in bold.

| Site characteristrics | Soil depth | Total (nmol $g^{-1}$ dw) | Bacteria (nmol $g^{-1}$ dw) | Gram-positive bacteria (nmol $g^{-1}$ dw) | Gram-negative bacteria (nmol $g^{-1}$ dw) | Actinomycetes (nmol $g^{-1}$ dw) | Fungi (nmol $g^{-1}$ dw) | AM fungi (nmol $g^{-1}$ dw) | Cyclo (nmol $g^{-1}$ dw) | Protozoa (nmol $g^{-1}$ dw) |
|---|---|---|---|---|---|---|---|---|---|---|
| Total litter (g dw $m^{-2}$) | 0 – 5 | 0.05 | 0.03 | 0.08 | 0.10 | 0.15 | -0.17 | 0.12 | -0.18 | -0.23 |
| *Fagus sylvatica* (%) | 0 – 5 | -0.63 | -0.58 | -0.43 | -0.62 | -0.50 | -0.40 | **-0.73*** | 0.00 | -0.45 |
| *Tilia spp.* (%) | 0 – 5 | 0.58 | 0.55 | 0.38 | 0.58 | 0.46 | 0.60 | **0.67*** | 0.00 | 0.60 |
| *Fraxinus excelsior* (%) | 0 – 5 | 0.33 | 0.42 | 0.47 | 0.38 | 0.54 | 0.02 | 0.32 | 0.28 | -0.10 |
| *Carpinus betulus* (%) | 0 – 5 | 0.51 | 0.39 | 0.31 | 0.39 | 0.22 | 0.15 | 0.59 | -0.10 | 0.22 |
| *Acer spp.* (%) | 0 – 5 | 0.47 | 0.42 | 0.33 | 0.55 | 0.41 | 0.42 | 0.55 | -0.07 | 0.37 |
| *Quercus spp.* (%) | 0 – 5 | 0.20 | 0.17 | -0.07 | 0.20 | 0.02 | 0.17 | 0.30 | -0.25 | 0.40 |
| pH (KCl) | 0 – 5 | 0.58 | 0.52 | 0.38 | 0.55 | 0.43 | 0.40 | **0.67*** | -0.03 | 0.37 |
| C (%) | 10 – 20 | 0.58 | **0.77*** | **0.85**** | 0.54 | **0.90***** | 0.13 | 0.58 | **0.67*** | **0.79*** |
| N (%) | 10 – 20 | **0.80**** | **0.92***** | **0.95***** | **0.80**** | **0.97***** | 0.08 | **0.81**** | **0.86**** | **0.72*** |
| Clay (%)[a] | 10 – 20 | **0.77*** | **0.87**** | **0.88**** | **0.73*** | **0.93***** | 0.25 | **0.66*** | **0.87**** | **0.76*** |
| P (mg $kg^{-1}$)[b] | 10 – 20 | **0.95***** | **0.90***** | **0.83**** | **0.97***** | **0.78*** | 0.30 | **0.90***** | **0.93***** | 0.54 |
| Fine root biomass (g $m^{-2}$)[c] | 10 – 20 | -0.35 | -0.50 | -0.58 | -0.30 | -0.65 | -0.07 | -0.37 | -0.43 | -0.51 |
| Fine root necromass (g $m^{-2}$) [c] | 10 – 20 | -0.17 | -0.27 | -0.37 | -0.13 | -0.48 | 0.10 | -0.14 | -0.25 | -0.25 |
| pH (KCl)[1] | 10 – 20 | 0.56 | **0.69*** | **0.74*** | 0.48 | **0.81**** | 0.34 | 0.31 | **0.70*** | **0.78*** |

[a] Calculation based on data from Guckland et al. (2009). [b] Calculation based on data from Talkner et al. (2009). [c] Calculation based on data from Meinen et al. (2009a)

After regression analysis with some explanatory variables comprising litter and soil characteristics, PC 1 was significantly and positively associated with the percentages of *Tilia* and *Acer* litter and soil pH values, and significantly negatively associated with the portion of *Fagus* litter and the values of fine-root biomass and fine-root necromass (Table 3.4). For the second axis, the PLFA 18:2ω6,9, 20:4, 20:2 and 20:3 had strong positive loadings on PC 2, and PLFA i17:0 exhibited strong negative loadings on PC 2. The second principal component displayed no significant association with any variable. Some bacterial PLFAs such as a15:0, i16:0, br19:0, a17:0 and the actinomycetes marker 10Me17:0 showed strong positive loadings on PC 4, which was significantly and positively correlated with the percentage of *Fraxinus* litter (data not shown) but explained only 0.08% of the variation in PLFA composition. The biplot of the deeper soil layer of 10-20 cm differed markedly from the upper layer (Fig. 3.2b). There, the PLFA 16:0, i15:0, cy19:0 and i16:0 had strong positive loadings on PC 1, and the PLFA 18:1ω7, i17:0, a17:0, cy17:0, br19:0, 16:1ω5, 16:1ω7, 20:0 had strong negative loadings on PC 1. Similarly to PC 2 in the uppermost soil layer, no significant association with any variable could be found. Along the second axis, the PLFA 18:0, 18:1ω9, 22:0, br17:0 and 18:2ω6,9 had strong positive loadings on PC 2, and PLFA a15:0, i14:0, 10Me17:0, 15:0, 20:2 had strong negative loadings on PC 2. According to regression analyses, PC 2 was significantly and positively associated with fine root biomass and significantly, but negatively associated with the soil pH, C, N and the clay content in the deeper soil layer as well as with the percentage of *Fraxinus* litter and with total leaf litter (Table 3.4).

**Table 3.4:** Linear regressions between the first two principal components (PC1 and PC2) of the microbial diversity in the diversity gradient and site characteristics (*** = p ≤ 0.001; ** = p ≤ 0.01; * = p ≤ 0.05). Significance is marked in bold. A negative *R* indicate a negative relationship between the variables otherwise the relationship is positive.

| | 0 – 5 cm | | | | | | 10 – 20 cm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PC1 | | | PC2 | | | PC1 | | | PC2 | | |
| | *R* | *F* | *p* | *R* | *F* | *p* | *R* | *F* | *p* | *R* | *F* | *p* |
| Total litter (g dw $m^{-2}$) | 16.2 | 1.4 | 0.283 | -8.6 | 0.7 | 0.443 | -23.5 | 2.2 | 0.186 | -50.8 | 7.2 | **0.031*** |
| *Fagus sylvatica* (%) | -75.3 | 21.3 | **0.002**** | -14.6 | 1.2 | 0.310 | 8.2 | 0.6 | 0.456 | 24.5 | 2.3 | 0.176 |
| *Tilia spp.* (%) | 64.7 | 12.9 | **0.009**** | 23.1 | 2.1 | 0.191 | -4.2 | 0.3 | 0.596 | -11.3 | 0.9 | 0.378 |
| *Fraxinus exc.* (%) | 14.1 | 1.2 | 0.319 | -5.4 | 0.4 | 0.546 | -16.1 | 1.3 | 0.285 | -54.8 | 8.5 | **0.023*** |
| *Carpinus betulus* (%) | 20.9 | 1.9 | 0.216 | 30.9 | 3.1 | 0.121 | -2.1 | 0.2 | 0.713 | 0.5 | 0.0 | 0.856 |
| *Acer spp.* (%) | 56.7 | 9.2 | **0.019*** | 4.5 | 0.3 | 0.584 | -13.3 | 1.2 | 0.334 | -20.0 | 1.6 | 0.227 |
| *Quercus spp.* (%) | 32.1 | 3.3 | 0.112 | 1.7 | 0.1 | 0.737 | 27.6 | 2.7 | 0.146 | 0.5 | 0.0 | 0.862 |
| pH (KCl) | 79.3 | 26.8 | **0.001**** | 8.3 | 0.6 | 0.452 | -9.7 | 0.8 | 0.415 | -53.8 | 8.2 | **0.024*** |
| C (%) | 8.5 | 0.7 | 0.447 | -39.3 | 4.5 | 0.071 | -5.7 | 0.4 | 0.537 | -76.8 | 23.2 | **0.002**** |
| N (%) | 28.0 | 2.7 | 0.143 | -20.3 | 1.8 | 0.224 | -15.3 | 1.3 | 0.297 | -75.8 | 22.0 | **0.002**** |
| Clay (%)[a] | 31.2 | 3.2 | 0.118 | -6.6 | 0.5 | 0.506 | -10.3 | 0.8 | 0.400 | -59.1 | 10.1 | **0.015*** |
| P (mg $kg^{-1}$)[b] | 7.9 | 0.6 | 0.463 | -1.0 | 0.1 | 0.801 | -40.3 | 4.7 | 0.066 | -25.7 | 2.4 | 0.163 |
| Fine-root biomass (g $m^{-2}$)[c] | -56.1 | 9.0 | **0.020*** | -1.6 | 0.1 | 0.749 | 3.2 | 0.2 | 0.646 | 49.7 | 6.9 | **0.034*** |
| Fine-root necromass (g $m^{-2}$) [c] | -49.8 | 6.9 | **0.034*** | -3.1 | 0.2 | 0.648 | 0.2 | 0.0 | 0.914 | 34.6 | 3.7 | 0.095 |

[a] data from Guckland et al. (2009).
[b] data from Talkner et al. (2009).
[c] data from Meinen et al. (2009a).

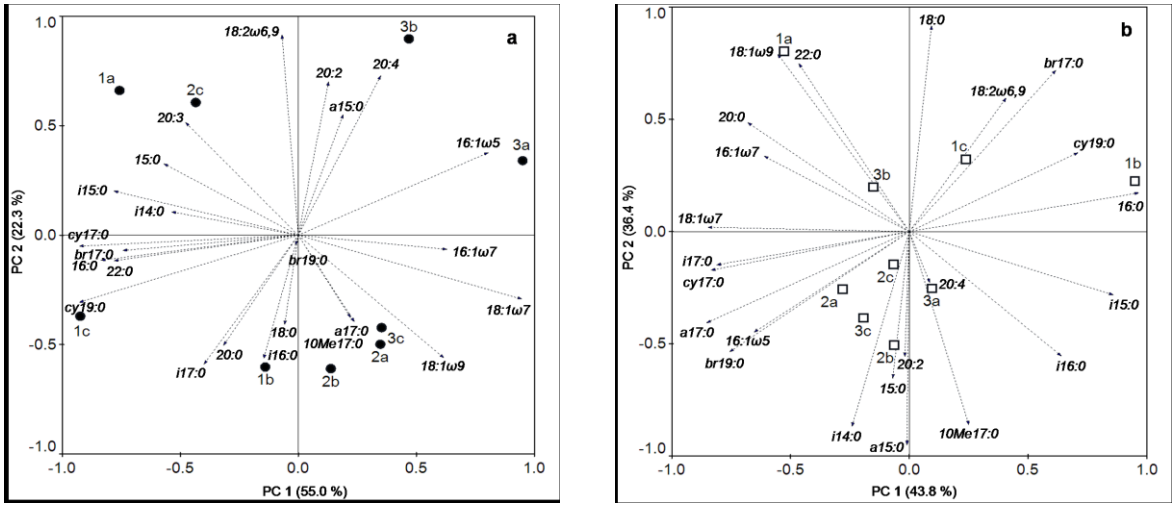


**Fig. 3.2:** Biplots from principal component analyses of the phospholipid fatty acids (PLFA) in soil depths of a) 0-5 cm and b) 10-20 cm showing loadings of individual PLFAs and sample points of the individual plots of all diversity levels along the first two principal components. Symbols ●, □, mark plots of the diversity levels 1, 2 and 3, the ensuing letters represent different replicates.

### 3.3.5 Direct gradient analysis

The first two axes of redundancy analysis (RDA) explained approximately 70% of the variation in both soil layers (Fig. 3.3). The RDA triplots show the PLFA loadings and the first five environmental variables after Monte Carlo permutation test in the form of arrows. In the soil depth 0-5 cm, only soil pH was significant ($F = 5.965$, $p < 0.002$); and in the soil depth of 10-20 cm, only the soil N content ($F = 3.689$, $p < 0.002$). In the deeper soil layers,

the litter variables were excluded from RDA because of their decreasing significance in the results from regression analyses (Table 3.4). PLFA loadings in the same direction of the arrowheads showed a high association of these environmental variables with the PLFA loadings.

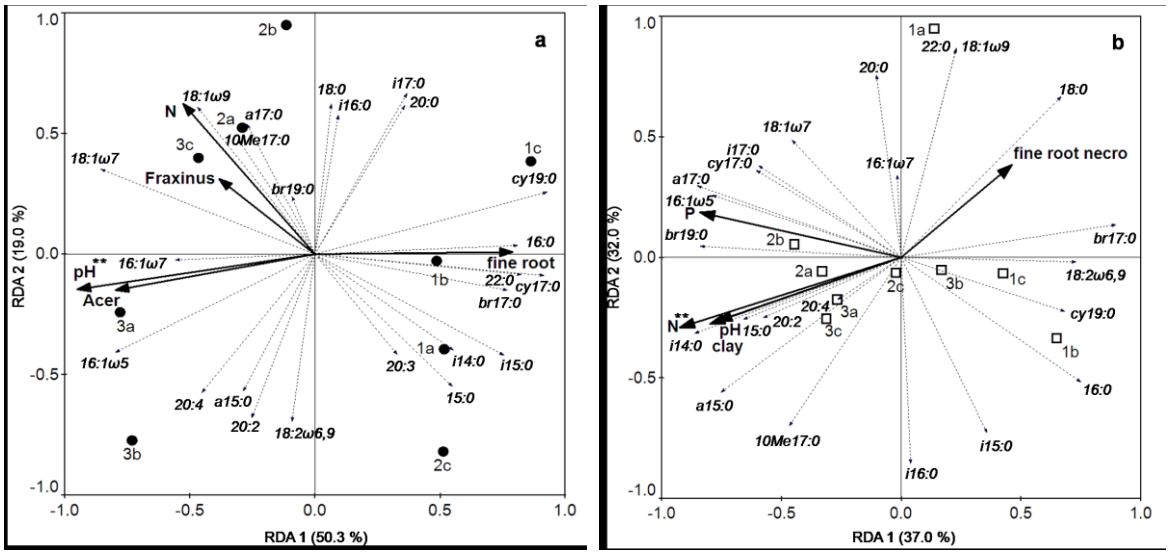


**Fig. 3.3:** Triplots from redundancy analysis (RDA) of the phospholipid fatty acids (PLFA) in soil depths of a) 0-5 cm and b) 10-20 cm showing loadings of single PLFAs, sample points of the individual plots of all diversity levels and the first five environmental variables after forward selection of Monte Carlo permutation test. Significance is marked with asterisks. Symbols ●, □, mark plots of the diversity levels 1, 2 and 3, the ensuing letters represent different replicates. Arrows: Acer, Fraxinus = litter amounts in the year 2005 (%), fine root = fine root biomass (g $m^{-2}$), fine root necro = fine root necromass (g $m^{-2}$), N = soil N content (%), P = soil P content (mg $kg^{-1}$), clay = soil clay content (%), pH = soil pH (KCl).

## 3.4 Discussion

The soil microbial community in the tree diversity gradient was influenced by both differences directly related to tree diversity, like litter composition, and differences only indirectly or not related to tree diversity, like soil pH, nutrient state and clay content.

### 3.4.1 Changes in microbial biomass and composition with increasing tree diversity

Our C/N analyses and topsoil pH measurements agree with findings of Guckland et al. (2009) and Jacob et al. (2009) that different tree litter, mainly the presence of *Fagus*, affected the pH and the nutrient status of the soil surface layers. The leaf litter types provide different initial nutrient concentrations, which influence the soil nutrient status of the topsoil layer. Jacob et al. (2009) found that leaf litter of *Fagus* had lowest, and

*Fraxinus* highest concentrations of N and P, whereas *Tilia*, *Carpinus* and *Acer* litter had intermediate concentrations. These low initial element concentrations of the *Fagus* leaf litter cause the low soil quality and soil pH in the diversity level 1. Bååth and Anderson (2003) found several PLFAs that sensitively react to differences in the soil pH. Our results were in accordance with these results: PLFAs 16:1ω5 and 16:1ω7 increased, and the PLFA cy19:0 decreased, with increasing soil pH. Kaur et al. (2005) described that low pH in soils can lead to an increasing modification of cis monounsaturated fatty acids to cyclo fatty acids, which are more stable but restrict the overall mobility of the microbes. Our results of PCA and RDA confirm a high association of cy19:0 with *Fagus* litter, which is positively correlated with root biomass ($r^2 = 0.78$, $F = 25.28$, $p < 0.01$). The cyclopropyl fatty acids:monoenoic precursors and total saturated:total monounsaturated fatty acids ratio, were higher in the plots of DL 1 than in plots with a higher tree diversity. This suggests that in the *Fagus*-dominated replicates, the microbial communities are more resource limited (Kieft et al., 1997; Bossio et al., 1998) than communities that benefit from a more diverse litter composition in higher diversity levels. Tree diversity can, consequently, indirectly via soil pH and nutrients drive the soil microbial community. However, further investigations are necessary clearly separating soil and biodiversity driven effects as in our experimental design biodiversity was not completely independent from clay content (Guckland et al., 2009).

In the topsoil of diversity level 3 the abundance of AM fungi was highest and PCA and RDA showed a correspondence to both, high soil pH and *Tilia* and *Acer* litter. However, *Tilia* species, which contribute more than 50% to the total canopy cover in some replicates (Jacob et al., 2009), are only little associated with AM fungi. We therefore presume that the combination of high pH values and higher proportions of trees such as *Fraxinus* and *Acer* having AM fungi result in a direct effect of tree species on the microbial community.

### 3.4.2 Changes in microbial composition with soil depth

Different microbial communities in surface and deeper soil layers are well described (Fritze et al., 2000; Blume et al., 2002; Steenwerth et al., 2008). Our sampling scheme offered a deeper insight into the upper soil layers that are still connected to the main rooting depth. Interestingly already a few centimeters below the soil surface, i.e., in a soil depth of 10-20 cm, the composition of the litter-decomposing microbial community has completely changed. At higher diversity levels significant higher proportions of

actinomycetes and other Gram-positive bacteria were found in soil depths below 10 cm. Fierer et al. (2003) stated that resource availability, which is mainly characterized by decreasing C concentration and by a reduction in C quality with increasing soil depth (Richter and Markewitz, 1995), is the principal factor governing the composition of the microbial community across soil profiles. It seems that in deeper soil horizons, only those microbial communities survive that are capable of using the available and - maybe limited - C resources. On the basis of stable C isotope analyses, Kramer and Gleixner (2008) found that Gram-negative bacteria preferably use C freshly derived from plants, and that Gram-positive bacteria are capable of additionally using older SOM-derived carbon. Our results show that in a soil depth of 10-20 cm mainly PLFAs of actinomycetes and other Gram-positive bacteria are negatively associated with soil C and N concentration and clay content. Soil microbes need C and N to cover their energy demand and, in particular, for their growth. How they use the C and N resources mainly depends on the nutrient that is limiting (Bardgett, 2005). In the subsoil the P content also varied the microbial diversity. This is not surprising because soil microbes are closely involved in the cycling of P (Bardgett, 2005). Microbes are capable of immobilizing P and often contain as much as 20-30% of the total organic P pool (Jonasson et al., 1999). We found a high association with soil P contents to the loading of PLFA 16:1ω5, which is characteristic for AM fungi. Mycorrhizal fungi support their host plants by the uptake of soil P via accessing P sources in deeper soil horizons with their mycelium (Bardgett, 2005). There are also results on enhanced decomposition of, and increased N capture from, complex organic material in the soil by AM fungi (Hodge et al., 2001). A comparative study shows that ectomycorrhizal (ECM) trees have greater rhizosphere effects on microbial activity and nutrient availability than arbuscular mycorrhizal (AM) trees (Phillips and Fahey, 2006). Our study also comprises ECM and AM tree species, but we could not distinct the two mycorrhizal types using PLFA patterns. The data obtained from a soil depth of 10-20 cm show a strong relationship between C, N, P and clay content. Possibly, this is due to a formation of stable organo-mineral complexes brought about by excretion of burrowing animals such as earthworms (Bardgett, 2005). Our results suggest that nutrient availability, which is also driven by the soil texture, has the greatest effect on microbial abundances and composition in the subsoil.

### 3.5 Conclusions

In conclusion, we found only few direct interactions between the microbial and plant communities like the presence of AM fungi. Most interactions were driven by abiotic factors such as the pH near the soil surface and the soil nutrient status of deeper soil layers. However, these abiotic factors were closely related to species-specific plant features. The pH of the topsoil is influenced by characteristics of the leaf litter, and the soil nutrient status, by interactions of the trees with their mycorrhizal fungi. Consequently, we conclude that the link between microbial diversity and the aboveground tree diversity is driven by indirect interactions with specific plant traits rather than by the tree species diversity itself.

# Chapter 4

## Seasonal differences in tree species' influence on soil microbial communities

Chapter source:
Thoms, C., Gleixner, G. (in review). Seasonal differences in tree species' influence on soil microbial communities. *Soil Biology & Biochemistry*.

**Abstract**

The linkage between tree diversity and the soil food web in temperate deciduous forest ecosystems remains uncertain. Using microbial phospholipid fatty acids (PLFAs), we analyzed the effect of tree species composition on microbial communities from topsoil collected in Hainich National Park, Germany. Previous results had shown minimal direct effects of tree species on the microbial community in autumn, most likely due to low plant activity and high nutrient and energy input from litterfall. However, microbial composition was affected indirectly through an influence of tree species on soil pH. In this study, we analyzed PLFA profiles in early summer and compared them with the results from autumn sampling. We hypothesized that plant-based traits would have stronger direct effects on the abundance and structure of the microbial community during the photosynthetically active period. The results showed that the soil microbial community differed more markedly between the tree diversity levels in early summer than in autumn. The acidifying character of the decaying beech litter strongly influenced the soil pH values and structured the soil microbial community indirectly in early summer as it had in autumn. However, the measured differences in the microbial composition in early summer could be attributed primarily to litter quality. This direct influence of plant traits appeared to be eclipsed in autumn because of the high nutrient supply from fresh litter input. Following litter decomposition in the topsoil, however, litter-based plant traits emerged as a factor structuring the soil microbial community in early summer. Our results suggest that the PLFAs i14:0 and i15:0, indicative of Gram-positive bacteria, are strongly involved in

decomposition processes and may be promoted by readily available nutrients. Furthermore, our results indicate that a dense root network in association with arbuscular mycorrhizal fungi strongly supported microbial growth in the more diverse forest stands. High proportions of arbuscular mycorrhizal fungi (PLFA 16:1ω5), root-associated microorganisms (PLFAs 16:1ω9, 16:1ω7, 17:1ω8 and 18:1ω7) and bacterial grazers (PLFA 20:5) characterized the microbial community in early summer on these study plots. We conclude that microbial communities are strongly influenced by abiotic controls. However, seasonal differences in litter decomposition rates and root activity should be considered in the analysis of the effects of tree diversity or species on soil microbial communities.

