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FINE ROOT TRAITS, BELOWGROUND INTERACTIONS AND COMPETITION EFFECTS ON THE RHIZOSPHERE OF *FAGUS SYLVATICA* AND *FRAXINUS EXCELSIOR* SAPLINGS

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"Look deep into nature,

and then you will understand everything better."

Albert Einstein

Summary

European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) are common tree species in Central European forests and of high ecological as well as economic value. However, knowledge about the structure and function of the ecologically important fine root system of beech and ash and its impact on rhizosphere processes is scarce. Moreover, little is known about the direct intra- and interspecific belowground competition effects of these two species. This thesis presents results from different greenhouse experiments on the species-specific effects of beech and ash saplings on key belowground dynamics as well as their root competition effects. The main objective was to disentangle species-specific effects from competition/biodiversity effects on rhizosphere and fine root properties.

In a competition experiment with saplings of beech and ash grown in different rhizobox-treatments (monoculture, mixture or single plant) we investigated morphological, C/N and $\delta^{13}C$ responses in the fine root system employing a root order-related analysis. We observed large differences in various root traits between the root order classes 1 to 4, which underscores the ecological significance of the position of roots in the root system, e.g. 1st order roots, i.e. root tips, had significantly higher specific root areas and contributed to 65-70% to the total length of the analysed root segments. While the species-specific fine root characteristics of beech and ash were obvious, no major root morphological or chemical (nitrogen concentration, C/N ratio) alterations in response to competition were found. This partly contradicts observations in mature stands, where fine roots of beech were shown to act very plastic in changing their specific root length in a competitive environment. Thus, adaptive root responses to competition may not be a universal phenomenon and are likely to vary with site conditions, species and plant age. In contrast to the fairly unaffected root morphological and chemical traits, fine root survival, which was analysed by sequential digital imaging of root growth through a root window, showed significant differences between competition treatments and species. Competition with conspecific or allospecific roots altered root longevity in both directions, either toward a shorter lifespan or

higher longevity. Mean root lifespan differed significantly among species with higher fine root longevity in ash and also depended on competition treatment. Fine root mortality increased in beech roots grown in mixture with ash and in beech monoculture compared to beech plants grown in isolation. Ash fine roots apparently profited from the presence of beech roots, while beech root growth and survival were negatively affected by ash. These results indicate size-asymmetric belowground competition. Thus, competition represents an important force influencing the fine root lifespan of beech and ash saplings.

In a rhizotron experiment with beech and ash saplings we investigated root-induced trace gas fluxes, microorganisms and root exudations. The results showed species-specific as well as root biomass effects on greenhouse gas fluxes. The CH_4 uptake of the soil planted with ash was higher and the N_2O emissions were lower than from soil under beech. In contrast, the CO_2 efflux was much higher in beech than in ash, although root biomass was smaller than that of ash. Thus, soil biological activity is not only quantitatively affected by root biomass, but also qualitatively related to the species identity of the tree. This qualitative effect is also supported by the findings of the species differences in the composition and concentration of organic acids measured in the closest proximity of fine roots. We additionally observed species-specific effects on soil microorganisms and total soil carbon content. In particular, the fine roots of beech altered carbon dynamics in the soil by reducing soil pH and thus decreasing the carbon use efficiency of bacteria, while more leaf litter-derived carbon was channeled into higher trophic levels in the presence of ash.

The last experiment dealt with the incorporation of plant carbon and microbial nitrogen into the rhizosphere food web of beech and ash. We conducted a 5-month $^{13}CO_2$ greenhouse labeling experiment to follow the flux of carbon from plant shoots to the rhizosphere and into the soil animal food web. In parallel, we used ^{15}N labeled mineral nitrogen to trace the flux of nitrogen via saprotrophic microorganisms and mycorrhiza into the soil animal food web. The litter and soil were minimally enriched in ^{15}N and ^{13}C whereas fine roots of beech and ash were highly enriched. Maximum values of ^{13}C were observed in the ectomycorrhiza of beech. The isotopic signature of soil animals was low, suggesting that the studied

animal species did not exclusively feed on mycorrhizal fungi. Furthermore, the isotopic signature of the soil animals did not significantly vary between beech and ash.

Overall, the comparison of the roots and rhizosphere of beech and ash and their interactions indicate that tree species identity needs to be considered in competition and biodiversity studies in the field. Further research on specific fine root traits and rhizosphere dynamics is needed for more tree species to predict realistic carbon models.

LIST OF ABBREVIATIONS

a.s.l.	above sea level
AM	arbuscular mycorrhiza
AMF	arbuscular mycorrhizal fungi
BAS	basal respiration
ANOVA	analysis of variances
CA	competition ability
CEC	cation exchange capacity
CH_4	methane
CO_2-eq	CO_2 equivalents
C_{mic}	microbial biomass
C_{org}	organic carbon
dw	dry weight
EM	ectomycorrhiza
EMF	ectomycorrhizal fungi
GC	gas chromatography
GHG	greenhouse gas
GLM	general linear model
HR	hazard ratio
MIRR	maximum initial respiratory response
N_2O	dinitrogen oxide
N_{total}	total nitrogen
PLFA	phospholipid fatty acid
qO_2	microbial specific respiration (BAS/C_{mic})
RCA	relative competition ability
RTD	root tissue density
SOM	soil organic matter
SRA	specific fine root area
SRL	specific fine root length

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CHAPTER

1

General Introduction

1.1 Fine roots and the rhizosphere

Root and rhizosphere research has long been neglected in plant science, owing to the difficult accessibility of the root system and methodological limitations in analysing root traits. It is not surprising that the understanding of belowground dynamics is still limited compared with the knowledge of aboveground vegetation factors (Schenk & Jackson, 2002). Roots are not without reason described as the 'hidden half' of plants (Waisel et al., 2002).

Nonetheless, the importance of the belowground plant organs has been known for a long time. For example, Weaver (1935) investigated several grasses in prairie ecosystems and found that they produced more organic matter belowground than aboveground; Dittmer (1937) excavated the roots and root hairs of a single rye plant (*Secale cereale*) and measured a total length of 11,000 km. Roots have multiple functions: they absorb and transport water and nutrients, anchor the plant in the ground, store carbohydrates and exchange material with mycorrhizal symbionts and microbes.

Beside these principal functions of anchorage, nutrient and water uptake, fine roots (conventionally defined as less than 2 mm in diameter) play an important role in soil carbon accumulation and in the regulation of biogeochemical cycles (Matamala et al., 2003; Norby et al., 2004; Fan & Guo, 2010). The recent climate change discussion and the rising awareness about carbon sinks in the soil have increased motivation for conducting research on the belowground dynamics of trees (Hertel & Leuschner, 2002; Pan et al., 2011; Reich, 2011). Forest ecosystems are estimated to contain about 80% of the aboveground and 40% of belowground terrestrial carbon (Dixon et al., 1994; McKinley et al., 2011). Hence, carbon dynamics in forest soils are increasingly recognized in the context of climate change mitigation as a consequence of increased atmospheric CO_2 (Gill & Jackson, 2000).

Furthermore, soil carbon pools contain considerably more carbon than the atmospheric pool (3.3 times) and the aboveground biomass (4 times) (Fitter, 2005; Lal, 2005; Hyvönen et al., 2007) and plenty of that is accumulated by fine roots (Richter et al., 1999). It is assumed that about 33% of the global net primary

production is directly provided to fine root production and functioning (Jackson et al., 1997).

One key pathway for carbon into soil is fine root turnover (i.e. growth and death of fine roots over a certain time period) and turnover of the associated mycorrhiza and their hyphae (Godbold et al., 2006; Brunner & Godbold, 2007). Due to their relatively short lifespan and therefore rapid production, senescence and decomposition, fine roots contribute significantly to soil carbon fluxes and nutrient cycling (Nadelhoffer et al., 1985; Joslin & Henderson, 1987; Hendrick & Pregitzer, 1993). The longevity of fine roots varies depending on the environmental conditions and species (Eissenstat & Yanai, 1997). The effects of drought (Gaul et al., 2008b; Meier & Leuschner, 2008b; Brunner et al., 2009), frost (Gaul et al., 2008a), ozone (Mainiero et al., 2009; Nikolova et al., 2010), nitrogen addition (Johnson et al., 2000; Rasse, 2002; Phillips et al., 2006) and elevated CO_2 (Iversen et al., 2008; Stover et al., 2010) on fine root survival have been subject to numerous studies; however, direct interaction effects between tree species have yet not been investigated (see section 1.3 and chapter 3).

Another channel of carbon release from fine roots to the soil is root exudation and root respiration (Norby et al., 1987; Matamala et al., 2003). Recently, root exudation was shown to strongly affect microbial activity in the rhizosphere (Pollierer et al., 2007; Pollierer et al., 2012) since carbon from rhizodeposits contains easily convertible amino acids, sugars and peptides and is thereby more available for soil organisms than the recalcitrant carbon from plant leaf litter (Bais et al., 2006; Dennis et al., 2010). Due to experimental constraints, root respiration is hard to distinguish from other carbon efflux sources in the soil (Hanson et al., 2000). Hence, estimations of the contribution of root respiration to total soil respiration vary enormously (Subke et al., 2006).

The rhizosphere, as a soil region surrounding the living fine roots, is a biologically, chemically and physically active region. Its effects extend far beyond the rhizosphere itself by influencing biogeochemical cycles, soil biota and plants (Cardon & Whitbeck, 2007). Consequently, more research on the species-specific as well as interaction effects of different tree species on the rhizosphere and key

functional root traits is crucial in order to better understand the belowground parts of plants and predict the impact of global environmental change.

1.2 Belowground competition as root structuring force

While aboveground plant organs compete for light, roots compete for soil resources. The reactions of a root system to the presence of neighbouring roots are versatile and include both avoidance (Schenk et al., 1999) and aggregation (Bartelheimer et al., 2006; Semchenko et al., 2007). Competition between adjoining plant species has been a controversial subject of numerous studies throughout the last decades (Winget and Koklowski, 1965; Weiner, 1990; Tilman, 1994; Grace, 1995; Goldberg, 1996; Holmgren et al., 1997; Schwinning and Weiner, 1998; Coomes and Grubb, 2000; Grams et al., 2002; Kozovits et al., 2005). However, belowground competition has been discussed to a lesser extent (Caldwell, 1987; Wilson, 1988; Casper and Jackson, 1997; Cahill and Casper, 2000; Leuschner et al., 2001; Zak et al., 2007; Rewald and Leuschner, 2009; Simon et al., 2010; Kalliokoski et al., 2010; Brassard et al., 2011; Lei et al., 2012; van Breugel et al., 2012), although it was shown that root competition can be more intense than shoot competition (Wilson, 1988). In contrast to aboveground competition, belowground competition is more demanding to investigate because of the inherent complexity of the root system.

There is a controversial debate among plant ecologists regarding whether belowground competition is more symmetric, with reference to root system size, than aboveground competition (Casper & Jackson, 1997; Schenk, 2006). Recent studies have shown that asymmetric competition occurs in temperate trees species (Hertel & Leuschner, 2006; Lei et al., 2012; Rewald & Leuschner, 2009) as well as in herbaceous species grown in unhomogenized field soil with nutrient-rich patches (Rajaniemi, 2003). Moreover, root system size alone may not be a sufficient indicator of competition as the root system is very heterogeneous and might react by changing root morphological and physiological traits or survival rates rather than altering total root biomass (Bolte & Villanueva, 2006; Hishi, 2007). Thus, competition can act as an important structuring force of the fine root

system (Wilson, 1988), but the effects on different root traits (apart from root biomass) have been poorly investigated until now. This information is needed to comprehensively understand the underlying mechanisms of belowground competition in mixed forest stands.

1.3 Analysis of key fine root traits – a comparison of methods

The analysis of fine root longevity and turnover has some severe restrictions and results can show discrepancies depending on the method used (Strand et al., 2008; Milchunas, 2009; Gaudinski et al., 2010). Fine root turnover can be estimated by employing destructive and indirect methods such as sequential coring, ingrowth mesh bags or carbon isotope analysis. Alternatively, the minirhizotron method can be used to estimate fine root turnover by direct observation. Minirhizotrons or root windows allow for repeated non-destructive measurements through visual analysis of root growth and decay (Hendrick & Pregitzer, 1996; Majdi, 1996). However, root longevity is overestimated by the minirhizotron technique because the data tend to be positively skewed (Tierney & Fahey, 2002). This is an inherent problem given that visual determination of the roots' vitality is difficult to assess and depends on a wide range of factors, such as climatic conditions, soil texture or chemical contents of the rhizodermis (Milchunas, 2009). Despite methodological limitations, the significance of fine root turnover and longevity should not be downplayed. These factors may have global consequences as they are a key constraint to quantify terrestrial carbon cycling (Guo et al., 2008a).

In addition to the above-mentioned problems, biomass analysis of fine roots has largely involved grouping the roots into the categories of coarse (> 2 mm in diameter) vs. fine (< 2 mm in diameter) roots. Yet, it has been argued that this common practice of classifying roots into arbitrary diameter classes does not reflect the roots' functionality. Instead, roots should be subdivided according to their position in the root system, i.e. root branch orders (Pregitzer et al., 1997; Pregitzer et al., 2002; Wells & Eissenstat, 2003). Here, root tips are specified as first-order roots and the root section from which two first-order segments ramify are defined as second order roots, and so forth. Over the past few years, root order-

related traits have been investigated in greater detail and numerous studies have verified the different functional roles of these orders (Sorgona et al., 2007; Guo et al., 2008b; Fan & Guo, 2010; Goebel et al., 2011; Huang et al., 2010; Jia et al., 2011; Sun et al., 2011; Picon-Cochard et al., 2012; Wang et al., 2012). Although dissecting the root system into branch orders is time consuming, it gives a more detailed view of the belowground dynamics and is a promising tool to underscore the distinction between species (Wang et al., 2006) and their functionality in dependence on e.g. nitrogen (Fan & Jiang, 2010), water uptake rates (Rewald et al., 2011) or carbon source/sink manipulations (Guo et al., 2004).

1.4 Study species: European beech vs. European ash

European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) are prevalent deciduous tree species in Central European forests and of high ecological as well as economic value. There are fundamental ecological differences between these species (Table 1.1). Field studies have shown the high belowground competitiveness of both species (Rysavy, 1992; Wagner, 1999). Therefore, the direct impacts of the species' competitiveness and rhizosphere effects are especially interesting and important to investigate.

The aboveground competitiveness of beech as a late-successional tree species is superior to ash owing to its shade tolerance and its vivid crown (Ellenberg & Leuschner, 2010; Petritan et al., 2009). Ash is only medium tolerant towards shade and therefore invests strongly in vertical growth when exposed to low light levels (Petritan et al., 2007; Petritan et al., 2009). Due to its vital height growth in the juvenile stage, ash often compete with beech when beech stands are rejuvenated (Rysavy, 1992; Wagner, 1999) and is admixed in natural beech forests (Ellenberg & Leuschner, 2010).

Belowground, the systematic differences between the species become even more obvious. Mature beech and ash vary considerably in their fine root morphology (e.g. higher root tip abundance of beech and thicker roots of ash),

leading to higher specific root length and specific root area of beech (Hölscher et al., 2002; Meinen et al., 2009a).

Table 1.1 Key functional and morphological traits of above- and belowground plant organs of beech and ash summarized from Hölscher et al. (2002), Meinen et al. (2009a), Jacob et al. (2010) and Ellenberg & Leuschner (2010)

Key species-specific traits	*Fagus sylvatica* L.	*Fraxinus excelsior* L.
Family	Fagaceae	Oleaceae
Successional status	late	early-mid
Aboveground		
Maximal height (m)	50	>40
Tree longevity (years)	350 (-900)	250-300
Leaf C/N ratio	>50	30-32
Xylem anatomy	diffuse-porous	ring-porous
Light demand	± low	± high
Belowground		
Type of mycorrhiza	ectomycorrhiza	arbuscular mycorrhiza
Specific fine root tip abundance (no. mg^{-1} dw)	40.2 ± 3.5	3.0 ± 0.05
Specific fine root surface area (cm^2 g^{-1} dw)	394 ± 25	289 ± 10
Average root diameter (mm)	0.38 ± 0.01	0.60 ± 0.02
Branching intensity	High	Low

Given are means ± SE, if indicated.

Beech is ectomycorrhized (EM) like most of the temperate tree species (Fig.1.1), while ash is colonized by arbuscular mycorrhiza (AM) (Fig.1.2).

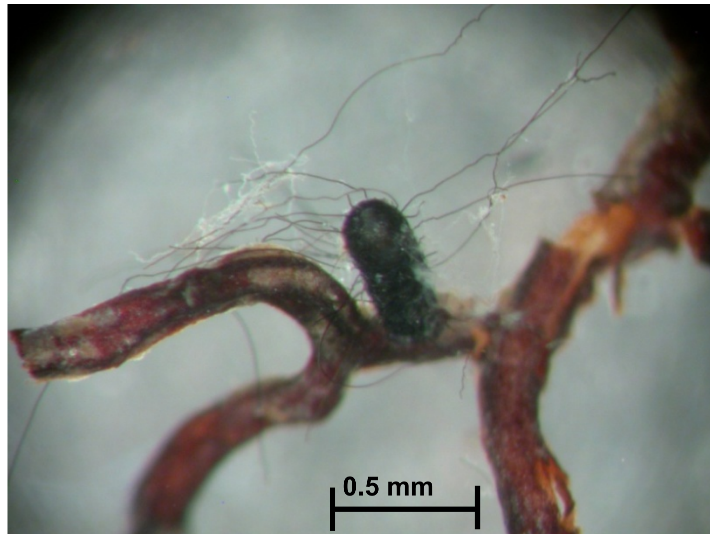

Fig. 1.1 Beech root with a mycorrhizal colonization of *Cenococcum geophilum* (Picture: F. Beyer).

The mycorrhizal strategy is linked to the carbon cycling (Cornelissen et al., 2001). For example, litter from VA-mycorrhized plants is more decomposable than those from ecto- or ericoid-mycorrhized ones.

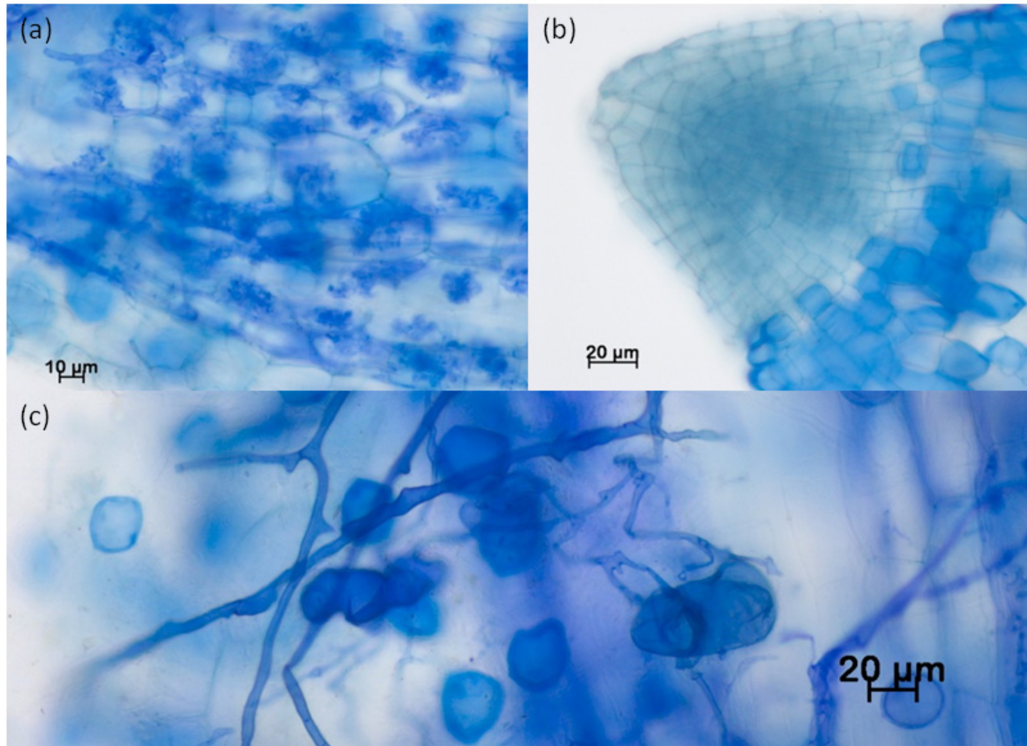

Fig. 1.2 Arbuscular mycorrhiza stained with lactophenol-blue in a squash preparation. Fungal tissues are stained in blue with: (a) arbuscles, (b) root tip with vesicles, (c) hyphae and vesicle (Picture: F. Beyer).

No differences in fine root biomass between monospecific and mixed forests have been found for beech (Bauhus et al., 2000; Meinen et al., 2009b). However, there is a lack of knowledge regarding the direct impact of intra- and interspecific competition on fine root morphology of beech and ash. Field observations and competition experiments between beech and ash have shown enhanced root growth of ash and slower growth of beech roots, which points to asymmetric root competition in favour of ash (Rust & Savill, 2000). Additionally, ash is becoming more important in forest management because of its drought resistance (Köcher et al., 2009; Rust & Savill, 2000), whereas beech suffers from summer droughts (Ammer et al., 2005; Löf et al., 2005; Meier & Leuschner, 2008a). Nevertheless, beech is highly competitive and adapted to a wide range of climatic and edaphic conditions, with the exceptions of extremely acidic and waterlogged soils

(Ellenberg & Leuschner, 2010; Jahn & Hübner, 1996; Leuschner et al., 1998). The direct mechanisms behind the belowground competitiveness of these two species remain unclear. Yet, this knowledge will be compulsory for a sound forest management, especially considering the predicted shift to hotter and drier summers in Central Europe.

1.5 Study objectives and hypotheses

Although significant progress has been made in recent years in the field of root ecology, open questions remain as to how inter- and intraspecific belowground competition affects the rhizosphere including direct effects on important root traits such as root survival, growth and morphology and how different tree species influence the rhizosphere.

This dissertation is subdivided into five studies. In chapter 2 and 3, we quantify intra- and interspecific competition intensity and changes in key fine root traits of European beech and European ash in a rhizobox experiment with tree saplings. We focus specifically on differences in root order-related characteristics and fine root survival rates. In chapter 4 and 5, we describe the results of a multidisciplinary rhizotron experiment, which was jointly conducted with five PhD students and embedded in the MicroRhizo: "*Biodiversity Manipulation in Rhizosphere and Soil*" research group within the Cluster of Excellence "*Functional Biodiversity Research*". The main objective of this study was to characterize species-specific effects of beech and ash on rhizosphere processes. Chapter 6 presents a long-term labelling experiment with ^{13}C and ^{15}N stable isotopes, which was a co-project with researchers from the Research Training Group GRK 1086: "*The role of biodiversity for biogeochemical cycles and biotic interactions in temperate deciduous forests*". Here, the research aim was to follow the incorporation of plant carbon and microbial nitrogen into the rhizosphere food web of beech and ash.

Our main hypotheses were:

(1) Belowground inter- and intraspecific competition affect root morphology and functional traits of fine roots, especially specific root length (Chapter 2).
(2) Belowground competition decreases fine root longevity (Chapter 3).
(3) Beech and ash fine roots differentially affect the structure of the microbial community, thereby modifying soil processes and plant nutrient capture (Chapter 4).
(4) CO_2 efflux from the soil is higher under ash than beech due to assumed higher root growth activity (Chapter 5).
(5) Plant carbon will be translocated via roots and mycorrhiza into fungal feeding soil invertebrates. The transfer of carbon and nitrogen into soil animals is more pronounced in beech with ectomycorrhiza (EM) than in ash with arbuscular mycorrhiza (AM) (Chapter 6).

1.6 Experimental design

The plant material for all experiments was obtained from the Hainich National Park, a mature temperate forest in the state of Thuringia, Germany (51°04' N 10°30' E, ca. 350 m a.s.l.). The forest is known for its species richness (Stellario-Carpinetum community, oak-hornbeam forests with up to 12 different tree species) on nutrient-rich soils (predominantly Stagnic Luvisol) developed from loess over Triassic limestone and is the largest cohesive deciduous forest in Germany (Leuschner et al., 2009). The Hainich National Park has only been extensively used in the last decades since it was used as a military area with low silvicultural impact (Schmidt et al., 2009). The mean annual temperature in this area ranges from 7.5 to 8.0°C and mean annual precipitation is 600-670 mm (Leuschner et al., 2009). Moreover, the Hainich National Park forest structure and its ecology was intensively studied by the Research Training Group GRK 1086: "*The role of biodiversity for biogeochemical cycles and biotic interactions in temperate deciduous forests*" since 2005 (see e.g. Meinen et al. 2009a).

The experiments comprised three different set-ups conducted under controlled greenhouse conditions:

1. The first two studies (chapter 2 and 3) were designed as a competition growth experiment with 32 rhizoboxes (30 cm length x 40 cm width x 4 cm depth) made of PVC. The rhizoboxes were equipped on the front sides with 30 cm x 40 cm Plexiglas windows covered with a black removable plastic tilt impenetrable to light. This feature guaranteed undisturbed root growth, but simultaneously enabled us to observe root dynamics. We established three treatments to test for effects of intraspecific and interspecific competition between beech and ash,: monospecific rhizoboxes (two plants of the same species), mixed boxes (one ash and one beech plant) and so-called 'iso boxes' with a single beech or ash plant. Sixteen monospecific boxes (8 beech and 8 ash), 8 mixed boxes and 8 iso boxes (4 beech and 4 ash) were established. Root growth was documented via monthly digital imaging (scanning) of the Plexiglas window. A final harvest gave results on relative growth rates and root order related key traits.

2. Another experiment (chapter 4 and 5) was established with 16 double split-root rhizotrons containing beech and ash saplings (4 x beech monoculture, 4 x ash monoculture, 4 x beech and ash in mixture and 4 control rhizotrons without any saplings). The root systems of the two tree saplings were separated into compartments with root strands of an individual sapling at each side and a shared root compartment in the centre where root strands of both tree saplings could interact. The rhizotrons (90 cm height x 64 cm width x 4 cm depth) were built from anodized aluminum covered at the front with a Perspex plate. Each rhizotron had 24 manipulation sites filled with soil or a soil-litter mixture to perform minimally invasive measurements.

3. The last experiment was conducted with 15 beeches and 14 ashes (mean height 60 cm). The juvenile trees were excavated with the surrounding

intact soil (depth 25 cm and 2-3 cm litter layer) from the Hainich National Park and placed into containers (25 cm diameter x 45 cm height). Thereafter, the plants were exposed to $^{13}CO_2$ enriched atmosphere (maximum CO_2 concentration 1,200 ppm) and irrigated with a ^{15}N labeled nutrient solution in a greenhouse for five months. Isotopic signatures were analysed after harvesting and fractionating all segments of the tree.

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CHAPTER

2

Competition effects on root morphological and functional traits in *Fagus sylvatica* and *Fraxinus excelsior* saplings: An analysis across different fine root orders

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Abstract

Aims The mechanisms of belowground competition are not well understood. Addressing literature reports on competition-induced changes in tree fine root morphology, we conducted an intra- and interspecific root competition experiment to investigate competition effects on important root morphological and functional traits in a root order-focused analysis.

Methods European beech and European ash saplings were grown for 34 months in containers under greenhouse conditions in monoculture (2 conspecific plants), in mixture (1 beech and 1 ash) or as single plants. The root system was fractionated according to root orders and eight morphological and functional properties were determined.

Results Root order was the most influential factor affecting the fine root traits (except for root diameter and $\delta^{13}C$); a significant species identity effect was found for root diameter, tissue density, N concentration and $\delta^{13}C$. Ash fine roots were thicker, but had lower tissue densities, contained more N and had systematically higher $\delta^{13}C$ values than beech roots. The competition treatments had no significant effect on morphological root traits but altered $\delta^{13}C$ in the 2nd root order.

Conclusion Neither intra- nor interspecific root competition affected fine root morphology significantly suggesting that competition-induced root modification may not be a universal phenomenon in temperate trees.

Keywords: *belowground competition response, root order analysis, N content, specific root area, $\delta^{13}C$, specific root length*

2.1 Introduction

Competition for shared soil resources may not only reduce productivity but could also lead to alterations in the size, distribution and morphology of the roots and root system of the competitors, because the plants may respond with morphological and/or functional adaptation to competition–induced alteration of nutrient and water supply. One intensely studied response to interspecific belowground competition is root system stratification in mixed forests, agro-forestry systems or grasslands (Belcher et al., 1995; Mordelet et al., 1997; Pechackova et al., 1999; Bauhus et al., 2000; Schmid & Kazda, 2002; Schmid, 2002; Moreno et al., 2005; Yanai et al., 2006; Kalliokoski et al., 2010) with species altering their depth distribution of roots in order to reduce competition. Various observational studies found a certain degree of belowground niche partitioning in terms of rooting depth and placing of roots in mixed stands of herbs and grasses (Parrish & Bazzaz, 1976; Mamolos et al., 1995; Fargione & Tilman, 2005) and in mixed stands of woody plants (Nambiar & Sands, 1993; Schmid & Kazda, 2002) while another study found no vertical root system segregation in a mixed forest with *Fagus sylvatica, Fraxinus excelsior*, *Acer* spp., *Tilia* spp. and *Carpinus betulus* (Meinen et al., 2009b). However, the roots of competing species could also respond to competition by increasing the specific root surface area or specific root length as was shown for beech grown in mixture with spruce (Grams et al., 2002; Bolte & Villanueva, 2006) and thus enhance their uptake capacity for water and nutrients in the shared soil volume. Competition could also influence the longevity of roots through altered resource availability (Lopez et al., 1998; Withington et al., 2006; Carswell et al., 2012). However, not much is yet known about competition-induced changes in the root morphology and function of trees. Such information is needed for a comprehensive understanding of the mechanisms underlying belowground competition in mixed forests stands.

In Central European temperate broad-leaved forests on fertile soil, European beech (*Fagus sylvatica* L.) is the dominant tree species; admixed species are European ash (*Fraxinus excelsior* L.), maple species (*Acer* spp.) and small-leaved linden (*Tilia cordata* Mill.) (Ellenberg & Leuschner, 2010). Late-

successional beech and early- to mid-successional ash often compete with each other in the juvenile stage of the forest dynamics cycle and when planted beech stands are rejuvenated (Rysavy, 1992; Wagner, 1999). Field observations and competition experiments between beech and ash have shown vigorous root growth of ash and slower growth of beech roots which points to asymmetric root competition in favor of ash and has raised concern among foresters about a possible suppression of beech offspring by ash saplings due to apparent belowground superiority in the gap phase of the forest dynamics cycle (Rysavy, 1992; Wagner, 1999). Mature beech and ash differ considerably in the morphology (diameter, root tip abundance) of their fine roots (Meinen et al., 2009a). In an attempt to achieve a deeper understanding of belowground competition between beech and ash, we examined the variability of important root morphological (diameter, tissue density, root length and surface area) and chemical traits ($\delta^{13}C$ signature, C and N content) across different root orders in the two species and incorporated this study in a root competition experiment. Main study goal was to compare a tree species effect with a putative competition effect on root traits. The growth experiment included three treatments (single-plant boxes, monospecific and mixed boxes) to test for differential effects of intraspecific and interspecific root competition. We analysed the root response on a root order basis, because this classification should reflect functional differences between different sections of a root system more precisely than arbitrarily chosen root diameter classes (Pregitzer et al., 2002; Guo et al., 2004; Goebel et al., 2011). Our approach should ease the detection of species and competition effects on root morphology and functions.

2.2 Material and methods

The growth experiment was conducted with ~3-yr-old saplings of European beech and European ash excavated from Hainich National Park, Thuringia, Germany (51°04' N 10°30' E, ca 350 m a.s.l.). The saplings were selected according to similarity in size (about 20 cm height) and growing conditions at the origin. The Hainich forest is a species-rich temperate broad-leaved forest (Stellario-Carpinetum community, oak-hornbeam forests) growing on base-rich soil (predominantly Stagnic Luvisol) developed from loess over Triassic limestone. Upon excavation, care was taken not to damage the root systems of the saplings. The adherent soil material was carefully washed off from the roots and the saplings were planted into boxes of 30 cm x 40 cm x 4 cm size (length x width x depth, volume 4800 cm^3) made of PVC that were filled with homogenized loamy soil of similar chemical and physical properties as found at the origin (Table 2.1).

Table 2.1 Soil properties in the rhizoboxes before planting the beech and ash saplings. Given are means ± SE (n=15).

Variable	Mean		SE
pH (H_2O)	8.14	±	0.23
pH (KCl)	7.50	±	0.04
C_{org} (g kg^{-1} dw)	36.10	±	0.20
N_{total} (g kg^{-1} dw)	1.62	±	0.01
C/N (g g^{-1})	22.36	±	0.18
P_{resin} (mg kg^{-1} dw)	14.24	±	2.29
N-NO_3^- (mg kg^{-1} dw)	3.47	±	0.33
N-NH_4^+ (mg kg^{-1} dw)	1.19	±	0.12

The experiment consisted of three treatments to test for effects of intraspecific and interspecific competition: monospecific rhizoboxes (two plants of the same species), mixed boxes (one ash and one beech plant) and so-called 'iso boxes' with a single beech or ash plant, i.e. in the absence of competition with conspecific or allospecific neighbours. Sixteen monospecific boxes (8 beech and 8 ash), 8 mixed

boxes and 8 iso boxes (4 beech and 4 ash) were established (32 in total). Each ten additional beech and ash saplings were harvested on the date of planting (July 22, 2008) to determine initial biomass for the two species at the experiments' beginning (Table 2.2).

Table 2.2 T-test table with *P*-values and means ± SE of the plant biomass (g DW plant^{-1}) of beech and ash saplings at the start of the experiment (n=5). Significant differences are printed in bold.

Fraction		**Beech**	**Ash**
	Initial biomass		
	P	Mean ± SE	Mean ± SE
Total	0.7708	2.06 ± 0.47	2.22 ± 0.23
Total aboveground	0.9818	1.26 ± 0.27	1.25 ± 0.15
Total belowground	0.4886	0.80 ± 0.21	0.97 ± 0.08
Leaves	0.1415	0.38 ± 0.05	0.52 ± 0.07
Fine roots	**0.0333**	**0.16 ± 0.05**	**0.52 ± 0.07**
Coarse roots	0.6773	0.64 ± 0.17	0.56 ± 0.06
Shoots	0.5528	0.88 ± 0.17	0.74 ± 0.09

The boxes were placed in a randomized arrangement in a climatized greenhouse where the plants grew for 34 months until harvest in late April/early May, 2011. The growth conditions were 13h photoperiod / 11h nighttime with additional constant light (besides sun light) being supplied by mercury fluorescent lamps (EYE CLEAN ARCTM, Eye Lightning International, OH, USA) with a photosynthetic photon flux density of 170 ± 10 µmol m^{-2} s^{-1}. Temperature was kept at 20°C during daytime and 10°C during night time.

2.2.1 Analysis of biometric and chemical data

The saplings were harvested between April 26 and May 15, 2011, i.e. after 34 months of growth in the boxes, applying a rotating harvesting scheme across the treatments. By carefully washing the roots under tap water, all adherent soil particles were removed. Three representative root segments per sapling of ca. 10 cm length were cut off and stored in tap water at 4°C until analysis for total root length and surface area on a flat-bed scanner (Epson Perfection V10) using

WinRhizo 2005c software (Régent Instruments Inc., Québec, QC, Canada). Subsequently, root orders were distinguished in root branches following Pregitzer et al. (2002) and the respective root sections cut at the ramification using a razorblade under a binocular at 20 x magnification. Root tips were counted as first order roots segments, the root section from which two first order segments ramified were defined as second order roots, and so forth. In the case of beech, the first order root segment with the ectomycorrhizal root tips were very short and thus combined in the analysis with the second order segment to gain enough material for C/N analysis; this material was classified as first order roots. Subsequently, the root material of every root order class was again analysed for length, mean diameter and surface area using the scanner and WinRhizo 2005c software which allowed quantifying root traits on a root order basis. The following root morphological parameters were measured: specific root area (SRA), specific root length (SRL), root tissue density (RTD), mean diameter per root order class, and total root length. The above- and belowground biomass was completely harvested, separated into the fractions leaves, shoots, coarse roots (> 2 mm in diameter) and fine roots (< 2 mm), oven-dried (70°C, 48 h) and weighed. Subsamples of root dry mass sorted by root order were ground for C/N analysis (Vario EL, elementar, Hanau Germany) and $\delta^{13}C$ analysis (elemental analyser, NA 1500, Carlo Erba, Milan, Italy coupled with a MAT 251 mass spectrometer, Finnigan, Bremen, Germany); the isotopic analyses were conducted in the Centre for Stable Isotope Research and Analysis (KOSI) of the University of Göttingen.

2.2.2 Analysis of mycorrhizal colonization

At harvest, three randomly selected terminal root sections of ca. 5 cm length per beech sapling were analysed under a binocular (16–40 x magnification) for the ectomycorrhizal colonization of about 500 root tips. Colonization rate was calculated as:

$$\left(\frac{n\ mycorrhizal\ root\ tips}{n\ vital\ root\ tips} \right) * 100$$

The colonization rate of the ash root with VA-mycorrhizal fungi was investigated in each three terminal root branches per sapling (ca. 3 cm length) that were stored in Eppendorf cups filled with 70% ethanol until analysis. For determining colonization rate, the endomycorrhizal structures were stained with lactophenol-blue (Schmitz et al., 1991) and the roots were bleached with 10% KOH at 90°C for 50 min. After rinsing them with distilled water, the roots were acidified with 3.7% HCl for 15 min at room temperature. After another cleaning process with distilled water, the roots were stained for 90-120 s in lactophenol-blue (1g L^{-1}, pH 2.3, Merck). To remove unspecific colourant from the plant tissue, the root mass was incubated for 60 min in an acidic glycerine solution (50 ml glycerine, 45 ml H_2O, 5 ml 1% HCl) at room temperature. The solution was renewed after 30 min. The roots were then stored in 50% glycerine. The root tips were subsequently mounted on microscope slides as squash preparation. The rate of mycorrhizal colonization (counting arbuscles and hyphae) was determined by the magnified intersection method following McGonigle et al. (1990) using an integrated raster in the ocular (à 10 x 10 = 100 boxes) at 200 x magnification.

