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Species-specific fine root biomass, morphology and dynamics of six co-occurring deciduous tree species in the Hainich National Park and a conifer tree species at the alpine treeline

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Forschen heißt zu sehen, was jeder sieht,
jedoch dabei zu denken, was noch niemand gedacht hat.

- Albert Szent-Györgyi –

Summary

This thesis is subdivided into two main research areas. The first two studies were conducted in a mature mixed temperate broad leaved forest with regard to species identity effects on fine root traits, while the third study of the thesis addresses the influence of soil temperature on fine roots of *Pinus cembra* at the alpine treeline.

In the species-rich broad leaved forest within the Hainich National Park, we assessed the role of taxonomic position and mycorrhiza type (EM and AM) on fine root biomass, fine root morphology (on root order level) and fine root dynamics of six coexisting tree species (*Fagus sylvatica* L., *Tilia cordata* Mill., *Carpinus betulus* L., *Fraxinus excelsior* L., *Acer pseudoplatanus* L. *Acer platanoides* L.). We detected similar trends of a decrease of specific root area and specific root length from the first (the root tips) to the fourth root order in all six species. Nevertheless, the root order traits differed strongly between the species, especially for the variables root tissue density and root nitrogen concentration. The highest root nitrogen contents were found in the root tips and decreased with root order. Comparing the species, *F. excelsior* contained the highest nitrogen content in all root orders. Fine root productivity differed strongly between the six species with the highest production in *C. betulus, F. sylvatica,* and *F. excelsior* (~ 150-170 g m^{-2} yr^{-1}). Most differences in fine root production and turnover between the species were found in the deeper soil layers compared to the upper soil layer at 0-10 cm. Root turnover varied up to fivefold among the species, with lowest values in *Acer pseudoplatanus* and highest values in its congener *A. platanoides.* Even these two congeneric species differed strongly in their branching traits in the same stand, suggesting that they use differing belowground foraging strategies (e.g. more root tips per biomass in *A. pseudoplatanus* vs. a higher root turnover in *A. platanoides*).
In general, species identity was found to be the most important determining factor for fine root morphology and biomass as well as for fine root productivity and turnover rates, whereas the influence of mycorrhiza type was only of secondary importance. Species differences in fine root traits were more pronounced for the respective root orders than in comparison for the whole root branches.

At the alpine treeline, soil temperature is assumed to be the key factor influencing root biomass, production and morphology of fine roots. So far, most studies have been conducted on temperature influences on fine roots of tree saplings and juvenile trees in ex-situ

experiments, and there is very little data on temperature dependent fine root traits of mature trees at the treeline. In this study we investigated fine root mass distribution, fine root morphology and fine root production and turnover around solitary *Pinus cembra* trees at the alpine treeline in the Central Eastern Alps in Austria. Those fine root data were linked to soil temperatures around the trees, measured at the time with maximal temperature deviations between shade and sun. The main objective was to determine whether soil temperature, lowered by the shade of the crown, impairs fine root growth around mature Pine trees.

In contrast to previous findings regarding fine root reactions to low temperatures, we found higher fine root masses (living and dead) in cooler areas around the tree stems during a sunny summer day. Additionally in cooler, shaded soil there was a higher fine root production and turnover, which leads us to the assumption that trees invest more carbon into fine roots of cooler soil areas to compensate fine root loss and maintain optimal resource acquisition, as nutrient accessibility is lower in cold soil areas. In general, our findings suggest that fine root traits and their dependency of soil temperatures might not be comparable between tree saplings and mature trees.

Zusammenfassung

Die vorliegende Arbeit setzt sich aus drei Studien zusammen. Die ersten beiden Studien wurden im temperaten Laubmischwald im Hainich Nationalpark durchgeführt. Feinwurzeln von sechs verschiedenen Laubbaumarten aus demselben Baumbestand wurden verglichen um die Abhängigkeit der Feinwurzeleigenschaften von der Artidentität zu ermitteln. Die dritte Studie behandelt die Fragestellung, in wie fern die Bodentemperatur und der Schattenwurf der Krone von *Pinus cembra* deren Feinwurzelverteilung, -morphologie und -produktivität an der alpinen Waldgrenze beeinflusst.