**Keywords:** seasons, tree diversity, site conditions, soil microorganisms, beech, *Fagus sylvatica*, forest, phospholipid fatty acids (PLFA), decomposition

## 4.1 Introduction

Soil microorganisms are important drivers of soil processes, and they play a key role in the decomposition of recent plant material (Bardgett, 2005). Because they rely on organic carbon (C) for growth, they are affected by any change in C input or C loss in soils (Zak et al., 2003). In forest ecosystems, C input is derived primarily from the decomposition of organic matter, such as leaf and root litter, plant exudates, woody plant debris and animal remains. The contribution of these components varies substantially depending on the tree species present (Gleixner et al. 2005), and root exudates, in particular, vary in quality and quantity among tree species (Grayston et al., 1997; Calvaruso et al., 2011). Additionally, litter quality measures, such as the amount of nutrients and tissue structure, as well as the relative proportions of C and N compounds of different decomposabililty, such as protein and lignin, vary between broadleaf tree species (Jacob et al., 2009; Gessner et al., 2010).
The total amount of organic matter input is highest in the topsoil. Consequently, this is the soil stratum in which the largest observed effects of tree species on soil chemical properties are found (Augusto et al., 2003; Hagen-Thorn et al., 2004; Thoms et al., 2010). In deciduous forests, the soils under *Fagus sylvatica* show lower soil pH values, lower base saturation and higher C/N ratios compared to forest soils under mixed deciduous forests consisting of *Fagus*, *Fraxinus*, *Tilia* and other deciduous tree species (Guckland et

al., 2009; Langenbruch et al., 2012). Additionally, both the quantity and quality of litter affect both the predators and the decomposer macrofauna (Weland, 2009). Studies that have investigated the relationships between plant species diversity and soil microbial communities using PLFA profiles in grasslands (Habekost et al, 2008; Breulmann et al., 2012) and in forests (Hackl et al., 2005; Merilä et al., 2010; Brockett et al., 2012; Wu et al., 2012; Myers et al., 2001) have underlined the importance of the quantity and quality of organic resources as well as abiotic factors such as soil pH, soil texture and soil moisture for the soil microbial community.

The life cycle of microbes in temperate broadleaf forests in Central Europe is strongly affected by the seasons through changes in biotic and abiotic factors. In early March, the vegetation starts to produce shoots, roots and leaves from C reserves, followed by a photosynthetically active period beginning typically in April to May. The growth period ends with litterfall in autumn, representing an excellent food input for the decomposer community in the topsoil. During winter, deciduous trees are generally inactive, and decomposition processes are also slow because of the decelerating effect of low temperatures on soil microbial metabolism. Soil temperatures increase in spring, and soil microbes accelerate their activity in conjunction with the beginning of the vegetation period (Kauri, 1982). In summer, the loss of precipitation can produce extremely dry soil conditions that again inhibit soil microbial activity (Moore-Kucera and Dick, 2008). Typically, studies in mature beech forests in Europe show two phases of increased microbial growth over the seasons with maxima in spring and autumn (Kauri, 1982; Kaiser et al., 2010). Unfortunately, studies considering the impact of tree species on soil microorganisms through seasonal cycles are rare (Collignon et al., 2011; Brant et al., 2006). For this reason, we have limited insight into seasonal effects on the influence of tree species on soil microorganisms. We expect differences to emerge during the active vegetation period, when trees might influence soil microorganisms more directly through root exudates and litter decomposition. As a function of litter quality, differences during the first year of decomposition can strongly alter the amounts of nutrients that are released into the soil and that can then be absorbed by microorganisms (Prescott et al., 2000). Meinen et al. (2009a) also reported different seasonal patterns in fine-root necromass in deciduous forest stands differing in tree species composition, with the highest fine root-necromass found in June and the lowest in January. Strong seasonality in the abundance of earthworm populations in mixed forest sites has also been demonstrated and has been

attributed to differences in litter decomposition and fluctuations in the soil carbon pool over the year (Cesarz et al., 2007).

In a previous study in Hainich National Park in Central Germany, we found few direct effects of tree features on the soil microbial community after extraction of PLFAs from soil samples following litterfall in autumn (Thoms et al., 2010). The group of arbuscular mycorrhizal fungi (AM fungi) was found in significantly higher concentrations on the plots with the highest tree species diversity. These plots were characterized by higher abundances of maple and ash, whose roots form symbioses with AM fungi. In the present study, we analyzed PLFA profiles from the same plots approximately 7 months after litterfall in early summer and compared all individual PLFAs separately with the autumn PLFA profiles. We hypothesize that a) differences in the microbial biomass and community structure between different tree diversity levels in early summer are greater than those in autumn and that b) direct plant-based traits have a stronger impact on the soil microbial community in early summer than in autumn.

## 4.2 Material and methods

### 4.2.1 Study site

The study was conducted in Hainich National Park, a deciduous forest in Thuringia, Central Germany. The unique land use history of Hainich National Park has created a species-rich temperate broadleaf forest that offers the opportunity to study the role of forest diversity in a variety of forest use types in species-poor to species-rich forest patches (Leuschner et al., 2009). In 2005, 12 study plots (50 m x 50 m each) representing three tree diversity levels (DL 1-3) with four replicates per level were established. A maximum distance of 5 km between the plots ensured that they were comparable in terms of homogenous climate and soil conditions (Guckland et al. 2009). Four pure beech forest (DL 1), four beech-ash-linden forest (DL 2) and four beech-ash-linden-hornbeam-maple forest (DL 3) plots form the diversity gradient (a map is shown in Guckland et al., 2009), comprising primarily the tree species *Fagus sylvatica L.*, *Fraxinus excelsior L.*, *Tilia* species (*T. cordata Mill.*, *T. platyphyllos Scop.*), *Carpinus betulus L.* and *Acer* species (*A. pseudoplatanus L.*, *A. platanoides L.*, *A. campestre L.*). All plots approached natural conditions without distinct anthropogenic impact (e.g., silvicultural management) over the

past several decades. The principal plot characteristics, including stem densities, litter amounts, litter nutrients, macrofauna and soil properties, are summarized in Table A1 (Appendix).

### 4.2.2 Soil sampling

On May 31 and June 1, 2006, soil samples were collected during a rainy period from all 12 plots using three randomly chosen transects of 30 m × 1 m within the plots. We took 12 samples per plot from soil depths of 0 - 5 cm of the mineral horizon (Ah horizon) with a split tube (Eijkelkamp, Giesbeek, Netherlands) 5.3 cm in diameter according to the sampling procedure previously used in November 2005 (Thoms et al., 2010). The samples were collected randomly at a minimum distance of 4 m and were mixed to obtain one composite sample per plot. After transport to the laboratory, all samples were immediately sieved < 2 mm to remove visible stones, animals, roots and plant material prior to lipid extraction. The water content was quantified using a subsample dried to 105 °C. Soil pH was measured in 1 M KCl solution. For C and N analyses, another subsample was air dried, ball milled and analyzed in an automated element analyzer (Elementaranalysator varioMAX, Elementar Analysensysteme GmbH, Hanau, Germany).

### 4.2.3 PLFA analysis

A total of 100 g of fresh soil was extracted according to the method of Bligh and Dyer (1959) and Zelles and Bai (1993). Soil lipids were extracted using a mixture of chloroform, methanol and phosphate buffer (1:2:0.8 v/v/v). Phospholipids were isolated on silica columns and hydrolyzed and methylated using a methanolic KOH solution. Fatty acid methyl esters (FAME) were separated into saturated, polyunsaturated and monounsaturated fatty acids using aminopropyl-modified and silver-impregnated SPE columns. The samples were quantified with a GC-AED System (GC: HP 6890 Series, AED: G 2350 A, Agilent Technologies, Wilmington, USA) using a BPX 70 column (50 m × 0.32 mm I.D., 0.25 μm film thickness) in the split mode (10:1). Helium was used as a carrier gas at a flow rate of 1.3 ml $min^{-1}$. The temperature program started at 100 °C (for 1 min). Thereafter, the temperature was raised to 135 °C at a rate of 4 °C $min^{-1}$, then to 230 °C at 2 °C $min^{-1}$. The final temperature of 260 °C was reached after further raising the temperature at 30 °C $min^{-1}$ and was kept constant for 1 min. PLFAs were identified based on a comparison with a standard mixture (Supelco) of saturated fatty acids and unsaturated

fatty acids and based on the mass spectral data in Gattinger et al. (2003). All saturated straight-chain fatty acids (n14:0 – n24:0) were used as a universal marker for microorganisms because they are very widespread. PLFAs i14:0, i15:0, a15:0, i16:0, 11Me17:0, i17:0, a17:0, br18:0 and br19:0 are derived primarily from Gram-positive bacteria and PLFAs cy17:0, cy19:0, 16:1ω9, 16:1ω7, 17:1ω8, 17:1ω7, 17:1ω5 and 18:1ω7 from Gram-negative bacteria (O'Leary and Wilkinson, 1988; Wilkinson, 1988), whereas fatty acids with a methyl group at position 10 are found in many species of the class Actinobacteria (Lechevalier, 1977; Kroppenstedt, 1985). The fatty acids 16:2, 18:3, 20:2, 20:3 and 20:4 were used to indicate eukaryotic organisms (Shaw, 1966; White et al., 1996). The fatty acids 18:2ω6,9 and 18:1ω9 were used as indicators for fungal biomass (Frostegård and Bååth, 1996; Tornberg et al., 2003), and the fatty acid 16:1ω5 was used as an indicator for arbuscular mycorrhizal fungi (Nordby et al., 1981; Olsson, 1999).

### 4.2.4 Statistical analysis

Statistical analyses were performed with R (version 2.13.0, R Development Core Team, 2011). To test for pairwise differences among the three diversity levels, we performed an analysis of variance (ANOVA) followed by a Tukey-Kramer HSD test for all pairwise comparisons of the means ($p < 0.05$ in all cases). The PLFA profiles were analyzed using CANOCO software (version 4.5, Microcomputer Power, Inc., Ithaca, NY). The results of the amounts of individual PLFAs were subjected to a principal component analysis (PCA) and a redundancy analysis (RDA) based on a covariance matrix. Both the absolute and relative abundances of the fatty acids were centered. For statistical analyses, sample DL 3a was weighted with a factor of 0.8 to overcome an analytical shortcoming. In this sample, all values for saturated PLFA were lost in the analyses and were calculated as the mean values from related plots (DL 3b-d). The PCA was used as an indirect gradient analysis to extract the major patterns from the variation in the PLFA data and to interpret their relevance in terms of the available environmental data in regression analyses. In the RDA used as a direct gradient analysis, the ordination axes represented aggregates of the environmental data that best explained the PLFA data. All environmental data were tested for the significance of their contribution ($p < 0.05$) to the explanation of the variation in the PLFA data using a Monte Carlo permutation test with 999 permutations.

## 4.3 Results

### 4.3.1 Microbial biomass

#### 4.3.1.1 Effects of tree species composition and season

We identified more individual PLFAs in early summer than in autumn (Fig. 4.1). The PLFA biomass, based on the absolute abundance of microbial phospholipid fatty acids, was up to sevenfold higher in DL 3 compared with the other diversity levels in early summer (Fig. 4.1b). This distribution was significant ($p < 0.05$) for most of the PLFAs. The strong difference in PLFA biomass between DL 3 and the other plots was maintained for all replicates of the diversity levels in early summer (Table A1).

The seasonal differences in PLFA biomass for individual PLFA for the studied diversity levels were only slight (data not shown). Significantly higher PLFA biomass in autumn was only found for PLFA i14:0 in DL 1 and PLFA n18:0 in DL 2, and significantly higher PLFA biomass in spring was only found for PLFA 20:2 in DL 3.

#### 4.3.1.2 Effects of tree and site characteristics

The results of a linear regression analysis for both seasons between the first principal component (PC 1) after the PCA and the different environmental variables considered are shown in Table 1 (see also section 4.3.2.3). Of the other principal components, only PC 2 of the relative PLFA abundance [mol%] in early summer was significantly correlated with the variable "Sd-*Fagus*" ($R^2 = 0.53$, $F = 10.1$, $p < 0.011$). All other variables were uncorrelated. PC 1 of the absolute PLFA abundance [nmol $g^{-1}$] explained approximately 70% of the data variance in autumn. After the regression analysis, PC 1 was weakly correlated with several tree parameters of *Tilia* (litter, stem density, fine-root necromass) and more strongly correlated with the fine-root necromass of *Acer* and *Carpinus*. In contrast, PC 1 of the absolute PLFA abundance [nmol $g^{-1}$] in early summer explained most of the data variability and was strongly correlated with litter- and nutrient-related variables (Tab. 4.1).

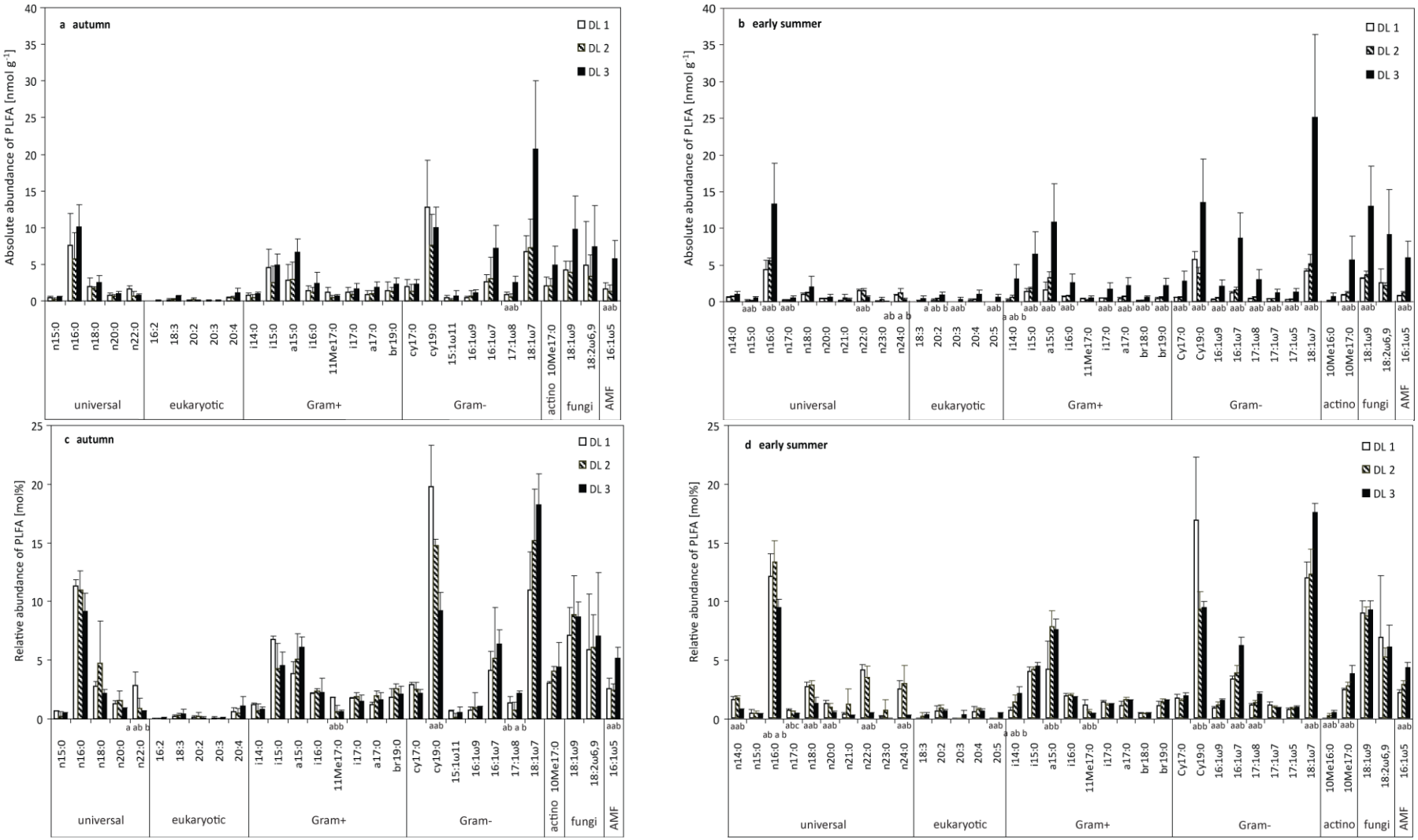


**Fig. 4.1:** Absolute abundance of PLFAs in a) autumn and b) early summer and relative abundance of PLFAs in c) autumn and d) early summer. Different letters indicate significant differences between the diversity levels (DLs) ($p < 0.05$)

**Table 4.1:** Linear regressions between the first principal component (PC 1) by principal component analysis of the absolute and relative abundances of PLFA in both seasons and different plant traits and site characteristics. Stars indicate a significant influence of the environmental variable on the data variation represented by PC 1 (*** = $p \leq$ 0.001; ** = $p \leq$ 0. 001; * = $p \leq$ 0.05). Data in bold mark for every PC 1 the five environmental variables with the highest *F*-value. A minus in brackets show a negative relationship between the variables otherwise the relationship is positive.

| | autumn [nmol $g^{-1}$] PC 1 (71.6 %) | | | early summer [nmol $g^{-1}$] PC 1 (96.7 %) | | | autumn [mol%] PC 1 (54.4 %) | | | early summer [mol%] PC 1 (49.7 %) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | *F* | *p* | $R^2$ | *F* | *p* | $R^2$ | *F* | *p* | $R^2$ | *F* | *p* |
| Sd-*Fagus*[1] | (-) 0.04 | 4.4 | 0.073 | (-) 0.22 | 2.5 | 0.148 | **0.85** | **40.1** | **0.000*****| 0.18 | 2.0 | 0.190 |
| Sd-*Fraxinus*[1] | 0.54 | 0.3 | 0.587 | 0.04 | 0.4 | 0.553 | (-) 0.18 | 1.5 | 0.260 | (-) 0.18 | 1.9 | 0.199 |
| Sd-*Tilia*[1] | **0.51** | **8.4** | **0.023*** | 0.36 | 5.0 | 0.053 | (-) 0.69 | 15.9 | 0.005** | (-) 0.73 | 23.8 | 0.001*** |
| Sd-*Acer*[1] | 0.43 | 7.4 | 0.030* | 0.27 | 3.4 | 0.100 | **(-) 0.80** | **28.4** | **0.001***** | (-) 0.73 | 24.1 | 0.001*** |
| Sd-*Carpinus*[1] | 0.00 | 5.2 | 0.057 | 0.51 | 9.4 | 0.013* | (-) 0.48 | 6.5 | 0.038* | (-) 0.53 | 10.4 | 0.011* |
| Litter-Total[2] | 0.40 | 0.0 | 0.894 | 0.35 | 4.9 | 0.054 | (-) 0.17 | 1.5 | 0.265 | (-) 0.39 | 5.7 | 0.041* |
| Litter-Total „h. q.“[2] | 0.48 | 4.7 | 0.066 | **0.64** | **15.7** | **0.003**** | (-) 0.68 | 14.9 | 0.006** | **(-) 0.80** | **35.4** | **0.000***** |
| Litter-*Fagus*[2] | (-) 0.08 | 6.5 | 0.038* | (-) 0.32 | 4.2 | 0.072 | **0.75** | **21.4** | **0.002**** | 0.76 | 29.3 | 0.000*** |
| Litter-*Fraxinus*[2] | 0.57 | 0.6 | 0.460 | 0.40 | 6.1 | 0.036* | (-) 0.15 | 1.2 | 0.306 | (-) 0.36 | 5.1 | 0.051 |
| Litter-*Tilia*[2] | **0.27** | **9.5** | **0.018*** | 0.13 | 1.4 | 0.273 | (-) 0.63 | 12.2 | 0.010* | (-) 0.55 | 11.0 | 0.009** |
| Litter-*Acer*[2] | 0.07 | 2.7 | 0.147 | 0.28 | 3.5 | 0.093 | (-) 0.57 | 9.4 | 0.018* | (-) 0.61 | 14.1 | 0.005** |
| Litter-*Carpinus*[2] | 0.01 | 0.5 | 0.507 | 0.30 | 3.9 | 0.080 | (-) 0.21 | 1.9 | 0.209 | (-) 0.33 | 4.5 | 0.063 |
| Litter C/N [3] | (-) 0.39 | 4.4 | 0.074 | (-) 0.38 | 5.6 | 0.042* | 0.65 | 13.0 | 0.009** | **0.80** | **35.8** | **0.000***** |
| Litter-C[3] | 0.03 | 0.2 | 0.667 | 0.35 | 4.8 | 0.055 | (-) 0.01 | 0.1 | 0.778 | (-) 0.22 | 2.5 | 0.151 |
| Litter-N[3] | 0.08 | 0.7 | 0.432 | 0.54 | 10.5 | 0.010* | (-) 0.30 | 3.0 | 0.126 | (-) 0.47 | 7.8 | 0.021* |
| Litter-K[3] | 0.17 | 0.6 | 0.459 | 0.51 | 9.3 | 0.014* | (-) 0.29 | 2.9 | 0.134 | (-) 0.45 | 7.3 | 0.024* |
| Litter-P[3] | 0.14 | 1.5 | 0.263 | **0.60** | **13.7** | **0.005**** | (-) 0.42 | 5.0 | 0.061 | (-) 0.56 | 11.3 | 0.008** |
| Litter-Ca[3] | 0.23 | 1.1 | 0.323 | 0.55 | 11.0 | 0.009** | (-) 0.39 | 4.4 | 0.073 | (-) 0.52 | 9.7 | 0.012* |
| Litter-Mg[3] | 0.10 | 2.1 | 0.189 | **0.63** | **15.6** | **0.003**** | (-) 0.53 | 8.0 | 0.025* | (-) 0.64 | 15.8 | 0.003** |
| Litter remains[4] | n.c. | n.c. | n.c. | 0.39 | 5.8 | 0.039* | n.c. | n.c. | n.c. | **0.82** | **40.0** | **0.000***** |
| Litter remains „h. q.“[4] | n.c. | n.c. | n.c. | 0.29 | 3.7 | 0.085 | n.c. | n.c. | n.c. | (-) 0.70 | 20.5 | 0.001*** |
| *Lumbricidae*[5] | 0.15 | 0.8 | 0.405 | 0.18 | 1.4 | 0.289 | (-) 0.45 | 5.8 | 0.047* | (-) 0.65 | 11.4 | 0.015* |
| *Isopoda*[5] | 0.02 | 1.2 | 0.306 | 0.18 | 1.4 | 0.277 | (-) 0.66 | 13.6 | 0.008** | (-) 0.44 | 4.7 | 0.074 |
| Clay (%)[6] | 0.40 | 0.1 | 0.733 | 0.41 | 6.2 | 0.034* | (-) 0.32 | 3.3 | 0.110 | (-) 0.49 | 8.5 | 0.017* |
| pH (KCl) | 0.03 | 4.6 | 0.069 | 0.41 | 6.2 | 0.035* | (-) **0.80** | **27.4** | **0.001***** | **(-) 0.84** | **46.0** | **0.000***** |
| Soil-C/N | (-) 0.11 | 0.8 | 0.392 | (-) 0.21 | 2.4 | 0.157 | 0.37 | 4.1 | 0.083 | 0.58 | 12.6 | 0.006** |
| Soil stock-C[7] | (-) 0.06 | 0.2 | 0.668 | 0.48 | 8.3 | 0.018* | (-) 0.02 | 0.2 | 0.700 | (-) 0.08 | 0.8 | 0.400 |
| Soil stock-N[7] | 0.05 | 0.5 | 0.524 | 0.49 | 8.7 | 0.017* | (-) 0.33 | 3.4 | 0.106 | (-) 0.45 | 7.4 | 0.024* |
| Soil stock-Ca[7] | 0.33 | 0.4 | 0.556 | **0.71** | **21.7** | **0.001***** | (-) 0.28 | 2.8 | 0.141 | (-) 0.46 | 7.7 | 0.022* |
| Soil stock-Mg[7] | 0.40 | 3.4 | 0.108 | **0.68** | **18.8** | **0.002**** | (-) 0.68 | 14.6 | 0.007** | (-) 0.71 | 22.2 | 0.001*** |
| FRB-*Fagus*[8] | (-) 0.07 | 4.6 | 0.069 | (-) 0.33 | 4.3 | 0.067 | 0.52 | 7.5 | 0.029* | 0.67 | 17.9 | 0.002** |
| FRB-*Fraxinus*[8] | 0.47 | 0.5 | 0.495 | 0.04 | 0.4 | 0.554 | (-) 0.08 | 0.6 | 0.453 | (-) 0.17 | 1.9 | 0.206 |
| FRB-*Tilia*[8] | 0.14 | 6.3 | 0.040* | 0.15 | 1.6 | 0.236 | (-) 0.67 | 14.1 | 0.007** | (-) 0.60 | 13.7 | 0.005** |
| FRB-*Acer*[8] | 0.49 | 1.2 | 0.314 | 0.37 | 5.2 | 0.048* | (-) 0.58 | 9.6 | 0.017* | (-) 0.57 | 12.1 | 0.007** |
| FRB-*Carpinus*[8] | 0.24 | 6.7 | 0.036* | 0.09 | 0.9 | 0.379 | (-) 0.44 | 5.6 | 0.050* | (-) 0.34 | 4.6 | 0.061 |
| Necro-*Fagus*[8] | (-) 0.06 | 2.3 | 0.176 | (-) 0.27 | 3.4 | 0.100 | **0.75** | **20.5** | **0.003**** | **0.83** | **42.6** | **0.000***** |
| Necro-*Fraxinus*[8] | 0.54 | 0.5 | 0.514 | 0.00 | 0.0 | 0.861 | (-) 0.01 | 0.1 | 0.754 | (-) 0.03 | 0.3 | 0.591 |
| Necro-*Tilia*[8] | **0.64** | **8.2** | **0.024*** | 0.00 | 0.0 | 0.986 | (-) 0.39 | 4.4 | 0.073 | (-) 0.18 | 2.0 | 0.194 |
| Necro-*Acer*[8] | **0.64** | **12.3** | **0.010*** | 0.00 | 0.0 | 0.955 | (-) 0.45 | 5.8 | 0.047* | (-) 0.19 | 2.1 | 0.181 |
| Necro-*Carpinus*[8] | **0.00** | **12.3** | **0.010*** | 0.00 | 0.0 | 0.878 | (-) 0.38 | 4.3 | 0.076 | (-) 0.19 | 2.1 | 0.181 |

n.c. = not calculated, because no explanation

[1] Stem density in n $ha^{-1}$ of the total stand, calculation based on data from Jacob et al. (2010a).