2.2.3 Statistical analysis

All study parameters were compared between treatments and root orders by one-way analysis of variance (ANOVA) or – in the case of unbalanced variances within groups – by general linear models (GLM). Subsequent pair-wise comparisons were performed using Tukey's HSD test. Normality of data distribution was checked with a Shapiro-Wilk test and variance homogeneity with a Levene test. Morphological traits and C/N values as affected by root order, treatment and tree species were additionally analysed with a Principal Components Analysis (PCA) to derive explanatory variables. All parameters were standardized (zero mean/unit variance). The PCA was carried out using the program CANOCO

4.5 for Windows. We calculated general linear models (GLM) for the root traits (SRL, SRA, RTD, diameter, length, N concentration, C/N ratio and $\delta^{13}C$ signature of root mass). The models included species (beech or ash), treatment (iso, mono, mix) and root order as well as all possible interactions as fixed effects. The significance level for all statistical tests was set to $P < 0.05$ unless stated otherwise. The statistical analyses were carried out with the software R (Version 2.13, The R Foundation for Statistical Computing; www.r-project.org).

2.3 Results

2.3.1 Species differences in root system size, morphology and chemistry

Beech and ash saplings of similar age and aboveground size, that were grown for 34 months in the iso-treatment, i.e. without con- or allospecific competitors, differed significantly in a number of functionally important root traits. While the two species were not different with respect to the fine and coarse root biomass per tree at the time of harvest (Fig. 2.1b) and thus had a similar belowground productivity, shoot biomass was generally higher in beech than ash (difference significant at $P < 0.05$ only in the iso-treatment, Fig. 2.1a). The beech saplings produced more shoot axes biomass than ash while leaf mass was similar. The fine root/leaf mass ratio tended to be larger in beech than ash (difference significant in the mono treatment, Fig. 2.1c). Microscopic inspection showed that the colonization rate with mycorrhizal fungi was significantly higher in beech roots (first order rootlets and root tips), the ectomycorrhizal (ECM) species, than in ash roots with arbuscular mycorrhizal (AM) (72 ± 2% vs 33 ± 3% respectively), but colonization rate was invariant across the treatments (Fig. 2.2).

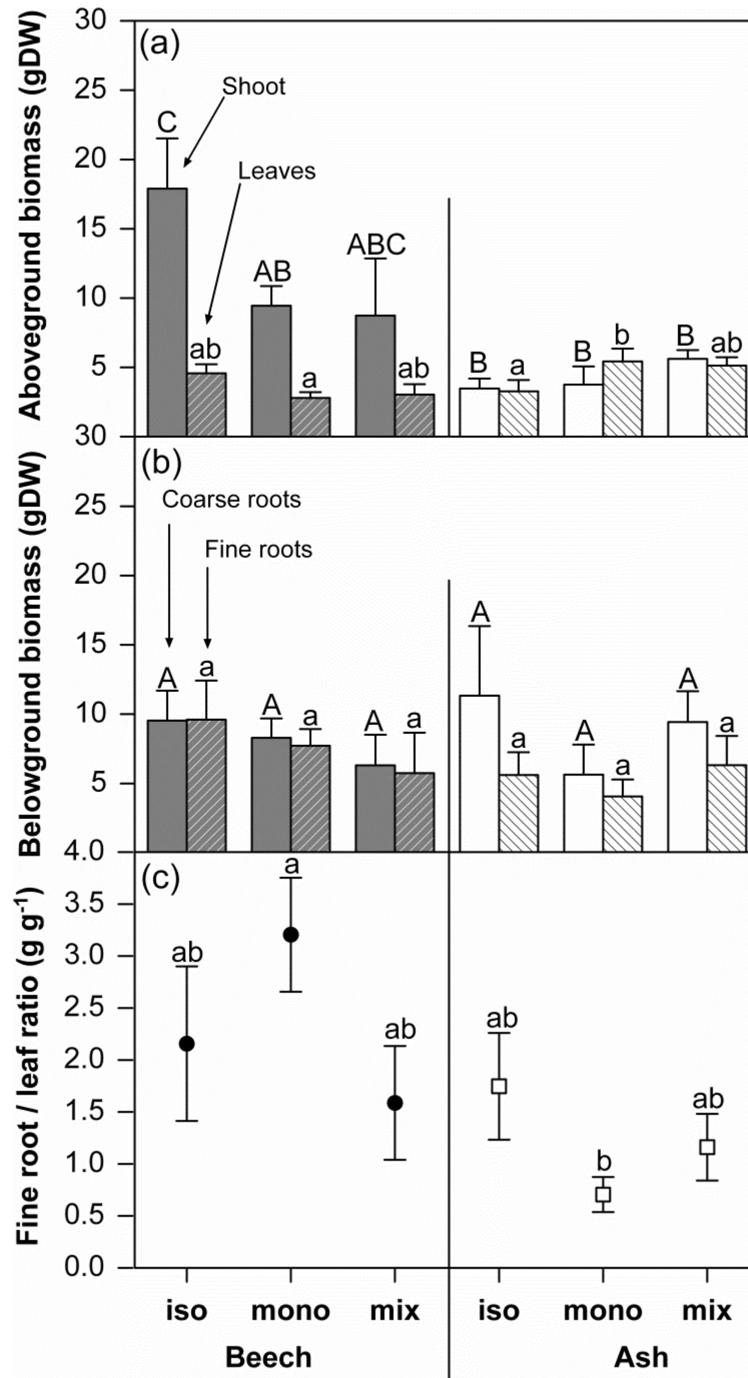

Fig. 2.1 (a) Aboveground biomass per tree in the fractions of leaves and shoots (axes and branches). (b) Belowground biomass per trees in the fractions of coarse and fine root biomass. (c) Fine root/leaf mass ratio in the different treatments. Given are means ± SE (n: beech iso=4, beech mono=16, beech mix=4, ash iso=3, ash mono=6, ash mix=4). The unbalanced number of replicates partly results from plant losses in the phase of establishment. Significant differences are indicated with different letters ($P < 0.05$) (iso=one sapling per rhizobox, mono=two saplings of beech or ash per rhizobox, mix=one beech and one ash sapling per rhizobox).

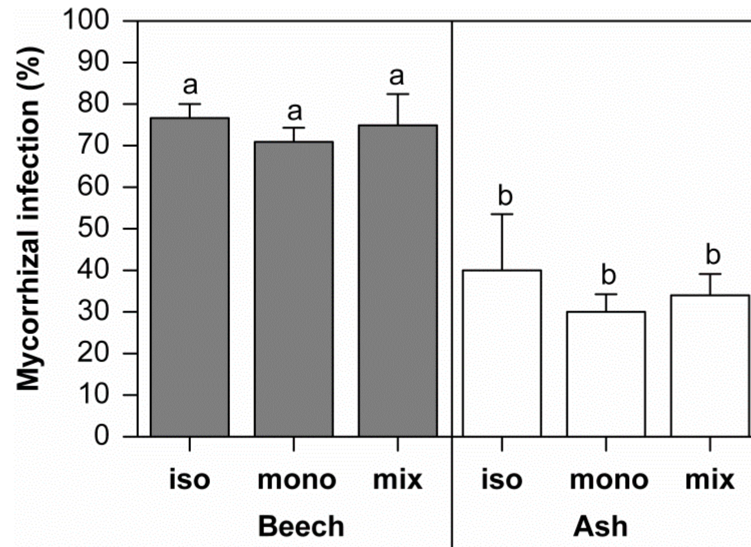

Fig. 2.2 Percent of root tips colonized by ectomycorrhiza (ECM; beech) or arbuscular mycorrhiza (AM; ash) in the three treatments (means ± SE). None of the treatment differences were significant at $P < 0.05$. The AM rate for ash is relatively low because only segments with arbuscles and hyphae were counted.

The species differences in root morphology and chemistry were more pronounced than those in total root biomass: ash fine roots were characterized by significantly higher average fine root diameters than beech roots and tended to have a smaller specific root length (SRL) and root tissue density (RTD) than beech, while specific root area (SRA) was not different (Fig. 2.3 and Fig. 2.4e). Ash fine roots tended to contain more N than beech fine roots (only marginally significant difference in 3rd order roots, $P < 0.1$). The $\delta^{13}C$ signature was less negative (by ca. 3‰) in the fine root biomass of ash roots as compared to beech roots (Fig. 2.4h). As is visible from the comparison of the mono and iso treatments, intraspecific competition decreased the shoot biomass of beech significantly, but resulted in no significant root biomass reduction (even though a tendency for a decrease was visible, Fig. 2.1a, b). In ash, neither aboveground nor belowground biomass production showed a significant competition-induced reduction.

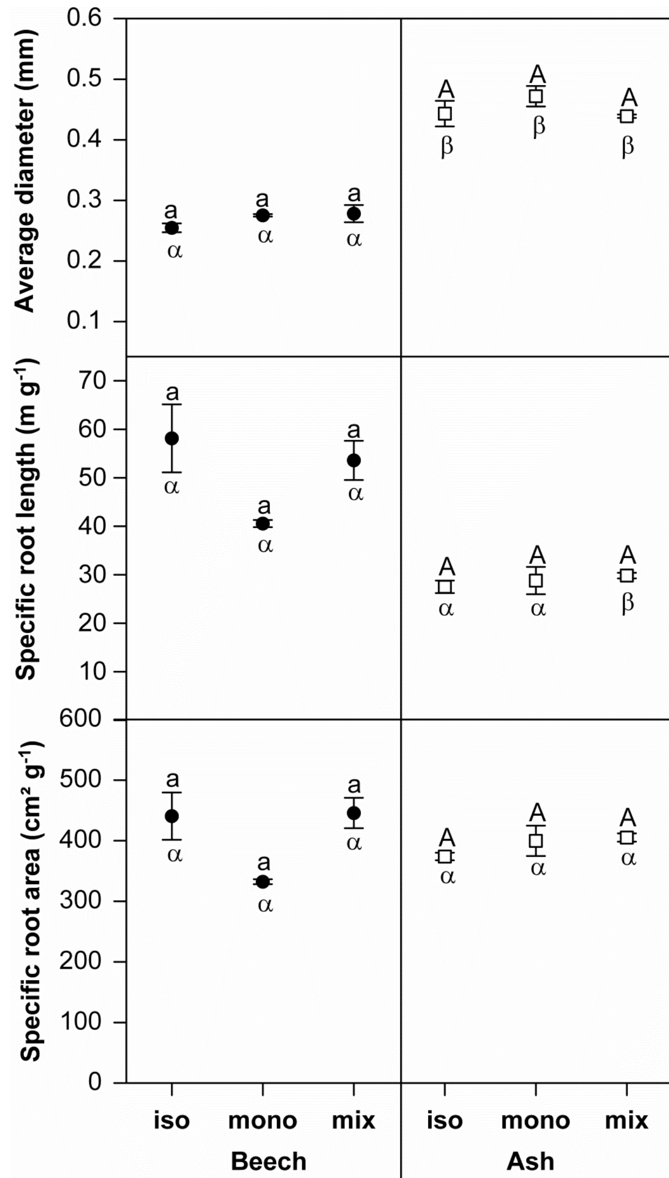

Fig. 2.3 Means (± SE) of fine root morphological traits of the beech and ash saplings in the three treatments (n: beech iso=4, beech mono=16, beech mix=4, ash iso=3, ash mono=6, ash mix=4). Significant differences between treatments are marked with different lower case letters for beech and with upper case letters for ash. Differences between tree species within a treatment are indicated by different Greek letters ($P < 0.05$).

2.3.2 Root functional traits as dependent on root order

The majority of investigated morphological and chemical traits depended on root order (Fig. 2.4), i.e. the age sequence of root segments from the root tip (1st order) to the oldest 4th order roots. While root diameter increased linearly

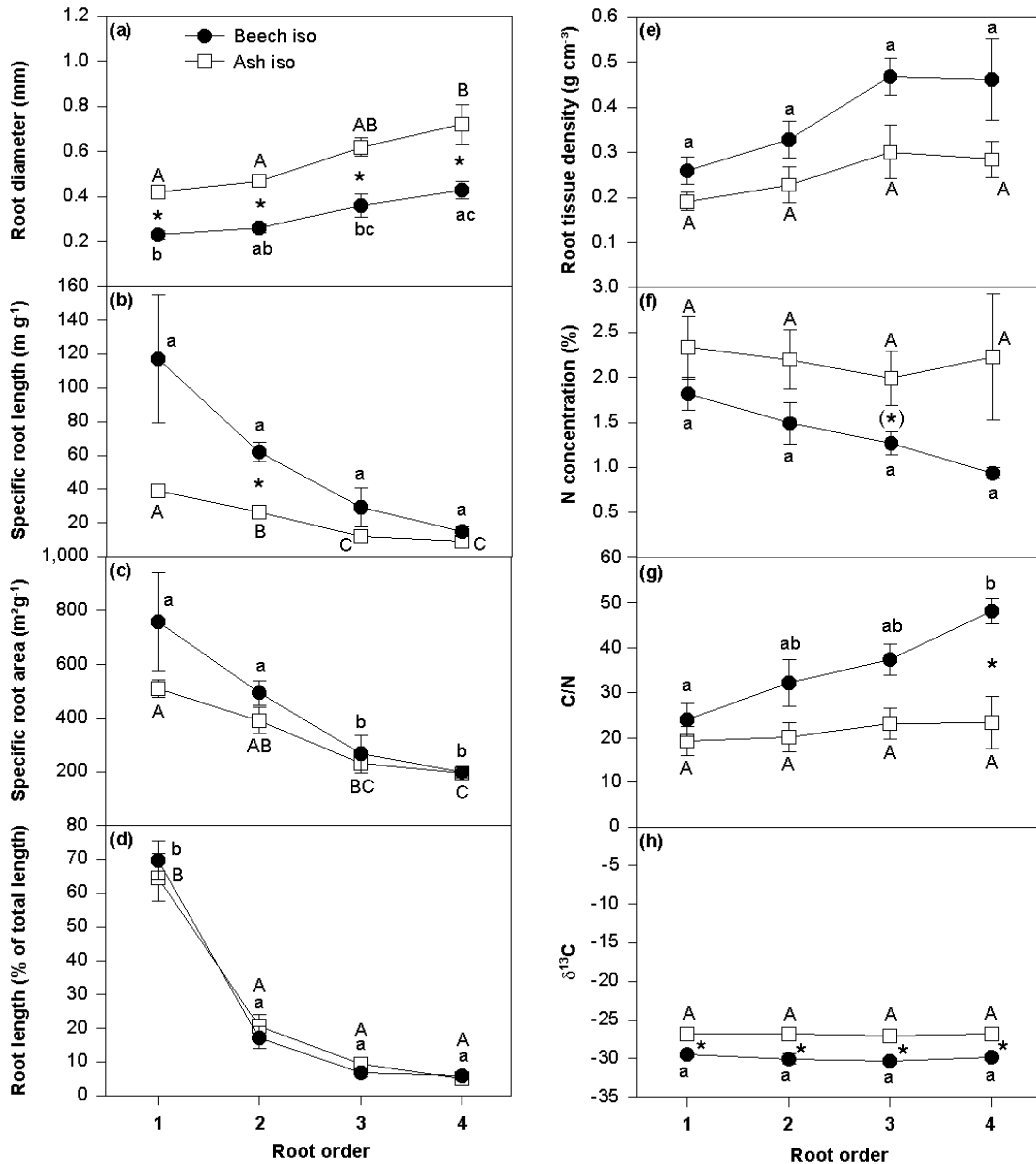

Fig. 2.4 Eight morphological and chemical traits of the root biomass of beech and ash sapling in the iso treatment differentiated by root order after 994 days of cultivation. Given are means ± SE (n: beech iso=4, beech mono=16, beech mix=4, ash iso=3, ash mono=6, ash mix=4). Different upper case letters indicate significant differences between root orders of ash plants, lower case letters for beech plants. Significant differences in a given root order class between ash and beech are marked with an asterisks ($P < 0.05$).

with order in both species (Fig. 2.4a), SRL and SRA decreased asymptotically towards higher orders (Fig. 2.4b and c) as did the relative proportion of root length per root order class in total root length (Fig. 2.4d). Root tissue density was lowest in the 1st order and showed a non-significant increase towards the 2nd and 3rd order classes in ash and beech (Fig. 2.4e). C/N ratio increased significantly with order in

beech roots while the N concentration decreased. In contrast, root N concentration and C/N ratio remained more or less stable across the root order classes in ash (Fig. 2.4f and g). Similarly, the $\delta^{13}C$ signature of root mass was remarkably constant across the four root orders in both species (Fig. 2.4h).

2.3.3 Effects of intra- and interspecific competition on root morphology and chemistry

By comparing root properties of ash and beech between the mono or mix treatments on the one side and the iso treatment on the other side, we were able to examine effects of intraspecific and interspecific root competition on root morphological and functional traits. In general, inter- and intraspecific belowground competition had only a small effect on root morphology and chemistry, despite a generally higher fine root density in the rhizoboxes in the mono and mix treatments with two saplings per box than in the iso treatment with only one sapling (Fig. 2.5).

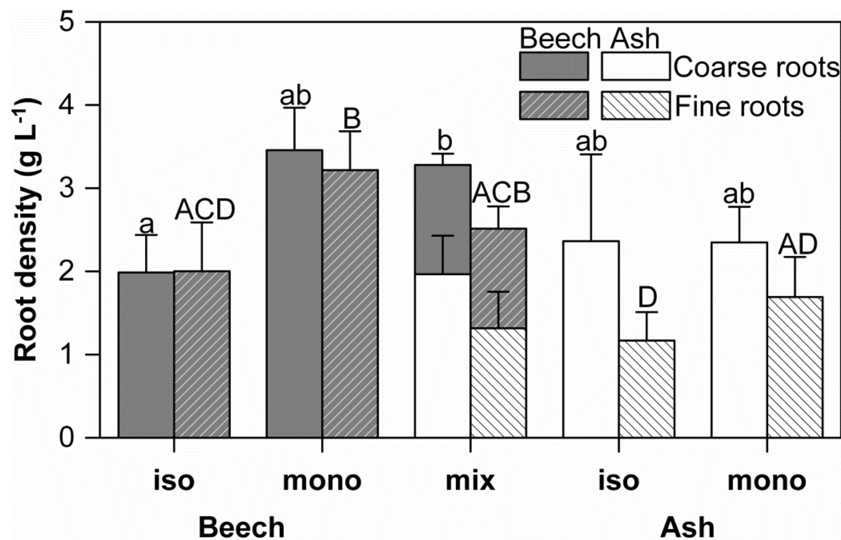

Fig. 2.5 Biomass of coarse and fine roots per soil volume in the rhizoboxes of the three treatments (see Fig. 2.1); means ± SE, n: beech iso=4, beech mono=8, mix=4, ash iso=3, ash mono=3). None of the treatment differences were significant at $P < 0.05$; different letters show marginally significant differences at $P < 0.1$.

With respect to beech roots, density increased by about 50% from the iso to the mono and mix treatments; ash behaved differently with no root density difference

between the iso and mono treatment and a non-significant increase towards the mix treatment.

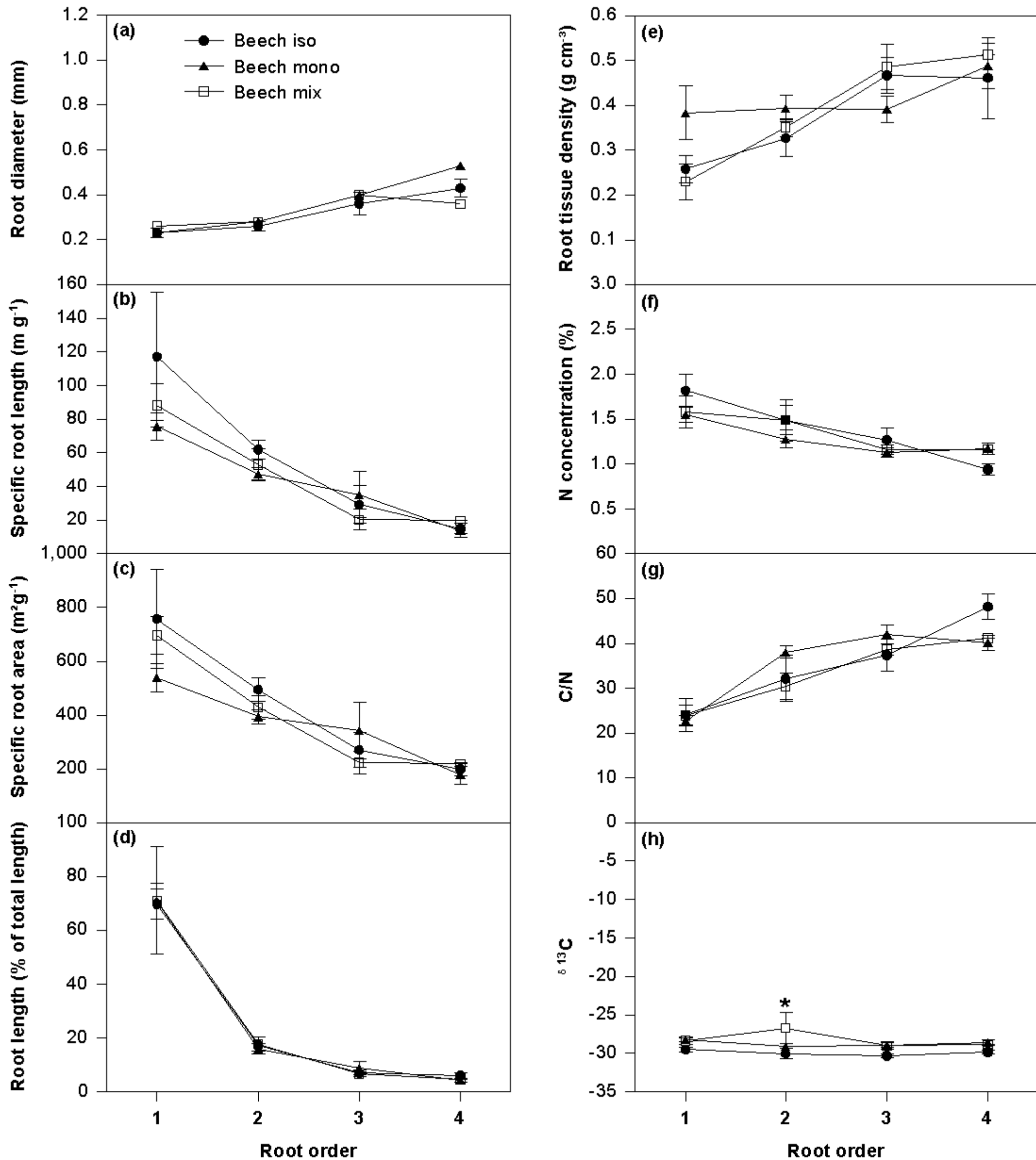

Fig. 2.6 Eight morphological and chemical traits of the root biomass of beech saplings in the three treatments differentiated by root order after 994 days of cultivation. Given are means ± SE (n: iso=4, mono=16, mix=4). No significant differences between treatments were found except for $\delta^{13}C$ in root order class # 2 (P<0.05).

Significant effects of the presence of competitors on root morphology or chemistry were only observed for the $\delta^{13}C$ signature of root mass with 2nd order roots in the mixed treatment showing for both species a significantly higher value than in the

iso and mono treatments ($P < 0.05$, Fig. 6 and 7). Neither mycorrhizal colonization rate nor mean fine root diameter, SRL, SRA or root N concentration were affected by competitive interactions.

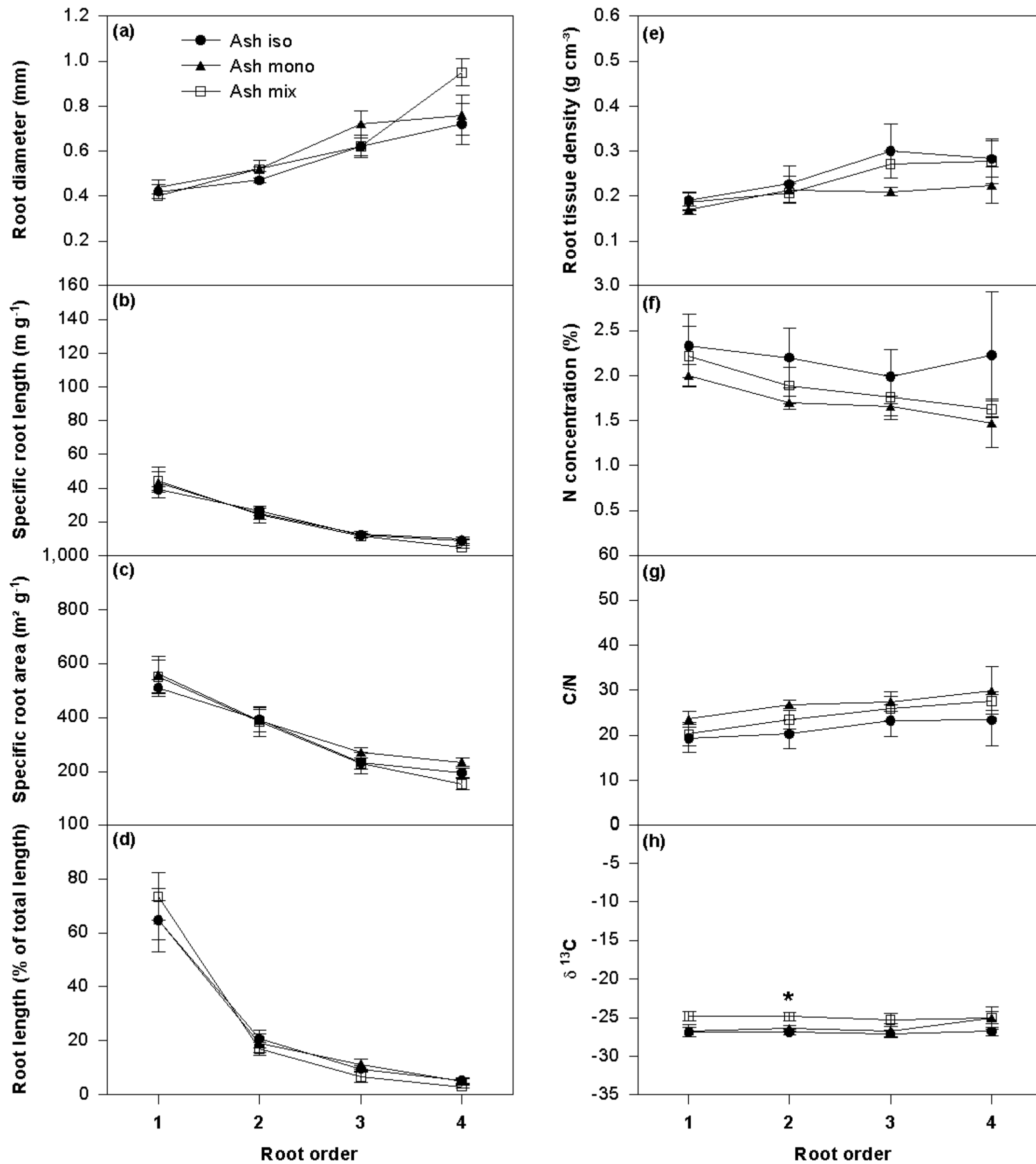

Fig. 2.7 Eight morphological and chemical traits of the root biomass of ash sapling in the three treatments differentiated by root order after 994 days of cultivation. Given are means ± SE (n: iso=3, mono=6, mix=4). No significant differences between treatments were found except for $\delta^{13}C$ in root order class # 2 ($P < 0.05$).

With PCA and multiple GLM analyses, we attempted to quantify the relative importance of the factors root order, species (beech vs. ash) and competition treatment (iso, mono, mix) on root morphological and chemical traits. The PCA analysis showed that root order was the single most important factor correlating on axis 1 (eigenvalue: 0.465) and it correlated with nearly all investigated root morphological and chemical traits except for root diameter and root $\delta^{13}C$ (Table 2.3).

Table 2.3 Results of a Principal Components Analysis (PCA) on the morphological and chemical parameters as affected by root order, species and competition treatment.

Variables		**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
	EV =	(0.465)	(0.352)	(0.100)	(0.030)
Treatment		0.035	-0.117	**0.985**	0.015
Species		0.207	**0.958**	0.107	-0.024
Root order		**-0.920**	0.222	0.053	0.157
Mean root diameter		-0.394	**0.851**	0.024	0.064
Root C/N		**-0.785**	**-0.543**	-0.075	-0.005
Length		**0.883**	-0.330	-0.105	0.256
Root N concentration		**0.681**	**0.623**	0.258	0.102
Specfic root area		**0.904**	-0.367	-0.023	-0.180
Specific root length		**0.716**	**-0.625**	-0.017	-0.161
Total surface area		**0.909**	-0.079	-0.067	0.366
Root tissue density		**-0.686**	**-0.638**	-0.049	0.255
$\delta^{13}C$ of root mass		0.224	**0.885**	-0.352	-0.025

Given are the loadings of the selected variables along four explanatory axes. Numbers in brackets indicate the eigenvalues of the axes # 1 to 4. Numbers in bold mark the variables with closest correlation to the respective axes.

The species effect had a high loading on axis 2 (eigenvalue: 0.352) with strong effects also by root diameter and $\delta^{13}C$, while the type of competition treatment reached a high loading only on axis 3 (eigenvalue: 0.100) with no other factor being closely related to this axis. The GLM analyses produced similar results. The root traits SRA, SRL, length proportion and root C/N ratio showed root order to be the most influential factor, while species effects had a dominant effect on the traits root tissue density, N concentration and $\delta^{13}C$ in root mass (and less clearly on root diameter, Table 2.4). The competition treatment played a certain role only in the

case of $\delta^{13}C$ and N concentration, but the effect was of only marginal importance with 9 and 4%, respectively, of the variation explained. Interactions of species and order effects were significant in case of the C/N ratio, root diameter and root length, but only with minor importance (6.6, 1.4 and 2.6%). All other interaction types were insignificant.

Table 2.4. Results of a multiple General Linear Model (GLM) on the influence of tree species identity (Species), competition treatment: (iso, mono or mix (Treatment)) and root order (1-4 (Order)) and their interactions on the C/N ratio, $\delta^{13}C$ signature and N concentration (N%) as well as morphological traits of the roots (specific root area (SRA), specific root length (SRL), root tissue density and root diameter).

		C/N			**$\delta^{13}C$**			**N concentration**			**Root Diameter**		
Source	DF	V.e.%	F	*P*	V.e.%	F	*P*	V.e.%	F	*P*	V.e.%	F	*P*
Species	1	**24.93**	**87.46**	***	**43.51**	**115.1**	***	**31.54**	**71.69**	***	**38.09**	**201.4**	***
Treatment	2	**2.08**	**3.65**	*	**9.26**	**12.25**	***	**4.20**	**4.78**	**	0.49	1.3	n.s.
Species*Treatment	2	0.54	0.94	n.s.	0.81	1.08	n.s.	1.13	1.28	n.s.	0.28	0.73	n.s.
Order	3	**31.36**	**36.67**	***	1.20	1.05	n.s.	**12.60**	**9.55**	***	**35.77**	**63.03**	***
Species*Order	3	**6.67**	**7.8**	***	0.41	0.36	n.s.	0.12	0.09	n.s.	**1.48**	**2.61**	*
Treatment*Order	6	1.78	1.04	n.s.	1.77	0.78	n.s.	0.42	0.16	n.s.	0.94	0.83	n.s.
Species*Treat.*Order	6	1.27	0.74	n.s.	1.46	0.64	n.s.	1.61	0.61	n.s.	1.96	1.73	n.s.
Model	23	**68.64**	**10.47**	***	**58.42**	**6.72**	***	**51.61**	**18.16**	***	**79.00**	**18.16**	***

		SRA			**SRL**			**Root tissue density**			**Root length**		
Source	DF	V.e.%	F	*P*	V.e.%	F	*P*	V.e.%	F	*P*	V.e.%	F	*P*
Species	1	0.53	1	n.s.	**10.09**	**21.91**	***	**29.59**	**59.04**	***	**2.06**	**11.73**	**
Treatment	2	0.19	0.17	n.s.	0.56	0.61	n.s.	0.11	0.11	n.s.	**1.84**	**5.23**	**
Species*Treatment	2	1.27	1.19	n.s.	0.77	0.83	n.s.	1.61	1.6	n.s.	0.28	0.79	n.s.
Order	3	**35.62**	**22.3**	***	**32.41**	**23.45**	***	**8.48**	**4.23**	*	**71.16**	**134.7**	***
Species*Order	3	0.27	0.17	n.s.	2.41	1.74	n.s.	0.56	0.37	n.s.	**2.63**	**4.98**	**
Treatment*Order	6	1.97	0.62	n.s.	1.48	0.53	n.s.	**3.92**	**1.3**	n.s.	1.92	1.82	n.s.
Species*Treat.*Order	6	1.07	0.33	n.s.	1.14	0.41	n.s.	1.10	0.37	n.s.	0.56	0.53	n.s.
Model	23	**40.91**	**3.34**	***	**48.86**	**4.61**	***	**45.37**	**0.377**	***	**80.45**	**19.87**	***

Shown are the degrees of freedom (DF), the percentage of variance explained (V.e.%), the F and *P* values (****P* < 0.0001, ** *P* < 0.001, * *P* < 0.05, n.s.: not significant) of the seven source variables and of the model itself. Variables with significant influence are printed in bold.

2.4 Discussion

By doubling the number of plants per rhizobox from one to two, we increased the density of fine roots per soil volume from the iso to the mono and mix treatment by 62 to 184% and thus created growth conditions with significant interspecific (mono treatment) or intraspecific (mix treatment) belowground competition. Fine root densities (biomass per soil volume) of 1.7-3.2 g dm^{-3} as found in the mono and mix treatments at the date of harvest are somewhat higher than fine root densities observed in the topsoil of mature beech or beech-ash-linden forests (ca.0.5-2.6 g dm^{-3}) on basic or acid soils (Meinen et al., 2009b). Thus, our experiment compares conditions of putative intensive intraspecific or interspecific root competition with the control where fine root density was markedly smaller in the iso treatments. Nevertheless, it is important to note that the two control treatments with mean root densities of 1.2 and 2.0 g dm^{-3} do not represent growth conditions without root competition but only with reduced competition intensity. As in all other experiments where belowground competition is measured against a control with single plants (iso treatment), the different fine root branches of this single plant are likely to compete with each other for resources as well. Thus, the root branches are exposed to a certain degree of self competition which however should be less intense than in the mono treatment with a putatively high intraspecific (between individual) competition. Because mutual shading between the two beech and/or ash shoots in the mono and mixed boxes was low, we assume that the observed reduction in aboveground production per plant in the mono and mix treatments as compared to the iso treatments (significant for beech) was primarily due to root competition, while shoot competition should have been less influential. Interestingly, we found no reduction in fine root biomass per plant in beech and ash with an increase in intra- or interspecific competition intensity from the iso to the mono or mix treatments; there was a tendency only in beech but no clear pattern in ash. Thus, beech apparently responded to increased root competition more sensitively with a reduction in shoot growth while root growth was maintained despite higher root densities. A possible explanation for the beech response is that root competition in the mono and mix treatments resulted in

reduced nutrient and/or water uptake per plant compared to the iso treatment which may have lowered total productivity and induced a shift to higher belowground allocation of resources in the beech mono treatment. Ash did not show such a response. The lack of a clear competition effect on the productivity of ash is astonishing; it might be explained by the generally lower aboveground growth rate of this species in the experiment. This growth experiment provided only weak evidence for the assumption that ash saplings should be superior over beech in root competition as suggested by field observations (Wagner, 1999; Rust & Savill, 2000; Jacob et al., 2012). In our mixed rhizoboxes, beech and ash maintained roughly similar fine and total root biomasses with no indication of competitive hierarchies. However, our data agree with field observations in mixed beech-ash stands that the two species are markedly different in their fine and finest root morphologies (Meinen et al., 2009a; Jacob et al., 2012) with much thicker mean root diameter, a lower specific root length and higher root N concentrations in ash than beech.

The detailed analysis based on root orders produced a precise picture of species differences in root morphology and chemistry. While the difference in specific root length and specific root area between the two species (higher values in beech) was larger in the 1st order roots and diminished towards the 4th order roots, the species difference in root tissue density and N concentrations increased from 1st to 4th order roots. Thus, the higher-order roots of beech are thinner than the corresponding ash roots but have a similar length and area development per root mass due to their much higher root tissue density. The higher tissue density may indicate a higher mean longevity of the higher-order roots of beech compared to the corresponding ash roots (Ryser, 1996). The fine 1st and 2nd order roots of beech developed a much larger surface area than the youngest ash rootlets despite a higher tissue density; they also possess more than 5-times higher fine root tip abundances per root mass than ash roots (Jacob et al., 2012). Accordingly, the 1st and 2nd order beech roots should achieve a much higher nutrient and water uptake capacity per unit of invested carbon or nitrogen due to the larger surface area. Surprisingly, this seems not to be the case. With ^{15}N tracer experiments in a mixed forest, Jacob et al. (unpubl.) found a significantly higher uptake of NH_4^+, NO_3^- and

glycine per fine root mass for ash than for beech, despite the much higher SRA and SRL of the latter. It appears that fine root morphology is not a good indicator of the root uptake capacity of tree roots. Beech and ash fine root systems were more or less similar with respect to the distribution of root length over the four root order classes with 65-70% being contributed by 1st order roots. This may be an expression of inherent constraints on the C allocation patterns in tree root systems where the task of maintaining a large absorbing surface area in the 1st order rootlets has to be balanced with the requirements of providing longer-lived more robust higher order roots with transportation, storage and anchorage functions (Pregitzer et al., 1997; Guo et al., 2008; Fan & Jiang, 2010).