Der Nationalpark Hainich ist durch eine relativ hohe Diversität an Baumarten gekennzeichnet. Die meisten Studien über Diversitätseinflüsse auf Feinwurzeln ergaben jedoch, dass Feinwurzeleigenschaften vermutlich eher durch die Artidentität und weniger durch die Diversität im Baumbestand beeinflusst werden. In unserer Studie untersuchten wir die Einflüsse der Artidentität und des Mykorrhiza-Typs (AM oder EM) auf die Biomasse, die Morphologie und die Produktivität von Feinwurzeln sechs verschiedener Baumarten (*Fagus sylvatica* L., *Tilia cordata* Mill., *Carpinus betulus* L., *Fraxinus excelsior* L., *Acer pseudoplatanus* L. und *Acer platanoides* L.). Die Feinwurzeln wurden bis auf Artniveau bestimmt und zugeordnet, in ihre einzelnen Verzweigungs-Ordnungen aufgeteilt und die Morphologie und Massenanteile wurden für jede einzelne Ordnung pro Art analysiert.

Die Morphologie der Feinwurzeln aller untersuchten Arten folgte zwar einem generellen Trend von der vierten zur ersten Ordnung (in Richtung Wurzelspitzen), nämlich einem Anstieg der spezifischen Wurzeloberfläche und der spezifischen Wurzellänge in Richtung Wurzelspitzen und einer Abnahme des Durchmessers. Dennoch unterschieden sich die morphologischen Parameter zwischen den Arten in den einzelnen Wurzelordnungen signifikant. Selbst die beiden Ahorn-Arten (*Acer pseudoplatanus* und *A. platanoides*) unterschieden sich sowohl in ihrer Feinwurzelbiomasse (höher in *A. pseudoplatanus*) und -produktivität (höher in *A. platanoides*), als auch in ihren morphologischen Eigenschaften.

Hainbuche, Rotbuche und Esche zeigten die höchste Feinwurzelproduktivität. Der Unterschied im Feinwurzelumsatz zwischen den Arten war am deutlichsten in den tieferen Bodenschichten erkennbar. *Acer pseudoplatanus* zeigte den geringsten Feinwurzelumsatz und *Acer platanoides* den höchsten, was bedeutet, dass die Feinwurzeln von *Acer pseudoplatanus* eine höhere Lebensdauer aufweisen. Aus dem Vergleich der beiden Ahornarten bezüglich Anzahl der Wurzelspitzen im Bodenvolumen, dem Wurzelumsatz und der morphologischen Eigenschaften lässt sich möglicherweise auf eine unterschiedliche Ressourcennutzung

schließen. Manche Arten wie z.B.: *A. pseudoplatanus* scheinen ihre Effektivität eher durch eine hohe Anzahl an Wurzelspitzen zu erreichen, während andere Arten wie z.B.: *A. platanoides* eher in einen hohen Wurzel-Umsatz investieren und dadurch viele „neue", aktivere Wurzeln zur Verfügung haben.

Im Allgemeinen hatte der Mykorrhiza-Typ nur einen geringfügigen Einfluss auf die Biomassen, die Morphologie und die Dynamiken der untersuchten Feinwurzeln. Tatsächlich scheint die Artzugehörigkeit die Eigenschaften von Feinwurzeln am Meisten zu beeinflussen, während die größten Unterschiede zwischen den Arten in den einzelnen Wurzelordnungen zu finden waren.

Neben der Artidentität werden die Feinwurzeleigenschaften von Bäumen an der Waldgrenze zum Großteil von extremen Temperaturen beeinflusst. Die meisten bisher durchgeführten Studien über Temperatureinflüsse auf Feinwurzelbiomasse, -morphologie und -produktivität wurden jedoch an Jungbäumen unter experimentellen Bedingungen durchgeführt. Aus diesen Studien geht hervor, dass niedrige Bodentemperaturen das Wachstum der Feinwurzeln hemmen. Untersuchungen an Altbäumen auf natürlichen Standorten sind jedoch selten. In unserer Studie befassten wir uns mit dem Einfluss des Schattenwurfes der Krone von *Pinus cembra* auf die Bodentemperaturen und deren Einflüsse auf die Feinwurzeln dieser Koniferen. Dazu wurden 2 mal 2 Meter Plots mit Rasterpunkten um Bäume an der Waldgrenze gelegt, in denen die Bodentemperaturen in einem tageszeitlichen Gradienten an einem sonnigen Tag im Hochsommer gemessen wurden. Die Bodentemperaturen während der maximalen Erwärmung am Nachmittag zeigten deutliche Unterschiede zwischen sonnigen und schattigen Bereichen. Die Feinwurzelbiomassen und -nekromassen in den Rasterpunkten korrelierten negativ mit diesen Bodentemperaturen wie auch die Feinwurzelproduktion und der Feinwurzelumsatz waren in den schattigen Bereichen höher als in den sonnigen.

