# Effects of tree species composition on fine root biomass and dynamics in the rhizosphere of deciduous tree stands in the Hainich National Park (Thuringia, Germany)

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Andreas Jacob

aus Berlin

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Betreuungsausschuss:

Anleiter: Dr. Dietrich Hertel, Pflanzenökologie und Ökosystemforschung, Georg-August-Universität, Göttingen

Erstbetreuer: Professor Dr. Christoph Leuschner, Pflanzenökologie und Ökosystemforschung, Georg-August-Universität, Göttingen

Zweitbetreuer: Professor Dr. Dirk Hölscher, Waldbau und Waldökologie der Tropen, Georg-August-Universität, Göttingen

Mitglieder der Prüfungskommission:

Referent: Professor Dr. Christoph Leuschner, Pflanzenökologie und Ökosystemforschung, Georg-August-Universität, Göttingen

Korreferent: Professor Dr. Dirk Hölscher, Waldbau und Waldökologie der Tropen, Georg-August-Universität, Göttingen

weitere Mitglieder der Prüfungskommission:

Professor Dr. Hermann Behling, Palynologie und Klimadynamik, Georg-August-Universität, Göttingen

Professor Dr. Erwin Bergmeier, Vegetationsanalyse und Phytodiversität, Georg-August-Universität, Göttingen

Professor Dr. Gerhard Gerold, Landschaftsökologie, Georg-August-Universität, Göttingen

Professor Dr. Markus Hauck, Pflanzenökologie und Ökosystemforschung, Georg-August-Universität, Göttingen

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*Meiner Familie*

## Table of contents

## List of figures

## List of tables

## List of abbreviations and acronyms

| | |
|---|---|
| Al | aluminium |
| AM | arbuscular mycorrhiza |
| ANOVA | analysis of variance |
| a.s.l. | above sea level |
| $BaCl_2$ | barium chloride |
| C | carbon |
| Ca | calcium |
| $C_2H_5O_2N$ | glycine |
| dl | diversity level |
| d.w. | dry weight |
| ECM | ectomycorrhiza |
| Fe | ferric |
| FRP | fine root production |
| FRT | fine root turnover |
| GLM | general linear model |
| K | potassium |
| $K_2SO_4$ | potassium sulfate |
| Mg | magnesium |
| Mn | manganese |
| N | nitrogen |
| Na | sodium |
| n | quantity |
| $^{14}N$ | stable nitrogen isotope |
| $^{15}N$ | stable nitrogen isotope |
| $NH_4^+$ | ammonium |
| $NH_4Cl$ | ammonium chloride |
| $NO_3^-$ | nitrate |
| NPP | netto primary production |
| $O_f$ | decayed organic layer |
| $O_l$ | organic litter layer |
| p | presence of a species |
| SRA | specific fine root area |
| SRL | specific fine root length |
| $V_{max}$ | maximum uptake velocity |

## Summary

During the past two decades, much research has focused on understanding the role of biodiversity for ecosystem functioning and the provision of ecosystem goods and services for humanity. While several experiments with artificial grasslands and herbaceous communities provided clear evidence for a positive relationship between plant species diversity and aboveground productivity in species-rich communities less is known as to whether these results also apply to natural or near-natural communities. Only few data exist on the biodiversity-productivity relation in natural and semi-natural forests and confirmation for a positive diversity effect on aboveground productivity of such ecosystems is rather weak. Moreover, only little information on the diversity-productivity relationship is available so far with regard to the belowground compartment. Some recent belowground studies found a higher standing fine root biomass and productivity in species-rich compared to species-poor stands or monocultures of temperate trees, pointing forward to complementarity in soil space exploration and resource use of the root systems of coexisting tree species.

By using a replicated tree cluster approach with 100 small mature tree groups with variable tree species composition (all possible monospecific, 2-species and 3-species combinations of the five tree species *Acer pseudoplatanus, Carpinus betulus, Fagus sylvatica, Fraxinus excelsior,* and *Tilia cordata*) in Hainich National Park (Thuringia, Germany), this study attempts for the first time to separate possible tree species diversity and tree species identity effects on fine root dynamics with a focus on aspects of spatial distribution, root morphology and nitrogen uptake in a mixed old-growth broad-leaved forest. The tested main hypotheses were that (i) tree species identity has a larger effect on standing fine root biomass than tree species diversity, (ii) identity effects on tree fine root productivity are more important than a diversity effect, and (iii) the coexisting five tree species differ in their preference for specific nitrogen (N) forms.

First, I conducted a fine root inventory that included tree roots ≤2 mm in diameter and analysed the fine root bio- and necromass in all cluster plots differing in tree species diversity and tree species composition. I used a key to distinguish between the fine roots on species level by their morphological attributes. In order to proof the evidence of belowground overyielding in terms of fine root productivity, an ingrowth core study was carried out in the 100 cluster plots. In additional monospecific study plots of the five tree species, I investigated species-specific differences in the preference for $NH_4^+$, $NO_3^-$ and glycine uptake using a $^{15}N$ tracer experiment.

The results revealed no evidence of a positive diversity effect on standing fine root biomass and thus of overyielding in terms of fine root biomass. Fine root necromass decreased from 136 g $m^{-2}$ in the monospecific cluster plots to 118 g $m^{-2}$ in the 3-species plots. Instead, there was evidence for a significant species identity effect on fine root biomass. An up to 10-20% higher fine root biomass was recorded in 2-species cluster plots with the presence of *A. pseudoplatanus* and *F. sylvatica* than in cluster plots with presence of *C. betulus*. A 100% higher fine root biomass was found for monospecific cluster plots of *F. sylvatica* and *F. excelsior* in comparison to plots of *C. betulus*. Fine roots of *F. excelsior* generally tended to be over-represented in the 2- and 3-species mixed cluster plots compared to the respective monospecific plots pointing at apparent belowground competitive superiority of *F. excelsior* in this mixed forest.

Fine root productivity on plot level was not significantly different between monospecific, 2- and 3-species cluster plots and ranged from 97 to 139 g $m^{-2}$ $yr^{-1}$ while fine root turnover increased from 0.39 $yr^{-1}$ in the 1-species plots to 0.64 and 0.56 $yr^{-1}$ in the 2- and 3-species plots (difference signifficant at $P < 0.1$). On the species level, large differences in the mean fine root growth rate were found among the five tree species in the monospecific cluster plots. Hence, *T. cordata* showed an up to five times higher fine root growth rate than *C. betulus* and about two times higher rates than *F. sylvatica* in the respective monospecific plots. Comparing the species-specific root growth rate in monospecific with the mixed-species cluster plots revealed a higher productivity in mixtures for *F. excelsior*, *A. pseudoplatanus* and *T. cordata*, but lower values for *C. betulus*. Fine root turnover was similar for the five species in the monospecific plots and tended to be higher for *F. excelsior*, *F. sylvatica* and *A. pseudoplatanus* in the mixed-species than monospecific plots. The presence of *F. excelsior* significantly influenced fine root productivity and turnover with accelerated root turnover in species-richer plots.

Apparent root nitrogen uptake rates of the five tree species were in the range of 5-46 µg N $g^{-1}$ root $h^{-1}$ for $NH_4^+$, 6-86 µg N $g^{-1}$ root $h^{-1}$ for $NO_3^-$ and 4-29 µg N $g^{-1}$ root $h^{-1}$ for glycine during the first hour after tracer application. *C. betulus, T. cordata* and *A. pseudoplatanus* seemed to prefer $NH_4^+$ over $NO_3^-$, while *F. excelsior* showed equal preference for both inorganic N forms and *F. sylvatica* apparently preferred $NO_3^-$.

This study found no evidence for spatial root system complementarity and belowground overyielding in the mixed stands of Hainich forest. Tree species identity effects on root productivity and turnover were much more important with a key role apparently played by *F. excelsior*.

# Chapter 1

## General introduction

## Biodiversity and ecosystem functioning

Biodiversity is the variation among species, habitats, functional groups and genetic and biological traits (Chapin III *et al.* 2000, Cardinale *et al.* 2012, Naeem *et al.* 2012). Today, an increasing threat to species and ecosystems is present at both global and local scale pictured by an alarming rate of species loss and ecosystem fragmentation, harvesting or land use intensification mainly caused by anthropogenic factors (Wardle *et al.* 2011).

Ecosystem functions can be described as ecological processes that control fluxes of energy, nutrients and organic matter through an environment (Cardinale *et al.* 2012). Examples for these ecological processes are primary plant production, carbon, nitrogen and water cycling and decomposition. Some of the ecosystem functions are directly controlled by biodiversity and are very essential for goods and services, which they provide to humanity (e.g. supply of food resources, carbon stocks).

Biodiversity or species richness must not necessarily stabilise or enhance ecosystem functioning (Loreau 1998). However, the majority of studies on biodiversity and ecosystem functioning found a positive relationship between species richness and productivity (e.g. Hector *et al.* 1999, Tilman 2001, Roscher *et al.* 2005, Balvanera *et al.* 2006, Cardinale *et al.* 2012). In case of a positive interaction between species diversity and ecosystem functioning and stability, this is due to two different types of effects: selection or complementarity (Loreau and Hector 2001, Morin *et al.* 2011). The selection or sampling effect comprises species-specific impacts on ecosystem-level processes, i.e. a higher probability of species-rich communities of including the most productive species in the assemblage (Aarssen 1997). In case of the complementarity effect, a higher productivity takes places through interspecific interactions (e.g. facilitation) or niche partitioning of the species present resulting in a more efficient use of available nutrients or water or soil space (Holmgren *et al.* 1997). Cardinale (2012) described a saturating effect of biodiversity on ecosystem processes when functionally redundant species can be lost with little or no impact on these processes. Only a few studies exists which report non-existent or even negative diversity-productivity relationships (e.g. Kahmen *et al.* 2005, Thompson *et al.* 2005, Grace *et al.* 2007, Rose and Leuschner 2012). Most of the cited experiments are related to natural and artificial communities (e.g. grasslands, herbaceous communities), but less is known about this relationship in forests.

## Biodiversity and forest ecosystem functioning

Forests are among the most productive terrestrial ecosystems and cover a total land area of c. 41.6 Miokm$^2$ with 42% in the tropics, 33% in the boreal and 25% in the temperate zone (Fischlin *et al.* 2007). One third of Germany's total area (i.e. c. 11.1 Mha) is stocked by forests with c. 40.1% and 57.6% being covered by deciduous and coniferous forests, respectively (Schmitz 2004). The most important climax tree species in Central Europe is beech (*Fagus sylvatica*). Germany is located in the centre of the European distribution area of beech forests and accounts for c. 26% of that area. However, at present only c. 5% of the country's land area is covered by beech forests (Bohn and Gollub 2007).

In addition to direct anthropogenic factors, forest ecosystems are highly vulnerable to climate/environmental change impacts such as droughts, floods, fires and insect pest outbreaks which may result in forest dieback (Fischlin *et al.* 2007). Due to increasing summer temperatures and declining precipitation, drought can be considered as a main stressor for European forests in future times. Drought increases tree mortality and can cause a reduced resilience against disturbances in forests (Chapin III *et al.* 2000). To avoid or reduce these threats in forest ecosystems, a change in forest management strategies has to emerge. Research on how biodiversity influences forest ecosystem functioning can help to optimize forest management and conservation under a changing climate.

While several authors reported a generally higher productivity of species-rich natural and near-natural forests or plantations than of monospecific stands in the boreal, temperate or tropical zone (e.g. Erskine *et al.* 2006, Potvin and Gotelli 2008, Lei *et al.* 2009, Pretzsch and Schütze 2009, Oelmann *et al.* 2010, Paquette and Messier 2011), others found no or negative relationships (e.g. Szwagrzyk and Gazda 2007, Jacob *et al.* 2010, Long and Shaw 2010, Unger *et al.* 2012). It is proposed that the diversity-productivity relationship in forests is influenced by environmental and site-specific conditions (e.g. water availability, soil fertility). It has been assumed that a positive diversity effect may only exist in artificial compositions of tree species with largely different functional traits or in natural forests of medium to low productivity (Jacob *et al.* 2013). Under conditions such as low temperature and infertile soils, interspecific interactions in terms of facilitation can result in increased productivity compared to the corresponding monocultures (Paquette and Messier 2011).

## Effects of tree species diversity and identity on belowground processes

Most of the biodiversity studies on the ecological relevance of tree species diversity focused on the aboveground tree compartment of forests while only few studies have considered diversity effects on the belowground compartment of temperate and tropical forests or plantations (e.g. Berrish and Ewel 1988; Cuevas *et al.* 1991; Hendriks and Bianchi 1995; Leuschner *et al.* 2001; Schmid 2002; Meinen *et al.* 2009a, b; Brassard *et al.* 2010). In most cases, the structure and productivity of the fine root system in two-species stands was compared with that of the monospecific stands. Mixed-species forests with more than three species were only exceptionally investigated (Berish and Ewel 1988; Meinen *et al.* 2009a, b).

Several authors found a higher fine root biomass in the mixtures than in monocultures (Berish and Ewel 1988, Cuevas *et al.* 1991, Hendriks and Bianchi 1995, Schmid 2002, Schmid and Kazda 2002), but there are also studies, which show no effect or even a decrease of fine root biomass in tree species mixtures (Morgan 1992, Hertel 1999, Bauhus *et al.* 2000, Leuschner *et al.* 2001). Vertical differences in the rooting patterns of different tree species may result in reduced interspecific belowground competition for resources and can explain a higher fine root biomass in mixed stands. Although, it remains still unclear whether this finding is caused by a true species diversity effect or due to a particularly large root biomass of one or more species, thus representing rather a species identity effect. Beside vertical stratification in the soil, complementarity in the use of belowground resources could also be achieved by species differences in the timing of resource uptake (Fitter 1986) or by utilising different chemical forms of nutrient elements (for example of N, von Felten *et al.* 2009). If different species (or functional types of mycorrhizae) were using different N forms, this could increase the total amount of N utilised by the community and thus might enhance productivity. Nitrogen partitioning in species-rich communities due to species differences in the vertical distribution of roots, the timing of uptake, and/or the preference of different N forms has been proven for arctic tundra communities and may also exist in synthetic temperate grasslands (e.g. McKane *et al.* 2002, Kahmen *et al.* 2006, von Felten *et al.* 2009). It is not known so far whether a significant N partitioning among temperate tree species exists in mixed stands, thereby facilitating their coexistence.

In the context of this doctoral thesis, I was able to distinguish fine roots of different tree species based on their root morphological traits, and it was possible to demonstrate for the first time that tree species diversity and tree species identity may separately affect tree fine root dynamics in a species-rich temperate old-growth forest in Hainich National Park (Thuringia, Germany). An approach with 100 small-

scale cluster plots representing all possible 25 combinations of five different tree species (fourfold replicated and differing in diversity levels from one to three species per plot) was used to analyse species-specific patterns of fine root abundance, productivity, and morphology.

## Study framework

Research Training Group (Graduiertenkolleg) 1086

This study was conducted as part of the Research Training Group (RTG) 1086 – '*The role of biodiversity for biogeochemical cycles and biotic interactions in temperate deciduous forests*' which is funded by 'Deutsche Forschungs-gemeinschaft (DFG)' for a period of nine years. The main focus of the program is to assess relationships between tree species diversity and ecosystem functioning (e.g. productivity, nutrient and water cycles, above- and belowground biotic interactions and ecosystem services) in an old-growth mixed forest of Hainich National Park. The first PhD student cohort (2005-2008) aimed to identify and quantify tree species diversity effects on biogeochemical cycles and interaction mechanism on the level of tree stands (based on study plots of 50x50 m size differing in tree species diversity from monospecific over 3-species to 5-species stands). Subsequently, the second cohort (2008-2011) intended to distinguish between tree species diversity and identity effects as well as interaction mechanisms on the level of small tree groups (based on tree cluster plots comprising all possible 1-, 2- and 3-species combinations of five selected tree species). The third cohort (2011-2014) will investigate tree species identity effects on the level of individual trees. About 14 PhD students within each cohort from the Faculties of Biology, Agricultural and Forest Sciences of the Georg-August University Göttingen, the Helmholtz Institute of Soil Ecology in Munich and the Max-Planck-Institute for Biogeochemistry in Jena are working together in three different project groups (A-Biodiversity analysis and biotic interactions, B-Turnover and C-Synthesis) all combined in this interdisciplinary program for young scientists.

My research work (sub-project B2) is embedded within the second cohort and related to the project group 'Turnover'. I focused on the analysis of different effects of different tree species compositions on fine root mass and dynamics in the rhizosphere of deciduous tree stands in the north-eastern part of Hainich National Park.

## Study site, species and design

Hainich National Park

All research work of the program was conducted in Hainich National Park in Thuringia, Central Germany. The Hainich forest is known as the largest unfragmented deciduous forest complex in Germany with an area of approx. 160 $km^2$ located in the western part of the Thuringian Basin. This mountain range reaches a length of 25 km from the southeast to the northwest with an altitude of 350 to 500 m above sea level (Nationalparkverwaltung Hainich 2012). In 1997, the semi-natural mixed forest stands in the south-eastern part of the Hainich gained the status of a national park (approx. 75 $km^2$) after long-term utilisation as military training ground combined with selective cutting regimes in former times. Additionally, parts of Hainich National Park were included in the ancient old-growth beech forests of the UNESCO-World Natural Heritage Sites in 2011. A remarkable diversity of species is accompanied by structural richness in that habitat. According to the current forest inventory, 5,576 species of invertebrates, 1,646 species of fungi, 813 species of vascular plants and ferns, 189 species of birds, 49 species of mammals and 18 species of amphibians and reptiles can be found in the national park (Nationalparkverwaltung Hainich 2012).

Both study sites of the second cohort of the RTG are located in the Lindig and Thiemsburg area (distance approx. 1.5 km) in the north-eastern part of the national park with an altitude of c. 350 m above sea level (Figure 1.1). The average annual temperature is 7.7°C and the average precipitation amounts 590 mm per year (data of the nearby weather station Weberstedt/ Hainich; period 1973-2004, Deutscher Wetterdienst 2005). The soil has been classified as Luvisol (IUSS 2007) developed from Triassic limestone and is covered by thick layers of loess (varying from 60 to 120 cm; Guckland *et al.* 2009). This soil type dominates the cluster plots. The soil shows stagnic properties in spring and winter and is drying out during summer. Due to differences in historic land ownership and management practises (coppice-with-standard system, selective cutting), the forest in this area forms a mosaic of at least 200-years-old stands differing in tree diversity, ranging from nearly monospecific beech stands to species-rich stands with up to 14 broad-leaved tree species per hectare (Leuschner *et al.* 2009). This closed forest area with an average tree height of 35 m and no larger canopy gaps has essentially been unmanaged for more than 40 years and therefore is of major interest for studies of old-growth temperate forests in Central Europe.

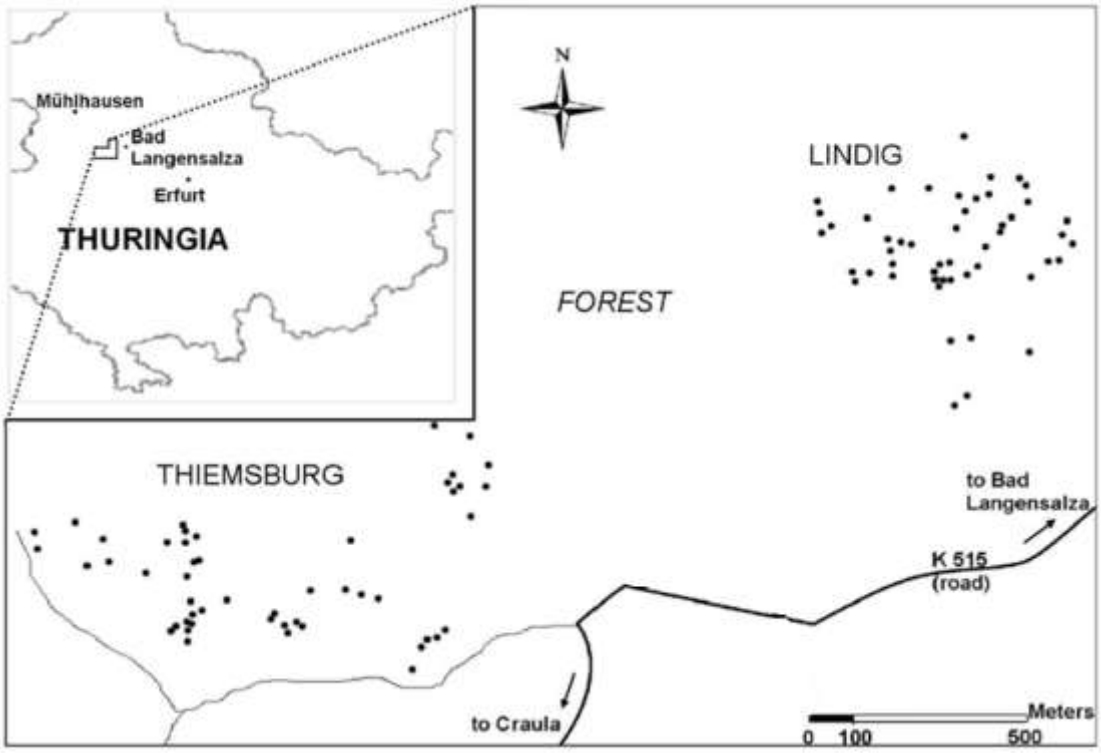


**Figure 1.1** Study area with the location of the 100 cluster plots (black dots) in the two forest regions Thiemsburg and Lindig in Hainich National Park, Thuringia (Germany) with both regions containing 50 clusters (Figure based on D. Seidel 2011).

Investigated tree species and their fine root system

Common ash (*Fraxinus excelsior* L.), European beech (*Fagus sylvatica* L.), hornbeam (*Carpinus betulus* L.), Small-leaved lime (*Tilia cordata* Mill.) and Sycamore maple (*Acer pseudoplatanus* L.) represent the five most abundant tree species in the north-eastern part of Hainich National Park. The focus of all research work within the RTG is mainly focused on these five deciduous tree species.

At the study site, fine roots (≤2 mm in diameter) of the five tree species are mainly located in the upper soil layer (<40 cm soil depth), where up to 85% of the fine roots can be found (Meinen *et al.* 2009a). In absence of vertical root system stratification, horizontal rooting patterns showed largely overlapping root systems (Meinen *et al.* 2009c, Jacob *et al.* 2013).

In the following, a short description and characterisation of each investigated tree species is given related to its fine root system and morphology.

A) Common ash (*Fraxinus excelsior* L.) belongs to the family of Oleaceae. This typical tree species of mixed hardwood alluvial forests occurs on a wide range of soil types, although it is mostly associated with basic soils on calcareous substrates. Due to the broad ecological amplitude of ash one can distinguish between so called 'chalk ash', which grows on drier shallower lime-rich soils and so called 'water ash' on more moist sites (Dobrowolska *et al.* 2008, Schütt *et al.* 2006). The fast-growing early- to mid-successional tree species has a relatively homogeneous and regular plate-root system (Rust and Savill 2000). The rooting pattern is even in horizontal direction with a concentration of the fine roots in the upper 20 cm soil layer (Korn 2004). Fine roots are beige to greyish-brown in colour, short and formed in bunches as well as typically infected with endomycorrhizae (e.g. *Glomus fasciculatus*, Roloff and Schütt 1994).

B) European beech (*Fagus sylvatica* L.) is a member of the family Fagaceae and a highly competitive tree species which often forms natural monospecific forest stands. This late-successional species is very variable in its ecological niche occupation and is also known as a climax tree species in Central European forests. Beech has a characteristic heart-root system (Rust and Savill 2000), which is very dense, and mostly concentrated close to the stem base (Korn 2004). Rooting structure, patterns and mycorrhization are depending on soil and nutrient conditions with a high sensitivity to changes in the rhizosphere. The proportion of fine roots is extremely high compared to other forest tree species (Köstler *et al.* 1968) and concentrated in the upper soil layers. Fine roots of beech are red to reddish-brown and have a rough surface structure. Root tips are numerous and infected by ectomycorrhizae (ECM) of many genera, e.g. *Lactarius* and *Russula*.

C) Hornbeam (*Carpinus betulus* L.) is a tree species of the family Betulaceae and known for its former high importance in the coppice-with-standards system in forest management. Hornbeam is a mid- to late-successional species with a deeper rooting heart-root system that is typically radially and uniformly structured (Korn 2004, Schütt *et al.* 2006). The fine root distribution is also very even; smaller diameter roots often insert directly at the main roots. Hornbeam fine roots are red to orange-red coloured and form a symbiosis with ECM-related fungi (e.g. *Inocybe corydalina*, Lang 2008).

D) Small-leaved lime (*Tilia cordata* Mill.) belongs to the family Tiliaceae and appears to be a strong competitor under dry conditions. Lime is similar to hornbeam a mid- to

late-successional tree species, but with an irregular, highly concentrated heart-root system (Korn 2004). The soil occupation by this species is less intense, but the fine root proportion and the probility to produce fine roots can be higher than in beech (Köstler *et al.* 1968). The colour of lime fine roots is varying from nearly black to reddish brown (Hölscher *et al.* 2002) and the root tips are typically infected by ECM fungi (e.g. *Inocybe geophylla*, Lang 2008).

E) Sycamore maple (*Acer pseudoplatanus* L.) is a species of the family Aceraceae and often dominant on sites where beech is underrepresented or absent (Ellenberg and Leuschner 2010). Maple prefers moist soils and increasing light availabilities with growing age. This mid- to late-successional species has a shallow intensively branched heart-root system; the main (horizontal) root mass is located close to the soil surface (Korn 2004). The fragile fine roots are dark brown to light beige in colour, but finest rootlets appear translucent and hairy. Root tips are also transparent and typically infected by endomycorrhiza forming vesicular-arbuscular (AM) fungi.

Cluster approach

In order to distinguish between tree species diversity and tree species identity effects, all possible combinations of the five most abundant tree species in the north-eastern part of Hainich National Park were investigated in cluster plots. A cluster plot consisted of a group of three neighbouring tree individuals (Figure 1.2).

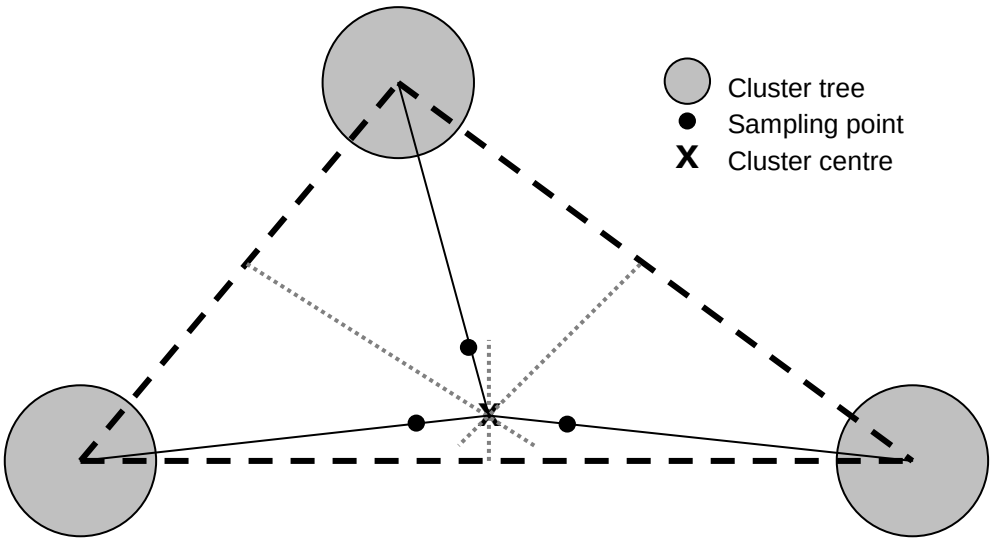


**Figure 1.2** Scheme of a tree cluster.

The approach contained mature trees of one, two or three different species and represented all 25 possible combinations of the five species resulting in five monospecific cluster types (all three trees of one species), ten 2-species cluster types and ten 3-species cluster types. All cluster types were selected in 4-fold replication, resulting in 100 cluster plots in total. Half of the cluster plots (50) were located in the Thiemsburg region of the forest, the other 50 in the Lindig region about 2 km distant. The mean distance between the plots was c. 99 m. Yet, several plots (23) were only 20-30 m distant from each other, which may have been sufficient to exclude fine root system overlap and horizontal water and nutrient fluxes between neighbouring plots in most of the cases. The approx. 2 $m^2$-fenced centre of each cluster triangle represented the study plot for most of the root-related studies except for the studies of chapter 4 ($^{15}N$ tracer study).

### $^{15}N$ tracer study

For analysing species-specific differences in N uptake rates in fine roots of the five tree species and their possible preference for nitrogen forms, additional study plots for each species were selected beside the cluster approach to avoid adverse effects through the $^{15}N$ tracer application on further investigations on the cluster plots. These plots were selected with species either occurring in small monospecific groups of three to six trees or well mixed with allospecific neighbours.

Three forest stand complexes were located in the Thiemsburg region of Hainich National Park. Each forest stand complex represented a replication in time (three different $^{15}N$ tracer applications) and consisted of five sub-plots with one sub-plot per tree species. Mature trees of specific target species dominated the sub-plots with a size of 2 $m^2$. The mean distance between the sub-plots within a forest stand complex was c. 50 m which may have been sufficient to exclude fine root system overlap and horizontal water and nutrient fluxes between neighbouring sub-plots.

## Study objectives and hypotheses

The overall aim of my PhD study was to separate tree species diversity and tree species identity effects on fine root dynamics in the rhizosphere of temperate mixed forest.

The specific aims of the work were

1) to quantify standing fine root bio- and necromass and the contribution of the five different tree species to total fine root mass in the stand
2) to identify differences in fine root morphological traits between the tree species
3) to quantify fine root productivity in various tree species compositions
4) to assess if fine root productivity and site conditions are determinants of the species-specific fine root morphology, and
5) to quantify the N uptake rate and preference of different N forms for the five tree species

In a first step, the standing fine root bio- and necromass of trees and herbs was investigated. In the root inventory, three sampling locations were selected randomly in the centre of each cluster plot (Figure 1.2). Morphological fine root parameters (e.g. mean diameter, specific root area and length, root tip abundance) and seasonal changes in fine root bio- and necromass were analysed. Moreover, several investigations on tree species-specific fine root activity (e.g. fine root growth, turnover and nitrogen uptake) were carried out. Fine root productivity was estimated by the ingrowth core approach (one installed core in the centre of every cluster plot). Additionally, different $^{15}N$ labelled N forms (inorganic and organic) were applied to analyse the species' N preference. In all studies, the fine root mass was not only separated into living and dead fractions, but also separated according to species on the basis of root morphological traits.

In chapter 2, I tested the hypotheses that (i) tree species identity has a larger effect on standing fine root biomass than species diversity, (ii) no significant 'overyielding' with respect to standing fine root biomass occurs in the 2- and 3-species mixed plots in comparison to the five monospecific plot, and (iii) the five tree species differ in their fine root biomass/basal area ratio which manifests in significant over- or under-representation of certain species in terms of their fine root biomass in mixtures. An

earlier study had found decreasing fine root densities with increasing soil depth in all tree species on the stand level (Meinen *et al.* 2009c). Additionally, no evidence was found for vertical and horizontal segregation of the root systems of the different tree species in the species-rich stands of the forest.

In chapter 3, I tested the hypotheses that iv) fine root productivity increases with a diversity increase from 1 to 3 species; v) a productivity increase is mainly a consequence of the presence of species with particularly high root productivity (selection effect); and consequently vi) species identity effects on root productivity are more important than a diversity effect; and vii) fine root turnover increases with increasing species richness due to more intense interspecific competition. The authors of a recent ingrowth core study in the Hainich forest found that fine root growth into the root-free soil increased with tree species diversity indicating a more rapid recovery of the root system after soil disturbances in the species-rich stands (Meinen *et al.* 2009b).

In chapter 4, I addressed three hypotheses about complementarity in the use of soil N: (1) the coexisting five tree species differ in the preferences for specific N forms, (2) this differentiation, if it exists, is related to species differences in the type of mycorrhization and fine root morphology, and (3) N partitioning with respect to the N form applied, increases the uptake rate of N by trees at the stand level.