An unexpected result is the remarkable constancy of $\delta^{13}C$ in the root bulk dry mass across all four root order classes in beech and ash and the by 3 ‰ lower $\delta^{13}C$ values in beech roots throughout the samples. Since both species shared the same soil and atmospheric environments, differences in drought exposure can be excluded. A more likely explanation of the systematic species difference in root $\delta^{13}C$ could be inherent species-specific characteristics of carbohydrate storage and supply for root growth (Epron et al., 2011). Constant $\delta^{13}C$ signatures in the root bulk dry mass across the four root order classes may primarily be a reflection of the constant growing conditions in our experiment where photosynthetic carbon gain and root water uptake should not have experienced greater variation over time and root growth, and ageing probably occurred more or less continuously. However, increases in root diameter and tissue density by more than 50% between the 1st and 4th order roots are associated with marked changes in root anatomy, periderm chemistry and also function which might well have affected the $\delta^{13}C$ signature of total dry mass in the root tissue. One possible explanation could be that fine root growth is most likely a high-priority C sink in these vigorously growing beech and ash saplings that absorbed a major part of the photosynthates assimilated in the leaves directly without previous storage and metabolisation in other organs. The constant growing conditions may have generated a uniform $\delta^{13}C$ signal in the carbohydrates used for root growth. The role of fine roots as high-priority C sinks might also explain why $\delta^{13}C$ was significantly higher in 2nd order beech fine roots in the mix treatment as compared to the other treatments; beech

was found to have shorter root longevity in mixture than in the mono and iso treatments (Beyer et al., unpubl. results). A higher C demand for root growth would decrease the discrimination against ^{13}C. This explanation, however, is not applicable for the elevated $\delta^{13}C$ values of ash in the mix treatment.

Our competition experiment started from the observation in field studies that beech trees may produce fine roots with significantly higher SRA and SRL when growing in mixture with Norway spruce (*Picea abies* (L.) Karst.) as compared to monospecific beech stands (Bolte & Villanueva, 2006). In contrast, our results show only weak evidence in support of competition-induced alteration of fine root morphology and chemistry in beech and ash saplings. Significant competition-induced modifications of root morphology were detected neither in beech nor in ash. The more detailed analysis based on root order classes also revealed no consistent treatment effects (except for a higher $\delta^{13}C$ value of beech and ash 2nd order roots in the mix treatment). In fact, the PCA and GLM analyses indicate that the influence of intra- and interspecific competition on root morphology is negligible or insignificant, and of minor importance only with respect to root N content and $\delta^{13}C$ signature. This is also true for the degree of mycorrhizal colonization that was not affected by competition intensity. The same analysis evidenced the large influence of root order on fine root morphology and chemistry which calls for a shift from a root diameter-focused to a root order-centred perspective in functional root ecology (Pregitzer et al., 1997; Eissenstat et al., 2000).

Alterations in root morphology in response to specific neighbours have been observed in *Fagus* and other trees, and also in grassland plants (van Hees, 1997; Huber-Sannwald et al., 1997; Curt et al., 2005). Our finding of a weak response of ash and beech roots to neighbour presence and the observed one-sided response of beech roots to spruce presence, but not of spruce to beech presence (Grams et al., 2002; Bolte & Villanueva, 2006), indicates that root morphological alteration in response to neighbours may not be a universal process, but rather a species-specific, and perhaps site- and age-specific reaction. No morphological change in response to allospecific neighbours was also found in a field growth experiment with 6-yr-old beech trees (Lei et al., 2012). The production of thinner

roots with a higher SRL and SRA in response to a specific neighbour could improve nutrient and water uptake under conditions of increased resource competition because a higher SRL is typically associated with higher root respiration and also elevated N uptake rates (Reich et al., 1998). On the other hand, thinner roots with lower tissue density are less costly and should achieve a better cost/benefit rate of root operation than thicker roots (Eissenstat, 1992), but their lifetime may also be shorter. We speculate that the apparently contrasting responses of beech roots in the field study of Bolte and Villanueva (2006) and in our experiment may be due to differences in tree age (adult vs. sapling) and growth conditions (acidic forest soil vs. base-rich laboratory soil system).

2.5 Conclusions

This root competition experiment with two morphologically and functionally largely different temperate tree species conducts a root order-focused analysis of morphological and chemical responses in the fine root system. Large differences in various root traits between the root order classes 1 to 4 underscore that comparison of root systems between different treatments and between species should focus on root orders instead of root diameters as has been widely done. We found no major root morphological or chemical alteration in the response to either intraspecific or interspecific competition which partly is contradicting earlier observations under field conditions in mature stands. We conclude that adaptive root responses to competition may not be a universal phenomenon and are likely to vary with site conditions, species and plant age. Fine root morphology may show considerable differences between coexisting temperate broad-leaved tree species but morphology does not allow interference on root functioning. While root morphology appeared to be rather unaffected by root competition in our experiment, further studies have to show whether root functioning and root longevity respond to the presence of belowground neighbours.

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CHAPTER

3

Inter- and intraspecific competition effects on plant growth and root survival of *Fagus sylvatica* and *Fraxinus excelsior*

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Abstract

Belowground competition has been identified as a major force structuring plant communities, but it is not well known how inter- and intraspecific root competition are influencing the survivorship of individual roots. We investigated the impact of inter- and intraspecific competition between European ash *(Fraxinus excelsior)* and European beech *(Fagus sylvatica)* on fine root survivorship, root system size and plant productivity in a competition experiment with direct fine root growth observation.

Ash and beech saplings were grown either in mixture, monoculture or in isolation (single plant) in rhizoboxes with a transparent observation window that allowed quantifying root growth as well as root longevity dependent on neighbour presence. Root survival was analysed using Cox proportional hazards regression and Kaplan-Meier estimations. Standing root biomass and root productivity were quantified at a final harvest, allowing the calculation of competition indices and biomass partitioning in the plant.

With competition indices indicating asymmetric competition in favour of ash, our experiment supports earlier findings on the competitive superiority of juvenile ash over beech plants. Mean root lifespan differed significantly among species (higher longevity of ash fine roots) and also in dependence of the competition treatment. The risk of fine root mortality increased when beech roots grew in mixture with ash or in beech monoculture as compared to beech plants growing in isolation. In contrast, ash fine roots had a lower mortality in mixture with beech than when grown in isolation.

Our data indicate that ash fine roots apparently profit from the presence of beech roots while beech root growth and survival are negatively affected, indicating size-asymmetric belowground competition. Competition may represent an important force influencing the fine root lifespan of these tree species.

Keywords: *survival analysis, belowground competition, root longevity, European ash, European beech, rhizobox*

3.1 Introduction

Fine roots represent only a small fraction of forest biomass but have been found to play an important role for carbon sequestration through rapid root turnover and subsequent decomposition (Jackson et al., 1997; Block et al., 2006). Numerous studies have investigated effects of environmental factors on fine root growth, turnover and longevity, among them effects of drought (Meier & Leuschner, 2008; Gaul et al., 2008b; Brunner et al., 2009), frost (Gaul et al., 2008a), ozone (Mainiero et al., 2009; Nikolova et al., 2010), nitrogen addition (Johnson et al., 2000; Rasse, 2002; Phillips et al., 2006) and elevated CO_2 (Iversen et al., 2008; Stover et al., 2010).

Another important factor likely influencing root growth and survival is root competition which is studied in forests or tree plantations mostly by examining changes in standing root biomass or root production in stands with different species composition (Rewald & Leuschner, 2009a; Lei et al., 2012) or by experimental approaches in the field (Hertel & Leuschner, 2006; Rewald & Leuschner, 2009b) . It has been shown that root competition can be more intense than shoot competition in certain ecosystems (Wilson, 1988) but the mechanisms of the interaction are not well understood. Root competition may be more or less symmetric but can also be asymmetric (de Kroon et al., 2003; Schenk, 2006; Rewald & Leuschner, 2009b) which may lead to the eventual competitive exclusion of inferior competitors. One possible consequence of belowground competition is altered resource availability in the soil which might affect the longevity of roots (Lopez et al., 1998; Withington et al., 2006; Carswell et al., 2012). Reduced root longevity could lower standing root biomass and/or increase root turnover. Similar to drought or frost, competition may act as a stressor that increases root mortality and decreases longevity.

However, our understanding of the effects of intra- and interspecific competition on fine root growth, death and root turnover in temperate broad-leaved trees is rudimentary. This is unfortunate because the role of fine root productivity for the carbon cycle and soil biological activity in forests is increasingly recognised (Eissenstat et al., 2000; Chapin & Ruess, 2001; Norby et

al., 2004; Brüggemann et al., 2011) while foresters are discussing the advantages of mixed forests over monocultures in Europe and North America (Pretzsch, 2005; Knoke et al., 2005; Pretzsch & Schutze, 2009; Richards et al., 2010). This demands for a mechanistic understanding of root competition in tree mono- and polycultures.

In Central European temperate broad-leaved forests, competition between young trees of European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) is a relevant phenomenon in forestry. Ash shows vigorous shoot growth in the seedling and sapling stage on base-rich soils where it may suppress beech saplings in the early stages of stand regeneration (Rust & Savill, 2000; Wagner et al., 2010). While beech is the dominant tree species of the natural forest vegetation of Central Europe (Ellenberg & Leuschner, 2010) and one of the economically most important tree species ash is a subordinate species which accompanies beech in various forest communities on fertile soils. The two species differ in light demand at the juvenile stage, successional status and drought tolerance (ash: early- to mid-successional, light-demanding with low drought sensitivity; beech: late-successional with relatively low light demand but higher drought sensitivity (Rust & Savill, 2000; Köcher et al., 2009; Ellenberg & Leuschner, 2010), traits that may alter the species' abundance in forest communities and their valuation in future forestry under a warmer and partly drier climate. While aboveground competition between beech and ash is determined by the rapid height growth of ash on the one hand and the superior shade production of beech on the other hand (Leuschner & Rode, 1999; Petritan et al., 2009), belowground competition between the two species may be equally, or more important, but is less well investigated.

Several studies in mixed stands with beech and ash on fertile soil provided evidence that ash may explore empty soil space faster than beech, produce a higher fine root density at maturity in mixture, and show a higher root turnover than co-occurring beech (Meinen et al., 2009b; Jacob et al., 2012). The belowground interactions of these two species might be particularly interesting because beech and ash are colonized by two different types of mycorrhizal fungi. Like most other temperate tree species, beech is associated with ectomycorrhizal fungi (EM) while

ash is colonized by arbuscular fungi (AM). The different mycorrhizal strategies may be linked to differences in root functioning (Jacob et al. unpubl.) and forest carbon and nutrient cycling (Cornelissen et al., 2001), which in turn could influence root competition between beech and ash. A deeper understanding of this interaction requires experiments addressing competition effects on root dynamics.

This study investigates the effects of inter- and intraspecific belowground competition on root growth and root survivorship of beech and ash saplings in a rhizobox experiment. Root dynamics were observed by repeated image analysis in combination with a root survivorship analysis. Growth in two-species mixture was compared with a monospecific treatment and a single-plant treatment in which the target plant competed only with its own roots (self competition). The objectives of the study were to examine (i) whether interspecific fine root competition between beech and ash is more intense than intraspecific competition in monoculture of the two species, (ii) interspecific competition is asymmetric in favour of ash as indicated by previous studies on beech and ash saplings, and (iii) inter- and intraspecific competition reduce the longevity of fine roots.

3.2 Material and methods

3.2.1. Plant material and rhizobox experiment

Beech and ash saplings were excavated in a random sampling scheme in Hainich National Park, a mixed temperate broad-leaved forest in Central Germany (51°04' N 10°30' E, about 350 m a.s.l). The soil type is defined as Stagnic Luvisol (IUSS Working Group WRB, 2007). The saplings of beech and ash were about 20 cm high and ca. three years old when excavated on July 22, 2008. The root system was carefully washed to remove all adhering soil material and the saplings were subsequently planted into boxes of 30 cm x 40 cm x 4 cm size (length x width x depth) filled with homogenized loamy soil which resembled the soil material at the plants' origin (Table 3.1).

Table 3.1 Soil properties in the rhizoboxes before the planting of the beech and ash saplings. Given are means and standard errors (n=15).

Variable	Mean		SE
pH (H_2O)	8.14	±	0.23
pH (KCl)	7.50	±	0.04
C_{org} ($g\ kg^{-1}$ dw)	36.10	±	0.20
N_{total} ($g\ kg^{-1}$ dw)	1.62	±	0.01
C/N ($g\ g^{-1}$)	22.36	±	0.18
P_{resin} ($mg\ kg^{-1}$ dw)	14.24	±	2.29
$N\text{-}NO_3^-$ ($mg\ kg^{-1}$ dw)	3.47	±	0.33
$N\text{-}NH_4^+$ ($mg\ kg^{-1}$ dw)	1.19	±	0.12

The rhizoboxes possessed a 30 cm x 40 cm large Plexiglas window on every front side that was covered with light impenetrable black removable plastic to guarantee undisturbed root proliferation but allow the observation of root growth. We established a growth experiment with three treatments to test for effects of intraspecific and interspecific competition between beech and ash: monospecific rhizoboxes (two plants of the same species), mixed boxes (one ash and one beech plant) and so-called 'iso boxes' with a single beech or ash plant.. Sixteen monospecific boxes (8 beech and 8 ash), 8 mixed boxes and 8 iso boxes (4 beech

and 4 ash) were established (32 in total). The boxes were placed in a randomized arrangement in a climatized greenhouse where the plants grew for 34 months until harvest in late April / early May, 2011. The plants were grown under constant conditions of a 13h photoperiod / 11h nighttime cycle with additional constant light (besides sun light) being supplied by mercury fluorescent lamps (EYE CLEAN ARCTM, Eye Lightning International, OH, USA) with a photosynthetic photon flux density of 170 ± 10 µmol m^{-2} s^{-1} andtemperature was kept at 20°C during daytime and 10°C during night time. Thereby, dormancy could not be prevented but was shortened to 4 month during the experiment. The boxes were watered every 2-3 days and volumetric soil water content was measured with a mobile TDR probe (Trime-FM, IMKO, Ettlingen, Germany), and kept at constant level (ca. 20 vol.%) to guarantee comparable non-limiting soil moisture conditions in all boxes. On the date of planting (July 22, 2008), ten additional beech and ash saplings were harvested to obtain the mean initial biomass of the two species at the experiments' beginning (Table 3.2).

Table 3.2. Biomass fractions of the beech and ash plants at the experiment's start (in g DW per plant).

	Initial Biomass		
	P	**Beech**	**Ash**
Total plant	0.77	2.06 ± 0.47	2.22 ± 0.23
Total aboveground	0.98	1.26 ± 0.27	1.25 ± 0.15
Total belowground	0.49	0.80 ± 0.21	0.97 ± 0.08
Leaves	0.14	0.38 ± 0.05	0.52 ± 0.07
Fine roots	**0.03**	**0.16 ± 0.05**	**0.52 ± 0.07**
Coarse roots	0.68	0.64 ± 0.17	0.56 ± 0.06
Shoot axis	0.55	0.88 ± 0.17	0.74 ± 0.09

T-test table with *P*-values and means ± SE (n=5). Significant species differences are printed in bold.

About 19% of the saplings (mostly ash plants) showed poor growth in the boxes from the beginning onwards and died in the first months. Therefore, only 22 boxes of the 32 original ones were included in the analysis (n: beech iso=4, beech mono=16, beech mix=4, ash iso=3, ash mono=6, ash mix=4).

3.2.2 Harvest

The trees were harvested in the time interval from 26 April to 12 May, 2011, i.e. over a period of 17 days by applying a rotating harvesting scheme alternating between the different treatments. The above- and belowground biomass was completely harvested and separated into the fractions leaves, shoots, coarse roots (> 2 mm in diameter) and fine roots (< 2 mm). Shoot length and shoot diameter at soil surface were measured. All soil particles adhering to the roots were removed by washing the roots carefully under tap water. Three randomly chosen fine root segments of about 10 cm length were taken from each tree and stored in tap water at 4°C until they were analysed on a flat-bed scanner with WinRhizo 2005c software (Régent Instruments Inc., Québec, QC, Canada). The following root morphological parameters were measured (including the ectomycorrhizal structures in the case of beech): specific root area (SRA), specific root length (SRL) and mean root diameter. Thereafter all compartments were oven-dried (70°C, 48 h) and weighed.

3.2.3 Calculation of competition indices

For comparing the performance of the two species in mixture with that in monoculture, we calculated the competitive ability index (CA) according to Rewald and Leuschner (2009) which relates a species' relative growth rate (RGR; in mg g^{-1} d^{-1} between the date of planting and date of harvest) in the mixed boxes (interspecific competition; RGR_{mix}) with that in the monospecific boxes (intraspecific competition, RGR_{mono}) as follows:

$$CA = (RGR_{mix} - RGR_{mono}) * RGR_{mono}^{-1}.$$

Positive CA values display a better growth in mixture with a different species than with a conspecific plant.

The relative competitive ability index (RCA) relates the growth of a single plant (only self competition; RGR_{iso}) to the growth in monospecific boxes (intraspecific competition, RGR_{mono}):

$RCA = (RGR_{iso} - RGR_{mono}) * RGR_{iso}^{-1}$.

If the RCA index reaches unity (RCA = 1), intraspecific competition is so strong that no growth of the target plant is possible, whereas a RCA value of 0 indicates no intraspecific competition effect on growth.

3.2.4 Root survivorship analysis

The windows of the rhizoboxes were scanned with a flat-bed scanner (Epson Perfection V10) at approximately monthly intervals from July 2009 to April 2011. The scan images (size 225 mm x 200 mm) were analyzed with WinRHIZO Tron software (Régent Instruments Inc., Québec, QC, Canada) for root length increment and the loss of root segments in order to measure dynamics of root growth and root death as inferred from the disappearance of root segments or marked tissue colour change. At each scanning event, root branches were hand marked on the screen and their diameters along the root indicated for root classification. The increase in root length (newly grown roots) and the loss in root length (assumed root death) were plotted against time. Because of the time-consuming process of root growth and turnover analysis, three boxes per treatment were randomly chosen for analysis. Ash roots could be distinguished from beech roots based on periderm colour and root branching patterns (ash: whitish periderm, relatively thick and typically very rough surface structure, beech: brown-red to dark-brown periderm, thinner roots with more delicate morphology (Hölscher et al., 2002; Meinen et al., 2009a). Because of the uncertain vitality status of the roots, we used a conservative classification system and considered a root segment as dead only when its colour deviated considerably from the vital status, the root tissue disintegrated or the root disappeared completely. The longevity of a root segment was expressed in days by calculating the period between the assumed date of death and the date of first

observation; the dates of first observation and death were assumed to be the mid-point between two subsequent scanning dates.

3.2.5 Statistical analyses

The statistical analyses were carried out with the software R (Version 2.13, The R Foundation for Statistical Computing, http://www.r-project.org). All studied growth-related and root morphological parameters were compared among the treatments and species by one-way analysis of variance (ANOVA) or – in cases of unbalanced variances within groups – by general linear models (GLM). Subsequent pairwise comparisons were performed using Tukey's HSD test. Normality of data distribution was checked by quantile-quantile plots, variance homogeneity was inspected in boxplots.

Root survivorship curves were produced with the Kaplan-Meier product limit method (Kaplan & Meier, 1958) and the package 'survival' in R. If the observed root segment was still alive at the time of the last scan, root longevity was right-censored. Roots present at the first scan were considered to have been produced at that time. The calculation of median longevity was not possible for the ash mono treatment because more than 50% of the fine roots were still alive at the harvest and the data thus censored. Therefore, survival rates were not only calculated for the full length of the scan monitoring period (622 days) but additionally for shorter periods of 250 and 500 days. We modeled root survival for each treatment with three-factorial Cox models using tree species, treatment (mix, mono, iso) and the box effect as covariables ('coxph' command in R). By using Cox proportional hazards regression, we determined the effect of tree species and tree mixture on root longevity (Cox, 1972; Wells & Eissenstat, 2001; Anderson et al., 2003). From the fitted Cox models, we derived the hazard ratio (HR). A HR value > 1 indicates a higher death risk, a HR < 1 a lower death risk of one parameter level against the other, e.g. beech root survival vs. ash root survival. The significance level for all statistical tests was set to $P < 0.05$.

3.3 Results

3.3.1 Treatment effects on root and shoot growth and competitive ability of beech and ash

Table 3.3 Biomass per plant individual (DW in g) in the fractions coarse roots (> 2mm diameter), fine roots (< 2 mm diameter), shoot and leaves.

		n	Coarse roots	Fine roots	Shoot	Leaves
Beech	iso	4	9.54 ± 2.16 a	9.60 ± 2.82 a	17.89 ± 3.63 **c**	4.58 ± 0.65 **ab**
Beech	mono	16	8.29 ± 1.40 a	7.72 ± 1.20 a	9.45 ± 1.42 **ab**	2.80 ± 0.42 **a**
Beech	mix	4	6.31 ± 2.21 a	5.75 ± 2.91 a	8.74 ± 4.12 **abc**	3.04 ± 0.74 **ab**
Ash	iso	3	11.34 ± 5.01 a	5.61 ± 1.64 a	3.47 ± 0.72 **b**	3.27 ± 0.81 **ab**
Ash	mono	6	5.64 ± 2.17 a	4.06 ± 1.22 a	3.75 ± 1.32 **b**	5.42 ± 0.92 **b**
Ash	mix	4	9.43 ± 2.23 a	6.32 ± 2.11 a	5.62 ± 0.63 **b**	5.12 ± 0.61 **ab**

Given are means ± SE of n individuals. Significant differences between the treatments are indicated with different lower case letters ($P < 0.05$). Treatments: iso = one sapling per rhizobox, mono = two saplings of beech or ash per rhizobox, mix = one beech and one ash sapling per rhizobox.

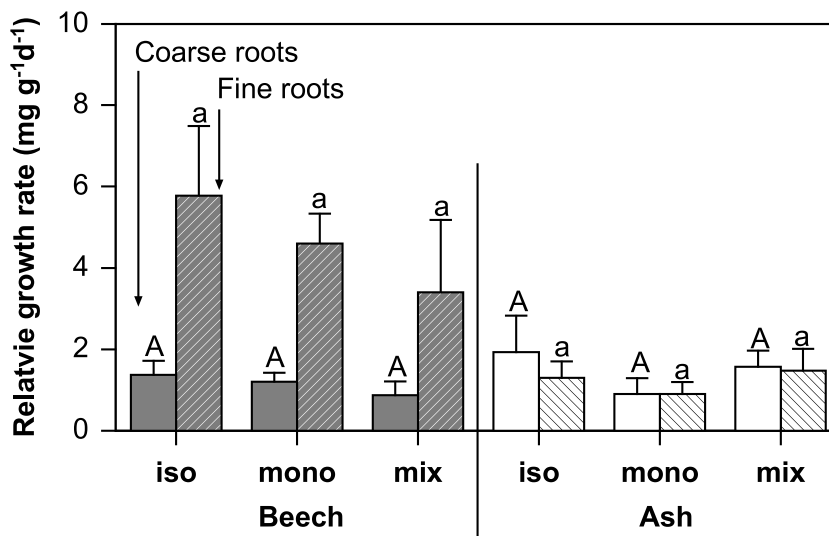

Fig. 3.1 Relative growth rate (RGR) (in mg g^{-1} d^{-1}) of fine roots of beech (grey bars with stripes) and ash (white bars with stripes) and coarse roots of beech (grey bars) and ash (white bars). Given are means ± SE of 3-16 replicate plants in the treatments. Significant differences between treatments are indicated by different upper case letters (RGR of coarse roots) or by different lower case letters (RGR of fine roots, $P < 0.05$).

The competition treatment had no influence on fine root diameter or SRA (Table 3.4). Specific root length tended to be higher in beech than ash in all treatments (differences not significant at $P < 0.05$).

Table 3.4 Fine root morphological traits (mean diameter, specific root area (SRA) and specific root length (SRL) of the beech and ash plants in the three treatments at the date of harvest.

		n	**Diameter** (mm)				**SRA** ($cm^2\ g^{-1}$)				**SRL** ($m\ g^{-1}$)			
Beech	iso	4	0.255	±	0.007	**a**	440.53	±	39.04	a	5813.1	±	701.4	**a**
Beech	mono	16	0.275	±	0.002	**a**	332.29	±	4.13	a	4055.1	±	73.7	**ab**
Beech	mix	4	0.278	±	0.014	**a**	445.66	±	25.14	a	5359.2	±	405.0	**ab**
Ash	iso	3	0.443	±	0.021	**b**	373.86	±	6.23	a	2751.1	±	129.4	**ab**
Ash	mono	6	0.472	±	0.017	**b**	399.78	±	25.12	a	2879.3	±	281.4	**b**
Ash	mix	4	0.439	±	0.003	**b**	405.22	±	6.76	a	2982.7	±	58.3	**ab**

Given are means (± SE). Significant differences between treatments are marked with different lower case letters ($P < 0.05$).

During the experiment, the total fine root length visible in the rhizobox windows (225 mm x 200 mm) increased by 63 – 205 cm in absolute terms in the studied boxes (Fig. 3.2a). Both the fine root length increase and fine root length loss were largest in the mixed treatment (Fig. 3.2a and b) with 261 ± 70 cm of cumulative root length being present immediately before the harvest and 180 ± 112 cm of root length lost since the start of the experiment, indicating relatively high turnover rates in this treatment. The other four treatments ranged below the fine root length growth of the mix treatment in a sequence from the ash mono (205 ± 97 cm) to the beech iso (166 ± 24 cm), beech mono (131 ± 53 cm) and finally the ash iso treatment (131 ± 33 cm) (Fig. 3.2a and b). Cumulative fine root loss was much smaller in all other four treatments than in the mix treatment but did not show systematic differences between the beech and ash mono and iso treatments (all treatments ~ 64 ± 1 cm during the whole study period).

The harvest data showed that neither the fine root nor the coarse root biomass produced per sapling during the experiment differed significantly between species or treatments (Table 3.3). As a consequence, the mean relative growth rate (RGR; given in $mg\ g^{-1}\ d^{-1}$) of fine roots during the experiment (duration: 994 days) did not differ significantly between the species and treatments, but showed a non-significant trend for a decrease from the iso to the mono and further to the mix treatment in beech while the differences among the ash treatments were small (Fig. 3.1). The well-known relatively large fine root diameter of ash was found in all

treatments, while specific root area (SRA, surface area per mass) was not different between the species due to the higher tissue density of beech roots. The competition treatment had no influence on fine root diameter or SRA (Table 3.4). Specific root length tended to be higher in beech iso than ash iso treatments (*P* = 0.0787).

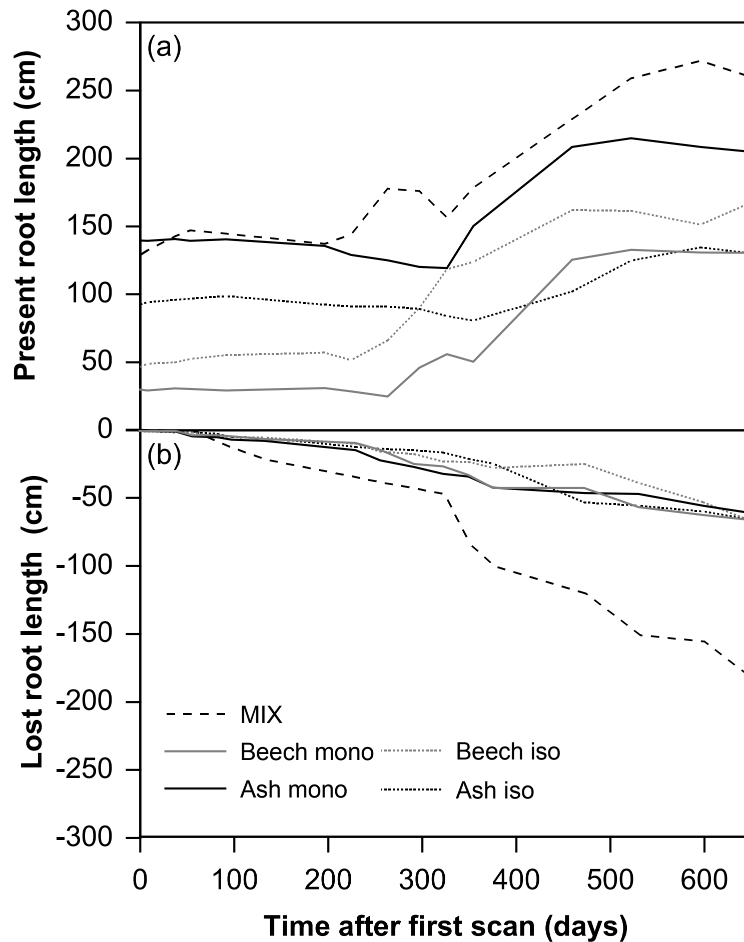

Fig. 3.2 Change in root length present (a) and loss of root length (b) over the scanned time period from July 2009 until April 2011 in the different treatments.

In the aboveground compartment, beech plants produced significantly more shoot mass (but not leaf mass) in the iso treatment than ash plants and also more than the beech mono treatment and all ash treatments. In contrast, ash was superior over beech with respect to leaf mass production in the mono treatment ($P < 0.05$).

Large differences between beech and ash were found for the competitive ability index (CA), which was calculated from the RGR of the target species in interspecific interaction (mix treatment) as compared to intraspecific interaction (mono treatment) for the aboveground, belowground and total biomass production (Table 3.5).

Table 3.5 Competition indices calculated for the beech and ash plants grown in the different treatments with interspecific, intraspecific or only self competition, differentiated for the belowground, aboveground and total plant biomass (n=3-16 plants).

CA	**Belowground**	**Aboveground**	**Whole plant**
Beech	-0.258	-0.229	-0.259
Ash	0.525	0.154	0.375
RCA			
Beech	0.179	0.478	0.334
Ash	0.423	-0.500	0.200

Given are mean values for the competition ability index (CA) which relates the growth with intraspecific competition to that with interspecific competition (mono vs. mix) and the relative competitive ability (RCA) which compares the growth of isolated plants with that of plants in monoculture with intraspecific competition (iso vs. mono).

From the decrease in beech fine root production from the mono to the mix treatment, it follows that the belowground competitive ability of beech in mixture was low (CA value of -0.258) compared to that of ash (CA value of +0.525) indicating asymmetric root competition in favour of ash (Tab. 3.5). A similar relation of the CA values between beech and ash with superiority of ash was found for the aboveground biomass and total biomass but the species difference was larger in the belowground compartment. The relative competitive ability index (RCA), which compares the RGR of a target species in intraspecific interaction (mono) with that in isolated growth (iso), showed lower values for beech (0.179) than for ash (0.423) in the belowground compartment, but a higher value for beech in the aboveground compartment and for total biomass (Table 3.5). Larger RCA indices indicate a higher intensity of intraspecific competition in the mono treatment. The negative RCA index of ash in the aboveground compartment reflects better growth of the plants in the mono than in the iso treatment in this species. The two competition indices indicate that ash was the superior competitor

in the mixed treatment below- and aboveground, which seemed to be related to relatively high belowground competition intensity in the ash mono treatment, while beech was exposed to higher aboveground competition intensity in the monospecific treatment than ash

3.3.2 Root survival as influenced by species and competition treatment

In the root survivorship analysis, a total of 11,996 root segments were monitored, of which 4209 (35%) could be traced until the time of disappearance or death. Roots which survived until the last scan were included into the equations as right-censored data. The root survival curves generated with these data show clearly different patterns between treatments and species (Fig. 3.3a and b). The median longevity of ash fine roots was not much different from that of beech roots when grown in isolation (483 vs. 565 days, Table 3.6), but ash roots showed a much higher longevity than beech in the mixed treatment (603 vs. 321 days) and also a higher median lifespan in the mono treatments (> 622 vs. 502 days). While beech decreased its root longevity from the iso to the mix treatment (medians: 687 and 313 days), ash increased it toward the mixture (and also to the mono treatment; 483 and 603 or > 622 days). The treatments also differed with respect to the temporal change of the survival rate during the experiment. While after 250 days of root lifetime, the lowest probability of survival was found in the beech mono treatment with 67.3%, the sequence of treatments changed and at the age of 500 days, by far the lowest root survival rate was faced by beech in the mix treatment (only 18.4% probability of survival, Fig. 3.2 a and Table 3.6).

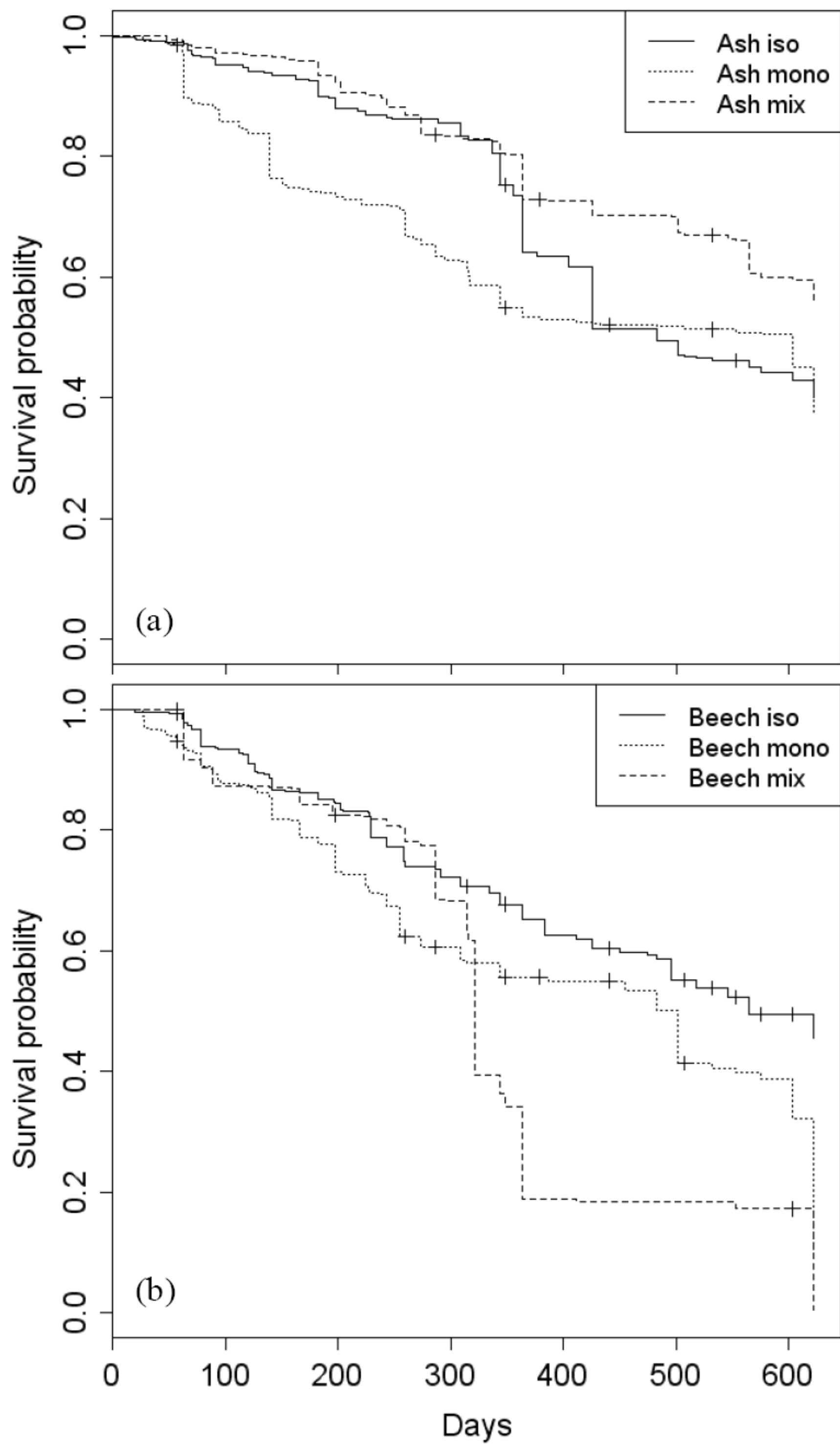

Fig. 3.3 Root survivorship curves with Kaplan-Meier estimates for the three treatments of ash (a) and beech (b) plants calculated from the presence/absence of roots according to continuous root observation on the box windows (right-censored data). Fine root appearance and loss events were assumed to have occurred midway between successive sampling dates.

Table 3.6 Survival data for the roots of the different treatments derived from the Kaplan-Meier equation for right-censored data. Given are the median longevity (point of time when 50% of the roots had died, in days) and the proportion of surviving roots after 250 or 500 days of lifetime.

Treatment	*n*	*n* uncensored	Median longevity	Probability of survival (%)	
				after 250 days	after 500 days
Beech iso	2266	687	565	77.2	55.1
Beech mono	1733	655	502	67.3	50.0
Beech mix	484	313	321	80.7	18.4
Ash iso	1723	613	483	86.1	49.5
Ash mono	2311	609	>622	88.2	70.1
Ash mix	3449	1323	603	71.8	51.9

Cox proportional hazard regressions show in all cases highly significant differences between the treatments and species (Table 3.7). Across all treatments, ash fine roots had a significantly lower risk of mortality than beech in the analysed time period (hazard ratio (HR) for ash vs. beech = 0.64, $P < 0.001$).