[2] Litter amounts in g DM $m^{-2}$ of the year 2005, litter total „high quality“ are litter total without *Fagus* litter, calculation based on data from Jacob et al. (2010a).

[3] Initial nutrient concentrations in mg $g^{-1}$ DM of the litter refered to the specific litter composition on the plots in autumn 2005, calculation based on data from Jacob et al. (2009).

[4] Litter remains in June 2006 after 7 months of decomposition of the fallen litter in 2005, litter remains „high quality“ are litter remains without *Fagus* litter, calculation based on data from Jacob et al. (2010a,b)

[5] Decomposer fauna abundances (individuals $m^{-2}$) from November 05 for autumn, and from May 05 for early summer, calculation based on data from Weland (2009).

[6] Calculation based on data from Guckland et al. (2009).

[7] Stock concentrations of the soil in kg $ha^{-1}$, calculation based on data from Guckland et al. (2009).

[8] Fine root biomass and necro fine root biomass in g $m^{-2}$, calculation based on data from Meinen et al. (2009a).

### 4.3.2 Microbial Composition

#### 4.3.2.1 Effects of tree species composition and season

The microbial composition, based on the relative abundance of microbial phospholipid fatty acids [mol%], differed more markedly between the tree diversity levels in early summer than in autumn (Fig. 4.1c, 4.1d). PLFAs indicative of Actinobacteria, AM fungi, protozoa (PLFA 20:5) and Gram-negative bacteria (PLFAs 16:1ω9, 16:1ω7, 17:1ω8, 18:1ω7) showed higher proportions in DL 3 than in DL 1 and 2 in early summer (Fig. 4.1d). Significantly higher proportions of PLFAs n22:0, 11Me17:0 and cy19:0 were found in DL 1 in early summer, as also found for the autumn sampling.

The seasonal comparison of individual PLFAs in terms of the diversity levels considered revealed a major change in the composition of the soil microbial communities. It can be deduced from significant differences in the microbial compositions between both seasons (Table 4.2) that the beech-dominated plots (DL 1 and DL 2) were characterized by lower proportions of bacteria (Gram-positive, Actinobacteria, Cyclo) in early summer. In contrast, only a few single PLFAs (20:2, 16:1ω9, i14:0) were more abundant on the DL 3 plots in early summer.

**Table 4.2:** Comparison of the microbial composition between the two seasons for each diversity levels (DL) (means ± 1 SE three plots per DL). Stars show significant differences ($p < 0.05$) between autumn and early summer (*** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$) and are in the column with the seasonal higher values.

| Individual PLFA [mol%] | Microbial group | DL 1 | | DL 2 | | DL 3 | |
|---|---|---|---|---|---|---|---|
| | | autumn | early summer | autumn | early summer | autumn | early summer |
| n15:0 | universal | 0.7 ± 0.0 | 0.4 ± 0.3 | 0.2 ± 0.4 | 0.4 ± 0.2 | 0.5 ± 0.1* | 0.3 ± 0.0 |
| n16:0 | universal | 11.3 ± 0.5 | 12.1 ± 1.9 | 11.0 ± 1.6 | 13.3 ± 1.8 | 9.2 ± 1.5 | 9.4 ± 0.7 |
| n18:0 | universal | 2.8 ± 0.4 | 2.7 ± 0.4 | 4.8 ± 3.6 | 2.8 ± 0.4 | 2.2 ± 0.4* | 1.2 ± 0.5 |
| n20:0 | universal | 1.3 ± 0.3 | 1.2 ± 0.3 | 1.5 ± 0.8 | 0.9 ± 0.3 | 0.9 ± 0.1** | 0.4 ± 0.1 |
| n22:0 | universal | 2.8 ± 1.2 | 4.1 ± 0.5 | 0.9 ± 0.9 | 3.4 ± 1.0* | 0.7 ± 0.0*** | 0.4 ± 0.0 |
| 18:3 | eukaryotic | 0.2 ± 0.1* | 0.0 ± 0.0 | 0.3 ± 0.2 | 0.1 ± 0.3 | 0.4 ± 0.4 | 0.3 ± 0.2 |
| 20:2 | eukaryotic | 0.1 ± 0.1 | 0.6 ± 0.5 | 0.2 ± 0.3 | 0.9 ± 0.2* | 0.1 ± 0.1 | 0.6 ± 0.1*** |
| 20:3 | eukaryotic | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.1 | 0.3 ± 0.3 |
| 20:4 | eukaryotic | 0.6 ± 0.3 | 0.5 ± 0.5 | 0.5 ± 0.4 | 0.7 ± 0.2 | 1.1 ± 0.9 | 0.6 ± 0.2 |
| i14:0 | Gram+ | 1.2 ± 0.1* | 0.6 ± 0.3 | 0.7 ± 0.6 | 1.4 ± 0.6 | 0.8 ± 0.2 | 2.1 ± 0.6* |
| i15:0 | Gram+ | 6.8 ± 0.2*** | 4.0 ± 0.4 | 4.2 ± 2.2 | 4.2 ± 0.2 | 4.6 ± 1.1 | 4.4 ± 0.3 |
| a15:0 | Gram+ | 3.9 ± 1.0 | 4.2 ± 2.4 | 5.1 ± 2.1 | 7.8 ± 1.4 | 6.1 ± 0.9 | 7.5 ± 0.9 |
| i16:0 | Gram+ | 2.1 ± 0.1 | 1.9 ± 0.2 | 2.4 ± 0.2* | 1.9 ± 0.1 | 2.2 ± 1.3 | 1.7 ± 0.1 |
| 11Me17:0 | Gram+ | 1.8 ± 0.0 | 1.1 ± 0.4 | 0.6 ± 0.5 | 0.5 ± 0.2 | 0.6 ± 0.1* | 0.4 ± 0.1 |
| i17:0 | Gram+ | 1.7 ± 0.1* | 1.3 ± 0.2 | 1.9 ± 0.4* | 1.1 ± 0.1 | 1.5 ± 0.5 | 1.2 ± 0.1 |
| a17:0 | Gram+ | 1.2 ± 0.2 | 1.0 ± 0.4 | 2.0 ± 0.4 | 1.5 ± 0.2 | 1.7 ± 0.6 | 1.5 ± 0.1 |
| br19:0 | Gram+ | 1.8 ± 0.7 | 1.0 ± 0.4 | 2.6 ± 0.4* | 1.4 ± 0.2 | 2.1 ± 0.6 | 1.5 ± 0.0 |
| cy17:0 | Gram- | 2.9 ± 0.2** | 1.7 ± 0.3 | 2.5 ± 0.6* | 1.5 ± 0.1 | 2.1 ± 0.4 | 1.9 ± 0.2 |
| cy19:0 | Gram- | 19.8 ± 3.5 | 16.8 ± 5.4 | 14.8 ± 0.5** | 9.2 ± 1.5 | 9.2 ± 1.6 | 9.4 ± 0.5 |
| 16:1ω9 | Gram- | 0.7 ± 0.2 | 0.8 ± 0.1 | 1.0 ± 1.2 | 1.1 ± 0.2 | 1.0 ± 0.1 | 1.5 ± 0.1*** |
| 16:1ω7 | Gram- | 4.1 ± 1.6 | 3.3 ± 0.3 | 5.1 ± 4.4 | 3.8 ± 0.6 | 6.4 ± 1.2 | 6.2 ± 0.7 |
| 17:1ω8 | Gram- | 1.4 ± 0.6 | 1.1 ± 0.1 | 0.8 ± 0.7 | 1.3 ± 0.2 | 2.2 ± 0.2 | 2.0 ± 0.2 |
| 18:1ω7 | Gram- | 11.0 ± 3.3 | 11.9 ± 1.4 | 15.2 ± 4.4 | 12.2 ± 2.1 | 18.3 ± 2.6 | 17.5 ± 0.8 |
| 10Me17:0 | actino | 3.0 ± 0.2** | 2.4 ± 0.1 | 4.1 ± 0.4** | 2.7 ± 0.3 | 4.4 ± 2.1 | 3.8 ± 0.7 |
| 18:1ω9 | fungi | 7.1 ± 2.3 | 9.0 ± 1.0 | 8.9 ± 3.4 | 8.7 ± 0.8 | 8.6 ± 1.3 | 9.2 ± 0.8 |
| 18:2ω6,9 | fungi | 5.9 ± 4.8 | 6.9 ± 5.2 | 6.1 ± 2.8 | 5.2 ± 0.8 | 7.1 ± 5.4 | 6.0 ± 1.9 |
| 16:1ω5 | AMF | 2.6 ± 0.9 | 2.1 ± 0.2 | 2.5 ± 0.5 | 2.9 ± 0.3 | 5.2 ± 1.0 | 4.3 ± 0.5 |

### 4.3.2.2 PLFAs related to tree diversity

We used biplots following the PCA to illustrate the differences between autumn and early summer for selected PLFA loadings along the tree diversity gradient (Fig. 4.2). Only study plots that were sampled on both sampling dates could be used in the analysis. The plots of the diversity levels were distinctly separated along the first axis. Several PLFAs were clearly linked to the tree diversity levels in early summer as well as in autumn: the PLFAs cy19:0, 11Me17:0 and n22:0 were strongly linked to DL 1, whereas the PLFAs 10Me17:0, 16:1ω9, 16:1ω7, 16:1ω5, 17:1ω8 and 18:1ω7 were linked to DL 3 (Fig. 4.2a). However, several PLFAs were not clearly linked to a certain tree diversity level: the PLFAs a15:0 and a17:0 had negative loadings on PC 1 and positive loadings on PC 2 in both seasons and were therefore highly connected with both DL 2 and DL 3 (Fig. 4.2a). Furthermore, certain PLFAs were unspecific in terms of the diversity levels in both seasons: the iso PLFAs i14:0 and i15:0; two PLFAs 20:2 and 20:3, indicating eukaryotic organisms; and the fungal PLFA marker 18:2ω6,9 (Fig. 4.2b).

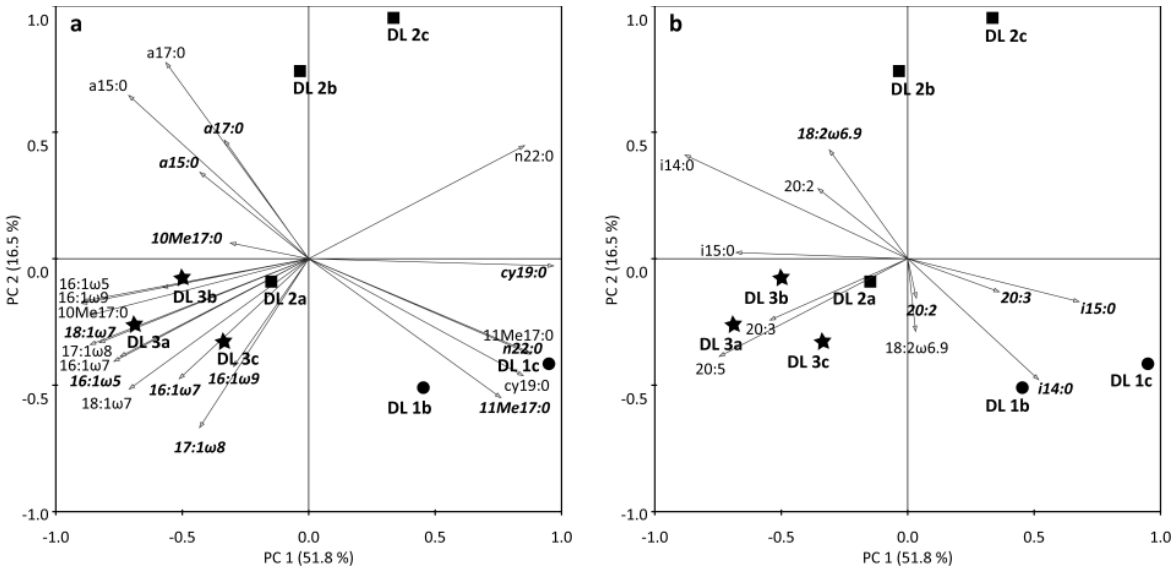


**Fig. 4.2:** Biplots from principal component analyses of phospholipid fatty acids (PLFAs) showing selected relative PLFA data from both seasons as loadings and the plots of the diversity levels as sample points. PLFA loadings in bold italic represent the autumn samples, and PLFA loadings in regular font represent the spring samples. The black symbols mark the sample points differentiating the diversity levels with circles for DL 1, squares for DL 2 and stars for DL 3; the ensuing letters represent different replicates. In a) only the PLFAs are shown that are linked to the same diversity level in both seasons, and in b) only the PLFAs are shown that are linked to different diversity levels in both seasons.

#### 4.3.2.3 Effects of tree and site characteristics

The results of a linear regression analysis between the first principal components (PC 1) of the relative PLFA abundances [mol%] after PCA and different environmental variables for both seasons corresponded to the results using the absolute PLFA abundances [nmol $g^{-1}$] (section 4.3.1.2). However, several remarkable differences were observed (Table 4.1). The microbial composition in autumn was heavily influenced by *Fagus* (stem density, litter and fine-root necromass) and the soil pH but also by *Tilia* and *Acer* (stem density, fine-root biomass). Similarily, the microbial composition in early summer was also closely correlated with *Fagus* (fine-root necromass) and the soil pH but also with variables such as "Litter remains", "Litter C/N" and "Litter total, high quality".

The first two axes of the RDA explained more than 70% of the data variance in the tree diversity levels in each season (Fig. 4.3). The arrows in the triplots show that the first six environmental variables after a Monte Carlo permutation test explained most of the variance. The PLFA loadings and sampling points for the diversity plots in the same direction as the arrowheads were strongly associated with these environmental variables. The RDA results confirmed the dominant influence of *Fagus* on the microbial community in both seasons. The tree parameters of *Fagus sylvatica* showed the strongest significant effect on the variation in the data. For example, the stem density ($F = 6.6$, $p < 0.001$ and $F = 5.2$, $p < 0.004$) was significant in both seasons, and the fine-root necromass was also significant in early summer ($F = 7.2$, $p < 0.001$). Several other factors may be important but were not judged significant by the analysis: in autumn, the litter of *Acer* and *Fagus*, decomposer fauna (*Isopoda*) and fine-root biomass of *Tilia* (Fig. 4.3a), and in early summer the fine-root biomass of *Fagus*, soil stock-C and initial litter-Mg (Fig. 4.3b). In both seasons, the influence of pH might have been masked by other factors: in early summer by the high correlation with the soil C/N-ratio ($R^2 = 0.80$, $F = 35.1$, $p < 0.000$) and in autumn by the high correlation with the litter of *Fagus* ($R^2 = 0.93$, $F = 97.5$, $p < 0.000$).

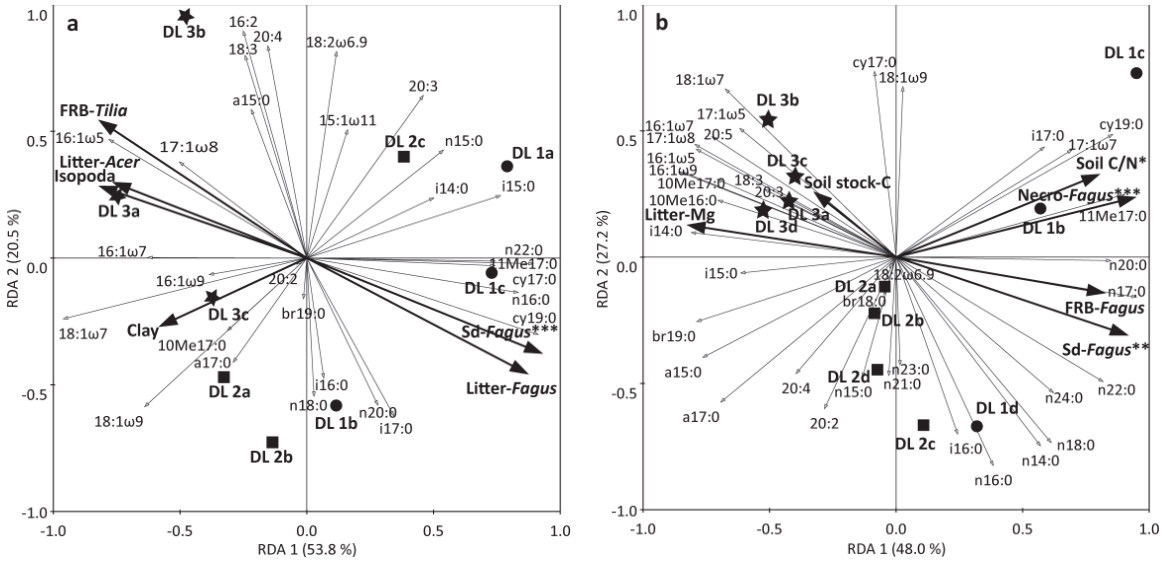


**Fig. 4.3:** Triplots from redundancy analysis of phospholipid fatty acids (PLFAs) in a) autumn and b) early summer showing relative PLFA abundances as loadings, the plots of the diversity levels as sample points and the first six environmental variables after forward selection based on a Monte Carlo permutation test. Significance is marked with asterisks. For explanation of symbols, see Fig. 4.2. Arrows: Sd-*Fagus* = stem density of *Fagus* of the total stand (n $ha^{-1}$); Litter-*Fagus,* Litter-*Acer* = litter amounts of *Fagus, Acer* for 2005 (g DM $m^{-2}$); FRB-*Tilia*, FRB-*Fagus* = fine-root biomass of *Tilia, Fagus* (g $m^{-2}$); Necro-*Fagus* = fine-root necromass of *Fagus* (g $m^{-2}$); *Isopoda* = individuals $m^{-2}$ in November 2005; Soil C/N = ratio from soil C and N (%) from PLFA soil samples; Soil-C = stock concentration of the soils in kg $ha^{-1}$; Clay = soil clay content (%) in 0-10 cm soil depth; Litter-Mg = Mg concentration of total litter (only five principal tree species) (g DM $m^{-2}$). Calculations based on data from Meinen et al. (2009a), Guckland et al. (2009), Jacob et al. (2009; 2010a,b) and Weland (2009).

## 4.4 Discussion

### 4.4.1 Effects of tree species composition on the microbial community

Litter fall in autumn provides large amounts of nutrients and energy for soil microorganisms in the topsoil, and the abundance of these microorganisms is strongly related to the amount of litter input (Prevost-Boure et al., 2011; Wu et al., 2012). In autumn 2005, the total amount of fallen litter (g DM $m^{-2}$) on our study plots was quite similar for DL 2 and DL 3 (391 ± 37.1 and 393 ± 59.4) and only slightly lower in DL 1 (324 ± 21.6) (Jacob et al., 2010a). At that time, we found almost no differences in the microbial biomasses and community structure between the tree diversity levels. Clearly, a fresh litter supply activated the soil microbial community at every tree diversity level in a similar manner. Our current analysis, based on a higher degree of variability of the tree and site factors, showed more details in the autumn sampling. Nevertheless, this analysis confirms the results presented in Thoms et al. (2010).

The PLFA biomass in autumn showed weak correlations with the measured variables and was only weakly driven by the supply of litter (leaves/ fine roots) from *Tilia* and the occurrence of tree species such as *Acer* associated with AM fungi. The structure of the microbial community in autumn was primarily influenced by *Fagus* and by the effect of this tree species on the soil pH values. This influence was confirmed by the higher occurrence of signature PLFAs (cy19:0, 11Me17:0, n22:0) for low pH values on the pure beech plots (DL 1), also observed in the early summer sampling. The season-independent influence of the soil pH in our study is in agreement with the established concept that soil pH strongly determines microbial community structure (Bååth and Anderson, 2003; Pietri and Brooks, 2009; Griffiths et al., 2011; Lucas-Borja et al., 2012; Rousk et al., 2010). However, several of the iso PLFAs (i14:0; i15:0) were strongly connected to the DL 1 plots in autumn and to the DL 3 plots in early summer. These findings suggest that they rely on readily available nutrients and that their activity is independent of soil pH.

In early summer, after seven months of seasonal changes in environmental conditions, the soil microbial communities in the topsoil showed clearer differences among the diversity levels. Specifically, we found significantly less PLFA biomass on the plots dominated by beech (DL 1 and 2) than on the DL 3 plots (Fig. 4.1b). Of all the tested environmental variables, litter- and soil-related nutrients best explained the high microbial abundances in DL 3, which can be attributed to the influence of the decomposition of "high quality" litter. The leaf litter of *Fagus* comprises lower initial nutrient concentrations of Ca, Mg, N and P than the leaf litter of *Fraxinus*, *Tilia, Carpinus* and *Acer* (Jacob et al., 2009). A soil analysis of our study plots showed that the long-term input of low-quality litter produced significantly lower soil nutrient stocks on the DL 1 and DL 2 plots (Guckland et al., 2009). In general, the soil texture increases the binding of the nutrients released as a result of decomposition. In this way, the texture of the soil can prevent the removal of nutrients. In our study, clayey soils were characterized by a higher soil stock of nutrients, but soil texture was not directly associated with microbial abundance. Our results indicate that in early summer, the microbial community was more directly influenced by tree species because the differences in the decomposability of the litter produced by these species influenced microbial abundance. In particular, higher microbial abundances occurred over the growth period on the plots with a high input of high-quality litter.

However, our results also indicate a strong rhizosphere effect. The higher PLFA biomass on the DL 3 plots was accompanied by higher proportions of Gram-negative bacteria, AM fungi, eukaryotic microorganisms, and Actinobacteria (Fig. 4.1d). Gram-negative bacteria

are associated with root activity (Kramer and Gleixner, 2006), a relationship that has been confirmed by several $^{13}$C-labeling studies showing a particular association of specific monounsaturated fatty acids such as PLFA 16:1ω5, 16:1ω7, 16:1ω9, 17:1ω8 and 18:1ω7 with highly enriched root-derived C (Treonis et al., 2004; Elfstrand et al., 2008; Esperschütz et al., 2009). Nutrients released by the roots and consumed by the bacteria present in the soil are only temporarily stored in the microbial biomass but can be successively mobilized by microfaunal grazing. The higher number of PLFAs indicative of protozoa (20:2-20:5) on the DL 3 plots, as well as the significant increase in the absolute and relative abundance of these PLFAs, verifies that good conditions were present for bacterial grazers and supports the impression of higher rhizosphere activity. Studies in mixed forests indicate that fine roots tend to be more concentrated near the soil surface (Schmid and Kazda, 2001; Rysavy and Roloff, 1994; Meinen et al., 2009a). Most likely, the horizontal distribution of the fine roots of *Acer* species contributed to a tendency for the roots to be compressed in the topsoil of the DL 3 plots. Meinen et al. (2009b) observed the spreading of *Acer* roots over a large area in the near-surface horizons with maximum stem distances of up to 20 m. *Acer* roots are associated with AM fungi. This association explains the higher abundances of AM fungi on the mixed forest stands with the highest tree species diversity. Mycelial exudates of AM fungi can also directly affect soil bacterial communities (Toljander et al., 2007). Most likely, more intense root competition in the uppermost soil layer on the DL 3 plots promoted the growth and spread of AM fungal hyphae. This might offer the possibility for trees to benefit more from nutrient sources in the rizosphere.