Bisherige Annahmen, dass niedrige Bodentemperaturen im Allgemeinen das Feinwurzelwachstum hemmen, treffen offensichtlich nicht für adulte Bäume zu. Wir fanden in den schattigen, kälteren Bodenbereichen eine deutlich erhöhte Feinwurzelmasse (lebend und tot) sowie einen erhöhten Feinwurzelumsatz bei *Pinus cembra.* Folglich ist anzunehmen, dass niedrige Bodentemperaturen eine schlechtere Nährstoffversorgung hervorrufen und die Bäume mehr Kohlenstoff in die Feinwurzeln investieren um den Mangel und die höhere Sterblichkeit der Feinwurzeln auszugleichen.

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1

General Introduction

1.1 The role of fine roots in forest ecosystems

Temperate forests represent economic and ecological important ecosystems providing wood, harboring relatively high biodiversity or in special cases protect alpine environments against avalanches. Both managed and unmanaged forests were often investigated for aboveground structures of economically important species while roots were often neglected due to their hidden way of life. Although their importance for forest ecosystems is known quite well, the role of species identity and the analysis of root functional traits in forests still require huge investigation effort.

In root research one has to differentiate between coarse roots and fine roots, as they differ completely in their functionality. Coarse roots with large diameters (>2 mm) are responsible for storage and transport as well as for stabilization of the trees in the ground (Fitter 1996, Pregitzer 2002). Fine roots (commonly defined with <2 mm in diameter) are short living and vitally important plant organs, responsible for water and nutrient uptake. Fine root litter represents besides leaf litter one of the most important sources for carbon and nutrients (Fogel 1983, Rumpel 2002). In general, the majority of worldwide forest carbon is stored belowground and roots build up a key part within carbon cycling (Schlesinger 1997). Around 20-40 % of net primary production is invested into fine root production and another ca. 30 % goes into leaf production (Keyes & Grier 1981, Vogt et al. 1996, Müller-Haubold et al. 2014). In fact it is much more complex to investigate fine root production compared to leaves, as fine roots grow belowground and are not shed as entities in the end of their lifetime, but progressively in more distal segments (Xia et al. 2010).

Although several studies investigated biodiversity and mixture effects on fine roots in temperate broad-leaved species, most of them were conducted in pot experiments on saplings (Withington et al. 2006, McCormack et al. 2014), while only few studies were conducted in mature forests (Meinen et al. 2009 abc, Jacob et al. 2012).

In mixed temperate broad leaved forests complementarity of different species is often discussed as well as a higher productivity with increasing biodiversity, but the majority of previous research did not focus on species identity as driving factor for fine root morphology and dynamics. However, recent studies on root performance, production and ecology of mature temperate forest areas (Hertel 1999, Leuschner 2001, Meinen 2009, Jacob 2012) found no effects or even a decrease of fine root biomass in species rich compositions compared to monocultures. Although no distinct correlation between biodiversity and fine root productivity was found, there is a strong evidence for a high influence of species identity

on fine root biomass and morphology in mixed forests (Jacob 2012). We assume that species react individually to soil properties, water and nutrient supply and climate and these species specific reactions cannot be described sufficiently by biomass investigations alone.

It is not well known how fine root morphology, biomass and dynamics as well as functionality vary with the taxonomic position and ecology of temperate broad leaved tree species or if there are common patterns in root traits for coexisting species. Many factors like phylogenetic relatedness, mycorrhiza type or successional status might influence fine root morphology. In addition, there might be strong variation in fine root morphology, biomass and belowground dynamics according to climatic influences, soil properties, nutrient supply and microbial activity (Gill & Jackson 2003, Leuschner 2003, Finer et al. 2007, 2011).