Table 3.7 Results of Cox proportional hazards regression analysis for individual root lifespan. Significant *P*-values are printed in bold (< 0.05). Significant interaction terms suggest greater (hazard ratio > 1) or lower (hazard ratio < 1) risk of mortality of the first-mentioned treatment.

			Hazard ratio	Confidence interval (95%)	*P*
All boxes					
Ash	vs.	Beech	0.64	[0.58-0.70]	**<0.001**
Mix	vs.	Iso	1.96	[1.75-2.20]	**<0.001**
Mono	vs.	Iso	1.38	[1.06-1.80]	**<0.05**
Mono	vs.	Mix	0.69	[0.58-0.83]	**<0.001**
Box effect			0.99	[0.98-1.00]	n.s.
Beech boxes					
Mix	vs.	Iso	7.71	[2.97-20.01]	**<0.001**
Mono	vs.	Mix	2.13	[1.64-2.78]	**<0.001**
Mono	vs.	Iso	103.30	[50.27-212.26]	**<0.001**
Ash boxes					
Mix	vs.	Iso	0.14	[0.11-0.19]	**<0.001**
Mono	vs.	Mix	0.002	[0.00-0.01]	**<0.001**
Mono	vs.	Iso	0.002	[0.00-0.01]	**<0.001**

When all treatments are pooled (category 'all'), root mortality risk decreased in general from the mono to the mix and finally to the iso treatments. However the hazard ratios of the three treatments also revealed the different behaviour of beech

and ash roots in this experiment. Beech roots faced the lowest mortality risk in the iso treatment and a significantly higher risk in the mono than the mix treatment (HR = 2.13, $P < 0.001$). The ash treatments showed the lowest risk in the mono treatment (HR for mono vs. mix and for mono vs. iso = 0.002, $P < 0.001$ in both cases; Table 3.7), while the iso treatment faced the highest risk (HR for mix vs. iso: 0.143, $P < 0.001$). The box effect (3 replicate boxes) was not significant.

3.4. Discussion

3.4.1 Asymmetric competition between ash and beech

Our competition experiment with ca. 3-yr-old ash and beech saplings support earlier findings on the competitive superiority of juvenile *Fraxinus* over *Fagus* plants (Rust & Savill, 2000; Saxe & Kerstiens, 2005) The Competitive Ability Index (CA), which compares the growth performance in mixture with that in monoculture, was much higher for ash than for beech, and the species difference was particularly large for the belowground compartment. Thus, root competition was asymmetric in favour of ash and this species profited from the presence of beech by increasing its productivity of coarse roots and fine roots in the mix treatment by about 60% over that in the mono treatment. The apparent stimulation of ash productivity was less in the aboveground compartment (c. 50% more shoot axis mass, but no more leaf mass), which shows that above- and belowground responses to a competitor can be largely different. On the other hand, when relating the growth performance in monoculture to that in isolation as is done in the RCA index, we found a higher intraspecific competition intensity for the ash mono boxes than for the beech mono boxes, i.e. a relatively small growth-reducing effect of intraspecific competition between the two neighbouring beech plants.

Surprisingly, this species difference was not caused by a higher fine root density of ash than beech in the mono boxes; on the contrary, the two beech plants achieved on average higher fine root densities (c. 3.3 g dm^{-3}) than the two ash plants (c. 2.0 g dm^{-3}; data not shown) in the mono treatments. Thus, other factors than the mere presence of a higher fine root biomass must be responsible for the

more intense intraspecific competition in the ash boxes. Possible explanations are higher root surface-specific nutrient or water uptake rates of ash as compared to beech or differences in the resource exploitation intensity of the AM and ECM fungal nets of ash and beech. The relatively high competition intensity in the ash mono boxes, in turn, offers an explanation for the increased productivity of ash roots in the mix treatment where they compete with beech roots. Ash roots apparently were released from the intense intraspecific competition when meeting an inferior competitor in the mix treatment.

However, the belowground success of competing plants does not only depend on the amount of root biomass produced but also on the capability to explore additional soil space. Ash saplings are known for their rapid vertical and lateral spread of roots (Rust & Savill, 2000; Fender et al., 2012) which is also visible from the large coarse : fine root ratio of *Fraxinus* in comparison to *Fagus* in our experiment; these thicker fine root elements enable efficient space exploration in the case of ash.

The finding of a size-asymmetric competitive interaction between beech and ash roots with superiority of ash agrees with the results of the container experiment of Rust and Savill (2000) which demonstrated that the overall mortality of beech saplings was strongly correlated with the proportion of ash plants (25 – 75%) being present in the mixture, i.e. that the mortality risk of beech saplings increased with density of ash plants in the neighborhood. However, the above mentioned study took only the survival of the total plant into account and did not analyse root survival in detail.

There has been a vital discussion about the high competitive ability of ash saplings in the regeneration stage of beech-ash mixed stands in Central Europe, a phenomenon termed beech regeneration dieback (Rysavy & Roloff, 1994; Wagner, 1999). According to the experiments of Rust and Savill (2000), asymmetric root competition for water is the decisive process in the beech-ash interaction at sites with limited water supply. As water supply was favourable across all treatments in our experiment, competition for water cannot be the only decisive factor promoting ash root growth in mixture with beech. Jacob et al. (unpubl. results) showed in an in situ ^{15}N trace study that ash fine roots had a considerably larger

ammonium and nitrate uptake capacity per unit fine root surface area than beech. We speculate that ash roots may have pre-empted the available N pools (and perhaps also soil moisture) in the shared soil volume which may have resulted in a reduction of beech root growth, but a stimulation of ash root growth in the mix treatment. N depletion could decrease root growth but may also alter root longevity since N addition has often been reported to alter fine root turnover of forest trees, either increasing or decreasing it (Brassard et al., 2009). Such a nitrogen-mediated mechanism would represent exploitation competition based on unequal resource acquisition by the two species and it could also explain the measured higher intraspecific competition in the ash mono boxes. We cannot exclude that interference competition, i.e. direct root-to-root interaction through self/non-self discrimination or the release of allelopathic exudates, is contributing to the asymmetric outcome of competition. For allelopathy, however, it was assumed not to be an important mechanism in northern forests (Brassard et al., 2009).

Beech has been found to be an inferior belowground competitor also in other tree species combinations, for example in stands of young beech-spruce (Grams et al., 2002; Wang et al., 2003), beech-Douglas fir (Hendriks & Bianchi, 1995) or beech-oak-spruce-Douglas fir mixtures (Lei et al., 2012). An observational study in a mature mixed stand indicated apparent competitive superiority of ash over four other broad-leaved species (Jacob et al., 2012). On the other hand, beech has also been found to be a superior belowground competitor, for example in mature stands of beech and oak (Leuschner et al., 2001), beech, oak, hornbeam and linden (Rewald & Leuschner, 2009a), beech and spruce (Schmid, 2002; Bolte & Villanueva, 2006) and beech and Douglas fir (Reyer et al., 2010). It appears that the outcome of root competition is dependent on the species combination, tree age and the environment; the same species may be a superior or inferior competitor in contrasting neighborhoods.

3.4.2 Effects of inter- and intraspecific belowground competition on root longevity

As far as we know, we are the first to show that belowground competition has a significant effect on the mortality risk of fine roots. Our data reveal that the competition treatment significantly altered the risk of beech and ash roots to die while other factors with a possible influence on root mortality such as drought stress and carbohydrate supply (as inferred from the uniform soil moisture and light regimes across the treatments) could be controlled in the experiment. The competition effect is visible in the significantly higher mortality risk of beech roots in the mix and mono boxes than when grown in isolation (hazard ratios of 7.7 and 2.1 for the mix vs. iso and mono vs. iso constellation, respectively) and also in the lower mortality risk of ash roots in the mix as compared to the iso treatment (HR = 0.14). Apart from the mortality risk, the change in survival rate over the life time of a root also revealed contrasting patterns for the different treatments which can only be explained by the interacting effects of competition and species.

The unexpectedly high mortality rates of ash roots in the iso treatment after day 350 of their individual lifetime are astonishing and are not easy to explain. One may assume that the different root branches of the same ash plant may have faced considerable self competition and/or reciprocal root inhibition in the boxes (Falik et al., 2003; Schenk, 2006) where root density was relatively high in the experiment's second half despite only one plant being present. However, such a response should also have occurred in the ash mono treatment with even higher root density. Alternatively, one may speculate that the relatively high root turnover in the iso treatment was driven by the carbon economy in these ash plants because the costs of establishing new roots may have been lower than maintaining existing roots.

Ash fine roots had across all treatments a lower mortality risk than beech roots which may partly be related to the larger mean fine root diameter of *Fraxinus* since root diameter was identified as the most influential factor on the hazard ratio across 18 Chinese temperate tree species (Gu et al., 2011). However, even though the thicker roots may have increased the competitive ability of ash in the mix

treatment by increasing root longevity, they cannot be the main explanation of the species' competitive superiority belowground.

The calculated competition indices for beech and ash imply a greater belowground competitive ability of ash over beech and a higher intensity of intraspecific competition in the ash than in the beech mono treatment. These findings are partly in line with the results of the root survival analysis, where the median longevity of ash was significantly higher than that of beech in mixture. However, it is important to note that the results of the root survivorship analysis cannot directly be compared to the production-based competition indices because the survival probability is calculated for root segments independent from their length or biomass. A vital discussion exists about the reliability of rhizotron observations of root survival; several authors assume a general overestimation of turnover rates by this technique (Guo et al., 2008; Strand et al., 2008). On the other hand, right-censoring of root observation data and consideration of roots with a longer history than the time span to the first scan must result in underestimation of root age (Strand et al., 2008). Due to the difficulty of determining the date of root death, we used the time of root disappearance or a marked change in root morphology as symptoms of death. This approach probably overestimates the longevity of beech roots in comparison to ash roots, as the latter have higher N concentrations and thus are decomposing and dissappearing faster (Scheu & Schauermann, 1994). These are inherent problems not only of minirhizotrons but also of the rhizobox approach; however, alternative methods without these shortcomings do not yet exist.

Our rhizobox experiment offers the opportunity to obtain simultaneously information on root dynamics throughout the experimental period in a constant environment and on root biomass production through the harvest at the end of the experiment. This is an advantage over minirhizotron measurements conducted under field conditions where the relation to root biomass density and thus biomass production is more difficult to establish and environmental conditions are difficult to control.

3.5 Conclusions

This root competition experiment with two morphologically and functionally largely different temperate tree species provided evidence for significant effects of inter- and intraspecific competition on root survival probabilities and relative root growth rates. Thus, apart from several abiotic (water and nutrient availability, temperature, CO_2 and O_3 concentrations) and biotic factors (age, root order, vitality and seasonality), competition has to be recognized as an additional factor influencing root longevity and thus turnover. We could show that competition with conspecific or allospecific roots can alter root longevity in both directions, either toward a shorter lifespan and thus a higher mortality rate, or toward a greater longevity and thus reduced mortality. In the first case, competition is experienced as a negative agent reducing vitality and/or growth, in the latter case competition acts like a facilitating factor. This situation was found when interspecific competition was highly asymmetric and led to an apparent stimulation of root growth of the superior species.

Several earlier competition experiments with potted herbaceous species suggested that a plant may produce greater root biomass in the presence of a competing plant due to self/non-self discrimination than when growing alone (Falik et al., 2003; Hess & De Kroon, 2007) to avoid wasteful allocation of resources to competition with its own roots. Our results with woody plants do not support this concept since we found (non-significant) tendencies for a decrease, rather an increase, in a plant's coarse and fine root biomass from the iso to the mix treatment. Thus, in beech and ash, the presence of allospecific (or conspecific) roots apparently did not stimulate increased resource allocation toward the roots to improve belowground competitive ability. Our data further support the conclusion that interspecific root competition can be highly size-asymmetric in spite of the earlier assumption that belowground competition should be more symmetric than aboveground competition (Weiner et al., 1997; Cahill & Casper, 2000). Further studies with laboratory systems and also in the field are needed to deepen our understanding about the dependence of root survival and root longevity on the intensity and type of belowground competition in trees.

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CHAPTER

4

Roots from beech (*Fagus sylvatica* L.) and ash (*Fraxinus excelsior* L.) differentially affect soil microorganisms and carbon dynamics

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Abstract

Knowledge about the influence of living roots on decomposition processes in soil is scarce but is needed to understand carbon dynamics in soil. We investigated the effect of dominant deciduous tree species of the Central European forest vegetation, European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.), on soil biota and carbon dynamics differentiating between root- and leaf litter-mediated effects. The influence of beech and ash seedlings on carbon and nitrogen flow was investigated using leaf litter enriched in ^{13}C and ^{15}N in double-split-root rhizotrons planted with beech and ash seedlings as well as a mixture of both tree species and a control without plants. Stable isotope and compound-specific fatty acid analysis (^{13}C-PLFA) were used to follow the incorporation of stable isotopes into microorganisms, soil animals and plants. Further, the bacterial community composition was analyzed using pyrosequencing of 16S rRNA gene amplicons. Although beech root biomass was significantly lower than that of ash only beech significantly decreased soil carbon and nitrogen concentrations after 475 days of incubation. In addition, beech significantly decreased microbial carbon use efficiency as indicated by higher specific respiration. Low soil pH probably increased specific respiration of bacteria suggesting that rhizodeposits of beech roots induced increased microbial respiration and therefore carbon loss from soil. Compared to beech $\delta^{13}C$ and $\delta^{15}N$ signatures of gamasid mites in ash rhizotrons were significantly higher indicating higher amounts of litter-derived carbon and nitrogen to reach higher trophic levels. Similar $\delta^{13}C$ signatures of bacteria and fine roots indicate that mainly bacteria incorporated root-derived carbon in beech rhizotrons. The results suggest that beech and ash differentially impact soil processes with beech more strongly affecting the belowground system via root exudates and associated changes in rhizosphere microorganisms and carbon dynamics than ash.

Keywords: *^{13}C, ^{15}N, bacteria, carbon cycling, decomposition, fungi, nitrogen, soil food web*

4.1 Introduction

Soils store twice as much carbon as plants and the atmosphere together thereby forming an important component of the global carbon cycle (Schlesinger and Andrews, 2000). However, the way carbon is processed and how carbon dynamics are controlled still is not well understood. Knowledge on factors changing the flux of carbon from plants into the soil and controlling its turnover is of significant importance especially in face to global warming (McKinley et al., 2011).

In terrestrial ecosystems 90% of the annual biomass produced by plants enters the dead organic matter pool forming the basis of the decomposer system in soil (Gessner et al., 2010). Plant carbon enters the soil via two pathways, dead organic matter (leaf litter and dead roots) and root exudates. Soil chemical properties are mainly influenced by parent material and mineralogy but also by leaf litter forming the major resource of soil biota responsible for decomposition processes (Reich et al., 2005; Jacob et al., 2009; Langenbruch et al., 2012). Litter quality strongly influences soil pH, as calcium and magnesium of the litter compete with H^+ and Al^{3+} for exchange sites on soil particle surfaces or organic matter (Reich et al., 2005). As a consequence, high pH often promotes higher microbial biomass resulting in higher soil respiration, mineralization and decomposition (Swift et al., 1979; Wardle, 1998). Low mineralization and decomposition rates are associated with high C-to-N ratios and high lignin contents as it is typical for recalcitrant litter. In contrast, Pollierer et al. (2007) highlighted that in temperate forests carbon does not enter the soil food web predominantly via litter but rather via roots. Rhizodeposits comprise labile exudates (e.g., sugars, amino acids and organic acids), but also complex molecules (e.g., polysaccharides, mucilage and proteins). Labile exudates control both community structure and activity of rhizosphere microorganisms (Paterson et al., 2009). Summarizing results of 95 plant ^{14}C labeling studies, Jones et al. (2004) estimated the loss of carbon by exudation to be equivalent to 5 - 10% of the net carbon fixed by plants and 25% of the carbon plants allocate to root growth. This supply of energy increases microbial biomass (Butler et al., 2004), acts as soil organic matter (SOM) priming agent (Bird et al., 2011) and alters the physical and

chemical soil environment (Gregory, 2006). Microbial communities in rhizosphere and bulk soil are therefore responsible for root exudate-mediated changes in soil processes (Söderberg et al., 2004; Paterson et al., 2007). Since plant species differ in the quality and quantity of exudates (Jones et al., 2004), soil carbon dynamics are likely affected by plant species identity and diversity (Grayston et al., 1998; Steinbeiss et al., 2008).

Decomposition studies report both effects of individual plant species (Jacob et al., 2009) and positive mixing effects (Gartner and Cardon, 2004; Hättenschwiler et al., 2005). Until today, however, studies investigating the influence of plant diversity on belowground dynamics in forests are scare (but see Meinen et al., 2009) and most often only consider the effect of aboveground plant residues (Hättenschwiler and Gasser, 2005; Jacob et al., 2009, 2010). To what extent belowground processes mediated by roots and root exudates affect soil organisms and thereby carbon dynamics remains largely unknown. This lack of knowledge is unfortunate as 60% of the terrestrial carbon is bound in forests and its contribution to global carbon cycling is of fundamental importance (McKinley et al., 2011).

To improve knowledge on carbon dynamics in forest soils from a root perspective we used the common temperate broad-leaved tree species European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) to differentiate between general and species-specific effects of living roots on soil organisms and decomposition of litter material in soil. Beech is the dominant tree species in many Central European deciduous forests. Ash often is associated with beech and is expected to increase in dominance in a warmer and drier climate (Broadmeadow and Ray, 2005). Life history traits of beech and ash differ strongly, e.g. speed of growth, root morphology, litter quality, mycorrhizal association, and nutrient, water and light use efficiency (Grime et al., 1997; Emborg, 1998). Beech has higher specific root tip abundance, specific fine root surface area (SRA) and specific fine root length (SRL), whereas ash roots are characterized by higher mean fine root diameter (Meinen et al., 2009). Roots of beech are colonized by ectomycorrhizal (EM) fungi and those of ash by arbuscular mycorrhizal (AM) fungi which differ in nutrient acquisition strategies (Smith and Read, 2008). Beech

tolerates soil pH from acid to highly alkaline, while ash is restricted to soils of high base saturation (Weber-Blaschke et al., 2002). Litter of beech at more acidic sites has high C-to-N ratio (>50) and high lignin content, while ash litter is regarded as high quality litter due to its low C-to-N ratio of about 28 and low lignin content (Jacob et al., 2010).

For allowing access to the root system and to investigate interactions between both tree species, beech and ash seedlings were planted into double split-root systems. The systems allowed dissecting root associated processes and belowground interactions between beech and ash. Carbon and nitrogen fluxes in soil were traced following the incorporation of ^{13}C and ^{15}N from labeled ash litter into soil, bacteria, fungi, soil animals and plants. Ash litter was used to follow the uptake of resources from high quality litter materials by beech and ash as compared to more recalcitrant soil resources.

We hypothesized that (1) beech and ash differentially affect the structure of the microbial community thereby modifying soil processes and plant nutrient capture. Differences in microbial community structure are expected to (2) result in differential decomposition of labeled ash litter and differential mobilization of nutrients from the litter. Further, we expected (3) modifications of the soil microorganism community and soil processes to be most pronounced in the mixed treatment with both tree species present due to complementary effects of the two tree species.

4.2 Material and methods

4.2.1 Rhizotrons

Double split-root rhizotrons were used to separate root systems of two tree seedlings into compartments with root strands of one individual seedling at each side and a shared root compartment in the center where root strands of both tree seedlings could interact (Fig. 4.1). We focused on the middle compartment where the two root strands grew together. The central compartment had a volume of 7.6 l and side compartments half the volume. Rhizotrons were 90 cm high and 64 cm

wide, and were built from anodized aluminum covered at the front with a 10-mm Perspex plate.

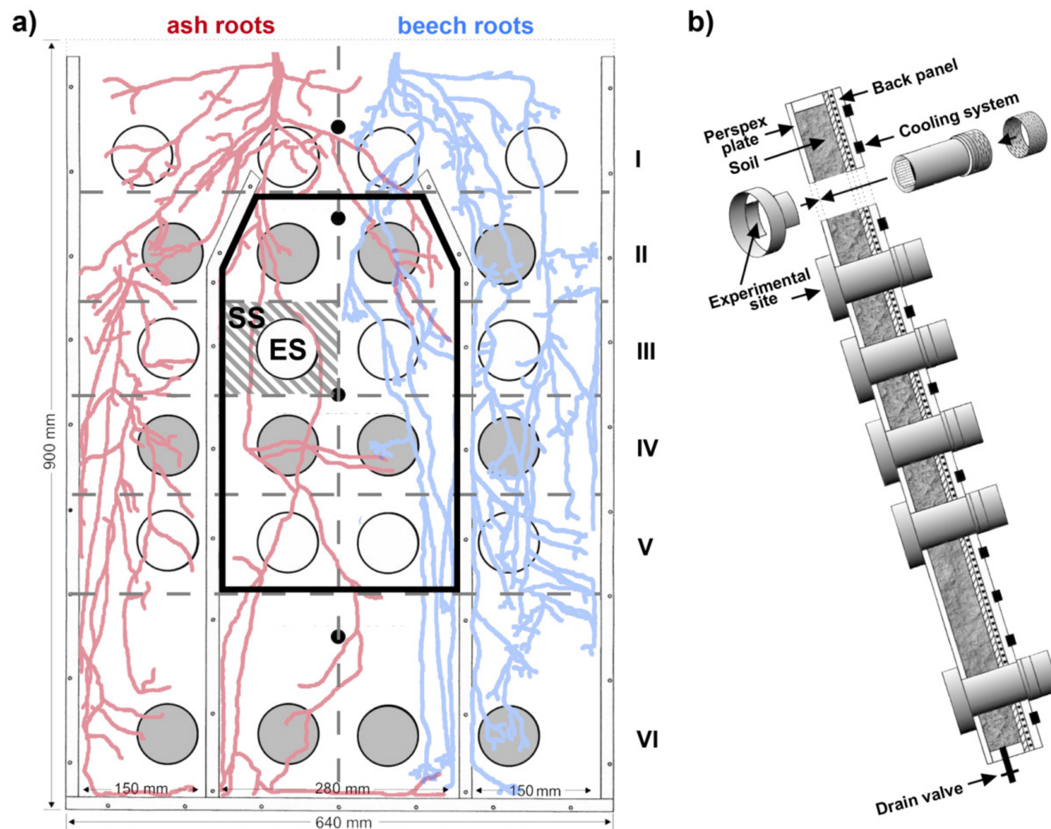

Fig. 4.1 Scheme of double split-root rhizotrons. (a) Front view of mixed species treatments with ash (left) and beech (right) roots interacting in the central compartment. Circles represent experimental sites (ES) with soil (open circles) or soil-litter mixture (grey circles). The shaded area refers to the surrounding sampling site (SS). Roman numerals indicate soil depths (I-VI). The bold rim in the central compartment from soil depth II to VI represents the sampling area. Black dots along the central dashed line refer to the position of temperature sensors. Dashed lines mark the sampling grid. (b) Side view of the double split-root rhizotron and assembly of ES. Tubes inside ES were withdrawn and the empty space filled with soil or soil-litter mixture allowing roots to grow into ES. A water flux based cooling system is installed at the back panel. A valve allowed drainage of the rhizotrons.

They were tilted at 35° to direct roots growing along the Perspex plate. The Perspex plate was covered with black scrim to ensure that roots grow in darkness. Rhizotrons were divided into six soil depth sections (I-VI). Each soil depth contained four experimental sites (ES), two in the center and two at the sides (Fig. 4.1). The back side of the rhizotrons was equipped with a cooling system keeping the temperature at a constant level of 20ºC over the whole soil column. Climate

conditions were set to 20°C air temperature, 70% relative air humidity and 10h daylight in winter and 14h in summer. The tree seedlings were illuminated (EYE Lighting, Clean Ace, Mentor, OH, USA) ensuring a minimum PPFD of 200 ± 10 µmol m^{-2} s^{-1} from June 2009 to October 2010. The experiment lasted for 475 days, i.e., plants were harvested after the second season.

4.2.1.1 Soil and plants

The soil was taken from a mixed temperate broadleaf forest dominated by *F. sylvatica, F. excelsior* and *Tilia cordata* in Central Germany (Hainich forest, 51°04' N 10°30' E, about 350 m a.s.l.) from a depth of 0 - 10 cm after removing the litter. The soil type was a Stagnic Luvisol (IUSS Working Group WRB 2007; 1.8% sand, 80.2% silt and 18.1% clay) and free of carbonate (< 0.02% of total carbon) with a pH (H_2O) of 4.56 ± 0.03 and a gravimetric water content at date of sampling of 22.7%. Initial total carbon amounted to 19.2 ± 0.3 g kg^{-1} dry weight, initial total nitrogen averaged 1.56 ± 0.01 g kg^{-1} dry weight and base saturation was 22.9 ± 1.3%. Each rhizotron was filled with 15.2 L of sieved soil (1 cm mesh) containing soil microflora and fauna. Volumetric soil water content was monitored three times a week with a TDR measurement device (Trime-FM, IMKO, Ettlingen, Germany), and kept at constant level by adding distilled water. Soil temperature was measured with NTC thermistors (Epcos, Munich, Germany), arranged vertically in the center of the rhizotrons at soil depths of 8, 20, 42.5 and 70.5 cm at a distance of 2 cm from the Perspex plate. Data were recorded in 15-min intervals with a CR1000 data logger (combined with two AM416 Relay Multiplexer, Campbell Scientific Inc., Utah, USA).

In spring 2009 beech (*F. sylvatica*) and ash (*F. excelsior*) seedlings with comparable root biomass were excavated in the Hainich forest with intact soil cores to preserve the root system. Initial shoot height was 23.1 ± 1.2 and 17.9 ± 1.1 cm, and root length was 12.1 ± 0.7 and 15.4 ± 1.2 cm for beech and ash seedlings, respectively. At the start of the experiment, ash had significantly higher fine root biomass than beech, but tree species did not differ significantly in total root and

total aboveground biomass (Table 4.1). Before planting, the soil material adhering to the root systems was removed by watering. The remaining soil-water mixture was used to equilibrate microbial communities in soil.

Table 4.1 Means ± 1 SE and *T*- and *P*-values of plant biomass of beech and ash saplings at the start of the experiment (in g plant^{-1}; n=5).

	Initial Biomass							
	Beech			Ash				
	Means		SE	Means		SE	*T*	*P*
Biomass								
Total	2.04	±	0.46	2.13	±	0.22	0.15	0.7122
Total aboveground	1.26	±	0.27	1.25	±	0.15	0.01	0.9294
Total belowground	0.78	±	0.20	0.88	±	0.08	0.81	0.3933
Shoots	0.88	±	0.22	0.74	±	0.09	0.27	0.6190
Leaves	0.38	±	0.05	0.52	±	0.07	2.49	0.1530
Fine roots	0.16	±	0.05	0.41	±	0.08	**6.49**	**0.0343**
Coarse roots	0.64	±	0.17	0.56	±	0.06	0.08	0.7866

Fifty-three days after planting, 1.5 g labeled ash litter was added to ES of each of the treatments, i.e., the control, beech, ash and mixed rhizotrons at every second soil depth (II, IV, VI; see Fig. 4.1). Therefore, tubes were withdrawn and the empty space filled with soil or soil-litter mixture. Prior to adding ash leaves (air dried, crushed to pieces < 1 cm) were mixed with 40 g soil (air dried). The litter was labeled with ^{13}C and ^{15}N by incubating ash trees in the green house for one vegetation period with the CO_2 concentration in air elevated by adding $^{13}CO_2$ (maximum concentration 1,200 ppm) and by watering the soil with nutrient solution containing $^{15}NO_3{}^{15}NH_4$ (both 99 atom %; Euriso-top, Saint-Aubin, Essonne, France). The solution contained 0.6 mM $CaCl_2$, 0.4 mM $MgSO_4$, 0.01 mM $FeCl_3$, 0.4 mM K_3PO_4, 1.8 µM $MnSO_4$, 0.064 µM CuCl, 0.15 µM $ZnCl_2$, 0.1 µM MoO_3, 5 mM NO_3NH_4 and 0.01 mM H_3BO_3. The stable isotope signature of the ash litter was 146.8 ± 0.3‰ for $\delta^{13}C$ and 13,139 ± 60‰ for $\delta^{15}N$ (Table 4.2, see Table S1 for atom% values).

Table 4.2 Isotopic signatures of the used soil, labeled ash litter and of the soil-litter-mixture in manipulation sites at the start of the experiment and at the end after 422 days of litter incubation (means ± 1 SE).

	Start			End	
	Soil	**Litter**	**Soil-litter mixture**	**Soil-litter mixture**	**Difference* [%]**
$\delta^{13}C$ [‰]	-26 ± 0.1	146.8 ± 0.3	69 ± 0.6	-17.44 ± 1.86	88.25
$\delta^{15}N$ [‰]	1.6 ± 0.2	13139 ± 59	6154 ± 0.4	577.4 ± 124.9	81.23
C [%]	1.92 ± 0	36.05 ± 0.1	5.93 ± 0.1	1.94 ± 0.06	65.34
N [%]	0.16 ± 0	1.85 ± 0	0.4 ± 0	0.18 ± 0.004	54.82
C/N	11.7 ± 0.1	19.5 ± 0.1	14.9 ± 0.1	10.98 ± 0.12	15.33

* Differences between the signatures from the start and the end of the experiment are displayed for soil samples from manipulation sites overall treatments (n=16) and were related to natural isotopic signatures of ash litter (V. Eißfeller, unpubl. data).

4.2.2 Experimental design

The experiment was set up in a factorial design with the factors beech (absence and presence) and ash (absence and presence), resulting in the following treatments with four replicates each: (a) two beech seedlings (BB), (b) two ash seedlings (AA), (c) a mixture with one beech and one ash seedling (BA or AB, depending on target tree species), and (d) an unplanted control (Co), resulting in rhizotrons without (B-: Co and AA) and with beech (B+: BB and BA), as well as rhizotrons without (A-: Co and BB) and with ash (A+: AA and AB).

4.2.3 Sampling

After 475 days rhizotrons were harvested. They were opened in horizontal position and a sampling grid was used to identify locations for sampling i.e., at ES and the surrounding of these sites (SS) (Fig. 4.1). Samples from the depth layers II, III, IV and V of the central compartment were analysed. Further, as we were not interested in effects of soil depth we pooled the data from the four layers. In addition to soil samples, plant shoots and roots from each of the soil layers were taken for measuring plant biomass.

4.2.3.1 Plants

At harvest shoot length and root collar diameter of saplings was measured. Roots were separated from soil, washed and cleaned from adhering soil particles. To obtain overall plant biomass fine root biomass estimated from MS for mycorrhizal analysis were combined with plant biomass data from SS. Whenever possible three intact root strands of ca. 7 cm length from each tree species per compartment and soil depths were taken and digitalised on a flat-bed scanner for image analysis carried out using WinRhizo 2005c software (Régent Instruments Inc., Québec, QC, Canada) to determine specific fine root area (SRA; cm^2 g^{-1} dry matter), specific fine root length (SRL; cm g^{-1} dry matter) and total fine root surface. Thereafter, samples were oven-dried (70°C, 48 h), weighed and milled for measurement of organic carbon (C_{org}), total nitrogen (N_{total}) as well as $\delta^{13}C$ and $\delta^{15}N$ signatures (Delta C, Finnigan MAT, Bremen, Germany).

4.2.3.2 Mycorrhiza

Colonization of roots at ES by mycorrhiza-forming fungi was determined. Fine roots were stored in Falcon tubes with moist tissue paper at 4°C until analysis. Fine roots of beech were analyzed with a stereomicroscope (Leica M205 FA, Leica Microsystems, Wetzlar, Germany). The percentage of EM fungi colonization was calculated using the following equation:

$$\text{EM fungi colonization [\%]} = \left(\frac{n\ mycorrhizal\ root\ tips}{n\ vital\ root\ tips}\right) \times 100$$

Fine roots of ash were stored in 70% EtOH at room temperature. For determining the colonization by AM fungi roots were stained with lactophenole-blue (Schmit et al., 1991) and stored at room temperature in 50% glycerol until microscopic inspection at 200 x magnification. AM fungi colonization was calculated with the magnified intersection method of McGonigle et al. (1990) using a 10x10 grid. The abundance of vesicles, arbuscles and hyphae was calculated as percentage of mycorrhizal structures of the total number of intersections. The percentage of

vesicles was taken as relative colonization rate of AM fungi and used for further calculations.

4.2.3.4 Soil properties

Soil pH was measured in a suspension of 10 g soil and 25 ml H_2O with a Vario pH meter (WTW GmbH, Weilheim, Germany). Soil water content was measured gravimetrically after drying at 105°C for 24h. Nitrate and ammonium concentrations were measured by extracting soil samples in 0.5 M K_2SO_4 solution (1:3 wet soil mass-to-solution ratio). Samples were shaken for 1 h and filtered through Sartorius folded filters (Sartorius Stedim, Aubagne, France). Nitrate and ammonium concentrations of filtered extracts were analyzed using continuous flow injection colorimetry (SAN+ Continuous Flow Analyzer, Skalar Instruments, Breda, Netherlands). Nitrate was determined by copper cadmium reduction method (ISO method 13395) and ammonium was quantified by Berthelot reaction method (ISO method 11732). C_{org}, N_{tota}l as well as $\delta^{13}C$ and $\delta^{15}N$ values were measured after grinding soil samples with a disc mill. Samples were analyzed with a coupled system consisting of an elemental analyzer (NA 1500, Carlo Erba, Mailand) and a mass spectrometer (Delta C, Finnigan MAT, Bremen, Germany).

4.2.3.5 Microbial respiration

Basal respiration (BAS), microbial biomass (C_{mic}), and specific respiration (qO_2) were measured by substrate-induced respiration (SIR), i.e., the respiratory response of microorganisms to glucose (Anderson and Domsch, 1978). Before measurement, roots were removed and soil samples were sieved (2 mm). Measurements were done using an automated O_2 microcompensation system (Scheu, 1992). BAS of microorganisms reflected their averaged oxygen consumption rate without the addition of glucose within 10-30 h after attachment of the samples to the analysis system. Subsequently, 4 mg glucose g^{-1} soil dry weight was added as aqueous solution to the soil samples. The mean of the three

lowest hourly measurements within the first 10 h was taken as the maximum initial respiratory response (MIRR). C_{mic} (µg C g^{-1}) was calculated as 38 x MIRR (µl O_2 g^{-1} soil dry weight h^{-1}) according to Beck et al., (1997). Microbial specific respiration qO_2 (µl O_2 mg^{-1} C_{mic} h^{-1}) was calculated as BAS/C_{mic}.

4.2.3.6 Fatty acid analysis

Before extraction of lipids, soil samples were sieved (2 mm) and root and litter pieces were removed. Lipid extraction followed Frostegård et al., (1991). Briefly, 4 g soil (wet weight) was mixed with 18.5 ml Bligh & Dyer solution and shaken for 2 h (Bligh and Dyer, 1959). Subsequently, samples were centrifuged at 2,500 rpm for 10 min at 8°C. Supernatants were transferred to new tubes. The remaining pellet was washed with 5 ml of the Bligh & Dyer solution and centrifuged as described above. The supernatants were combined and 6.2 ml chloroform and 6.2 ml citrate buffer were added. Two ml of the lipid containing lower phase was transferred to a new tube. The organic phase was evaporated at 40°C for 40 min. Columns containing silic acid fractionated the lipid material into phospholipids by adding methanol. The phospholipid-methanol solution was evaporated at 40°C for 90 min. Each sample was dissolved in 1 ml methanol-toluene-solvent (1:1) and 30 µl internal Standard (5.77 mg methylnondecanoat in 25 ml isooctane) was added. Basic methanolysis of lipids was conducted in 1 ml 0.2 M methanolic KOH (2.8 g KOH in 250 ml methanol) incubated at 37°C for 15 min. Afterwards, 2 ml hexane, 0.3 ml acetic acid and 2 ml deionized water were added, vortexed and centrifuged as described above. The upper phase was transferred to new tubes and evaporated at 40°C for 45 min. The remaining extract was solved with 100 µl isooctane and filled into 1.5 ml vials for analysis. Bacterial biomass was estimated using the following PLFAs: a15:0, i15:0, i16:0, 16:1ω7, i17:0, cy17:0 and cy19:0; the PLFA 18:2ω6,9 was used as fungal biomarker (Ruess and Chamberlain, 2010). A gas-chromatography-combustion-isotope-ratio-monitoring-mass spectrometer (GC-C-IRM-MS) using Thermo Finnigan Trace GC coupled via a GP interface to a Delta Plus mass spectrometer (Finnigan, Bremen, Germany) was used to determine the

isotopic composition of individual PLFAs. Fatty acid identification was verified by GC-MS using a Varian CP-3800 chromatograph coupled to a 1200L mass spectrometer and a fused silica column (Phenomenex Zebron ZB-5MS, 30 m, 0.25 µm film thickness, ID 0.32 mm) and helium as carrier gas.

4.2.3.7 Pyrosequencing

DNA and RNA were co-isolated from 2 g soil using the RNA PowerSoilTM Total RNA Isolation Kit and DNA Elution Accessory Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). Residual DNA contaminations in RNA extracts were removed using the TURBO DNA-free™ Kit (Ambion Applied Biosystems, Darmstadt, Germany). RNA was concentrated using the RNeasy MiniElute Kit (QIAGEN, Hilden, Germany). The nucleic acid concentration was estimated using a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany).