In summary, our analysis showed that the activity of soil microorganisms was primarily influenced by the soil pH in autumn, whereas other direct plant traits were generally eclipsed because of the overall nutrient supply furnished by the freshly fallen litter. The advanced decomposition of the leaf and root litter in the topsoils occurring until early summer produced greater differences between the microbial communities of the studied diversity levels. Additionally, our results suggest that a higher release of exudates in the rhizosphere promoted the activity of root-associated microorganisms and their grazers in the stands with the highest tree species diversity in early summer. Unfortunately, our current analysis cannot determine whether litter decomposition or root/mycelial exudates are primarily responsible for the large increase in the PLFA biomass in DL 3. We assume that the combination of both factors is important. Our results clearly indicate that direct

plant traits, in addition to abiotic factors such as the soil pH, strongly affect the soil microbial community.

### 4.4.2 Seasonal effects on the microbial community

The PLFA biomass in both seasons was highly similar, confirming our expectations (Kauri, 1982; Kaiser et al., 2010). In contrast, the microbial community structure shifted toward lower proportions of bacterial PLFAs (Gram-positive, Actinobacteria, Cyclo) on the beech-dominated plots (DL 1 and 2) (Table 4.2). Apparently, the decomposition of recalcitrant beech litter caused a decrease in certain bacterial species after 7 months of decomposition. Studies by Kauri et al. (1982) and Baum et al. (2009) support the suggestion that bacterial groups benefit most from the decomposition of freshly fallen litter in beech forest soils. $^{13}C$ labeled leaf litter studies have also identified Gram-positive bacteria as the most abundant microbial group with the highest incorporation of litter-derived C (Elfstrand et al., 2008; Rubino et al., 2010). Additionally, Yang et al. (2009) confirmed the importance of Actinobacteria in lignin degradation.

The different decomposition rates of the leaf litter types in the forest stands of the tree diversity gradient are due to differences in litter quality (Jacob et al., 2010b). Decomposition rates generally increase with a decreasing ratio of carbon to nitrogen (C/N ratio) (Taylor et al., 1989). Therefore, the C/N ratio is an important indicator of litter quality. However, other measures are also correlated with the leaf litter decomposition rates along the diversity gradient: the initial N and Ca concentrations (positively) as well as the C/P ratio and the lignin/N ratio (negatively) (Jacob et al., 2010b). The litter remaining after 7 months of decomposition, calculated from the rates of litter decomposition (Jacob et al., 2010b), furnished evidence that the lower proportions of “high quality” litter on the beech-dominated plots (DL 1 and 2) were already completely decomposed in June and that the ongoing decomposition of the “high quality” litter on the DL 3 plots may provide a continuous nutrient source for the soil microbes. The anteiso PLFAs a15:0 and a17:0, also indicative of Gram-positive bacteria, were strongly linked to the DL 2 and 3 plots independent of season. Accordingly, these PLFAs characterize microorganisms that, most likely, prefer litter of higher quality or their effect on the pH and the abundance of the soil fauna. The activity of fungi was affected neither by tree diversity nor by season. This result suggested that the fungi are active year-round.

Additionally, we found little evidence of changes in the community of root-associated microorganisms and AM fungi between the seasons. Significantly higher proportions were found only for PLFA 16:1ω9 on the DL 3 plots in early summer. This result suggests that root-associated microorganisms are equally supported in both seasons. Consequently, the denser root network with higher abundances of AM fungi, not root exudation, is the most likely cause of the overall higher abundance of root-associated microorganisms at higher diversity levels. However, the higher abundance and number of PLFAs indicative of protozoa in early summer than in autumn suggests that DL 3 furnished better conditions for bacterial grazers. This result indicates that the additional production of root-associated microorganisms might be grazed immediately by higher organisms.

### 4.5 Conclusion

The comparison of the soil microbial communities between two seasons in the topsoils of a temperate deciduous forest with different abundances of *F. sylvatica* emphasizes the significant role of direct plant traits related to litter quality (leaves/ fine roots) and root activity (exudates, networks, density) for the abundance and composition of these communities. Advanced leaf litter decomposition and higher root activity in early summer corresponded to more differences in the microbial community, whereas fewer differences were found in the autumn sampling. Our results highlight the seasonally independent effect of soil pH on microbial composition. Above all, however, these results highlight the seasonal dynamics of microbial communities in forest ecosystems. Future studies examining the influence of tree diversity on soil microbial communities should consider the time of sampling because the relevant processes linking above- and belowground diversity are best viewed in terms of this framework of seasonal dynamics.

# Chapter 5

## Evidence for deep soil water uptake by *Fagus sylvatica* L. in a temperate mixed forest from hydrogen isotopes of leaf lipids after summer drought

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**Abstract**

The predicted increase of more frequent and severe summer droughts in Central Europe may affect mono-specific and mixed forests and the species therein differently. We investigated the drought response of *Fagus sylvatica*, the most common tree species in Central Europe, in three forest stands (50 x 50 m) with different tree species diversity in the Hainich National Park, Central Germany. We used the hydrogen isotopic signature (δD, deuterium) of throughfall, soil water and *n*-alkanes from sun and shade leaf lipids to investigate the water use strategy of *Fagus sylvatica* during a period of drought. The samples were taken during the growing season 2006, which was characterized by two long droughts in the period from May to July. As expected, we observed a strong evaporative enrichment of deuterium in the soil water up to a depth of 50 cm. This effect was highest on the plot with the highest tree diversity and driven by the lack of a closed litter layer during summer time. Previous studies from the same site already suggested that mixed forest plots exhaust the soil water faster than mono-specific beech plots in the beginning of a drought period. Most interestingly, the δD values in the *n*-alkanes ($C_{27}$) of the sun leaves were significantly lower on the plot with the lowest abundance of *Fagus*. This indicated the uptake of deep soil water (> 70 cm soil depth), which was more depleted in deuterium than the near-surface soil water during the drought in the mixed plot. In addition, we found evidence that under water stress *Fagus* can regulate metabolic processes targeted in certain crown areas like the reduction or cessation of the renewal of *n*-alkanes in the shade leaves to maintain the function of the sun leaves. We conclude that, *Fagus* tries to overcome its drought sensitivity in gaining deep soil water in response to soil drought, in particular when grown in combination. This may be seen as a drought avoidance strategy and an advantage when faced with interspecies competition.

**Keywords:** hydrogen isotopes, leaf lipids, sun leaves, drought, *Fagus sylvatica*, beech, forest, diversity, *n*-alkanes, deep soil water, complementary resource use

## 5.1 Introduction

The occurence and productivity of tree species in forest ecosystems is strongly influenced by seasonal climate conditions (Leuschner et al., 2001; Stribley and Ashmore, 2002; Bréda et al., 2006). In the context of a higher frequency of summer droughts (Meehl and Tebaldi, 2004) it may be important for some tree species to withstand water shortage in mono-specific or mixed forest stands. There is evidence that complementary resource use might be beneficial in high diverse forest stands and is facilitated by a higher fine root production (Brassard et al., 2011; Lei et al., 2012). However, the exhaustion of soil water by drought tolerant species is predicted to lead to increased water stress during prolonged droughts (Bittner et al., 2010) and may damage drought sensitive species in mixed-species stands. In general, *Fagus sylvatica* represents a drought-sensitive tree species, especially with respect to soil drying (Leuschner et al., 2001; Hölscher et al., 2005; Köcher et al., 2009), and responds early with closing of the stomata (Jonard et al., 2011; Aranda et al., 2000). The response to extreme water stress in *Fagus sylvatica* has been well studied by Lemoine et al. (2002). Interestingly, shade leaves of *Fagus* responded earlier and faster with the closing of stomata to reduced water potentials, when compared with sun leaves. A drought-tolerating strategy is used by tree species like *Fraxinus exelsior* with a high maximum leaf conductance ($g_L$) and a low sensitivity of sap flow to water shortage (Köcher et al., 2009).
Protection against water loss and excessive ultraviolet radiation is provided by the waxy outer surfaces of plants and *n*-alkanes are important chemical constituents of these epicuticular wax layers (Eglinton and Hamilton, 1967). The hydrogen isotopic composition of *n*-alkanes has been found to integrate signals from both the environmental water and biochemical processes within the plant (Ziegler et al., 1976; Liu and Yang, 2008). Changes in environmental factors such as air temperature and relative humidity have an effect on the evapotranspiration in forest ecosystems and consequently on the fractionation of hydrogen isotopes in the plant-soil system (Farquhar et al., 2007; Smith and Freeman, 2006). Leaf wax *n*-alkanes are synthesized early in the ontogeny of a tree leaf (Kahmen et al., 2011), however, they are also regenerated over the whole growing season depending on

loss through weathering or a greater need for protection (Lockheart et al, 1997; Sachse et al., 2009). Thus, limited soil water and a low relative humidity during summer droughts can be expected to have a short-term effect on the δD values of *n*-alkanes.

In 2006, there was a prolonged drought in Central-Germany, where a stronger extraction of soil water was found in forest stands with high tree species diversity compared to less diverse forest stands or mono-specific beech stands (Krämer and Hölscher, 2010). The increased soil water extraction already in early summer in the mixed forests was attributed to certain tree species with a greater exhaustion of soil water, notably by *Tilia* species (Gebauer et al., 2012). In this study we compared the δD values of soil water and the leaf-wax *n*-alkanes of *Fagus sylvatica* between forest stands classified in three tree diversity levels. We hypothesise that a) stronger soil evaporation and ecosystem water use during the summer drought in the forest stand with the highest tree diversity (DL 3a) resulted in higher hydrogen isotope ratios of the soil water, and consequently b) higher hydrogen isotopic ratios of the leaf-wax *n*-alkanes of *Fagus sylvatica* in this forest stand. Furthermore, based on the results of Lemoine et al. (2002), we also hypothesise that the leaf-wax *n*-alkanes in the shade leaves will have higher hydrogen isotopic ratios than in the sun leaves of *Fagus sylvatica* directly after drought.

## 5.2 Material and methods

### 5.2.1 Meteorological measurements

Meteorological and soil data were continuously measured by an eddy covariance flux tower at the Hainich site (51°04'46''N, 10°27'08''E, 440 m a.s.l.). A detailed description of the measurement equipment is given in Knohl et al. (2003). Briefly, the flux tower was equipped with instruments to measure air temperature and relative humidity (HMP45D and HMP35D, Vaisala, Helsinki, Finland). Precipitation was collected with rain gauges (type 5.4032.30.008 Thies, Göttingen) at 3 m height and about 800 m away in a forest clearing. Volumetric soil water content ($\theta$ in %) was measured at 5, 15 and 30 cm soil depth using Theta-probes (ML-2x, DeltaT, Cambridge, UK).

### 5.2.2 Leaf sampling and lipid extraction

Beech leaves were sampled on 1$^{st}$ of August 2006, after the prolonged desiccation period in June and July by tree climbers. Fifteen leaves each from both the upper sun-crown and

the lower shadow-crown from five beech trees on each of the three intensive plots (DL 1a, DL 2c, DL 3a) were collected. The exact location of the trees and some features are shown in Table A2 (Appendix). DL 1a belongs to the replicates of the almost pure beech stands of diversity level 1 (*Fagus sylvatica*). DL 2c and DL 3a are replicates from diversity level 2 and 3, and are dominated by beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and lime (*Tilia* species), where DL 3a comprises also hornbeam (*Carpinus betulus*) and maple (*Acer* species). A detailed description of the study site and the diversity levels is given in chapter 2. The leaf samples were dried and ground. Soluble organic matter was extracted from 30-150 mg of dried leaf powder using an accelerated solvent extractor (ASE 200, Dionex Corp., Sunnyvale, USA) with dichloromethane: methanol mixture (9:1) at 100°C and 2000 psi (=138 bar) for 15 min in 2 cycles. The total extract was separated using a solid phase extraction (SPE) on silica gel in accordance to the method described by Garcin et al. (2012). Glass columns (6 ml glass reaction tubes, Supelco) were filled with 2 g of silica gel previously dried at 50°C over night. After sample transfer the *n*-alkanes were eluted with 12 ml hexane.

### 5.2.3 Quantification and δD measurements of the *n*-alkanes

The compounds of the aliphatic fraction were identified and quantified with a GC-FID System (TraceGC, ThermoElectron, Rodano, Italy) using a Rxi-1ms column (30 m × 0.32 mm ID, 0.5 µm film thickness, Agilent) and an external *n*-alkane standard mixture. The injector was operated at 300 °C in splitless mode. Helium was used as a carrier gas at a flow rate of 2.0 ml $min^{-1}$. The temperature program started at 90 °C (for 1 min). Thereafter, the temperature was raised to 300 °C at a rate of 10 °C $min^{-1}$, and was kept constant for 9 min. The final temperature of 335 °C (hold for 5 min.) was reached after further raising the temperature at 30 °C $min^{-1}$.

For the δD measurements of the *n*-alkanes an aliquot of 1 µl of each sample was injected into a HP5890 GC (Agilent Technologies, Palo Alto, USA) equipped with a Rxi-1ms column (30 m × 0.32 mm ID, 0.5 µm film thickness, Agilent). The injector was operated at 280 °C in splitless mode. The temperature program started at 60 °C (for 1 min). Thereafter, the temperature was raised to 140 °C at a rate of 30 °C $min^{-1}$, then to 300 °C at 5 °C $min^{-1}$. The final temperature of 340 °C was reached after further raising the temperature at 30 °C $min^{-1}$, and was kept constant for 3 min. The column flow was held constant at 1.5 ml/min throughout the run. Following the GC separation, the hydrogen bound in the *n*-alkanes was

quantitatively converted into $H_2$ gas prior to analysis in the isotope ratio mass spectrometer (IRMS) (Delta$^{plus}$XL, Finnigan MAT, Bremen, Germany). The quantitative conversion is achieved by high temperature conversion at > 1400°C (Hilkert et al., 1999), and was operated in this system at 1440°C. Each sample was measured in triplicate. The δD values were normalized to the VSMOW scale using a mixture of *n*-alkanes (*n*-$C_{20}$ to *n*-$C_{33}$) which were calibrated against international reference substances (NBS-22, IAEA-OH22) using the offline TC/EA technique (Gehre et al., 2004). After the measurement of two samples (6 GC runs), the standard mixture was measured three times. A drift correction was applied (Werner and Brand, 2001). The $H_3^+$ factor was determined once a day and it fluctuated slightly between 8 and 10 (SD = 0.91) over the 18-day measurement period.

### 5.2.4 Sampling and δD measurement of throughfall and soil water

For the period from January until July 2007 we used subsamples from throughfall and soil water collection for hydrogen isotopic analysis. The throughfall water was collected in storage rain gauges as described in Krämer and Hölscher (2009) and Talkner et al. (2010). In brief, the gauges were placed at a height of 1 m, and consisted of a plastic bottle and a funnel 10.5 cm in diameter attached to a metal rod. A table tennis ball was placed in the funnel to reduce evaporation. Each study plot was equipped with 15 rain gauges along the 3 randomly chosen transects. The five gauges of each transect were pooled, resulting in three replicate samples per date for each plot. The water was collected manually every two weeks but each second sample was used for isotopic analysis. In winter, snow was sampled with buckets 25 cm in diameter with the same sampling design.

The soil water was collected using suction cup lysimeters with a vacuum > 500 mbar. Each transect of the investigated plots was equipped with two clusters of lysimeters with two at 10 cm, and two at 50 cm soil depth, for a total of 24 lysimeters per plot. As for gross precipitation, the water was collected manually every two weeks but each second sample was used for isotopic analysis. After collection the water samples were stored at 8 °C until isotopic analysis was performed.

The δD values of all water samples were measured in the Isolab at MPI-BGC using a high temperature reactor (TC/EA) coupled on-line with a ConFlo III interface to a Delta$^+$XL isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) (Gehre et al., 2004).

## 5.3 Results

### 5.3.1 Climate and site conditions

The first half of May, the time of leaf shoot for some tree species, was characterized by low precipitation, which resulted in a significant reduction of soil moisture in the uppermost soil layers in the Hainich region (Fig. 5.1). At the end of May and the beginning of June, large rain events saturated the soil with water. In the following drought period between the 2nd of June and 26th of July only isolated and sporadic summer rain events occurred, which led to a noticeable dehydration of the topsoil to a depth of at least 30 cm. This second period of drought had on average a daily temperature of 19 °C with a temperature maxima on July 19 and 20 with 27 °C combined with low relative humidity.

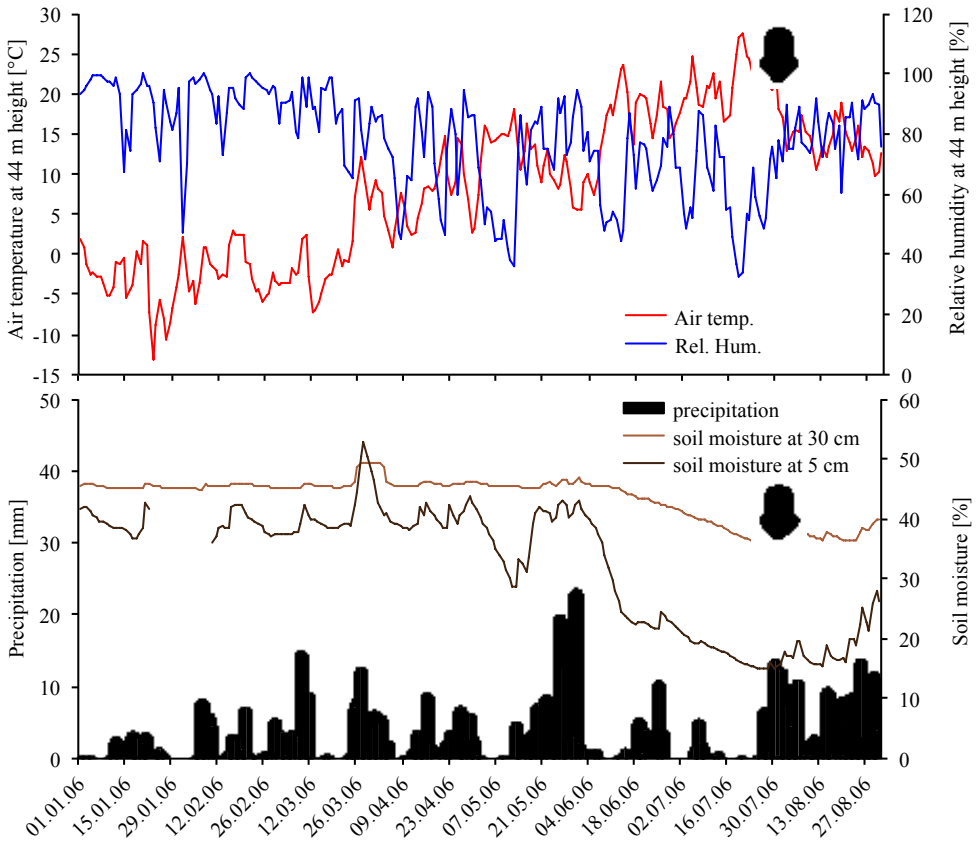


**Fig. 5.1:** Meteorological parameters and soil moisture at two soil depths (5 and 30 cm) from the Hainich eddy covariance flux tower between January and July 2006. The black arrows indicate the time of leaf sampling.

### 5.3.2 δD values of throughfall and soil water

The available throughfall water for the three study plots between February and June corresponded to the global meteoric water line (GMWL, $\delta D = 8.13\ \delta^{18}O + 10.8$) (Fig. 5.2). The outlier of plot DL 3a (April sampling) can be attributed to evaporation processes in the rain gauges between the samplings. However, a comparison of the ratio of δD to $\delta^{18}O$ in the soil water of the study plots for the period January to June with the GMWL showed differences between the study plots (Fig. 5.3). The soil water of plot DL 1a and DL 2c also corresponded to the GMWL, but for plot DL 3a a strong evaporative enrichment was observed caused by the sampling on 29th of June.

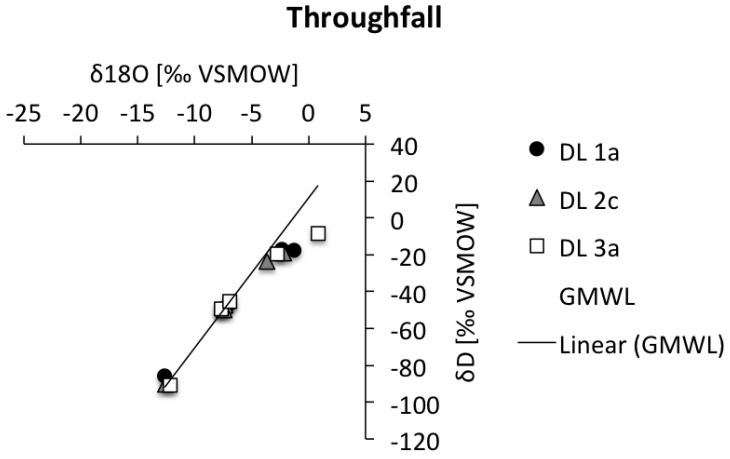


**Fig. 5.2:** Comparison of the ratios between δD and $\delta^{18}O$ values of throughfall from the study plots with the global meteoric water line (GMWL) in the period from February to June 2006.

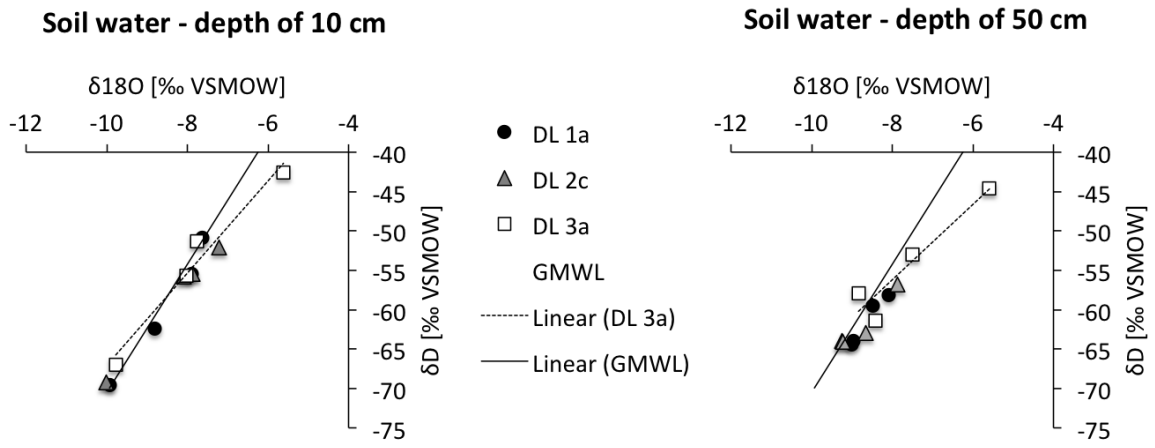


**Fig. 5.3:** Comparison of the ratios between δD and $\delta^{18}O$ values of soil water at a soil depth of 10 cm and 50 cm between the study plots with the global meteoric water line (GMWL) in the period from January to June 2006.

Changes in the hydrogen isotope ratios in the soil water at depths of 10 and 50 cm over this period is shown separately in Figure 5.4. Here, at a soil depth of 10 cm, the δD values of the soil water from April to June increased in all study plots. The increase was strongest on plot DL 3a but with no significance due to a high variability in June. At a soil depth of 50 cm, the δD values of the soil water only increased from May to June on plot DL 2c and DL 3a. However, despite a similar level of variability in June, deuterium enrichment in the soil water of 50 cm soil depth on plot DL 3a was significantly different to plot DL 1a by approximately 20‰. Missing values in the measuring phase from January to July were due to soil freezing (February, March) and a lack of rainfall (July).