1.2 Morphology and function of fine roots in temperate broad leaved species

Fine roots are the most distal parts of the root system and are usually defined as root branches with a diameter smaller than 2 mm (Fitter 1996, 2002). They include the root tips which are characterized by a high plasticity against environmental influences and are often involved into fungal symbiosis. Most tree species in cool temperate broad-leaved forests live in symbiosis with ectomycorrhizal fungi (EM) and only a few with arbuscular mycorrhiza (AM) (McGuire et al. 2008, Lang et al. 2011). Mycorrhization can enhance nutrient acquisition and leads to better resource exploitation. Depending on the mycorrhization type, species have a better access to inorganic Phosphorus in case of AM or to organic nitrogen compounds in case of EM, as well as to other nutrient compounds (George et al. 1995, Read & Perez-Moreno 2003, Smith et al. 2003, Lang et al. 2011). Besides enhanced resource gain through mycorrhization, every tree species has individual root traits, such as different periderm color, surface cell structure, ramification and root tip morphology (Figure A 2.1) as well as differing chemical properties (Guo et al. 2008, Pregitzer et al. 2002). Based on those traits, some economically important broad leaved species were described within a morphological key in former studies (Hölscher et al. 2002, Meinen et al. 2009bc and Jacob et al. 2012). This key enabled us to identify fine roots until species level to assess species-specific fine root dynamics of mature trees in mixed stands.

Leaves and roots represent organs with high functional activity for resource acquisition and underlie temporary fluctuations, thus the variations of fine root morphology in temperate tree species might be as high as observed for leaf morphology. Alternatively, coexisting tree species from different genera and families could develop convergent patterns of fine root

morphology (Withington et al. 2006), because a common selective force controls root development, at least at the same stand.

Fine root morphology can be analyzed in different ways depending on the research goal. One possibility is to measure morphological traits on the whole fine root individuals (<2 mm in diameter) which is more or less superficial regarding functional traits of the respective species (Pregitzer et al. 1997, 1998, Pregitzer 2002, Guo et al 2008). Those functional traits of fine roots correspond mostly with root branching patterns and thus, a more precise method is to analyze morphological patterns on branching order level (Pregitzer et al. 1997, 1998, Pregitzer 2002, Guo et al. 2008). Morphological root properties like specific root length, specific root area, nitrogen content and root diameter change with their distance from the root tips (McCormack 2015) and thus, root functionality changes with root order. In a morphological study of 23 temperate tree species Guo et al. (2008) concluded that the transition between resource uptake and long distance transport lies between 3rd and 4th root order. In fact, there are general trends in fine root branching patterns and anatomy for all species. However, considerable differences in morphology and chemical properties of the individual root orders were found between several studied species (Pregitzer et al. 2002, Guo et al. 2008, McCormack 2015). The understanding of differences in root morphology between tree species, mycorrhization groups or even congeners can help us to evaluate species foraging strategies in mixed forests.

1.3 Fine root dynamics and nutrient cycling

To assess fine root dynamics, the lifespan of fine roots (inverse of fine root turnover) must be considered to assess fine root activity and carbon investment into fine roots as well as their contribution to the nutrient cycle. Trees consume a huge part of the annually produced carbohydrates in order to build and maintain fine roots, and hence, reduce timber production (Fogel 1983, Hertel et al. 2013). However, fine root dieback and the following root litter is one of the most important contributions to the carbon and nutrient cycle in forests (Fogel 1983, Rumpel et al. 2002, Fan & Guo 2010). Fine roots are organs with, lower C/N proportion in more distal parts (Pregitzer et al. 1997, 2002) and have a short life span. The finest root segments of the root branches are shed and rebuilt gradually (Xia et al. 2010) and contain high nitrogen contents which are correlated negatively with fine root lifespan (McCormack 2012). A cost- benefit approach to predict fine root lifespan assumes that a plant maintains a root only until the efficiency of resource acquisition is maximized (see review by

Eissenstat et al. 2000). The soil nutrient cycle thus is largely influenced by a dieback (input of carbon and nitrogen to the soil) and turnover of short living fine roots.

Besides several studies on fine root dynamics in greenhouse experiments, only a few studies analyzed fine root lifespan and productivity of mixed mature forests (e.g. Tierney & Fahey 2001, Meinen et al. 2009ab) but most of them did not focus on species identity. Eissenstat et al. (2015) investigated fine root turnover in relation to root branching orders and in their study of six AM species fine root morphology, productivity and foraging strategy were connected They suggest that thin-rooted species forage more by root proliferation, whereas thick-rooted species forage more by mycorrhizal fungi. Nevertheless, it is not known how different mycorrhiza types influence morphology and foraging strategies as Eissenstat et al. (2015) compared only AM tree species in their study.

The comparison of EM and AM tree species in their fine root dynamics and possible differences could help us to explain the dominance of EM species in cool temperate and boreal forests while in tropical and subtropical forests AM species are more common.