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# Chapter 2

## On the significance of belowground overyielding in temperate mixed forests: separating species identity and species diversity effects[1]

**Andreas Jacob, Dietrich Hertel & Christoph Leuschner**

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## Abstract

1. Complementary soil exploration by the root systems of coexisting tree species has been hypothesised to result in a higher root biomass of mixed forests than of monocultures but the existing evidence for a belowground diversity effect in forests is scarce and not conclusive.
2. In a species-rich temperate broad-leaved forest, we analysed the fine root biomass (roots ≤2 mm) and necromass in 100 plots differing in tree species diversity (one to three species) and species composition (all possible combinations of five species of the genera *Acer, Carpinus, Fagus, Fraxinus,* and *Tilia*) which allowed us to separate possible species diversity and species identity effects on fine root biomass.
3. We found no evidence of a positive diversity effect on standing fine root biomass and thus of overyielding in terms of root biomass. Root necromass decreased with increasing species diversity at marginal significance.
4. Various lines of evidence indicate significant species identity effects on fine root biomass (10-20% higher fine root biomass in plots with presence of maple and beech than in plots with hornbeam; 100% higher fine root biomass in monospecific beech and ash plots than in hornbeam plots; differences significant). Ash fine roots tended to be over-represented in the 2- and 3-species mixed plots compared to monospecific ash plots pointing at apparent belowground competitive superiority of *Fraxinus* in this mixed forest.
5. Our results indicate that belowground overyielding and spatial complementarity of root systems may be the exception rather than the rule in temperate mixed forests.

Key words: *Acer pseudoplatanus,* belowground complementarity, *Carpinus betulus, Fagus sylvatica, Fraxinus excelsior,* fine root biomass, old-growth forest, root competition, *Tilia cordata*

## Introduction

During the past two decades, significant progress has been made in understanding the role of biodiversity for ecosystem functioning. Experiments with artificial grasslands and herbaceous plant communities frequently have demonstrated a positive relationship between plant species diversity and aboveground productivity (Hector *et al.* 1999, Roscher *et al.* 2005, Tilman *et al.* 2001). Such growth-promoting effects have been related to 'selection effects' (the increasing probability to include highly productive species in species-richer mixtures, Aarssen 1997) and to 'complementarity effects' (the assumed complementary use of resources by different species due to niche partitioning or facilitative interactions among species) (Holmgren *et al.* 1997, Loreau 1998, Cardinale *et al.* 2007). A meta-analysis has shown that species mixtures are more productive than the average of all monocultures in about 80% of the investigated 44 diversity-productivity experiments while in only 12% of all experiments do diverse polycultures achieve greater biomass than their single most productive species (Cardinale *et al.* 2007). However, these findings have been criticised because they seem to have only limited relevance for patterns found in natural or semi-natural grassland communities where, in most cases, no or even negative diversity-productivity relationships are found (Kahmen *et al.* 2005, Thompson *et al.* 2005, Grace *et al.* 2007).

Even more uncertainty exists with respect to the functional significance of tree species diversity for ecosystem processes in forests where experimental approaches are far more difficult than in grasslands and observational studies necessarily are a main source of information (Scherer-Lorenzen *et al.* 2005). The few data on the biodiversity-productivity relation in forests are contradictory and might, in several studies, be confounded by incomplete control of other influential factors (Vilà *et al.* 2005). While several authors reported a generally higher productivity of species-rich forests or plantations than of monospecific stands in the temperate or tropical zone (Erskine *et al.* 2006, Vilà *et al.* 2007, Lei *et al.* 2009, Oelmann *et al.* 2010, Paquette and Messier 2011), others found no relationship or a negative one (Jacob *et al.* 2010a, Long and Shaw 2010). In a survey of Central European natural forest stands, Szwagrzyk and Gazda (2007) detected no relationship between tree diversity and aboveground biomass. It may turn out that the relationship between species diversity and productivity in forests is dependent on climate, water availability and soil fertility and that a positive diversity effect is occurring only in certain types of plantations composed of trees with largely different functional traits or in natural forests of medium to low productivity. Under conditions of low temperature, infertile soils or drought, facilitation can be a relevant force

resulting in enhanced productivity compared to the corresponding monocultures (Paquette and Messier 2011).

Most research on the role of tree species diversity for forest productivity (or biomass as a rough estimate of productivity) has focused on the aboveground compartment while only few studies have considered diversity effects on forest belowground productivity or root biomass (Hendriks and Bianchi 1995, Leuschner *et al.* 2001, Schmid 2002, Meinen *et al.* 2009a, Brassard *et al.* 2010 for temperate forests, and Berish and Ewel 1988 and Cuevas *et al.* 1991 for tropical forests or plantations). In most cases, the biomass or production of fine roots (i.e. roots ≤2 mm in diameter) in two-species stands was contrasted with that of the respective monospecific stands; more species-rich forests (>three species) were only exceptionally investigated (Berish and Ewel 1988, Meinen *et al.* 2009a).

If root production (or root biomass) were generally higher in mixed forests than in monospecific stands, this could be relevant for the carbon storage and sequestration potential of forests (Scherer-Lorenzen *et al.* 2005). Mixed stands should maintain a larger root biomass and have a higher root production rate than monocultures when species-specific root-related traits are allowing different tree species to exploit belowground resources in a complementary manner. This may occur when the co-existing species differ in the depth distribution and/or maximal extension of their fine root systems, the fine root mass or length density (mass or length per soil volume) is different, the fine roots have different specific uptake capacities for water and nutrients or show contrasting phenologies of proliferation, or the fine roots utilise different types of resources (e.g. different N forms). Whether the use of belowground space, water or nutrients in mixtures is complementary depends on the existence of inherent tree species differences in root system morphology and functionality which can lead to the occupation of diverging belowground niches. Another force which could lead to complementary resource use might be root competition when its outcome is markedly asymmetric as was observed by Rewald and Leuschner (2009) in a temperate mixed forest. The inferior competitor could respond with a niche shift towards previously unoccupied niche space (Schenk 2006).

Many of the plant traits and processes relevant for the development of belowground niche complementarity are difficult to investigate and consequently only poorly understood. A promising exception is, for example, the study of Guo *et al.* (2008) who investigated the relationship between fine root anatomy and assumed resource absorption capacity in 23 temperate tree species. A relatively well studied phenomenon is vertical root system stratification in mixed stands of trees or woody

crops which sometimes is associated with a higher standing root biomass in the mixture as compared to the respective pure stands (hereafter termed belowground 'overyielding' with respect to root biomass). In the cases, where root biomass overyielding was found (Schmid 2002), it remained unclear whether the biomass increase was caused by a species diversity effect involving root system complementarity or facilitative interactions, or alternatively was produced by a particularly large root biomass of one or more species, thus representing a selection effect.

This study attempts for the first time to separate possible species diversity and species identity effects on tree fine root biomass in a species-rich temperate old-growth forest, i.e. for a natural forest with long continuity. The studied forest with up to 14 tree species per hectare offers nearly optimal conditions for analysing the functional role of tree diversity under natural conditions because it consists of a small-scale matrix of patches with low to high tree species richness and includes a broad variety of species combinations which are found in close proximity to each other under more or less homogeneous soil and climate conditions (Leuschner *et al.* 2009). This setting enabled us to conduct comprehensive root inventories in a matrix of monospecific, 2-species and 3-species plots with all possible combinations of five target tree species being available under the conditions of a natural forest. Thus, this approach allows combining the advantages of a biodiversity experiment, which uses defined diversity levels and replicated species combinations, with the strengths of observational studies in natural systems at near-equilibrium.

In a previous study on large-scale plots in this forest, Meinen *et al.* (2009a) obtained evidence for a dominant influence of tree species identity on standing fine root biomass, but a true diversity effect could not be shown because the study used a dilution gradient of tree species diversity with only one matrix tree species due to lacking monospecific plots of the other species. In the present study, much smaller plots were used to study monospecific and mixed plots composed of five common broad-leaved tree species differing considerably in important functional traits. The study bases on a root morphological key developed for distinguishing the fine roots of the five species. This allowed us to analyse species-specific patterns of fine root abundance across a matrix of 100 small-scale plots representing 25 tree species combinations of variable diversity (one to three species). We tested the hypotheses that 1) tree species identity has a larger effect on standing fine root biomass than species diversity, 2) no significant 'overyielding' with respect to standing fine root biomass occurs in the 2- and 3-species mixed plots in comparison to the five monospecific plot types, and 3) the five tree species differ in their fine root

biomass/basal area ratio which manifests in significant over- or under-representation of certain species in terms of their fine root biomass in mixture. Evidence in support of the last hypothesis would be interpreted as indication of a significant species identity effect on fine root biomass in this mixed stand.

## Material and methods

*Study site and plot selection*

The study was conducted in 2008 in a species-rich temperate broad-leaved forest in Hainich National Park, Thuringia, Germany. This conservation area was established in 1997 to protect one of the largest non-fragmented old-growth deciduous forests in Central Europe. All investigations were done in a forest stand in the Thiemsburg and Lindig forest regions (north-eastern part of the National Park) at ca 350 m a.s.l. in level terrain (Supplementary material, Appendix Figure 2.A1). The species-rich forest of the Stellario-Carpinetum community (oak-hornbeam forests) consists of 27-32 m tall trees of >100 to about 200 years in age with the most common species being Common ash *(Fraxinus excelsior* L.), European beech (*Fagus sylvatica* L.*)*, hornbeam (*Carpinus betulus* L.), Small-leaved lime *(Tilia cordata* Mill.) and Sycamore maple (*Acer pseudoplatanus* L.). The study region has a mean annual temperature of 7.7°C and a mean annual precipitation of 590 mm $yr^{-1}$ (period 1973-2004; station Weberstedt; Deutscher Wetterdienst, 2005). In the study year 2008, an average precipitation of 612 mm $yr^{-1}$ and a mean annual temperature of 8.6°C were recorded.

For the study, 100 plots were selected each consisting of a group of three neighbouring mature trees of variable species identity (hereafter termed 'tree clusters'). The clusters consisted of trees of one, two, or three different species and represented all possible combinations of the five species, thus resulting in five

**Table 2.1** Tree species combinations represented in 1-, 2- and 3-species clusters. A - Ash, B - Beech, H - Hornbeam, L - Lime, M - Maple. All 25 combinations were replicated fourfold.

| **Diversity level** | | | | |
|---|---|---|---|---|
| 1-species | 2-species | | 3-species | |
| A | AB | BL | ABH | ALM |
| B | AH | BM | ABL | BHL |
| H | AL | HL | ABM | BHM |
| L | AM | HM | AHL | BLM |
| M | BH | LM | AHM | HLM |

monospecific cluster types (only one tree species present), ten 2-species cluster types and ten 3-species cluster types (Table 2.1; Supplementary material, Appendix Figure 2.A2). All cluster types were selected in fourfold replication, resulting in 100 cluster plots in total. Half of the plots (50) were located in the Thiemsburg region of the forest, the other 50 in the Lindig region about 2 km to the East (Supplementary material, Appendix Figure 2.A1); one of the regions alone would have been too small to select all 100 plots. Since the plots of the two forest regions were statistically not different with regard to stand structural properties and soil chemical properties (P ≤0.05, see below), they were pooled in the subsequent analysis. Mean distance between the plots was 99 m, minimum distance 20-30 m (in 23 plots). A distance of 20-30 m was accepted because earlier root studies had shown that the maximum horizontal extension of the fine roots of a tree was typically <15 m (extremes to 19 m) in this stand (Meinen *et al.* 2009b) and significant horizontal water and nutrient fluxes between neighbouring plots can be excluded in the level terrain. The tree clusters were selected with the aim of maximum comparability between the 1-, 2- and 3-species plots in terms of aboveground forest structure (stand basal area, canopy closure) and edaphic conditions (Table 2.2). While the five species showed species-specific differences in tree dimensions and stem distance, the three diversity levels (20 x 1-species, 40 x 2-species and 40 x 3-species plots) differed not significantly in their cumulative basal area of the three trees per plot area (0.025 – 0.031 $m^2$ $m^{-2}$ cluster area) and the breast height diameters (0.43 – 0.46 m, Table 2.2). The three cluster-building trees were on average 7.5 to 7.8 m distant to each other in the plots of all three diversity levels.

The most widespread soil types are partly stagnic Luvisols (IUSS 2007) with topsoil acidification developed in a base-rich Pleistocene loess cover over Triassic

**Table 2.2** Characteristics of aboveground stand structure of the each three trees in the 100 cluster plots with a species richness of one to three species (means ± SE, n= 20 plots per 1-species combination and n= 40 plots per 2- and 3-species combination). Different letters indicate significant differences between the three diversity levels (P ≤0.05). Data on basal area, tree diameter and distance between the three cluster-building trees were provided by D. Seidel (unpubl.), cover values of juvenile trees by E.A. Vockenhuber (unpubl.).

| | **Diversity level** | | |
|---|---|---|---|
| | 1-species | 2-species | 3-species |
| **Stand characteristics** | | | |
| Cumulative basal area ($m^2$ $m^{-2}$ cluster area) | 0.025 ± 0.003 a | 0.031 ± 0.005 a | 0.030 ± 0.003 a |
| Mean breast height diameter (m) | 0.43 ± 0.03 a | 0.43 ± 0.01 a | 0.45 ± 0.01 a |
| Mean distance between the three cluster trees (m) | 7.5 ± 0.6 a | 7.6 ± 0.4 a | 7.8 ± 0.4 a |
| Groundcover of juvenile trees (% $m^{-2}$) | 6.2 ± 0.7 a | 8.8 ± 0.7 a | 8.4 ± 0.7 a |

limestone. On top of the mineral soil, a mull-type humus layer of about 1 cm depth was present in all tree clusters except for plots with two or three beech trees, where thicker, less decomposed moder layers were found. The soil texture of the mineral soil (0-30 cm) is characterised by high silt (about 74%) and low sand (< 5%) contents (Guckland *et al.* 2009). The clay content was somewhat lower in the 2-species cluster plots (mean: 53.4%) than in the 1- and 3-species plots (57.2 and 58.2%; all soil chemical data after C. Langenbruch and M. Meissner, unpubl.). Important soil chemical parameters differed not significantly between the plots of the three diversity levels (pH ($H_2O$): 5.4 to 5.6; C/N ratio: 12.7 to 13.1 g $g^{-1}$ in 0-20 cm depth; base saturation at the cation exchangers: 85-90%). The soil water content reached similar means in the three cluster types during the growing season 2008.

*Soil sampling and root analysis*

Fine root sampling was conducted on three occasions in May, September and November 2008 in all 100 cluster plots. For avoiding interference between the three sequential sampling events, we selected three locations in the centre of a cluster plot by random but at a minimum distance of 50 cm to each other. Each one soil core was extracted from the 0-20 cm layer of the soil profile (including the organic layer) using a cylindrical steel corer of 35 mm in diameter. Earlier root coring studies to greater depth showed that >60 to 77% of the fine root biomass in the 0-40 cm profile are located in the upper 20 cm and that no conspicuous vertical root system stratification among the species is occurring in the stand (Meinen *et al.* 2009a, b). All samples were transferred to polyethylene bags and stored at 4°C in the dark until processing took place within ten weeks. Earlier investigations had shown that tree fine root material stored under these conditions did not alter its vitality status (Leuschner *et al.* 2001, Meinen *et al.* 2009a). The soil samples were soaked in water and sieved (mesh size 0.25 mm) to separate the fine root mass (≤2 mm in diameter) from soil material. Coarse tree roots (≥2 mm in diameter) and roots of forest floor herbs and grasses were discarded. Fine root fragments longer than 10 mm were collected manually using a pair of tweezers; all root fragments were separated into live and dead mass and sorted by species under a stereomicroscope (6-40x). Tree species identification in the fine root fraction was conducted with the help of a classification system based on a set of fine root morphological criteria (branching patterns, colour and surface structure of the periderm, size and shape of root tips, diameter of fine and finest roots) established by Hölscher *et al.* (2002) and Meinen *et al.* (2009a). For separating live and dead root material, fine root characteristics such as root elasticity, toughness, cohesion of cortex and stele, and

periderm colour were used (Persson 1978, 1983; Meinen *et al.* 2009a; Brassard *et al.* 2010; Leuschner *et al.* 2001). After the extraction of fine root fragments ≥10 mm length from the samples, the residual material was subjected to a detailed microscopic analysis of smaller root necromass particles (≤10 mm) that may account for a large proportion of fine root necromass (Leuschner *et al.* 2001). This approach was applied to half of the root samples collected which allowed us to extrapolate from the ≥10 mm fraction to total necromass in all those samples that were not investigated in detail (for more details see Leuschner *et al.* 2001 and Meinen *et al.* 2009a). The fine root bio- and necromass samples were dried at 70°C for 48 h and weighed. The root mass data were expressed as dry mass per square meter of ground area (for the 0-20 cm soil profile).

*Statistical analyses*

Probability of fit to normal distribution was tested by a Shapiro-Wilk test. Data sets showing no Gaussian distribution were log-transformed to reach normal distribution. A multi-factorial analysis of variance was used to test for significant effects of the cluster diversity level or the presence of a given tree species, or the interaction between diversity level and species presence, on fine root biomass, necromass and biomass/necromass ratio. In pair-wise comparisons, single-factorial analysis of variance (GLM) accompanied by a Scheffé post-hoc test was applied; this was done for all variables except for the parameters related to stand aboveground structure and soil chemistry for which normal distribution could not be achieved by data transformation. Here, a non-parametric Mann-Whitney *U*-test was applied. Linear Pearson correlation analyses were employed to analyse the relation between fine root biomass and selected stand structural and soil chemical parameters, and species diversity expressed as species richness in stand basal area or in a root sample. All mentioned analyses were carried out with the software package SAS, ver. 9.1 (SAS Inst.). The influence of tree species diversity on standing fine root biomass or necromass in the tree clusters was additionally analysed with a principal components analysis (PCA) in which species diversity was introduced through Shannon's *H* using the relative contribution of a species to the cluster basal area as a measure of relative abundance. Several soil chemical (pH, C/N ratio, base saturation, clay content) and hydrological parameters and the distance to the nearest stem as possible explaining variables were also considered in the PCA analysis. The PCA analyses were conducted with the package CANOCO, ver. 4.5. Subsequently, single-factor linear regression analyses were conducted to quantify the influence of the first three PCA axes on fine root biomass or necromass at the

three sampling dates, and on fine root biomass/necromass ratio. Significance was determined in most tests at P ≤0.05; in a few cases, marginally significant differences (P ≤0.1) are also indicated.

## Results

*The relationship between fine root mass and tree species diversity and species identity*

Tree fine root biomass in the upper 20 cm of the soil was not significantly different in cluster plots with one, two or three species (Figure 2.1A). A diversity effect was absent irrespective of the measure of species richness used (contribution of different species to stand basal area or to standing fine root biomass, Table 2.3). On the contrary, average fine root biomass tended to be lower in 2- and 3-species plots than in monospecific plots, but this trend was not significant. This result is supported by the PCA analysis (Table 2.4). The results of the multi-factorial analysis of variance support our finding of a lacking diversity effect on fine root biomass. Moreover, a model including the possibly influencing factors diversity and species identity (reflected by the presence or absence of a given species in a plot) and the interaction between diversity and species identity showed no significant influence on fine root biomass (Table 2.5). In all clusters, lowest fine root biomass totals (summing up all species present) were recorded in September.

Dead fine root mass (necromass) showed a marginally significant decrease (P ≤0.1) from the monospecific plots (136 g $m^{-2}$) to the 3-species plots (118 g $m^{-2}$), when averaged over the whole growing season (Figure 2.1B). While fine root necromass tended to increase with increasing diversity level in May, a marginally

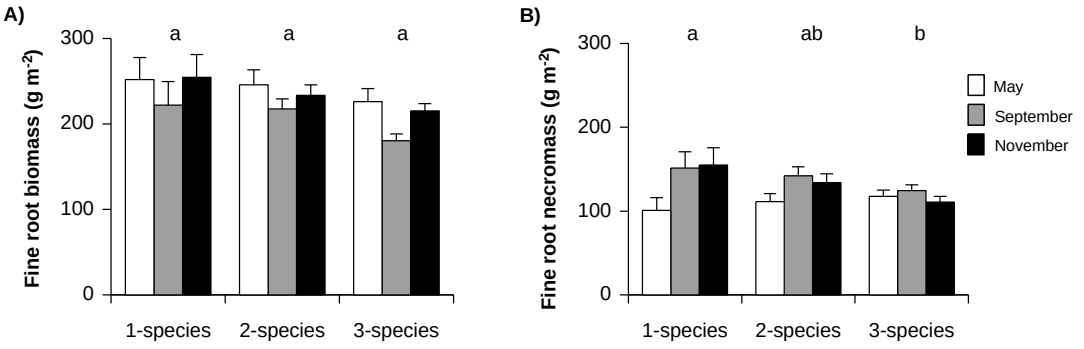


**Figure 2.1** Fine root biomass (A) and fine root necromass (B) in May (white filled bars), September (grey filled bars) and November (black filled bars) 2008 in the upper 20 cm of the soil in the centre of 1-, 2- and 3-species clusters (means ± SE, each four replicate plots per species combination, five to ten combinations per diversity level, 100 plots in total). Different letters indicate significant differences between the three diversity levels (in the case of fine root necromass: the significance level is P ≤0.1).

**Table 2.3** Results of linear Pearson correlation analyses relating selected stand structural and soil chemical variables, and tree species diversity in a root sample to total tree fine root biomass in the clusters (0-20 cm soil depth). All data were log-transformed prior to analysis. All 25 cluster types (and all three diversity levels) were included in the analysis. *H*'-Shannon-Wiener diversity index.

| Variable | $r_P$ | P |
|---|---|---|
| Cumulative basal area ($m^2$ $m^{-2}$ cluster area) | 0.088 | ns (0.38) |
| Mean distance between the three cluster trees (m) | -0.115 | ns (0.25) |
| pH ($H_2O$) | 0.034 | ns (0.74) |
| Tree diversity (*H*' for species richness in stand basal area) | -0.160 | ns (0.11) |
| Tree diversity (*H*' for species richness in fine root biomass) | -0.118 | ns (0.24) |
| Soil C/N ratio (g $g^{-1}$) | 0.038 | ns (0.71) |
| Clay content (%) | -0.171 | ns (0.09) |
| Soil water content (% of d.w.) | -0.117 | ns (0.25) |
| Base saturation (%) | -0.075 | ns (0.46) |

significant decrease from the 1- to the 3-species plots was observed in November. However, analysis of variance revealed no significant species diversity effect on mean fine root necromass, while the presence of ash, hornbeam and lime (and the interaction diversity level x ash presence) exerted a significant effect (Table 2.5). In most plots, fine root necromass reached its highest value in September in coincidence with the seasonal minimum of fine root biomass.

Mean fine root biomass/necromass ratio was not significantly different between the plots of the three diversity levels when species richness was measured as the contribution to stand basal area (Figure 2.2). A similar result was obtained when expressing species richness as the species' contribution to stand fine root biomass

**Table 2.4** Pearson correlation coefficients and P values for the relationship between the PCA axes (cf. Supplementary material, Appendix Figure 2.A3) and fine root biomass or necromass in the tree clusters (n= 100). Correlations are for sample scores.

| | Axis 1 | | Axis 2 | | Axis 3 | |
|---|---|---|---|---|---|---|
| | r | P | r | P | r | P |
| Fine root biomass | | | | | | |
| May | -0.14 | ns | -0.07 | ns | 0.14 | ns |
| September | -0.09 | ns | -0.05 | ns | 0.23 | 0.02 |
| November | -0.03 | ns | 0.10 | ns | 0.10 | ns |
| Mean fine root biomass | -0.11 | ns | -0.01 | ns | 0.22 | 0.03 |
| Fine root necromass | | | | | | |
| May | 0.12 | ns | -0.05 | ns | 0.13 | ns |
| September | 0.01 | ns | -0.02 | ns | 0.24 | 0.01 |
| November | -0.13 | ns | 0.04 | ns | 0.27 | 0.01 |
| Mean fine root necromass | 0.001 | ns | -0.01 | ns | 0.28 | 0.01 |
| Fine root biomass/necromass ratio | 0.14 | ns | 0.06 | ns | 0.07 | ns |

**Table 2.5** Results of a multi-factorial analysis of variance on the influence of the diversity level of the tree clusters ('dl'), the presence of the five tree species in the plots ('p_Ash', 'p_Beech', 'p_Hornbeam', 'p_Lime', 'p_Maple'), and the interaction between diversity level and the presence of one of these species on fine root biomass, fine root necromass, or the fine root biomass/necromass ratio in the 100 clusters. Given are the F- and P-values of the source variables and the coefficient of determination ($r^2$) of the model.

| | **Dependent variable** | | |
|---|---|---|---|
| **Source** | Fine root biomass | Fine root necromass | Fine root biomass/ necromass ratio |
| dl | 1.45 (n.s.) | 1.02 (n.s.) | 0.93 (n.s.) |
| p_Ash | 0.97 (n.s.) | 35.65 (P ≤0.001) | 17.46 (P ≤0.001) |
| p_Beech | 0.09 (n.s.) | 0.39 (n.s.) | 0.60 (n.s.) |
| p_Hornbeam | 9.66 (P ≤0.01) | 6.51 (P ≤0.05) | 1.33 (n.s.) |
| p_Lime | 0.21 (n.s.) | 10.77 (P ≤0.01) | 0.28 (n.s.) |
| p_Maple | 2.83 (n.s.) | 2.76 (n.s.) | 5.79 (P ≤0.05) |
| dl x p_Ash | 0.00 (n.s.) | 7.11 (P ≤0.05) | 0.00 (n.s.) |
| dl x p_Beech | 0.60 (n.s.) | 1.30 (n.s.) | 0.93 (n.s.) |
| dl x p_Hornbeam | 0.00 (n.s.) | 2.70 (n.s.) | 1.59 (n.s.) |
| dl x p_Lime | 0.04 (n.s.) | 0.00 (n.s.) | 0.49 (n.s.) |
| dl x p_Maple | 1.98 (n.s.) | 0.34 (n.s.) | 3.20 (P ≤0.05) |
| Model $R^2$ | 0.20 (n.s.) | 0.50 (P ≤0.001) | 0.33 (P ≤0.01) |

(data not shown). However, we found the root biomass/necromass ratio to be influenced by the presence of ash and maple (and the interaction diversity level x maple presence) in the analysis of variance (Table 2.5).

Linear Pearson correlation analyses conducted separately for the species in all 25 species combinations did not reveal significant relations between the species'

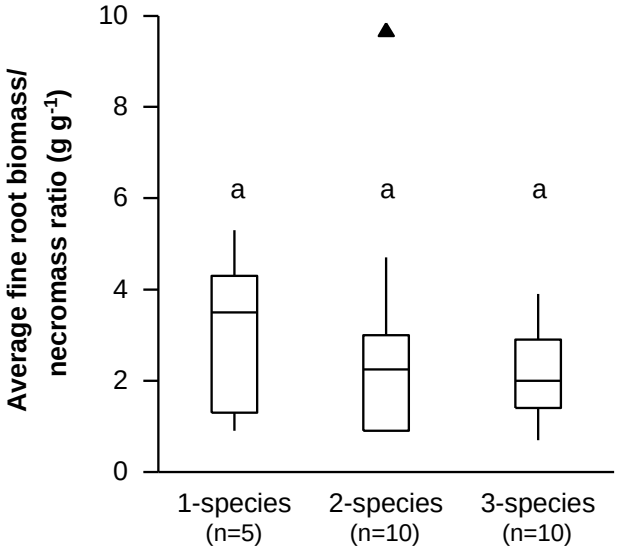


**Figure 2.2** Average fine root biomass/necromass ratio in soil cores from tree clusters representing different diversity levels. No significant differences existed between the means of the three diversity levels (P ≤0.05). The black triangle indicates a 'far outside value' (value larger than upper quartile plus 3x quartile distance).

**Table 2.6** Pearson correlation coefficients (P-values in brackets) for the dependence of the fine root biomass (in g $m^{-2}$) of the five tree species in the cluster plots on selected stand structural and soil chemical variables. Only the 20 monospecific clusters were included in the analysis. All data were log-transformed. None of the relationships was significant at P ≤0.05; marginally significant ones in bold (P ≤0.1).

| Variable | Ash | Beech | Hornbeam | Lime | Maple |
|---|---|---|---|---|---|
| Cumulative basal area | 0.799 | 0.760 | **-0.908** | 0.131 | 0.817 |
| ($m^2$ $m^{-2}$ cluster area) | (0.20) | (0.24) | **(0.09)** | (0.87) | (0.18) |
| Mean distance between the | 0.056 | -0.280 | 0.898 | 0.720 | -0.888 |
| three cluster trees (m) | (0.94) | (0.72) | (0.10) | (0.28) | (0.11) |
| | -0.484 | 0.157 | 0.597 | **0.914** | 0.366 |
| pH ($H_2O$), 0-20 cm | (0.52) | (0.84) | (0.40) | **(0.08)** | (0.63) |
| | 0.499 | 0.264 | 0.510 | 0.765 | 0.180 |
| Soil C/N ratio (g $g^{-1}$), 0-20 cm | (0.50) | (0.74) | (0.49) | (0.23) | (0.82) |
| | -0.140 | -0.137 | -0.834 | 0.574 | **0.929** |
| Clay content (%), 0-20 cm | (0.86) | (0.86) | (0.17) | (0.43) | **(0.07)** |
| Soil water content (% d.w.), | -0.228 | 0.407 | 0.255 | 0.766 | -0.741 |
| 0-20 cm | (0.77) | (0.59) | (0.74) | (0.23) | (0.26) |
| | -0.459 | -0.007 | -0.023 | 0.900 | **0.916** |
| Base saturation (%), 0-20 cm | (0.54) | (0.99) | (0.98) | (0.10) | **(0.08)** |

fine root biomass and several stand structural and soil chemical variables (basal area, mean distance between the three cluster trees, pH ($H_2O$), C/N ratio, soil water content and base saturation, Table 2.3). This finding is supported by the results of the PCA analysis (Supplementary material, Appendix Figure 2.A3, Table 2.4). Only clay content had a marginally significant effect (P =0.09) on fine root biomass in the single-factor correlation analyses. Similarly, we did not detect a significant influence of these stand structural and soil chemical variables on the fine root biomass of the five species, when only the respective monospecific (1-species) plots were analysed (Table 2.6).

*Belowground versus aboveground abundance of the tree species*

While species diversity did not have a positive effect on the fine root biomass total in the mixed-species plots, we obtained weak evidence of a possible diversity effect on the relative contribution of a tree species to the stand total of fine root biomass (0-20 cm profile). In monospecific clusters, the target species reached on average from 65% (monospecific hornbeam clusters) to 94% (monospecific ash clusters) of the fine root biomass total. The remaining fine root biomass was contributed by other tree species which were present with stems outside the cluster plots and extended their fine roots into the plots (Supplementary material Appendix 2 Table 2.A1). In the 2-species plots, the respective target species was present by definition with only one or two trees and the species' contribution to the fine root biomass total ranged

**Table 2.7** Fine root biomass (0-20 cm profile), basal area of the cluster trees and the ratio of the two parameters for the five tree species in the respective 1-species clusters (n= 4 plots). Given are means ± SE. Different letters indicate significant differences between the tree species (P ≤0.05).

| | Fine root biomass (g $m^{-2}$) | Basal area ($m^2$ $m^{-2}$ cluster area) | Fine root biomass/ basal area ratio (kg $m^{-2}$) |
|---|---|---|---|
| Ash | 246.7 ± 23.2 a | 0.031 ± 0.005 a | 8.4 ± 1.2 a |
| Beech | 255.6 ± 30.0 a | 0.028 ± 0.008 a | 11.5 ± 3.7 a |
| Hornbeam | 114.8 ± 25.5 b | 0.017 ± 0.005 a | 10.2 ± 4.2 a |
| Lime | 191.7 ± 52.9 ab | 0.025 ± 0.007 a | 8.1 ± 1.3 a |
| Maple | 191.6 ± 60.2 ab | 0.026 ± 0.010 a | 9.7 ± 2.9 a |

between 21 and 71%. In the most diverse 3-species plots, the single tree of a target species held root biomass proportions of 12 to 47% of the total. When comparing the fine root biomass proportion of a species in the 1-, 2- and 3-species clusters, ash, beech, hornbeam and maple showed the expected trend of a decreasing proportion with increasing species diversity, while lime tended to increase its relative contribution to total fine root biomass from the 2-species to the 3-species clusters from 28 to 33% despite a decreasing stem number (Supplementary material, Appendix Table 2.A1); however, this difference was not significant.