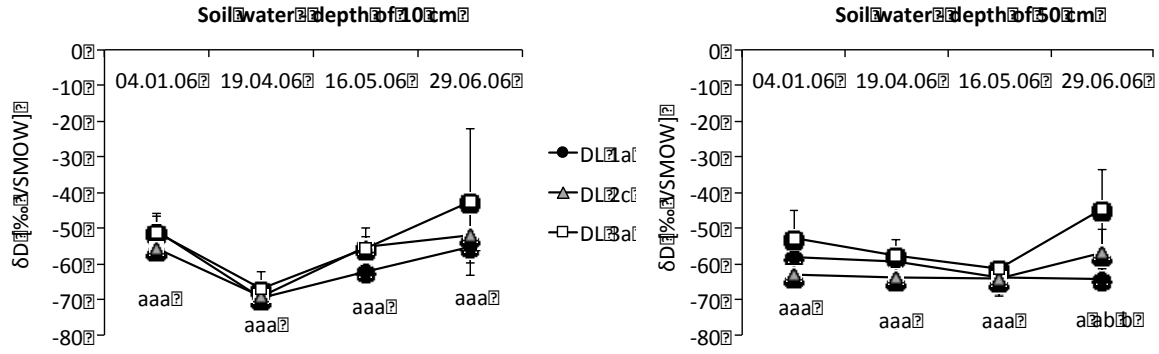


**Fig. 5.4:** Comparison of the δD values of soil water at 10 cm and 50 cm soil depth between the study plots in the period from January to June 2006. Different letters indicate significant differences between the soilwater δD values of the diversity plots in the order DL 1, DL 2, DL 3 ($p < 0.05$).

### 5.3.3 Concentrations and δD values of leaf-wax lipids

The distribution of the major *n*-alkanes in both sun and shade beech leaves showed the dominance of the *n*-alkane *n*-$C_{27}$, an example of which is shown in a chromatogram for a sun leaf in Figure 5.5. Of the other *n*-alkanes, which were quantified, only the concentrations of *n*-$C_{25}$ and *n*-$C_{29}$ were notable. In general, the total concentration of the *n*-alkanes was higher in sun leaves and ranged between 853 and 993 ng per mg dry weight, in comparison to a dry weight between 615 and 914 ng per mg for the shade leaves (not shown).

The following results refer only to the main *n*-alkane *n*-$C_{27}$, since reliable hydrogen isotope values could only be measured for this *n*-alkane. Unfortunately, no sampling of beech leaves was conducted before or during the drought. Therefore, only differences in the sun and shade leaves between beech trees in a different tree species composition on the diversity plots can be compared.

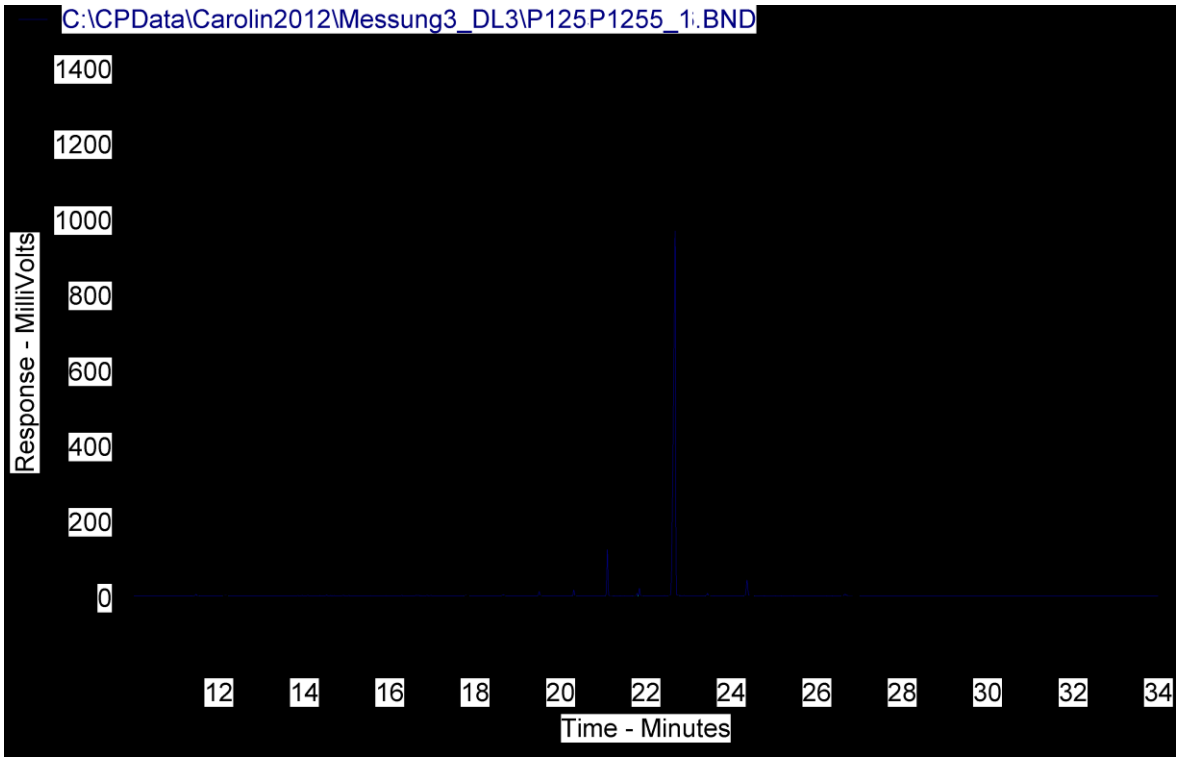


**Fig. 5.5:** Chromatogram of a beech leaf sample (sun leaf, Bu 1, plot DL 3a) after quantification with a GC-FID System.

We observed no significant differences in the concentrations of *n*-$C_{27}$ between the diversity plots, neither for the sun nor the shade leaves (Fig. 5.6). In a similar fashion, δD measurements were also not significantly different between the diversity plots for the shade leaves. However, the sun leaves showed a significant difference on plot DL 3a with approximately 20% lower δD values for *n*-$C_{27}$ compared to the other plots (DL 1a, DL 2c).

The comparison of the *n*-$C_{27}$ concentrations in both leaf types showed the greatest similarity to the mean for DL 1 (Fig. 5.6). On the mixed forest stands (DL 2c, DL 3a), the mean *n*-$C_{27}$ concentrations of the shade leaves were much lower than that of the sun leaves, but only differed significantly for plot DL 3a.

The hydrogen isotope ratios of beech leaves on plots DL 1a and DL 3a showed significantly higher δD values for *n*-$C_{27}$ in the shade leaves compared to the sun leaves, whereas beech leaves on the DL 2c plot tended to have higher δD values for *n*-$C_{27}$ in the sun leaves.

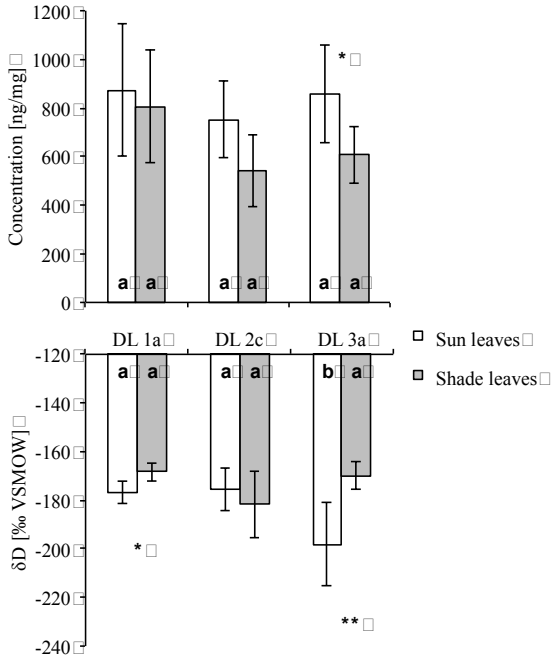


**Fig. 5.6:** δD values and concentrations of the main n-alkane *n*-$C_{27}$ in the sun and shade leaves of the beech trees on the diversity plots (n = 5). Different letters indicate significant differences between the diversity plots for each leaf type ($p < 0.05$). Stars show significant differences between sun and shade leaves for each diversity plot (*** = $p \leq 0.001$; ** = $p \leq 0.001$; * = $p \leq 0.05$).

### 5.3.4 Isotopic fractionation between δD of soil water and δD of leaf-wax lipids ($\varepsilon_{apparent}$)

The calculation of $\varepsilon_{apparent}$ in table 5.1 includes the δD of the soil water at a soil depth of 50 cm as the uptake of soil water by beech roots was preferentially at soil depths from 30 to 50 cm during the moderate desiccation period in 2009 in the same forest, irrespective of tree species mixture (Meißner et al., 2012). The results of $\varepsilon_{apparent}$ of the shade leaves are in the range between -104‰ and -125‰, wherein $\varepsilon_{apparent}$ is very similar for the plots with a higher tree-species diversity (DL 2c and DL 3a), and approximately 20 % lower for plot DL 1a. The values of $\varepsilon_{apparent}$ for the sun leaves on plots DL 1a and DL 2c are similar to the $\varepsilon_{apparent}$ values for the shade leaves. However, $\varepsilon_{apparent}$ for the sun leaves on plot DL 3a showed a strong deviation from the range with a decrease of approximately 29 %.

**Table 5.1:** The apparent fractionation ($\varepsilon_{apparent}$) of the sun and shade leaves on the diversity plots (means ± 1 SE of three lysimeter measurements or five beech trees, respectively).

| | Sun leaves | | | Shade leaves | | |
|---|---|---|---|---|---|---|
| | DL 1a | DL 2c | DL 3a | DL 1a | DL 2c | DL 3a |
| δD of the soil water in a depth of 50 cm | -64.4 ± 3.1 | -56.8 ± 6.5 | -44.6 ± 11.0 | -64.4 ± 3.1 | -56.8 ± 6.5 | -44.6 ± 11.0 |
| δD *n*-alkane *n*-$C_{27}$ | -176.2 ± 4.6 | -175.4 ± 8.8 | -198.1 ± 17.1 | -168.4 ± 3.8 | -181.6 ± 13.8 | -169.9 ± 5.7 |
| $\varepsilon_{apparent}$ | -111.8 ± 4.6 | -118.6 ± 8.8 | -153.5 ± 17.1 | -104.0 ± 3.8 | -124.8 ± 13.8 | -125.3 ± 5.7 |

## 5.4 Discussion

### The isotopic signature of the soil water

Our results clearly demonstrate that during the drought end of June, the soil evaporation led to an enrichment of deuterium in the soil water even down to a soil depth of 50 cm on the plot with lowest abundance of *Fagus sylvatica* (DL 3a). The deviation of the $\delta D/\delta^{18}O$ ratios of the soilwater on plot DL 3a from the meteoric water line (GMWL) indicated a substantial contribution of evaporative water loss. This observation could not be made for the plots DL 1a and DL 2c, and suggest that a year-round soil cover with a strong litter layer can significantly reduce evaporation of soil water especially during prolonged droughts. The protective effect of the litter layer has already been observed in other studies (Sharafatmandrad et al., 2010; Quijano et al., 2012), in particular that a soil cover of 2 cm, regardless of the type of coverage, led to reduced soil evaporation (Zhou et al., 2008). In addition, the lack of a closed litter layer on plot DL 3a seems to be responsible for the higher variability of the soil water δD values.

The δD of soil water on plot DL 1a in soil depth of 50 cm corresponded to the δD of soil water at the Hainich site observed in summer 2009 in the same soil depth (Meißner et al., 2012). In general, deeper soil layers are barely influenced by short periods of rainfall during summer time, as it is mainly the more D-depleted winter precipitation that reaches deeper soil layers in Central Germany (Lonschinski et al., 2011). We are not aware of studies showing a fractionation of soil water by soil evaporation in such a soil depth. Important for our study are the strong differences in the δD of the soil water between the plots of the diversity levels through which isotopically different source water for the synthesis of *n*-alkanes was provided.

**Differences in sun and shade leaves of *Fagus sylvatica***

Our results suggest that under water stress *Fagus* is able to regulate the shade leaves, while probably maintaining the functions of the sun leaves as long as possible, confirming the results of Lemoine et al. (2002). The trend for lower concentrations of *n*-alkanes in the shade leaves and the differences in the δD values of the *n*-alkane $C_{27}$ between both leaf types on the mixed forest stands (DL 2c and DL 3a) indicate that *Fagus* reduced or even stopped the renewal of *n*-alkanes in the shade leaves in the mixed stands. Consequently, the *n*-alkanes of the shade leaves do not reflect the δD signal of the extracted soil water and/or evapotranspiration processes during the drought but during the time of their ontogeny or a short time after. However, on plot DL 1a we observed similar concentrations of the *n*-alkane $C_{27}$ for both leaf types indicating a similar synthesis or renewal rate of the *n*-alkanes over the entire period of drought. The significantly higher δD values of the *n*-alkane $C_{27}$ in the shade leaves when compared to the sun leaves on plot DL 1a can then consequently be attributed to transpiration or closing of stomata.

Sachse et al., (2009) observed the maximum concentration of $C_{27}$ in the shade leaves of *Fagus* during the time of the leaf shoot (ontogenity). Over the whole vegetation period the *n*-alkane concentrations varied greatly in this study, probably depending on the renewal rate. It seems that the renewal rate of leaf-wax lipids in the shade leaves of *Fagus sylvatica* fluctuates over the growing season and may be affected by certain environmental parameters such as water stress during periods of drought.

The deviation of the δD values of leaf-wax $C_{27}$ of the sun leaves towards lower values compared to the shade leaves on the forest stand with the highest tree diversity (DL 3a) exclude soil water from the upper soil layers (< 50 cm soil depth) as water source for the *n*-alkane synthesis as well as an influence of other processes (evapotranspiration) on the δD values. However, the tendency to higher δD values of *n*-$C_{27}$ in the sun leaves compared to the shade leaves on plot DL 2c suggest that on this forest stand the more enriched soil water from the upper soil layers (< 50 cm soil depth) was used as source water for the *n*-alkane synthesis in the sun leaves.

**Differences in the δD values of leaf-wax lipids of *Fagus sylvatica***

Contrary to our hypothesis, the deuterium-enriched soil water on plot DL 3a did not lead to higher δD values in the *n*-alkanes ($C_{27}$) of the beech leaves in comparison to the other plots (DL 2c and DL 1a). Instead we observed significantly lower δD values for $C_{27}$ in the sun

leaves on plot DL 3a. Moreover, the values of the apparent fractionation ($\varepsilon_{apparent}$) for the sun leaves in DL 3a were not comparable to values for the apparent fractionation known for beech at this site (Sachse et al., 2009). Thereby, the $\delta$ D values of leaf-wax lipids on plot DL 3a suggest other conditions or processes in the synthesis of *n*-alkanes in the leaves of these beech trees.

A depletion in δD for $C_{27}$ was unexpected because a higher rate of transpiration or even the closing of the stomata will inevitably lead to an enrichment of deuterium in the leaf water and thus to higher δD values in the leaf lipids. Lower δD values in the leaf lipids can occur if the source water was strongly depleted in δD or by a change in the biosynthesis. Evidence of altered biosynthesis of *n*-alkanes in trees under water stress has not yet been found, however, a relationship is discussed for mangroves under high salinity (Ladd and Sachs, 2012).

However, a correction of the apparent fractionation ($\varepsilon_{apparent}$) on plot DL 3a (-154 ‰) to the minimum value for the apparent fractionation observed by Sachse et al. (2009) (-125 ‰) results in a calculated value of -74 ‰ for the soil water used for *n*-alkane synthesis in the sun leaves during this drought. This corrected δD value for the soil water does not seem to be unlikely and corresponds to measured δD values of soil water around -70 ‰ at a soil depth of 50-70 cm at the Hainich site during summer 2009 (Meißner et al., 2012). Consequentliy, these data suggest that during the drought *Fagus* was able to exhaust soil water from a depth of more than 70 cm. Several previous studies have shown that *Fagus* is able to move their roots preferably into deeper soil layers, when in competition with other tree species (Rysavy and Roloff, 1994; Hendriks and Bianci, 1995; Schmid and Kazda, 2001; Bolte and Villanueva, 2006). With the exploitation of deeper soil layers *Fagus* might be able to overcome the early soil drying in the near-surface soil layers in mixed stands in the beginning of a drought caused by *Tilia* (Gebauer et al., 2012). It can be assumed that functional traits of *Fraxinus* and *Tilia* led to a more intense water shortage in the upper soil layers during the drought indicating a complementary use of water resources in the more diverse forest stands. Apparently there was no need for *Fagus* to extract soil water in deeper layers in mono-specific stands since water reserves were available for longer during droughts (Krämer and Hölscher, 2010). Evidence for enhanced fine root production of *Fagus* in subsoil layers at the Hainich site after the big summer drought in 2003 support our findings (Tefs and Gleixner, 2012). The death of fine roots during dry periods probably induces the growth of new fine roots, which penetrate deeper and deeper into the soil. Furthermore, higher earthworm densities on plot DL 3a observed by Weland (2009) could

have favored the growth of roots in deeper soil layers. Don et al. (2008) found earthworm burrows up to a soil depth of 140 cm with a maximum in soil depth of 110 cm in a grassland site not far from our study area. Likewise, the results of an intensive crown transpiration calculated from sap flow measurements confirm that *Fagus* must have access to sufficient water reserves in the soil during summer 2006 (Gebauer et al., 2012). Even other periods in the past suggest that beech grew better in a mixed than in a monospecific neighborhood at the Hainich site (Mölder et al., 2011). This would imply that *Fagus* not only can ensure their survival in mixed forest stands, but are also able to increase productivity. However, no evidence of complementary resource use in association with biomass production was found on our study plots (Jacob et al., 2010). Recently, a study showed a high drought sensivity for *Fagus* in a young mixed broad-leaved stand in France during the same summer drought in 2006 (Zapater et al., 2013). We attribute these results to the young age of the mixed forest, so that drought-induced root growth of *Fagus* in deep soil layers could not be adequately implemented. Another explanation is the dominant deep rooting of *Quercus* and *Salix* species, which may have supressed *Fagus* growth. Finally, our results can not be generalized for temperate mixed beech forests, as interspecies competition depends on functional traits of the present trees species.

### 5.5 Conclusion

Our results suggest that *Fagus* is able to exploit deeper soil layers during their growth in forests with high tree species diversity. There is evidence of resource use complementarity in mixed forest stands, in particular if such stands exhaust the available soil water in the upper soil layers faster than the mono-specific stands during periods of droughts. Consequently, *Fagus* is probably less restricted by water shortage in mixed forests even during prolonged droughts at the Hainich site. Moreover, it seems that under water stress *Fagus* can regulate targeted metabolic processes in the upper and the lower crown through the reduction or cessation of the renewal of *n*-alkanes in the shade crown. We are aware that our suggestions require confirmation by further studies, possibly in combination with compound-specific $^{13}C$ analyses of leaf *n*-alkanes.

# Chapter 6

## Synthesis

In our study site, the plant-soil system of beech forest ecosystems was strongly influenced by differences in tree species diversity. To address the first hypothesis of our study it can be concluded that **complementary resource use** is an essential process in plant coexistence in forest ecosystems with high tree species diversity. The results of the hydrogen isotope analyses gave strong evidence that during the dry periods in summer 2006 *Fagus* had access to soil water in deep soil layers of at least 70 cm in the mixed forest stand with highest tree species diversity (DL 3a). It seems that during droughts an increased competitive pressure in the rhizosphere of mixed forests results in complementary use of soil water with a rapid reduction in soil water reserves of the uppermost soil layers. As a consequence, drought sensitive tree species like *Fagus* are affected by increased death of fine roots. As time progresses, this most likely induces greater formation of fine roots preferentially in deeper soil layers. After the big summer drought of 2003 enhanced fine root production of *Fagus* in subsoil layers was observed (Tefs and Gleixner, 2012). This 'water stress avoiding strategy' seems to be vital for *Fagus* in forest stands with high tree diversity and indicates spatial niche differentation.

Additionally, it seems that competitive pressure in the rooting zone could have also induced resource uptake strategies in other plant species. For example, *Acer* species and/or the herb-layer might have condensed the root network in the topsoils in plots of DL 3 increasing the competitive pressure. The vertical root distribution of *Acer* suggests this to be the case (Meinen et al., 2009c) as well as a higher biomass and diversity of the herb-layer in plots of DL 3 (Mölder et al., 2008). Consequently, the more compact root network in the upper soil layer makes it necessary to optimize the aquisition of nutrients and promote growth of mycorrhizal fungi. We found in both sampling dates a distinct higher abundance of **arbuscular mycorrhizal fungi (AM fungi)** in the topsoil of DL 3. This suggest a more intense or complementary use of resources in the forest stands with highest tree species diversity due to AMF activity. Several studies confirm that AM fungi have a significant role in promoting plant coexistence and, therefore, maintain plant diversity

(Van der Heijden et al., 1998; Wagg et al., 2011a,b). An interesting aspect here is that AMF might increase the functional redundancy of plant species by decreasing plant species-specific constraints in resource uptake (Klironomos et al., 2000). The coexistence of functionally redundant plant species during early years of diversity experiments might be necessary to develop a functionally unique and highly productive plant community (Reich et al., 2012). Biodiversity experiments in grassland ecosystems confirm an increase of AMF biomass with plant species richness (Hedlund et al., 2003). The abundance of AM fungi was positively correlated to the soil P content (see Fig. 3.3b) and supports the widely cited ability of AM fungi to aquire phosphorus for their host plant. This strong relationship strengthens the significant role of AM fungi in aquiring limited nutrients for the trees on plots of DL 3. Morover, AM fungal biomass in turn can also affect the growth of soil bacteria in direct contact with AMF hyphae (Scheublin et al., 2010; Lecomte et al., 2011), and could therefore support a higher abundance of microbial communities. Therefore, we conclude that complementary resource use can contribute to increased microbial biomass in highly diverse plant communities.

The forest ecosystems with the highest tree species diversity promoted the highest abundance of soil microorganisms in our study site. This relationship was strongest in the topsoil during the spring/early summer sampling. In autumn the 0 to 5 cm soil layer did not show this correlation, however, the relationship was obvious when looking at the entire soil layer from 0 to 20 cm (see Fig. 3.1b). In this context, the second hypothesis of this study, namely that **the effect of „high quality“ litter** was a possible reason for the correlation between tree species diversity and increased soil microorganism abundance, will be discussed. In our study the litter of *Fagus sylvatica* was contrasted to litter of other deciduous tree species. Non-beech litter on our study sites comprised litter from *Tilia* species, *Fraxinus excelsior*, *Carpinus betulus* and *Acer* species. Higher initial nutrient contents (Ca, Mg, P, N, K), lower lignin contents, lower nutrient ratios (C/N, C/P) and a lower acidification in comparison to *Fagus* litter characterize these litter types (Jacob et al., 2009; Guckland et al., 2009), and led to more rapid decomposition rates (Jacob et al., 2010b). The more acidifying character of beech litter was able to strongly reduce the soil pH (from 5.3 to 3.2) and affected the soil microbial structure indirectly. Our results suggest that decomposition of high quality litter, combined with fast nutrient cycling and a rapid turnover contributed to higher bacterial populations throughout the year. The decomposition of high quality litter, also called „labile“ litter, is often described as a

bacterial energy channel (Wardle and Yeates, 1993; Wardle et al., 2004). On the other hand, the fungal food chain dominates when recalcitrant material with a high C/N ratio predominates the soil input, however, both channels cannot be separated clearly (Scheu et al., 2005).