The V2-V3 region of the 16S rRNA was reverse transcribed using the SuperScriptTM III reverse transcriptase (Invitrogen, Karlsruhe, Germany). As template 100 ng of the DNA-free RNA were applied. The resulting cDNA as well as the extracted DNA was amplified in triplicate using the Phusion® Hot Start High-Fidelity DNA polymerase (FINNZYMES, Espoo, Finland) as described by Nacke et al., (2011).

The following barcoded primer set was used for reverse transcription and amplification, containing the Roche 454 pyrosequencing adaptors (underlined):
V2for 5’-
CTATGCGCCTTGCCAGCCCGCTCAGAGTGGCGGACGGGTGAGTAA-3’
and V3rev 5’-
CGTATCGCCTCCCTCGCGCCATCAGCGTATTACCGCGGCTGCTG-3’
modified from (Schmalenberger et al., 2001).

The PCR products were treated and purified as described by Nacke et al., (2011). All kits were used as described in the manufacturer´s instructions. The Göttingen

Genomics Laboratory determined the sequences of the partial 16S rRNA genes using a Roche GS-FLX 454 pyrosequencer (Roche, Mannheim, Germany) according to the manufacturer´s instructions for amplicon sequencing.

Sequences shorter than 300 bp were removed from the dataset. To minimize the bias introduced by pyrosequencing due to decreasing read precision at the end of the reads denoising was carried out using Denoiser 0.91 (Reeder and Knight, 2010). OTU determination was performed using uclust OTU picker 1.2.22q (Edgar, 2010) at genetic divergence of 3%, 5% and 20% according to Schloss and Handelsman (2005). The resulting datasets have been deposited in the GenBank short-read archive under accession number SRA050002.

4.2.3.8 Soil animals

Soil not needed for other analysis was taken to extract soil animals by heat (Kempson et al., 1963). Animals were conserved in saturated NaCl solution and kept at -10°C until analysis. The gamasid mite *Hypoaspis aculeifer* (G. Canestrini, 1884) was taken for stable isotope analysis as it occurred in sufficient numbers for the analysis. Twenty adult mites were weighed into tin capsules and dried at 40°C for 24 h. Samples were analysed as described above.

4.2.4 Statistical analysis

Two-way ANOVA was used to test for main effects of beech (B- and B+), ash (A- and A+) and their interactions with data of the four soil depths pooled. To detect differences in plant biomass and mycorrhizal colonization contrasts were calculated in a GLM using pairwise *t*-test to account for dependence in mixed rhizotrons. U-Test was used for analyzing the number of root tips. Treatments in beech-only rhizotrons (BB) were compared to ash-only (AA) and beech-ash mixture (BA). Ash (AA) was also compared with beech-ash mixture (AB). Statistical analyses were done using SAS 9.2 (SAS Institute; Cary, NC, USA).

Discriminant function analysis (DFA) was used to analyze pyrosequencing data as well as fatty acid patterns combined with microbial respiration and soil chemical data. Differences of the bacterial composition in beech and ash rhizotrons and the control were calculated using multi-dimensional scaling (MDS) to reduce dimensions in the dataset. DFA and MDS were calculated using STATISTICA 7.0 for Windows (StatSoft, Tulsa, USA, 2001).

Means were compared using Tukey's Honestly Significant Difference test ($P < 0.05$). Data on plant biomass, isotopic signatures, SRA, SLR, number of fine root tips, NO_3^-, NH_4^+, C_{org}, N_{total}, microbial respiration and PLFA content were log-transformed and percentage data, i.e., colonization rate of mycorrhiza, were arcsine-square root transformed prior to statistical analyses to improve homogeneity of variance. Means given in text and tables are based on non-transformed data.

4.3 Results

4.3.1 Plants and mycorrhiza

After 475 days, total biomass of tree seedlings in BB rhizotrons was significantly lower than in AA and BA rhizotrons (Table 4.3). Fine and coarse root biomass were significantly lower in BB rhizotrons compared to that of seedlings in AA (-69%) and BA rhizotrons (-62%) resulting in significantly lower total root biomass. Total biomass, total root biomass and coarse root biomass of seedlings in mixtures exceeded that of seedlings in monocultures, but this increase was only significant for beech (60%, 62%, 70%, respectively); biomass of ash seedlings in mixture increased by 11%, 17% and 23%, respectively.

$\delta^{13}C$ and $\delta^{15}N$ signatures in fine roots were significantly lower in BB than those in AA rhizotrons (Table 4.3; see Table S2 for atom% values). SRA and SRL did not differ significantly between tree species but tended to be higher in beech (BB vs AA: +6% and +68%, respectively), especially in the mixture (BA vs AB: +24% and +79%, respectively). Generally, fine root tips of tree seedlings increased in mixed rhizotrons, especially beech in mixed rhizotrons had a significantly

higher number of root tips than beech in monoculture by +89% compared to ash in mixed rhizotrons and by +54% compared to ash in monoculture. Mycorrhizal colonization of roots of beech in BB rhizotrons was significantly lower than that of roots of ash in AA rhizotrons, however, as beech and ash are colonized by different types of mycorrhiza the differences have to be interpreted with caution. Beech did not influence the colonization rate of ash by arbuscular mycorrhiza (AA vs AB; +2%), whereas ash increased the colonization of beech by ectomycorrhiza (BB vs BA; +45%) although the effect was not significant (Table 4.3).

4.3.2 Soil properties

In general, the studied soil properties were strongly affected by beech and not by ash with interactions between tree species also being not significant (Table 4.4). Soil pH was significantly lower in B+ (4.54 ± 0.08) than in B- rhizotrons (4.80 ± 0.06). In presence of beech C_{org} and N_{total} were significantly decreased by -7% and -6%, respectively, but NO_3^- and NH_4^+ concentrations remained unaffected (Table 4.4, see Table S3 for atom% values). Further, $\delta^{13}C$ and $\delta^{15}N$ of bulk soil were significantly lower in B+ (-24.46 ± 0.32‰ and 127.04 ± 19.95‰, respectively) compared to B- rhizotrons (-22.24 ± 0.78‰ and 265.25 ± 48.79‰, respectively). Generally, after 422 days of litter incubation, the signatures of $\delta^{13}C$ and $\delta^{15}N$ within the soil-litter-mixtures decreased strongly by 86 and 5576 delta units, respectively (Table 4.2; see Table S1 for atom% values).

Table 4.3 GLM table of contrasts between rhizotrons planted with beech (BB), ash (AA), beech mixed with ash (BA) and ash mixed with beech (AB) for plant parameters of rhizotrons planted with beech, ash or both after 475 days as well as means ± 1 SE of the respective parameters (n=4). Significant effects are given in bold. Atom% values of plant compartments are given in Table S2.

	BB vs AA		**BB vs BA**		**AA vs AB**		**BB** (pure beech)		**AA** (pure ash)		**BA** (beech in mixture)		**AB** (ash in mixture)	
	F	***P***	***F***	***P***	***F***	***P***	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Biomass [g dw] per plant														
Total	**8.82**	**0.0117**	**6.39**	**0.0266**	0.00	0.9518	4.52	± 0.79	12.08	± 1.69	11.33	± 2.03	13.50	± 2.33
Total aboveground	4.20	0.0629	2.91	0.1138	1.11	0.3128	1.85	± 0.38	3.34	± 0.86	4.29	± 1.01	3.03	± 0.43
Total root	**9.52**	**0.0094**	**6.96**	**0.0217**	0.28	0.6035	2.67	± 0.50	8.74	± 1.08	7.04	± 1.08	10.47	± 2.15
Shoot	1.24	0.2876	3.43	0.0889	0.12	0.7300	1.38	± 0.25	1.78	± 0.32	3.20	± 0.73	2.64	± 0.41
Leaves	**5.14**	**0.0426**	0.53	0.4809	**7.50**	**0.0180**	0.46	± 0.14	1.56	± 0.56	1.08	± 0.34	0.39	± 0.22
Fine roots	**9.14**	**0.0106**	4.60	0.0532	0.09	0.7669	0.78	± 0.18	2.38	± 0.30	1.80	± 0.27	2.27	± 0.44
Coarse roots	**7.95**	**0.0154**	**6.50**	**0.0255**	0.59	0.4557	1.89	± 0.35	6.36	± 0.87	5.24	± 0.82	8.21	± 1.91
$\delta^{13}C$ [‰] plant compartments														
Shoot	**5.14**	**0.0426**	**7.00**	**0.0214**	2.12	0.1708	-29.09	± 0.32	-28.07	± 0.28	-27.90	± 0.22	-27.40	± 0.26
Leaves	0.30	0.5955	0.25	0.6287	0.75	0.4029	-29.62	± 0.56	-29.26	± 0.27	-29.29	± 0.44	-29.83	± 0.20
Fine roots	**8.27**	**0.0139**	0.04	0.8402	0.01	0.9395	-27.64	± 0.34	-25.60	± 0.85	-27.49	± 0.19	-25.56	± 0.23
Coarse roots	**12.86**	**0.0037**	2.78	0.1215	0.06	0.8162	-28.35	± 0.31	-25.74	± 0.76	-27.15	± 0.31	-25.92	± 0.32
$\delta^{15}N$ [‰] plant compartments														
Shoot	0.87	0.3701	0.07	0.8018	2.15	0.1682	171.27	± 30.67	260.05	± 66.16	154.54	± 18.34	154.40	± 26.76
Leaves	**5.34**	**0.0394**	0.55	0.4741	1.98	0.1853	192.42	± 32.67	316.50	± 43.37	166.67	± 23.49	228.28	± 15.10
Fine roots	**4.77**	**0.0496**	1.35	0.2674	4.07	0.0666	209.02	± 41.75	396.07	± 99.34	148.85	± 17.63	214.48	± 22.80
Coarse roots	**9.34**	**0.0100**	0.10	0.7630	2.81	0.1196	193.66	± 27.78	390.78	± 78.87	178.50	± 12.60	257.86	± 19.23
SRA§ [m²/g]														
Fine roots	0.23	0.6385	0.05	0.8271	0.78	0.3950	485.16	± 15.36	456.49	± 42.70	509.00	± 54.07	410.65	± 64.00
SRL§ [m/g]														
Fine roots	2.89	0.1150	0.50	0.4947	0.20	0.6596	2374.80	± 221.17	1414.42	± 168.82	3235.44	± 848.14	1810.83	± 450.85
Fine root tips														
Number	-0.48	0.9970	**-13.16**	**0.0000**	2.13	0.1750	1623.50	± 230.01	2299.00	± 419.58	3072.50	± 207.37	3543.75	± 107.79
Mycorrhiza [%]														
Colonization†	**27.50**	**0.0002**	3.07	0.1053	0.04	0.8481	37.81	± 8.58	81.82	± 5.17	54.80	± 6.51	83.54	± 2.87

§SRA, specific root area; SRL, specific root length.

†Note that the different type of mycorrhiza in beech and ash demanded for special counting techniques, thus direct comparisons have to be treated with caution but allow comparison with trees in mixture.

Table 4.4 ANOVA table of *F*- and *P*-values on the effect of beech and ash on soil and microbial parameters, and signatures in gamasid mites as well as means ± 1 SE of the respective parameters in rhizotrons planted with beech (B) and ash (A) after 475 days (n=4). Significant effects are given in bold. Atom% values of soil C and N, PLFA and gamasid mites are given in Table S2.

	Beech		Ash		Beech × Ash		B-						B+					
							A- (Control)			A+ (Ash)			A- (Beech)			A+ (Mixture)		
	F	**P**	**F**	**P**	**F**	**P**	**Mean**		**SE**	**Mean**		**SE**	**Mean**		**SE**	**Mean**		**SE**
Soil data																		
pH (H_2O)	**5.77**	**0.0334**	0.11	0.7436	0.02	0.8944	4.78	±	0.12	4.83	±	0.05	4.53	±	0.14	4.55	±	0.11
N-NO_3^- [mg kg^{-1} dw]	1.00	0.3387	0.04	0.8532	0.62	0.4487	41.31	±	4.96	42.88	±	3.20	39.32	±	3.83	35.14	±	5.93
N-NH_4^+[mg kg^{-1} dw]	0.01	0.9422	0.41	0.5360	0.38	0.5477	2.46	±	0.93	1.47	±	0.63	1.88	±	0.72	1.86	±	0.69
C_{org} [mg kg^{-1} dw]	**15.02**	**0.0022**	0.08	0.7829	0.02	0.8980	1.89	±	0.04	1.91	±	0.05	1.76	±	0.03	1.77	±	0.02
δ^{13}C soil [‰]	**7.54**	**0.0177**	1.73	0.2129	1.40	0.2604	-23.27	±	0.58	-21.21	±	1.35	-24.51	±	0.57	-24.41	±	0.40
N_{total} [mg kg^{-1} dw]	**7.82**	**0.0162**	0.24	0.6297	0.00	0.9687	0.18	±	0.00	0.17	±	0.00	0.17	±	0.00	0.16	±	0.00
δ^{15}N soil [‰]	**7.42**	**0.0185**	0.83	0.3816	0.31	0.5907	212.18	±	55.44	318.33	±	78.47	126.29	±	37.64	127.79	±	20.99
C-to-N ratio	0.56	0.4677	0.98	0.3406	0.00	0.9932	10.78	±	0.24	10.94	±	0.08	10.66	±	0.16	10.82	±	0.14
CEC [µmolc g^{-1} dw]	0.06	0.8162	1.33	0.2726	0.06	0.8109	189.78	±	3.98	185.12	±	2.33	191.98	±	9.94	201.36	±	7.84
Base saturation [%]	1.39	0.2638	0.04	0.8518	1.13	0.3108	20.21	±	0.29	20.80	±	0.65	19.90	±	0.99	20.92	±	0.47
Microbial parameters																		
BAS [µl O_2 h^{-1} g]§	4.04	0.0674	0.09	0.7674	0.19	0.6701	1.18	±	0.09	1.18	±	0.05	1.41	±	0.07	1.36	±	0.15
C_{mic} [µg C g^{-1}] §	0.03	0.8643	0.48	0.5019	0.40	0.5365	150.03	±	13.65	134.32	±	5.93	139.79	±	6.62	140.86	±	13.38
qO_2 [µl O_2 mg^{-1} C_{mic} h^{-1}] §	**9.00**	**0.0111**	0.14	0.7178	1.59	0.2311	0.008	±	0.001	0.009	±	0.000	0.010	±	0.001	0.010	±	0.001
PLFA [nmol g^{-1}dry weight]																		
Total	0.75	0.4025	0.00	0.9619	1.11	0.3130	7.22	±	1.32	6.03	±	1.36	6.57	±	0.55	8.19	±	0.97
Bacteria	0.53	0.4801	0.01	0.9377	1.05	0.3262	6.95	±	1.20	5.85	±	1.29	6.25	±	0.52	7.66	±	0.95
Fungi	3.36	0.0916	0.18	0.6757	1.20	0.2955	0.27	±	0.16	0.18	±	0.07	0.33	±	0.05	0.53	±	0.15
Fungi-to-bacteria ratio	**5.17**	**0.0422**	0.33	0.5755	0.85	0.3752	0.032	±	0.017	0.026	±	0.010	0.050	±	0.008	0.073	±	0.019
PLFA δ^{13}C [‰]																		
Total	2.43	0.1454	1.40	0.2590	0.30	0.5944	-22.80	±	2.37	-21.49	±	2.09	-27.14	±	0.60	-23.55	±	2.51
Bacteria	2.01	0.1818	0.49	0.4960	0.51	0.4871	-24.38	±	1.47	-24.43	±	1.05	-27.25	±	0.45	-25.31	±	1.89
Fungi	**7.48**	**0.0181**	0.08	0.7807	0.16	0.6941	-21.01	±	6.61	-17.06	±	4.53	-31.59	±	0.92	-28.27	±	4.01
Gamasid mites																		
δ^{13}C [‰]	**20.59**	**0.0008**	**159.43**	**<.0001**	**7.80**	**0.0175**	-23.37	±	0.86	-13.89	±	0.31	-20.19	±	1.40	-8.78	±	0.43
δ^{15}N [‰]	**25.75**	**0.0004**	**148.88**	**<.0001**	**11.93**	**0.0054**	130.14	±	23.08	713.33	±	43.37	339.07	±	37.35	1121.26	±	26.97

§BAS, basal respiration; C_{mic}, microbial biomass; qO_2, specific respiration.

4.3.3 Microorganisms

C_{mic} was not significantly affected by tree species and averaged over all treatments 141.25 ± 4.93 µg C g^{-1}. However, qO_2 was significantly higher in B+ (0.0101 ± 0.003 µl O_2 mg^{-1} C_{mic} h^{-1}) than in B- rhizotrons (-16%, Table 4.4), which was due to marginally higher BAS in B+ (1.39 ± 0.08 µl O_2 h^{-1} g^{-1}) as compared to B- rhizotrons (-15%). The ratio of fungal-to-bacterial marker PLFAs was significantly higher in B+ (0.061 ± 0.007) than in B- rhizotrons (-53%) as the fungal biomass was higher in B+ (0.43 ± 0.08 nmol g^{-1}dry weight) than in B- rhizotrons (-47%), whereas bacterial biomass remained unaffected.

Bacterial and total PLFA content were not significantly affected by the treatments and averaged 6.67 ± 1.67 and 7.00 ± 0.53 nmol g^{-1}dry weight, respectively. The $\delta^{13}C$ values of the fungal marker PLFA 18:2ω6,9 were significantly lower in B+ (-29.93 ± 2.00‰) than in B- rhizotrons (-18.75 ± 3.60‰). Also, weighted $\delta^{13}C$ values of bacterial PLFAs were lower in B+ (-26.28 ± 0.97‰) than in B- rhizotrons (-24.40 ± 0.84‰), whereas in A+ rhizotrons (-24.87 ± 1.01‰) they tended to be higher than in A- rhizotrons (-25.82 ± 0.89‰). In general, ash did not significantly influence $\delta^{13}C$ values of marker PLFAs (Table 4.4; see Table S3 for atom% values).

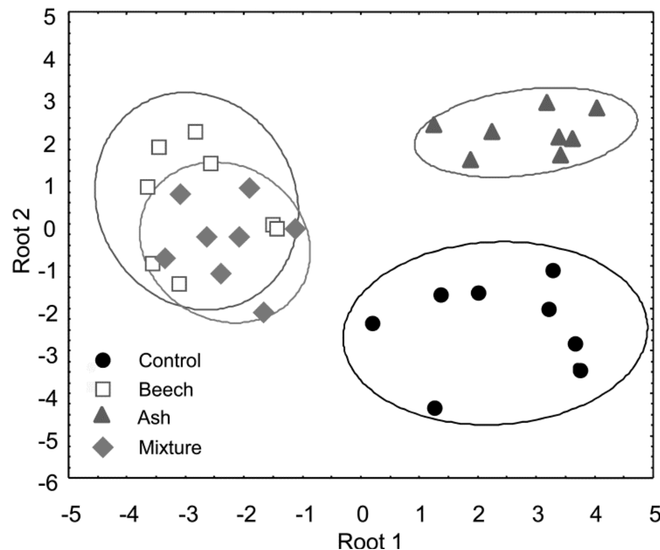

Fig. 4.2 Discriminant function analysis (DFA) of microbial PLFAs, microbial respiration and soil properties in rhizotrons without trees (control), with beech, ash and a mixture of beech and ash. Wilks' Lambda: 0.016480, F (54,33) = 1.85, P = 0.0296. Ellipses represent confidence intervals at P = 0.05.

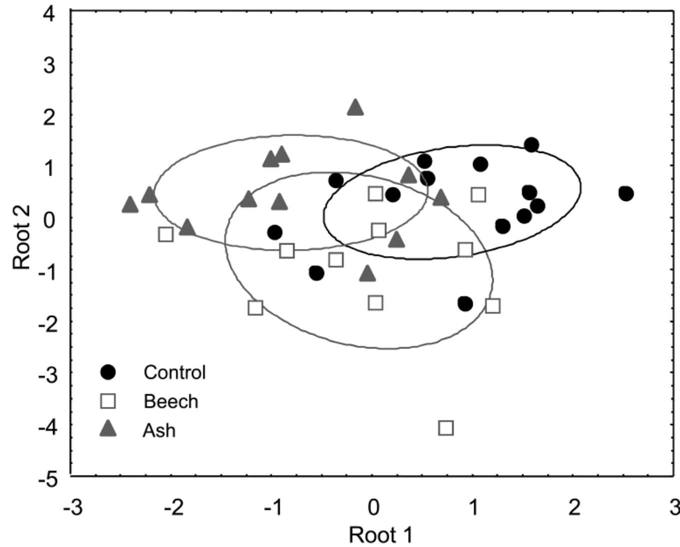

Fig. 4.3 Discriminat function analysis (DFA) of bacterial phyla based on pyrosequencing of 16S rRNA in rhizotrons without trees (control) and with beech and ash saplings after reducing data to six dimensions by multidimensional scaling (MDS). Wilks' Lambda: 0.499576; $F_{(12,60)}$ = 2.07; P = 0.0325. Ellipses represent confidence intervals at P = 0.05.

Table 4.5 Summary of input variables of the discriminant function analysis (DFA), i.e. data on PLFA, soil properties and microbial respiration.

		Wilks' Lambda	**F (3,11)**	***P*-level**
Gram$^+$ bacteria	i15:0	0.0175	0.2171	0.8825
	a15:0	0.0242	1.7284	0.2188
	i16:0	0.0237	1.6062	0.2441
	i17:0	**0.0430**	**5.8991**	**0.0119**
Gram$^-$ bacteria	**cy17:0**	**0.0390**	**5.0135**	**0.0198**
	cy19:0	0.0239	1.6448	0.2358
Unspecified bacteria	16:1ω7	0.0250	1.8939	0.1891
Fungi	18:2ω6:9c	0.0298	2.9597	0.0792
Microbial respiration	BAS	0.0178	0.2972	0.8267
	C_{mic}	0.0179	0.3145	0.8146
	qO_2	0.0175	0.2325	0.8719
Soil properties	pH	0.0320	3.4554	0.0549
	NO_3^-	0.0211	1.0298	0.4170
	NH_4^+	0.0188	0.5116	0.6825
	C_{org}	0.0182	0.3726	0.7745
	N_{total}	0.0261	2.1450	0.1524
	$\delta^{13}C$	0.0221	1.2510	0.3384
	$\delta^{15}N$	0.0173	0.1733	0.9122

DFA suggested strong similarity in the composition of PLFAs in BB and BA rhizotrons. Both treatments differed strongly from AA and control treatments (Fig. 4.2). Differences were mainly due to low amounts of gram-negative (cy17:0) and gram-positive bacteria (i17:0) in beech rhizotrons. Higher fungal biomass and low pH in beech and mixed rhizotrons also contributed to the separation of these treatments but to a lower extent (Tables 4.5, 4.6). Pyrosequencing of the bacterial community revealed high overlap of bacterial phyla and species with little differences between the treatments (Fig. 4.3).

Table 4.6 Means ± 1 SE of PLFA markers of the microbial community in rhizotrons after 475 days.

	Beech absent (B-)				Beech present (B+)			
	Ash absent (A-) (Control)		Ash present (A+) (Ash)		Ash absent (A-) (Beech)		Ash present (A+) (Mixture)	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Gram^{+} bacteria								
i15:0	0.92	± 0.22	0.81	± 0.36	1.05	± 0.21	1.59	± 0.35
a15:0	1.41	± 0.29	1.04	± 0.34	1.40	± 0.24	1.93	± 0.24
i16:0	0.70	± 0.08	0.66	± 0.12	0.80	± 0.06	0.87	± 0.06
i17:0	0.62	± 0.05	0.74	± 0.14	0.42	± 0.04	0.70	± 0.09
Gram^{-} bacteria								
cy17:0	0.72	± 0.16	0.77	± 0.13	0.63	± 0.06	0.84	± 0.16
cy19:0	1.22	± 0.58	1.13	± 0.46	1.13	± 0.22	0.74	± 0.26
Unspecified bacteria								
16:1ω7	1.35	± 0.37	0.70	± 0.35	0.81	± 0.21	0.98	± 0.44
Fungi								
18:2ω6,9	0.27	± 0.16	0.18	± 0.07	0.33	± 0.05	0.53	± 0.15

4.3.4 Soil fauna / gamasid mites

The $\delta^{13}C$ and $\delta^{15}N$ from the added ash litter was incorporated into basal species of the soil food web as indicated by the label in the predatory mite *H. aculeifer* (Table 4.4; see Table S3 for atom% values). The signatures suggest that incorporation of label was most pronounced in mixed rhizotrons (significant

interaction between beech and ash) followed by AA, BB and control rhizotrons. Abundances of soil animal taxa extracted by heat, i.e., collembolans, gamasid and oribatid mites as well as earthworms, generally did not differ between treatments (Table S4).

4.4 Discussion

4.4.1 Changes in the microbial community due to rhizodeposition

Lower pH in the rhizosphere of beech likely contributed to favoring soil fungi supporting our hypothesis (1) that beech and ash differentially affect the structure of the microbial community. Acidification of the soil by beech is well known (Holzwarth et al., 2011; Langenbruch et al., 2012), however, commonly it has been ascribed to low concentrations of calcium and magnesium and high concentrations of recalcitrant compounds such as lignin in beech leaf litter (Reich et al., 2005; Hobbie et al., 2006; Hansen et al., 2009). As we excluded leaf litter fall from seedlings to the rhizotron soil surface and uniformly placed high quality ash litter in each of the treatments, the observed differences must have been due to the activity of beech roots. Indeed, in the vicinity of beech roots concentrations of formate and acetate were increased as compared to control rhizotrons in the same experiment, whereas in the vicinity of ash roots only the concentration of acetate increased (Fender et al., 2013). The release of organic acids increases nutrient availability and this is facilitated by low pH (Jones et al., 2004); presumably, beech employs this strategy to increase nutrient mobilization and uptake. Low pH in the soil, however, predominantly is caused by the release of H^+ by roots rather than by dissociation of organic acids (Neumann and Römheld, 1999). Notably, acidification of the soil by beech roots occurred despite a comparatively lower root biomass in beech than ash rhizotrons. However, SRA and SRL were higher in B+ rhizotrons as compared to A+ rhizotrons. This suggests that the observed modifications were partly due to changes in root physiology rather than root biomass and number of fine root tips (Lehmann, 2003). Differences in the release rates of specific exudates of the two species presumably also contributed to the observed changes.

Bacterial community composition was little affected by tree roots as indicated by analysis of 16S rRNA. The ratio of fungal-to-bacterial biomass measured via fatty acid analysis increased in B+ rhizotrons and reflected the general pattern of increasing fungal dominance at low pH accounting for

differences in soil processes (Aciego Pietri and Brookes, 2008; Rousk et al., 2009). Fungal biomass was measured using 18:2ω6,9 as marker PLFA (Ruess and Chamberlain, 2010; Frostegård et al., 2011) which includes EM and saprotrophic fungi (Kaiser et al., 2010). We suggest the change in fungal biomass to refer not to AM fungi since the PLFA 18:2ω6,9 is only found in very low densities in this type of fungi (Olsson and Johansen, 2000) and since the AM colonization rate did not change. Colonization by EM fungi in beech was relatively low (46 ± 6%, pooled data from BB and BA rhizotrons). This corresponds to low colonization rates in other greenhouse and rhizotron experiments (Dučić et al., 2009; Reich et al., 2009; Winkler et al., 2010) when compared to field data (Leuschner et al., 2004; Lang et al., 2011). Low EMF colonization rate and a stronger depletion of $\delta^{13}C$ of PLFA 18:2ω6,9 in B+ rhizotrons point to SOM decomposition suggesting that saprotrophic rather than EM fungi increased in beech rhizotrons as fine root tips and mycorrhiza were shown to have relatively similar signatures, whereas soil is stronger depleted in $\delta^{13}C$ (Eissfeller et al. submitted to SBB, Nr. 7189). We therefore suggest saprotrophic fungi to substantially contribute to changes in the fungal PLFA marker.

Combined data on PLFAs, soil properties and microbial respiration revealed high similarity of beech and mixed rhizotrons in DFA with these differing significantly from ash and control rhizotrons. The fatty acids i17:0 and cy17:0 contributed most to this separation, with lesser contribution by pH and fungal biomass. The fatty acid i17:0 is regarded as marker for gram-positive bacteria whereas cy17:0 characterizes gram-negative bacteria, the former considered to dominate in microorganisms being present in bulk soil whereas the latter in rhizosphere soil processing labile root derived carbon (Söderberg et al., 2004; Paterson et al., 2007). The relative abundance of both was lowest in BB rhizotrons suggesting that both suffered from the presence of beech roots, presumably due to beech increasing the competitive strength of saprotrophic fungi.

4.4.2 Changes in decomposition due to different tree species

Hypothesis (2) assuming that litter decomposition is differentially affected by tree species was supported by our data. Generally, stable isotope values of the litter-soil mixture in ES decreased strongly during incubation. Ash litter is known to decompose fast; in the field it disappears entirely after two years (Jacob et al., 2009). High and constant temperatures within the climate chambers (20°C) contributed to fast decomposition of the litter in the rhizotrons. Data on higher qO_2 (this study) and higher cumulative heterotrophic CO_2 production in beech as compared to ash rhizotrons (Fender et al., 2013) suggest an overall higher stimulation of litter decomposition in beech root affected soil, i.e., higher carbon loss due to microbial respiration. High H^+ concentrations have been shown to limit bacterial growth, while low concentrations limit fungal growth (Rousk et al., 2009). The fact that qO_2 increased whereas bacterial biomass did not change suggests that the metabolic costs of rhizosphere bacteria increased at least at the end of the experiment. Presumably, lower soil pH in beech rhizotrons decreased the efficiency of bacteria to use carbon for biomass production by increasing respiratory losses.

$\delta^{13}C$ values in fungal and bacterial PLFAs were depleted most in B+ rhizotrons suggesting that bacteria and fungi incorporated less litter carbon in presence of beech roots than of ash also indicating a faster turnover of litter carbon. Further, the more depleted $\delta^{13}C$ values in fungi compared to beech fine roots suggest that fungal carbon originated from soil organic matter in beech rhizotrons, whereas higher $\delta^{13}C$ values in bacteria rather suggest bacteria to depend on root-derived carbon as their signatures resembled that of beech fine roots (Bowling et al., 2008).

Several studies found plant species identity to have stronger effects than plant diversity (De Deyn et al., 2004; Hättenschwiler and Gasser, 2005; Ball et al., 2009), with certain plant species acting as key species (Jacob et al., 2009). The strong effect of beech in this study is mediated by roots whereas ash had no effect suggesting that rhizodeposition in ash is of minor importance. Despite this low rhizosphere changes ash incorporated more litter nitrogen than beech (Lang and

Polle, 2011; Schulz et al., 2011); potentially, ash is more effective in exploiting resources from fast decomposing litter such as ash leaves or by virtue of the higher root biomass production of ash in our experiment. Notably, ash seedlings incorporated more litter ^{15}N than beech seedlings supporting the conclusion that the reduced N_{total} in B+ rhizotrons was due to increased SOM decomposition and not due to plant uptake by beech. Notably, the uptake of ^{15}N declined in mixture with ash. This corresponds to field observations where the N concentrations in ash declined in mixtures with other tree species and their ectomycrrhizal diversity (Lang and Polle, 2011). A higher uptake of N by ash roots was also found in a ^{15}N tracer study in the Hainich forest where ash fine roots showed a significantly higher mass-specific uptake of labeled NH_4^+ and glycine (but not of NO_3^-) than beech roots (A. Jacob, unpubl.results).

4.4.3 Channeling of litter-derived carbon into higher trophic levels

Hypothesis (3) assuming that mixing of both tree species beneficially affects microorganisms thereby stimulating carbon turnover is supported in part by our data. Generally, mixing of tree species increased plant biomass, fine root tips, SRA, SRL and mycorrhizal colonization especially that of beech seedlings but did not affect soil chemistry and microbial biomass. However, soil chemistry and microbial data are point measures and do not reflect fluxes over the whole period of the experiment. As the plants are sinks for resources made available over the whole experimental time higher plant growth in mixed rhizotrons suggests that the gross flux of resources was greater in mixed rhizotrons.

Isotope analyses of food web components are a net measure over the long experimental period. Here, we measured $\delta^{13}C$ and $\delta^{15}N$ being incorporated within the predatory mite *H. aculeifer*. $\delta^{13}C$ and $\delta^{15}N$ values of *H. aculeifer* were significantly increased in mixed and ash rhizotrons suggesting that more litter-derived carbon and nitrogen entered basal species of the soil food web which served as prey for gamasid mites, such as nematodes and collembolans feeding on bacteria and fungi. In contrast, in control and beech rhizotrons $\delta^{13}C$ values of *H. aculeifer* resembled those in Hainich beech forests ($\delta^{13}C$: -23.9 ± 0.76‰; $\delta^{15}N$:

+2.0 ± 2.11‰; Klarner et al., 2013) suggesting low incorporation of litter-derived carbon (and nitrogen) into the prey of *H. aculeifer.* However, the turnover of belowground C in unplanted soil, i.e., the control, was numerously shown to be lower compared to planted soil (Kuzyakov, 2010; Bird et al., 2011), i.e., soil with beech trees. Low incorporation of litter resources in BB rhizotrons may point to the fast decomposition of ash litter and to the dominance of root derived resources as basis of the soil animal food web in beech forests as suggested earlier (Pollierer et al., 2007). Of course, measurements of a single species, i.e., *H. aculeifer*, do not allow to predict carbon and nitrogen cycling through the whole soil food web. However, since the soil fauna composition within the rhizotrons did not differ, we suggest tree species to significantly affect the amount and the way carbon is channeled through the soil food web.

4.5 Conclusions

The results suggest that the effect of living roots on litter decomposition, SOM dynamics and energy channels varies with tree species identity. Rhizodeposits have the potential to change soil pH with the potential to affect the metabolic activity of microorganisms. This propagates to higher trophic levels as tree species can impact the amount of litter-derived resource entering the soil food web and on energy channels. Effects of living roots are notoriously understudied and have to be included into studies on soil C dynamics to understand carbon and nutrient cycling as well as soil food web functioning of forests.

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Supplementary data

Table S1 Means ± 1 SE of atom% values of soil C and N, PLFA and gamasid mites as influenced by beech (B) and ash (A) in rhizotrons after 475 days.

	BB (pure beech)			**AA** (pure ash)			**BA** (beech in mixture)			**AB** (ash in mixture)		
	Means		**SE**	**Means**		**SE**	**Means**		**SE**	**Means**		**SE**
atom% ^{13}C												
Shoot	1.07383513	±	0.00017420	1.07495600	±	0.00015455	1.07514475	±	0.00017318	1.07568600	±	0.00019963
Leave	1.07326575	±	0.00030884	1.07365156	±	0.00015001	1.07362325	±	0.00034224	1.07303038	±	0.00015562
Fine roots	1.07465313	±	0.00018487	1.07750778	±	0.00046296	1.07596411	±	0.00014480	1.07730790	±	0.00017922
Coarse roots	1.07542997	±	0.00016796	1.07765847	±	0.00041694	1.07558569	±	0.00023790	1.07770568	±	0.00024885
atom% ^{15}N												
Shoot	0.42876468	±	0.00559039	0.46109969	±	0.01205151	0.42266801	±	0.00472628	0.42261393	±	0.00689963
Leave	0.43647468	±	0.00595216	0.48166205	±	0.00789685	0.42708961	±	0.00605381	0.44954088	±	0.00388855
Fine roots	0.43692704	±	0.00760428	0.50868574	±	0.01805715	0.43140529	±	0.00454258	0.46031359	±	0.00587501
Coarse roots	0.44251012	±	0.00506091	0.51054955	±	0.01435028	0.42058708	±	0.00324815	0.44450474	±	0.00495442

Table S2 Means ± 1 SE of atom% values of soil C and N, PLFA and gamasid mites as influenced by beech (B) and ash (A) in rhizotrons after 475 days.

	B-						**B+**					
	A- (Control)			**A+** (Ash)			**A-** (Beech)			**A+** (Mixture)		
	Mean		**SE**	**Mean**		**SE**	**Mean**		**SE**	**Mean**		**SE**
soil data												
atom% ^{13}C	1.08291691	±	0.00068504	1.08691934	±	0.00267118	1.08061441	±	0.00267118	1.07997306	±	0.00267118
atom% ^{15}N	0.4995646	±	0.02435464	0.57079355	±	0.05107642	0.44495352	±	0.05107642	0.43470143	±	0.05107642
PLFA												
Total (atom% ^{13}C)	1.08072624	±	0.00041531	1.08215226	±	0.00067208	1.07597482	±	0.00038549	1.07989923	±	0.00226927
Bacteria (atom% ^{13}C)	1.07898895	±	0.00120338	1.07894481	±	0.00082096	1.07585318	±	0.00033924	1.07797283	±	0.00043242
Fungi (atom% ^{13}C)	1.08268228	±	0.00023938	1.08700051	±	0.00061554	1.07110968	±	0.00036185	1.07473461	±	0.00054633
gamasid mites												
atom% ^{13}C	1.08010350	±	0.00093649	1.09026840	±	0.00034029	1.08358067	±	0.00152939	1.09606000	±	0.00046706
atom% ^{15}N	0.41377425	±	0.00841666	0.62595061	±	0.01575008	0.48989230	±	0.01359816	0.77384461	±	0.00976060

Table S3. ANOVA table of *F*- and *P*-values as well as means ± 1 SE for soil animal taxa extracted by heat from rhizotrons influenced by beech (B) and ash (A) after 475 days.