Tree species identity influenced standing fine root biomass in different ways. Ash and beech reached more than two times higher absolute fine root biomasses in the respective monospecific plots than hornbeam, while intermediate biomass figures were recorded for lime and maple (difference to the other three species not significant, Table 2.7). This species sequence in fine root biomass seems in part to be a consequence of cluster differences in the basal area of the three trees which tended to be smaller in the pure hornbeam clusters. The corresponding ratio of fine root biomass to basal area in the plots varied only moderately among the five species (8.1 – 11.5 kg $m^{-2}$) with no significant species differences. When comparing all 44 plots (1-, 2- and 3-species), in which a species was present, we found a

**Table 2.8** Fine root biomass (0-20 cm profile; sum of all species present) in plots where ash, beech, hornbeam, lime or maple trees were present (each 44 plots). Given are means ± SE. Different letters indicate significant differences between the five plot types (P ≤0.05).

| Species present | Fine root biomass (g $m^{-2}$) |
|---|---|
| Ash | 215.1 ± 10.9 ab |
| Beech | 227.1 ± 11.0 a |
| Hornbeam | 195.3 ± 9.2 b |
| Lime | 220.0 ± 14.0 ab |
| Maple | 240.0 ± 13.9 a |

significantly higher fine root biomass in plots with presence of maple or beech than in plots with hornbeam (means of 240 and 227 g $m^{-2}$ *versus* 195 g $m^{-2}$; Table 2.8). Plots with presence of ash and lime reached intermediate values.

We quantified the relative over- or under-representation of a tree species in terms of fine root biomass in mixture by relating the fine root biomass/basal area ratio in 2- and 3-species plots to the ratio of this species in its monospecific plots. Only in four of the 50 investigated species combinations, a species differed significantly in its root biomass/basal area ratio in mixed (2- or 3-species) plots from the ratio in the respective 1-species plots. The deviation was negative in all four cases, thus indicating a relative under-representation of this species in mixture (Figure 2.3). However, in 27 of the studied 50 cases, a non-significant tendency of an increase (and not decrease) in the ratio appeared which may suggest that over-representation of a species in mixture is also existing but was not detectable due to the limited number (four) of replicate plots per species combination investigated. The variation in the fine root biomass/basal area ratio of a species was large among the different 2-species or 3-species plot types in which this species was present. When analysing the species means of the deviation of the observed from the expected ratio for the each ten cluster types investigated per species (including the non-significant deviations), it appears that ash produced in most cases more fine root biomass in mixture than in its monospecific plots (on average 60 and 120% over-representation in 2- and 3-species plots, respectively). Over-representation in mixed plots was less pronounced in the case of lime, beech, hornbeam and maple (always <40%; difference ash to beech, hornbeam and maple significant at $P \leq 0.05$, Figure 2.3). Maple fine roots tended to be under-represented in several of the 2-species clusters while beech and hornbeam fine roots reached rather small biomasses in 3-species clusters relative to the monospecific plots. When comparing the mean deviation factors for 2- and 3-species plots, it appeared that ash and maple fine roots occurred with a higher percentage in 3-species than in 2-species plots which does not reflect the abundance of stems in the two diversity levels.

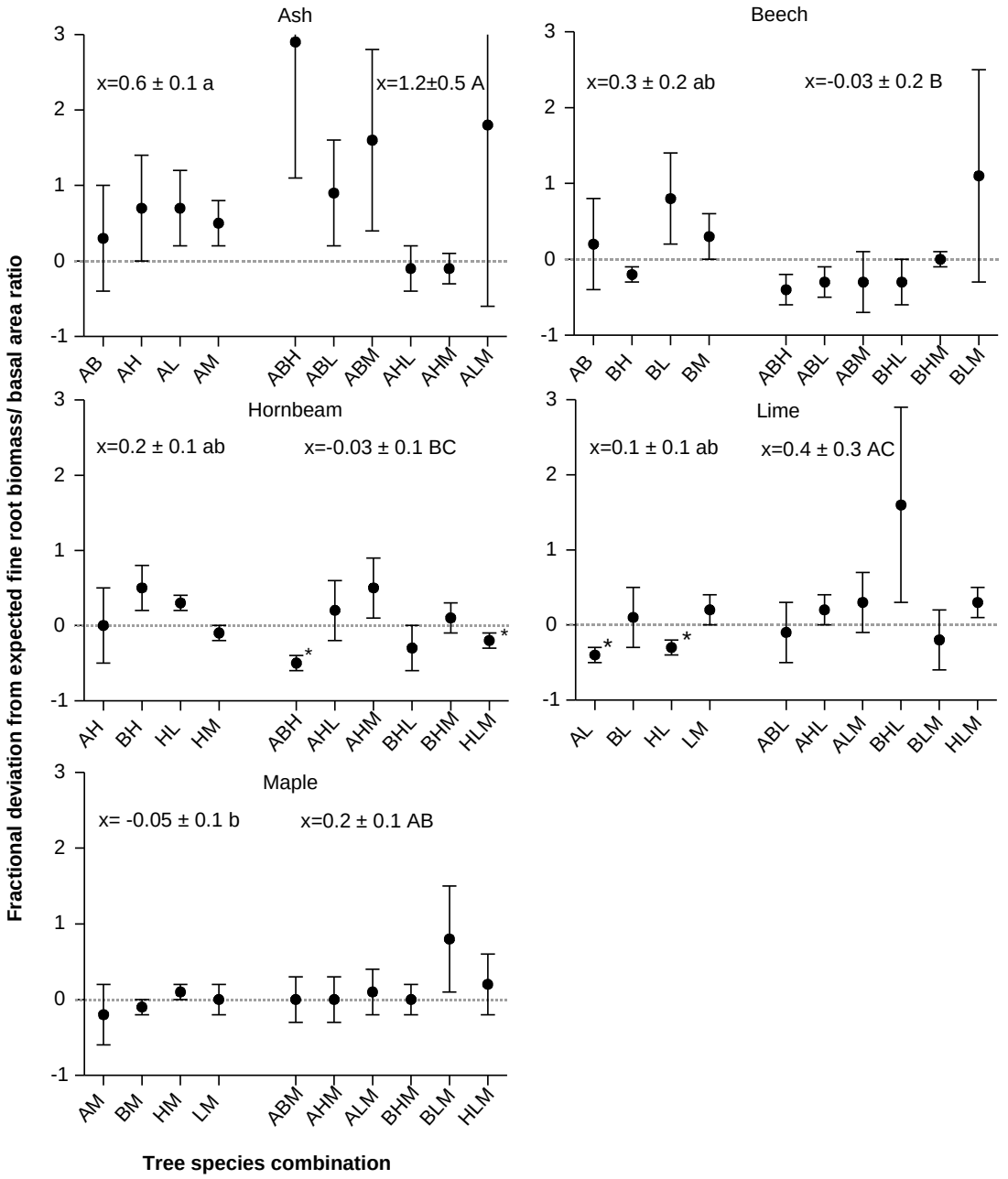


**Figure 2.3** Deviation of the observed fine root biomass/basal area ratio of a tree species (A – Ash, B – Beech, H – Hornbeam, L – Lime, M – Maple) from the expected ratio in the various species combinations (deviation expressed as a fraction by relating the observed ratio to the ratio expected from the respective species' monospecific plots; mean ± SE of four plots per species combination). Positive values stand for a larger observed root biomass/basal area ratio than expected, i.e. an over-representation of the species in terms of fine root biomass in the mixed plots, negative values for a smaller than expected ratio, i.e. under-representation. Significant deviation in a given cluster type from the expected ratio is marked by an asterisk. Given is also the mean fractional deviation (± SE) of all 2-species and 3-species clusters of a species (x – values above the figure). If the five species differ significantly in their mean ratio deviation in the 2-species clusters from the expected value, the x – values are marked with different small letters; if such a species difference exists in the 3-species clusters different capital letters are used (P ≤0.05). Ash had a significantly larger mean ratio deviation than beech, hornbeam and maple when all 2- and 3-species clusters of a species are pooled.

## Discussion

*No evidence of a species diversity effect on stand fine root biomass*

A main objective of this study in 100 forest patches with defined tree species composition was to separate a possible diversity effect from the likely dominating species identity effect on fine root biomass. Our results from 40 3-species and 40 2-species mixed plots and 20 monospecific plots are clear evidence against a diversity effect because standing fine root biomass tended to decrease, and not increase, toward the more diverse plots and this (non-significant) trend persisted through the entire growing season. This result agrees well with the findings of a previous study on 12 nearby large-scale (50 m x 50 m) plots with variable tree species diversity (one, three or five dominant tree species) where no significant difference in fine root biomass between the three diversity levels could be detected (Meinen *et al.* 2009a). The conclusion of a lacking diversity effect on fine root biomass in this forest seems to be robust because these two independent root inventories produced similar results despite marked differences with respect to spatial scale (size of sampled plot area: 2500 *versus* c. 25 $m^2$ in average), sampling depth (> 40 *versus* 20 cm) and timing (different sampling years: 2005/2006 *versus* 2008).

Comparative inventories in other monospecific and mixed temperate forests produced controversial evidence with respect to fine root biomass 'overyielding' showing either higher (Schmid 2002), lower (Morgan *et al.* 1992) or equal stand totals of fine root biomass in the mixtures (Brandtberg *et al.* 2000, Leuschner *et al.* 2001, Rewald and Leuschner 2009). The scarce existing information seems to indicate that a higher fine root biomass in mixed forests is not the rule but may occur only under certain conditions, in a similar manner as it was found for aboveground biomass 'overyielding' in tree mixtures by Pretzsch and Schütze (2009) and Pretzsch *et al.* (2010). Globally, diversity effects on fine root biomass and production in forests cannot be an important force, a conclusion which is supported by the fact that tropical forests with their high species diversity have on average very similar fine root biomasses as temperate forests with only one to about five tree species (Finér *et al.* 2011).

'Overyielding' in terms of fine root biomass in mixed stands might occur when species with largely different growth and rooting strategies (e.g. early- *versus* late-successional or broad-leaved *versus* coniferous species) are combined. Meinen *et al.* (2009a) found no vertical fine root biomass stratification among the five species in the Hainich forest mixed stands; however, this finding contrast with observations in several other temperate mixed forests (Büttner and Leuschner 1994, Hendriks and Bianchi 1995, Schmid and Kazda 2002, Bolte and Villanueva 2006). Species

mixtures such as *Populus/Picea* or *Betulus/Pinus/Fagus* with markedly different tree functional attributes were indeed found to produce pronounced root system stratification (Curt and Prevosto 2003, Legare *et al.* 2005). In our stand, the lower clay-rich soil horizons contained only small fine root densities (typically <0.3 g $L^{-1}$, Meinen *et al.* 2009a) which most likely is a consequence of the high bulk density of the soil that may have hindered the development of significant vertical root system stratification in the mixed plots.

From our finding that 1-species, 2-species and 3-species plots had a very similar fine root biomass and thus did not differ in fine root density in the upper soil (0-20 cm), we assume that the available rooting space in the relatively shallow soils of the Hainich forest must pose an upper limit to the production and maintenance of fine root biomass at this site; this limit is apparently not exceeded neither in the monospecific nor in the mixed plots. With about 1.5 g fine root biomass per $L^{-1}$ in the 0-20 cm layer, root biomass density is relatively high in the Hainich forest pointing a strong root competition, probably because suitable rooting space is limited. In fact, fine root density has been found to rarely exceed values of 1-2 g biomass $L^{-1}$ soil volume in the densely rooted topsoil horizons of temperate broad-leaved forests (Hertel 1999).

The amount of fine root necromass in the soil tended to decrease, rather than increase, with increasing tree diversity and the decrease was apparently driven by the presence of ash, lime and hornbeam roots. The amount of necromass present in the soil depends on the balance between fine root mortality (supply rate) and root necromass decomposition (loss rate). In the absence of data on fine root decomposition, it can only be speculated that root mass may decompose more rapidly when species such as ash, lime or hornbeam are present. These species were found in the Hainich forest to produce more rapidly decomposing leaf litter as compared to beech (and partly also maple) (Jacob *et al.* 2010b). Root litter decomposition studies are needed to test this assumption.

*Species differences in rooting patterns and indication for possible functional complementarity in the root systems of mixed stands*

Observational studies allow sound conclusions about diversity and species identity effects on ecosystem processes only when other factors such as stand structural properties and soil conditions can be sufficiently controlled (Leuschner *et al.* 2009). The 100 tree cluster plots of our study were carefully selected in order to exclude possible gradients in aboveground biomass (approximated from stem density and breast-height diameter data) and soil physical and chemical properties (notably clay

content, pH, base saturation and soil water status) as much as possible. A principal components analysis showed neither fine root biomass nor root biomass/necromass ratio to be influenced by stand structural or edaphic factors in the 100 Hainich plots.

While tree species diversity had no effect on fine root biomass and showed a weak negative influence on fine root necromass, standing fine root biomass was found to be significantly influenced by species identity. Monospecific beech and ash plots had more than two times higher fine root biomass totals than hornbeam plots and 25% larger amounts than plots of lime and maple, and the ANOVA showed a significant effect of hornbeam. One may argue that these figures base on a rather limited number of root cores (12 per species) and may partly be biased by the ingrowth of a certain amount of root biomass from trees outside the clusters. However, inherent tree species differences in fine root biomass are supported by the fact that the each 40 plots with presence of maple and beech had a significantly (by 10-20%) higher standing fine root biomass than plots where hornbeam was present. Such species differences are most likely the result of inherent differences in the rooting characteristics of the species. From the rich literature on fine root biomass in forest stands, it is safe to conclude that temperate tree species differ not only in the structure of their coarse and large root systems (Kutschera *et al.* 2009) but also in the stand totals of fine root biomass, in fine root density (root mass per soil volume) and the depth extension of fine roots. These species characteristics are modified by edaphic, climatic and stand structural factors.

For Central European tree species, it is known from extensive literature surveys that the fine root biomass of monospecific mature stands can differ up to twofold between different species under comparable conditions which points at species-specific differences in the root/shoot ratio (or fine root/leaf area ratio) at maturity (Leuschner and Hertel 2003, Finér *et al.* 2007). For example, the fine root biomass of beech is in temperate regions significantly higher than that of spruce and pine (Finér *et al.* 2007); no precise data are available for the less common tree species. The variation in the fine root biomass/basal area ratio found among the five species of this study in the monospecific plots (8.1-11.5 kg $m^{-2}$) also points at species differences in rooting characteristics.

The reasons for inherent species differences in fine root system size and spatial structure remain unclear but may perhaps relate to species differences in total leaf area and/or growth rate and associated differences in water and nutrient demand. That the differences in fine root biomass were caused by differences in stem density can be excluded in our study. Mixing tree species with different fine root biomass/basal area ratios and thus fine root biomasses could lead to a higher

standing fine root biomass than in the respective pure stands if several species with high ratios are present, which would represent a selection effect. Such a phenomenon did not occur in our plots where no signs of biomass 'overyielding' were found.

Empirical evidence for the existence of marked tree species differences in root water and nutrient uptake was recently obtained by *in situ* sap flux measurements in small-diameter roots and $^{15}N$ tracer studies in the Hainich forest. Accordingly, the five investigated species of this study are differing not only with respect to the profile total of fine root biomass but also in their specific water and nitrogen uptake capacities. Korn (2004) found a more than twofold difference among the five investigated tree species in the simultaneously measured water absorption rate per root surface area in 2-5 mm roots. Furthermore, the species showed up to fivefold differences in the net $^{15}N$ accumulation rate per fine root mass under *in situ* conditions in the forest soil. In addition, the five species also differed with respect to the preferred N form: Three species seemed to prefer ammonium over nitrate, one species nitrate over ammonium, while another species took up both N forms at similar rates in relation to the available pool (A. Jacob, unpubl.). Differential preference of $NH_4^+$ and $NO_3^-$ by co-existing plant species has been interpreted as an indication of complementarity in the use of N resources (Kahmen *et al.* 2006, von Felten *et al.* 2009). Thus, while our data provide evidence against significant complementarity in the utilisation of rooting space by the species, a certain degree of root functional complementarity may exist in the Hainich forest which deserves further study.

*Can root competition induce belowground competition?*

Strong root competition may lead to partial or full competitive exclusion of inferior belowground competitors or to root system segregation with reduced competition intensity where the soil physical conditions allow deeper rooting (Rajaniemi *et al.* 2003, Schenk 2006). Our analysis of the species' relative belowground presence, which we expressed as the observed deviation from the expected fine root biomass/basal area ratio, showed a non-significant tendency toward over-representation of ash fine roots in mixed plots as compared to monospecific plots. Tendencies for over- or under-representation in terms of fine root biomass per basal area in the mixed plots were also observed in the other tree species but in a less consistent manner than for ash. Despite the weak statistical significance, the fine root abundance patterns of ash may point at belowground competitive superiority of *F. excelsior* over the other four species in the Hainich forest, because our

observation matches with experimental data on root competition between ash and beech saplings by Rust and Savill (2000) that showed superiority of ash over beech. A similar asymmetric outcome of root competition was observed between the fine roots of beech and Sessile oak (*Quercus petraea*) trees in a root chamber competition experiment under field conditions (Leuschner *et al.* 2001) and Rewald and Leuschner (2009) measured a significant over-representation of beech roots relative to the species' basal area, but under-representation of oak roots, in a 4-species broad-leaved forest. In the beech-oak stand studied by Leuschner *et al.* (2001), the assumed beech superiority was associated with root system segregation. Interestingly, the apparent superiority of ash root systems in the Hainich mixed forest did not lead to significant vertical stratification of the root systems among the five species; rather, mutual replacement between the species' root systems was the apparent consequence.

## Conclusions

In the studied species-rich old-growth forest, complementarity in soil space exploration and a higher fine root biomass of mixed than monospecific stands were not detected. This matches with a lacking aboveground overyielding effect in terms biomass and productivity in this forest (Jacob *et al.* 2010a). These findings have consequences for the assessment of mixed forests in terms of carbon storage. A missing complementarity in belowground space exploration does not preclude complementarity among the co-occurring species in terms of water and nutrient uptake rates. Ongoing experiments on root water and N absorption in the Hainich forest point in this direction. If complementarity were indeed a relevant factor leading to a higher water and/or N consumption of mixed than monospecific stands in this forest, it is likely that this effect is mostly a result of the largely different morphology and physiology of the species (such as ecto- *versus* arbuscular mycorrhizal and diffuse- *versus* ring-porous species with different root system morphologies), which would emphasise the prominent role of species identity, and not species diversity, for the size and function of tree root systems.

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## Supplementary material (Appendix)

**Figure 2.A1** is conforming to **Figure 1.1!**

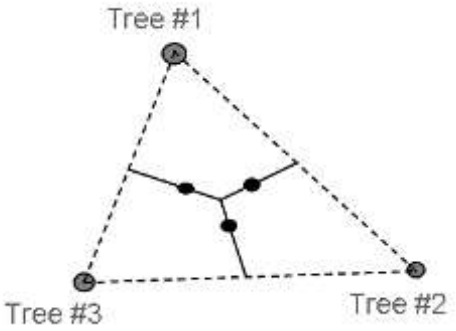


**Figure 2.A2** Sketch of a cluster of three mature tree individuals of variable species identity. Sequential soil coring for root analysis (black dots) took place in May, September and November 2008 at three locations close to the fenced cluster centre.

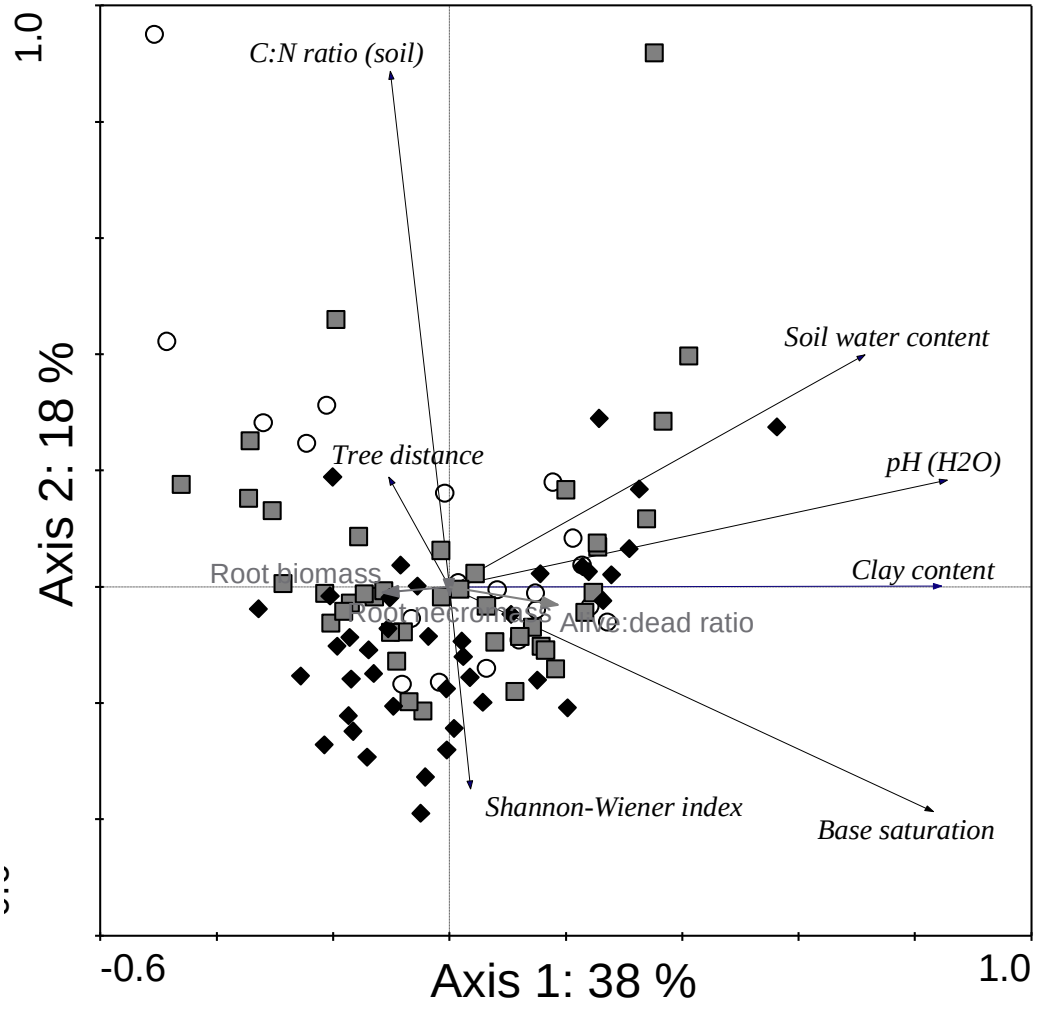


**Figure 2.A3** Principal components analysis (PCA) of environmental variables in 1-species, 2-species or 3-species clusters: biplot of environmental variables and fine root biomass or fine root necromass (first axis: Eigenvalue 0.38, loading of pH ($H_2O$) 0.86, clay content 0.85, base saturation 0.83; second axis: Eigenvalue 0.18, loading of C/N ratio 0.89; third axis: Eigenvalue 0.15, loading of tree distance -0.76, Shannon-Wiener index -0.67). Symbols are for 1-species (○), 2-species (■) or 3-species clusters (◆).

**Table 2.A1** Relative contribution of the five tree species to standing fine root biomass in the upper (0-20 cm) soil profile in the 25 tree species combinations represented in the clusters (in percent of tree fine root biomass total, four plots per combination). Given are means ± SE. Others tree species refer to *Prunus* sp. and *Populus* sp. growing their roots into the cluster plots from beyond the plot border. Values in bold refer to the root abundance of those species being present in the respective tree clusters. Different small letters indicate significant differences between the three diversity levels for a given species ($P \leq 0.05$).

| | A - Ash | B - Beech | H - Hornbeam | L - Lime | M - Maple | Other tree species |
|---|---|---|---|---|---|---|
| A | **94.0 ± 2.3** a | 3.6 ± 2.1 | 2.5 ± 2.5 | 0.0 | 0.0 | 0.0 |
| B | 0.0 | **88.5 ± 4.6** a | 0.7 ± 0.7 | 9.4 ± 4.3 | 1.4 ± 1.0 | 0.0 |
| H | 11.8 ± 6.1 | 10.0 ± 3.9 | **64.8 ± 9.4** a | 9.8 ± 3.8 | 3.5 ± 3.2 | 0.0 |
| L | 2.5 ± 2.3 | 3.6 ± 3.3 | 0.6 ± 0.6 | **87.0 ± 4.4** a | 6.4 ± 1.1 | 0.0 |
| M | 17.4 ± 11.5 | 2.0 ± 1.7 | 0.2 ± 0.2 | 5.5 ± 5.2 | **74.9 ± 8.7** a | 0.0 |
| AB | **48.0 ± 18.0** | **39.1 ± 16.4** | 2.4 ± 2.4 | 2.8 ± 2.8 | 7.8 ± 5.1 | 0.0 |
| AH | **33.1 ± 2.4** | 3.9 ± 2.4 | **35.6 ± 12.6** | 4.2 ± 2.9 | 18.4 ± 12.8 | 4.7 ± 4.7 |
| AL | **70.3 ± 7.7** | 0.2 ± 0.2 | 0.0 | **24.7 ± 5.6** | 4.8 ± 2.7 | 0.0 |
| AM | **70.5 ± 8.0** | 0.0 | 0.0 | 0.3 ± 0.3 | **29.2 ± 8.1** | 0.0 |
| BH | 0.1 ± 0.1 | **44.5 ± 3.7** | **38.7 ± 2.6** | 7.6 ± 2.6 | 8.4 ± 3.2 | 0.7 ± 0.7 |
| BL | 0.4 ± 0.2 | **59.3 ± 7.7** | 1.1 ± 1.1 | **35.4 ± 6.9** | 3.9 ± 3.8 | 0.0 |
| BM | 11.6 ± 10.1 | **42.2 ± 7.5** | 0.2 ± 0.2 | 3.0 ± 1.7 | **43.0 ± 3.7** | 0.0 |
| HL | 1.5 ± 1.1 | 6.3 ± 3.8 | **55.3 ± 9.3** | **21.2 ± 5.3** | 15.7 ± 7.7 | 0.0 |
| HM | 20.7 ± 11.9 | 2.9 ± 2.0 | **25.0 ± 6.6** | 2.3 ± 2.0 | **49.1 ± 9.1** | 0.0 |
| LM | 20.5 ± 10.3 | 1.1 ± 0.6 | 5.9 ± 4.1 | **31.9 ± 4.2** | **40.7 ± 10.8** | 0.0 |
| **Mean**$_{Species}$ | **55.5 ± 9.1** b | **46.3 ± 4.5** b | **38.6 ± 6.3** b | **28.3 ± 3.2** b | **40.5 ± 4.2** b | |
| ABH | **45.6 ± 5.0** | **16.6 ± 8.5** | **12.0 ± 2.9** | 8.5 ± 5.5 | 17.4 ± 5.9 | 0.0 |
| ABL | **28.5 ± 9.8** | **25.9 ± 8.8** | 6.3 ± 4.8 | **26.3 ± 13.1** | 12.9 ± 4.1 | 0.0 |
| ABM | **46.9 ± 7.0** | **13.8 ± 5.6** | 0.0 | 10.2 ± 6.8 | **28.9 ± 4.7** | 0.2 ± 0.2 |
| AHL | **31.6 ± 10.9** | 5.3 ± 4.2 | **24.0 ± 5.3** | **30.1 ± 1.7** | 9.0 ± 4.2 | 0.0 |
| AHM | **32.0 ± 8.3** | 5.3 ± 1.9 | **35.3 ± 9.1** | 1.3 ± 0.6 | **26.1 ± 1.7** | 0.0 |
| ALM | **24.1 ± 10.0** | 0.6 ± 0.4 | 0.2 ± 0.2 | **44.0 ± 8.4** | **31.1 ± 6.7** | 0.0 |
| BHL | 9.7 ± 9.7 | **19.1 ± 9.8** | **16.3 ± 6.9** | **36.2 ± 13.0** | 18.7 ± 5.1 | 0.0 |
| BHM | 20.5 ± 9.5 | **26.6 ± 2.9** | **21.9 ± 6.8** | 4.2 ± 2.0 | **26.8 ± 5.6** | 0.0 |
| BLM | 0.8 ± 0.8 | **31.8 ± 14.5** | 0.6 ± 0.6 | **23.8 ± 5.6** | **43.0 ± 16.4** | 0.0 |
| HLM | 4.9 ± 3.4 | 3.2 ± 1.7 | **27.1 ± 3.5** | **34.8 ± 4.9** | **29.9 ± 3.4** | 0.0 |
| **Mean**$_{Species}$ | **34.8 ± 3.8** c | **22.3 ± 2.8** c | **22.8 ± 3.3** c | **32.5 ± 3.0** b | **31.0 ± 2.5** c | |

# Chapter 3

## Diversity and species identity effects on fine root production and turnover in a species-rich temperate broad-leaved forest[1]

**Andreas Jacob, Dietrich Hertel & Christoph Leuschner[1]**

[1] Under revision in: Functional Plant Biology

## Abstract

We investigated the evidence of belowground overyielding in a species-rich temperate broad-leaved forest with an ingrowth core study in 100 plots containing five common tree species (beech, lime, maple, hornbeam, ash) in monospecific and 2-species or 3-species combinations. This design allowed separating diversity and species identity effects on root dynamics in a mature forest with long continuity. Fine root production was not significantly different between monospecific and 2- or 3-species plots. For fine root turnover, a marginally significant ($P < 0.1$) increase from the monospecific to the mixed stands was found. Species identity effects on root turnover and root productivity were important. A key role is apparently played in this forest by ash which had in the mixtures the highest fine root production and root turnover of all species. However, evidence in support of a diversity effect on fine root production and turnover was weak.

Key words: belowground overyielding, *Fagus sylvatica, Fraxinus excelsior*, ingrowth cores, mixed stands, monospecific stands, root longevity

## Introduction

Much research has addressed the assumed positive effect of plant diversity on terrestrial productivity, but the bulk of studies considered only the aboveground compartment and ignored root production (Hector *et al.* 1999, Tilman 2001, Roscher *et al.* 2005). Given that roughly a third or more of global terrestrial net primary production is assumed to occur belowground (Fogel 1985, Jackson *et al.* 1997) and root production is often increased at the expense of aboveground production when soil resources are short in supply, wrong conclusions on the diversity-productivity relationship may be drawn when root growth and turnover are not considered. The last decade has seen increasing interest in the diversity-productivity relationship not only in grasslands and herbaceous communities but also in forests, including tree plantations and natural forests (Scherer-Lorenzen *et al.* 2005, Scherer-Lorenzen *et al.* 2007, Morin *et al.* 2011). Two hypotheses have been formulated explaining a positive diversity-productivity relationship (Loreau and Hector 2001). The first assumes that a mixture of species differing in functional traits can achieve a higher productivity than monocultures due to complementary resource use and/or facilitation between the species, with both mechanisms enhancing growth ('complementarity effect'). The second explanation refers to species-specific impacts on ecosystem processes since a more diverse plant community should have a higher probability of containing highly productive species than species-poor assemblages ('selection or sampling effect').

From forest growth trials and recent biodiversity experiments with planted trees it is known that certain tree species combinations can show transgressive aboveground overyielding, i.e. a higher productivity of the poly-cultures than the most productive mono-culture (Kelty 1992, 2006; Pretzsch and Schütze 2009; Potvin and Gotelli 2008). This situation may exist when species with markedly different growth and resource capture strategies are combined. A less clear picture emerged from observational studies in natural or near-natural forests, where various authors reported a generally higher productivity of species-rich forests than of monospecific stands (Erskine *et al.* 2006, Vila *et al.* 2007, Lei *et al.* 2009a, Oelmann *et al.* 2010, Paquette and Messier 2011, Gamfeldt *et al.* 2013, Vilà *et al.* 2013), while others found no relationship or a negative one (Jacob *et al.* 2010, Long and Shaw 2010). In a survey of Central European temperate forests, Szwagrzyk and Gazda (2007) detected no relationship between tree diversity and aboveground biomass which may serve as a rough proxy of productivity. Some of the cited observational studies may be confounded by incomplete control of influential factors such as variable soil conditions (Vila *et al.* 2005). It may turn out that the relationship

between species diversity and aboveground productivity in forests is dependent on climate, water availability and soil fertility and that a positive diversity effect is occurring only in certain types of plantations composed of trees with largely different functional traits or in natural forests of medium to low productivity. Under conditions of low temperature, infertile soils or drought, facilitation can be a relevant force resulting in enhanced productivity compared to the corresponding monocultures (Paquette and Messier 2011). Paquette and Messier (2011) explained the observed positive diversity effect on aboveground productivity in Canadian boreal forests mainly with positive interactions between neighbouring trees that increased productivity through facilitation in this harsh environment. A positive diversity effect on productivity was less significant in Canadian temperate forests according to these authors. It is still under debate whether diversity effects should be stronger under less favourable conditions, or rather under more benign conditions.