1.4 Temperature as an influencing factor for fine roots and tree growth

There are several controversial approaches to explain the causes for the formation of alpine treelines and the limitation of tree growth. Aboveground decrease of tree growth is supposed to be attributed to reduced carbon gain via photosynthesis (‘carbon-source limitation hypothesis’) under cold conditions (see reviews by Troll 1973, Tranquillini 1979, Stevens and Fox 1991) and a short vegetation period. Another more recent theory (Körner 1998, 2012ab, Hoch et al. 2002, Hoch & Körner 2003) presumes a hampered metabolic activity for cell division and tissue growth at low temperatures as the reason for treeline (‘carbon-sink hypotheses’). In addition to those theories, other local stressors like wind, and a high snow charge play a role for the development of alpine treelines (data reviewed by Stevens & Fox 1991, Sveinbjörnsson 2000, Holtmeier 2009).

A number of studies showed that the alpine treelines coincide better with soil temperatures than with air temperatures (Sveinbjörnsson 2000, Körner & Hoch 2006, Körner 2012). On a global scale, Körner & Paulsen (2004) found the alpine treeline position to correlate with a mean soil temperature of 6.7 °C in 10 cm soil depth during the growing season. A physical explanation for the better relation between tree growth and soil temperature compared to tree growth and air temperature might be the fact that soil temperatures show fewer and less transient fluctuations than air temperatures and are buffered by soil texture and understory vegetation (Körner & Paulsen 2004).

Root growth seems to benefit more from this mitigated soil temperature variations than aboveground tree growth as roots were found to be able to grow earlier in the year at still low temperatures (2 or 3 °C) compared to the other plant parts (Holtmeier 2009). Thus, root growth might be additionally facilitated by an isolating snow cover in the Alps during winter which keeps soil temperatures more constant at a higher level. For tree seedlings however, several studies indicated that low soil temperatures (below 5 °C) impair fine root production and activity (Turner & Streule 1983, Häsler et al. 1999, Alvarez-Uria & Körner 2007, 2011, Körner 2012ab, Schenker et al. 2014). Studies about mature trees at treeline sites are still rare regarding the influence of root-zone temperature on fine roots.

Given that temperature decreases with increasing elevation and low soil temperatures hamper above and belowground tree performance (Dang 2004, Hoch & Körner 2003) additional temperature reduction by crown shading is one of the explanatory variables for the transition from closed forests to loose patches and single trees towards the treeline. It is discussed that less shade might result in a more suitable soil thermal condition and therefore favors tree growth and survival of more isolated trees (Körner 1998, Körner 2012).

Contradictory to those experiments on tree saplings, recent investigations on elevational transects within worldwide treeline stands documented a disproportional increase in fine root biomass with increasing elevation. As aboveground biomass and tree height decrease with elevation an allocation shift from aboveground biomass to belowground biomass seems to occur towards the treeline (Kitayama & Aiba 2002, Leuschner et al. 2007, Hertel & Wesche 2008, Hertel et al. 2008, Hertel & Schöling 2010, Hertel & Schöling 2011). Latter might be an adaptation to unfavorable conditions such as impaired nutrient supply at low temperatures (Gaul et al. 2008).

Although the alpine treeline has attracted studies by many researchers over decades, the ecological causes for the treeline formation are still under contradicting debate.

1.5 Study framework and experimental design

This thesis is subdivided into two main sections. In the first section results of research on species specific fine root traits in a diverse temperate mature broad leaved forest with a focus on mycorrhiza type, root branching patterns and productivity (Chapter 2 and Chapter 3) are presented. We compared two groups of different mycorrhization types (ecto- (EM) and arbuscular-mycorrhizal (AM) symbiosis) of six co-occurring broad-leaved tree species and assessed possible differences in research acquisition strategies of coexisting species. This part

of the study was integrated in a DFG research training group 1086 “The role of Biodiversity for Biogeochemical Interactions in Temperate Deciduous forests”.

The second section (Chapter 3) deals with temperature influences on fine root biomass, growth and morphology of a conifer tree species (*Pinus cembra* L.) at the alpine timberline. The aim of our study was to systematically analyze the influences of shade on soil temperatures and fine root performance of Stone pine (*Pinus cembra* L.) at the alpine treeline of an avalanche forest in the Central Alps in Austria.