The positive effect of litter quality on soil fertility was shown for several soil nutrient stocks like Ca, Mg, N and K (Guckland et al., 2009) and for organically bound P (Talkner et al., 2009) in the soils of DL 3. In **deeper soil layers,** at a soil deph of 10 to 20 cm, the PLFA biomass was strongly correlated with edaphic factors, such as the soil N, C and P content. However, soil N and C content were also highly associated with clay content at our study sites. Possibly, higher clay contents in the soils of the mixed forests supported the availability of nutrients for the soil microbial community as well as the growth and spreading of AMF hyphae. Several studies have shown a strong interaction between clay particles and fungal hyphae (Tisdall and Oades, 1979; Dorioz et al., 1993) which can enhance soil aggregation (Rillig et al., 2002). Moreover, higher litter quality also promotes the establishment of large populations of lumbricids, which prefer litter with high Ca concentrations and small C/N ratios (Weland, 2009). A high earthworm activity can increase the formation of macro- and microaggregates (Shipitalo and Protz, 1989; Barois et al., 1993), and stable organo-mineral complexes (Fiedler, 2001) as well as deeper rooting depth (Don et al., 2008). Unfortunately, the soil aggregation on the study plots was not further examined, but the plots of DL 3 demonstrate the interplay between a favourable soil texture, soil fertility and trophic networks leading to higher microbial abundances even in deeper soil layers.

As already implicated, **seasonal changes** are important when considering tree species effects on the soil microbial community and confirm the third main hypothesis of our study. The seasonal comparison demonstrated how strong soil microbes are regulated by easy available C-sources through litter decomposition and exudation in the rooting zone. In autumn an increased supply of easily available C-sources from freshly fallen litter reduced the differences in litter quality. Additionally, the lack of exudates in the rooting zone during this photosynthetically inactive time made the microbial communities more similar. Only significant differences in the abundance of AM fungi between the diversity levels indicated a tree species influence in this season. The PLFA-analsysis of the soil sampling in early summer confirmed our expectations, showing distinct differences between the diversity levels with more direct effects mainly from litter traits on the soil microbial

community. However, we conclude that besides litter trait effects, exudation in the rooting zone also directly affected the soil microbial community during the photosynthetically active time. An increased abundance of Gram-negative bacteria and protozoa on plots of DL 3 in this season supported this assumption. Protozoa are important grazers of soil microbes (Bonkowski, 2004). Nutrients released by roots and consumed by microbes are only temporarily stored in the bacterial biomass, but can be successively mobilized by microfaunal grazing. This effect was clearly observed in a $^{13}C$-labeling experiment, in which saplings of *Fagus* and *Fraxinus* in pots were exposed to labeled $CO_2$ in a greenhouse during the growth period. In the soil sampling taken shortly before litterfall in October, the only microbial group in which the label was still detectable was the group of protozoa (Fig. A19/Appendix). The interactions between plants, microbes and grazers form a loop, often described as a „microbial loop“ (Clarholm, 1994). Grazing can be very important through changing the community structure of the microflora with impacts for organic matter turnover and nutrient cycling (Klironomos et al., 1992).

**Finally,** our study design did not provide the opportunity to prove a tree diversity effect *per se* on the soil microbial community. But it has been shown that some tree characteristics can act as key features in promoting or hampering soil microorgansims. In comparison with other tree species, *Fagus sylvatica* were able to reduce the soil pH most strongly and gave the most recalcitrant litter. Therefore, in comparison to other tree species *Fagus* had the largest effect on the microbial communities in the beech forest stands in this study. Possibly, the dominance of another deciduous tree species with similar acidifying, recalcitrant litter, like *Quercus* species, would have mitigated or masked the effect of *Fagus* on the soil microbial community. Ultimately tree species create their own environment in forest stands with a microbial community that is adapted to this environment.

Beech mixed forests stands with a higher proportion of other deciduous tree species providing litter of higher quality should be favored in forestry. This helps to counteract the acidification of the soil and increases soil fertility. However, forestry should also consider access to deep soil water or ground water when increasing the proportion of tree species in beech forests. If the potential rooting zone is restricted due to water logging, acidification or stony subsoil material, the cultivation of *Fagus* in monospecific stands should be favored alternatively to reduce the danger of severe water shortage during droughts.

**Future research** should strengthen the focus on further understanding the link between biodiversity and ecosystem functioning in forests. There are many open questions concerning complementary species and complementary resource use in high diverse forests. For example, further insights in the question how contribute complementarity to the stabilization of ecosystems (resistance and resilience). Molecular biomarkers also in combination with labelling techniques provide new insights investigating biogeochemical cycles and biotic interactions in ecosystem research. Our study could show that the isotopic content of sedimentary *n*-alkanes provides not only information about past climate, but might also be used to study plant physiological processes over the growing season, and possibly how plants adapt to the current climate change. However, more studies are necessary in order to strengthen the reliability of the *n*-alkanes in different plant species. Furthermore, it should be noted that the greatest limitation of the PLFA-method is the missing informational content at the species level. In future research, we need new techniques to enhance the visibility of microbiological processes in soil. However, it is also essential to combine as many as possible scientific fields/views with each other for understanding the link between biodiversity and ecosystem functioning entirely.

Earth's ecosystems are more and more affected by human impact (Vitousek et al., 1997). Biodiversity research now agrees that a high diversity of species is important and regulates several processes that are essential to the functioning of ecosystems (Cardinale et al., 2011). In view of the fact that "we are changing Earth more rapidly than we are understanding it" (Vitousek et al., 1997) human activity should be legislated to protect and to increase biodiversity in earth ecosystems.

# Chapter 7

## Summary

The increasing loss of biodiversity has contributed to the increased importance and popularity of biodiversity research in the last two decades. To understand the fundamental relationships between biodiversity and ecosystem function, biodiversity experiments have previously been conducted mainly in grassland ecosystems. However, biodiversity in forest ecosystems is more complex and biodiversity experiments are complicated and can run across generations. Comparative studies in natural forest ecosystems offer an alternative, but with several advantages and disadvantages. In Germany, beech forests (*Fagus sylvatica*) represent the primary type of natural forest vegetation. In the past, monocultures of spruces (*Picea*) were preferred in forestry with consequences for biodiversity and stability of these forest ecosystems. A key objective in today's forestry is to increase the proportion of mixed forests with various species of deciduous trees.

The present work pursued the **goal** of investigating if increased proportions of deciduous tree species in beech forest ecosystems lead to a higher abundance of soil microorganisms due to an increased proportion of high quality litter and complementarity effects in the rooting zone. It was assumed that different species-specific tree characteristics (primarily characteristics of leaf and root litter) would directly influence the dynamic of the microbial community, however with differences during the seasons. Furthermore, this study was also focused on investigating whether the observed increase in complementarity effects, including complementary resource use in the narrower sense, with increasing plant diversity in long-term grassland experiments also plays a role in mixed forest ecosystems.

The **study area** was the Hainich National Park, a temperate deciduous forest on loess over limestone in the northwest of Thuringia. Within the framework of the Graduate School 1086 of the German Research Foundation 12 study plots (50 x 50 m) were established in the first phase from 2005 to 2008. The study plots represented a diversity gradient of three levels, each with 4 replicates and a decreasing proportion of *Fagus sylvatica* in the tree species. In the three diversity levels the following tree species or genera were mainly

found: in the first diversity level (DL 1) *Fagus sylvatica*, in the second diversity level (DL 2) *Fagus sylvatica*, *Fraxinus excelsior* and *Tilia* species, and in the third diversity level (DL 3) *Fagus sylvatica*, *Fraxinus excelsior*, *Tilia* species, *Acer* species and *Carpinus betulus*.

In the three studies two molecular biomarkers were applied. For the characterization of soil microbial communities **phospholipid fatty acids (PLFA)** were extracted and analyzed from the upper soil layer in different soil depths. Two sampling campaigns were performed, one in autumn after the litterfall of *Fagus* (middle of Novemver), and one in early summer (early June) when the trees are fully photosynthetically active.
To compare the water use strategy of *Fagus sylvatica* between the three diversity levels ***n*-alkanes** were extracted from sun and shade leaves and their hydrogen isotope ratios (δD) were determined. In addition, the isotope ratios (δD, $\delta^{18}O$) of soil water at two soil depths (10 and 50 cm) and of the water of throughfall were determined and analyzed.

Referring to the four main hypotheses outlined in the introduction it could be stated:

- In species-rich forest ecosystems complementary resource use plays a crucial role in maintaining the coexistence of different trees species. Using the example of a long dry period in summer 2006 it can be concluded that *Fagus sylvatica* was able to extract very deep soil water of at least 70 cm on the investigated plot with the highest tree species diversity. An intensive withdrawal of soil water on plots of DL 3 led to a significant reduction of soil water resources already in spring compared to the forest stands with lower tree species diversity (DL 1 and 2), and illustrates complementary resource use in species-rich forests.
- Faster decomposition rates and higher nutrient contents of high quality litter promote the abundance of microorganisms in the soil. In addition, AM fungi play an important role in the complementary use of soil nutrients, they provide a dense root network and can thus promote microbial communities in the soil.
- In soil depths of 10 to 20 cm, an influence on the microbial biomass (PLFA) by edaphic factors such as pH, clay content and soil nutrient content (C, N and P) was identified. This demonstrates the supportive influence of litter quality on the abundance of microorganisms in the entire upper soil layer.

- The abundance and structure of the microbial communities showed clear differences between the seasons with the largest observed differences between the diversity levels in early summer. This is due to differences in species-specific tree characteristics that affected the microbial community mostly in the photosynthetically active period when decomposition rates and root activity is highest.

The study demonstrated that mixed beech forests with a high proportion of high quality litter promoted higher abundances of microorganisms, and further that the complementary use of resources in tree species-rich mixed beech forests is more pronounced than in monospecific beech forests.

# Chapter 8

## Zusammenfassung

Der zunehmende Verlust an Biodiversität hat dazu beigetragen, dass die Biodiversitätsforschung in den letzten zwei Jahrzehnten an Bedeutung und Popularität gewonnen hat. Zum Verständnis grundlegender Zusammenhänge zwischen Biodiversität und dem Funktionieren von Ökosystemen wurden Biodiversitätsexperimente hauptsächlich in Grasslandökosystemen durchgeführt. Waldökosysteme dagegen sind komplexer und Biodiversitätsexperimente aufwendiger und können generationenübergreifend sein. Vergleichende Studien an naturnahen Wäldern bieten eine Alternative, jedoch mit verschiedenen Vor- und Nachteilen. In Deutschland repräsentieren Rotbuchenwälder (*Fagus sylvatica*) den Hauptteil der potentiell natürlichen Waldvegetation Deutschlands. In der Vergangenheit wurden vorrangig Monokulturen aus Fichten (*Picea*) in der Forstwirtschaft bevorzugt mit Konsequenzen für die Biodiversität und Stabilität der Waldökosysteme. Ein Leitziel in der heutigen Forstwirtschaft ist die Erhöhung des Anteils an Mischwäldern mit verschiedenen Laubbaumarten.

Die hier vorliegenden Arbeit verfolgte das **Ziel** inwieweit sich ein steigender Anteil von Laubbaumarten in Buchenwaldökosystemen positiv auf die Abundanz der mikrobiellen Gemeinschaften im Boden auswirkt unter Bezugnahme eines steigenden Anteils an Streu mit höherer Qualität sowie der Zunahme an Komplementarität im Wurzelraum. Es wurde angenommen, dass verschiedenen Baumarteneigenschaften (vorrangig Merkmale der Blatt- und Wurzelstreu) direkt die Dynamik der mikrobiellen Gemeinschaft beeinflussen jedoch mit saisonalen Unterschieden. Weiterhin sollte untersucht werden, ob die in Langzeit-Grasslandexperimenten festgestellte Zunahme von Komplementarität bzw. im engeren Sinne komplementärer Ressourcennutzung mit steigender Pflanzendiversität auch in Waldökosystemen mit steigender Baumartendiversität eine zunehmende Rolle spielt.

**Das Untersuchungsgebiet** war der Hainich Nationalpark, ein temperater Laubmischwald auf Löss über Muschelkalk im Nordwesten von Thüringen. Im Rahmen des Graduiertenkollegs 1086 der Deutschen Forschungsgemeinschaft wurden in der ersten

Phase im Zeitraum von 2005 bis 2008 12 Untersuchungsflächen (50 x 50 m) eingerichtet. Die Flächen repräsentierten einen Diversitätsgradienten in 3 Stufen mit jeweils 4 Replikaten und einem abnehmenden Anteil an *Fagus sylvatica* im Baumartenbestand. Hierbei waren in den 3 Diversitätsstufen folgende Baumarten bzw. –gattungen hauptsächlich vorzufinden: in der ersten Diversitätstufe (DL 1) *Fagus sylvatica*, in der zweiten Diversitätsstufe (DL 2) *Fagus sylvatica*, *Fraxinus excelsior* und *Tilia* species, und in der dritten Diversitätsstufe (DL 3) *Fagus sylvatica*, *Fraxinus excelsior*, *Tilia* species, *Acer* species und *Carpinus betulus*.

In den Studien kamen zwei molekulare Biomarker zur Anwendung. Zur Charakterisierung der mikrobiellen Gemeinschaften wurden **Phospholipidfettsäuren (PLFA)** aus dem Oberboden in verschiedenen Tiefenstufen extrahiert und analysiert. Es fanden insgesamt 2 Probenahmekampagnen statt, eine im Herbst nach dem Streufall der Buche (Mitte November), und eine im Frühsommer (Anfang Juni) als die Bäume photosynthetisch voll aktiv waren.
Für den Vergleich der Wassernutzungstrategie von Buchen zwischen den 3 Diversitätsstufen wurden ***n*-Alkane** von Sonnen- und Schattenblättern extrahiert und deren Wasserstoffisotopenverhältnisse (δD) bestimmt. Zusätzlich wurden die Isotope des Bodenwassers (δD, $\delta^{18}O$) in zwei Tiefenstufen (10 und 50 cm) und des Kronendurchlasses auf den Untersuchungsflächen bestimmt und ausgewertet.

Bezugnehmend auf die in der Einleitung gestellten vier Haupthypothesen konnte im Ergebnis festgestellt werden:

- In artenreichen Waldökosystemen spielt die komplementäre Nutzung von Ressourcen eine wichtige Rolle um die Koexistenz verschiedener Baumarten zu ermöglichen. Am Beispiel einer langen Trockenperiode im Sommer 2006 konnte geschlussfolgert werden, dass *Fagus sylvatica* im Bestand mit der höchsten Baumartendiversität sehr tiefes Bodenwasser von mindestens 70 cm Tiefe aufgenommen hat. Eine intensive Bodenwasserentnahme in den Beständen von DL 3 führte bereits im Frühjahr zu einer deutlichen Reduzierung der Bodenwasserreserven im Vergleich zu den Beständen mit geringerer Baumartendiversität (DL 1 und 2) und verdeutlicht komplementäre Ressourcennutzung in artenreichen Waldbeständen.

- Schnellere Zersetzungsraten und höhere Nährstoffgehalte von Streu mit hoher Qualität fördern die Abundanz von Mikroorganismen im Boden. Zusätzlich spielen AM-Pilze eine wichtige Rolle in der komplementären Nutzung von Bodennährstoffen, sie ermöglichen ein dichtes Wurzelnetzwerk und können somit mikrobielle Gemeinschaften im Boden fördern
- In Bodentiefen von 10 bis 20 cm konnte ein Einfluss auf die mikrobielle Biomasse (PLFA) von edaphischen Faktoren wie pH-Wert, Tongehalt und Bodennährstoffgehalten (C, N und P) festgestellt werden. Dies verdeutlicht den fördernden Einfluss der Streuqualität auf die Abundanz der Mikroorganismen in der gesamten oberen Bodenschicht.
- Die Abundanz und Struktur der mikrobiellen Gemeinschaften war in beiden untersuchten Jahreszeiten sehr unterschiedlich und zeigte im Frühsommer die größten Unterschiede zwischen den Diversitätsstufen. Dies ist zurückzuführen auf Unterschiede in Baumarteneigenschaften, die am stärksten zur photosynthetisch aktivsten Zeit die mikrobielle Gemeinschaft beeinflussten wenn Zersetzungsraten und Wurzelaktivität am höchsten sind.

Die Studie konnte aufzeigen, dass Buchenmischwälder mit einem hohen Anteil an hoch qualitativer Streu die Abundanz von Mikroorganismen fördert und das die komplementäre Nutzung von Ressourcen in baumartenreichen Buchenmischwäldern ausgeprägter ist als in monospezifischen Buchenwäldern.

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# Appendix

**Table A1:** Tree and site characteristics of the 12 study plots of the three diversity levels (n.a. = not available; l.m. = lost measurement).

| | Diversity Level 1 | | | | Diversity Level 2 | | | | Diversity Level 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | a | b | c | d | a | b | c | d |
| Stem density (n ha$^{-1}$) | 428 | 216 | 220 | 224 | 436 | 532 | 776 | 660 | 392 | 332 | 468 | 484 |
| Sd - *Fagus sylvatica* (n ha$^{-1}$) | 400 | 180 | 220 | 196 | 208 | 316 | 572 | 400 | 12 | 8 | 196 | 64 |
| Sd - *Fraxinus excelsior*(n ha$^{-1}$) | 8 | 0 | 0 | 0 | 60 | 176 | 100 | 160 | 28 | 44 | 76 | 136 |
| Sd - *Tilia sp.* (n ha$^{-1}$) | 12 | 0 | 0 | 8 | 144 | 20 | 84 | 80 | 264 | 212 | 160 | 184 |
| Sd - *Acer sp.* (n ha$^{-1}$) | 8 | 4 | 0 | 20 | 24 | 12 | 20 | 16 | 32 | 24 | 20 | 32 |
| Sd - *Carpinus betulus* (n ha$^{-1}$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 36 | 36 | 16 | 44 |
| Sd - other species (n ha$^{-1}$) | 0 | 32 | 0 | 0 | 0 | 8 | 0 | 0 | 20 | 8 | 0 | 24 |
| Litter total (g DM m$^{-2}$) | 331.2 | 299.2 | 340.4 | n.a. | 388.7 | 429.2 | 355.1 | n.a. | 349.2 | 368.2 | 460.2 | n.a. |
| Litter total „high quality“ (g DM m$^{-2}$) | 24.0 | 1.2 | 0.2 | n.a. | 198.6 | 102.6 | 148.5 | n.a. | 270.3 | 296.9 | 304.7 | n.a. |
| Litter - *Fagus sylvatica* (%) | 92.8 | 91.9 | 99.8 | n.a. | 48.1 | 73.5 | 57.3 | n.a. | 10.0 | 13.1 | 33.7 | n.a. |
| Litter - *Fraxinus excelsior*( %) | 5.3 | 0.0 | 0.0 | n.a. | 20.4 | 19.7 | 22.2 | n.a. | 14.6 | 1.4 | 39.0 | n.a. |
| Litter - *Tilia sp.* (%) | 1.7 | 0.1 | 0.0 | n.a. | 20.3 | 3.4 | 15.7 | n.a. | 47.3 | 35.1 | 15.1 | n.a. |
| Litter - *Acer sp.* (%) | 0.2 | 0.1 | 0.1 | n.a. | 10.4 | 0.8 | 4.0 | n.a. | 7.6 | 8.5 | 6.2 | n.a. |
| Litter - *Carpinus betulus* (%) | 0.0 | 0.1 | 0.0 | n.a. | 0.0 | 0.0 | 0.0 | n.a. | 7.9 | 35.7 | 5.8 | n.a. |
| Litter C/N ratio | 52.9 | 55.9 | 56.0 | n.a. | 41.4 | 46.1 | 42.6 | n.a. | 37.2 | 40.3 | 36.9 | n.a. |
| Litter – C (g DM m$^{-2}$) | 153.4 | 128.6 | 158.2 | n.a. | 174.1 | 191.5 | 159.9 | n.a. | 136.4 | 154.4 | 205.2 | n.a. |
| Litter – N (g DM m$^{-2}$) | 2.9 | 2.3 | 2.8 | n.a. | 4.2 | 4.2 | 3.7 | n.a. | 3.7 | 3.8 | 5.6 | n.a. |
| Litter – P (g DM m$^{-2}$) | 0.1 | 0.1 | 0.1 | n.a. | 0.2 | 0.2 | 0.2 | n.a. | 0.2 | 0.2 | 0.3 | n.a. |
| Litter – K (g DM m$^{-2}$) | 1.9 | 1.6 | 1.9 | n.a. | 2.7 | 2.7 | 2.4 | n.a. | 2.4 | 2.5 | 3.4 | n.a. |
| Litter – Ca (g DM m$^{-2}$) | 5.8 | 4.7 | 5.7 | n.a. | 8.7 | 8.2 | 7.6 | n.a. | 7.9 | 8.2 | 11.1 | n.a. |
| Litter – Mg (g DM m$^{-2}$) | 0.4 | 0.3 | 0.4 | n.a. | 0.8 | 0.7 | 0.7 | n.a. | 0.8 | 0.9 | 1.0 | n.a. |
| Litter remains (%) | 79.2 | 83.8 | 84.0 | n.a. | 53.3 | 67.5 | 57.5 | n.a. | 32.8 | 38.4 | 42.3 | n.a. |
| Litter remains „high quality“ (%) | 1.3 | 0.1 | 0.0 | n.a. | 12.6 | 4.1 | 8.9 | n.a. | 23.1 | 26.7 | 14.0 | n.a. |
| *Lumbricidae* autumn (indiv. m$^{-2}$) | 70.0 | 156.8 | 56.0 | n.a. | 93.3 | 91.0 | 70.0 | n.a. | 126.0 | 182.0 | 151.2 | n.a. |
| *Lumbricidae* early summer (indiv. m$^{-2}$) | 100.8 | 147.0 | 42.0 | n.a. | 172.7 | 116.7 | 112.0 | n.a. | 261.3 | 284.7 | 168.0 | n.a. |
| *Isopoda* autumn (indiv. m$^{-2}$) | 233.3 | 121.3 | 84.0 | n.a. | 406.0 | 485.3 | 201.6 | n.a. | 480.7 | 672.0 | 476.0 | n.a. |
| *Isopoda* early summer (indiv. m$^{-2}$) | 322.0 | 56.0 | 224.0 | n.a. | 238.0 | 312.7 | 210.0 | n.a. | 368.7 | 728.0 | 312.7 | n.a. |
| Soil clay content (%) | 17.5 | 13.6 | 14.7 | 24.4 | 25.2 | 32.8 | 20.3 | 31.3 | 22.8 | 21.6 | 32.3 | 42.2 |
| Soil pH (KCl) in 0 - 5 cm autumn | 3.5 | 3.5 | 3.2 | n.a. | 4.3 | 4.4 | 4.2 | n.a. | 5.3 | 5.3 | 5.1 | n.a. |
| Soil pH (KCl) in 0 - 5 cm early summer | 3.8 | 3.5 | 3.4 | 4.3 | 5.0 | 4.7 | 4.3 | 4.0 | 5.1 | 4.9 | 4.9 | 5.3 |
| Soil C/N ratio 0 - 5 cm autumn | 14.0 | 16.6 | 17.2 | n.a. | 12.4 | 12.5 | 12.9 | n.a. | 13.4 | 12.4 | 12.0 | n.a. |
| Soil C/N ratio 0 - 5 cm early summer | 13.4 | 15.8 | 17.0 | 13.0 | 12.4 | 12.2 | 13.0 | 14.8 | 13.2 | 12.7 | 12.4 | 12.5 |
| Soil stock-C (kg ha$^{-1}$) | 2.2 | 3.2 | 3.3 | 2.7 | 3.0 | 3.6 | 2.9 | 3.8 | 2.2 | 3.2 | 5.0 | 4.1 |
| Soil stock-N (kg ha$^{-1}$) | 0.14 | 0.18 | 0.19 | 0.18 | 0.21 | 0.27 | 0.22 | 0.29 | 0.24 | 0.22 | 0.34 | 0.29 |
| Soil stock-Ca (kg ha$^{-1}$) | 271.0 | 171.6 | 250.3 | 1352.2 | 1634.1 | 2799.7 | 1152.7 | 2288.4 | 1469.3 | 1340.3 | 4591.4 | 5787.8 |
| Soil stock-Mg (kg ha$^{-1}$) | 28.1 | 20.6 | 22.7 | 66.2 | 83.2 | 125.0 | 85.6 | 137.7 | 200.7 | 232.1 | 197.9 | 360.9 |
| Total PLFA biomass (nmol g$^{-1}$) autumn 2005 | 105.6 | 46.7 | 44.4 | n.a. | 61.6 | 19.5 | 72.9 | n.a. | 143.7 | 74.9 | 110.8 | n.a. |
| Total PLFA biomass (nmol g$^{-1}$) early s. 2006 | l.m. | 36.6 | 29.6 | 40.6 | 46.3 | 44.2 | 38.4 | 39.9 | 72.6[1] | 104.0 | 201.4 | 199.8 |

Data on stem density (tree individuals >7 cm dbh) and litter amounts from Jacob et al. (2010a). Litter nutrients are calculated based on data from Jacob et al. (2009). Data on clay content and soil nutrient stocks (soil depth 0 - 10 cm) from Guckland et al. (2009). Litter remains based on litter bag data sampled in June 2006 after 7 months of decomposition of the total fallen litter of *Fagus*, *Fraxinus*, *Tilia*, *Acer* and *Carpinus* in the year 2005, Litter remains „high quality“ are litter remains without *Fagus* litter, calculation based on data from Jacob et al. (2010b). Data on decomposer macrofauna abundances (sampled in spring on 11/05/05 and in autumn on 23/11/05) from Weland (2009). For the data on fine root biomass and fine root necromass (sampled between june 2005 and june 2006 in soil depth 0 – 10 cm) see Meinen et al. (2009a). Data on soil pH and soil C/N were measured on the soil samples for PLFA analysis.
[1] Values for saturated PLFA were discarded and calculated from related plots (DL 3b-d).