All cited studies considered only aboveground production (wood growth and in certain cases fine litter production), while diversity effects on belowground production have been examined in only a few recent studies. Based on ingrowth core experiments in temperate broad-leaved forests or plantations, Meinen *et al.* (2009a), Brassard *et al.* (2010, 2013) and Lei *et al.* (2012a) found evidence for a higher production of fine roots (roots ≤2 mm in diameter) in species-rich than species-poor stands or monocultures. One mechanism leading to higher fine root production of mixtures than pure stands is greater soil volume filling of fine roots due to species-specific differences in root placement, as it was shown by Brassard *et al.* (2013) for various boreal forests. However, the existing studies comparing mixed forests and monocultures were unable to distinguish between diversity and species identity effects on belowground productivity. In a replicated study in 100 plots with variable tree species composition in a temperate mixed forest, Jacob *et al.* (2013) could separate species diversity and identity effects on standing fine root biomass and found considerable root mass variation among patches dominated by different tree species, but no species diversity effect. However, fine root production was not investigated in this study.

Recent carbon (C) budget studies in forests show that the growth and turnover of fine roots represents an important source of organic matter in temperate forest soils which may exceed the C input with aboveground litter fall (Rasse *et al.* 2005). If fine root biomass and productivity were higher in mixed forests (or poly-cultures) than monocultures, increasing tree species richness could be a means of increasing the C storage and sequestration potential of the soil in managed forests and tree plantations. Through rhizodeposition and root death and subsequent

decomposition, fine roots can also have a profound effect on soil biological activity and soil chemistry (Uselman *et al.* 2012); if mixed forests were indeed more productive belowground than mono-specific stands, tree diversity could also exert an indirect positive effect on soil nutrient availability. Alternatively, increased root productivity could also lead to higher nutrient uptake, thus decreasing nutrient availability in the soil, which could outbalance the positive effect via organic matter input.

This study combines the merits of a replicated biodiversity experiment with all possible tree species combinations available with the realism of an observational study in a mature forest with long continuity to search for evidence of belowground overyielding in a species-rich temperate broad-leaved forest. The studied forest harbours a relatively high tree species diversity (up to 14 species per hectare) and consists of a mosaic of patches with low to high diversity which offers the opportunity to study forest productivity along gradients of tree species richness under comparable soil and climate conditions (Leuschner *et al.* 2009). The main objective of the study was to separate for the first time possible tree species identity and diversity effects on fine root production in a mature forest. The study design consists of three diversity levels (1-species, 2-species and 3-species plots) and all possible combinations of five common target tree species being available in fourfold replication under the conditions of a natural forest which allows a statistical analysis of diversity and species identity effects in a matrix of 100 small-scale plots.

In previous studies in the same forest (Meinen *et al.* 2009a, b; Jacob *et al.* 2013); we established a morphological key for tree species identification on the fine root level which allowed us to measure root growth in mixed stands for individual species. A comprehensive fine root inventory in the same plot matrix as used here showed marked species identity effects on standing fine root biomass but no diversity effect on biomass (Jacob *et al.* 2013). Fine root production was addressed in the study by Meinen *et al.* (2009a) which showed a significant increase in productivity from 1-species to 5-species stands in this forest, but a true diversity effect on root productivity could not be shown because the study was restricted to only 12 plots and used a dilution gradient of tree species diversity with only one tree species being present in monospecific plots.

Based on the earlier findings in this old-growth forest, we searched with the recent study for evidence of belowground overyielding and attempted to disentangle diversity and species identity effects on fine root productivity and root turnover. We applied an ingrowth core approach to all 100 plots of the diversity matrix to test the hypotheses that (i) fine root production increases with a diversity increase from 1 to

3 species, (ii) a production increase is mainly a consequence of the presence of species with particularly high root productivity (selection effect) and thus species identity effects on root production are more important than a diversity effect, and (iii) fine root turnover increases with increasing species richness due to more intense interspecific competition which may decrease fine root longevity (Beyer *et al.* 2013). Since earlier studies in this forest revealed considerable differences in fine root morphology among the species (Meinen *et al.* 2009*a*), we further hypothesized that (iv) assumed species identity effects on root production are mainly caused by differences in the specific root area and length and of mean diameter of the fine roots.

## Material and methods

*Study site*

The study was conducted in Hainich National Park (Thuringia, Germany) in the Thiemsburg and Lindig sections in the North-East of the park. The Hainich forest consists of species-rich temperate broad-leaved forest communities with European beech (*Fagus sylvatica* L.*),* Common ash *(Fraxinus excelsior* L.), hornbeam (*Carpinus betulus* L.), Small-leaved lime *(Tilia cordata* Mill.), Sycamore maple (*Acer pseudoplatanus* L.) and other species occurring either in mixture or in mono-specific patches in close proximity to each other (Leuschner *et al.* 2009). The study area has a mean annual temperature of 7.7°C and a mean annual precipitation of 590 mm $yr^{-1}$ (period 1973-2004; station Weberstedt; Deutscher Wetterdienst, 2005). All plots are located on partly Stagnic Luvisol with topsoil acidification (IUSS 2007) derived from base-rich Pleistocene loess which covers Triassic limestone as bedrock. Soil texture and the thickness of the weathered mineral soil layer on top of the bedrock were principally similar among the study plots.

Hundred small-scale plots each consisting of a group of three neighbouring trees of variable species identity (hereafter termed ‘tree clusters’) were selected on level terrain (c. 350 m a.s.l.). The clusters consisted of mature trees of one, two, or three different species which grew at a mean distance of 7.5 to 7.8 m to each other and represented all possible combinations of the five above-mentioned tree species, thus resulting in five 1-species plot types (only one tree species present), ten 2-species plot types and ten 3-species plot types (diversity levels DL1, DL2 and DL3). The mean area enclosed by the triangle of the three central trees was 21 $m^2$. Root coring was conducted in the centre of a plot in the middle of the three trees; this area (2 $m^2$) was fenced to exclude disturbance by wild boar and trampling. The

distance of this coring area to the nearest trees outside the triangle was typically >10 m which ensured that only roots of the three plot trees were present in the extracted soil samples (see also Jacob *et al.* 2013).

All plot types were replicated fourfold, resulting in 100 plots in total (20 1-species, 40 2-species and 40 3-species plots). Half of the plots (50) were located in the Thiemsburg region of the forest, the other 50 in the Lindig region about 2 km eastwards. Because the soil and stand properties were not systematically different between the two regions, all plots were pooled in the analysis. The mean minimum distance between the plots was c. 100 m, excluding any interference between neighbouring plots in terms of root system overlap or water and nutrient fluxes.

The trees in the 100 plots were all mature canopy trees of 27-32 m in height and 100-200 years in age which formed a closed canopy. The tree dimensions (diameter at breast height, tree height) were comparable between the 1-, 2- and 3-species plots with cumulative basal areas of the three cluster trees between 0.025 and 0.031 $m^2$ per $m^2$ plot area and mean breast height diameters of 0.43 - 0.45 m; the cover of juvenile trees varied between 6.2% in the 1-species plots and 8.8% in the 2-species plots (Table 3.1). Important soil chemical properties (pH, base saturation, C/N ratio) were not significantly different between the three diversity levels (Table 3.1) and the soil water content showed means of 45.7 - 46.4 vol% in summer 2009 in all diversity levels. A mull-type humus layer of about 1 cm depth was present on top of the mineral soil in all tree clusters except for plots with two or three beech trees, where thicker, less decomposed moder layers were found. The

**Table 3.1** Characteristics of stand structure and soil in 1-species, 2-species and 3-species plots (one-factorial ANOVA or Mann-Whitney *U*-test). Means ± SE (n= 20 replicate plots in the 1-species category, 40 in the 2-species and 40 in the 3-species categories). Data on basal area and tree diameter were provided by D. Seidel (unpubl.), cover values of herb layer and juvenile trees by E.A. Vockenhuber (unpubl.) and all soil chemical data are after C. Langenbruch and M. Meissner (unpubl.). None of the parameters showed significantly different means between the three diversity levels (P <0.05), as indicated by the small 'a' letters behind the figures.

| **Parameters** | **Diversity level** | | |
|---|---|---|---|
| | 1-species plots | 2-species plots | 3-species plots |
| Cumulative basal area ($m^2$ $m^{-2}$ plot area) | 0.025 ± 0.003 a | 0.031 ± 0.005 a | 0.030 ± 0.003 a |
| Mean breast height diameter (m) | 0.43 ± 0.03 a | 0.43 ± 0.01 a | 0.45 ± 0.01 a |
| Cover herbaceous layer (%) | 36.2 ± 5.0 a | 33.5 ± 3.6 a | 31.0 ± 3.1 a |
| Groundcover of juvenile trees (%) | 6.2 ± 0.7 a | 8.8 ± 0.7 a | 8.4 ± 0.7 a |
| Tree fine root biomass (g $m^{-2}$) in 0-20 cm | 242.7 ± 26.7 a | 232.1 ± 13.9 a | 207.1 ± 10.7 a |
| Herb fine root biomass (g $m^{-2}$) in 0-20 cm | 34.9 ± 5.4 a | 33.4 ± 3.6 a | 36.2 ± 4.3 a |
| pH ($H_2O$), 0-20 cm | 5.5 ± 0.2 a | 5.6 ± 0.1 a | 5.4 ± 0.1 a |
| C/N ratio (g $g^{-1}$), 0-20 cm | 12.8 ± 0.3 a | 13.1 ± 0.2 a | 12.7 ± 0.1 a |
| Base saturation (%), 0-20 cm | 84.9 ± 5.1 a | 87.7 ± 2.7 a | 89.7 ± 1.7 a |
| Mean soil temperature (°C), in 5 cm (Sept 2008-2010) | 8.33 ± 0.06 a | 8.27 ± 0.04 a | 8.30 ± 0.04 a |

soil texture of the mineral soil (0-30 cm) is characterised by high silt (about 74%) and low sand (<5%) contents (Guckland *et al.* 2009). The standing fine root biomass recorded in root inventories in early, mid and late summer 2008 in all 100 plots (Jacob *et al.* 2013) decreased slightly (but non-significantly) from the 1-species plots (242.7 g $m^{-2}$ for the 0-10 cm horizon) to the 3-species plots (207.1 g $m^{-2}$; Table 3.1); these data were used for calculating fine root turnover rate by relating measured fine root growth to standing fine root biomass. Mean daily soil temperature was continuously monitored for 24 months (September 2008-2010) in all plots at 5 cm soil depth using temperature data loggers (DS 1921 Thermochrom iButtons, Fa. Dallas Semiconductor, USA). Mean soil temperature ranged between 8.27 and 8.33°C in the three diversity levels.

*Fine root sampling*

We used the ingrowth core approach for estimating fine root production and fine root turnover in all 100 plots (Persson 1980, Powell and Day 1991, Majdi 1996, Meinen *et al.* 2009a). The shortcomings of this method, which may underestimate true fine root production, have been discussed repeatedly (e.g. Fahey *et al.* 1999, Hertel and Leuschner 2002, Hendrick *et al.* 2006). Since our study aimed at comparing different stands on similar soil with data comparability across the stands being more important than the absolute error in the root production estimates, the ingrowth core approach appeared as an appropriate technique. In April 2009, 100 ingrowth cores (one per plot) were installed within the fenced plot centres and re-sampled after 18 months in September 2010. Because the root material had to be sorted by species, the total number of ingrowth cores had to limited to 100. We preferred a replication of root sampling on the plot level (n= 4 plots) over replicated root sampling within a plot while reducing the plot number. With a sharp soil corer (diameter 3.5 cm), soil cores were extracted from the topsoil (0-20 cm), the soil material cleaned by hand from all macroscopically visible live and dead rootlets using a pair of tweezers, and the cores subsequently re-inserted into the hole. Thus, the material inside the cores was the root-free original soil. The structure and density of the extracted soil was conserved as much as possible. The core edges were marked accurately with plastic sticks at the soil surface. The start of fine root regrowth into the root-free soil in the cores after the initial disturbance was estimated by harvesting single ingrowth cores in 2-month intervals. Accordingly, fine root growth started in the bulk of the ingrowth cores around April 2010, i.e. after a 12-month lag period. Upon harvest in September 2010, the cores were collected and transferred to the laboratory for fine root extraction. The samples were soaked in water and all ingrown roots washed out

over a fine sieve (mesh size 0.25 mm). Fine roots ≥10 mm length and ≤2 mm in diameter were picked out with a pair of tweezers, sorted by tree species and separated into live and dead roots under a stereo-microscope at 6-40x magnification. For smaller root fragments, we could not be sure that they were produced during the observation period. Ignorance of this fraction must have resulted in a certain underestimation of root production. Criteria for distinguishing between live and dead rootlets were tissue elasticity, toughness, cohesion between periderm and stele and colour (Persson 1978, 1983; Leuschner *et al.* 2001; Meinen *et al.* 2009a, b; Brassard *et al.* 2010). We refrained from analysing different root order classes as suggested by Pregitzer *et al.* (2002) because the young ingrown rootlets represented mainly 1$^{st}$ and 2$^{nd}$ order rootlets. Tree species identification in the fine root fraction was labour-intensive and achieved by a classification system for the five species that bases on a set of morphological root characteristics including root colour, periderm surface structure, and branching patterns (Persson 1978, 1983; Leuschner *et al.* 2001; Meinen *et al.* 2009*a*, *b*; Brassard *et al.* 2010; Lei *et al.* 2012*a*). This detailed approach was only applied to fine root fragments ≥10 mm length that were extracted from the samples; the residual material (≤10 mm length, mostly referring to small dead root particles) was discarded. The fine root bio- and necromass fractions were dried at 70°C for 48 h and weighed. The mass of larger root segments (≥10 mm in length; live and dead) found in the cores at harvest was used as an estimate of fine root production. The figures were multiplied by a factor of 259.98 to transform core volume (3.5 cm diameter, 20 cm depth) to 1 m$^2$ ground area and 20 cm depth. Based on the assumption that root growth occurred mostly in the 6 month-period April – September 2010, we doubled the production figures to achieve fine root production per 12 months. Fine root turnover rate (unit: yr$^{-1}$) was derived by dividing annual fine root growth rate (in g m$^{-2}$ yr$^{-1}$) by the mean standing fine root biomass in the growing season (unit: g m$^{-2}$) as had been determined by Jacob *et al.* (2013) in undisturbed soil in close vicinity to the ingrowth cores in all 100 plots on three sampling dates in 2008 (Table 3.1). The root production data are expressed either in absolute figures scaled to ground area (in g m$^{-2}$ yr$^{-1}$) or were related to the cumulative basal area of the one, two or three trees of the respective species in the plot (given as production/basal area ratio). To facilitate the comparison of the three diversity levels, we then normalized the ratios to that of the 1-species plots (= 1.0).

*Statistical analyses*

All data sets were tested for normal distribution with a Shapiro-Wilk test. Data with non-normal distribution were log-transformed if possible. Comparison of means was done with one-way analysis of variance (ANOVA), followed by post-hoc comparison with Scheffe´s test in unbalanced data sets and Tukey's test in balanced data sets. If log-transformation was not possible, means of fine root growth, root turnover and fine root growth-basal area ratio were compared among species using Kruskal-Wallis single factor analysis of variance followed by a non-parametric Mann-Whitney one-sample *U*-test. To analyse the influence of tree species diversity on fine root growth or turnover, we used a Principal Components Analysis (PCA) in which species diversity was introduced through Shannon's *H'* using the relative contribution of a species to the basal area or to the standing fine root biomass in a plot as alternative measures of the relative aboveground and belowground abundance of the species. Several stand structural and soil chemical parameters as possible explaining variables were also considered in the PCA analysis. The PCA analyses were conducted with the package CANOCO, version 4.5. Subsequently, linear regression analyses were done on the dependence of fine root growth and fine root turnover on stand and soil characteristics and various fine root morphological traits. Multiple regression analyses were conducted to partition the variance in fine root growth and turnover to various soil variables (pH, C/N ratio, standing fine root biomass of a species or of all species) and fine root morphological properties (fine root diameter, specific fine root length, specific fine root tip abundance). In addition, these relationships were also analysed with linear regression analyses. To test for the dependence of fine root productivity and turnover on the diversity level and the presence of certain tree species, a Spearman rank correlation analysis and a multi-factorial analysis of variance was conducted. All analyses were performed with the software package SAS, version 9.1 (SAS Institute Inc., Cary, NC, USA). Significance was determined at $P < 0.05$ throughout; in certain cases, marginally significant differences ($0.1 < P < 0.05$) were also indicated.

## Results

*Effects of tree species diversity and species identity on fine root production and turnover*

The fine root growth rate into the cores in the 0-20 cm horizon was not significantly different between plots containing one, two or three tree species (means of 96.9 to 138.5 g $m^{-2}$ $yr^{-1}$, sum of all species present; Figure 3.1A and Table 3.2). A diversity

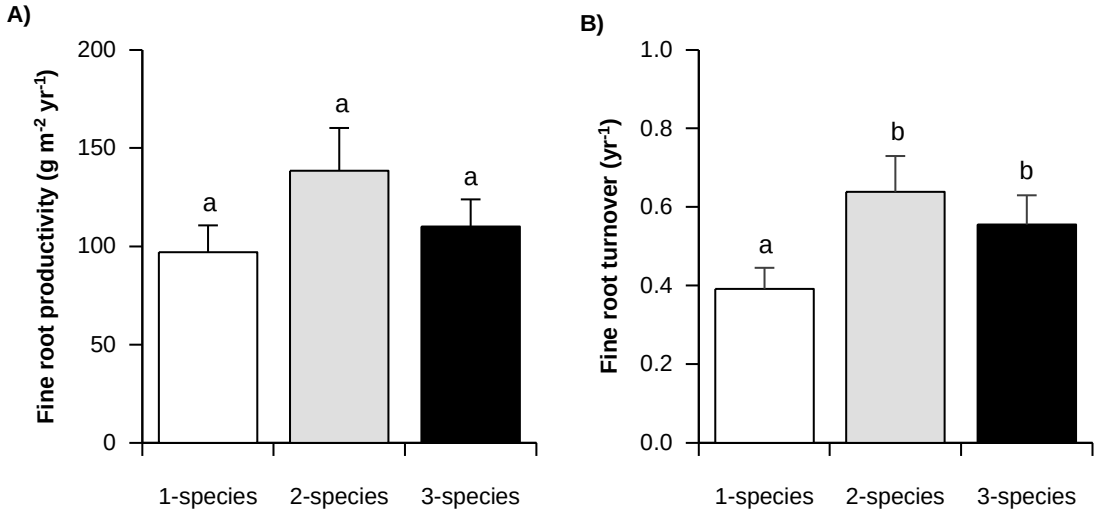


**Figure 3.1** Ingrowth of tree fine roots into root-free soil (ingrowth cores, A) and fine root turnover rate (B) in 1-species, 2-species and 3-species plots. Given are means ± SE (each four replicate plots per species combination, five to ten combinations per diversity level, 100 plots in total). The data are profile totals (all species present) of the upper 20 cm of the soil in the centre of 1-species, 2-species and 3-species plots. None of the differences were significant at P <0.05. Different letters indicate marginally significant differences (P <0.1).

effect on root productivity was also absent when species richness was measured as the number of species present in a sample's fine root biomass and not with respect to the diversity of stems in a plot (Table 3.2). This result is supported by the PCA analysis which revealed a close positive correlation of fine root growth with the first axis while the diversity measures correlated negatively with the second axis (Table 3.3). Fine root turnover (annual production divided by mean standing root biomass) was lowest in the 1-species plots (mean: 0.39 $yr^{-1}$) tended to be higher in the 2- and 3-species plots (0.64 and 0.56 $yr^{-1}$, respectively; Figure 3.1B) indicating that mean fine root longevity decreases with an increase in species richness or because certain species with high turnover were present in the more diverse plots. However, the difference between monospecific and 2- or 3-species stands was significant only at P <0.1.

**Table 3.2** Linear regression analyses between fine root growth into ingrowth cores (productivity) or fine root turnover (0-20 cm soil depth, n= 25) as dependent variables and eight stand and soil parameters of the plots. In contrast to Table 3.7, the calculations are done with the total root biomass of all species in a plot. All data were log-transformed prior to analysis. Given are $r^2$, P value and the slope b. Values in bold indicate significant relationships at P <0.05; whereas values in italics indicate marginally relationships at P <0.1. (*Spearman rank correlation analysis).

| **Variable** | **Fine root productivity** ($g\ m^{-2}\ yr^{-1}$) | | | **Fine root turnover** ($yr^{-1}$) | | |
|---|---|---|---|---|---|---|
| | $r^2$ | P | b | $r^2$ | P | b |
| Cumulative basal area ($m^2\ m^{-2}$ plot area) | <0.001 | 0.91 | 0.03 | 0.002 | 0.68 | 0.07 |
| Mean breast height diameter (m) | 0.002 | 0.67 | -0.32 | 0.002 | 0.68 | -0.21 |
| Groundcover of juvenile trees (%) | 0.010 | 0.34 | 0.13 | 0.016 | 0.21 | 0.11 |
| Tree diversity (*H*' for species richness in fine root biomass) | 0.017 | 0.21 | -0.50 | 0.021 | 0.16 | -0.40 |
| Diversity level* | -0.116 | 0.58 | - | 0.267 | 0.20 | - |
| pH ($H_2O$), 0-20 cm | 0 | 0.97 | 0.04 | <0.001 | 0.80 | -0.19 |
| C/N ratio ($g\ g^{-1}$), 0-20 cm | **0.059** | **0.01** | **-5.00** | **0.107** | **0.001** | **-4.43** |
| Base saturation (%), 0-20 cm | 0.004 | 0.54 | 0.37 | *0.030* | *0.08* | *0.68* |

At the species level, large differences in mean fine root growth rate existed among the five species in the mono-specific plots (29 - 159 g $m^{-2}\ yr^{-1}$) with lowest values in hornbeam and highest in lime (differences only significant at 0.1 > P > 0.05; Table 3.4: rows in first bloc). Root growth of lime was about five times higher than that of hornbeam and about twice as high as that of beech in the respective plots even though the species occurred with each three stems in the respective plots. Maple and ash reached intermediate growth rates. Comparing the root growth of a species in mono-specific plots with that in 2-species- and 3-species plots showed a tendency toward a higher root production in mixture for ash, maple and lime, but a lower production in hornbeam, when growth was normalized by standing fine root biomass (relative growth rate) and related to the species' basal area (difference between diversity levels significant in hornbeam, marginally significant in ash; Table 3.4: rows in second bloc). Fine root turnover showed rather similar rates for the five species in the 1-species plots (means of 0.4 to 0.7 $yr^{-1}$; species differences not significant; Table 3.4: rows in third bloc), but revealed significant species differences (P <0.05) in the 2-species- and 3-species plots (ash > beech in 2-species plots; ash > all other species, and lime and maple > hornbeam in 3-species plots). Moreover, turnover

tended to be higher in ash, beech and maple in 2- and 3-species plots than in the respective 1-species plots, but the differences were not significant (Table 3.4: rows in third bloc).

**Table 3.3** Results of a Principal Components Analysis (PCA) on the differentiation of the 100 cluster plots with respect to tree species diversity, stand structural characteristics and root growth-related traits. Given are the loadings of the selected variables along the first four explanatory axes. Numbers in brackets below the axes indicate the eigenvalues (EV) of the axes. Figures in bold mark variables with closest correlation to the respective axis. FRP – fine root productivity, FRT – fine root turnover, BA – basal area.

| Variable | Axis 1 (EV 0.147) | Axis 2 (EV 0.120) | Axis 3 (EV 0.093) | Axis 4 (EV 0.087) |
|---|---|---|---|---|
| DIVERSITY PARAMETERS | | | | |
| Diversity level | 0.491 | **-0.825** | -0.144 | 0.075 |
| Tree diversity (*H*' for species richness in cumulative basal area) | 0.516 | **-0.821** | -0.138 | 0.056 |
| Tree diversity (*H*' for species richness in fine root biomass) | 0.413 | **-0.836** | -0.149 | 0.059 |
| STAND STRUCTURAL PARAMETERS | | | | |
| Cumulative basal area | 0.067 | -0.084 | 0.358 | -0.097 |
| Mean breast height diameter | 0.002 | -0.149 | -0.196 | -0.049 |
| Groundcover of juvenile trees | 0.012 | -0.136 | 0.093 | 0.036 |
| SOIL PROPERTIES | | | | |
| C/N ratio | -0.277 | -0.087 | -0.065 | -0.163 |
| Base saturation | 0.364 | 0.031 | 0.126 | 0.297 |
| ROOT-RELATED PARAMETERS | | | | |
| Standing fine root biomass | -0.159 | 0.108 | 0.353 | 0.026 |
| Fine root growth rate | **0.682** | 0.419 | 0.291 | -0.072 |
| Fine root turnover rate | **0.811** | 0.328 | 0.148 | -0.194 |
| FRP_Ash | **0.608** | 0.314 | -0.035 | 0.249 |
| FRP_Beech | -0.281 | -0.073 | 0.038 | -0.470 |
| FRP_Hornbeam | 0.495 | 0.306 | -0.408 | **-0.505** |
| FRP_Lime | 0.089 | 0.281 | 0.130 | 0.489 |
| FRP_Maple | 0.167 | -0.047 | **0.754** | -0.304 |
| FRP/BA ratio_Ash | 0.437 | -0.174 | -0.051 | 0.311 |
| FRP/BA ratio_Beech | -0.243 | -0.182 | -0.263 | -0.334 |
| FRP/BA ratio_Hornbeam | -0.073 | 0.101 | -0.477 | -0.230 |
| FRP/BA ratio_Lime | -0.016 | -0.119 | -0.139 | 0.389 |
| FRP/BA ratio_Maple | 0.088 | -0.333 | 0.430 | -0.385 |
| FRT_Ash | **0.646** | 0.283 | -0.288 | -0.123 |
| FRT_Beech | -0.059 | -0.210 | 0.148 | -0.301 |
| FRT_Hornbeam | 0.471 | 0.246 | -0.408 | -0.470 |
| FRT_Lime | 0.257 | 0.154 | 0.129 | 0.475 |
| FRT_Maple | 0.299 | -0.181 | **0.625** | -0.287 |

The significant influence of species identity on fine root growth and turnover was underscored by the results of the PCA analysis that showed a close positive correlation of the production of ash roots, and of plot-level root production and turnover with the first PCA axis (eigenvalue 0.147); less tight was the correlation of

**Table 3.4** Fine root growth into ingrowth cores (productivity), fine root production/basal area ratio normalised to the 1-species plots, and fine root turnover rate of the five tree species in the upper soil profile (0-20 cm) in the three plot categories (diversity levels) (Kruskal-Wallis single-factor analysis of variance followed by a non-parametric Mann-Whitney one-sample *U*-test). Given are means ± SE (number of replicate plots in brackets). Different small letters indicate significant differences between the three diversity levels for a given species, different capital letters significant differences between the species in a diversity level ($P < 0.05$); letters in italics: $P < 0.1$.

| | Ash | Beech | Hornbeam | Lime | Maple | Mean of the five species |
|---|---|---|---|---|---|---|
| FINE ROOT GROWTH (g $m^{-2}$ $yr^{-1}$) | | | | | | |
| 1-species plots | 98.1 ± 36.5 a *B* (n= 4) | 79.7 ± 11.6 c *B* (n= 4) | 29.2 ± 9.6 b *A* (n= 4) | 158.9 ± 99.7 a *AB* (n= 4) | 101.6 ± 65.6 *ab AB* (n= 4) | 93.5 ± 24.3 *c* |
| 2-species plots | 118.3 ± 33.7 a *C* (n= 16) | 41.8 ± 12.4 b *AB* (n= 16) | 32.2 ± 11.3 b *A* (n= 14) | 44.2 ± 13.7 a *AB* (n= 16) | 63.7 ± 21.1 *b BC* (n= 14) | 60.7 ± 9.7 *b* |
| 3-species plots | 70.5 ± 15.3 a *D* (n= 23) | 17.6 ± 5.3 a *B* (n= 21) | 14.9 ± 8.0 a *A* (n= 20) | 23.8 ± 5.5 a *BC* (n= 22) | 33.7 ± 9.0 *a C* (n= 22) | 32.9 ± 4.7 *a* |
| NORMALISED FINE ROOT PRODUCTION/ BASAL AREA RATIO | | | | | | |
| 1-species plots | 1.0 *a* (n= 4) | 1.0 ab (n= 4) | 1.0 b (n= 4) | 1.0 a (n= 4) | 1.0 a (n= 4) | 1.0 a |
| 2-species plots | 1.2 ± 0.3 *a* AB (n= 16) | 1.3 ± 0.2 b B (n= 16) | 0.7 ± 0.2 b A (n= 14) | 1.3 ± 0.3 a AB (n= 16) | 1.2 ± 0.3 a AB (n= 14) | 1.2 ± 0.1 a |
| 3-species plots | 2.6 ± 0.5 *b D* (n= 23) | 0.8 ± 0.2 a *B* (n= 21) | 0.3 ± 0.1 a *A* (n= 20) | 1.3 ± 0.4 a *BC* (n= 22) | 1.4 ± 0.3 a *C* (n= 22) | 1.3 ± 0.2 a |
| FINE ROOT TURNOVER ($yr^{-1}$) | | | | | | |
| 1-species plots | 0.4 ± 0.2 a A (n= 4) | 0.3 ± 0.03 a A (n= 4) | 0.4 ± 0.2 *b* A (n= 4) | 0.7 ± 0.4 a A (n= 4) | 0.4 ± 0.2 a A (n= 4) | 0.4 ± 0.1 *a* |
| 2-species plots | 1.7 ± 0.8 a B (n= 16) | 0.6 ± 0.3 a A (n= 16) | 0.6 ± 0.2 *b* AB (n= 14) | 0.9 ± 0.3 a AB (n= 16) | 0.7 ± 0.2 a AB (n= 14) | 0.9 ± 0.2 *a* |
| 3-species plots | 1.2 ± 0.3 a C (n= 23) | 0.9 ± 0.4 a BC (n= 21) | 0.3 ± 0.1 *a* A (n= 20) | 0.6 ± 0.2 a B (n= 22) | 0.7 ± 0.3 a B (n= 22) | 0.7 ± 0.1 a |

maple root growth to axis 3 (eigenvalue 0.093; positive association) and hornbeam root growth to axis 4 (eigenvalue 0.087; negative association; Table 3.3). The species identity effect on root growth and turnover is also evident from the multi-factorial analysis of variance which showed no diversity effect, but a significant effect of the presence of ash in a plot (but not of the other species), on root production and turnover (Table 3.5). Fine root production in a plot was particularly high when ash roots were present (148 g $m^{-2}$) as compared to 98 – 122 g $m^{-2}$ in all plots with presence of beech, hornbeam, lime or maple (Table 3.6).

**Table 3.5** Results of a multi-factorial analysis of variance on the influence of the diversity level in the plots (dl), the presence of the five tree species in the plots (p_Ash, p_Beech, p_Hornbeam, p_Lime, p_Maple) and the interaction between diversity level and the presence of one of these species on fine root productivity or fine root turnover in the 100 plots. Given are the P and F values of the source variables and the coefficient of determination ($r^2$) of the model.

| **Source** | **Dependent variable** | |
|---|---|---|
| | Fine root productivity (g $m^{-2}$ $yr^{-1}$) | Fine root turnover ($yr^{-1}$) |
| dl | 0.79 (n.s.) | 1.62 (n.s.) |
| p_Ash | 3.82 (P <0.05) | 5.25 (P <0.05) |
| p_Beech | 0.15 (n.s.) | 1.01 (n.s.) |
| p_Hornbeam | 0.02 (n.s.) | 0.38 (n.s.) |
| p_Lime | 1.72 (n.s.) | 1.41 (n.s.) |
| p_Maple | 0.99 (n.s.) | 0.79 (n.s.) |
| dl x p_Ash | 2.45 (P <0.1) | 2.73 (P <0.1) |
| dl x p_Beech | 2.74 (P <0.1) | 4.78 (P <0.01) |
| dl x p_Hornbeam | 1.59 (n.s.) | 0.80 (n.s.) |
| dl x p_Lime | 1.95 (n.s.) | 1.19 (n.s.) |
| dl x p_Maple | 1.47 (n.s.) | 1.23 (n.s.) |
| Model $r^2$ | 0.23 (P <0.05) | 0.27 (P <0.01) |

With respect to root turnover, the presence of ash tended to increase the root turnover of the community (or decrease mean root longevity) as is visible in the higher turnover in all plots containing ash (Table 3.6). Nevertheless, the overall influence of species identity on fine root growth and turnover was relatively small compared to other biotic and abiotic factors despite the significant ash effect on production and turnover. Models describing the effects of tree species presence and of diversity on fine root production and turnover had coefficients of determination of only 0.23 and 0.27, respectively (Table 3.5).