	Beech		Ash		Beech × Ash		B-						B+					
							A- (Control)			A+ (Ash)			A- (Beech)			A+ (Mixture)		
	F	P	F	P	F	P	Mean		SE	Mean		SE	Mean		SE	Mean		SE
Soil taxa																		
Total	1.84	0.1994	0.02	0.8840	2.67	0.1281	294.75	±	130.36	93.00	±	20.99	131.50	±	26.88	141.25	±	25.02
Collembola†	0.20	0.6614	0.70	0.4177	0.20	0.6597	61.00	±	37.94	46.00	±	15.63	59.50	±	30.63	65.00	±	9.35
Sminthurida	0.63	0.4418	2.70	0.1263	4.41	0.0576	54.00	±	13.36	14.50	±	5.48	12.25	±	6.84	21.50	±	12.07
Gamasida	2.89	0.1148	0.00	0.9889	2.22	0.1624	167.25	±	116.19	21.50	±	4.03	49.50	±	17.29	43.50	±	9.46
Oribatida	0.15	0.7023	0.28	0.6059	0.82	0.3820	10.50	±	2.50	8.50	±	2.99	9.75	±	2.29	10.25	±	0.48
Lumbricidae	0.35	0.5626	2.40	0.1474	0.08	0.7884	2.00	±	0.91	2.50	±	1.19	0.50	±	0.29	1.00	±	0.41

†Collembola without Sminthuridae

CHAPTER

5

Root-induced tree species effects on the source/sink strength for greenhouse gases (CH_4, N_2O and CO_2) of a temperate deciduous forest soil

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Abstract

Through their leaf litter and throughfall water, tree species can have a pronounced influence on soil chemistry. However, there is little knowledge of species-specific root effects on greenhouse gas fluxes between forest soils and the atmosphere. By growing saplings of beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*) in monoculture or mixture at defined atmospheric and soil conditions in rhizotrons, we tested four hypotheses related to potential root-induced tree species effects on the uptake of CH_4 and the emission of N_2O and CO_2 from the soil. This design excluded putative effects of leaf litter mineralisation on trace gas fluxes. Gas fluxes were measured biweekly using the closed chamber technique; the CO_2 derived from root respiration was estimated, and the concentration of organic acids in the rhizosphere solution was analysed. Rhizotrons planted with ash took up significantly more CH_4 and emitted less N_2O than control rhizotrons without plants. CH_4 and N_2O fluxes from beech rhizotrons did not differ from the root-free control but were significantly smaller (CH_4) or higher (N_2O) than the fluxes from the ash treatment. While root respiration of ash was higher than of beech, root-induced soil respiration was higher in the rhizosphere of beech roots. The concentration of organic acids tended to be higher in the rhizosphere of beech and also the composition was different from that of ash. We conclude that tree species identity may substantially alter the soil source/sink strength for greenhouse gases through root-related processes.

Keywords: *Fagus sylvatica, Fraxinus excelsior; Greenhouse gas exchange; Methane oxidation; Organic acids; Root growth.*

5.1 Introduction

The net greenhouse gas (GHG) balance of European forest soils was recently estimated at -19 ± 11 g Ceq-CO_2 m^{-2} yr^{-1}, indicating an on average higher net uptake of GHG by forest soils than by grasslands, peatlands, and croplands (Schulze et al., 2010). Regarding CH_4, temperate forest soils contribute with an estimated amount of 3 to 5.7 Tg CH_4 yr^{-1} to the most important terrestrial sink for atmospheric CH_4, i.e. the oxidation of methane in soils (Curry, 2007; Dutaur and Verchot, 2007; Ishizuka et al., 2009). In contrast, temperate forest soils typically are sources of atmospheric N_2O (Kesik et al., 2005). Due to the complex source/sink function of soils in the global C and N cycles, specific interest is currently paid to the quantification of gas fluxes between the soils of deciduous forests and the atmosphere, and how they can be influenced for mitigating global warming.

Recent studies found a significant tree species effect on the CH_4, N_2O and CO_2 fluxes from the soils of European deciduous forests (Borken and Beese, 2006; Degelmann et al., 2009). The CH_4 uptake was found to be higher in beech-dominated stands compared to stands of spruce (Butterbach-Bahl et al., 2002). Comparative studies on the N_2O emission from forest soils reported predominantly higher effluxes in broad-leaved than needle-leaved forests (Butterbach-Bahl and Kiese, 2005; Ambus et al., 2006). Regarding methane Guckland et al. (2009) detected no difference in the CH_4 fluxes of the soil among forest patches with varying abundance of beech in a mixed broad-leaved deciduous forest. Whether GHG exchange is dependent on the species identity of broad-leaved trees, is not well known. For example, Vesterdal et al. (2012) measured significantly higher CO_2 emissions under 30-year old ash than under beech trees in a common garden experiment with six broad-leaved tree species, but we are not aware of comparative studies on the effects of beech and ash on N_2O and CH_4 fluxes.

The bulk of abiotic factors affecting the GHG exchange between soil and atmosphere are well studied, among them soil temperature, soil bulk density, soil acidity, soil moisture and N deposition (Davidson et al., 1998; Smith et al., 2000; Le Mer and Roger, 2001; Jungkunst and Fiedler, 2007; Bagherzadeh et al., 2008;

Ciarlo et al., 2008). More recently, the influences of biotic factors such as tree species identity, and the activity of soil fauna, fungi and the soil microbial community on soil processes, that may affect gas fluxes, have received increased attention (Binkley and Menyailo, 2005). Nevertheless, it is not well understood how changes in the biotic components of forest ecosystems affect GHG fluxes (Hanson et al., 2000; Silver et al., 2005; Paterson et al., 2009).

It is undisputed that the effects of trees on the C and N cycling in the soil are larger than that of most other organism groups (Brady and Weil, 2002). The influence has been explained by changes in physical and chemical properties of the soil as a consequence of litter input, stemflow, throughfall and root activity (Menyailo and Huwe, 1999; Erickson et al., 2002; Guckland et al., 2009; van Haren et al., 2010). In most cases, tree species effects on CH_4 uptake or N_2O release have been thought to occur mainly through the input of litter and its specific properties (Hagen-Thorn et al., 2004; Vesterdal et al., 2008).

However, it is clear that roots may influence the soil via exudates, the depletion of water and nutrient reserves, decaying root material, respiration and physical changes caused by root growth and root architecture (Rovira, 1965; Cheng and Gershenson, 2007). These processes may stimulate or inhibit certain groups of biota and biochemical processes in the soil (Fig. 5.1). For example, in a comparative field study in a deciduous broad-leaved forest in Central Germany (Hainich forest), higher pH and higher contents of organic carbon (C_{org}) and total nitrogen (N_{total}) were found in the mineral soil under ash trees as compared to profiles under nearby beeches (Holzwarth et al., 2011; Langenbruch et al., 2011). From such field studies it is usually difficult to establish causal links between observed differences in gas fluxes under different tree species and tree functional traits (Butterbach-Bahl et al., 2002; von Arnold et al., 2005; Vesterdal et al., 2008; Christiansen and Gundersen, 2011). Thus, our knowledge about tree species-specific rhizosphere effects on greenhouse gas fluxes is very poor.

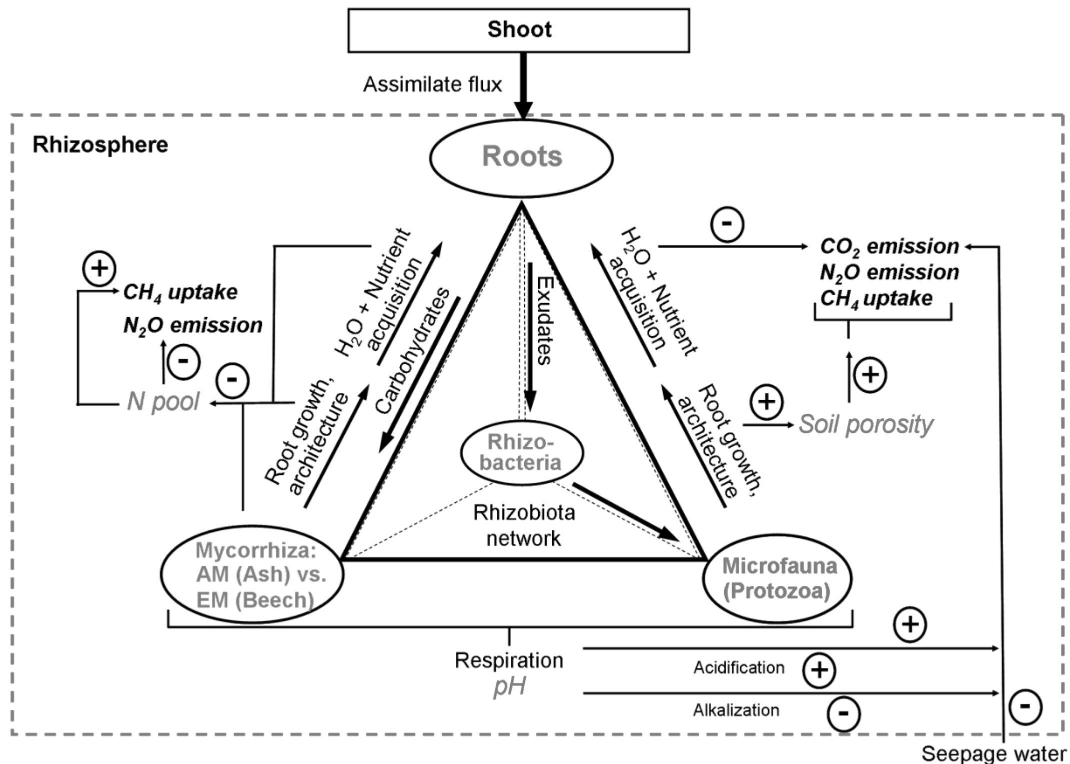

Fig. 5.1 Schematic representation of the biochemical rhizosphere processes and pools involved in the gas exchange of CH_4, CO_2 and N_2O between soil and atmosphere.

More recently, evidence has accumulated that the root systems of tree species growing in mixture may utilise soil resources in a complementary way, i.e. exhaust the available water and/or nutrients to a larger extent than the same species are doing in monoculture (Kelty, 1992; Brassard et al., 2011). So far, no information exists whether tree species mixtures are deviating from the respective monocultures with respect to gas fluxes between soil and atmosphere. Whether tree species effects and root system interactions are relevant forces influencing the source/sink function of forest soils for C and N is best examined in manipulative laboratory studies using soil columns and pot experiments with defined tree species composition and soil conditions.

We introduced a novel double-split-root rhizotron system for studying species effects on the C and N cycling and related gas fluxes in the rhizosphere of tree saplings grown in neighbourhood of either a conspecific (monoculture) or an allospecific sapling (mixture). The aims of this study were to identify species-specific root-induced differences in CH_4, N_2O, and CO_2 fluxes between atmosphere and soil planted with two common Central European broad-leaved tree

species, European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) under controlled soil conditions, and to examine the evidence for a putative effect of species interaction (*Fagus* x *Fraxinus*) on the GHG fluxes. We chose two species with largely different morphology, physiology and phylogeny that are co-occurring in several broad-leaved forest communities of Central Europe and are of moderate to high economic importance for forestry (Ellenberg and Leuschner, 2010). The two species represent different families (Fagaceae and Oleaceae), contrast in their successional status (late-successional vs. early-/mid-successional) and differ with respect to root morphology and mycorrhization (beech: thin fine roots with many root tips and ectomycorrhiza vs. ash: thick fine roots with only few root tips and arbuscular mycorrhiza). According to the considerable differences in root morphology, root system size, and mycorrhiza comprising the rhizobiota network, we hypothesised that (1) the N_2O emissions are higher from soils under ash due to a higher C and N supply in the soil than under beech, (2) the CO_2 efflux from the soil is higher under ash than under beech due to an assumed higher root growth activity, while (3) CH_4 uptake is not different between soil planted with ash or beech saplings, and (4) the interaction of beech and ash roots affects the GHG fluxes in a way that is not simply an additive effect of the fluxes in the monocultures. In order to investigate only root-induced effects on GHG fluxes, we excluded the decomposition of leaf litter as a confounding process in our experimental design.

5.2 Materials and methods

5.2.1 Plant and soil material

Plant and soil material was collected in a temperate mixed broad-leaved forest in western Thuringia, Germany, the Hainich National Park (51°04' N 10°30' E, about 350 m a.s.l). *Fagus sylvatica* and *Fraxinus excelsior* form species-rich mixed stands together with other broad-leaved tree species of the genera Tilia, Acer and Carpinus (Leuschner et al., 2009). The stands are 27 to 32 m tall and about 80 to 120 years old. Both target species show a vital rejuvenation in the stands. The

climate has a sub-continental character with a mean annual temperature of 7.5°C, and a mean annual precipitation of 590 mm (Deutscher Wetterdienst, 2005). The soil type is a Stagnic Luvisol (IUSS Working Group WRB, 2007). The mineral soil is composed of 1.8% sand, 80.2% silt, and 18.1% clay.

The rhizotrons were filled with mineral soil material taken from the upper 10 cm of the profile in the Hainich forest right below the organic layer. After sampling, the fresh soil was homogenised and coarse-grained soil particles and roots were removed by sieving with a mesh size of 10 mm, preserving most of the mesofauna. The chemical properties of the soil at the start of the experiment are given in Table 5.1.

Table 5.1 Chemical properties of the uppermost 20 cm of the soil in the rhizotrons at the start of the experiment and after 475 d of growth of either two beech, two ash, or one beech and one ash sapling (means ± 1 SE; n = 4).

	Start of the experiment		**End of the experiment**							
			Control		**Beech**		**Ash**		**Mixed**	
Bulk density [g cm^{-3}]	nd		1.19	± 0.10	1.03	± 0.12	1.05	± 0.04	0.94	± 0.05
pH (H_2O)	4.56	± 0.03	4.68	± 0.10	4.40	± 0.14	4.75	± 0.06	4.54	± 0.15
CEC [µmolc g^{-1} dw]	191.7	± 11.8	190.7	± 15.7	182.3	± 6.1	184.4	± 8.7	198.2	± 16.7
Base saturation [%]	22.9	± 1.3	17.5	± 1.8	7.8	± 1.1	20.1	± 0.5	21.3	± 1.1
NO_3^- [mg N kg^{-1} dw]	6.39	± 0.28	42.1*	± 10.8	34.9*	± 3.9	39.0*	± 2.6	33.9*	± 6.6
NH_4^+ [mg N kg^{-1} dw]	7.85	± 0.28	4.42	± 1.58	2.20*	± 0.80	1.66*	± 0.57	1.56*	± 0.50
C_{org} [g kg^{-1} dw]	19.2	± 0.3	18.3	± 0.5	17.5*	± 0.5	17.0*	± 0.4	16.4*	± 0.5
N_{total} [g kg^{-1} dw]	1.64	± 0.01	1.68*	± 0.07	1.63	± 0.04	1.56*	± 0.02	1.54*	± 0.06
C:N ratio [g g^{-1}]	11.7	± 0.14	10.9	± 0.3	10.8	± 0.2	10.9	± 0.2	10.6	± 0.1

None of the nine parameters differed significantly ($P < 0.05$) between the four treatments at the end of the experiment (Tukey-Kramer test); significant differences between a treatment's final state and the conditions at the start of the experiment are indicated by * ($P < 0.05$, paired t test); nd = not determined.

The saplings were excavated close to the soil sampling site in spring 2009. Immediately before the onset of the experiment, the saplings were carefully dug out with intact soil cores to extract the complete root system with only minimal damage. Adherent coarse soil particles were carefully removed from the roots. In order to allow for a complete colonization of the roots by the local mycorrhiza community, the sapling root systems and the surrounding soil material were stored together in watered pots overnight. The saplings of beech and ash had an initial shoot height of 23.1 ± 1.2 cm and 17.9 ± 1.1 cm (means ± 1 SE), and a mean tap root length of 12.1 ± 0.7 cm, and 15.4 ± 1.2 cm, respectively. At the beginning of the experiment, the beech and ash saplings had 2 to 5 leaf buds, but no unfolded leaves. The initial root biomass of the two species is given in Table 5.2. Initial aboveground biomass was 1.26 ± 0.27 g for beech and 1.25 ± 0.15 g for ash and it increased to 2.66 ± 0.60 and 3.24 ± 0.59 g, respectively until harvest after 475 days of cultivation. During winter, the beech and ash saplings were leafless for 50 and 110 d, respectively, which is considerably shorter than in nature, especially in the case of beech.

Table 5.2 Fine, coarse and total root biomass at the beginning and end of the experiment (day 475) in rhizotrons planted either with two beech, two ash or one beech with one ash sapling. Given are means ± 1 SE (n = 4).

	Start of the experiment					**End of the experiment**				
	Beech	**Ash**	**Mixed (total)**	**Mixed (beech)**	**Mixed (ash)**	**Beech**	**Ash**	**Mixed (total)**	**Mixed (beech)**	**Mixed (ash)**
Root $mass_{fine}$	0.33 ± 0.10	0.81 ± 0.16	0.46 ± 0.09	0.16 ± 0.05	0.41 ± 0.08	1.56^{a} ± 0.54	4.76^{b*} ± 0.91	4.07^{ab} ± 0.76	1.80^{*} ± 0.37	2.27 ± 0.63
Root $mass_{coarse}$	1.28 ± 0.33	1.12 ± 0.12	1.20 ± 0.15	0.64 ± 0.17	0.56 ± 0.06	3.79^{aA} ± 1.07	11.93^{b*} ± 2.94	13.45^{b} ± 2.23	5.24^{B*} ± 1.16	8.21 ± 2.70
Root $mass_{total}$	1.61 ± 0.42	1.94 ± 0.16	1.77 ± 0.18	0.80 ± 0.21	0.97 ± 0.08	5.34^{aA} ± 1.54	16.69^{b*} ± 3.65	17.52^{b} ± 2.59	7.04^{B*} ± 1.53	10.47 ± 3.04

Different lower case letters indicate significant differences between treatments ($P < 0.05$, Tukey-Kramer test), upper case letters signed differences for a species between monospecific and mixed rhizotrons ($P < 0.05$, Tukey-Kramer test); significant differences between start and end of the experiment are indicated by an asterisk after the values at the end of the experiment ($P < 0.05$, paired t test).

5.2.2 Experimental setup

The novel double-split-root rhizotrons are made of anodised aluminium plates with a transparent 10 mm acrylic glass front to observe root growth and death. The total volume of a rhizotron is 16.2 L (600 mm x 900 mm x 30 mm, w x h x d, Fig. 5.2), which is divided by two vertical bars in three compartments at a volume ratio of 1:2:1. The rhizotrons can be thermally regulated by a pipe system of circulating water installed in the back plate and driven by a water pump (Master DW 5500e, Sicce S.p.A., Pozzoleone, Italy). Thereby, thermal homogeneity can be maintained in the entire soil volume of each rhizotron as well as among all 16 rhizotrons used in the experiment. The rhizotrons are equipped with 24 raster access ports (RAP) located in six soil depths to allow for minimal-invasive sampling of bulk soil and rhizosphere solution via steel capillaries as described by Blossfeld et al. (2011). Each RAP field consists of a raster plate of 34 x 34 multiple-step holes (d = 0.7 / 0.5 mm, distance 1 mm) for inserting steel capillaries at defined depths which serve as microsuction cups. By rotation and perpendicular movement of a RAP between the front and back plate, the microcapillary raster fields can be positioned close to the surface of target roots (distance 0.5 mm) in order to allow sampling of rhizosphere solution at variable distances to the root surface and along the root axis. The RAPs are equipped with sterile filter membranes (cyclopore track-etched membranes of 47 mm diameter and a pore width of 0.2 µm, Whatman, Piscataway, NJ, USA) to guarantee for sterile sampling of rhizosphere and soil solution.

Opposite to each RAP, observation and manipulation windows (d = 52 mm) made of acrylic glass of reduced thickness (1 mm) are installed in the front plate. The rhizotrons are tilted by 35° in forward direction to induce root growth along the transparent front plate. During the experiment, the front plates were kept covered with black scrim to exclude light penetration to the soil which could have influenced root growth and soil fauna activity.

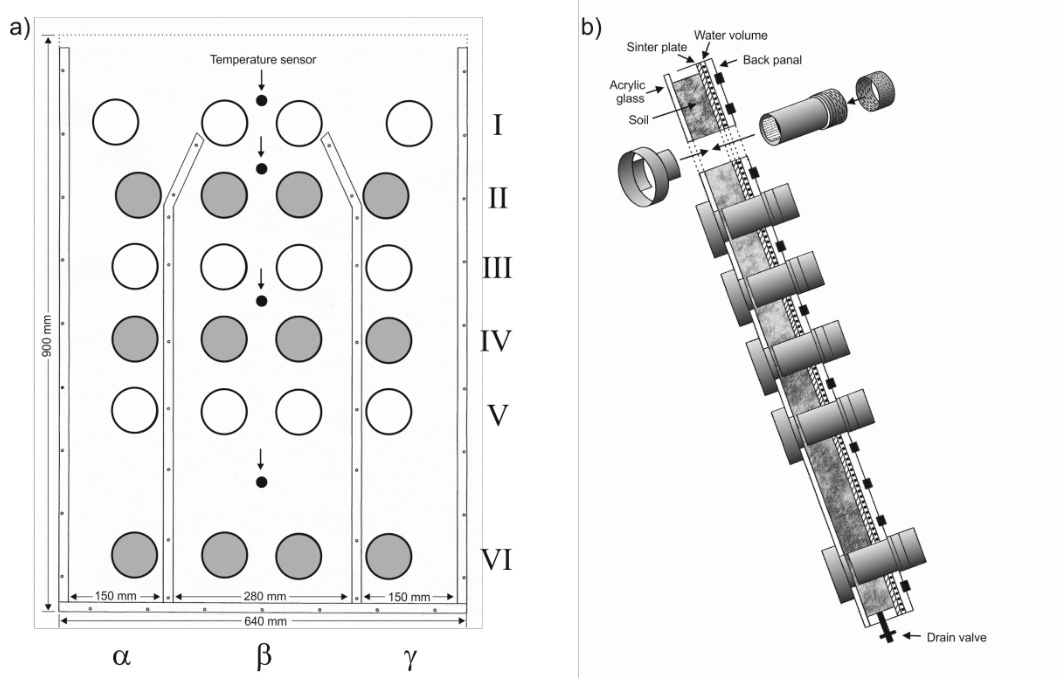

Fig. 5.2 Front view of a double-split-root rhizotron. Two metal bars separate the soil volume into thee compartments (α, β, γ) at a ratio of 1:2:1. Roman numerals mark the six soil layers in the rhizotron that were accessible by each four cylindrical openings for inserting litter material as a stimulant of local soil biological activity. Black dots mark the position of temperature sensors. The circles mark the position of the raster access ports (upper surface of rhizotron) and the corresponding openings for material addition at the lower surface that could also be used for direct observation through the acryl glass window. In the shaded circles, 1.5 g of ash litter were added to stimulate soil biological activity; no litter was added in the clear circles. b) Cross-section of a rhizotron. The soil layer in the rhizotron has a wide of 30 mm. For the uppermost soil layer, the design of a raster access port (upper side) and the front ring of 1 mm thick acrylic glass of the observation window (lower side) are shown in detail. The black squares symbolise the position of the water circulation system for thermal regulation of the soil.

A one-factorial fully randomised experiment with two blocks and sixteen double-split-root rhizotrons was set up with species composition being varied (ash, beech, mixed). Each eight rhizotrons were installed in a climate chamber (blocks) of the Experimental Botanical Garden of the University of Göttingen with a controlled temperature regime, relative air humidity, and light supply. The experiment consisted of four treatments, each replicated four times: mono-specific beech rhizotrons planted with two beech saplings, mono-specific ash rhizotrons planted with two ash saplings, mixed rhizotrons planted with one beech and one ash sapling, and an unplanted (root-free) control (bare soil).

All rhizotrons were homogeneously filled with 16.2 L of fresh, sieved soil material (gravimetric water content: 22.7%) before planting. Each of the two saplings per rhizotron was planted right above one separating aluminium bar in the boxes which created three soil compartments (α, β, γ compartment); thus, the roots of the two saplings had a free choice of growing into the three soil compartments (Fig 1a). For simulating the patchy nutrient distribution which is typical for many forest soils, we created local well-defined hot spots of nutrient availability in the soil volume of the rhizotrons by adding a total of 18 g ash leaf litter to every rhizotron on August 22, 2009, i.e. 53 d after planting. This was done by inserting each 1.5 g of litter, diluted by 38.5 g of soil material, to 12 systematically distributed patches of the rhizotron that were accessible by the observation and manipulation windows opposite to the RAPs (layer I: at 0 – 192 mm soil depth, without litter; layer II: 192 – 305 mm depth with litter, layer III: 305 – 417 mm depth without litter; layer IV: 417 – 530 mm depth with litter; layer V: 530 – 698 mm depth without litter; layer VI: 698 – 900 mm depth with litter, Fig. 5.2).

The experiment was conducted under constant climate conditions (20°C air temperature, 70% relative air humidity) and 10 to 14 h daylight with 203 ± 10 µmol m^{-2} s^{-1} PPFD (MT400DL/BH, Iwasaki Electric Co., Tokyo, Japan) from June, 30, 2009 until October, 18, 2010 (475 d). The volumetric soil water content was monitored three times a week with a mobile TDR probe (Trime-FM, IMKO, Ettlingen, Germany), and kept at constant level by adding distilled water if a deviation from the target value (21 vol.-%) was observed. Soil temperature was measured with four NTC thermistors per rhizotron (Epcos, Munich, Germany), positioned vertically in the centre of the rhizotrons (compartment β) at soil depths of 80, 200, 425, and 705 mm with 20 mm distance to the acrylic glass front plate. Data were recorded in 15 min-intervals with a CR1000 data logger (combined with two AM416 Relay Multiplexers, Campbell Scientific Inc., Utah, USA).

5.2.3 Measurement and analysis of gas fluxes

The rhizotrons were established three months before starting gas flux measurements in order to adjust the soil to the experimental conditions and to balance the gas exchange after disturbing the natural soil structure. Gas fluxes were measured biweekly at the soil surface for a period of 324 days until harvest applying the closed-chamber technique. A chamber was positioned between the two saplings of each rhizotron (soil surface of compartement β). To create a sufficiently large headspace volume of 1.75 L we used brass chambers with dimensions of 350 mm x 170 mm x 29.5 mm (h x w x d). During the gas flux measurements, the chambers were closed for 1 h. After 0, 20, 40 and 60 min, gas samples were taken from the chamber headspace by flushing gas-tight 50 mL-sample syringes with headspace air, using a cannula and two three-way valves. The gas concentrations were analysed by a gas chromatographic system. A detailed description of the GC configuration is presented in Loftfield et al. (1997). The fluxes were calculated from the linear concentration change during the time of chamber closure.

5.2.4 Sampling of rhizosphere solution and analysis of organic acids by capillary electrophoresis-UV

Before the final harvest, rhizosphere solution was collected to identify organic acids originating from root exudates of beech or ash saplings. Each four transverse transects to beech and ash fine roots were investigated at distances of 1, 6 and 11 mm to the root surface. The roots used for this investigation all grew within the upper 650 mm of the soil column. For comparison, soil solution was also extracted at three randomly selected locations in the soil of the (root-free) control rhizotrons. In order to generate sufficient solution for sampling, the soil was irrigated with 1.67 L m^{-2} of distilled water 1 h before sampling. Capillaries of stainless steel (do = 0.6 mm, di = 0.4 mm, length = 1000 mm, SWS Edelstahl GmbH, Emmingen, Germany) were inserted into the RAPs. Before sampling rhizosphere solution, the system was flooded with 70% ethanol to sterilise it. To avoid a blockage of the

capillaries by particles, a steel wire (d = 0.3 mm, wiped with 70% ethanol) was inserted into each capillary during the process of positioning into the raster plates. The wire was removed before collecting the rhizosphere solution. The capillaries were connected to a vacuum sampling chamber (Blossfeld et al. 2011). The pressure in this chamber was lowered by 320 hPa to a level of 650 hPa with a diaphragm pump (Typ MZ 2, Vacuubrand GmbH, Wertheim/Main, Germany). We collected soil solution volumes in the range from a few µL to 1.5 mL in Eppendorf caps of 1.5 mL volume over 60 min.

All samples were analysed for organic acids (oxalate, formate, acetate, lactate) using capillary electrophoresis with a salicylate electrolyte (Bazzanella et al., 1997). A capillary electrophoresis system G1600A (Agilent, Böblingen, Germany) was used, equipped with a built-in diode-array detector. Fused silica capillaries (Polymicro, Phoenix, USA) of 75 µm I.D. x 64.5 cm total length (56 cm to detector) were used. The electrolyte solution contained 7.5 m*M* salicylic acid, 15 m*M* TRIS, 0.5 m*M* dodecyltrimethylammonium hydroxide and 0.3 m*M* $Ca(OH)_2$. A voltage of 30 kV was applied during all separations, with temperature maintained at 25°C. Injections were carried out hydrodynamically with a pressure of 50 mbar for 30 s. The separated compounds were detected by indirect UV-detection at a wavelength of 232 nm. Quantification was performed using external calibration with aqueous standard solutions (5, 10, 20 µ*M*) and internal standardization using phenylacetic acid as internal standard. The rhizosphere samples (12.5 µL/ash, 10 µL/beech) were diluted with deionized water (77.5 µL/ash, 80 µL/beech) and 10 µL of a 100 µ*M* internal standard solution prior to analysis. Calibration lines for the organic acids investigated were linear in the range between 5 and 20 µ*M* with correlation coefficients from 0.9965 to 0.9991. The limits of detection (LOD, 3σ) were about 0.5 – 1.0 µ*M*.

5.2.5 Plant harvest and soil analysis

At the first day of harvest (475 d after planting), the shoot length and root collar diameter of each sapling were measured. The roots were carefully excavated from

the soil, washed and cleaned from adherent soil particles. Where possible, three representative root branches per species and soil compartment were isolated in all six soil layers of the rhizotrons and digitalised on a flat-bed scanner for image analysis to determine specific fine root area (SRA, $cm^2\ g^{-1}$ dry matter), specific fine root length (SRL, cm g^{-1} dry matter) and total fine root surface area using WinRhizo 2005c software (Régent Instruments Inc., Québec, QC, Canada). All biomass samples were oven-dried (70°C, 48 h) and weighed for dry weight determination. For quantifying the vertical distribution of root biomass in the rhizotrons, we calculated the relative cumulative root biomass in the six soil depth layers of the boxes and described the depth distribution by the exponential function $y = 1 - \beta d$ given by Gale and Grigal (1987) which expresses the cumulative proportion of root biomass y as a function of soil depth d and a specific factor β. The relative growth rate of the roots (RGR, mg $d^{-1}\ g^{-1}$ root mass) was estimated by subtracting the initial root mass (determined in five saplings per species at the day of planting) from the root mass of the harvested saplings divided by the duration of the experiment and relating to initial root mass. The plant material was ground with a disc mill and the C and N concentrations detected in a mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany). The colonisation with AM and EM was determined as described previously (Lang et al., 2011), and differed between ash (85%) and beech (44%), but not between mono or mixed systems. Compared to field observations, both ectomycorrhizal and arbuscular mycorrhizal colonization rates were within typical ranges (Pena et al., 2010; Lang et al., 2011).

For estimating the root-induced respiratory activity in immediate vicinity of the roots, we estimated root respiration by calculating root growth respiration from the expression Rg = (RGRroot+73.7)/4.31 given by Reich et al. (1998) with RGRroot being the relative growth rate of the roots, and on the assumption that root maintenance respiration is approximated by Rm = 0.106 x N following Ryan (1991) with N being the nitrogen concentration of root dry mass. By subtracting the calculated root respiration (growth plus maintenance respiration) and the pure soil respiration, measured in the root-free rhizotrons, from the measured total net CO_2 efflux, we obtained an estimate of the root-induced additional soil respiration

in the rhizosphere. The root respiration rates of ash calculated from root RGR and root N content were checked against independent in situ measurements conducted with miniature root cuvettes (1.5 mL microcentrifuge tubes) placed around root segments of ash saplings (n = 5) using miniature planar CO_2 optodes (Presens, Regensburg, Germany) for online optoanalytical measurement of net CO_2 release over 180 min at one minute intervals..

During the harvest soil samples from the upper 20 cm-layer located below the gas flux sampling area were extracted for chemical analysis. To exclude an effect of soil depth on soil properties additional samples were taken in each soil layer. The soil pH was analysed in a suspension with 10 g soil and mixed with 25 mL H_2O using a Vario pH meter (WTW GmbH, Weilheim, Germany). The gravimetric soil water content was determined by weighing the soil samples before and after drying at 105°C for 24 h. The nitrate (mg N-NO_3^- kg^{-1} dw) and ammonium (mg N-NH_4^+ kg^{-1}dw) concentrations were estimated by extracting soil samples in 0.5 M K_2SO_4 solution (1:3 wet soil mass to solution ratio) directly after collection. The samples were shaken for 1 h and passed through folded filters (150 mm in diameter, 65 g m^{-2}, Sartorius Stedim, Aubagne, France). The $NO_3^-$and NH_4^+ concentrations of the filtered extracts were analysed using continuous flow injection colorimetry (SAN+ Continuous Flow Analyzer, Skalar Instruments, Breda, The Netherlands). Nitrate was determined by the copper cadmium reduction method (ISO method 13395) and NH_4^+ by the Berthelot reaction method (ISO method 11732). The contents of C_{org} and N_{total} were determined in a mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany) after grounding the dry soil in a disc mill. The bulk density of the material was determined in 5 cm soil depth under the gas flux sampling area using plastic cores with a defined volume of 10.8 cm3 after Schlichting et al. (1995). The particle size distribution of the soil material in the rhizotrons was analysed in five replicate samples using the sieving and pipette method (Schlichting et al., 1995).

5.2.6 Data analysis

All statistical analyses were carried out with SAS 9.1 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA). Cumulative gas fluxes were calculated by summing up all measurements done in a rhizotron considering the number of measurements taken and the length of the entire measuring period (324 d). The gas fluxes varied considerably between the different measurement days as it is common for GHG fluxes from soil, so that we refrained from showing the time course. All data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variances applying the Levene test. The bloc effect of the two climate chambers was tested with a two-factorial ANOVA considering the two factors “treatment” and “bloc” and an interaction term (“treatment x block”). For the various soil chemical properties, the gas fluxes and the biological parameters, no bloc (chamber) effect was detected. To investigate the effects of beech and ash roots on various parameters, one-way ANOVA with a post hoc Tukey-Kramer test was used to locate significant differences among treatment means for data showing normal distribution. If the data were not normally distributed or variances were not homogeneous, the non-parametric Kruskal-Wallis test was used to test for significant differences between means. The significance of differences between two treatments was subsequently investigated with the Wilcoxon U-test. A paired t test was used to test for significant differences in normally distributed soil parameters between the soil state at harvest and the experiment’s beginning. Linear regression analysis was conducted to relate the chemical soil properties of the uppermost 20 cm to various biological parameters (listed in Tables 5.1 and 5.2), and to relate gas fluxes to soil chemical properties and biological parameters. In all analyses, significance was determined at $P < 0.05$.

5.3 Results

5.3.1 Gas fluxes

The uptake of CH_4 by the soil was found to be 173% higher in ash rhizotrons than in the root-free control rhizotrons with significant differences to both the control and to the mono-specific beech rhizotrons (Fig. 5.3a). Beech and control rhizotrons showed comparable CH_4 fluxes (-46.8 ± 5.0 and -38.5 ± 9.1 mg C m^{-2} 324 d^{-1}). In the mixed rhizotrons, intermediate uptake rates were measured. We found a close negative correlation between CH_4 uptake rate and the extractable NH_4^+ concentration in the soil across the four treatments (R^2 = 0.33; P = 0.020, Fig. 5.4a) while the CH_4 fluxes were positively related to fine root biomass (R^2 = 0.38; P = 0.032, Fig. 5.4b).

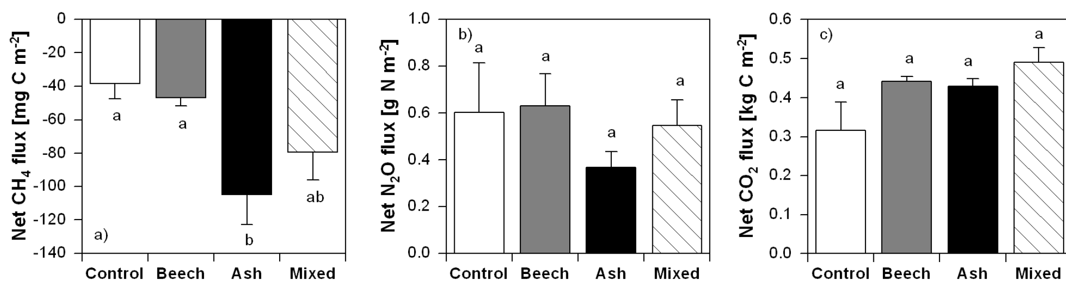

Fig. 5.3 Fluxes of CH_4, CO_2 and N_2O measured at the soil surface of rhizotrons planted with beech and/or ash saplings, cumulated for 324 d (biweekly measurements). Given are data for rhizotrons planted with no saplings (bare soil, control), or rhizotrons with two beech (beech), two ash (ash), or one beech and one ash sapling (mixed); n = 4; means ± 1 SE. Different lower case letters indicate significant differences between the treatments (for CH_4: Tukey-Kramer test; for N_2O and CO_2: Wilcoxon U-test, each with $P < 0.05$).