**Table A2:** Coordinates and features of the sampled beech trees in 2006. (BHD = breast height diameter)

| Plot | Tree-ID | x | y | Tree height | Crown base | First branch | BHD |
|---|---|---|---|---|---|---|---|
| DL 1a | Bu 7 | 4395363.0 | 5661830.2 | 36.9 | 20.5 | 13.1 | 58.4 |
| DL 1a | Bu 15 | 4395369.1 | 5661826.1 | 35.3 | 15.3 | - | 69.6 |
| DL 1a | Bu 32 | 4395368.3 | 5661802.0 | 30.9 | 11.6 | - | 48.2 |
| DL 1a | Bu 90 | 4395342.4 | 5661815.3 | 32.8 | 20.0 | 16.7 | 41.4 |
| DL 1a | Bu 94 | 4395377.3 | 5661796.8 | 32.6 | 17.8 | 16.4 | 47.8 |
| DL 2c | Bu 67 | 4395490.6 | 5661717.1 | 32.8 | 12.8 | 12.5 | 41.4 |
| DL 2c | Bu 103 | 4395470.8 | 5661714.7 | 24.0 | 15.5 | 11.0 | 37.6 |
| DL 2c | Bu 104 | 4395473.8 | 5661720.9 | 30.2 | 14.9 | 12.1 | 39.2 |
| DL 2c | Bu 143 | 4395475.4 | 5661745.2 | 29.0 | 14.0 | 6.3 | 42.7 |
| DL 2c | Bu 175 | 4395509.8 | 5661699.6 | 27.5 | 11.7 | 8.8 | 40.7 |
| DL 3a | Bu 1 | 4396662.7 | 5662585.5 | 30.6 | 14.4 | 10.6 | 58.4 |
| DL 3a | Bu 3 | 4396656.9 | 5662574.9 | 27.5 | 11.3 | - | 51.2 |
| DL 3a | Bu 4 | 4396641.9 | 5662624.7 | 30.0 | 14.1 | 12.0 | 37.1 |
| DL 3a | Bu 31 | 4396663.3 | 5662639.3 | 29.8 | 14.7 | - | 64.5 |
| DL 3a | Bu 34 | 4396635.3 | 5662635.8 | 28.6 | 10.7 | 9.2 | 58.2 |

Data (unpublished) provided by Mascha Brauns.

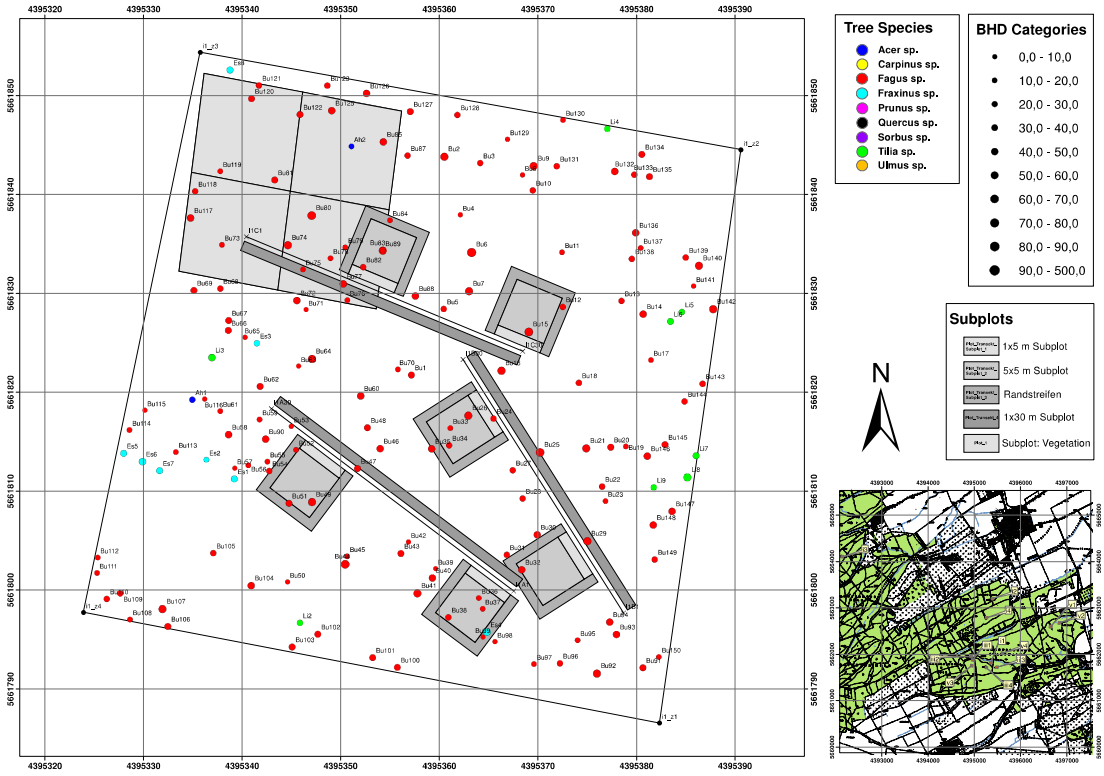


**Fig. A1:** The distribution of tree species and transects on plot DL 1a (made by M. Daenner).

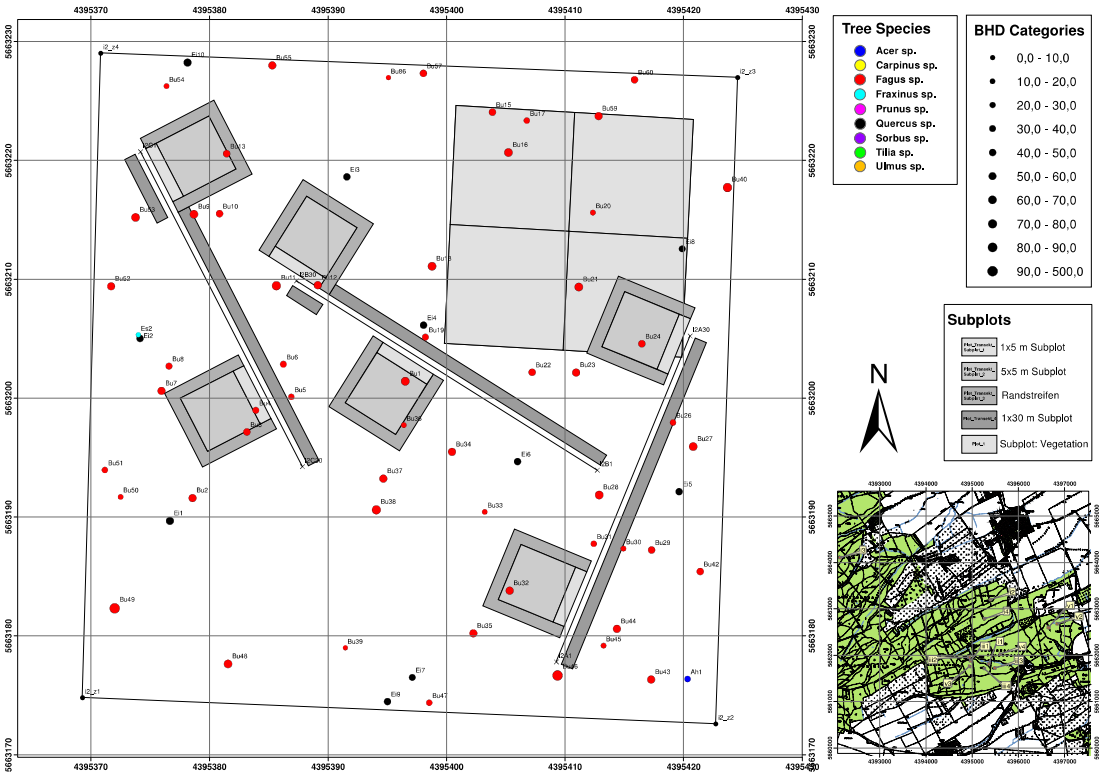


**Fig. A2:** The distribution of tree species and transects on plot DL 1b (made by M. Daenner).

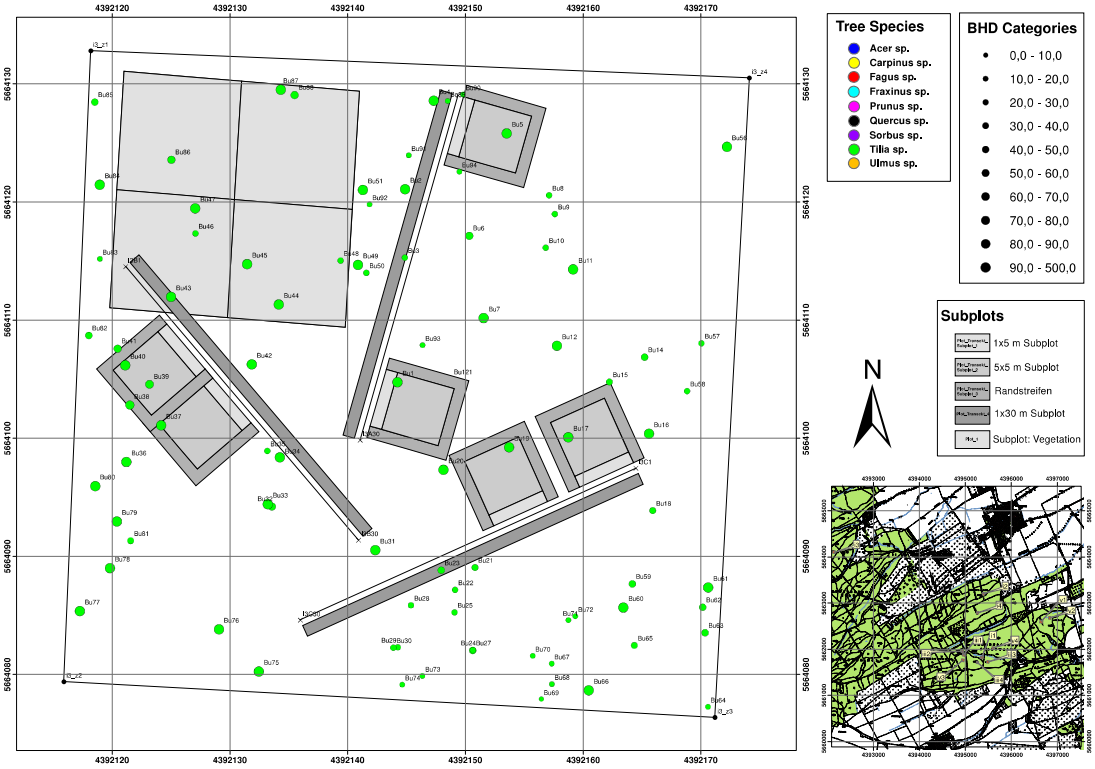


**Figure A3:** The distribution of tree species,and transects on plot DL 1c (made by M. Daenner). Error in the color, the trees have to be red indicating *Fagus* sp.