**Table 3.6** Fine root productivity and turnover (0-20 cm profile; sum of all species present) in plots where ash, beech, hornbeam, lime or maple trees were present. Given are means ± SE (Kruskal-Wallis single factor analysis of variance followed by a non-parametric Mann-Whitney one-sample *U*-test; n = no. of plots). Different letters indicate significant differences between the five plot types (P <0.05).

| Species | n | Fine root productivity ($g\ m^{-2}\ yr^{-1}$) | Fine root turnover ($yr^{-1}$) |
|---|---|---|---|
| Ash | 43 | 147.5 ± 19.8 b | 0.8 ± 0.1 b |
| Beech | 41 | 97.5 ± 10.0 a | 0.5 ± 0.1 a |
| Hornbeam | 38 | 114.2 ± 15.9 ab | 0.6 ± 0.1 ab |
| Lime | 42 | 114.6 ± 19.2 a | 0.6 ± 0.1 a |
| Maple | 40 | 121.6 ± 13.5 ab | 0.6 ± 0.1 ab |

*Environmental and biological determinants of fine root productivity and turnover*

The growth of fine roots into the ingrowth cores was neither dependent on the size or density of the trees in the plots (mean DBH and cumulative basal area per plot) nor on soil pH or base saturation (Table 3.2); topsoil C/N ratio was identified as the only influential abiotic factor (P= 0.01; negative relation). A similar pattern was found for fine root turnover with a significant effect on C/N ratio (P <0.001; negative relation) and soil acidity (P >0.08; positive relation) but no relation to tree size and density (Table 3.2). When analysing the five species separately in the respective 1-species plots, the dominant effect of soil C/N ratio was also visible, but it was significant only for hornbeam (P= 0.05; negative relation; Table 3.7). In the case of root turnover, the negative C/N effect existed only for hornbeam and was only marginally significant (P= 0.1).

The five species varied with respect to important root morphological traits and it appears that this variation was larger in mixed plots with interspecific root competition than in monospecific plots where intraspecific competition was the dominant force (Supplementary material, Appendix Table 3.A1). Mean fine root diameter was largest in ash (0.56-0.60 mm; difference significant to hornbeam and maple in 1-species plots and to all other species in 2- and 3-species plots), but more or less similar in the other four species (0.35-0.53 mm). Large species differences existed also with respect to the abundance of fine root tips with means of only 2.6-4.5 tips $mg^{-1}$ in ash and much higher frequencies in hornbeam, lime and maple (10.5-16.8); beech fine roots were characterized by significantly larger frequencies in the mixed plots (21.6-29.0). The species differences in specific fine root length (SRL) and area (SRA) were less pronounced. It appears that higher species richness led to altered fine root morphology in some of the species.

**Table 3.7** Linear regression analyses between fine root growth into ingrowth cores (productivity, FRP) or fine root turnover (FRT, 0-20 cm soil depth) as dependent variables and six stand structural and soil chemical parameters for the five tree species (each four replicate monospecific plots per species). All data were log-transformed. Given are $r^2$, P value and the slope b. Values in bold indicate significant relationships at P <0.05 (relationships at 0.1 > P > 0.05 are printed in italics).

| | | Ash | | Beech | | Hornbeam | | Lime | | Maple | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | | FRP | FRT | FRP | FRT | FRP | FRT | FRP | FRT | FRP | FRT |
| Cumulative basal area ($m^2$ $m^{-2}$ plot area) | $r^2$ | 0.614 | 0.578 | 0.092 | 0.093 | *0.833* | *0.814* | 0.502 | 0.022 | 0.592 | 0.679 |
| | P | 0.22 | 0.24 | 0.69 | 0.69 | *0.08* | *0.10* | 0.29 | 0.84 | 0.23 | 0.18 |
| | b | -0.23 | -2.61 | 0.16 | -0.12 | *1.01* | *1.58* | -3.69 | 0.41 | 1.63 | 1.21 |
| Mean breast height diameter (m) | $r^2$ | 0.633 | 0.667 | 0 | 0.011 | 0.255 | 0.166 | 0.662 | 0.009 | 0.519 | 0.439 |
| | P | 0.21 | 0.18 | 0.99 | 0.89 | 0.50 | 0.59 | 0.19 | 0.90 | 0.28 | 0.34 |
| | b | 10.30 | 12.75 | -0.002 | -0.10 | 2.61 | 3.34 | 35.47 | -2.25 | -3.90 | -2.49 |
| Groundcover of juvenile trees (%) | $r^2$ | 0.282 | 0.283 | 0.255 | 0.010 | 0.001 | 0.027 | 0.152 | 0.064 | 0.348 | 0.334 |
| | P | 0.47 | 0.47 | 0.50 | 0.68 | 0.97 | 0.83 | 0.61 | 0.74 | 0.41 | 0.42 |
| | b | 0.27 | 0.32 | -0.46 | -0.20 | -0.02 | -0.18 | 1.16 | -0.40 | -1.18 | -0.81 |
| pH ($H_2O$), 0-20 cm | $r^2$ | 0.240 | 0.264 | 0.111 | 0.191 | 0.012 | 0.040 | 0.306 | 0.624 | 0.051 | 0.111 |
| | P | 0.49 | 0.49 | 0.66 | 0.56 | 0.89 | 0.79 | 0.45 | 0.21 | 0.77 | 0.66 |
| | b | 4.68 | 5.92 | -1.14 | -1.07 | -0.66 | -1.93 | 14.78 | 11.23 | 3.85 | 3.94 |
| C/N ratio (g $g^{-1}$), 0-20 cm | $r^2$ | 0.254 | 0.239 | 0.696 | 0.790 | **0.901** | *0.803* | 0.014 | 0.588 | 0.023 | 0.045 |
| | P | 0.50 | 0.49 | 0.17 | 0.11 | **0.05** | *0.10* | 0.88 | 0.23 | 0.84 | 0.78 |
| | b | -18.90 | -22.08 | 2.84 | 2.17 | -17.6 | *-26.27* | -13.61 | 47.57 | 13.41 | 12.96 |
| Base saturation (%), 0-20 cm | $r^2$ | 0.234 | 0.265 | 0.264 | 0.324 | 0.325 | 0.217 | 0.018 | 0.784 | 0.755 | *0.837* |
| | P | 0.48 | 0.49 | 0.49 | 0.43 | 0.43 | 0.47 | 0.86 | 11.44 | 0.13 | *0.08* |
| | b | 18.46 | 23.70 | -0.23 | -0.18 | 2.34 | 3.03 | 6.01 | 21.02 | 194.42 | *142.31* |

The mean fine root diameter of beech and lime significantly decreased from the 1-species to the 2- and 3-species plots and, in beech, this change was associated with increases in SRL (significant) and SRA (not significant; Supplementary material, Appendix Table 3.A1). Ash and lime significantly increased the number of root tips per unit root mass from 1-species to 2- and 3-species plots. The apparent diversity effect on fine root morphology is visible also from correlation analyses between fine root diameter and diversity level (beech: $P < 0.001$), SRL (beech: $P < 0.05$) and root tip abundance (ash: $P < 0.05$; Supplementary material, Appendix Table 3.A1).

The results of a multiple regression analysis showed that the studied ~10 root morphological, stand structural and soil chemical parameters had only a small influence on the fine root growth and turnover of the five species (Table 3.8: $r^2$ mostly <0.25); specific root length and the presence of ash roots were identified as the most influential parameters. The model for the whole data set (all five species) indicated the important role played by the presence of ash for root productivity but did not show significant effects of the species' root morphology.

**Table 3.8** Results of multiple linear regression analyses (forward selection procedure) on the influence of important soil chemical, root morphological and stand structural parameters on the fine root productivity and fine root turnover of the five species. Given are the coefficients of determination (model $r^2$, partial $r^2$) for each model as well as parameter estimates for the variables with significant influence that were included in the models, and the F and P values for these predictors. Selected predictor variables were the total basal area of the plot (BA), the species presence in a plot (p_species), the fine root biomass of a species (normalized to the species' basal area in the plot), pH, soil C/N, and the fine root diameter (dia_species), root tip frequency (tips_species) or specific root length of the species (SRL_species). For species not listed in the table, a model with $P < 0.1$ could be developed.

| | Model $r^2$ | Partial $r^2$ | Predictor | Parameter estimate | F value | P value |
|---|---|---|---|---|---|---|
| FINE ROOT PRODUCTIVITY (g $m^{-2}$ $yr^{-1}$) | | | | | | |
| Ash | 0.13 | 0.13 | tips_Ash | -14.53 | 6.01 | <0.05 |
| Beech | 0.23 | 0.23 | BA | 587.0 | 11.68 | <0.01 |
| Hornbeam | 0.08 | 0.08 | pH | 16.43 | 3.34 | <0.1 |
| Lime | 0.14 | 0.14 | dia_lime | 272.1 | 6.68 | <0.05 |
| | 0.30 | 0.16 | SRL_Lime | 5.34 | 8.66 | <0.01 |
| All species | 0.09 | 0.09 | p_Ash | 51.48 | 19.83 | <0.0001 |
| FINE ROOT TURNOVER ($yr^{-1}$) | | | | | | |
| Ash | 0.07 | 0.07 | pH | 0.86 | 2.99 | <0.1 |
| | 0.21 | 0.06 | SRL_Ash | 0.15 | 3.08 | <0.1 |
| Beech | 0.53 | 0.53 | SRL_Beech | 0.059 | 44.00 | <0.0001 |
| Hornbeam | 0.12 | 0.12 | pH | 0.341 | 4.56 | <0.05 |
| | 0.19 | 0.07 | SRL_Hornbeam | 0.019 | 2.93 | <0.1 |
| Maple | 0.13 | 0.07 | dia_Maple | -2.74 | 3.05 | <0.1 |
| All species | 0.08 | 0.08 | SRL_Species | 0.04 | 16.36 | <0.0001 |
| | 0.13 | 0.05 | p_Ash | 0.82 | 11.34 | <0.001 |
| | 0.14 | 0.01 | pH | 0.23 | 3.07 | <0.1 |
| | 0.15 | 0.01 | dia_Species | 1.83 | 3.35 | <0.1 |

## Discussion

*Diversity effects on fine root production*

The ingrowth core study in 100 plots differing in tree species diversity (one to three species) did not reveal a significant positive diversity effect on fine root production neither in the multiple regression analysis nor in the PCA. These findings match with the results of an inventory of standing fine root biomass in the 100 plots conducted in the growing season 2008 showing no influence of diversity on mean fine root biomass (Jacob *et al.* 2013). From a theoretical point of view, root production should increase with diversity when co-occurring tree species have largely different root system architectures and soil space exploration strategies or different root growth phenologies resulting in the complementary use of soil resources. However, the few existing studies on the diversity - fine root productivity relationship in mixed forests or plantations produced controversial results. A comparison of mixed (4- to 5-species) and monospecific boreal forests showed a significantly higher fine root production of the mixed stands (Brassard *et al.* 2010) while a mixed stand of Sitka spruce and Scots pine had a by 50% smaller fine root production than the respective pure stands (McKay and Malcom 1988). Brassard *et al.* (2013) reported higher fine root production in evenly-mixed than single-species boreal forests, but they found no effect of tree species richness. Two studies with mixtures of planted young (3- to 6-yrs-old) trees found higher root production in poly-cultures of two to four species than in the monocultures (Fredericksen and Zedaker 1995, Lei *et al.* 2012a), but the increase was only weak and partly insignificant in one of the studies. The latter results from young trees with expanding root systems can probably not be transferred to mature forests. Meinen *et al.* (2009a) applied two independent techniques of fine root production measurement to a tree diversity gradient in the Hainich forest in close proximity to this study and found a significant increase in fine root production with both approaches. These results are not fully in agreement with the findings of the recent study in the Hainich forest where no significant trend, but only a non-significant tendency, for a productivity increase from the 1-species to the 2-species plots was found. However, plot size and sampling design were largely different between the two approaches (50 m x 50 m *versus* 2 m x 2 m plot size, 12 *versus* 100 plots, 24 *versus* one sample per plot) and the data were collected in two different summers.

From the existing information on the diversity – root productivity relation in forests, we conclude that a productivity increase with increasing tree species richness seems not to be the rule. Positive diversity effects on belowground

productivity do most likely exist in certain tree plantations and agroforestry systems (tropical and temperate), when largely different tree functional types with contrasting rooting patterns are present. Further, in the harsh boreal climate, it is not unlikely that the root production of mixed stands is somewhat higher than that of monospecific stands due to facilitation effects (Brassard *et al.* 2010) or as a consequence of more complete soil space filling in mixed forests (Brassard *et al.* 2013). Facilitative interactions which favour belowground production are less likely in temperate and tropical forests, unless moisture is limiting. Thus, the significance of belowground overyielding in forests may also depend on climate. Further, young stands with expanding root systems could well differ from mature stands where the rooting space is fully explored (Uselman *et al.* 2007, Brassard *et al.* 2009, Yuan and Chen 2012).

A major shortcoming of most studies on belowground overyielding is that the design does not allow to separate species identity and diversity effects. This can only be achieved in experiments with planted tree species combinations (Lei *et al.* 2012a, b) or in observational studies in mixed forests with careful selection of tree neighbourhood constellations (Leuschner *et al.* 2009) as was done in this study. To our knowledge, our investigation is the first that separated species diversity and identity effects in a mature forest with long stand continuity.

An interesting finding of our study is the apparent increase in fine root turnover rate with increasing tree species richness, i.e. an about 40-60% higher turnover (or shorter mean root longevity) in the 2- and 3-species plots than in the mono-specific plots (difference marginally significant at $P < 0.1$). It appears that in three of the five species, the fine roots tended to live longer in plots where only roots of the same species were present as compared to mixed plots. This agrees with the observation of Meinen *et al.* (2009a) in 1-, 3- and 5-species stands in the Hainich forest that turnover was significantly higher in the species-richer stands. Similarly, Lei *et al.* (2012a) measured a higher root turnover in mixed than monospecific plots of a plantation of juvenile temperate broad-leaved trees. A closer look on the turnover rates obtained for the five tree species in the 1-, 2- and 3-species plots in Hainich forest shows that the acceleration of turnover appears to be a consequence of root growth and survival responses to the presence of certain allospecific roots in the plots, in other words, is caused by species-specific effects and not by the influence of species diversity *per se*. Neither the regression analyses (linear and multiple) nor the PCA showed a positive diversity effect on turnover. The mechanism causing the apparent reduction in root lifespan in the mixed stands must necessarily remain speculative. One possible explanation, which is supported by

some empirical evidence in the Hainich forest, is that interspecific belowground competition for water may have been more intense than intraspecific competition, resulting in a greater depletion of soil moisture reserves in part of the growing season in the mixed as compared to the mono-specific stands. Gebauer *et al.* (2012) measured a 50% higher canopy transpiration of a mixed lime-ash-maple-hornbeam-beech stand than of two nearby stands with moderate to high beech abundance in the Hainich forest in summer 2005 with average rainfall which was attributed to a particularly large soil moisture extraction of the lime trees in the mixed stand; lime was found to have a relatively high transpiration rate per unit ground area. Data on soil moisture variation in these stands support the view that the studied Hainich mixed forests are depleting the soil water reserves under certain conditions more completely than the beech monocultures (Krämer and Hölscher 2010). Temporarily drier soils in the mixed stands may well be a reason for a shorter fine root lifespan and higher root turnover, if increased root mortality is leading to the compensatory increase in fine root growth as it was observed in several temperate broad-leaved mixed forests (Pregitzer *et al.* 1993, Rewald and Leuschner 2009).

*Species identity effects on fine root production*

In contrast to species diversity, the effect of species identity on fine root productivity was pronounced. When comparing all 38-43 plots (1-species, 2-species and 3-species) in which a species was present, we found a more than 30% higher productivity in plots with ash presence than in plots where ash was absent (difference significant at $P < 0.05$; data not shown). The presence of ash seemed to increase plot-level fine root production in the 2-species and 3-species plots. The PCA analysis revealed a close association of total fine root production (all species in a plot) with the production of ash roots; both parameters had high loadings on the first PCA axis (eigenvalue 0.147). A similar relation existed for root turnover indicating that a high presence of ash fine roots in a plot increased fine root production through a decreased root longevity of certain species when growing in mixture. The specific effect of ash roots in the mixed plots was also visible in the multiple regression analysis, which contrasts with the influence of the other four species: neither lime nor maple or hornbeam significantly impacted on plot-level root production or turnover; for beech, a significant effect on root turnover was found.

Root production studies in mixed forests with species identification conducted by sequential coring or with ingrowth cores have repeatedly found hints on significant species differences in fine root production when root growth was related to the species' abundance in the stand. For example, Hertel (1999) and

Leuschner *et al.* (2001) found a higher fine root production per basal area of European beech than of Sessile oak (*Quercus petraea*) in a mixed stand on sandy soil. Pronounced species differences in fine root production are also indicated by the fact that, in Hainich forest, the species differences in root production largely exceeded the differences in stand basal area in the mono-specific plots.

Since the five early-/mid- to late-successional tree species of the Hainich forest differed markedly in their fine root morphology, we asked whether fine root productivity depended of root morphological traits and whether the traits varied between monospecific and mixed plots in response to increasing interspecific competition. While it has been assumed that the fine roots of fast-growing trees are thinner with a higher specific root length and larger number of root tips than those of slow-growing trees (Comas *et al.* 2002, Comas and Eissenstat 2004), our root morphological data do not fit into this picture. Early-/mid- successional ash with rapid growth at young age had the thickest roots in the ≤2 mm diameter class with smallest specific root length and a very small number of tips while maple roots were relatively thin, even though this species is not a typical fast-growing early-successional species. In an attempt to identify root growth-controlling environmental and biotic factors for the five species, we regressed important root morphological, soil chemical and stand structural variables on fine root growth and turnover. The multiple regression analysis gave only low coefficients of determination for the models indicating that plant-internal factors such as carbohydrate supply (that was not measured) must be more influential for fine root growth than the parameters considered. The influence of fine root morphology on root productivity and turnover was generally weak. The expected increase in turnover with an increase in specific root length or a decrease in mean fine root diameter was found in four of the five species (except for lime) while root morphology influenced root production in only one species (lime).

In a common garden experiment, Withington *et al.* (2006) found median fine root life span as estimated from minirhizotron observation to vary from 0.5 to 2.5 $yr^{-1}$ among eleven temperate tree species. Comas and Eissenstat (2004) stated that the fine roots of fast-growing tree species generally have higher turnover rates than the roots of slow-growing species. Thus, we expected that the five species should also differ with respect to root longevity. However, we found more or less similar turnover rates in the monospecific plots (0.3–0.7 $yr^{-1}$), irrespective of the species' height growth rates (relatively fast growing ash versus slow growing beech).

Ingrowth core measurements of fine root production represent well-defined disturbances of the root system that may be used to assess the belowground

resilience of trees and forests. Accordingly, Meinen *et al.* (2009a) interpreted the increase in turnover rates from the 1-species to the 5-species plots (from 0.26 to 0.51 $yr^{-1}$) as an indication that a forest with a higher tree species diversity recovers more rapidly in its fine root system after a topsoil disturbance than a species-poor stand, thus supporting the predictions of the insurance hypothesis of biodiversity (Yachi and Loreau 1999). While our results tend to support this conclusion, an alternative explanation has also to be considered. With three of the five species apparently facing a decrease in their fine root longevity in the presence of allospecific belowground competitors as compared to growth in monospecific stands, we hypothesize that the speeding-up of root turnover in the mixed stands could also in part be a stress response of certain tree species in response to the presence of roots of other species as was found in a growth chamber experiment by Beyer *et al.* (2013). Well-designed root growth experiments with variable species combinations are needed to understand fine root growth responses to allospecific competitors. Such studies should preferably be conducted under field conditions in the forest using experimental tools such as the *in situ* root growth chamber (Hertel and Leuschner 2006) and they best should examine changes in root lifespan for root segments defined by their root order (Pregitzer *et al.* 2002).

## Conclusions

Our study is probably the first that examined the diversity – belowground productivity relationship in a mature forest with long continuity and that could separate between diversity and species identity effects. In support of our hypothesis #2, we found several indications that certain tree species with specific characteristics in terms of root morphology, root growth, root physiology and, perhaps, also responsiveness to root competition have an over-proportional influence on the fine root productivity at the community level. The presence of such a species (in our case ash) may increase fine root production and turnover in more species-rich communities through a selection effect, supporting hypothesis #2. In contradiction to our hypothesis #4, the specific morphology of the species' fine roots was apparently not an important determinant of fine root productivity. A key finding is that diversity *per se*, either through complementary use of soil resources and/or belowground facilitation, was not a factor enhancing root productivity in the Hainich forest, contradicting our hypothesis #1. One reason is that spatial root system segregation of the competing species does not play an important role at this site (Meinen *et al.* 2009c, Jacob *et al.* 2013). It has to be kept in mind that some of the results of this study may perhaps

be confounded with some variation in site conditions; this is a widespread limitation in observational studies in forests, but may also apply to large-scale diversity experiments with planted trees. By selecting the plots in a stratified random procedure, we tried to reduce this factor.

One cause of the recent interest in studies on possible belowground overyielding in forests is its possible relevance for the forest carbon cycle. If higher species richness would enhance the root-mediated C flux from the trees to the soil, and the soil C store were higher in mixed than in monospecific forests, this would represent an additional incentive in the light of trace gas emission reduction goals to prefer mixed stands over the widely planted mono-specific stands in forestry. Our study provides some evidence in support of this idea since fine root turnover increased by ~40-50% from the 1-species to the 2- and 3-species (though only at marginal significance), while standing fine root biomass remained roughly stable. The turnover increase with diversity was significant and larger in the study of Meinen *et al.* (2009*a*) in this forest, indicating an enhancement of belowground C sequestration with increasing tree species richness. However, even if root production and turnover were significantly higher in species richer stands in all years, the additional flux of carbon to the soil is not larger than ~10-40 g C $m^{-2}$ $yr^{-1}$ in excess of the monospecific stands, which is in the range of c. 1-6% of total (above- and belowground) NPP (Ellenberg and Leuschner 2010). Moreover, the organic carbon stocks in the soil were significantly influenced by the species (higher forest floor stores under beech) but not by diversity (Guckland *et al.* 2009; Langenbruch *et al.* 2011). Thus, the C storage and sequestration potential of a somewhat increased fine root production and turnover in temperate broad-leaved mixed forests would in any case be of only marginal relevance for the trace gas balance of European temperate forests. Other possible belowground consequences of higher tree species diversity might be of greater relevance for the functioning of forests. Further investigation has to show whether more diverse forests also possess a higher diversity of tree root functionality in terms of nutrient and water uptake strategies, harbour more diverse communities of mycorrhizal fungi and soil microbes, and show a greater variability of root exudate fractions.

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**Table 3.A1** Fine root morphological characteristics of the five tree species in the 1-species- (n= 4), 2-species- (n= 16) and 3-species plots (n= 24). Given are means ± SE for the three diversity levels and the r and P values of linear Pearson ($r_P$) or Spearman ($r_s$) correlation analyses for the relationships between morphological root traits and diversity level. Significant relationships (P <0.05) are printed in bold. Different capital letters indicate significant differences between species, different small letters significant differences between the three diversity levels for a given species (P <0.05).

| **Species** | **Diversity level (DL)** | | | | |
|---|---|---|---|---|---|
| | 1-species plots | 2-species plots | 3-species plots | r | *P* |
| FINE ROOT DIAMETER (mm) | | | | | |
| Ash | 0.60 ± 0.08 a A | 0.56 ± 0.01 a A | 0.58 ± 0.03 a A | -0.090 | 0.56 |
| Beech | 0.49 ± 0.04 a ABC | 0.39 ± 0.02 b B | 0.35 ± 0.01 c B | **-0.525** | **<0.001** |
| Hornbeam | 0.40 ± 0.02 a BC | 0.41 ± 0.01 a BC | 0.45 ± 0.02 a C | 0.235 | 0.13 |
| Lime | 0.53 ± 0.09 a AB | 0.40 ± 0.01 b B | 0.42 ± 0.01 b CD | -0.075 | 0.63 |
| Maple | 0.36 ± 0.02 a C | 0.36 ± 0.01 a C | 0.40 ± 0.01 a BD | 0.097 | 0.53 |
| SPECIFIC FINE ROOT LENGTH (m $g^{-1}$) | | | | | |
| Ash | 11.3 ± 2.9 a A | 15.1 ± 1.4 a A | 14.7 ± 0.8 a A | 0.159 | 0.30 |
| Beech | 12.5 ± 0.8 a A | 16.5 ± 0.6 ab AB | 26.0 ± 1.9 b BC | **0.292** | **<0.05** |
| Hornbeam | 12.4 ± 1.5 a A | 16.9 ± 1.4 a AB | 14.8 ± 0.8 a A | -0.060 | 0.70 |
| Lime | 13.9 ± 1.9 a A | 17.7 ± 1.0 a AB | 16.8 ± 0.8 a AB | 0.029 | 0.85 |
| Maple | 15.7 ± 2.4 a A | 19.4 ± 1.1 a B | 20.7 ± 0.8 a C | 0.197 | 0.20 |
| SPECIFIC FINE ROOT AREA ($cm^2$ $g^{-1}$) | | | | | |
| Ash | 185.0 ± 31.3 a A | 241.3 ± 18.7 a A | 239.5 ± 8.8 a A | 0.151 | 0.33 |
| Beech | 163.0 ± 12.0 a A | 190.9 ± 6.8 a AB | 237.3 ± 11.9 a AC | 0.105 | 0.50 |
| Hornbeam | 150.1 ± 9.4 a A | 176.3 ± 8.5 a B | 164.8 ± 9.2 a B | -0.138 | 0.37 |
| Lime | 179.6 ± 22.7 a A | 195.4 ± 8.4 a B | 186.4 ± 6.5 a C | -0.046 | 0.77 |
| Maple | 171.5 ± 21.1 a A | 184.3 ± 7.2 a B | 202.2 ± 4.6 a AC | 0.232 | 0.13 |
| SPECIFIC FINE ROOT TIP ABUNDANCE (no. of tips $mg^{-1}$) | | | | | |
| Ash | 2.6 ± 0.4 a A | 3.9 ± 0.3 ab A | 4.5 ± 0.9 b A | **0.306** | **<0.05** |
| Beech | 14.6 ± 1.5 a B | 21.6 ± 2.6 a B | 29.0 ± 3.0 a B | 0.203 | 0.19 |
| Hornbeam | 12.9 ± 1.1 a B | 16.2 ± 1.0 a BC | 15.4 ± 1.0 a C | 0.030 | 0.85 |
| Lime | 10.5 ± 2.0 a B | 16.8 ± 0.8 b BC | 15.2 ± 1.0 ab C | 0.000 | 1.00 |
| Maple | 11.7 ± 2.5 a B | 14.8 ± 1.1 a C | 15.2 ± 0.7 a C | 0.041 | 0.79 |



Supplementary material (Appendix)

**Table 3.A2** Dependence of the fine root productivity and fine root turnover of the five tree species on four fine root morphological traits (linear regressions with the correlation coefficients (r), P values and total number of observations (n= no. of plots with a species' presence). Significant relationships (P <0.05) are printed in bold, relationships at 0.1> P >0.05 in italics.

| Species | | **Fine root productivity ($g\ m^{-2}\ yr^{-1}$)** | | **Fine root turnover ($yr^{-1}$)** | |
|---|---|---|---|---|---|
| | n | r | P | r | P |
| FINE ROOT DIAMETER (mm) | | | | | |
| Ash | 43 | 0.122 | 0.222 | 0.018 | 0.453 |
| Beech | 41 | -0.064 | 0.346 | **-0.344** | **0.013** |
| Hornbeam | 38 | 0.159 | 0.172 | 0.044 | 0.395 |
| Lime | 42 | **-0.314** | **0.020** | **-0.264** | **0.043** |
| Maple | 40 | -0.100 | 0.272 | -0.092 | 0.289 |
| SPECIFIC FINE ROOT LENGTH ($m\ g^{-1}$) | | | | | |
| Ash | 43 | *-0.210* | *0.086* | -0.090 | 0.287 |
| Beech | 41 | 0.005 | 0.487 | 0.043 | 0.393 |
| Hornbeam | 38 | 0.061 | 0.358 | 0.152 | 0.183 |
| Lime | 42 | 0.081 | 0.307 | 0.051 | 0.373 |
| Maple | 40 | 0.070 | 0.334 | 0.096 | 0.282 |
| SPECIFIC FINE ROOT AREA ($cm^2\ g^{-1}$) | | | | | |
| Ash | 43 | *-0.236* | *0.062* | -0.141 | 0.185 |
| Beech | 41 | -0.039 | 0.401 | -0.011 | 0.471 |
| Hornbeam | 38 | 0.042 | 0.400 | 0.132 | 0.217 |
| Lime | 42 | 0.005 | 0.486 | -0.025 | 0.434 |
| Maple | 40 | 0.050 | 0.380 | 0.062 | 0.352 |
| SPECIFIC FINE ROOT TIP ABUNDANCE (no. of tips $mg^{-1}$) | | | | | |
| Ash | 43 | **-0.299** | **0.024** | -0.176 | 0.129 |
| Beech | 41 | -0.065 | 0.344 | -0.042 | 0.394 |
| Hornbeam | 38 | *-0.229* | *0.082* | -0.098 | 0.283 |
| Lime | 42 | 0.057 | 0.361 | 0.002 | 0.493 |
| Maple | 40 | 0.101 | 0.271 | 0.130 | 0.215 |

# Chapter 4

## Complementarity in the use of nitrogen forms in a temperate broad-leaved mixed forest[1]

**Andreas Jacob & Christoph Leuschner**

[1]Under revision in: Plant Ecology & Diversity

## Abstract

Background: The complementary use of different forms of soil nitrogen (N) might lead to a higher productivity of mixed forests than monocultures, but convincing evidence for temperate mixed forests is scarce.

Aims: To search for species differences in N uptake rates and the preference for $NH_4^+$, $NO_3^-$ or glycine among five temperate broad-leaved tree species (*Acer pseudoplatanus, Carpinus betulus, Fagus sylvatica, Fraxinus excelsior, Tilia cordata*) in a mature mixed stand.

Methods: $^{15}N$ tracer was added to the soil and its accumulation in fine root biomass was analysed after 10 min, 1 h and 1 d.

Results: The species' estimated root uptake rates were in the range of 5-46 µg N $g^{-1}$ root $h^{-1}$ for $NH_4^+$, 6-86 µg N $g^{-1}$ root $h^{-1}$ for $NO_3^-$ and 4-29 µg N $g^{-1}$ root $h^{-1}$ for glycine during the first hour after tracer application. C*arpinus, Tilia* and *Acer* tended to prefer $NH_4^+$ over $NO_3^-$, while *Fraxinus* showed equal preference for both N forms and *Fagus* seemed to prefer $NO_3^-$.

Conclusions: The five co-existing tree species differed in uptake rates and partly in their N form preference, but complementarity in the use of different N forms seems to be of minor importance in this forest because tree species appear to be rather flexible in their N form preference.


Key words: *Acer pseudoplatanus,* ammonium, *Carpinus betulus, Fagus sylvatica, Fraxinus excelsior,* glycine, nitrate, root uptake, *Tilia cordata,* $^{15}N$ trace

## Introduction

The role of biodiversity for ecosystem functioning and the provision of ecosystem services has been one of the focal topics of ecological research in the past 15 years. Much research has been conducted in synthetic grasslands differing in species richness (e.g. Cardinale *et al.* 2007, Hector *et al.* 1999, Tilman *et al.* 2001). Less information exists on the role of diversity for ecosystem functions in forests and the majority of studies considered only aboveground biomass or productivity. While it is well documented that certain tree plantations can achieve a higher stem wood production than the respective monocultures (e.g. Kelty 2006, Pretzsch *et al.* 2010, and see review by Zhang *et al.* 2012), a positive diversity-productivity relationship has been found only rarely along diversity gradients in natural temperate and tropical forests (e.g. Vilà *et al.* 2007, Erskine *et al.* 2006, Paquette and Messier 2011). Several comparative studies in temperate and tropical forests failed to prove a positive diversity-aboveground productivity (or biomass) relationship (e.g. Jacob *et al.* 2010, Long and Shaw 2010, Szwagrzyk and Gazda 2007, Unger *et al.* 2012).

It has been predicted that a positive diversity effect on productivity bases on species complementarity in resource use, or/and facilitative effects between co-occurring species (Loreau and Hector 2001). It is not yet clear, however, whether light, water and nutrient levels are indeed depleted to a greater extent in species-rich forest stands due to niche complementarity than in the corresponding mono-specific stands. Complementarity in the use of belowground resources could be achieved through different mechanisms, (i) by vertical stratification of the root systems of co-occurring species (e.g. Berendse 1982; Gebauer and Ehleringer 2000; Kalliokoski *et al.* 2010; Yanai *et al.* 2006, 2008; Brassard *et al.* 2013), thereby reducing interspecific competition for water and/or nutrients, (ii) by species differences in the timing of resource uptake (e.g. Fitter 1986; Köcher *et al.* 2012), or (iii) by utilising different chemical forms of a nutrient element, in the case of nitrogen (N) the uptake of ammonium, nitrate or organic N compounds (e.g. von Felten *et al.* 2009). If different species were using different N form, this could increase the total amount of N utilised by the community and thus might enhance productivity. Nitrogen partitioning in species-rich communities by species differences in the vertical distribution of roots, the timing of uptake, and/or the preference of different N forms has been proven for arctic tundra communities and may exist in synthetic temperate grasslands (e.g. McKane *et al.* 2002, Kahmen *et al.* 2006, von Felten *et al.* 2009).