1.5.1 Species specific fine root traits in a temperate mixed broad leaved forest

Study site description

In order to compare fine root traits like biomass, morphology and productivity of six temperate broad leaved species concerning the influence of two different mycorrhization types, we chose the Hainich National Park as study area. It is situated in Thuringia, Central Germany (350 m a.s.l.; 51° 04’ N, 10° 30’ E; Figure 1.1) and protects 7500 ha old grown forest which is mostly dominated by European beech (*Fagus sylvatica* L.), but additionally contains relatively species-rich patches with up to 14 different tree species.

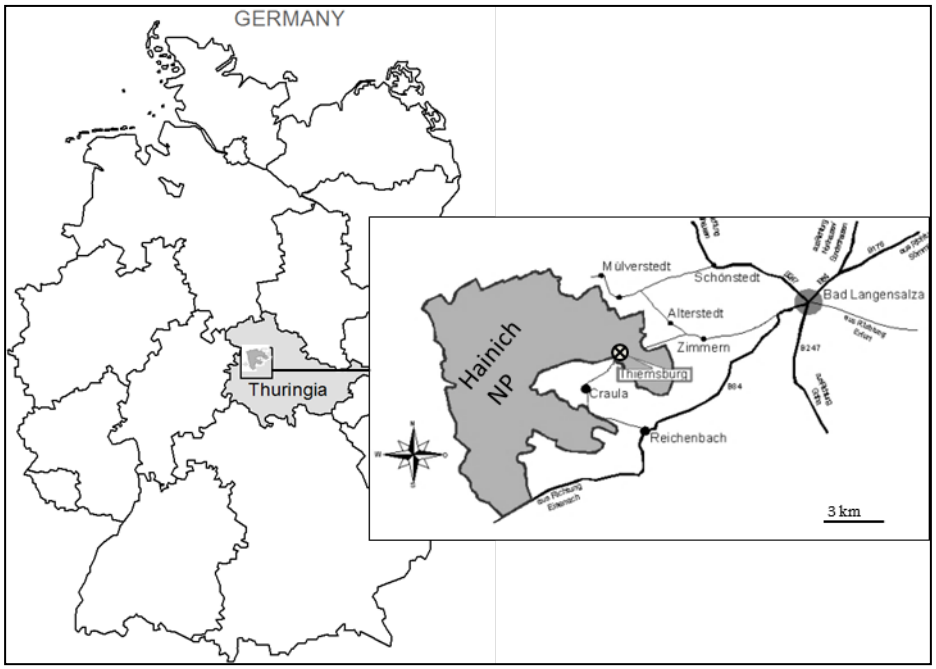

Figure 1.1: Location of the study area in Germany and within the National Park Hainich (Thiemsburg area is marked with ⊗).

The relatively high tree age of around 90-150 years in this forest (Schmidt et al. 2009) is caused by only minor management practices like selective logging in the past 50 years and one part of the forest had been used as military training area. All activities were ceased completely with declaration of national park in 1997.

Suitable plots for our study were selected in a mixed 'Stellario-Carpinetum' association in the north-eastern part of the National Park, the so called 'Thiemsburg area' (Figure 1.1). Our studies in Chapter 2 and 3 focused on 6 different species: European beech (*Fagus sylvatica* L.), Small-leaved lime *(Tilia cordata* Mill.), European hornbeam (*Carpinus betulus* L.), European ash (*Fraxinus excelsior* L.), Sycamore maple (*Acer pseudoplatanus* L.) and Norway maple (*Acer platanoides* L.). These are the most abundant species in this area while half of them form EM *(F. sylvatica, C. betulus, T. cordata*) and the other half form AM (*A. pseudoplatanus, A. platanoides, F excelsior*; Lang et al. 2011). Dominant trees were about 27-32 m high and the forest did not show larger canopy gaps (average canopy openness 5.7 %, Seidel et al. 2012).

The region has semi-humid climate with an annual precipitation of ~ 590 mm yr^{-1} and a mean annual temperature of 7.5 °C (period 1973-2004, Deutscher Wetterdienst 2005). In the study years 2012 and 2013, precipitation totals of 603 mm (2012) and 598 mm (2013) and mean annual air temperatures of 9.7 °C (2012) and 8.5 °C (2013) were recorded at the nearest weather station Weberstedt/ Hainich (Deutscher Wetterdienst 2009).