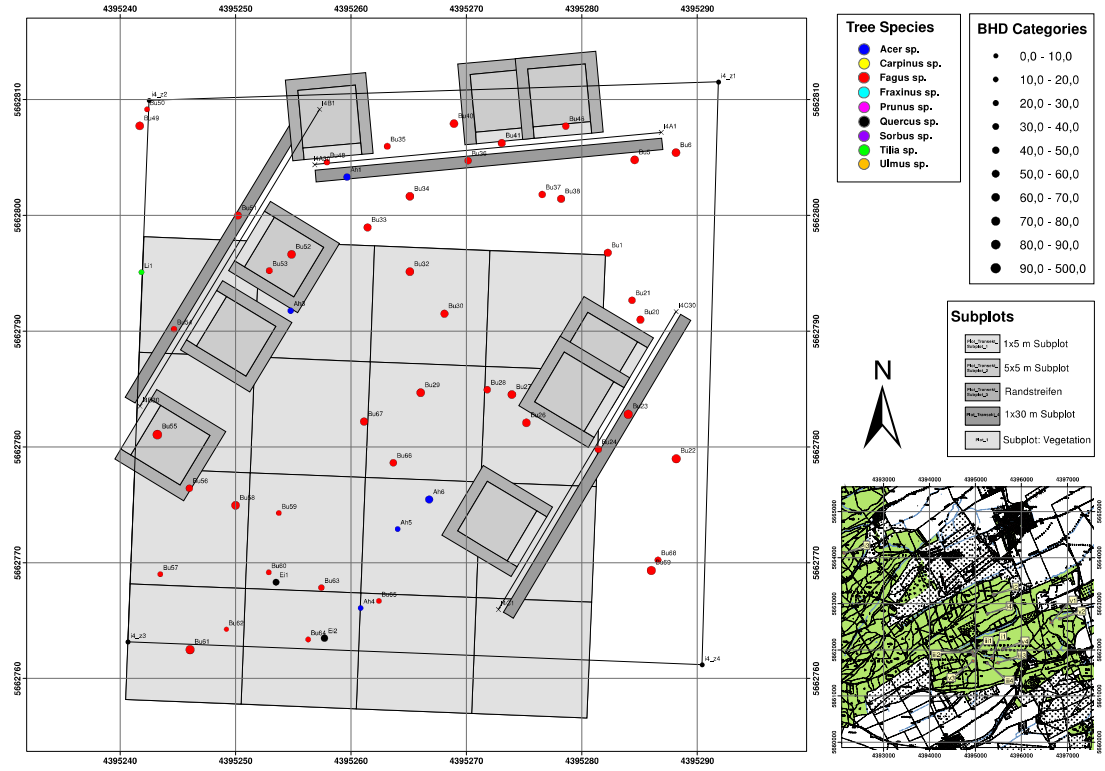


**Figure A4:** The distribution of tree species and transects on plot DL 1d (made by M. Daenner).

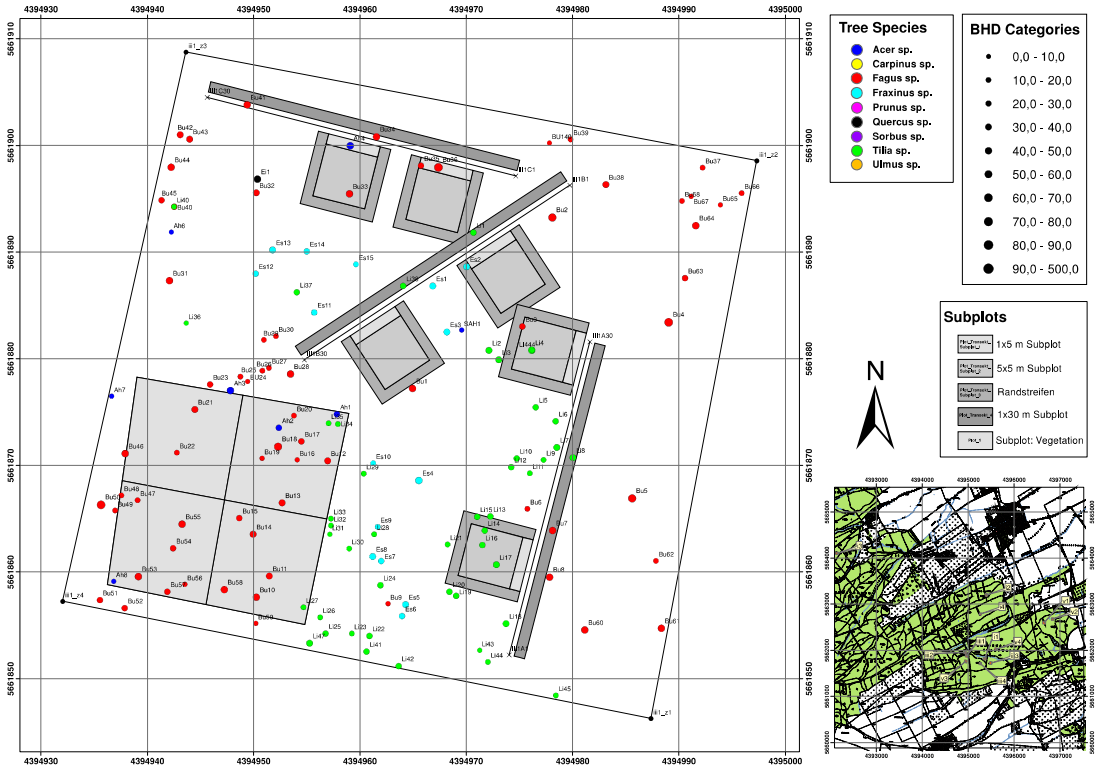


**Fig. A5:** The distribution of tree species,and transects on plot DL 2a (made by M. Daenner).

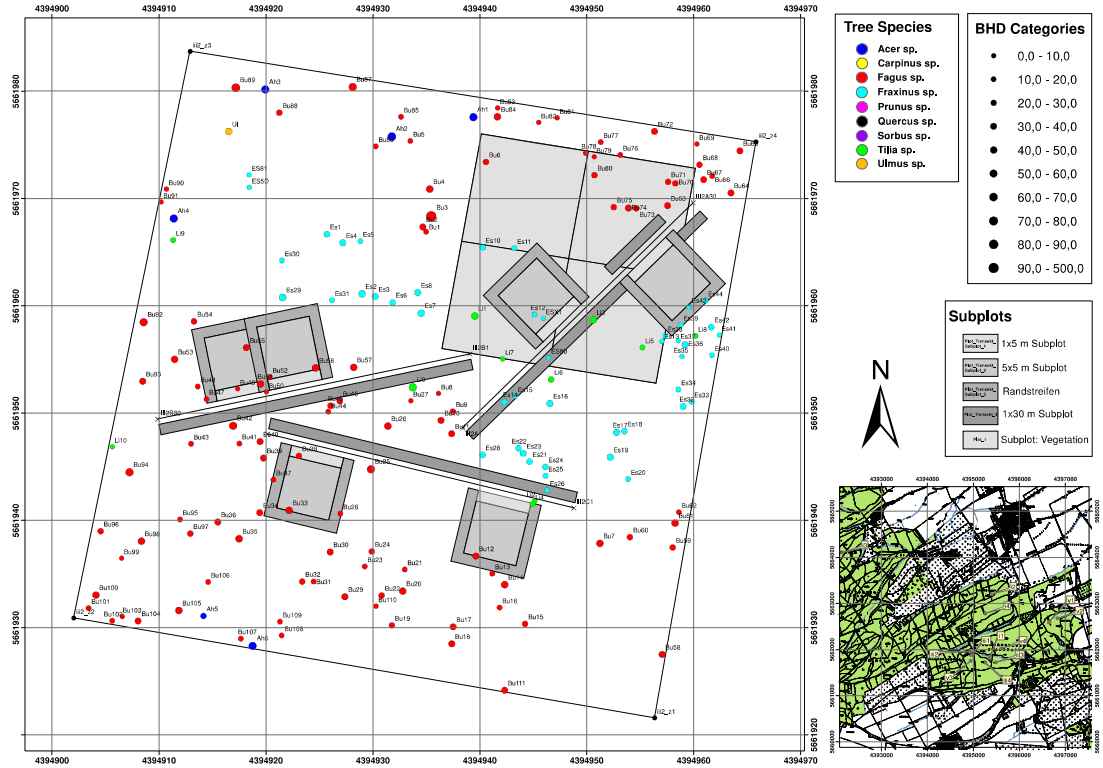


**Fig. A6:** The distribution of tree species and transects on plot DL 2b (made by M. Daenner).

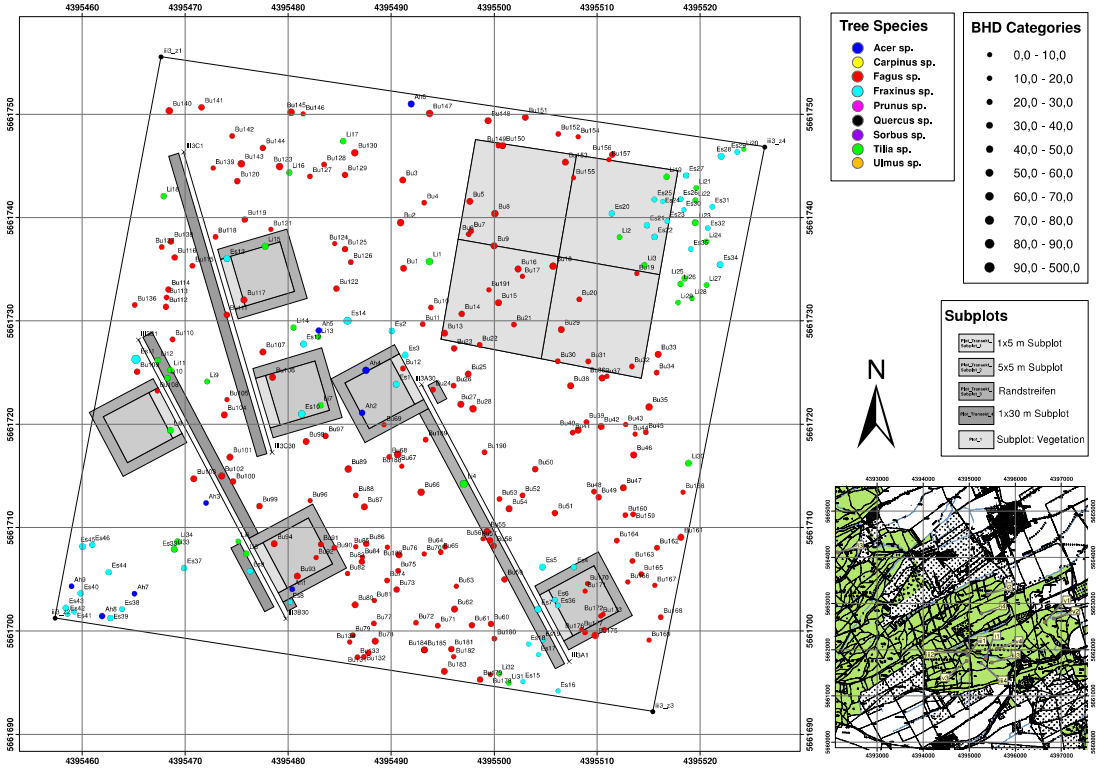


**Fig. A7:** The distribution of tree species,and transects on plot DL 2c (made by M. Daenner).

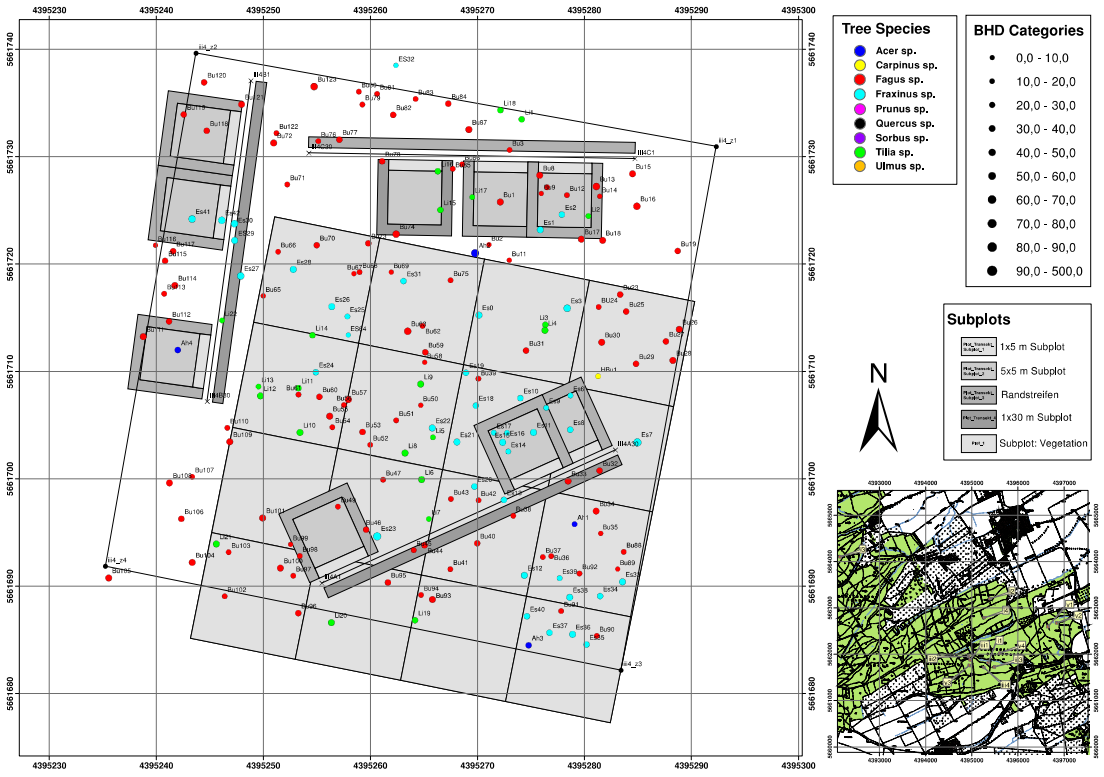


**Fig. A8:** The distribution of tree species and transects on plot DL 2d (made by M. Daenner).

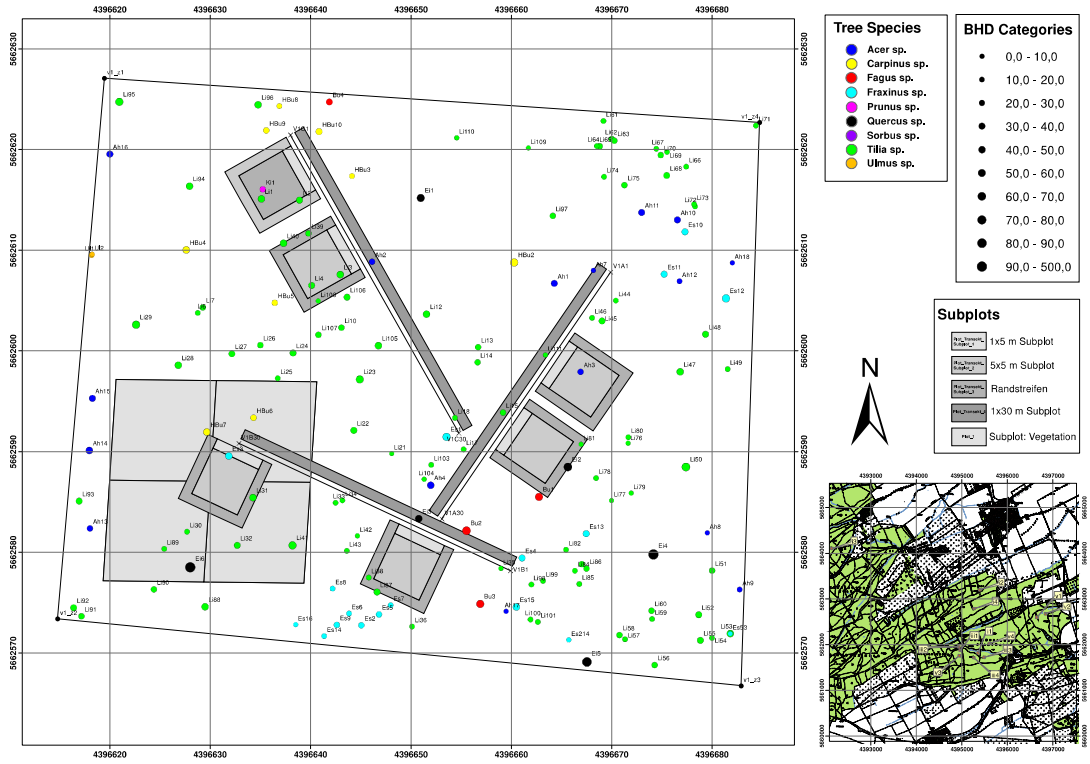


**Fig. A9:** The distribution of tree species,and transects on plot DL 3a (made by M. Daenner).

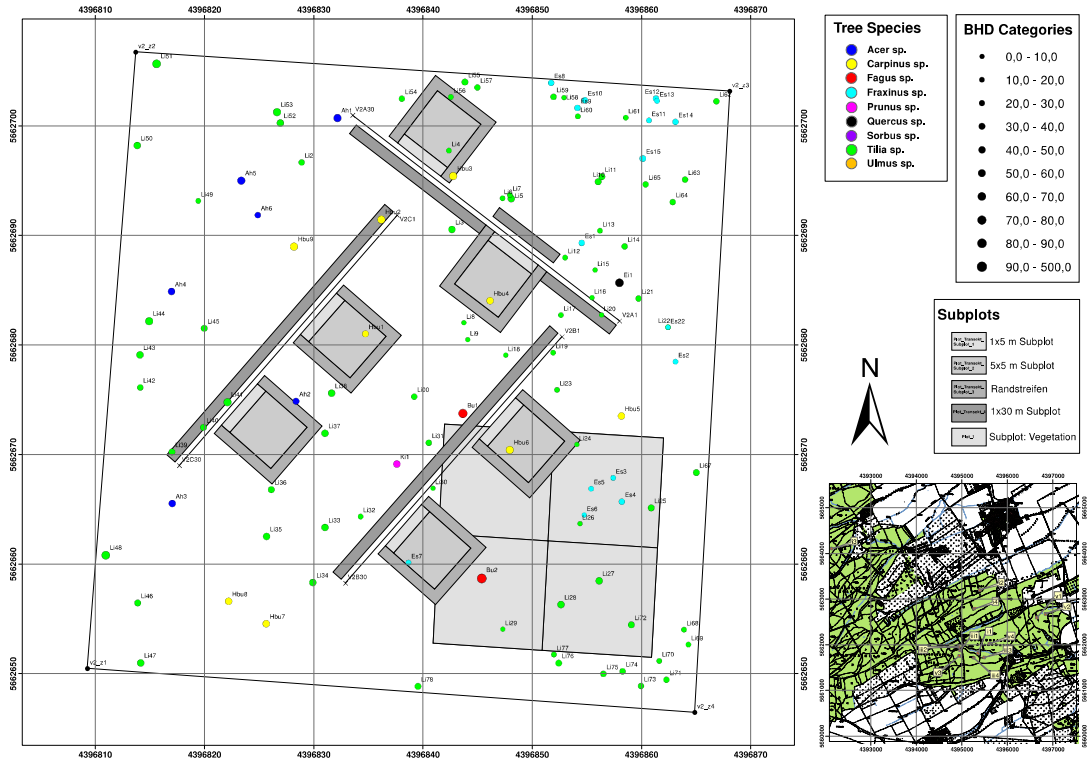


**Fig. A10:** The distribution of tree species and transects on plot DL 3b (made by M. Daenner).

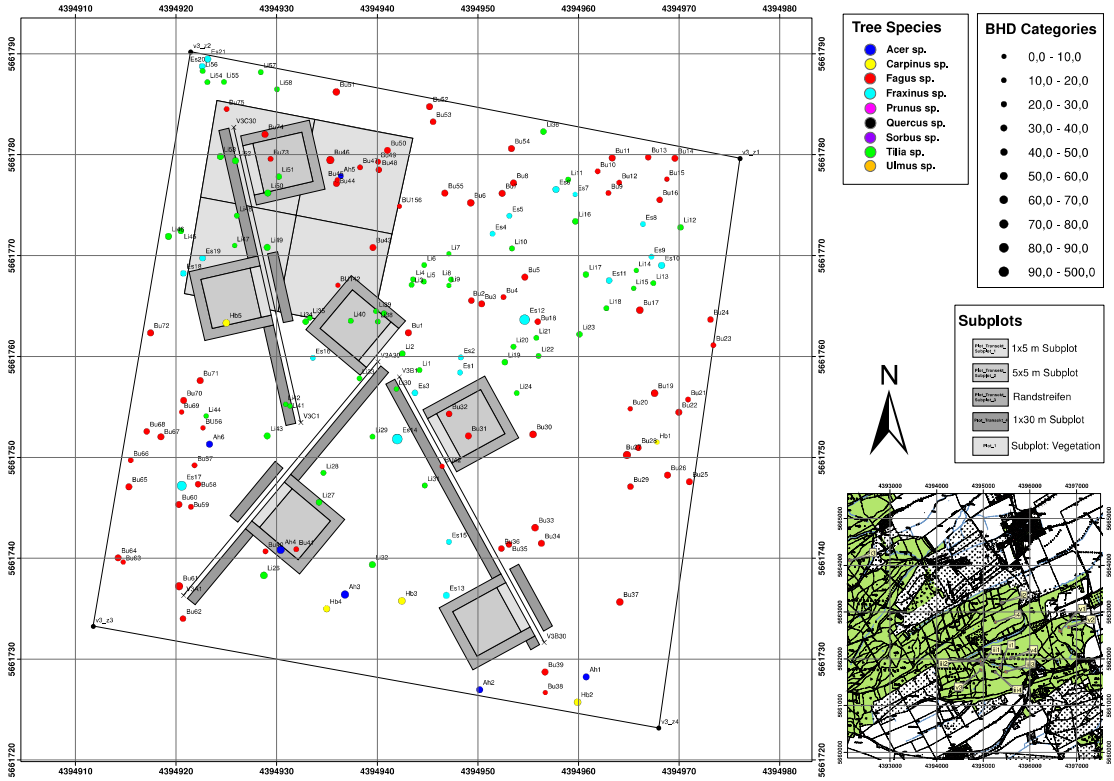


**Fig. A11:** The distribution of tree species,and transects on plot DL 3c (made by M. Daenner).

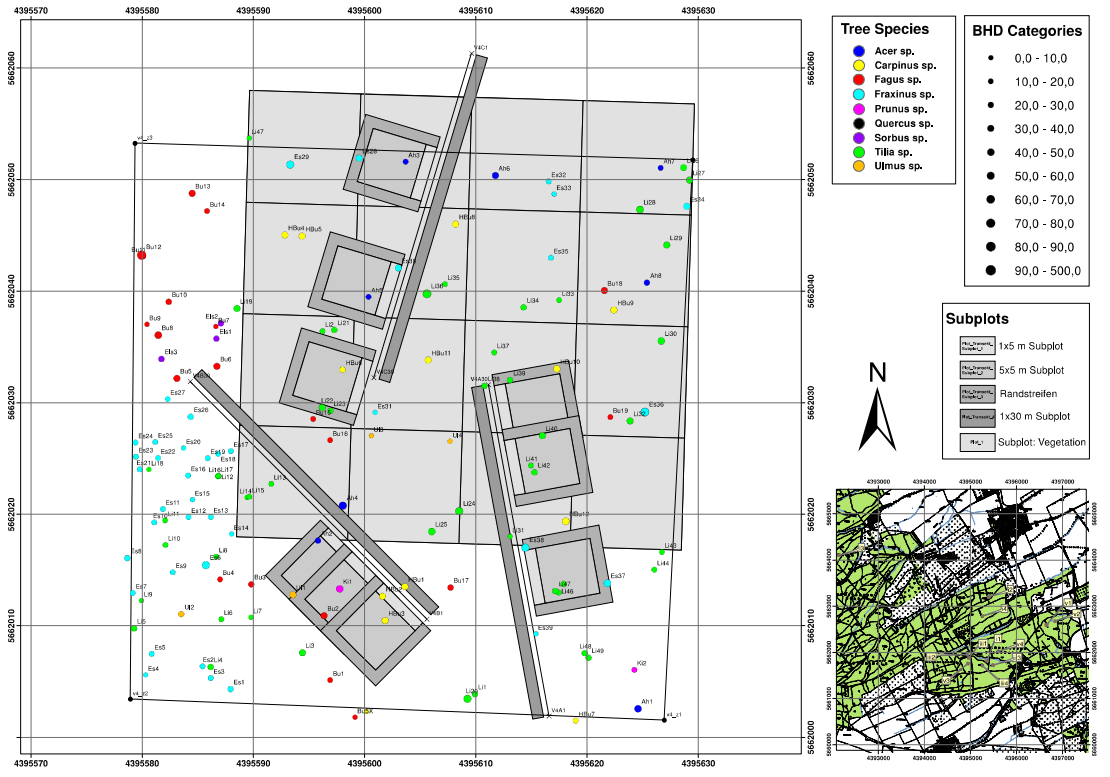


**Fig. A12:** The distribution of tree species and transects on plot DL 3d (made by M. Daenner).


**Fig. A13:** Transect B of plot DL 1a in June 2005.


**Fig. A14:** Transect B of plot DL 2a in June 2005.


**Fig. A15:** Transect B of plot DL 3c in June 2005.

**Fig. A16:** Soil sampling in a subplot of plot DL 3c in November 2005.

**Fig. A17:** Removel of soil from the split tupe in November 2005.

**Fig. A18:** Soil sampling on June 1, 2006.

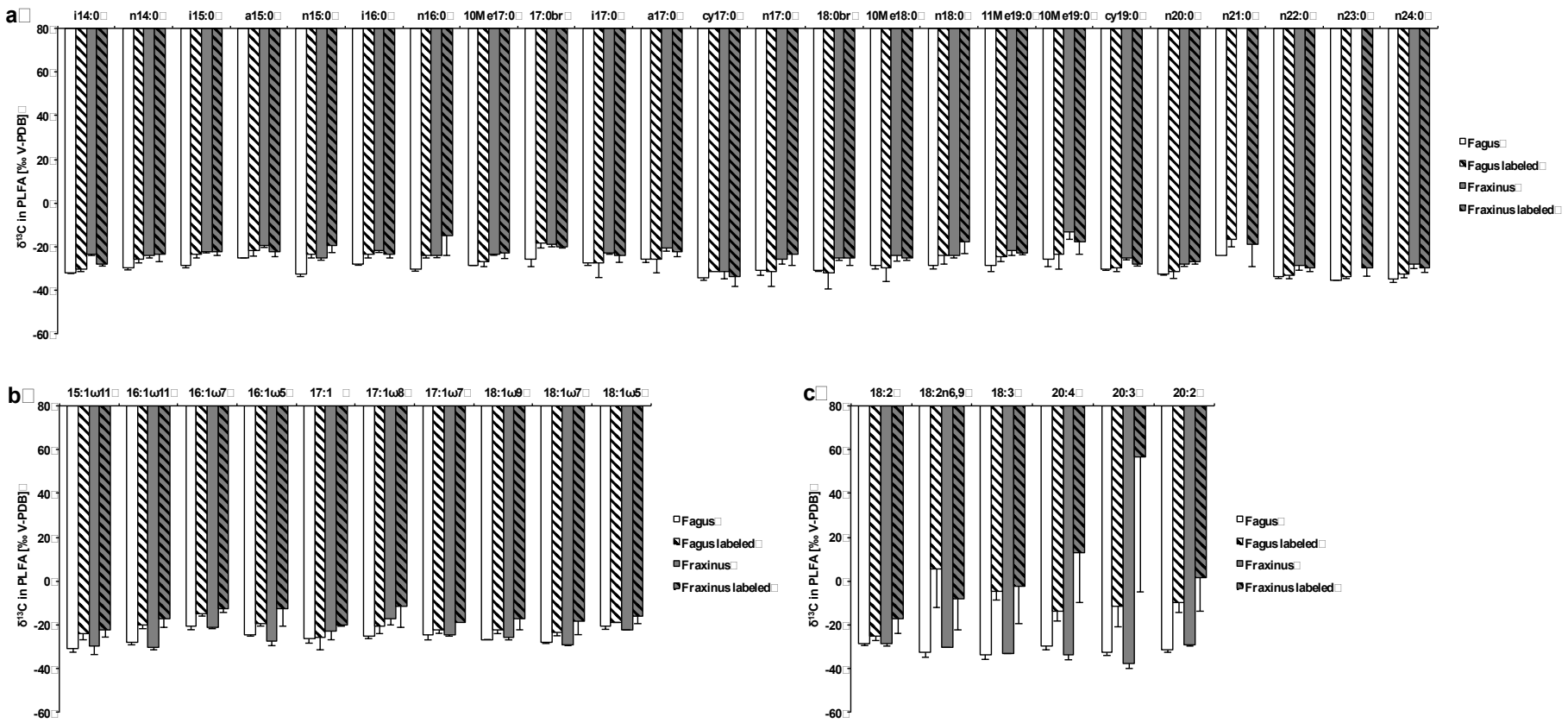


**Fig. A19:** $\delta^{13}C$ values (mean ± stdv; n = 4) in identified PLFA divided in a) saturated fatty acids (SATFA), b) monounsaturated fatty acids (MUFA), c) polyunsaturated fatty acids (PUFA). The several columns represent the replicates differing in treatment and tree species, respectively. Eight saplings of each beech (*Fagus sylvatica*) and ash (*Fraxinus exelsior*) were gouged from the Hainich National Park, transferred into pots (approximately 30 cm in diameter; one plant per pot) and placed in a greenhouse from July until October 2008. Four pots of each tree species were grown under unlabeled conditions and served as control. The other four pots of each tree species were grown under labeled conditions with $^{13}C$-labelded $CO_2$. Soil sampling was conducted after trashing the pots just before litter fall in the middle of October 2008.

## Authors' contributions to the manuscripts:

**Manuscript 1:**

Thoms, C., Gattinger, A., Jacob, M., Thomas, F.M., Gleixner, G., 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest, *Soil Biology & Biochemistry* 42, 1558-1565.

• **Carolin Thoms** is the first author. She carried out the soil sampling and the laboratory work including PLFA extraction and the measurements on the GC and the GC-MS system. She evaluated the data statistically and wrote the paper.

• **Dr. Andreas Gattinger** actively contributed to the identification of PLFA after GC-MS measurements. He gave many suggestions for PLFA analyses and interpretation.

• **Dr. Mascha Jacob** provided the data on litter amounts and made several corrections to the manuscript drafts.

• **Prof. Frank Thomas** was the supervisor of M. Jacob and made several corrections to the manuscript drafts.

• **apl. Prof. G. Gleixner** is responsible for initiating the study, for the sampling design and gave general objectives. He reviewed the manuscript and made several corrections to the drafts.

**Manuscript 2:**

Thoms, C., Gleixner, G. Seasonal differences in tree species' influence on soil microbial communities, in review.

• **Carolin Thoms** is the first author. She planned and carried out the soil sampling, supervised (due to pregnancy) the PLFA extraction in the laboratory and made the measurements on the GC and the GC-MS. She combined the datasets of other projects of the graduate school for statistical analyses to interpret the PLFA data. She evaluated the datasets statistically and wrote the paper.

• **apl. Prof. G. Gleixner** gave general objectives. He critically reviewed the manuscript and made many corrections to the drafts.

**Manuscript 3:**

Thoms, C., Hölscher, D., Gleixner, G. Evidence for deep soil water uptake by *Fagus sylvatica* L. in a temperate mixed forest from hydrogen isotopes of leaf lipids after summer drought, in progress.

• **Carolin Thoms** is the first author. She is responsible for the research idea. She acquired the water samples (throughfall, soil water) and leaf samples for isotopic measurement. She performed the extraction of *n*-alkanes in the laboratory and made the measurements on the GC and the GC-MS system. She evaluated the data statistically and wrote the paper.

• **Prof. Dirk Hölscher** provided the samples from throughfall measurements. He made some corrections to the manuscript draft and will give further suggestions for submission.

• **apl. Prof. G. Gleixner** gave suggestions for the general objectives. He already made several corrections on the drafts.

## Lebenslauf - Curriculum vitae

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**Persönliche Daten**

| | |
|---|---|
| Name: | Carolin Thoms (geb. Fornaçon) |
| Geburtsdatum: | 28. 04. 1980 |
| Geburtsort: | Halle (Saale) |
| Staatsangehörigkeit: | deutsch |
| Familienstand: | verheiratet, 2 Kinder |

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**Ausbildung**

| | |
|---|---|
| Juli 1998 | Abitur am Albert-Schweitzer-Gymnasium, Halle (Saale) |
| Okt 1998 - Jan 2005 | Studium der Geographie an der Martin-Luther-Universität Halle-Wittenberg, Nebenfächer: Geologie and Bodenkunde |
| 25. Januar 2005 | Diplom (1.5), Thema der Diplomarbeit: „Vergleich landwirtschaftlich genutzter Flächen mit Aufforstungsflächen unterschiedlichen Alters und Bestandes bezüglich der Schwermetalldynamik im Wirkungsbereich der ehemaligen August-Bebel-Hütte in Helbra“ |
| seit Mai 2005 | Dissertation am Max-Planck-Institut für Biogeochemie in Jena, finanziert von der Deutschen Forschungsgemeinschaft innerhalb des Graduiertenkollegs 1086 “Die Bedeutung der Biodiversität für Stoffkreisläufe und biotische Interaktionen in temperaten Laubwäldern”. |

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**Familie**

| | |
|---|---|
| Apr 2007 - Mai 2008 | Elternzeit |
| Jan 2010 - Apr 2011 | Elternzeit |

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**Internationale Konferenzen**

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|---|---|
| April 2009 | *European Geosciences Union*, Wien, Östereich<br>**Vortrag:** „Effects of tree diversity on the soil microbial community in three soil depth in a Central European beech forest“. |
| Juli 2012 | *Eurosoil, Bari, Italien*<br>**Poster:** „Seasonal impact of plant traits and site characteristics on soil microorganisms in a temperate deciduous forest with different abundance of European beech (*Fagus sylvatica* L.)”. |

**Veröffentlichungen**

(1) **Fornaçon, C.**, Srugies, A., Hartmann, K.-J. (2002). Zur standortkundlichen Kennzeichnung von Bodentypen in Landschaften des Schwarzerdegebietes. *Hallesches Jahrb. Geowiss.*, 24, 89-96.

(2) Sauerwein, M., **Fornaçon, C.** (2002). Geoökologische Kartierung und Verwendung von Stadtstrukturtypen in Halle (Saale). *Hallesches Jahrb. Geowiss.*, 24, 29-40.

(3) **Fornaçon, C.**, Frühauf, M., Schumann, H. (2005). Beitrag zur Schwermetalldynamik auf aufgeforsteten Standorten im Einflussbereich der ehemaligen August-Bebel-Hütte in Helbra (Mansfelder Land). *Hercynia N. F.*, 38, 197-207.

(4) **Thoms, C.**, Gattinger, A., Jacob, M., Thomas, F.M., Gleixner, G. (2010). Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. *Soil Biology & Biochemistry*. 42, 1558-1565.

Jena, den 26. 03. 2013

**Selbständigkeitserklärung**

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

Jena, den 26. 03. 2013

Carolin Thoms