Temperate tree species have been found to differ substantially with respect to leaf phenology, rooting depth, type of mycorrhiza and apparently also preference

of N form (e.g. Lyr *et al.* 1992, Prentice and Leemans 1990, Ellenberg and Leuschner 2010), but it is not known whether this differentiation leads to significant N partitioning among the species when growing in mixed stands, thereby facilitating their coexistence. Experimental evidence for the complementary use of nitrogen by co-existing tree species would represent support for the assumption that diversity is indeed enhancing productivity in mixed forests because a higher stand N uptake is generally a pre-requisite of a higher net primary production.

Oak-hornbeam forests are among the most species-rich broad-leaved forests in Central Europe, where three to five or more tree species from different families with largely different light demand, drought sensitivity, canopy and root system morphologies and mycorrhiza types are coexisting (Köcher *et al.* 2009, Legner *et al.* 2013). This poses the question as to whether the species are possessing different or broadly overlapping belowground resource niches. In earlier studies in a species-rich old-growth oak-hornbeam forest on fertile relatively base-rich soil, we studied the size and spatial distribution of the fine root systems of five co-occurring tree species and detected no significant vertical root system stratification among the species (Meinen *et al.* 2009). Given that the five investigated tree species differ significantly with respect to total fine root biomass, fine root branching patterns and root surface/mass ratio, and are infected either by ectomycorrhizal or arbuscular fungi, we hypothesized that the species are partitioning the available soil N pool by different preferences for the three main N forms ($NH_4^+$, $NO_3^-$, organic N compounds), thereby separating their belowground niches in a functional rather than spatial manner.

Here, we conduct a $^{15}N$ tracer study to address three questions: (i) Do the coexisting tree species differ in their N uptake rates per root mass and surface area and in their preference of the three N forms, (ii) are these differences, if they exist, related to species differences in mycorrhiza type and fine root morphology, and (iii) does nitrogen partitioning with respect to N form increase the uptake rate of N by the trees at the stand level? We attempted to quantify the uptake capacity of $NH_4^+$, $NO_3^-$ and the amino acid glycine of mature tree individuals of five tree species growing in close vicinity within a representative patch of this old-growth mixed forest. In order to measure N uptake under more or less natural conditions without major N fertilisation and irrigation effects, we focused on the $^{15}N$ uptake of fine roots of the target species and recorded the accumulation of the tracer *in situ* in short time steps (10 min, 1 h, 1 d) immediately after the application of small amounts of either $^{15}N$-$NH_4^+$, $^{15}N$-$NO_3^-$ or $^{15}N$-glycine solutions to the topsoil. The amount of $^{15}N$ added to the soil was ~1.5 kg N $ha^{-1}$ in each treatment which is equivalent to about 1% of the

estimated annual N net mineralization rate at this site (~120-150 kg N $ha^{-1}$ $yr^{-1}$, cf. Ellenberg and Leuschner 2010) and not more than 2‰ of the total N pool in this horizon.

## Material and methods

*Study site and tree species*

The study was conducted in August 2009 in a broad-leaved mixed forest of Hainich National Park in Thuringia, Germany, where up to 14 tree species (among them *Tilia, Acer, Fagus, Carpinus, Fraxinus, Ulmus* and *Quercus* species) are coexisting (Leuschner *et al.* 2009). This conservation area was established in 1997 to protect one of the largest non-fragmented old-growth deciduous forests in Central Europe. All investigations were done in a forest stand in the north-eastern part of the national park (Thiemsburg forest region, 350 m a.s.l.; 51° 04' N, 10° 30' E) where the majority of trees are about 90-150 years old (Mölder *et al.* 2008) and the five tree species Common ash *(Fraxinus excelsior* L.), European beech (*Fagus sylvatica* L.*)*, Hornbeam (*Carpinus betulus* L.), Small-leaved lime *(Tilia cordata* Mill.) and Sycamore maple (*Acer pseudoplatanus* L.) are the most abundant species. These five species from different families (Oleaceae, Fagaceae, Betulaceae, Tiliaceae, Aceraceae) were selected for study. The stand belongs to the Stellario-Carpinetum forest community (oak-hornbeam forests) with the species either occurring in small monospecific groups of three to six trees or well mixed with allospecific neighbours. The closed forest has a tree height of 27-32 m with no larger canopy gaps (average canopy openness 5.7%, Seidel *et al.* 2012). As an average for the study plots, the herb layer had a mean cover of about 17% (Vockenhuber *et al.* 2011). The forest was affected by only minor management activities in the past 50 years (selective tree cutting) because part of the stand was used as a military training area and all activities ceased in 1997 with the declaration of a national park.

The region has a mean annual temperature of 7.7°C and a mean annual precipitation of about 590 mm $yr^{--1}$ (period 1973-2004; Deutscher Wetterdienst, 2005). In the study year 2009, a mean annual temperature of 8.9°C and an average precipitation of 773 mm $yr^{--1}$ were recorded (data of the nearby weather station Weberstedt/ Hainich; Deutscher Wetterdienst, 2009).

The most widespread soil type in the study region is a eutrophic Luvisol (FAO taxonomy 2006) with a profile depth of 60 to 70 cm which developed in a base-rich Pleistocene loess layer over Triassic limestone. The soil can dry out

strongly in summer and shows partly stagnant properties during spring and winter. Patches with dominance of *Fagus* trees showed the accumulation of Ol and Of layers on top of the mineral soil, resulting in the formation of an up to 3 cm thick moder layer while patches with the other four species were characterised by mull-type humus layers (about 1 cm thick) with more rapid litter decomposition. The soil texture of the mineral soil (0-30 cm) is characterised by high silt (about 74%) and low sand (< 5%) contents (Guckland *et al.* 2009).

*Study plots*

We investigated the incorporation of three N forms ($NH_4^+$, $NO_3^-$, glycine) by the fine roots of the five main tree species occurring in this mixed forest. Each three plots of 2 $m^2$ size were selected in the centre of mono-specific tree groups consisting either of *Fraxinus, Acer, Carpinus, Tilia* or *Fagus* trees, fifteen plots in total. The plots were surrounded by three to more than six individuals of the target tree species. Minimum distance between the plots was ~15 m, maximum distance more than 500 m. The 2-$m^2$ plots were positioned by random in the tree groups at a distance of 0.5 m to the stem of one of the target trees. The tree groups were carefully selected for maximum comparability in terms of stand structure and edaphic properties of the mineral soil. All trees around the plots were 25-33 m tall and were present in the upper canopy. The five plot types (species) differed not significantly with respect to the mean diameter at breast height (DBH) and basal area of the target trees (Table 4.1); however, the *Fraxinus* and *Carpinus* trees tended to have a larger DBH than the individuals of the other three species. Variation in soil chemical and physical properties across the five plot types was only moderate to low (Table 4.1), but a number of significant differences between the plot types existed due to local soil heterogeneity and putative species effects on soil properties. The pH ($H_2O$) of the topsoil was significantly higher in the *Fraxinus* and *Acer* plots, and the base saturation was higher in the *Fraxinus* plots and lower in the *Fagus* plots. In contrast, the concentration of $K_2SO_4$-extractable inorganic nitrogen in the topsoil was not different between the five plot types; however, the ratio of extractable ammonium ($NH_4^+$) to nitrate ($NO_3^-$) was higher under *Fagus* than under the other four species. The concentration of free amino acids was significantly higher in the *Fagus* and *Tilia* plots than in the *Fraxinus* and *Acer* plots (Table 4.1). The content of organic N in the mineral soil was smallest under *Fagus* and highest under *Fraxinus* (difference significant). The means of gravimetric soil water content were more or less similar in the five plot types (23.7 – 29.0%, Table 4.1).

**Table 4.1** Stand structural and soil chemical characteristics in the plots with *Fraxinus, Acer, Carpinus, Tilia* or *Fagus* trees in which the N-uptake experiments took place (MANN-WHITNEY *U* test; means ± SD of each 3 plots à 2 $m^2$; 0-20 cm soil depth). In addition, fine root biomass data and root morphological traits are give for the five species (stand structural characteristics: ANOVA/GLM with post-hoc *Tukey* test; other parameters: MANN-WHITNEY *U* test; means ± SD, root biomass: n= 38 sampling locations per species in close proximity of the $^{15}N$ study plots, root morphological traits: data from branch fine root samples taken in each four monospecific plots near the $^{15}N$ plots). Most of the data were log-transformed before analysis. Different letters indicate significant differences between tree species (stand structure and soil chemistry: $P< 0.05$; root morphology: $P< 0.1$). AM - arbuscular mycorrhiza, ECM - ectomycorrhiza.

| | *Fraxinus excelsior* | *Acer pseudoplatanus* | *Carpinus betulus* | *Tilia cordata* | *Fagus sylvatica* |
|---|---|---|---|---|---|
| **STAND STRUCTURAL CHARACTERISTICS** | | | | | |
| Mean basal area of target tree ($m^2$ $m^{-2}$ plot area) | 1.52 ± 0.72 a | 0.80 ± 0.17 a | 1.34 ± 0.13 a | 0.73 ± 0.18 a | 0.58 ± 0.10 a |
| Mean breast height diameter of target tree (m) | 1.84 ± 0.17 a | 1.41 ± 0.06 a | 1.84 ± 0.03 a | 1.35 ± 0.06 a | 1.21 ± 0.04 a |
| Mean distance to surrounding tree individuals (m) | 5.05 ± 0.37 a | 5.94 ± 0.54 a | 8.74 ± 0.69 b | 6.00 ± 0.52 a | 5.27 ± 0.65 a |
| Fine root biomass of 0-20 cm layer (g $m^{-2}$) | 321 ± 37 b | 182 ± 25 a | 195 ± 23 a | 225 ± 33 a | 244 ± 34 a |
| **SOIL CHARACTERISTICS** | | | | | |
| pH ($H_2O$) | 6.0 ± 0.1 c | 5.9 ± 0.1 bc | 4.9 ± 0.2 a | 5.1 ± 0.2 a | 5.2 ± 0.1 a |
| Soil C/N ratio (g $g^{-1}$) | 13.2 ± 0.2 ac | 13.0 ± 0.2 a | 13.7 ± 0.2 b | 13.7 ± 0.3 bc | 13.8 ± 0.4 abc |
| $N_{inorganic}$ (µg N $g^{-1}$ d.w.) | 1.10 ± 0.07 a | 1.53 ± 0.19 a | 1.06 ± 0.10 a | 1.43 ± 0.18 a | 1.57 ± 0.56 a |
| $N\text{-}NH_4^+$ (µg N $g^{-1}$ d.w.) | 0.77 ± 0.05 ab | 1.01 ± 0.11 bc | 0.73 ± 0.05 a | 1.00 ± 0.11 c | 1.35 ± 0.53 abc |
| $N\text{-}NO_3^-$ (µg N $g^{-1}$ d.w.) | 0.33 ± 0.05 b | 0.51 ± 0.10 b | 0.33 ± 0.07 ab | 0.43 ± 0.10 b | 0.22 ± 0.05 a |
| Amino acid-N (µg N $g^{-1}$ d.w.) | 17.3 ± 14.1 a | 22.7 ± 5.2 a | - | 29.6 ± 7.8b | 40.3 ± 13.0 c |
| $N_{organic}$ (mg N $g^{-1}$) | 4.10 ± 0.25 b | 3.02 ± 0.17 a | 3.41 ± 0.30 ab | 3.26 ± 0.22 a | 2.75 ± 0.14 a |
| CEC (µ$mol_c$ $g^{-1}$ d.w.) | 401 ± 27 b | 365 ± 19 ab | 348 ± 25 ab | 343 ± 23 ab | 326 ± 15 a |
| Base saturation (%) | 69.6 ± 2.7 d | 58.3 ± 1.6 b | 50.3 ± 6.9 abc | 57.2 ± 3.5 bc | 45.1 ± 4.5 a |
| Soil water content (% of d.w.) in study period | 25.0 ± 1.8 ab | 29.0 ± 1.2 b | 26.4 ± 1.7 ab | 26.7 ± 1.7 ab | 23.7 ± 1.2 a |
| **ROOT MORPHOLOGICAL TRAITS** | | | | | |
| Type of mycorrhiza | AM | AM | ECM | ECM | ECM |
| Mean root diameter in ≤ 2 mm class (mm) | 0.60 ± 0.05 e | 0.36 ± 0.02 a | 0.40 ± 0.02 b | 0.53 ± 0.10 d | 0.49 ± 0.07 bd |
| Specific fine root length (m $g^{-1}$) | 11.3 ± 2.0 a | 15.7 ± 1.9 b | 12.4 ± 1.3 a | 13.9 ± 3.3 ab | 12.5 ± 1.8 ab |
| Specific fine root surface area ($cm^2$ $g^{-1}$) | 185.0 ± 23.1 ab | 171.5 ± 15.4 b | 150.1 ± 10.0 a | 179.6 ± 31.1 ab | 163.0 ± 17.8 ab |
| Specific root tip abundance (n $mg^{-1}$) | 2.6 ± 0.3 a | 11.7 ± 1.9 bd | 12.9 ± 1.5 c | 10.5 ± 2.8 b | 14.6 ± 2.2 cd |
| Fine root C/N ratio (g $g^{-1}$) | 35.0 ± 1.8 a | 54.2 ± 3.3 c | 46.0 ± 4.5 b | 58.4 ± 3.4 c | 56.1 ± 4.3 c |

*Soil chemical analyses*

Soil samples were collected in the topsoil (0-20 cm) of all plots. Total carbon and nitrogen were measured in dried (70°C, 48 h) and ground soil material by dry combustion (vario EL, elementar, Hanau, Germany), the concentration of exchangeable $NH_4^+$ and $NO_3^-$ by percolation of fresh soil with 0.5 M $K_2SO_4$ and subsequent analysis of the extract using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, The Netherlands) with the Berthelot reaction for $NH_4^+$ detection and the copper-cadmium reduction method for $NO_3^-$ detection. The concentration of exchangeable K, Mg, Ca, Na, Al, Mn and Fe was determined by percolation with 0.2 M $BaCl_2$ solution and subsequent element analysis in the extract by ICP-OES (Perkin Elmer Optima 5300 DV). The methodology follows mainly König and Fortmann (1996). The ninhydrin colorimetric analysis according to Rosen (1957) was applied for measuring the concentration of free amino acids in the topsoil (0-20 cm). Briefly, the $K_2SO_4$ extracts were mixed with a 3% ninhydrin solution and a cyanide-acetate buffer, heated for 15 min at 100°C and the extinction measured in a colorimeter at 570 nm. The standard curve was established with glycine solutions ranging from 0 to 0.75 mM. The soil pH was measured in a suspension of 10 g fresh soil in 25 ml deionized water (pH($H_2O$)).

*$^{15}N$ tracer application*

In all 15 plots, $^{15}N$ tracer was applied synchronously during a rainless period of about 14 days in August 2009. Each one plot per species was used to measure the incorporation rate of $^{15}N$-$NH_4^+$, $^{15}N$-$NO_3^-$ or $^{15}N$-glycine into the fine roots (diameter $\leq$2 mm) of the target species in the topsoil under undisturbed *in situ* conditions with intact root systems. The experiment investigated the immediate response of the fine roots of mature trees to the localised addition of inorganic or organic nitrogen in a dose that resulted neither in a significant fertilisation nor irrigation effect. With a gravimetric soil water content of 23-29%, equivalent to about 50-60% of field capacity (M. Meissner, unpublished data), the topsoil of the study plots was moderately dry before the commencement of the experiments, which guaranteed that all tracer compounds remained in the topsoil (0-20 cm). We selected a rainless period with moderately dry soil for the experiments, because (i) this situation reflects the typical pulsed availability of inorganic N in this soil where mineralisation is driven by short rainfall events in summer (Guckland 2009), and (ii) the added nitrogen (N) solutions percolated in the dry soil only to a soil depth of at most 20 cm which avoided tracer loss to deeper horizons and allowed to budget the amount of tracer

added. The applied $^{15}N$ containing solutions were equivalent to 1.5 mm rainfall and increased the soil moisture on average by 1.5 vol.% in this uppermost horizon. In every 2-$m^2$ plot, all leaf litter was removed from the soil surface and 3 L of deionised water with 1.15 g $^{15}NH_4Cl$ (95 atom% $^{15}N$) or 2.16 g $K^{15}NO_3$ (98 atom% $^{15}N$) or 1.61 g $C_2H_5O_2{}^{15}N$ (95 atom% $^{15}N$) was added evenly to the soil surface using a watering can. The solutions contained 1.42 kg $^{15}N$ $ha^{-1}$ or ~1.5 kg total N $ha^{-1}$ in all three treatments. Subsequently, the removed litter material was returned to the soil surface of the plots.

*Root analyses*

At intervals of 10 min, 1 h and 1 d after tracer application, each four soil samples per plot were extracted at random locations in the 2 $m^2$ plot area with a cylindrical steel corer (diameter 52 mm, length 20 cm) to examine the rate of apparent net incorporation of $^{15}N$ in fine root biomass immediately after application. For avoiding interference with coring locations used in earlier sampling rounds, a minimum distance of 15 cm to the nearest sampling location was adopted in the sampling after 1 h and 1 d. We are aware that analysing root $^{15}N$ concentrations cannot give the true $^{15}N$ uptake rate of a fine root but only the $^{15}N$ concentration in fine root biomass at the time of harvest which results from the net influx of $^{15}N$ (uptake minus efflux) since application minus the loss due to $^{15}N$ translocation in axial direction out of the root segment. Thus, interference between N uptake and allocation is to be expected in particular for the third sampling after 1 d. Even though we are displaying the $^{15}N$ accumulation data of all three measuring intervals (10 min, 1 h, 1 d), we discuss only the first two time steps which should be less affected by N allocation. We refer to the concentration data as 'approximate $^{15}N$ incorporation' and, by considering the dilution of the tracer by the inorganic N already present in the soil, as 'approximate N uptake', because the final $^{15}N$ concentration in the soil solution is not precisely known (see below). For characterising the background level of $^{15}N$ abundance in the fine root biomass of the five species, four root samples per plot were collected in 0-20 cm soil depth at random locations before tracer application. This $^{15}N$ signature of live fine root biomass of the target tree species was used as reference in the root uptake study.

All soil samples with the roots contained in it were transferred to polyethylene bags and stored at 4°C in the dark. Processing of the root samples started immediately after collection but took several weeks in certain samples due to the very labour-intensive separation of fine root mass by species and into live and dead mass fractions. Earlier investigations had shown that tree fine root material stored

under these conditions did not experience alterations in the vitality status of the roots (e.g. Meinen *et al.* 2009; A. Jacob, unpublished data). We also tested for a possible effect of storage duration on the measured mass-specific N uptake rate but found no dependence on time in 344 investigated root samples that were investigated after 2 to 22 days (r= -0.08, P =0.15) (M. Bartels, personal communication). The collected soil material with water contents < c. 60% of field capacity was relatively dry with most soil pores filled with air which prevented significant diffusion of N compounds across the bulk soil during storage.

For collecting the roots and identifying their species identity, the soil samples were carefully soaked in water and sieved (mesh size 0.25 mm) to separate the fine roots (≤2 mm in diameter) of the target tree species from all adherent soil material. Coarse tree roots (≥2 mm in diameter) and roots of other tree species and herbaceous plants (mainly forest floor herbs and grasses) were discarded. Fine root fragments longer than 1 cm were collected manually using a pair of tweezers; all live root fragments were picked out and sorted by species under a stereomicroscope (6-40x). During five years of root research in the Hainich forest, a key for species identification based on fine root morphological and architectural traits has been developed (D. Hertel, C. Meinen, A. Jacob, unpublished data), which was used to identify the five tree species in the root samples. After careful washing and fractionation, the fine root biomass samples were dried at 70°C for 48 h, ground and the $^{15}N$ signature and the total N concentration in the fine root mass analysed using an elemental analyzer (NA 2500, CE-Instruments, Rodano, Milano, Italy) coupled with an isotope mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany).

*Estimation of root N uptake*

The specific $^{15}N$ enrichment in the root dry mass of a species was calculated as atom% $^{15}N_{excess}$ of a root sample which is atom% $^{15}N$ of the root sample collected after labelling minus the atom% $^{15}N$ in natural abundance in the root dry mass of that species (background level):

$$\text{atom\% } ^{15}N_{excess} = \text{atom\% } ^{15}N_{sample} - \text{atom\% } ^{15}N_{background\ level} \quad (1)$$

The uptake of $^{15}N$ by the fine roots after intervals of 10 min, 1 h and 1 d ($U_{15N}$; expressed as a mean rate per hour in µg $^{15}N$ $g^{-1}$ root $h^{-1}$) was calculated as the product of the atom% $^{15}N_{excess}$ value at this time and the N concentration in fine root biomass (in µg N $g^{-1}$ root), given as hourly rate. For calculating the mass-specific rate of N uptake for each N form, the dilution of the applied tracer by the

concentration of that N form in the soil was taken into account, assuming that the tracer applied did not influence the $^{15}N/^{14}N$ ratio of the N taken up by the roots, i.e. that the $^{15}N/^{14}N$ uptake ratio was equal to the $^{15}N/^{14}N$ ratio of available N in the soil. From infiltration experiments, we assumed that all tracer did remain within the 0-20 cm soil horizon and calculated a dilution factor for each N form by relating the added amount of $^{15}N$ ($R_{15N\ label}$) to the amount of $K_2SO_4$-extractable N of this N form in the 0-20 cm horizon ($R_{available\ N}$). N uptake per root dry mass was then calculated as

$$U_N = U_{15N} \times (R_{available\ N}/R_{15N\ label}) \quad (2)$$

This calculation was conducted for $NH_4^+$ and $NO_3^-$ using the extractable ammonium and nitrate concentrations in the soil measured in the plots in July 2009. For glycine, no data on the background concentration in the soil are available for 2009. Instead, we used the free amino acid concentration measured in *Fraxinus, Acer, Tilia* and *Fagus* plots in July and August 2012 as a calculation basis. Thus, the reference data are less precise in the case of amino acid concentrations, because they were measured in a different year and do not cover the *Carpinus* plots.

For calculating the total amount of N taken up per hour by the fine roots of a species in the topsoil (0-20 cm horizon), we multiplied the mass-specific uptake rate of a species with its fine root biomass in this horizon in monospecific patches of the species.

*Statistical analysis*

All data sets were analysed for normal distribution using a Shapiro-Wilk test. Normally distributed data (partly achieved by data transformation) were tested for significant differences between means using an ANOVA/GLM procedure followed by pair-wise comparison with a post-hoc Tukey test. Data sets with non-normal distribution of the residuals were analysed with a non-parametric Mann-Whitney *U* test. The relationship between $^{15}N$ enrichment in the roots (relative to the background level) and selected stand structural and soil chemical parameters was examined with linear PEARSON correlation analyses. All calculations were done with the software package SAS (version 9.1, SAS Institute, Cary, NC, USA). In most cases, significance was determined at $P < 0.05$; however, due to the small number of replicate samples (4) and the large variation in apparent uptake rates, we also tested in certain cases for marginally significant differences ($P < 0.1$) between means.

## Results

*Soil N fractions and rooting patterns*

The total quantity of inorganic N ($K_2SO_4$-extractable $NH_4^+$ and $NO_3^-$) ranged in August 2009 between means of 1.06 and 1.57 µg N $g^{-1}$ dry soil in the plots of the five species with no significant differences between the species (Table 4.1). The $NH_4^+/NO_3^-$ ratio varied between ~2 and 6 with highest ammonium content under *Fagus* and lowest under *Carpinus*. The concentration of free amino acids (data from summer 2012) was ~15-25 times higher than that of inorganic N (Table 4.1).

Tree fine root biomass in the 0-20 cm soil horizon was highest in the monospecific patches of *Fraxinus* (322 g $m^{-2}$) and lowest in those of *Acer* and *Carpinus* (182 and 195 g $m^{-2}$, respectively; Table 4.1). The fine root systems of the five species differed not only with respect to the type of mycorrhiza (two AM and three ECM species) but also in several root morphological traits; however, these traits were not systematically different between AM and ECM tree species. The mean diameter of roots in the ≤2 mm-class was highest in *Fraxinus* (600 µm) and lowest in *Acer* (360 µm), the two AM species, with both differences being significant to the other three species which had intermediate mean diameters in this class (Table 4.1). The particularly thick or thin fine roots of *Fraxinus* and *Acer* were related to low and high specific root lengths (SRL) in these species (11.3 and 15.7 m $g^{-1}$), while specific root area (SRA) was not related to mean root diameter or SRL. A striking morphological characteristic of the relatively thick *Fraxinus* fine and finest roots was the very low number of root tips (mean of 2.6 tips $mg^{-1}$ root *versus* 10.5-14.5 tips $mg^{-1}$ root in the other species) and the low C/N ratio of fine root biomass in this species (Table 4.1).

*Tracer incorporation into fine root biomass*

In all five plot types, the $^{15}N$ tracer was detected in the fine root biomass of the 0-20 cm soil horizon within minutes after applying the tracer to the soil surface. We found atom% $^{15}N_{excess}$ values in the range of 0.04 – 0.26 in the root biomass after 10 min with no significant differences between the five species and between the three N forms applied ($NH_4^+$, $NO_3^-$, glycine; Table 4.2). Species and N form differences were more pronounced in the atom% $^{15}N_{excess}$ values measured 1 h after tracer application with higher $^{15}N$-$NO_3^-$ incorporation in *Fraxinus* than in *Acer*, *Carpinus* and *Tilia*, and higher $^{15}N$-glycine incorporation in *Fraxinus* and *Acer* than in *Carpinus* and *Fagus* (significant at $P < 0.1$). With respect to N form differences, we found in *Fagus* higher values for $^{15}N$-$NO_3^-$ than for $^{15}N$-$NH_4^+$ and $^{15}N$-glycine incorporation ($P < 0.1$).

**Table 4.2** Enrichment of $^{15}N$ (atom% $^{15}N_{excess}$ values) in the fine roots of the five tree species depending on the $^{15}N$ source. Given are means ± SD (Mann-Whitney *U* test; n= 4). Different letters indicate differences significant at P <0.1 for species contrasts (Latin capital letters; same N form and sampling interval), N form contrasts (Greek letters; same species and sampling interval) and sampling interval contrasts (10 min – 1 h – 1 d; Latin lower case letters; same species and N form). All data were log-transformed before analysis.

| **Nitrogen form** | **Species** | | | | |
|---|---|---|---|---|---|
| | *Fraxinus* | *Acer* | *Carpinus* | *Tilia* | *Fagus* |
| $^{15}N$-$NH_4^+$ | | | | | |
| 10 min | 0.24 ± 0.15 | 0.18 ± 0.04 β | 0.15 ± 0.06 | 0.11 ± 0.03 | 0.06 ± 0.03 |
| 1 h | 0.29 ± 0.13 | 0.15 ± 0.08 | 0.12 ± 0.05 | 0.12 ± 0.03 | 0.04 ± 0.003 α |
| 1 d | 0.27 ± 0.14 | 0.09 ± 0.03 | 0.14 ± 0.04 | 0.16 ± 0.05 | 0.11 ± 0.06 |
| $^{15}N$-$NO_3^-$ | | | | | |
| 10 min | 0.17 ± 0.08 | 0.14 ± 0.03 β | 0.10 ± 0.03 | 0.12 ± 0.04 | 0.26 ± 0.09 |
| 1 h | 0.46 ± 0.12 B | 0.08 ± 0.05 A | 0.04 ± 0.004 A | 0.09 ± 0.06 A | 0.12 ± 0.04 β AB |
| 1 d | 0.14 ± 0.07 | 0.16 ± 0.05 | 0.14 ± 0.06 | 0.17 ± 0.04 | 0.13 ± 0.06 |
| $^{15}N$-Glycine | | | | | |
| 10 min | 0.18 ± 0.13 | 0.04 ± 0.02 α | 0.05 ± 0.02 | 0.04 ± 0.02 | 0.07 ± 0.02 b |
| 1 h | 0.16 ± 0.05 B | 0.12 ± 0.04 B | 0.03 ± 0.01 A | 0.05 ± 0.02 AB | 0.03 ± 0.005 α A a |
| 1 d | 0.09 ± 0.04 | 0.09 ± 0.06 | 0.07 ± 0.03 | 0.07 ± 0.04 | 0.03 ± 0.004 ab |

Mass-specific incorporation rates estimated from the $^{15}N$ enrichment in fine root biomass, fine root N concentration and N availability in the soil (approximated from the amount of tracer applied and tracer dilution) were by far highest during the first sampling interval (10 min after application) and dropped to 15-50% of this rate 1 h after application. We calculated incorporation (uptake) rates in the range of 5-47 µg N $g^{-1}$ root $h^{-1}$ for $NH_4^+$, 6-86 µg N $g^{-1}$ root $h^{-1}$ for $NO_3^-$ and 4-29 µg N $g^{-1}$ root $h^{-1}$ for glycine for the first hour after tracer application (grey bars in Figure 4.1).

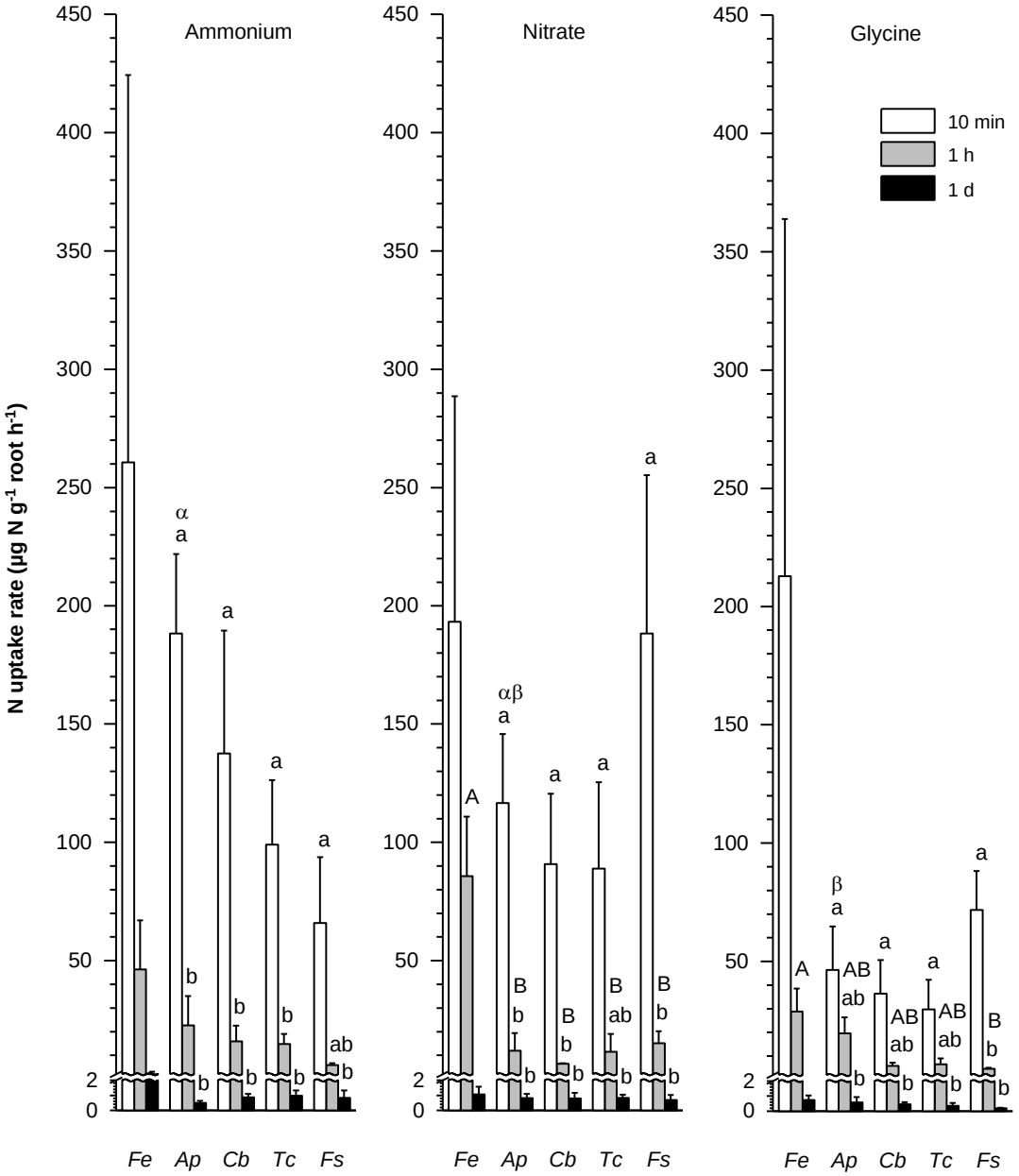


**Figure 4.1** Apparent mass-specific uptake rate of ammonium, nitrate or glycine of roots of the five tree species 10 min, 1 h or 1 d after application of the tracer. Note different scale of y-axis at very low rate to demonstrate the low rates measured after 1 d. Given are means ± SD (ANOVA/GLM with post-hoc Tukey test; n = 4). Different letters indicate significant differences (P <0.05) for species contrasts (Latin capital letters; same N form and sampling interval), N form contrasts (Greek letters; same species and sampling interval) and sampling interval contrasts (10 min – 1 h – 1 d; Latin lower case letters; same species and N form).