The cumulative N_2O emission over the experimental period was approximately 30% smaller from the rhizotrons with ash saplings (0.40 ± 0.09 g N m^{-2} 324 d^{-1}) than from the other three treatments (means of 0.55, 0.63 and 0.60 g N m^{-2} 324 d^{-1} in the mixed, mono-specific beech and root-free control rhizotrons, respectively, Fig. 5.3b). However, the variation in N_2O fluxes among the replicate rhizotrons (n = 4) was large with values ranging from 0.15 to 1.03 g N m^{-2} 324 d^{-1} in the control. As a consequence, the difference between the ash and the beech rhizotrons was only marginally significant (P = 0.056). We found a highly significant negative relation between the cumulative N_2O emission and the salt-extractable NO_3^-

concentration in the soil across the four treatments (R^2 = 0.74, P < 0.0001, Fig. 5.4c). A negative correlation also existed between the N_2O flux and total fine root area in a rhizotron (R^2 = 0.42; P = 0.020, Fig. 5.4d).

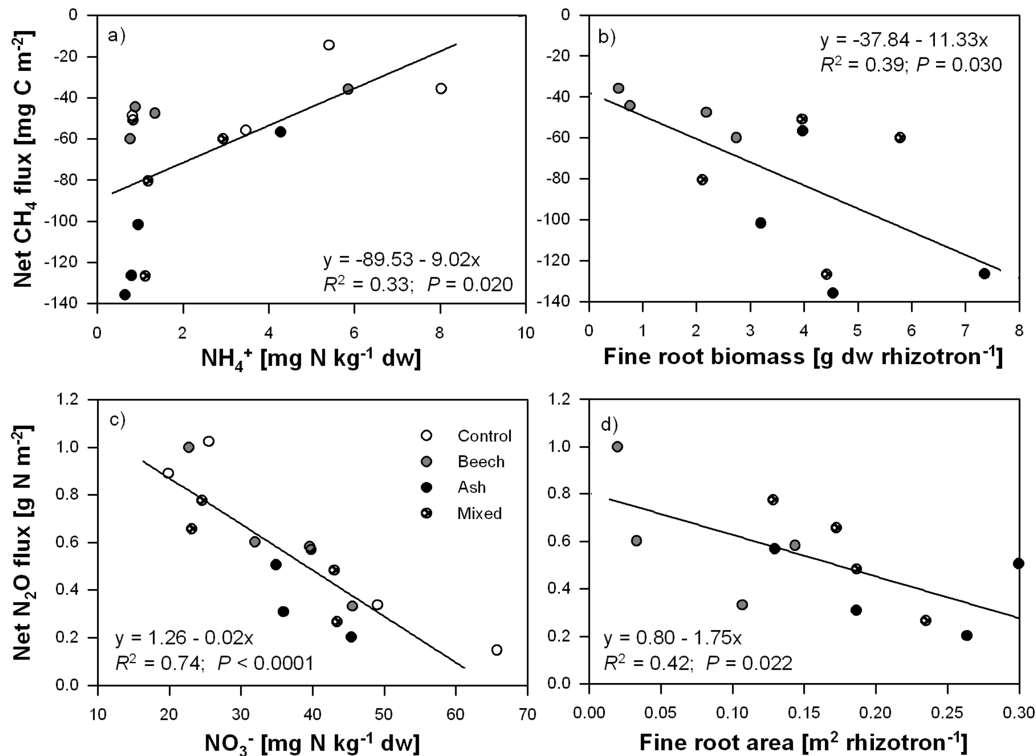

Fig. 5.4 Relationships between cumulative N_2O fluxes (period: 324 d) in rhizotrons planted either with beech or ash saplings or a beech/ash mixture and (a) the NH_4^+ concentration in the uppermost 20 cm of the soil or (b) total fine root biomass in a rhizotron. Relationships between the cumulative CH_4 uptake of the soil (period: 324 d) and (c) the NO_3^- concentration in the uppermost 20 cm of the soil or (d) the total fine root surface area in a rhizotron; n = 4 rhizotrons per treatment in all cases.

The cumulative net release of CO_2 from the soil tended to be higher by 40% and 36% in the mono-specific beech and mono-specific ash rhizotrons than in the control rhizotrons, but the difference was not significant (Fig. 5.3c). The highest CO_2 emission was measured in the mixed rhizotrons that contained beech and ash roots (0.55 ± 0.04 kg C m^{-2} 324 d^{-1}); the differences to the root-free control (55% higher) was marginally significant (P = 0.056). Mass-specific root respiration as calculated from relative root growth rate and root N concentration was higher in the mono-specific ash rhizotrons (26.7 ± 0.6 nmol CO_2 g dw^{-1} s^{-1}) than in the beech rhizotrons (22.0 ± 0.6 nmol CO_2 g dw^{-1} s^{-1}). A similar species ranking was

found for root respiration in the mixed rhizotrons (27.9 ± 1.2 nmol CO_2 g dw^{-1} s^{-1} for ash and 24.8 ± 0.9 nmol g dw^{-1} s^{-1} for beech; Table 5.3). The net CO_2 release was not related to any of the chemical properties of the topsoil or root morphological parameters listed in Table 5.1 (data not shown).

Table 5.3 Partitioning of measured net CO_2 efflux from the soil (cumulated over 324 d from biweekly measurements) into the components soil respiration (respiration of root-free control), root respiration and root-induced soil respiration (in kg C m^{-2} 324 d^{-1}: left columns, or % of total respiration: right columns; n = 4 rhizotrons).

	Control		**Beech**		**Ash**		**Mixed (total)**	
Total respiration	0.317[a] ± 0.072	100	0.442[a] ± 0.013	100	0.429[a] ± 0.020	100	0.490[a] ± 0.038	100
Soil respiration	0.317[ab] ± 0.072	100	0.317[a] ± 0.072	71.9 ± 2.0	0.264[b] ± 0.030	61.6 ± 6.8	0.271[ab] ± 0.030	54.9 ± 3.5
Root respiration	-	-	0.040[a] ± 0.012	9.0 ± 2.7	0.152[b] ± 0.036	35.5 ± 8.5	0.162[b] ± 0.029	33.8 ± 8.3
Root-induced soil respiration	-	-	0.085[a] ± 0.010	19.1 ± 2.0	0.013[b] ± 0.013	2.9 ± 2.9	0.058[ab] ± 0.039	10.2 ± 6.9

Root respiration was estimated from relative root growth rate (growth respiration) and root N concentration (root maintenance respiration); see text. Root-induced soil respiration was calculated as the difference between net CO_2 efflux and soil plus root respiration. Lower case letters show significant differences between the respiration rates of each treatment (Wilcoxon U-test, $P < 0.05$).

5.3.2 Organic acids in the rhizosphere solution

According to the micro-capillary extraction at 1 – 11 mm distance to the root surface, the average concentration of organic acids (oxalate, formate, acetate and lactate) tended to be higher in the vicinity of beech roots than close to ash roots (432 ± 103 µ*M* vs. 314 ± 101 µ*M*). The analysis revealed higher concentrations of acetate in direct contact (1 mm distance) to ash roots as compared to beech roots. In the rhizosphere of ash roots, the acetate concentration rapidly decreased with root distance from > 70 µ*M* at 1 mm to 30 µ*M* at 11 mm (Fig. 5.5). Both oxalate and formate decreased (from 20 µ*M* to 7 µ*M* and from 47 µ*M* to 20 µ*M*, respectively) with increasing distance from ash roots while the concentrations of oxalate and formate remained more or less invariant between 1 and 11 mm

distance to beech roots. Formate was the most abundant organic acid found near beech roots (53 – 87 µ*M*). The qualitative differences in the composition of the soil solutions near beech and ash roots are illustrated by the fact that the acetate concentration contributed by 47% to the total concentration of organic acids in the vicinity of ash roots, while 45% of the organic acids were formate near beech roots. In root-free bulk soil, the soil solution contained exclusively oxalate and formate (32 ± 21 and 45 ± 15 µ*M*, respectively) while acetate and lactate was solely detected in the vicinity of roots (Fig. 5.5).

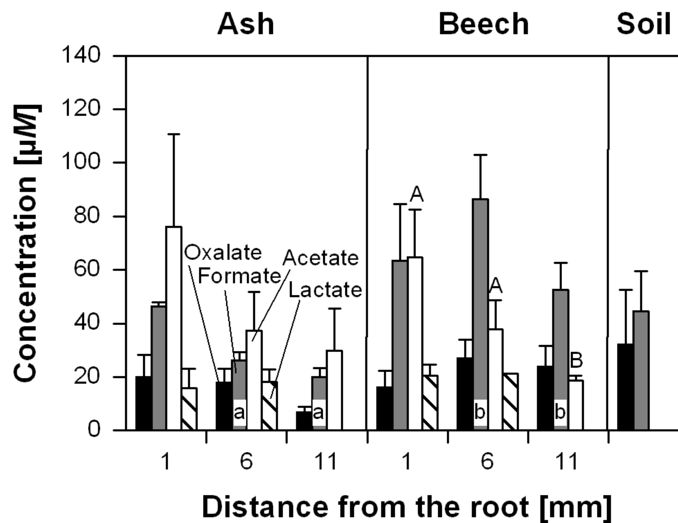

Fig. 5.5 Variation in organic acid concentration (oxalate, formate, acetate and lactate) in the rhizosphere solution at three distances from the root surface, measured in orthogonal direction from fine root branches of beech or ash saplings (n = 4, means ± 1 SE) and control measurements in root-free rhizotrons (n = 3). Significant differences between beech and ash for the corresponding distances were marked with lower case letters, differences within a transect marked by upper case letters (Wilcoxon U-test, $P < 0.05$) To keep the figure readable we omitted letters indicating non-significant differences. For the lactate concentration in 6 mm distance from the beech root, no SE could be calculated due to values below the detection limit.

5.3.3 Root biomass and soil properties

Ash saplings produced about three times as much root biomass (fine, coarse and total) during the experimental period of 475 d than beech saplings (Table 5.2). In the mixed rhizotrons, root biomass was similarly large as in the mono-specific ash rhizotrons showing no evidence of belowground 'overyielding' in terms of standing root mass. However, the beech saplings grown in mixture produced on

average significantly more fine and coarse root biomass than beech saplings planted in mono-specific culture, while ash root biomass was not significantly affected by the neighbour identity (Table 5.2). Most of beech root biomass, i.e. 82% to 98%, was located in the upper 30 cm of the rhizotrons, whereas the corresponding relative proportion of ash roots was 59 to 91%, evidencing a more deep-reaching soil exploration in ash (Fig. 5.6). None of the investigated soil chemical and physical parameters of Table 5.1 showed a significant relation to the fine or total root biomass of the two species in the rhizotrons (data not shown).

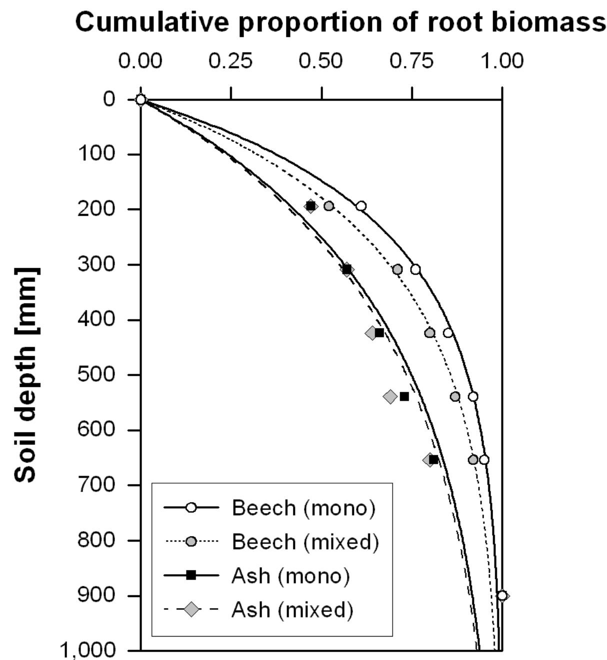

Fig. 5.6 Cumulative amount of root biomass as a function of soil depth in the rhizotrons planted either with two beech, two ash, or one beech and one ash sapling (relative units, means of n = 4 rhizotrons). Each seven measurements were conducted per rhizotron along the profile. The shape of the curve is described by the β-value of the regression equation $y = 1 - \beta^d$ after Gale and Grigal (1987, see text). Mono-specific beech rhizotrons: $\beta = 0.954$, $R^2 = 0.999$, $P < 0.001$; mono-specific ash rhizotrons: $\beta = 0.973$, $R^2 = 0.978$, $P < 0.001$; beech in mixed rhizotrons: $\beta = 0.962$, $R^2 = 0.998$, $P < 0.001$; ash in mixed rhizotrons: $\beta = 0.974$, $R^2 = 0.966$, $P < 0.001$.

At the end of the experiment, the chemical properties of the soil in the rhizotrons differed not significantly between the four treatments (Table 5.1). However, C_{org} decreased in the rhizotrons by 0.9 g C kg^{-1} to 2.8 g kg^{-1} dw during the experimental period and the decrease tended to be largest in the treatment with beech/ash mixture. The reduction led to C concentrations that were by 10% lower

than in the control rhizotrons where plants were absent ($P = 0.056$). The pH (H_2O) value tended to be 0.4 units higher under ash than under beech saplings ($P = 0.056$) indicating that root-induced acidification was more pronounced by beech than by ash. Large changes occurred over the experimental period in the soil content of salt-extractable inorganic nitrogen: the NH_4^+ concentration decreased and the NO_3^-concentration increased in the 475 d period; these changes were influenced by the presence of the tree saplings. At the end of the experiment, the rhizotrons with saplings contained by 50% to 65% smaller NH_4^+ concentrations than the control soil. The NO_3^-concentration increased by 399% to 526% during the experiment with a relatively small increase found in the mixed rhizotrons and a large increase in the control (Table 5.1). No changes in soil properties with soil depth were observed. Regression analyses showed that root-related parameters (total fine root area, fine root biomass per rhizotron) had no influence on the extractable NO_3^- and NH_4^+ concentrations in the soil ($P > 0.05$).

5.4 Discussion

While field studies in mature forests provide valuable information on the average source or sink strength of the soils and seasonal flux dynamics, it is more difficult to understand the controlling factors of CH_4 uptake and N_2O release in different forest types because differences in tree species composition are typically associated with differences in soil physical and chemical conditions which complicates the separation of tree species from soil effects on GHG exchange (Fig. 5.1). By excluding litter fall and controlling for temperature, soil moisture, soil bulk density and initial soil N and C content, our investigation focused on possible root-induced effects of different tree species on GHG fluxes in order to disentangle biotic and abiotic controls of N_2O emission and CH_4 uptake in temperate mixed forests.

5.4.1 CH_4 uptake

The recorded CH_4 uptake rates in the rhizotrons (0 – 40 µg C m^{-2} h^{-1}) showed a similar magnitude as CH_4 fluxes measured under field conditions in the Hainich forest (0 – 78 µg C m^{-2} h^{-1}, Guckland et al., 2009). The specific effect of ash roots was visible in the cumulative rate of CH_4 uptake which was by 173% higher in rhizotrons planted with ash saplings than in the control soil, and by 124% larger than in beech rhizotrons. Thus, the observed stimulation of CH_4 oxidation in our experiment was mainly a consequence of the presence of ash roots, while beech roots increased the uptake by only 22% (non-significantly) compared to the control. Beside this species effect, we found a close positive relation between CH_4 uptake and the amount of fine root biomass in the rhizotrons across all treatments. It is known that CH_4 uptake is particularly susceptible to variation in soil moisture and gas diffusivity (Smith et al., 2000). Because soil moisture and soil bulk density were similar among the treatments, differences in oxygen supply cannot explain the higher CH_4 oxidation in rhizotrons with tree saplings compared to the root-free control, and in the ash treatment in particular. This conclusion is supported by the occasional measurement of O_2 partial pressure in the bulk soil using O_2-sensitive optodes which showed that the O_2 pressure in the soil (about 200 hPa) was close to atmospheric O_2 partial pressure in all rhizotrons (data not shown, the optodes consisted of a PSt1 sensor with a Microx TX3 device, Presens, Regensburg, Germany).

The oxidation of CH_4 depends on the initial CH_4 concentration in the soil volume (Le Mer and Roger, 2001). In our rhizotrons, the concentrations were at an ambient atmospheric level between 1640 and 1890 ppb in all treatments. For each measurement date, these initial concentrations did not differ among the treatments (tested with Wilcoxon U-test). Methane oxidation is known to be sensitive to NH_4^+ fertilisation either through competitive inhibition of methane monooxygenase by NH_4^+ or through a negative salt effect in fertilisation experiments (Steudler et al., 1989; Bodelier, 2011). We found a significant negative relation between CH_4 uptake rate and extractable NH_4^+ concentration in our experiment ($R^2 = 0.33$, $P = 0.02$), which is hardly explicable by a salt effect on the methanotrophs because the

inorganic N concentrations were rather small. Reduced NH_4^+ concentrations in the planted rhizotrons compared to root-free soil (means of 1.56 – 2.20 vs. 4.42 mg NH_4^+-N kg^{-1} dw, respectively) can be one possible explanation for the observed higher CH_4 uptake rates in the treatments with tree saplings than in bare soil.

The particularly high CH_4 uptake rates in the ash treatment might well be a consequence of the deeper reaching root system of this species in comparison to beech which rarely exceeded a maximum rooting depth of 60 cm (0 – 3% of fine root biomass), while 1 – 14% of ash root biomass was located below 60 cm. As has also been observed in field studies (Rust and Savill, 2000; Meinen et al., 2009), ash saplings showed a higher production of fine and also coarse roots, explored the subsoil in the rhizotrons more rapidly and reached higher densities of fine root mass per soil volume (0.60 ± 0.05 and 0.26 ± 0.10 g L^{-1} in the upper 20 cm of the mono-specific and mixed rhizotrons, respectively) than beech saplings (0.22 ± 0.05 and 0.23 ± 0.05 g L^{-1}, respectively). We assume that deep-reaching roots create channels of higher gas diffusivity that facilitate the downward transfer of CH_4 in soils.

Finally, the composition and concentration of root exudates may also affect CH_4 oxidation through specific promoting or inhibiting effects. Morphological and physiological differences between ectomycorrhiza (*Fagus*) and arbuscular mycorrhiza (*Fraxinus*) on soil chemistry and related effects on gas fluxes might also be important. This deserves further study.

5.4.2 N_2O emission

The N_2O fluxes measured in the rhizotrons were higher than emission rates recorded under field conditions in the soils of the Hainich forest (19 – 124 vs. < 10 µg N m^{-2} h^{-1}, (Guckland et al., 2010). This is a common outcome of experiments (Jungkunst et al., 2008) and can be related to the destruction of soil aggregates leading to a higher bio-availability of C and N, continuously favourable soil moisture and higher temperatures in the laboratory than in the field (20°C vs. 10 – 20°C). Overall, the initial N_2O concentrations were between 290 and 510 ppb and

did not differ among treatments for each measurement date (tested with Wilcoxon U-test). The cumulative N_2O emissions from rhizotrons planted with ash were on average by 50 – 60% smaller than those from rhizotrons with beech (mono-specific and mixed), and also than from the root-free control, which points at a suppressing effect of ash roots on the release of N_2O from the soil. Since the variation of gas flux among each of the four replicate rhizotrons was large, which is a characteristic outcome of N_2O flux measurements (Jungkunst et al., 2006, 2008), we found only marginally (P = 0.056) or non-significant differences between the four treatments. Therefore, we discuss the likely trends only briefly.

Across all 16 rhizotrons, we found no relation of N_2O flux to the NH_4^+ concentration but a negative one to NO_3^-concentration.. The latter is best explained by a more rapid NO_3^-depletion with higher denitrification rates, which is a main source of the N_2O released (Davidson et al., 2000; Bateman and Baggs, 2005). A negative correlation was also detected between total fine root surface area (and fine root biomass) in the rhizotrons and the cumulative N_2O emission (R^2 = 0.42, P = 0.020 and R^2 = 0.30, P = 0.065 (data not shown), respectively). Ash with a more rapid root and shoot growth rate must have taken up more N than the slower growing beech (Table 5.4), but a trend for a greater depletion of the $NO_3^-$and N_{total} pools in the soil by ash as compared to slower growing beech was not found. However, in a ^{15}N tracer field experiment, Jacob et al. (unpublished data) found a larger uptake of NH_4^+ and glycin in ash compared to beech, maple, lime and hornbeam.

Table 5.4 Nitrogen net accumulation in the root or total biomass of the tree saplings in the mono-specific beech, mono-specific ash and mixed rhizotrons at the end of the experiment (in mg N per rhizotron; means ± 1 SE; n = 4; each two saplings per rhizotron).

	Beech		**Ash**		**Mixed**	
N accumulation in root biomass	0.067^a	± 0.028	0.317^b	± 0.053	0.295^b	± 0.039
N accumulation in total plant biomass	0.113^a	± 0.036	0.473^b	± 0.059	0.478^b	± 0.060

Therefore, it can be suggested that the ash saplings growing in the rhizotrons took up more N as well. The NO_3^- concentration in the soil was not related to root mass and area, and it did not significantly differ between the treatments. Nevertheless, our results indicate that certain broad-leaved tree species can have a substantial influence on the emission of N_2O from forest soils through their root systems. A root-induced influence on the N_2O release can occur independently from a leaf litter effect, and in the absence of significant alterations in pH, total soil N content or soil C:N ratio, which typically characterize soil patches under beech as compared to ash trees in mixed stands (Neirynck et al., 2000; Holzwarth et al., 2011; Langenbruch et al., 2011).

5.4.3 CO_2 emission

It has been found notoriously difficult to partition the measured net CO_2 efflux from soils to the relevant sources, i.e. autotrophic respiration (root maintenance and growth respiration), the respiration of bacteria, fungi and animals in the soil matrix, and additional microbial respiration in the immediate proximity of roots that is stimulated by root exudation (root-induced respiration). The CO_2 measurements in this study showed that CO_2 efflux from the treatments with tree saplings was by 36 to 55% higher than from root-free soil, which agrees well with empirical data on the relative importance of autotrophic respiration in beech forests in Central Germany (30 – 35% of total soil respiration in the vegetation period or 50% in August, Brumme et al. 2009). We attempted to obtain a rough quantification of root respiration and root-induced respiration in the rhizosphere by calculating theoretical figures of root respiratory activity from established relations between root growth rate and root N concentration and subsequently relating it to the 'background' respiratory activity in root-free soil. The calculated respiration rates for beech roots (22 and 25 nmol CO_2 g dw^{-1} s^{-1} in mono-specific and mixed rhizotrons) are in the range of rates measured in situ in the roots of 10-yr-old beech trees in a beech forest using a cuvette technique applied to isolated root branches (16 nmol CO_2 g dw^{-1} s^{-1}, Gansert 1994). For the ash roots in the rhizotrons, we

calculated 27 and 28 nmol CO_2 g dw^{-1} s^{-1}, which is somewhat higher than rates determined by in situ measurements in the rhizotrons using planar CO_2 optodes (mean of 19 nmol CO_2 g dw^{-1} s^{-1}, n = 5 roots, data not shown). Since fine and coarse root biomass were about three times larger in the ash rhizotrons than in the beech treatment, the CO_2 release from root respiration must have been much larger in the former with more vigorous root growth. However, from the comparable rates of total soil respiration measured in the two treatments over the 324 d-experimental period (cumulative values of 0.44 and 0.43 kg C m^{-2} ground area in the beech and ash rhizotrons), it follows that beech roots must be responsible for a much higher root-induced soil respiration in the rhizosphere than ash roots, given that the 'background' soil respiration (adopted from the rates measured in root-free soil) was indeed similar in the two treatments as assumed here. According to this calculation, root respiration contributed with a much larger proportion (about 40%) to total CO_2 efflux in ash rhizotrons than in beech rhizotrons (< 10%), whereas soil respiration and root-induced soil respiration must be relatively more important in the latter. The calculated larger root-induced heterotrophic respiration under beech saplings matches with the larger basal respiration (BAS) in beech compared to ash rhizotrons detected by substrate-induced respiration analysis (data not shown). In the proximity of beech roots, we found on average higher concentrations of organic acids. Because they are a growth substrate for many types of soil bacteria (Brimecombe et al., 2007; Walker et al., 2003), the higher concentration of organic acids might translate into a higher microbial activity in the rhizosphere of this species as compared to ash. This assumption is supported by higher rates of root-induced soil respiration in the rhizosphere of beech than ash roots, as they appeared from our investigation of root and soil respiration. It is remarkable that beech roots apparently stimulated the soil biological activity in the rhizosphere much more than ash roots despite a smaller root growth rate and consequently lower density of roots per soil volume. If this finding is of more general validity, it points at large tree species differences in the effect of roots on rhizosphere processes.

5.5 Conclusions

The present investigation of root-induced trace gas fluxes using novel double-split-root rhizotrons shows that broad-leaved tree species may substantially alter the source/sink strength of forest soil for greenhouse gases (GHG) via root-related processes. The comparison of beech and ash indicates that tree species identity needs to be considered as controlling factor of GHG fluxes in temperate forests. We found differing effects of beech and ash on CH_4 uptake, thus our results did not support hypothesis (3). Furthermore, the apparent root effects on GHG exchange occurred without marked changes in bulk soil C and N pools, pH and soil moisture conditions, contradicting hypothesis (1). The significant stimulation of CH_4 oxidation by ash roots was positively related to fine root biomass but the apparent reduction of N_2O release by ash was not. This indicates that it is not simply a quantitative root effect (more roots lead to lower GHG fluxes) but a qualitative root effect on soil biological activity. The CO_2 efflux data show that roots are capable of influencing soil biological activity through species-specific effects on root-induced soil respiration, which was much higher in beech than in ash. Species differences in the composition and concentration of organic acids in close proximity to fine roots seem to support the proposed qualitative effect, but further analyses are needed. Further, our results provide evidence that beech and ash significantly differ in root respiration under identical ambient conditions; the calculated rates were much higher in ash with faster root growth (hypothesis 2). We found no indication of synergistic effects in the allospecific treatment, contradicting hypothesis (4); the fluxes of N_2O, CH_4 and CO_2 in the mixed rhizotrons could all be explained by adding the activities of the two species.

The calculation of the greenhouse gas balance (total sum of CO_2, CH_4, and N_2O fluxes in CO_2-eq) of our soil from a temperate broad-leaved forest planted with beech and ash saplings under controlled climatic conditions revealed a tendency to a more favourable balance in the presence of ash than of beech (5.4 ± 0.2 vs. 5.9 ± 0.2 g CO_2-eq m^{-2} d^{-1}). Clearly, we carried out these measurements under constant climatic conditions without diurnal and annual variation; nevertheless, the calculations indicate that the stimulation of CH_4 uptake and the

reduction of N_2O emissions by ash saplings can compensate higher CO_2 emissions due to more vigorous root growth.

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CHAPTER

6

Incorporation of plant carbon and microbial nitrogen into the rhizosphere food web of beech and ash

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Abstract

We labeled tree saplings of beech and ash with ^{15}N and ^{13}C in a greenhouse. Carbon (C) was applied as $^{13}CO_2$ to plants and nitrogen (N) was added as $^{15}NH_4{}^{15}NO_3$ to the soil. We hypothesized that C will be transferred from plants to the rhizosphere, subsequently in beech to ectomycorrhiza (EM), in ash to arbuscular mycorrhiza (AM) and finally to soil animals. We expected the C signal to be more effectively transferred to soil animals in EM as compared to AM systems since EM forms more extensive extrametrical mycelia as compared to AM. For ^{15}N we hypothesized that it will be taken up by both saprotrophic microorganisms and mycorrhizal fungi and then channeled to soil animals. After five month, $\delta^{13}C$ and $\delta^{15}N$ signatures of soil animals, EM and fine roots of beech and ash were measured. Litter and soil were hardly enriched in ^{15}N whereas fine roots of beech and ash were highly enriched suggesting that nitrogen in $^{15}NH_4{}^{15}NO_3$ was predominantly taken up by plants and mycorrhizal fungi but little by saprotrophic microorganisms. Roots of beech and ash were highly enriched in ^{13}C with maximum values in EM proving that ^{13}C was translocated into roots and mycorrhizal fungi. Soil animals were a priori assigned to primary decomposers, secondary decomposers and predators. Generally, signatures of soil animals did not significantly vary between beech and ash and therefore were pooled. Primary decomposers had low $\delta^{13}C$ and $\delta^{15}N$ signatures similar to litter and soil confirming that rhizosphere C and microbial N are of limited importance for primary decomposer taxa. $\delta^{13}C$ and $\delta^{15}N$ signatures of secondary decomposers were higher than those of primary decomposers and spanned a large gradient indicating that certain secondary decomposers rely on root derived C and microbial N, however, none of the secondary decomposers had signatures pointing to exclusive feeding on EM. Unexpectedly, $\delta^{13}C$ and $\delta^{15}N$ signatures were highest in predators suggesting that they heavily preyed on secondary decomposer species such as the litter dwelling Collembola species *Lepidocyrtus cyaneus* and species not captured by the heat extraction procedure used for capturing prey taxa, presumably predominantly root associated nematodes. Overall, the results highlight that in particular higher trophic levels rely on carbon originating from other resources

than litter with these resources channeled to dominant predators via litter dwelling Collembola species.

Keywords*: Soil food web, labeling experiment, fine roots, mycorrhizal fungi, fungal energy channel, bacterial energy channel*

6.1 Introduction

In forest ecosystems most of the net primary production enters the decomposer community as detritus. This dead organic material usually is assumed to be the main source of nutrients for soil microbes (Swift et al., 1979; Berg and McClauthery, 2008) and decomposer animals (Hättenschwiler and Gasser, 2005; Scheu, 2005). However, this view has been challenged recently by documenting that soil animals strongly rely on root-derived carbon (Ruf et al., 2006; Albers et al., 2006; Pollierer et al., 2007, 2012). In fact, a large fraction of plant fixed carbon enters the belowground system via roots and root exudates (Bardgett et al., 2005; Leake et al., 2006) and this carbon is more easily available for soil organisms than the recalcitrant carbon in plant litter since it comprises predominantly amino acids, sugars and peptides (Bais et al., 2006; Dennis et al., 2010).

Most plant roots are closely associated with mycorrhizal fungi channeling plant carbon to the outer rhizosphere (Smith and Read, 1997; Wallander et al., 2009). In temperate forest ecosystems ectomycorrhizal fungi (EMF) dominate (e.g., in beech, oak, lime and hornbeam), but some tree species are associated with arbuscular mycorrhizal fungi (AMF; e.g., ash and acer; Lang and Polle, 2011; Lang et al., 2011). The transfer of carbon from the plant to the rhizosphere likely is more effective in the well-dispersed extrametrical mycelium of the EMF (Högberg et al., 2008; Cairney et al., 2012) than in AMF which do not form intensive extrametrical mycelia (Smith and Read, 1997).

Nitrogen is of crucial importance for soil microorganisms and plants. During decomposition of litter material and for microbial growth in general microorganisms immobilize mineral nutrients in soil thereby competing with plants for these resources (Chapman et al., 2006; Geissler et al., 2010). Tree roots take up nitrogen from soil, but in temperate forests most nitrogen is channeled to plants via EMF (Hobbie and Hobbie, 2006; van der Heijden et al., 2008). In soil food webs carbon is channeled along two main energy pathways, the fungal and bacterial energy channel (Moore and Hunt, 1988; Moore et al., 2005; Crotty et al., 2011). In temperate forests litter quality typically is low and litter is mainly processed by saprotrophic fungi (Wardle et al., 2004). Together with EMF

saprotrophic fungi form the main source of N for the fungal energy channel of soil food webs (Moore-Kucera and Dick, 2008). In contrast, bacteria predominantly consume root exudates and serve as source for N (and other elements) for the bacterial energy channel (Crotty et al., 2011).

From a trophic level point of view the soil food web might be separated into primary decomposers, secondary decomposers and predators (Scheu and Falca, 2000; Scheu, 2002). Primary decomposers, such as Diplopoda, and certain species of Oribatida and Lumbricidae, are assumed to feed mainly on litter material (Pollierer et al., 2009). Secondary decomposers, such as most Oribatida, Collembola and certain species of Isopoda and Lumbricidae, are assumed to feed predominantly on fungi and microbial residues (Maraun et al., 1998; Scheu and Falca, 2000). Predators, such as Lithobiidae or Araneida, have been assumed to rely predominantly on secondary decomposers as food (Scheu, 2002; Pollierer et al., 2012; Ferlian et al., 2012).

Natural variations in stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) have been shown to be a powerful tool for investigating nutrient fluxes and trophic interactions in soil food webs (Scheu and Falca, 2000; Illig et al., 2005; Tiunov, 2007; Pollierer et al., 2009). However, labeling experiments with enriched ^{13}C and ^{15}N compounds are indispensable for tracing carbon and nitrogen fluxes through decomposer systems (Ruf et al., 2006; Pollierer et al., 2007; Sticht et al., 2008; Högberg et al., 2010).

We conducted a $^{13}CO_2$ labeling experiment in the greenhouse to follow the flux of carbon from plant shoots to the rhizosphere and into the soil animal food web. In parallel, we used ^{15}N labeled mineral nitrogen (NH_4NO_3) to follow the flux of nitrogen via saprotrophic microorganisms and mycorrhiza into the soil animal food web. Saplings of European beech and European ash were excavated in the field, potted into mesocosms including rhizosphere soil and the associated soil animal community. After five months of labeling i.e., after one vegetation period, $\delta^{13}C$ and $\delta^{15}N$ signatures of beech and ash roots, ectomycorrhiza and soil animals were measured.

We investigated the following hypotheses: (1) Plant carbon is translocated via roots and mycorrhiza into fungal feeding soil invertebrates. (2) Carbon as well

as nitrogen is transferred mainly to lower trophic levels and is diluted towards higher trophic levels due to predators incorporating prey relying in part on root and in part on litter carbon. (3) Carbon and nitrogen transfer into the soil animal food web is more pronounced in beech than in ash due to the more extensive extrametrical mycelium in EMF than in AMF. (4) Mineral nitrogen is translocated to higher trophic levels via both saprotrophic microorganisms and mycorrhizal fungi and subsequently into soil animals.

6.2 Material and methods

6.2.1 Study site and experimental setup

Tree saplings were collected at two locations (Thiemsburg and Lindig) in the south east of the Hainich National Park, Thuringia, Germany (51°05′28″N, 10°31′24″E). The Hainich is the largest cohesive deciduous forest in Germany and was declared National Park in 1997. In the sampling area, forest cover was present since the mid 18th century. In the last four decades, the area was used for military training and has been managed extensively (Schmidt et al., 2009). The dominating tree species at the study sites is beech (*Fagus sylvatica* L.), but ash (*Fraxinus excelsior* L.), maple (*Acer pseudplatanus* L.) and lime (*Tilia platyphyllos* Scop. and *Tilia cordata* P. Mill.) are interspersed. The herb layer of the Hainich is dominated by *Allium ursinum* (L.), *Anemone nemorosa* (L.) and *Galium odoratum* (L.) (Vockenhuber et al., 2011). The mean annual temperature ranges from 7.5 to 8.0°C and the mean annual precipitation is 600 mm (Leuschner et al., 2009). The area represents a slightly sloping plateau of the Triassic Upper Limestone formation covered by Pleistocene loess (Leuschner et al., 2009).

At the study sites 15 saplings of *F. sylvatica* and 14 saplings of *F. excelsior* (height ca. 60 cm) were excavated together with the surrounding intact soil (depth 25 cm and 2-3 cm litter layer) and placed into containers (diameter 25 cm, height 45 cm) equipped with drainage at the bottom. For ^{13}C labeling tree saplings were exposed to $^{13}CO_2$ enriched atmosphere (maximum CO_2 concentration 1,200 ppm) in a greenhouse for five months at 23°C and 70% humidity. For ^{15}N labeling the

mesocosms were irrigated daily with a Hoagland-based nutrient solution containing 0.1 mM $^{15}NO_3{}^{15}NH_4$ and 0.6 mM $CaCl_2$, 0.4 mM $MgSO_4$, 0.01 mM $FeCl_3$, 0.4 mM K_3PO_4, 1.8 µM $MnSO_4$, 0.064 µM CuCl, 0.15 µM $ZnCl_2$, 0.1 µM MoO_3, 0.01 mM H_3BO_3 and 5 mM NO_3NH_4 (Euriso-top, Saint-Aubin, Essonne, France). The soil was moistened at regular intervals by adding tap water.

6.2.2 Sampling of soil, litter, plant and ectomycorrhiza

At the end of the experiment the soil was divided into two horizons, 0-10 cm (A1 horizon including litter) and 10-25 cm (A2 horizon). Aliquots of soil material for stable isotope analyses were collected from the A1 horizon, dried and stored in plastic bags until analysis. From the litter layer and A1 horizon large soil animals were picked by hand. From the A1 and A2 layer roots were washed, divided in coarse (> 2 mm) and fine roots (< 2 mm), dried (48 h, 70°C) and weighed. Aliquots of the litter were taken, dried and stored in plastic bags until stable isotope analysis. Root caps of beech with EMF were collected and twenty samples were analyzed for stable isotope ratios.