The apparent rates calculated for the first 10 min varied between 65 and 261 µg N $g^{-1}$ root $h^{-1}$ ($NH_4^+$), 88-194 µg N $g^{-1}$ root $h^{-1}$ ($NO_3^-$) and 29-213 µg N $g^{-1}$ root $h^{-1}$ (glycine). Uptake rates had dropped to very low values (mostly <2 µg N $g^{-1}$ root $h^{-1}$) when calculated for the first 24 hours after tracer application. Significant species differences (*P*< 0.05) were detected for the 1 h-sampling for $NO_3^-$ uptake (higher in *Fraxinus* than in *Acer Carpinus, Tilia* and *Fagus*) and glycine uptake (higher in

*Fraxinus* than in *Fagus*). Significant N form-differences (P <0.05) in the 1 h-sampling interval existed only for *Acer* ($NH_4^+$ higher than glycine).

By multiplying the root mass-specific uptake rates with the fine root biomass of the species in monospecific forest patches (0-20 cm soil profile), we obtained rough estimates of stand-level N uptake rates for the five species (Figure 4.2).

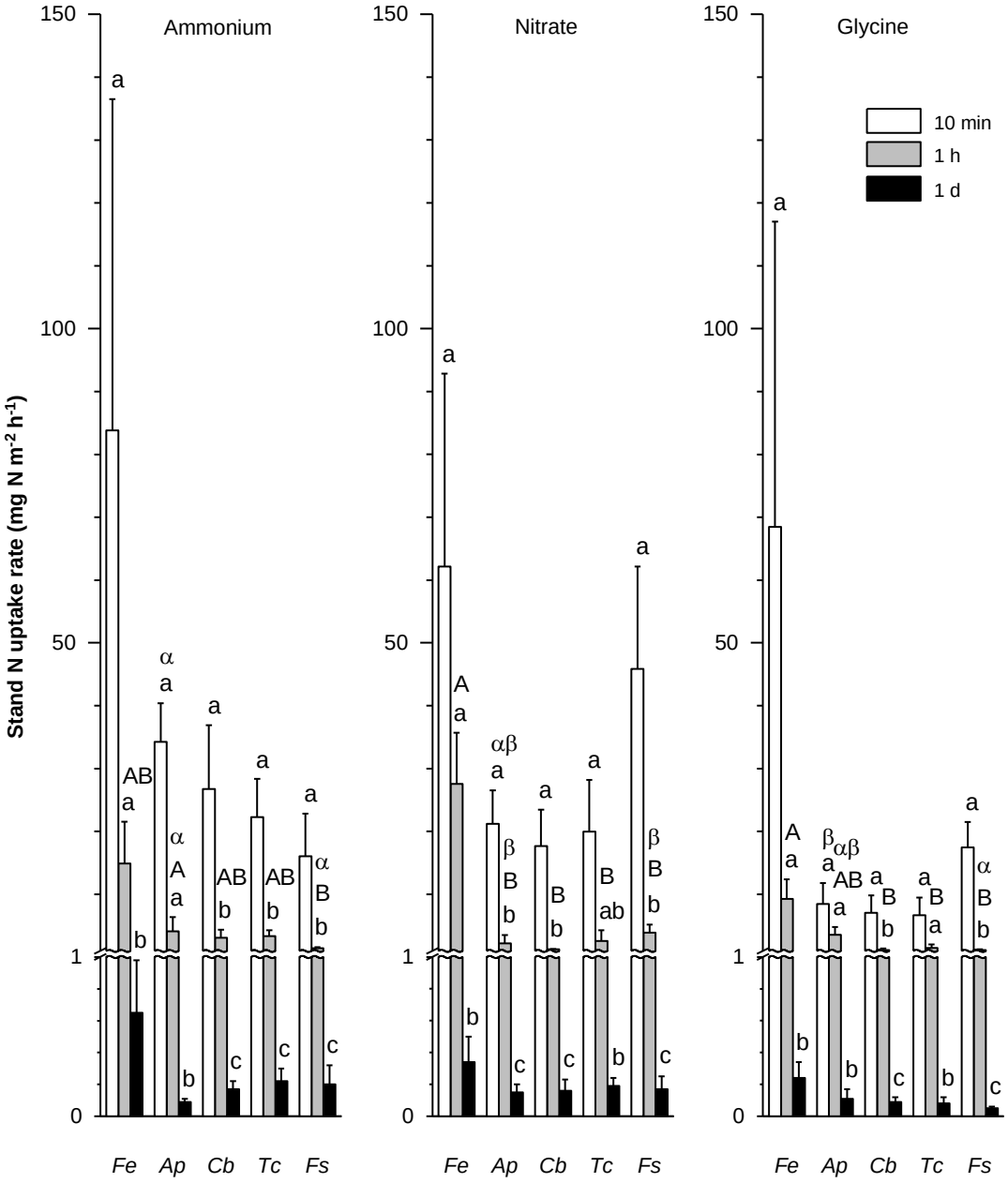


**Figure 4.2** Apparent stand-level uptake rate of ammonium, nitrate or glycine of roots of the five tree species 10 min, 1 h or 1 d after application of the tracer. The mass-specific rates were extrapolated to the stand level using the fine root biomass of the species (0-20 cm) in monospecific patches of the stand. All data were log-transformed before analysis. Given are means ± SD (ANOVA/GLM with post-hoc Tukey test; n= 4). Different letters indicate significant differences (P <0.05) for species contrasts (Latin capital letters; same N form and sampling interval), N form contrasts (Greek letters; same species and sampling interval) and sampling interval contrasts (10 min – 1 h – 1 d; Latin lower case letters; same species and N form).

We calculated uptake rates of 16-84 mg N $m^{-2}$ ground area $h^{-1}$ for $NH_4^+$ for the first sampling interval (10 min) in the five species, 17-63 mg N $m^{-2}$ ground area $h^{-1}$ for $NO_3^-$ and 6-69 mg N $m^{-2}$ ground area $h^{-1}$ for glycine. Species differences in stand-level uptake rate (1 h-sampling interval) were detected only for two species in the ammonium treatment, where *Acer* showed higher uptake rates than *Fagus*. However, *Fraxinus* revealed significantly higher nitrate and glycine uptake rates per unit ground area in the stand 1 h after tracer application than *Acer*, *Carpinus*, *Tilia* and *Fagus* due to both higher root mass-specific uptake rates and a high fine root biomass total of this species. Stand-level nitrate uptake after 1 h was significantly higher than ammonium and glycine uptake in *Fagus* patches and $NH_4^+$ uptake higher than $NO_3^-$ uptake in *Acer* patches.

*Factors influencing root N uptake*

Pearson correlation analyses testing for relationships between mass-specific N uptake rate and selected root morphological traits (mean fine root diameter, specific fine root length, specific fine root surface area, specific root tip abundance and fine root C/N ratio) showed a positive effect of specific root area on the $NH_4^+$ and $NO_3^-$ uptake across the five species (higher specific uptake of thinner fine roots), and a negative effect of root C/N ratio on the uptake of all three N forms (higher uptake of N-richer roots, Table 4.3).

**Table 4.3** Pearson correlation coefficients $r_P$ (P values in brackets) for the relationship between mass-specific uptake rates (in µg N g root dry $mass^{-1}$ $h^{-1}$ measured after 1 h) and selected root morphological traits depending on N forms (morphological data from n= 20 monospecific patches). All data were log-transformed before analysis. Significant relationships (P <0.05) are printed in bold, marginally significant ones (P <0.1) in italics.

| | **Nitrogen form** | | |
|---|---|---|---|
| **Variable** | $NH_4^+$ | $NO_3^-$ | Glycine |
| Mean fine root diameter (mm) | 0.055 (0.68) | 0.150 (0.25) | 0.192 (0.14) |
| Specific fine root length (m $g^{-1}$) | 0.173 (0.19) | 0.104 (0.43) | 0.049 (0.71) |
| Specific fine root surface area ($cm^2$ $g^{-1}$) | **0.320 (0.01)** | **0.291 (0.02)** | *0.240 (0.07)* |
| Specific root tip abundance (n $mg^{-1}$) | 0.170 (0.19) | 0.170 (0.19) | 0.076 (0.56) |
| Fine root C/N ratio (g $g^{-1}$) | **-0.379 (0.003)** | **-0.410 (0.001)** | **-0.421 (0.001)** |

N uptake expressed per unit specific root length (SRL) or specific root area (SRA) or scaled to a single fine root tip varied considerably among the five species (Table 4.4). The four species *Acer, Carpinus, Tilia* and *Fagus* differed by factors up to four in their approximated specific ammonium, nitrate and glycine incorporation rates with highest root area- and root tip-specific ammonium and glycine uptake rates

mostly found in *Acer*, but highest nitrate uptake rates in *Fagus*. *Fraxinus* differed from the four other species by having three-fold to more than ten-fold higher length-, area- and root tip-specific uptake rates for all three N forms than the average of these species.

## Discussion

*Species differences in N uptake rate*

The upper mineral soil of the Hainich mixed forest is characterized by largely overlapping fine root systems of five or more tree species (Meinen *et al.* 2009). We thus assumed that interspecific competition for N should be strong and the species might have developed physiological and/or morphological adaptations to reduce the negative consequences of belowground resource competition (e.g. Parrish and Bazzaz 1976, von Felten *et al.* 2009). We searched for differences in specific N uptake rates and N form preference among the five co-occurring tree species under as realistic field conditions as possible by investigating mature trees with natural mycorrhization and by adding tracer in a low dose to the rhizosphere. In support of our first hypothesis, the results show that significant species differences in N uptake per root mass, root surface area, per individual root tip and also per ground area in the stand do exist. *Fraxinus* had a significantly higher rate of tracer incorporation per root mass than the other species in the case of $NO_3^-$ and for glycine (only significant against *Fagus*; 1 h-measurement, $P < 0.05$). This was also true for uptake per root length and surface area, and per root tip. On the stand level, the higher N uptake of ash was further enhanced by the higher standing fine root biomass of *Fraxinus*.

Correlation analysis showed that the main determinant of root N uptake capacity was tissue N content (C/N ratio) followed by specific root surface area. This result indicates that interspecific differences in root N uptake capacity seem to depend more on root physiological and chemical properties than on root morphology because *Fraxinus* had a high uptake even though SRA was not particularly high. Ash has relatively thick and N-rich fine roots with a low number of root tips but with similar SRL and SRA as the other species. That uptake by *Fraxinus* roots was high despite the low number of root tips, may relate to the arbuscular mycorrhiza of this species in which hyphae infect larger root segments than in ECM-forming roots. The significantly higher uptake rates of nitrate and glycine per surface area in ash roots support our assumption of a large species-specific physiological effect, which might

**Table 4.4** Apparent N uptake rates of the fine roots of the five tree species for the three N forms expressed on a root length or root surface area basis or per individual fine root tip. The data base on the atom%-$^{15}$N excess values of root mass (n = 4) measured 10 min, 1 h or 1 d after tracer application. Given are means ± SE (ANOVA/GLM with post-hoc *Tukey* test). Significant differences between the species are marked by different small Latin letters, whereas significant differences between the N forms for a given species and time interval are indicated by different Greek letters ($P < 0.05$, only indicated for significant differences).

| N-Form | Species | | | | |
|---|---|---|---|---|---|
| | *Fraxinus* | *Acer* | *Carpinus* | *Tilia* | *Fagus* |
| **N UPTAKE RATE PER ROOT LENGTH** (µg N m$^{-1}$ root h$^{-1}$) | | | | | |
| $N\text{-}NH_4^+$ | | | | | |
| 10 min | 22.9 ± 14.4 | 12.1 ± 2.2 β | 11.1 ± 4.2 | 7.1 ± 2.0 | 5.3 ± 2.2 |
| 1 h | 4.07 ± 1.83 | 1.45 ± 0.80 | 1.29 ± 0.54 | 1.07 ± 0.30 | 0.47 ± 0.06 α |
| 1 d | 0.18 ± 0.09 | 0.03 ± 0.01 | 0.07 ± 0.02 | 0.07 ± 0.02 | 0.07 ± 0.03 |
| $N\text{-}NO_3^-$ | | | | | |
| 10 min | 17.0 ± 8.4 | 7.5 ± 1.9 αβ | 7.4 ± 2.4 | 6.4 ± 2.6 | 15.1 ± 5.4 |
| 1 h | 7.54 ± 2.22 b | 0.76 ± 0.48 a | 0.52 ± 0.01 a | 0.83 ± 0.54 a | 1.28 ± 0.41 ab β |
| 1 d | 0.09 ± 0.04 | 0.05 ± 0.02 | 0.07 ± 0.03 | 0.06 ± 0.02 | 0.06 ± 0.03 |
| N-Glycine | | | | | |
| 10 min | 18.7 ± 13.3 | 3.0 ± 1.2 β | 2.9 ± 1.2 | 2.1 ± 0.9 | 5.7 ± 1.3 |
| 1 h | 2.54 ± 0.86 b | 1.25 ± 0.44 ab | 0.48 ± 0.11 a | 0.47 ± 0.18 a | 0.37 ± 0.04 a α |
| 1 d | 0.07 ± 0.03 | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.002 |
| **N UPTAKE RATE PER ROOT SURFACE AREA** (ng N cm$^{-2}$ root h$^{-1}$) | | | | | |
| $N\text{-}NH_4^+$ | | | | | |
| 10 min | 1410 ± 886 | 1097 ± 197 β | 910 ± 344 | 552 ± 152 | 404 ± 171 |
| 1 h | 250.4 ± 37.9 | 131.7 ± 72.5 | 105.2 ± 43.9 | 82.6 ± 23.5 | 36.0 ± 4.6 α |
| 1 d | 10.93 ± 1.65 | 2.92 ± 0.78 | 5.70 ± 1.58 | 5.44 ± 1.96 | 5.15 ± 2.95 |
| $N\text{-}NO_3^-$ | | | | | |
| 10 min | 1045 ± 516 | 680 ± 170 αβ | 601 ± 197 | 495 ± 203 | 1154 ± 411 |
| 1 h | 463.6 ± 136.3 b | 69.5 ± 43.6 a | 42.7 ± 1.1 a | 64.0 ± 42.1 a | 98.4 ± 31.6 ab β |
| 1 d | 5.73 ± 2.74 | 4.72 ± 1.69 | 5.32 ± 2.37 | 4.64 ± 1.19 | 4.23 ± 2.04 |
| N-Glycine | | | | | |
| 10 min | 1152 ± 817 | 271 ± 107 α | 241 ± 94 | 166 ± 70 | 440 ± 101 |
| 1 h | 155.9 ± 52.7 b | 114.1 ± 39.7 ab | 38.9 ± 8.9 a | 36.2 ± 13.7 a | 28.3 ± 3.1 a α |
| 1 d | 4.01 ± 1.63 | 3.43 ± 2.05 | 2.97 ± 1.00 | 1.96 ± 1.11 | 1.17 ± 0.17 |
| **N UPTAKE RATE PER ROOT TIP** (ng N root tip$^{-1}$ h$^{-1}$) | | | | | |
| $N\text{-}NH_4^+$ | | | | | |
| 10 min | 99.06 ± 62.21 b | 16.09 ± 2.89 ab α | 10.66 ± 4.02 a | 9.43 ± 2.59 a | 4.51 ± 1.91 a |
| 1 h | 17.58 ± 7.90 b | 1.93 ± 1.06 ab | 1.23 ± 0.51 a | 1.41 ± 0.40 a | 0.40 ± 0.05 a α |
| 1 d | 0.77 ± 0.39 b | 0.04 ± 0.01 a | 0.07 ± 0.02 ab | 0.09 ± 0.03 ab | 0.06 ± 0.03 a |
| $N\text{-}NO_3^-$ | | | | | |
| 10 min | 75.16 ± 37.12 | 9.97 ± 2.49 αβ | 7.04 ± 2.30 | 8.46 ± 3.47 | 12.89 ± 4.60 |
| 1 h | 32.57 ± 9.58 b | 1.02 ± 0.64a | 0.50 ± 0.01 a | 1.09 ± 0.72 a | 1.10 ± 0.35 a β |
| 1 d | 0.40 ± 0.19 | 0.07 ± 0.02 | 0.06 ± 0.03 | 0.08 ± 0.02 | 0.05 ± 0.02 |
| N-Glycine | | | | | |
| 10 min | 80.90 ± 57.39 b | 3.97 ± 1.57 a β | 2.82 ± 1.10 a | 2.04 ± 0.86 a | 4.91 ± 1.13 ab |
| 1 h | 10.95 ± 3.70 c | 1.67 ± 0.58 b | 0.46 ± 0.10 ab | 0.62 ± 0.23 ab | 0.32 ± 0.03 a α |
| 1 d | 0.28 ± 0.11 b | 0.05 ± 0.03 a | 0.03 ± 0.01 a | 0.03 ± 0.02 a | 0.01 ± 0.002 a |

be explained by a higher density of specific transporters in the root membranes and/or kinetic constants of ion uptake in *Fraxinus* roots reflecting a higher substrate affinity. The elevated N concentration in ash roots would fit into this picture.

With respect to our second hypothesis about the dependence of N uptake capacity on root morphology and mycorrhiza type, we thus found some evidence for an important physiological effect while fine root morphology, the number of infected root tips, or the type of mycorrhiza seem to be less influential. This matches with results of a comparative examination of six tree species by Schulz *et al.* (2011), but it seems to contrast with the study in conifer roots of Yanai *et al.* (2009). One must also consider the possibility that uptake rates related to root orders would reveal clearer effects of root morphology, because root order is a better predictor of species differences in root function than root diameter (Guo *et al.* 2008).

The three ECM tree species differed not systematically in their N uptake patterns from the two AM species. The capture of organic N has mostly been associated with ECM (and ericoid-mycorrhizal) fungi because they possess proteolytic and chitinolytic capacities (e.g. Chalot and Brun 1998, Hodge *et al.* 2000). While AM fungi are thought to be more effective in the capture and transfer of inorganic nutrients to the plant (Smith and Read 1997), evidence exists that fungi of the order Glomales can also access N from complex organic material (Hodge *et al.* 2001), and grasses and shrubs with arbuscular mycorrhiza have been found to take up intact amino acids as well (e.g. Näsholm *et al.* 2000, Rains and Bledsoe 2007). The latter findings are in line with our result that estimated root mass-based uptake of glycine tended to be highest in *Fraxinus* and *Acer*, the two AM species of our species sample. This may indicate that organic N compounds may be captured by the roots of certain plants independently from their mycorrhizal status.

*Species differences in N form preference*

The third goal of our experiment was to search for assumed complementarity in the use of different N forms by the co-existing tree species. By relating the amount of ammonium-N, nitrate-N and glycine-N taken up per soil volume to the amount of available $NH_4^+$-N, $NO_3^-$-N and glycine-N in that soil volume and comparing the three N forms for a species, one may obtain a rough estimate of the relative preference of the N forms by the species. With this simple calculation, we obtained approximate $NH_4^+/NO_3^-$ uptake ratios of about 2 to 4 for *Tilia, Acer* and *Carpinus*, of ~1 for *Fraxinus,* and of 0.5 for *Fagus*. These estimates base on the assumption that the applied $^{15}N$ was equally available for root uptake throughout the soil core and no $NH_4^+$ was adsorbed to soil particles, which may not be realistic. Moreover, we have

only estimates of the $^{15}N$ pool size in the soil but no precise concentration data, which also weakens the calculation of 'uptake rates'. While these methodological shortcomings certainly have biased the absolute values of calculated mass-based N uptake rates, the relation between species in terms of N acquisition should not be influenced by this effect.

Based on these approximate uptake figures, we found a significant preference for $NH_4^+$ over $NO_3^-$ for *Carpinus.* In the other species, only tendencies were visible, in two species for an ammonium preference (*Acer* and *Tilia*), in one species (*Fagus*) apparently for a nitrate preference. *Fraxinus* took up both N forms at similar rates in relation to the available pool. Despite the weaknesses of our approach, the contrast between *Carpinus* and *Fagus* was large (nearly 8-fold difference in apparent $NH_4^+/NO_3^-$ preference ratio: 3.8 *versus* 0.5) suggesting that at least these two species are in fact preferring different inorganic N forms in the Hainich mixed stand.

While it is generally assumed that coniferous trees are primarily taking up $NH_4^+$ (e.g. Marschner *et al.* 1991; Flaig and Mohr 1992; Buchmann *et al.* 1995; Schmidt *et al.* 1996; Kronzucker *et al.* 1997, 2003; Malagoli *et al.* 2000; Hangs *et al.* 2003; Gessler *et al.* 1998; Yanai *et al.* 2009), which is more economic than the uptake of $NO_3^-$, temperate broad-leaved tree species have been found to prefer either $NO_3^-$ or $NH_4^+$, or show no clear preference for the two N forms. Examples of reported ammonium preference in broad-leaved trees, when both N forms were equally available, are *Acer rubrum, A. saccharum, A negundo and A. platanoides,* and *Quercus rubra, Q. bicolor* and *Q. macrocarpa* (seedlings or mature trees; e.g. Stadler *et al.* 1993, BassiriRad *et al.* 1999, Zerihun and BassiriRad 2001, Templer and Dawson 2004, Gallet-Budynek *et al.* 2009, Jin *et al.* 2010, Socci and Templer 2011). Gessler *et al.* (1998) observed that *F. sylvatica* is mainly using ammonium when N deposition is high.

The uptake of $NO_3^-$, despite being more costly, has the advantage that this ion is more readily available for uptake at a given soil concentration, because the effective diffusion coefficient of nitrate is one to two orders of magnitude larger than that of $NH_4^+$ (Krom and Berner 1980) and immobilization through adsorption to the solid phase is of minor importance. Our finding of a possible preference of $NO_3^-$ over $NH_4^+$ by *Fagus* is consistent with results of laboratory or field studies in stands of *F. sylvatica* and *F. grandifolia* by May (1999), Templer and Dawson (2004), Dannenmann *et al.* (2009), Schulz *et al.* (2011) and Simon *et al.* (2011). In contrast, Paar (1994) found that juvenile *F. sylvatica* plants grew best with mixed $NH_4^+/NO_3^-$ nutrition, while Wallenda *et al.* (2000) found evidence for $NH_4^+$ preference in a field

study in a beech forest. In line with these contradicting results, *F. sylvatica* grows well not only on calcareous soils, where $NH_4^+$ is mostly oxidised to $NO_3^-$ by nitrifiers, but the species is equally productive on highly acidic soils where most inorganic N is supplied as $NH_4^+$ (Leuschner *et al.* 2006). In general, it can be expected that ammonium uptake becomes increasingly important at lower temperatures (Glass and Siddiqi 1995) and when autotrophic nitrifier activity is hampered by low pH and hypoxia. Averill and Finzi (2011) found that plants can shift from inorganic to organic N sources when temperature decreases. Thus, it appears that the preference of temperate forest trees for a certain N form is not a constant but may vary with growing conditions as indicated by the seasonal changes in uptake patterns observed by Socci and Templer (2011) and by the extremely broad tolerance of soil chemical conditions by *F. sylvatica* (Leuschner *et al.* 2006). It may well be that many tree species are using the different N forms primarily in dependence of their relative abundance in the soil. If so, N preference should vary with season and also between years due to variable temperature and moisture conditions which has to be examined by further study.

An assessment of the ecological meaning of apparent tree species differences in N form preference has to consider that (i) $NH_4^+$ and $NO_3^-$ were added at equal amounts in the different treatments of our experiment (~1.5 kg $NH_4^+$-N or $NO_3^-$-N $ha^{-1}$ equivalent to ~10.7 mol N $m^{-2}$), even though the concentration of free amino acids was by an order of magnitude higher than that of inorganic N, and $K_2SO_4$-extractable $NH_4^+$-N was 2 to 6-times more abundant than that of $NO_3^-$-N, and (ii) the *Fagus* plots had relatively high and the *Fraxinus* plots relatively low concentrations of amino acids and $NH_4^+$ (which is only partly explained by differences in pH). Thus, we cannot exclude that experiments simulating precisely the actual $NH_4^+/NO_3^-$ availability in the rhizosphere of the five tree species would lead to different results with respect to the N form preference.

A probably more influential factor in our approach is the fact that the labelled $NH_4^+$ and $NO_3^-$ solutions were applied through independent experiments in different plots, thus ignoring the inhibitory effect of ammonium on nitrate uptake, that is known from physiological and also field experiments (e.g. Lee *et al.* 1992, Kreuzwieser *et al.* 1997, Gessler *et al.* 1998, Templer and Dawson 2004). Absorbed $NH_4^+$ is converted to glutamine which blocks the $NO_3^-$ transport at the transcript level, but there is also indication of a direct inhibiting effect of $NH_4^+$ on the $NO_3^-$ transporter in root cell membranes (Glass 2005). Correspondingly, field studies found a strong inhibition of $NO_3^-$ uptake by the roots of *Fagus* trees when they were exposed to $NH_4^+$ (Kreuzwieser *et al.* 1997). Thus, a simple extrapolation of uptake rates measured in

independent experiments with added $NH_4^+$ and $NO_3^-$ solutions to the field situation, where the root can select between the two N forms, might be misleading because it ignores the interactions between the $NH_4^+$ and $NO_3^-$ uptake systems.

Our results indicate that all five species are using not only $NH_4^+$ and $NO_3^-$ but glycine as well. Organic N compounds such as various amino acids and also oligopeptides have been shown to represent relevant N sources of tundra and boreal forest plants (e.g. Näsholm *et al.* 1998, Nordin *et al.* 2001, Persson *et al.* 2003, Finzi and Berthrong 2005, Kielland *et al.* 2006, Näsholm *et al.* 2009) and transporters mediating amino acid uptake have been identified both in mycorrhizal fungi and in plant roots (Näsholm *et al.* 2009). More recently, evidence has been found that amino acids are also used by coniferous and broad-leaved trees in temperate forests (e.g. Finzi and Berthrong 2005, Dannenmann *et al.* 2009, Gallet-Budynek *et al.* 2009). Wallenda and Read (1999) found that ectomycorrhizal roots of *Fagus* were able to take up amino acids at rates similar to those for ammonium, but it is not fully clear whether the ability is linked to the presence of the ectomycorrhizal mycelium (Wallenda *et al.* 2000). Recent field studies with the *in situ* depletion technique indicate that beech trees growing at low N availability seem to prefer amino acids (glutamine) over $NO_3^-$ (e.g. Dannenmann *et al.* 2009, Simon *et al.* 2011), highlighting the importance of organic N sources for temperate broad-leaved trees. In relation to ammonium uptake, the importance of glycine uptake seems to increase with decreasing net N mineralization rate from cool-temperate forests (glycine/$NH_4^+$ ratio: 0.19-0.53, Finzi and Berthrong 2005), to boreal coniferous forests (1.3, Näsholm *et al.* 1998) and arctic vascular and non-vascular plants (2.1 and 5, respectively; Kielland 1994, 1997). Our results, which consider only glycine, do not allow conclusions on the relative importance of organic N as a nitrogen source in this forest because plants may use other amino acids as well (Inselsbacher and Näsholm 2012) which were not investigated.

*The role of complementarity*

Complementarity in the use of available N pools among the five species would imply that the species differ not only with respect to the use of different N pools, but that the stand total of N uptake is higher than it would be if no niche separation with respect to N uptake does occur. Our data provide some evidence that the first condition is partially fulfilled in this mixed forest, but it remains unclear whether apparent differences in N form preference are indeed leading to relevant complementarity in the use of soil N as postulated in our third research question. A simple summing-up of the calculated approximate mass-specific $NH_4^+$, $NO_3^-$ and

glycine uptake rates with the aim to obtain the relative contribution of the respective N form to total N uptake may not be correct, because none of the species avoided one of the three N forms completely and the literature suggests a considerable flexibility of trees in the use of the different N forms. Thus, if one N form is depleted through excessive uptake by a certain tree species (e.g. nitrate by *Fagus* in our stand), it is likely that the species would shift their preference partly to other N forms to meet their demand. Moreover, ammonium is in general rapidly oxidised to nitrate in this fertile Luvisol if not immobilised by microbial and plant uptake or desorption. Thus, a higher $NH_4^+$ uptake must reduce the $NO_3^-$ pool and its utilization by biota. Both processes suggest that the trees' niches with respect to the use of different N forms are not clear-cut ones. Rather, they appear to be more or less flexible, thus reducing the potential for niche segregation and complementary N use by a mixed stand. We would expect significant niche complementarity to occur when tree species with the capability of using considerable amounts of organic N are present, while other species are not using this N form. In our mixed stand, this may perhaps be the case in *Acer pseudoplatanus* with a calculated low glycin uptake in comparison to $NH_4^+$. However, further field and laboratory studies are needed to confirm these apparent species differences.

Our data cannot give a clear answer to the question whether species-richer stands are exhausting the pool of available N in the soil to a larger extent than species-poorer stands, which would imply a larger ratio of N taken up to N supplied by mineralization and input with throughfall and stemflow water in the mixed compared to the monospecific forest patches. Stand-level N uptake can in our study only be estimated from data on N flux with annual leaf litter fall and the amount of N accumulated in annual stem wood growth. This was calculated for the Hainich forest by M. Jacob (personal communication) for monospecific *Fagus* stands and 3- or 5-species mixed stands consisting of *Fagus, Tilia, Acer, Carpinus* and *Fraxinus*. These minimum estimates of N demand indicate that the mixed stands had a 30-40% higher N uptake than the monospecific stand (4.1-4.6 *versus* 3.3 g N $m^{-2}$ $yr^{-1}$) due to higher N concentrations in the leaf litter. On the other hand, litter decomposition and gross N mineralisation were higher in the mixed stands due to the more nutrient-rich leaf litter than in the nearby monospecific *Fagus* stands (Jacob *et al.* 2009, Guckland *et al.* 2010). It is thus difficult to prove increased resource exploitation with respect to N availability in this mixed forest.

We principally would expect that complementarity in N uptake from different soil N pools, as was shown to occur in temperate grasslands and arctic tundra (Kahmen *et al.* 2006, McKane *et al.* 2002), should be of lower importance in

temperate broad-leaved forests, where N-fixing tree species are the exception. Complementary N use is more likely to occur due to possible seasonal differences in N uptake activity of the species resulting from species-specific growth cycles. This aspect requires attention by future studies on the seasonal and inter-annual variability of N uptake in temperate trees.

*Methodological considerations*

Because the isotopic signal would not have been detectable in the leaves of tall trees when $^{15}N$ is added in low doses, we focused on fine roots and chose very short re-sampling intervals (10 min to 1 d). This approach has several advantages; the mycorrhizal status of the roots remained intact, it enabled us to measure $NH_4^+$ incorporation without the need to apply possibly harmful nitrification blockers, and it minimised the risk of glycine being transformed to $NH_4^+$ before uptake. Further, axial transport of N in the roots (e.g. Lazof *et al.* 1992, Engels *et al.* 2000) should be negligible after 10 min and of minor importance after 1 h (e.g. Schmidt and Stewart 1999; Jones *et al.* 2005a, b; Persson 2006; Näsholm *et al.* 2009). The amount of $^{15}N$ solution added (~1.5 kg N $ha^{-1}$ in 1.5 mm of water) was relatively small. Yet, it roughly doubled the concentrations of ammonium and nitrate in the soil and thus significantly altered N availability. The tracer application simulates a small rainfall event, as it is frequently occurring during summer dry spells in this forest, when mineral N released by biological and physical mineralisation processes is transported through the rhizosphere with pulses of infiltrating water (e.g. van Schreven 1967, Ladd *et al.* 1977).

A major challenge of this mixed forest study was the sorting of fine root biomass by tree species which was time consuming and bases on earlier work on the fine root morphology of the species in this stand (Meinen *et al.* 2009, Jacob *et al.* 2013). Most root samples contained fine branch roots of three to five species which ensures that our N uptake data are reflecting the field situation with assumed intensive competition for N between roots of different tree species and between plants and the microbial community. A clear disadvantage of this approach is that it took several days to weeks to sort the root mass by species.

An alternative technique of measuring the uptake of different N forms by tree roots in short time intervals (hours) under field conditions is the *in situ* depletion technique described by Gessler *et al.* (1998). This approach uses artificial soil solutions into which isolated, but intact, fine root branches are inserted for 2 h. The technique is probably more precise with respect to the measured mass-specific

uptake rate than our soil coring approach because it focuses on a single well-defined root segment, but it is more artificial since the mycorrhizal net is destroyed, the rhizosphere is altered and competition with soil bacteria and other roots is excluded (Yanai *et al.* 2009). Despite largely different experimental settings, the two techniques nevertheless gave mass-related N uptake rates that were in the same order of magnitude for the first 1 or 2 h after tracer application. For example, Simon *et al.* (2011) reported $NH_4^+$, $NO_3^-$ and glutamine uptake rates with the *in situ* depletion technique for fine roots of *Fagus* trees of about 15-30, 30-85 and 70-150 µg N $g^{-1}$ root fresh weight $h^{-1}$, respectively. These figures compare well with our results measured in intact rhizosphere patches of beech trees during the first 10 min after tracer application (65, 188 and 71 µg N $g^{-1}$ root dry weight $h^{-1}$ for $NH_4^+$, $NO_3^-$ and glycine, respectively) considering that Simon *et al.* (2011) calculated on a root fresh weight instead of dry weight basis. However, the uptake rates calculated from our experiment were lower than the figures of Simon *et al.* (2011) when longer incubation periods (1 h or 1 d instead of 10 min) were considered. This is a likely consequence of the fact that the *in situ* depletion technique guarantees more or less constant N supply to the roots during the measuring period while the tracer had to move by mass flow or diffusion through the bulk soil in our coring experiment. We assume that the rates calculated by Simon *et al.* (2011) represent maximum uptake rates achievable under ambient N concentrations while our figures are influenced by processes such as N depletion in the immediate root neighbourhood and sorptive interactions with the soil (in particular of ammonium) which may reduce N supply to the roots and hence uptake (Comerford 2005).

Competition between roots and soil microbes for the added N tracer could also influence the results of uptake experiments because soil microorganisms typically are superior competitors shortly after inorganic N becomes available (Kuzyakov and Xu 2013). Subsequent studies should quantify N immobilisation in microbial biomass in the course of $^{15}N$ root uptake studies.

A very rough extrapolation of our root-specific uptake rates to the stand level and the whole vegetation period suggests that the average mass-related uptake rates must be markedly smaller in the field than the rates measured 10 min or 1 h after tracer application (typical rates of 109 and 9 µg N $g^{-1}$ beech root dry mass $h^{-1}$), but substantially higher than the low rates measured 1 d after application (<1 µg N $g^{-1}$ $h^{-1}$): A simple calculation may assume root uptake activity on 15 h of the day (predominantly in the daytime hours, Lucash *et al.* 2007), a length of the vegetation period of 200 d and a stand total of fine root biomass of 350 g $m^{-2}$ in the beech-dominated stand (Meinen *et al.* 2009), thus giving totals of 114 and 9 g N $m^{-2}$ taken

up per year when the incorporation rates measured after 10 min and 1 h are used, respectively. However, only 1 g N $m^{-2}$ are obtained when calculating with the 1 d-rates. Given that a mature stand of European beech is requiring about 6-7 g N $m^{-2}$ $yr^{-1}$ for maintaining its annual production of wood and leaf litter (Ellenberg *et al.* 1986, Matzner 1988, Sah 1990) and adding another ~3 g N $m^{-2}$ $yr^{-1}$, which likely is consumed by root growth and turnover, the first two extrapolated uptake figures are much higher than the estimated stand level uptake of c. 10 g N $m^{-2}$ $yr^{-1}$. The low calculated uptake rate based on the measurements after 1 d is clearly not sufficient to meet the annual demand of the stand. We conclude that field studies with $^{15}N$ tracer application and rapid root sampling may provide insights into N form preferences and uptake kinetics, but neither the *in situ* depletion technique of Gessler *et al.* (1998) and Simon *et al.* (2011) nor our intact root coring approach seem to give root N uptake rates that are close to the long-term mean N uptake of the stand.

One reason for this discrepancy is that short-term tracer application experiments often are overestimating uptake because part of the tracer, in particular $NH_4^+$, may be adsorbed in the first few minutes in the rhizodermis or periderm of the root, thus not entering the xylem (Kronzucker *et al.* 1995). Further, uptake experiments do not, or not adequately, simulate the large spatial and temporal variability in N availability in the soil, which is caused by uptake-driven nutrient depletion in the rhizosphere, sorption-desorption processes in the bulk soil and the shift between mass flow- and diffusion-mediated N supply to the roots (Comerford 2005). This could be relevant in particular for the sampling after 1 d. Finally, the sampling after 1 d (and perhaps in part also that after 1 h) likely is affected by axial N transport in the roots leading to underestimation of uptake.

Nitrogen availability may modify the uptake capacity of plant roots. In general, higher specific $NH_4^+$ uptake rates were measured when N concentrations were low (Wallenda *et al.* 2000), indicating up-regulation of maximum uptake velocity ($V_{max}$) when $NH_4^+$ is short in supply (e.g. Wang *et al.* 1993a, b). For assessing the reliability of N uptake figures derived from tracer experiments under field conditions, it would be necessary to compare different measuring approaches in the rhizosphere of mature trees and to monitor temporal change in apparent uptake rates immediately after tracer application (1-15 min). Such an approach was applied by Lazof *et al.* (1992) in corn plants by exposing intact growing roots for short periods to $^{15}N$ tracer solution.

## Conclusions

Broad-leaved trees co-existing in temperate mixed forests may well differ with respect to their preference for $NH_4^+$, $NO_3^-$ and glycine, when offered at equal abundances, but there are several reasons to believe that niche complementarity for N is not a major force in this type of forest. This would distinguish mixed temperate broad-leaved forests from temperate grasslands and arctic tundra, where niche complementarity through the use of different N forms was shown to be relevant. First, N-fixing tree species do rarely occur in these forests which narrows the maximum range of accessible N sources. Second, temperate tree species may be rather flexible with respect to the preference of different N forms, which should lead to considerable niche overlap among co-occurring species, as is demonstrated by the contrasting reports on $NO_3^-$, $NH_4^+$ or amino acid preference of *Fagus sylvatica*. The rapid oxidation of $NH_4^+$ to $NO_3^-$ in many temperate forests on base-rich fertile soils suggests a less rigid niche separation in these communities. Third, concepts on niche complementarity for N use in species-rich plant communities have rarely considered the well known physiological interaction between $NH_4^+$ and $NO_3^-$ uptake systems which implies a strong influence of ammonium or nitrate availability on the N form taken up. Finally, it still has to be shown that mixed stands with a larger diversity in N uptake strategies are indeed exhausting the total available soil N pool more completely, as should be expected when the N pools are used in a complementary manner. It may also be possible that soil microbes are immobilising any surplus in available N in less diverse stands, rendering it difficult to prove increased N use in more diverse forests. A larger reduction of the available soil N concentration in mixed as compared to monospecific stands might be expected in forest communities, where certain tree species are capable of using organic N sources, while others are not, but supporting evidence is not yet available.

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## Supplementary material (Appendix)

**Table 4.A1** Apparent preference of fine roots of the five species for the different N forms. Given is the measured apparent N uptake as ammonium, nitrate or glycine of the roots of a species (0-20 cm, 1 h after tracer application) as a fraction of the available $NH_4^+$, $NO_3^-$ or glycine in the soil volume (means ± SE of four replicate plots). Differences significant at P <0.1 are marked by different letters (species contrasts: Latin lower case letters, N form contrasts: Latin capital letters).

| | **N form** | | |
|---|---|---|---|
| **Species** | $NH_4^+$ | $NO_3^-$ | Glycine |
| *Fraxinus excelsior* | 0.018 ± 0.008 b | 0.021 ± 0.006 b | 0.009 ± 0.003 b |
| *Acer pseudoplatanus* | 0.005 ± 0.003 ab | 0.002 ± 0.001 a | 0.003 ± 0.001 ab |
| *Carpinus betulus* | 0.004 ± 0.002 ab B | 0.001 ± 0.00003 a A | 0.001 ± 0.0002 a A |
| *Tilia cordata* | 0.004 ± 0.001 ab | 0.002 ± 0.001 a | 0.001 ± 0.0005 a |
| *Fagus sylvatica* | 0.002 ± 0.0002 a AB | 0.003 ± 0.001 a B | 0.001 ± 0.0001 a A |

**Table 4.A2** Percental contribution of ammonium, nitrate and glycine uptake to the estimated total N uptake of the five tree species on the stand level. The calculation bases on the apparent uptake rates of a species measured 1 h after tracer application assuming that total N uptake is the sum of the uptake of the three N forms.

| | **N form** | | |
|---|---|---|---|
| | $NH_4^+$ | $NO_3^-$ | Glycine |
| *Fraxinus excelsior* | 28.8 | 53.3 | 17.9 |
| *Acer pseudoplatanus* | 41.8 | 22.1 | 36.2 |
| *Carpinus betulus* | 56.3 | 23.0 | 20.8 |
| *Tilia cordata* | 45.2 | 35.0 | 19.8 |
| *Fagus sylvatica* | 23.0 | 59.0 | 18.0 |

**Table 4.A3** Published field and laboratory studies on the apparent preference of various tree species (mature trees, saplings or seedlings) from temperate and boreal forests for ammonium, nitrate or organic N.

| N forms applied | Study approach | Species | Preferred N form | Authors |
|---|---|---|---|---|
| FIELD STUDIES | | | | |
| $NH_4^+$, $NO_3^-$, glycine | $^{13}C$ and $^{15}N$ tracer application using intact fine root system | mixed-forest, *Abies balsamifera, Betula alleghaniensis, Picea rubens, Sorbus americana* | glycine > $NH_4^+$ > $NO_3^-$; use of $N_{organic}$ by hardwood and coniferous trees increases with elevation | Averill and Finzi 2011 |
| $NH_4^+$, $NO_3^-$, arginine, glutamine | depletion technique using intact fine roots | mature stand of *Fagus sylvatica* | preference for organic N > $NO_3^-$ > $NH_4^+$ | Dannenmann et al. 2009 |
| $NO_3^-$ | depletion technique and $^{15}N$ tracer application using intact fine roots | 72-yr-old stand, *Acer rubrum, Acer saccharum, Fagus grandifolia, Fraxinus americana, Liriodendron tulipifera, Picea abies, Pinus strobus, Quercus rubra* | $NO_3^-$ uptake differs among species with low values for *Fagus* and highest for *A. saccharum* and *Pinus* | Fahey and Yavitt 2005 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using intact fine root system | 72-yr-old stand of *Picea abies* | recovery of $NH_4^+$ in roots of the organic layer > $NO_3^-$; more $NO_3^-$ than $NH_4^+$ recovered in the subsoil | Feng et al. 2008 |
| $NH_4^+$, $NO_3^-$, glycine | $^{13}C$ and $^{15}N$ tracer application using intact fine root system | 2 mixed forests, *Acer saccharum, Fraxinus americana, Fagus grandifolia, Quercus rubra, Tsuga canadensis* | intact glycine uptake in both stands; glycine > $NH_4^+$ > $NO_3^-$ on acidic soil, $NO_3^-$ > $NH_4^+$ > glycine on base-rich fertile soil | Gallet-Budynek et al. 2009 |
| $NH_4^+$, $NO_3^-$ | depletion technique using intact fine roots | 88-yr-old stand of *Picea abies* and 95-yr-old stand of *Fagus sylvatica* | uptake of $NH_4^+$ > $NO_3^-$ in *Picea* and *Fagus* | Gessler et al. 1998 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using intact fine root system | 50-75-yr-old mixed forest, *Acer saccharum, Fagus grandifolia Quercus rubra, Ulmus rubra* | higher recovery of $NO_3^-$ than $NH_4^+$ in well-drained soil, no differences between N forms in poorly drained soil | Groffman et al. 1993 |
| $NH_4^+$, glycine | $^{15}N$ tracer application using intact fine root system | seedlings of *Acer rubrum* and *Pinus palustris* | $NH_4^+$ > $NO_3^-$; no uptake of $N_{organic}$ as intact amino acid, but enrichment in roots of N mineralized from labeled glycine | Jin et al. 2010 |
| $NH_4^+$, $NO_3^-$ | *in situ* depletion technique using intact fine roots (and *ex situ* depletion technique using intact non-mycorrhizal fine roots) | 60-yr-old stand and 4-yr-old saplings of *Picea abies* | preferential uptake of $NH_4^+$ as compared to $NO_3^-$ | Marschner et al. 1991 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using intact fine root systems | 30-yr-old stand of *Fagus sylvatica* and *Quercus petraea* | preference for $NO_3^-$ over $NH_4^+$ | May 1999 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using intact fine root system | 140-yr-old stand of *Picea abies* | preference for $NH_4^+$ over $NO_3^-$ | Schmidt et al. 1996 |
| $NH_4^+$, $NO_3^-$, glutamine | depletion technique using intact fine roots | mature stand of *Fagus sylvatica* | similar uptake of glutamine and $NO_3^-$, but lower uptake of $NH_4^+$ | Simon et al. 2011 |
| $NH_4^+$, $NO_3^-$ | *in situ* depletion technique using intact fine roots (and *ex situ* $^{15}N$ tracer application using excised fine roots) | mixed forest, *Acer saccharum, Picea rubens* | uptake of $NH_4^+$ > $NO_3^-$ for both methods and species | Socci and Templer 2011 |
| $NH_4^+$, $NO_3^-$ | depletion technique using intact fine roots | 200+ yr-old stand of *Abies lasiocarpa* and 400+ yr-old spruce stand of *Picea engelmanni* | higher $NH_4^+$ than $NO_3^-$ uptake | Yanai et al. 2009 |

| N forms applied | Study approach | Species | Preferred N form | Authors |
|---|---|---|---|---|
| $NH_4^+$, $NO_3^-$, glycine | $^{15}N$ tracer application using intact fine root system | 146-yr-old mixed forest, *Acer pseudoplatanus, Carpinus betulus, Fagus sylvatica, Fraxinus excelsior, Tilia cordata* | preference for $NH_4^+$ in *Carpinus* (and *Acer* and *Tilia*), preference for $NO_3^-$ in *Fagus*, no difference in $NH_4^+$ *vs.* $NO_3^-$ uptake in *Fraxinus*, all species use small amounts of glycine | This study |
| LABORATORY STUDIES | | | | |
| $NH_4^+$, $NO_3^-$ | depletion technique using intact fine roots | 1-yr-old saplings of *Acer rubrum, A. saccharum* | higher $V_{max}$ for $NH_4^+$ uptake than $NO_3^-$ uptake | BassiriRad et al. 1999 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using excised fine roots | *Alnus crispa, Betula papyrifera, Larix laricina, Picea glauca, Picea mariana, Populus balsamifera, Populus tremuloides* | uptake of $NH_4^+$ > $NO_3^-$, very low $NO_3^-$ absorption capacity of all species | Chapin et al. 1986 |
| $NH_4^+$, $NO_3^-$, glycine | $^{15}N$ tracer application using excised fine roots | *Acer rubrum, A. saccharum., Betula sp., Carya ovata, Fraxinus americana, Pinus strobus, Populus grandi-dentata, Prunus serotina, Quercus rubra, Tsuga canadensis* | uptake of $NH_4^+$ > glycine > $NO_3^-$; preferation of inorganic N or organic N according to their availability in the soil | Finzi and Berthrong 2005 |
| $NH_4^+$, $NO_3^-$ | depletion technique using intact fine roots | seedlings of *Pinus sylvestris* | $NH_4^+$ as preferred N-form | Flaig and Mohr 1992 |
| $NH_4^+$ | *ex situ* $^{15}N$ and $^{86}Rb$ tracer application using excised fine roots | 40-yr-old stands of *Fagus sylvatica, Picea abies, Quercus robur* | $NH_4^+$ uptake of spruce and beech not differing with soil depth, reduced uptake with increasing soil depth in oak | Göransson et al. 2006, 2008 |
| $NH_4^+$, $NO_3^-$ | depletion technique using intact fine roots | seedlings of *Picea glauca*, *Pinus banksiana*, *Populus tremuloides* | *Picea* and *Pinus* with lower maximal uptake rates and lower affinity to $NH_4^+$ and $NO_3^-$ than *Populus* | Hangs et al. 2003 |
| $NH_4^+$, $NO_3$ | depletion technique using intact fine roots | seedlings of *Picea glauca* | higher $NH_4^+$ than $NO_3^-$ uptake | Kronzucker et al. 1997 |
| $NH_4^+$, $NO_3^-$ | depletion technique using excised fine roots | seedlings of *Betula alleghaniensis, Carya ovata, Fagus sylvatica, Fraxinus americana, Liriodendron tulipifera, Prunus serotina, Quercus phellos* | uptake of $NO_3^-$ not principally different from $NH_4^+$ in all species despite differences in shade-tolerance and relative growth rate | Lajtha 1994 |
| $NH_4^+$, $NO_3^-$ | depletion technique using intact fine roots | seedlings of *Larix decidua* and *Pinus sylvestris* | higher $NH_4^+$ than $NO_3^-$ uptake | Malagoli et al. 2000 |
| $NH_4^+$, $NO_3^-$, arginine, glycine | $^{13}C$ and $^{15}N$ tracer application using intact fine root system | seedlings of *Picea abies* and *Pinus sylvestris* | intact arginine and glycine and $NH_4^+$ taken up at equal rates, much higher than $NO_3^-$ uptake | Öhlund and Näsholm 2001, 2004 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using intact fine root system | 2-yr-old saplings of *Fagus sylvatica Fraxinus excelsior, Picea abies, Pinus sylvestris Quercus petraea, Tilia cordata* | all species except for *Picea* tended to take up $NO_3^-$ at higher rates than $NH_4^+$ | Schulz et al. 2011 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using intact fine root system | 2-yr-old saplings of *Fraxinus excelsior* and *Quercus robur* | slight preference for $NH_4^+$ | Stadler et al. 1993 |
| $NH_4^+$, $NO_3^-$ | $^{15}N$ tracer application using intact fine root system | seedlings of *Acer saccharum Fagus grandifolia, Quercus rubra, Tsuga canadensis* | preference for $NH_4^+$ in *Acer*, *Tsuga* and *Quercus*, preference for $NO_3^-$ in *Fagus* | Templer and Dawson 2004 |
| $NO_3^-$ | $^{15}N$ tracer application using intact fine root system | saplings of *Quercus serrata* | $NO_3^-$ uptake in midwinter in the absence of leaves | Ueda et al. 2010 |
| $NH_4^+$, $NO_3^-$ | depletion technique using intact fine root system | seedlings of *Acer negundo, A. platanoides, Pinus retinosa, P. taeda, Quercus bicolor, Qu. macrocarpa* | $NH_4^+$ > $NO_3^-$ uptake capacity; higher $K_m$ or $NO_3^-$ uptake in *A. negundo* and *A. platanoides* | Zerihun and Bassirirad 2001 |

# Chapter 5

## Synthesis

The main objective of the present study was to investigate fine root biomass and dynamics in monospecific and mixed cluster plots composed of all possible combinations of the five most abundant tree species in the Hainich mixed forests (chapter 1). Investigating the role of biodiversity in ecosystem functioning, the aim of this study was to identify tree species diversity and identity effects on standing fine root biomass, fine root growth and turnover, and fine root physiology in terms of soil N uptake strategies.

### *Fine root bio- and necromass*

In chapter 2, the existence of possible tree species diversity and identity effects on fine root bio- and necromass was examined in the cluster plots differing in species composition. No evidence has been found for a positive diversity effect on standing fine root biomass and hence of overyielding in terms of fine root biomass. In contrast, fine root necromass decreased slightly with increasing number of tree species resulting in a negative influence of tree species diversity on the necromass. This fits in part with the results of Meinen (2008) where fine root bio- and necromass at the stand level were not significantly influenced by tree species diversity. Both independent root inventories showed similar results despite marked differences with respect to spatial scale (size of a sampled plot area: 2500 *versus* c. 25 $m^2$ in average), sampling depth (0-40 *versus* -20 cm) and sampling timing (different years of investigation: 2005/06 *versus* 2008). We assume that the available rooting space in the relatively shallow soils of the Hainich forest must pose an upper limit to the production and maintenance of fine root biomass at this site. The fine root biomass density of 1.5 g $l^{-1}$ in the 0-20 cm soil layer is relatively high pointing to strong root competition because suitable rooting space is apparently limited. Fine root density has been found to rarely exceed values of 1-2 g fine root biomass per litre soil volume in the densely rooted topsoil horizons of temperate broad-leaved forests (Hertel 1999). The investigations of Meinen (2008) on rooting patterns to a depth of 40 cm, found no evidence for vertical fine root stratification among the five tree species in the Hainich mixed forest stands as a consequence of interspecific competition. These findings are contradictory to several other earlier studies in boreal and temperate mixed forests where species mixtures with markedly different tree functional types were found to generate root system stratification (e.g. Schmid and Kazda 2002, Curt and Prevosto 2003, Bolte and Villanueva 2006). We agree with the assumptions of Meinen (2008) that the soil-physical conditions in Hainich National Park (relatively shallow soils with a high clay content, stagnic soil properties during late winter and spring and drought events in summer) could make deeper

root penetration and spatial root segregation difficult for the tree species resulting in a marked concentration of fine roots (more than 80%) in the upper soil layer.

Standing fine root biomass was more influenced by species identity. When comparing all cluster plots, in which a species was present, we found that each cluster plot with presence of maple and beech showed higher standing fine root biomass compared to plots where hornbeam was present. In terms of the fine root biomass to basal area ratio of a tree species, fine roots of ash were over-represented in mixed cluster plots compared to the monospecific plots. The other four tree species showed only tendencies for an over- (lime and maple) or under-representation (beech and hornbeam) in the mixed plots. The fine root abundance patterns of ash may draw the picture of a belowground competitive superiority of this tree species over the other four species in the Hainich mixed forests. A root competition experiment between saplings of both species showed similar results with a superiority of ash over beech (Rust and Savill 2000). It seems that the belowground strategy of ash is more oriented to a fast developing root system for a greater benefit in e.g. water and nutrient uptake or soil space occupation. Moreover, we suppose that ash may be better adapted to the harsh soil conditions in the forest stands of the Hainich as mentioned above in contrast to beech. For the same study site, Köcher (2012) identified a higher drought tolerance of ash compared to the other species, which also suggests a better adaptation to increasing drought or heat events as predicted by climate change scenarios.

In conclusion, we can confirm all three hypotheses that (i) tree species identity has a larger effect on standing fine root biomass than species identity, that (ii) no significant overyielding with respect to standing fine root biomass exists in the mixed-species cluster plots in comparison to the monospecific plot types and that (iii) the five tree species differ in their over- or under-representation in terms of their fine root biomass in a mixture.

### *Belowground productivity*

In chapter 3 in extension of the findings of chapter 2, we focused on whether tree species diversity and identity influence fine root production in terms of fine root growth and turnover. We found no diversity effect on fine root growth into the root-free ingrowth cores whereas fine root turnover increased from monospecific to 2- and 3-species cluster plots (marginally significant). Results from other studies were often controversial and depending on the type of biome and study site conditions. For example, Brassard *et al.* (2010) compared mixed and monospecific boreal forest stands and found higher fine root production in the species mixtures (up to five tree

species) than in the respective monocultures. In contrast, McKay and Malcom (1988) could show higher production values in pure stands of two coniferous tree species compared to a mixture of both species. We believe that a positive effect on the belowground productivity–diversity relationship most likely exists in tree or agroforestry systems (e.g. in the tropics), where selected tree species with largely different functional traits are planted together. In addition, positive interactions related to fine root production might be less important in temperate and tropical forests, because these ecosystems form more closed canopies than boreal forests and the climate is more favourable.

Interestingly, we found a positive relation between tree species diversity and fine root turnover in our study. Despite the lack of minirhizotron data, it appears that the fine roots of some of the five species tended to have a higher longevity in cluster plots where only fine roots of the same species were present as compared to mixed plots. However, we suppose more a species-specific effect than an influence of species diversity, because the presence of allospecific roots in the cluster plot could reduce the growth and survivorship of certain roots (e.g. through allelopathy). Meinen (2008) found similar results with significantly higher fine root turnover rates in species-richer stands of the Hainich. The author interpreted this finding as an indication for a more rapid recovery of the fine root system of a mixed forest after soil disturbances (e.g. installation of ingrowth cores, wild boars) than in a species-poor stand. Another possible explanation could be that interspecific belowground competition for resources (e.g. water) is more intense than intraspecific competition resulting in greater soil moisture depletion in mixtures as compared to monocultures. Gebauer (2010) measured an up to 50% higher canopy transpiration rate in 5-species stands than in beech-dominated stands in the Hainich forests under certain conditions. Furthermore, Krämer (2009) observed a high variability in soil moisture in these stands, which may indicate that the studied Hainich mixed forests are depleting soil water reserves stronger than the pure beech stands.

Ash showed repeatedly an apparent species identity effect through increased plot-level fine root production in the mixed-species cluster plots in contrast to the other four tree species. Several other studies on root production in mixed forests, based on species identification, also revealed productivity differences between tree species. For example, both Hertel (1999) and Leuschner *et al.* (2001) observed a higher fine root production of European beech when compared to Sessile oak in a mixed stand on sandy soil.

In absence of a tree species diversity effect on fine root production, we can reject hypothesis (i) that fine root production increases from 1-species to 3-species

cluster plots. An increase in fine root production and turnover in mixtures may exist through the presence of a highly productive tree species (selection effect) supporting hypothesis (ii). In support of hypothesis (iii), there is clear evidence for a higher importance of tree species identity on root production than tree species diversity. While standing fine root biomass remained stable, we assume that the increase in fine root turnover with increasing species richness is linked with an intensification of belowground competition between the tree species supporting our last hypothesis (iv).

### *Fine root physiological activity in terms of N uptake strategy*

In chapter 4 we discussed the possible differences in uptake strategies of different forms of N ($NH_4^+$, $NO_3^-$ and glycine) among the five tree species based on analyses of short-time sampling of fine root material of the species after tracer application to the soil. All species partly differed in their preference of $NH_4^+$, $NO_3^-$ and glycine. While hornbeam, lime and maple tended to prefer $NH_4^+$ over $NO_3^-$, ash showed equal uptake rates for both inorganic nitrogen forms and beech preferred $NO_3^-$. In contrast to our results, the literature suggested that most coniferous tree species may primarily take up $NH_4^+$ whereas temperate broad-leaved tree species should prefer either $NO_3^-$ or $NH_4^+$ or show no clear preference for the two N forms. Thus, we infer that the N-form assimilated by trees (including organic N compounds) mainly depends on their relative abundance or availability in the fertile base-rich soil of the investigated Hainich forest stands.

Regarding organic N, these compounds can play an important role as an N source for arctic plants and boreal forest ecosystems (e.g. Näsholm *et al.* 1998, Kieland *et al.* 2006). Our results also indicate that the uptake of glycine by all five tree species contributes to the amount of total N taken up by each species. This suggests a significant role of amino acids as N source in temperate forests even though the importance of organic N might be smaller than that of inorganic N. Two recent studies conducted in beech forests under low N availability support this assumption with a clear preference for amino acids (Dannenmann *et al.* 2009, Simon *et al.* 2011). However, if an N form is depleted through the strong uptake by a particular tree species, it is likely that the species will shift its preference in favour of other N forms available to meet its demand. In relation to the type of mycorrhiza, the three ECM-infected tree species showed no differences in their N uptake strategy compared to the two species with AM mycorrhiza. The influence of fine root morphology on N uptake rates was remarkably small among the five tree species. Thus, we assume a higher influence of root physiological properties (e.g. uptake

kinetics) on specific N uptake rates than effects of species-specific differences in root morphological traits. Definitely, more root samples and replications are needed to proof and to confirm these findings and assumptions with respect to species differences in N form preference in the mixed forests of Hainich National Park.

We can summarize in support of our hypotheses that (i) the coexisting tree species differ in their preference for $NH_4^+$, $NO_3^-$ and glycine, but niche complementarity for N form uptake appears not as a major force in the Hainich forests. Further, (ii) large differences in fine root morphology existing between the five tree species seem to be less important for N uptake than root physiological traits, and (iii) in the absence of nitrogen partitioning with respect to N form there was apparently no increase of the N uptake rate by the tree species at the stand level.

### *Final conclusions*

The whole study design of the RTG, ranging from stand-, over tree group- to single tree level, enables to draw conclusions about influencing factors or effects on ecosystem functioning and processes. A few recent studies under the framework of the RTG already revealed that the identity of a single tree species in a stand with its functional and morphological traits could have a higher impact on the following compartments or functions of the mixed Hainich forest than tree species richness (Table 5.1): soil properties (Guckland 2009, Langenbruch 2012); water use strategy, transpiration rate and drought tolerance (Gebauer 2010, Köcher 2012); aboveground productivity and nutrient relations (Jacob 2010) and the soil fauna community and leaf litter decomposition (Weland 2009).

In relation to tree fine root systems, the same findings as those of the present study were first provided by the PhD study of Meinen (2008) conducted on large-scale plots (50 m x 50 m) in the Hainich forest stands. The author proposed a dominant influence of tree species identity on standing fine root biomass, because a diversity effect could not be shown. This stand-level study used a gradient of tree species diversity with one, three or five dominant tree species, but only beech was the sole matrix tree species due to the lack of monospecific plots of the other four tree species. In the present thesis, the used matrix of 100 small-scale cluster plots selected under natural conditions was used to analyse monospecific and mixed plots composed of all possible combinations of the five tree species allowing separating diversity from identity effects on standing fine root biomass and productivity. In accordance with the results of Meinen (2008), the prominent role of species identity and not species diversity could definitely be supported, which

determines the size and function of the root systems of the broad-leaved trees. Not complementarity effects but large differences in fine root morphology (such as type

**Table 5.1** PhD studies in the RTG addressing the influence of tree species diversity and identity effects on ecosystem functioning and processes in the Hainich mixed forests.

| Study design | Investigated parameter | Conclusions | Author |
|---|---|---|---|
| ***Tree species diversity effect*** | | | |
| Stand level | Insect diversity and multitrophic interactions | Complex diversity-functioning relationships | Sobek (2008) |
| Tree group level | Herb layer characteristics, fly communities and trophic interactions | Positive diversity-functioning relationships | Vockenhuber (2012) |
| Stand level | Herb layer community | Positive diversity-functioning relationships in the herb layer | Mölder (2008) |
| ***Tree species diversity and identity effects*** | | | |
| Stand level | Soil water dynamics, rainfall partitioning and ion deposition | Key-role of both selection and complementarity effects | Krämer (2009) |
| ***Tree species identity effect*** | | | |
| Stand level | C and N content in the soil | Influence of beech on soil properties and soil related processes | Guckland (2009) |
| Tree group level | C and N content in the soil | Positive influence of ash on the C stocks in the topsoil | Langenbruch (2012) |
| Stand level | Water turnover (xylem sap flow and canopy transpiration) | Species identity and related functional traits are more important for water consumption than species diversity | Gebauer (2010) |
| Individual tree level | Water use strategy (hydraulic traits) | Large differences in hydraulic traits between co-existing species resulting in different growth performance | Köcher (2012) |
| Stand level | Tree fine root distribution and dynamics | Absence of complementarity effects (no mass-related overyielding, no spatial root system segregation) | Meinen (2008) |
| Stand level | Nutrient cycle, aboveground productivity | Ecosystem functions are more influenced by functional traits than species diversity | Jacob (2010) |
| Stand level | Soil fauna community, leaf litter decomposition | Species' traits influence soil and habitat properties and soil fauna communities more than species richness | Weland (2009) |
| Tree group level | Tree fine root distribution and dynamics | Tree species identity more important in root-related functions (e.g. fine root productivity and turnover) | This study |

of mycorrhiza and hydraulic architecture) are leading to higher water and nutrient consumption rates of mixed than monospecific stands in the Hainich forest under certain conditions. More investigations have to be carried out to proof these findings.

*Outlook*

Further studies are needed to investigate the adaptation of a tree species' root system to different site/ stand and climatic conditions in more detail in order to better predict the effects of anthropogenic impacts (e.g. nitrogen addition) or climate change (e.g. increasing drought). Moreover, species identity effects should be addressed in deeper analysis on how tree species interact with or influence their environment in the soil via rhizodeposits (e.g. organic acids, phytohormones, allelochemicals or other chemical compounds) or compete for resources. Another topic of interest could be the question whether the plasticity of the fine root architecture of tree species exists as an outcome of intra- and interspecific belowground competition or indicates the adaption of these roots to special site or environmental conditions.

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