6.2.3 Sampling of soil animals

Animals of the litter and A1 layer were extracted by heat using a high-gradient canister method effectively extracting mobile soil animals such as arthropods and (non-dormant) earthworms (Kempson et al., 1963). Soil animals were transferred into 70% ethanol and sorted to groups. Individuals were counted and determined to family, genus or species level (see Appendix). Based on natural variations in stable isotope ratios ($^{15}N/^{14}N$), feeding experiments, analyses of fatty acids and gut content analyses soil animal species were classified into primary decomposers, secondary decomposers and predators (see Appendix). Primary decomposers included twelve species i.e., *Octolasion tyrtaeum* (Lumbricidae), two species of Diplopoda and nine taxa of Oribatida. Secondary decomposers comprised 15 taxa, i.e., four taxa of Lumbricidae, four taxa of Isopoda, *Craspedosoma* sp. (Diplopoda), four taxa of Oribatida, and *Sinella*/*Pseudosinella* spp. and

Lepidocyrtus cyaneus (Collembola). Fifteen soil arthropod taxa were classified as predators including *Neobisium carcinoides* (Pseudoscorpionida), six taxa of Chilopoda, three taxa of Araneida, three taxa of Opilionida and *Acrogalumna longipluma* and *Hypochthonius rufuls* (Oribatida).

6.2.4 Stable isotope analyses

Dry plant tissues of leaves, stems, coarse roots and fine roots, aliquots of litter and non-rhizosphere soil of the A1 horizon were dried and milled with a ball mill (Type MM 2, Retsch, Haan, Germany), dried again at 70°C for 24 h and kept in a desiccator until analysis. Aliquots of the samples and of EM root tips (ca. 1 mg) were weighed into tin capsules for stable isotope analysis ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). For stable isotope analyses of soil animals, individual or bulked specimens corresponding to a minimum of 5 µg N were used. Large species were dried, fragmented mechanically and a subsample was analyzed. The capsules were dried at 60°C for 24 h and stored in a desiccator prior to the analysis.

Stable isotope ratios were analyzed with a coupled system consisting of an elemental analyzer (NA 1500, Carlo Erba, Mailand) and a mass spectrometer (MAT 251, Finnigan, Bremen, Germany). Abundances of ^{13}C and ^{15}N are expressed using the δ notation with δ_{sample} [‰] = [(R_{sample} - $R_{standard}$) / $R_{standard}$] x 1000; R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of samples and standard, respectively. For ^{13}C PD Belemnite (PBD) and for ^{15}N atmospheric nitrogen served as the primary standard. Acetanilide (C_8H_9NO, Merck, Darmstadt) was used for internal calibration.

6.2.5 Statistical analyses

Differences in $\delta^{13}C$ and $\delta^{15}N$ signatures of the three groups of soil animal taxa, i.e., primary decomposers, secondary decomposers and predators, were analyzed with single factor analysis of variance (ANOVA) with the general linear model (GLM) procedure using SAS 9.13 (SAS Institute, Cary, NC, USA). Homogeneity of variances was inspected using Levene test. For post-hoc comparison of means, Scheffé test was used. Differences in $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of soil animals

between beech and ash trees were tested with single factor ANOVA. As data from beech and ash generally did not differ significantly animal taxa of the two tree species were pooled. Data given in text and figures represent means and standard errors.

6.3 Results

6.3.1 Soil, plants and ectomycorrhiza

$\delta^{13}C$ values in litter and soil (-20.1 ± 2.2 and -23.1 ± 0.5‰, respectively) were slightly increased compared to natural variations in the field (respective values of -26.8 ± 0.1 and -27.8 ± 0.2‰). In contrast, $\delta^{15}N$ values of litter and soil (744.1 ± 164.8 and 230.3 ± 38.6‰, respectively) were markedly increased compared to natural variations (respective values of -1.8 ± 1.7 and -0.2 ± 0.3‰). In saplings both $\delta^{13}C$ and $\delta^{15}N$ signatures were markedly increased with $\delta^{13}C$ and $\delta^{15}N$ signatures increasing from stems (41.3 ± 3.6‰ and 5,434 ± 327.8‰, respectively) to leaves (80.4 ± 8.2‰ and 3,506 ± 322‰) to coarse roots (48.0 ± 6.6‰ and 6,152 ± 676.8‰) to fine roots (113.3 ± 7.7‰ and 9,328 ± 1,066‰).

On average 96.0 ± 3.5‰ of vital root tips of beech were colonized by EMF. $\delta^{13}C$ and $\delta^{15}N$ values averaged 114.6 ± 8.2‰ and 13,484 ± 1,929‰, respectively (Fig. 6.1).

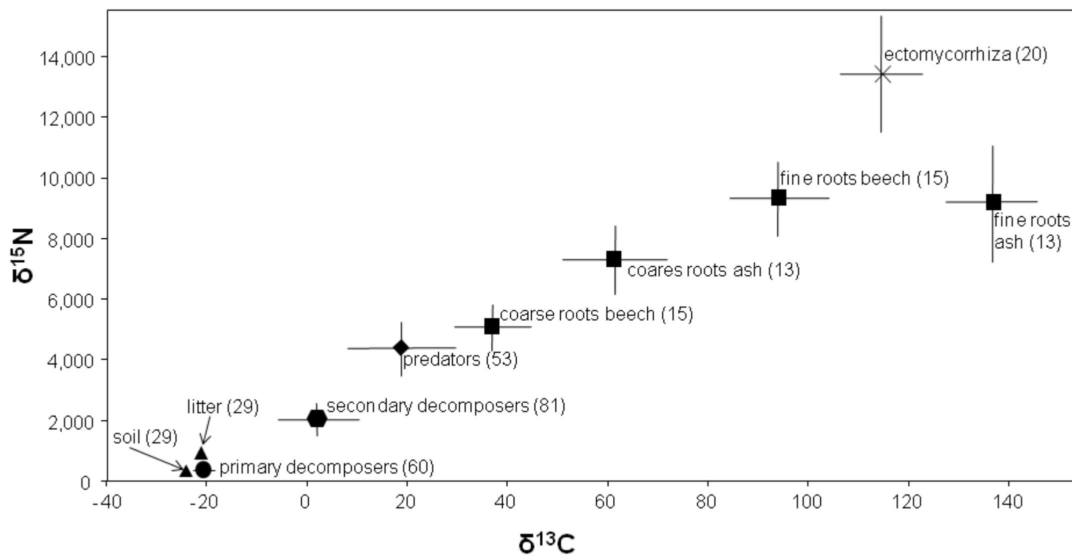

Fig. 6.1 Mean (± standard error) $\delta^{13}C$ and $\delta^{15}N$ value of primary decomposers (circle), secondary decomposers (hexagon) and predators (diamond). Means (± standard error) of $\delta^{13}C$ and $\delta^{15}N$ signatures of the soil and leaf litter (triangles) and coarse roots and fine roots of *Fagus sylvatica* and *Fraxinus excelsior* (squares) and of ectomycorrhiza (cross). Numbers of replicates are given in brackets.

6.3.2 Soil animals

In total 42 taxa of soil animals were analyzed (see Appendix). The overall mean $\delta^{13}C$ and $\delta^{15}N$ signatures of soil arthropods were -0.2 ± 6.6‰ and 2,282 ± 507.5‰, respectively, markedly exceeding those of the soil and litter layer, but being lower than those of plant roots and in particular EMF (Fig. 6.1). Notably this was true for each of the three trophic groups including predators with the highest stable isotope signatures. $\delta^{13}C$ and $\delta^{15}N$ signatures spanned from for *Eupelops plicatus* (-28.0 ± 0.7‰ and 82.9 ± 57.8‰, respectively) to *Clubiona compta* (76.0 ± 11.7‰ and 13,673 ± 1,522‰).

Primary decomposers included 12 taxa with mean $\delta^{13}C$ and $\delta^{15}N$ values of -21.6 ± 4.8‰ and 666.8 ± 584.9‰ (Appendix, Fig. 6.2). $\delta^{13}C$ and $\delta^{15}N$ values ranged from *E. plicatus* (-28.0 ± 0.7‰ and 82.9 ± 57.8‰, respectively) to *Glomeris undulata* (-11.2 ± 3.7‰ and 1,928 ± 465.1‰) (Fig. 6.2).

Secondary decomposers included 15 taxa with mean $\delta^{13}C$ and $\delta^{15}N$ values of 2.2 ± 8.0‰ and 2,105 ± 504.8‰, respectively. $\delta^{13}C$ and $\delta^{15}N$ values were lowest in *Nothrus palustris* with -23.8 ± 1.5‰ and 951.8 ± 285.4‰, respectively, and highest in *Craspedosoma* sp. with respective values of 38.8‰ and 7,383‰ (both single measurements; Fig. 6.2).

Predators included 15 taxa with mean $\delta^{13}C$ and $\delta^{15}N$ values of 18.8 ± 10.8‰ and 4,443 ± 876.8‰, respectively, differing significantly from respective values of primary and secondary decomposers ($F_{2,38} = 35.47$, $P < 0.0001$ and $F_{2,38} = 17.36$, $P < 0.0001$, respectively). $\delta^{13}C$ and $\delta^{15}N$ values were lowest in *Necrophloeophagus longicornis* with -9.2 ± 3.6‰ and 1,331 ± 391.3‰, respectively, and highest in *C. compta* with respective values of 76.0 ± 11.7‰ and 13,673 ± 1,522‰.

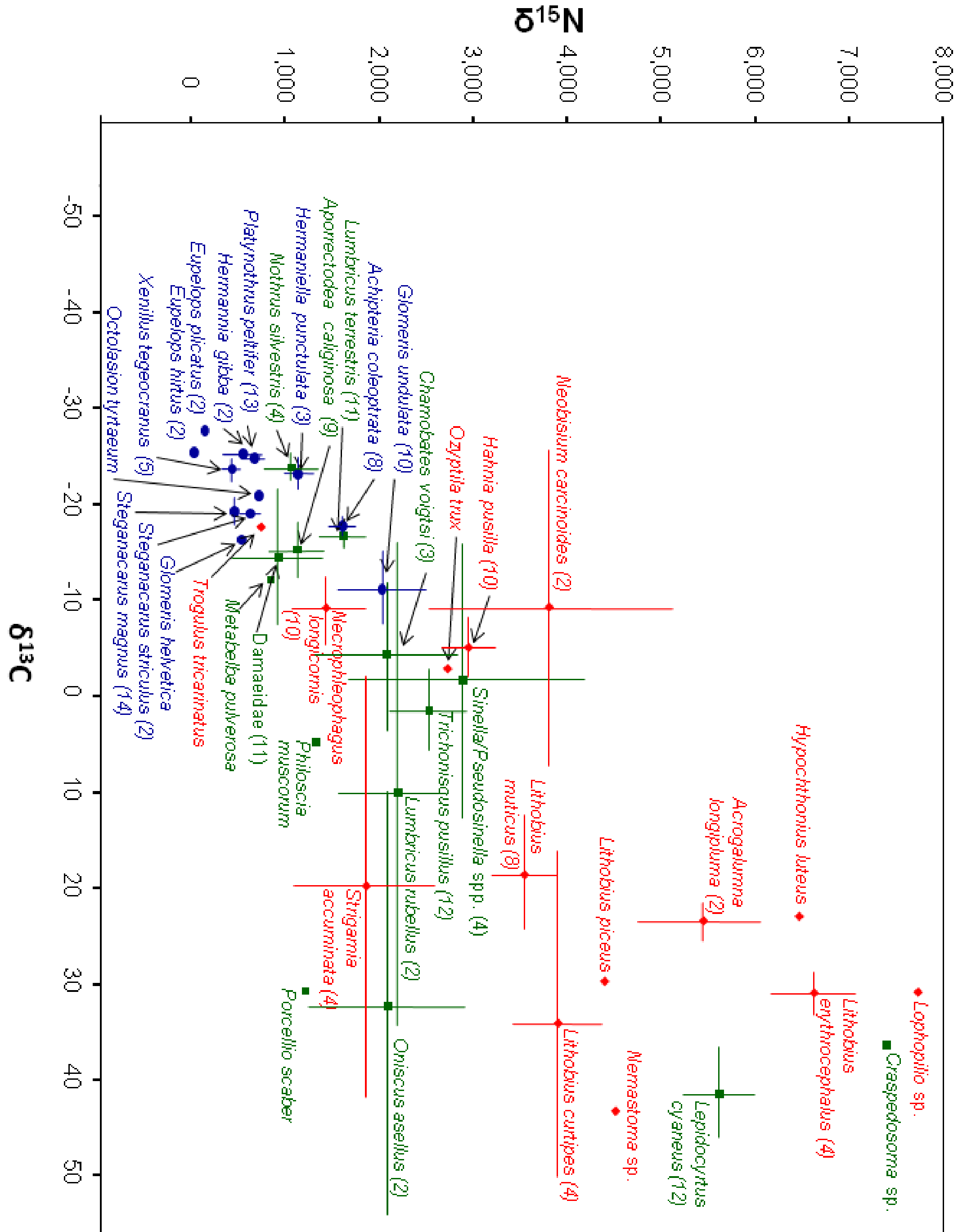

Fig. 6.2 Mean (± standard error) δ^{13}C and δ^{15}N signatures of primary decomposers (blue circles), secondary decomposers (green squares) and predators (red diamonds) (for details see Appendix). δ^{13}C and δ^{15}N values of *Clubiona compta* were 76.0 ± 11.7‰ and 13,673 ± 1,522‰, respectively (not shown). Numbers of replicates are given in brackets. Dots without standard error represent single measurements.

6.4 Discussion

The main objective of this study was to follow the flux of plant carbon and soil mineral nitrogen into the soil animal food web of temperate forests. Therefore, we labeled ash and beech tree saplings with $^{13}CO_2$ and added $^{15}NO_3{}^{15}NH_4$ to their rhizosphere. Ash and beech saplings were used for investigating the food web in the rhizosphere of plants colonized by EMF (beech) as compared to AMF (ash). The plants assimilated the $^{13}CO_2$, translocated the label to roots and in beech transferred it to EMF but little ^{13}C was transferred into soil and litter. Mineral nitrogen ($^{15}NO_3{}^{15}NH_4$) added to soil was transported via mycorrhizal fungi to plant roots as indicated by the signature of EMF exceeding that of beech fine roots. Similar to plant carbon, mineral nitrogen was only little incorporated into the soil but to some extent into litter probably by unspecific soaking during irrigation but $\delta^{15}N$ values in fine roots exceeded those in litter by more than a factor of twelve indicating that $^{15}NO_3{}^{15}NH_4$ was primarily assimilated by mycorrhizal fungi and transported to plant roots rather than immobilized by saprotrophic microorganisms and incorporated into litter (Lummer et al., 2012). Incorporation of label into higher trophic levels therefore likely was mainly via animals feeding on roots and/or AMF or EMF. However, in part $^{15}NO_3{}^{15}NH_4$ may also have been assimilated by algae potentially contributing to increased litter $\delta^{15}N$ signatures.

In contrast to our expectations, $\delta^{13}C$ and $\delta^{15}N$ signatures of soil animal species did not differ significantly between beech and ash treatments. The similar stable isotope signatures of soil animal species suggest that morphological and structural differences between the EMF rhizosphere of beech and the AMF rhizosphere of ash little affected the incorporation of label into higher order consumers. Potentially, stronger incorporation of label into soil animals via EMF in beech treatments was compensated by stronger transfer of label into soil animals via rhizosphere bacteria in ash treatments (Cesarz et al., 2013).

6.4.1 Primary decomposers

As expected, plant C and microbial N were little incorporated into primary decomposers supporting the assumption that they almost exclusively rely on litter and soil organic matter resources rather than root derived C and microbial N. This is consistent with findings of Pollierer et al. (2007) who also suggested that *Steganacarus magnus* and *Glomeris* sp. function as primary decomposers. However, primary decomposers were not trophically uniform. Rather, they formed a gradient of taxa that incorporated virtually no plant C and microbial N [*E. hirtus*, *E. plicatus*, *S. magnus, S. striculus*, *Platynothrus peltifer*, *Hermannia gibba*, *Xenillus tegeocranus* (all Oribatid mites), *Glomeris helvetica* (Diplopoda) and *O. tyrtaeum* (Lumbricidae)] to those also incorporating plant C and microbial N [*Hermaniella punctulata*, *Achipteria coleoptrata* (both Oribatid mites) and *Glomeris undulata* (Diplopoda)]. Presumably, in addition to dead organic matter the latter species to some extent also digested microorganisms that colonized these resources.

6.4.2 Secondary decomposers

Secondary decomposers incorporated more ^{13}C and ^{15}N than primary decomposers supporting the hypothesis that secondary decomposers essentially rely on plant C and microbial N. However, ^{15}N and ^{13}C signatures of some secondary decomposer species overlapped with those of primary decomposers reflecting that in fact decomposer soil invertebrates form a gradient from species exclusively incorporating litter C to those exclusively feeding on microorganisms (Scheu and Falca, 2000). In fact, species rich taxa previously assumed to predominantly feed on fungi, such as Collembola and Oribatida, have been shown to partition resources ranging from plant litter to microorganisms to even higher order animal consumers (Schneider et al., 2004; Chahartaghi et al., 2005). In the present study, secondary decomposers of the lower end of this gradient included Damaeidae, *M. pulverosa*, *N. palustris* (Oribatida), *Aporrectodea caliginosa*, *Lumbricus terrestris* (Lumbricidae), *Philoscia muscorum* and *Porcellio scaber* (Isopoda) whereas those

at the higher end included *Chamobates voigtsi* (Oribatida), *Sinella/Pseudosinella* spp. (Collembola), *Lumbricus rubellus* (Lumbricidae), *Trichonicus pusillus* and *Oniscus assellus* (Isopoda). δ^{15}N signatures of two secondary decomposers, i.e., *L. cyaneus* (Collembola) and *Craspedosoma* sp. (Diplopoda) were exceptionally high pointing to specific food resources. *L. cyaneus* is known to feed on algae (Scheunemann et al., 2010) and this may explain its high signature as algae on litter presumably directly incorporated ^{13}C and ^{15}N from the labeled atmospheric CO_2 and NH_4NO_3 in irrigation water. Unfortunately, measuring stable isotope signatures of algae growing on leaf litter is virtually impossible. For high stable isotope signatures of Craspedosomatidae the same as for *L. cyaneus* may apply. Notably, ^{13}C and ^{15}N signatures of secondary decomposers were considerably lower than those of EMF or roots indicating that none of them exclusively fed on mycorrhizal fungi; rather, the data suggest that they fed on a combined diet of mycorrhizal and saprotrophic fungi.

6.4.3 Predators

Contrary to our expectations, predators incorporated the highest amount of ^{13}C and ^{15}N. We hypothesized that predators predominantly feed on secondary decomposers, such as Collembola and Isopoda, as suggested earlier (Scheu, 2002). In part this hypothesis is supported as δ^{15}N and δ^{13}C signatures of e.g., *N. carcinoides* (Pseudoscorpionida), *Hahnia pusilla* and *Ozyptila trux* (both Araneida) were similar to secondary decomposers, indicating that these predators predominantly feed on secondary decomposers such as *Sinella/Pseudosinella* spp. (Collembola), *T. pusillus* (Isopoda) and *C. voigtsi* (Oribatida). However, both ^{15}N and ^{13}C signatures of most predator taxa, including *Lithobius muticus*, *Lithobius curtipes*, *L. piceus*, *L. erythrocephalus* (all Chilopoda), *Lophopilio* sp., *Nemastoma* sp. (Opilionida) *Hypochthonius luteus* and *Acrogalumna longipluma* (Oribatida), considerably exceeded that of the great majority of secondary decomposers indicating that they fed on higher labeled prey species such as the two highly labeled secondary decomposers *L. cyaneus* (Collembola) and *Craspedosoma* sp.

(Diplopoda) and potentially other species not measured in this study, such as small Collembola, Nematoda and Enchytraeidae. Lithobiidae predominantly hunt in the litter layer (Poser, 1990) which is colonized by epigeic Collembola such as *L. cyaneus*. High $\delta^{13}C$ and $\delta^{15}N$ signatures of *Lophopilio* sp. and *Nemastoma* sp. presumably are related to the wide feeding strategies of many Opilionida including intraguild predation and cannibalism (Martens, 1978). Further, Lithobiidae and Opilionda likely also fed on as the highly labeled secondary decomposers *L. cyaneus* and *Craspedosoma* sp. The high $\delta^{13}C$ and $\delta^{15}N$ signatures of *H. luteus* and *A. longipluma* (Oribatida) likely are due to feeding on prey closely connected to the rhizosphere and the high label of roots. Hypochthoniidae are known to rely on belowground carbon and presumably predominantly prey on nematodes (Pollierer et al., 2012) and this also applies to Galumnidae (Rockett and Woodring, 1966; Muraoka and Ishibashi, 1976). Therefore, high $\delta^{13}C$ and $\delta^{15}N$ signatures of *H. luteus* and *A. longipluma* likely resulted from feeding on nematodes which either directly fed on roots or on mycorrhizal fungi. High stable isotope signatures in predators therefore presumably resulted from incorporation of the label via two different pathways, the one based on algae and algal feeders the other based on root derived resources and associated nematodes. Potentially, the first pathway was more pronounced as in the field since the canopies of the tree seedlings were rather open thereby allowing more light entering the soil surface resulting in more pronounced algal growth.

Two predator taxa, *N. longicornis* and *Strigamia accuminata* (both Geophilomorpha), had rather low $\delta^{15}N$ signatures indicating that they fed on prey with low $\delta^{15}N$ signature, potentially a mixture of Lumbricidae and Isopoda. Indeed, Geophilomorpha are known to hunt for Lumbricidae by following them in large soil pores (Poser, 1990; Wolters and Ekschmitt, 1997). Low $\delta^{15}N$ signatures of *S. accuminata* may also be related to feeding on earthworms; however, high $\delta^{13}C$ signatures exceeding those of Lumbricidae suggest that they included also other prey, potentially Isopoda such as *O. asellus* and *P. scaber*.

6.5 Conclusions

Results of this study showed that primary and secondary decomposers comprise a gradient of species relying to different degrees on root C and microbial N. High stable isotope incorporation into EMF and considerably lower signatures in soil animals suggest that the animal species studied do not exclusively feed on mycorrhizal fungi, but long-term studies exceeding the life span of the animals are needed to prove this assumption. Surprisingly, predators were most intensively labeled with plant C and root N. Presumably, this high label was due to both feeding on algal consumers, such as the Collembola species *L. cyaneus*, and on plant rhizosphere associated root or mycorrhiza feeding nematodes. The results indicate that predators in soil animal food webs rely on very different carbon resources including algae, roots and microorganisms which are channeled to higher trophic levels predominantly via Collembola, Nematoda and Lumbricidae. Notably, dominant predators of temperate forests such as Lithobiidae appear to predominantly prey on species of litter dwelling Collembola such as *L. cyaneus.*

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Appendix

Soil animal species studied as assorted to trophic groups (primary decomposers, secondary decomposers, predators).

Species	Taxonomic group	Trophic group	References
Achipteria coleoptrata	Oribatida	Primary decomposer	Scheider et al. (2004, 2005); Pollierer et al. (2009, 2012); Maraun et al. (2011)
Eupelops plicatus	Oribatida	Primary decomposer	Schneider et al. (2004)
Eupelops hirtus	Oribatida	Primary decomposer	Maraun et a. (2011)
Hermannia gibba	Oribatida	Primary decomposer	Schuster (1956); A´Bear et al. (2010), Maraun et al. (2011)
Hermaniella punctulata	Oribatida	Primary decomposer	-
Platynothrus peltifer	Oribatida	Primary decomposer	Scheider et al. (2004, 2005); Pollierer et al. (2009, 2012); Maraun et al. (2011); Heidemann et al. (2011)
Steganacarus magnus	Oribatida	Primary decomposer	Maraun and Scheu (2000); Scheider et al. (2004, 2005); Pollierer et al. (2009, 2012); A´Bear et al. (2010); Heidemann et al. (2011)
Steganacarus striculus	Oribatida	Primary decomposer	Scheider et al. (2004)
Xenillus tegeocranus	Oribatida	Primary decomposer	-
Glomeris helvetica	Diplopoda	Primary decomposer	Scheu and Falca (2000)
Glomeris undulata	Diplopoda	Primary decomposer	Pollierer et al. (2009); Oelbermann and Scheu (2010); Semenyuk and Tiunov (2011)
Octolasion tyrtaeum	Lumbricidae	Primary decomposer	Marhan and Scheu (2005); Butenschön et al. (2007); Curry and Schmidt (2007); Scheunemann et al. (2010)
Chamobates voigtsi	Oribatida	Secondary decomposer	Riha (1951); Schuster (1956); Luxton (1972), Kaneko (1988); Schneider et al. (2004); Maraun et al. (2011)

Damaeidae	Oribatida	Secondary decomposer	Maraun et al. (1998); Schneider et al. (2004)
Metabelba pulverosa	Oribatida	Secondary decomposer	Schneider et al. (2004)
Nothrus silvestris	Oribatida	Secondary decomposer	Schneider et al. (2004, 2005)
Lepidocyrtus cyaneus	Collembola	Secondary decomposer	Chaharthaghi et al. (2005); Scheunemann et al. (2010); Crotty et al. (2011); Pollierer et al. (2012)
Sinella/Pseudo-sinella spp.	Collembola	Secondary decomposer	Scheunemann et al. (2010); Crotty et al. (2011)
Craspedosoma sp.	Diplopoda	Secondary decomposer	-
Trichoniscus pusillus	Isopoda	Secondary decomposer	Kautz et al. (2000); Scheu and Falca (2000); Pollierer et al. (2012)
Philoscia muscorum	Isopoda	Secondary decomposer	Scheu and Falca (2000)
Porcellio scaber	Isopoda	Secondary decomposer	Ihnen and Zimmer (2008); Crowther et al. (2011)
Oniscus assellus	Isopoda	Secondary decomposer	Oelbermann and Scheu (2010); Crowther et al. (2011)
Apporrectodea longa	Lumbricidae	Secondary decomposer	Curry and Schmidt (2007); Pollierer et al. (2009, 2012)
Lumbricus terrestris	Lumbricidae	Secondary decomposer	Gunn and Cherret (1983); Bonkowski et al. (2000), Curry and Schmidt (2007), Pollierer et al. (2009, 2012); Scheunemann et al. (2010)
Aporrectodea caliginosa	Lumbricidae	Secondary decomposer	Bonkowski et al. (2000), Scheu and Falca (2000)
Aporrectodea rosea	Lumbricidae	Secondary decomposer	Bonkowski et al. (2000); Pollierer et al. (2009)
Lumbricus rubellus	Lumbricidae	Secondary decomposer	Bonkowski et al. (2000)
Acrogalumna longipulma	Oribatida	Predator	Rockett and Woodring (1966); Rockett (1980); Wunderle (1992); Schneider et al. (2004)

Hypochthonius luteus	Oribatida	Predator	Riha (1951); Schneider et al. (2004); Ruf et al. (2006); Pollierer et al. (2009, 2012); Maraun et al. (2011), Heidemann et al. (2011)
Hahnia pusilla	Araneida	Predator	Scheu and Falca (2000)
Ozyptila trux	Araneida	Predator	Bellmann (2001)
Clubiona compta	Araneida	Predator	Bellmann (2001)
Necrophleo-phagus longicornis	Geophilo-morpha	Predator	Poser (1988), Poser (1990), Ferlain (2012)
Strigamia accuminata	Geophilo-morpha	Predator	Poser et al. (1990), Wolters and Eckschmitt, (1997), Ferlian et al. (2012)
Lithobius muticus	Lithobio-morpha	Predator	Poser (1988), Scheu and Falca (2000), Pollierer et al. (2009, 2010)
Lithobius piceus	Lithobio-morpha	Predator	Poser (1988), Scheu and Falca (2000), Pollierer et al. (2009, 2010)
Lithobius erythro-cephalus	Lithobio-morpha	Predator	Poser (1988), Scheu and Falca (2000), Pollierer et al. (2009, 2010)
Lithobius curtipes	Lithobio-morpha	Predator	Poser (1988), Scheu and Falca (2000), Pollierer et al. (2009, 2010)
Trogulus tricarinatus	Opilionida	Predator	Martens et al. (1978)
Lophopilio sp.	Opilionida	Predator	Oelbermann and Scheu (2010)
Nemastoma sp.	Opilionida	Predator	Martens et al. (1978)
Neobisium carcinoides	Pseudoscor-pionida	Predator	Scheu and Falca (2000), Oelbermann and Scheu (2010), Pollierer et al. (2012)

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CHAPTER

7

Synopsis

7. Synopsis

The previous chapters described the morphology and functioning of beech (*Fagus sylvatica* L.) and ash (*Fraxinus excelsior* L.) fine roots, their competitive interactions and their influence on the rhizosphere and trace gas fluxes analysed in greenhouse experiments with tree saplings. Intra- and interspecific competition effects were investigated with rhizoboxes (chapters 2 and 3). The species-specific effects of beech and ash on the rhizosphere and greenhouse gas (GHG) fluxes were observed in a rhizotron experiment (chapters 4 and 5). With beech and ash saplings in intact soil cores, we analysed the transfer of carbon and nitrogen into plant compartments and soil biota in a long-term isotopic labelling experiment (chapter 6). The following chapter sections integrate the information from our five studies about fine roots and the rhizosphere and put it into a wider context of belowground dynamics and the putative consequences for forest management and carbon budgeting of ecosystems.

7.1 Competition influences fine root survival

Numerous studies have investigated effects of environmental factors on fine root morphology, growth and longevity (e.g. Gaul et al. 2008; Meier & Leuschner 2008; Brunner et al. 2009; Mainiero et al. 2009), but competition among woody plants has been mainly studied in biomass-related experiments in the field, primarily by the comparison of changes in standing fine root biomass or fine root morphology from mixed forests to monocultures. Thus, the direct impacts of belowground competition on fine root traits and survival are not well understood.

In the chapters 2 and 3 of this thesis we described how root interactions, and especially belowground competition can act as structuring force of key functional fine root traits. For beech and ash, fine root survival was significantly affected by intra- and interspecific competition, while fine root morphology and nitrogen content were not influenced. The latter is partly contradicting earlier results by Bolte and Villanueva (2006) in mature forests with beech and spruce, where beech increased its specific fine root length and specific fine root area in

mixed stands compared to pure beech stands. This was described as a flexible 'foraging' strategy of beech in order to enlarge the soil exploitation and thus the water and nutrient uptake capacity. Our experiment with beech and ash saplings did not find such flexible strategies in altering morphological traits. Hence, adaptive root responses to competition may not be a universal phenomenon and can vary with species identity and plant age.

In contrast, significant differences in survival rates in dependence on intra- and interspecific competition were found. Mean root lifespan differed significantly among species (higher longevity of ash fine roots in comparison to beech roots) and also between the competition treatments. The risk of fine root mortality increased when beech roots grew in mixture with ash or in beech monoculture as compared to beech plants growing in isolation. On the contrary, ash fine roots had a lower mortality in mixture with beech than when grown in isolation. Thus, ash fine roots apparently profit from the presence of beech roots while beech root growth and survival are negatively affected by ash. In addition, competition indices indicated asymmetric competition in favour of ash. These findings support the results on the belowground superiority of juvenile ash over beech plants (Rust & Savill 2000; Saxe & Kerstiens 2005).

Norby and Jackson (2000) stated that a key constraint to quantify terrestrial carbon cycling and to predict the impacts of global environmental change is the limited knowledge about fine root turnover, i.e. the growth and decay of fine roots over a certain time period. In the present study intra- and interspecific competition was identified as an important force that influences fine root lifespan. In conclusion, for predicting forest ecosystem carbon sequestration we have to consider not only site-specific and climatic conditions, but also competition effects, as these can have significant effects on fine root longevity.

7.2 Species-specific fine root traits related to root order

Owing to the complexity and heterogeneity of the fine root system, the species-specific fine root traits are difficult to assess. We analysed morphological and chemical fine root traits in regard to the position in the root system, i.e. root

branching order, which was recently shown to be a helpful tool to investigate the root system and species-specific traits in detail (Pregitzer et al. 1997).

In our study root order was found to be the most influential factor affecting the studied fine root traits (except for $\delta^{13}C$); while a significant species identity effect was found for tissue density, root diameter, N concentration and $\delta^{13}C$. Beech and ash fine root systems were similar with respect to the distribution of root length from 1st to 4th root order class, with 65-70% of total root length of the analysed root segments being contributed by 1st order roots. Differences were shown for specific fine root length (SRL) and specific fine root area (SRA), with higher values in beech throughout the root orders and a decrease from the root tips to higher root orders for both species. Guo et al. (2004) assumed that the 1st and 2nd order roots may be disproportionately important in carbon and nitrogen fluxes at ecosystem scale due to their large proportions of fine root biomass, high N concentrations, high turnover, and potentially high decomposition rates. This is also supported by our results with significantly highest N concentration and a high SRL in the 1st order roots in beech and ash. Both the high N concentrations and the high SRL suggest high respiration rates of the first order roots (Reich et al. 1998). Thus, information about species-specific root order traits is especially important for the understanding of belowground dynamics in regard to nutrient and carbon cycles.

7.3 Species-specific effects on the rhizosphere

The root-soil interface is the region where most interactions between the plant and its environment occur (Waisel et al. 2002). Beech and ash showed significant differences in their influence on the microbial soil community and the trace gas fluxes between soil and atmosphere. Species-specific effects were observed on soil microorganisms and total soil carbon content. The fine roots of beech changed carbon dynamics in soil by reducing soil pH and decreasing the carbon use efficiency of bacteria. In the presence of ash more litter-derived ^{13}C was channelled into higher trophic levels. In addition, ash fine roots were significantly enriched in ^{15}N, indicating that they recycled the ^{15}N labeled leaf-litter more

effectively than beech fine roots. Higher uptake rates of ^{15}N in ash fine roots in comparison to beech were also found in a ^{15}N tracer experiment in the Hainich forest, where ash fine roots took up significantly more NH_4^+ and glycine than beech (Jacob, unpubl.).

The CH_4 uptake of the soil planted with ash was higher and the N_2O emissions were lower than from soil under beech. A general relation to the fine root biomass was observed. In contrast, the CO_2 efflux was much higher in beech than in ash, although root biomass was smaller than that of ash. Qualitative effects of beech and ash roots were likely related to the differences in the composition and concentration of organic acids exuded from the fine roots.

In our labelling experiment with beech and ash in intact soil cores the litter and soil were only minimally enriched in ^{15}N and ^{13}C, while fine roots of beech and ash were highly enriched. Maximum stable isotope values were observed in the ectomycorrhiza of beech, which supports the findings of Högberg et al. (2008) and Cairney et al. (2012) that the transfer of assimilated carbon from the plant to the rhizosphere is more effective in the widely dispersed extramatrical mycelium of ectomycorrhizal fungi than in arbuscular-mycorrhiza, which forms no such intensive extramatrical mycelia (Smith and Read 1997). Nevertheless, the isotopic signature of soil animals did not significantly vary between beech and ash, contradicting our hypothesis that fungal feeders prefer ectomycorrhiza. In summary, beech and ash vary considerably in their belowground performance and their influence on rhizosphere and soil functioning, which should be taken into account in biodiversity studies in the field.

7.4 Concluding remarks

The present work gave detailed information on the species-specific dynamics in the root system and rhizosphere of *Fagus sylvatica* L. and *Fraxinus excelsior* L. and their interactions. The large differences of these species in their response to competition as well as their rhizosphere effects call for more investigations in the field and for more species-specific knowledge of other tree species; only then is the belowground functional role of each tree species in mixed-species forests

comprehensible. Supplementary studies on the effects on turnover, growth and loss of fine roots under various conditions will help to determine the carbon fluxes and pools in forests and will improve the information for global carbon budgeting. In conclusion, detailed research on the response of fine roots to different environmental factors, including competition and biodiversity, as well as species-specific root traits is highly pertinent to guarantee accurate carbon cycle models at ecosystems scale and thus give sound guidelines for a climate change conscious forestry and land use management.

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Declaration of the author's own contribution to manuscripts with multiple authors

Chapter 2. I analyzed and collected all data, wrote the manuscript and plotted all graphs and tables. All co-authors contributed to the final version of the manuscript.

Chapter 3. I analyzed and collected all data, wrote the manuscript and plotted all graphs and tables. Ann-Catrin Fender provided data on soil parameters. All co-authors contributed to the final version of the manuscript.

Chapter 4. This study was conducted in the framework of the Cluster of Excellence "Functional Biodiversity Research" (FBR) as a joint project of Ann-Catrin Fender (data on soil chemistry), Simone Cesarz (soil animals), Kerttu Valtanen (mycorrhiza data) and Birgit Pfeiffer (pyrosequencing). I took samples and analyzed data on plant material, and provided data on biomass and morphology. Simone Cesarz wrote the manuscript and all co-authors contributed to the idea development and the final version of the manuscript.

Chapter 5. This study was conducted in the framework of the Cluster of Excellence "Functional Biodiversity Research" (FBR) as a joint project of Ann-Catrin Fender (data on soil chemistry and gas fluxes), Kerttu Valtanen (mycorrhiza data) and me. I took samples and analyzed data on plant material, and delivered data on biomass, morphology and the depth distribution of roots. Sabine Fiedler provided data on organic acids in the rhizosphere. Ann-Catrin Fender wrote the manuscript and all co-authors contributed to the idea development and the final version of the manuscript.

Chapter 6. The study design was developed by Verena Eißfeller and me. It was a joint project in the framework of the Cluster of Excellence "Functional Biodiversity Research" (FBR) and the Graduate school 1086 "The Role of Biodiversity for Biogeochemical Cycles and Biotic Interactions in Temperate Deciduous Forests". Data collection was done by Verena Eißfeller, Kerttu Valtanen and me. I analyzed the plant related data. All co-authors contributed to the final version of the manuscript.

Eidesstattliche Erklärung

Hiermit versichere ich, die vorliegende Arbeit mit dem Titel "Fine root traits, belowground interactions and competition effects on the rhizosphere of *Fagus sylvatica* and *Fraxinus excelsior* saplings" selbstständig und unter ausschließlicher Verwendung der angegebenen Literatur, Verweise und Hilfsmittel erstellt zu haben. Verwendete Quellen wurden als solche gekennzeichnet.

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