The forest grows on a base-rich Eutric Luvisol (FAO taxonomy 2006) over Triassic limestone bedrock. Profile depth is about 60-70 cm and the soil developed of clay-rich Pleistocene loess. The mineral soil structure shows a high silt proportion (~74%) and low sand (<5%) contents (Guckland et al. 2009). Marginal variation in soil chemistry between the study plots is caused by individual leaf litter chemistry of the species influencing C/N ratio, base saturation and pH values below the trees (Rothe & Binkley 2001, Guckland et al. 2009). Those differences are negligible and still the soil chemistry of the plots is comparable for fine root investigations. The soils in this area can dry out strongly in summer, while in spring and winter soil properties are mostly stagnant.

Study objectives and hypotheses

In order to investigate the fine root biomass, morphology and dynamics of the six species, we chose randomly selected circular plots (diameter 12 m; area 113 m^2) containing a mature tree pair or a single standing mature tree of one of the six target species in dominant canopy

position in the center. Diameter at breast height (dbh) of the selected trees ranged from 40 to 60 cm.

This plot selection scheme in the mixed forest was chosen to minimize possible species effects on soil chemistry, which would have been more pronounced in larger monospecific stands. In addition, the main bulk of fine roots in the plot soil belonged to the particular target species (typically >80 %). All stems (dbh >10 cm) in the 6 m plot around the target trees were investigated for their species identity, dbh, basal area and tree height. We sampled eight plots per species resulting in 48 plots in total.

To analyze species specific fine root morphology, biomass and productivity with respect to their type of mycorrhiza in a mature temperate broad leaved forest we conducted following investigations within the first two chapters.

CHAPTER 2

1. Soil samples were taken to assess standing fine root biomass and morphology.
2. All fine roots were undergone an elaborate species identification process using several criteria like surface structure, mycorrhiza type and branching patterns (Hölscher et al. 2002, Meinen et al. 2009a, Kubisch et al. 2015).
3. Living and dead fine roots were separated using criteria like color and elasticity of the stele (Leuschner et al. 2004, Meinen et al 2009a, Rewald & Leuschner 2009).
4. Fine root morphology of the living bulk fine roots (length >10 mm) was determined by scanning the fine roots and by analyzing them with WinRhizo software.
5. All living fine root branches were undergone a detailed root tip (mycorrhization rate) and root branching order analysis (weight and morphology). Each root branching order was scanned and analyzed separately per species.
6. SRL (specific root length), SRA (specific root area), MD (mean diameter), RTD (root tissue density), the most relevant morphological parameters for root function were analyzed for the whole branches and for each root branching order.
7. Fine root dry biomass and necromass where measured for each species.
8. A chemical analysis of the fine roots was conducted for every root branching order.

HYPOTHESES:

(i) Co-occurring species develop similar patterns of fine root system branching irrespective of phylogenetic relatedness.

(ii) EM and AM trees differ systematically in fine root morphology and functionality with a focus on fine root tip abundance, specific fine root surface area and branching patterns as we assume a differing functionality between mycorrhiza types.

(iii) Root order traits have a strong influence on fine root morphology and thus show larger differences between the species than bulk root morphology in shared soil.

CHAPTER 3

1. Fine root biomass production and fine root turnover was measured for all species using the ingrowth cores technique.
2. Fine root length and area production within the study period was measured (morphological analysis using WinRhizo software).
3. Species specific aboveground woody biomass production (calculated of dbh and tree height) and the possible interrelation with individual fine root properties was calculated.
4. Aboveground structure and species composition of all trees within the 6 m clusters around the dominant target trees was perceived.

HYPOTHESES

(i) Coexisting AM and EM tree species differ in fine root turnover and root productivity, reflecting different nutrient acquisition strategies.

(ii) Fine root productivity increases with decreasing mean fine root diameter of the species (Eissenstat 1991).

(iii) Fine root productivity is higher, and root lifespan shorter, in tree species with higher aboveground productivity.

1.5.2 Soil temperature effects on fine roots of Stone pine at the timberline

Study site description

In order to assess the influence of summer soil temperature (maximal temperature fluctuations between day and night) on fine root biomass and morphology, we chose a loose forest stand at the timberline, where *Pinus cembra* L. trees and their roots are directly exposed to the shade of their own crown. The study area is situated in the Central Eastern Alps in Tyrol (Austria) in 2025 m a.s.l. The study site is northerly of the upper Sellrain valley near the village St. Sigmund on the south exposed slope of the 'Haggener Sonnberg' (47°12'42" N, 11°5'04" E).

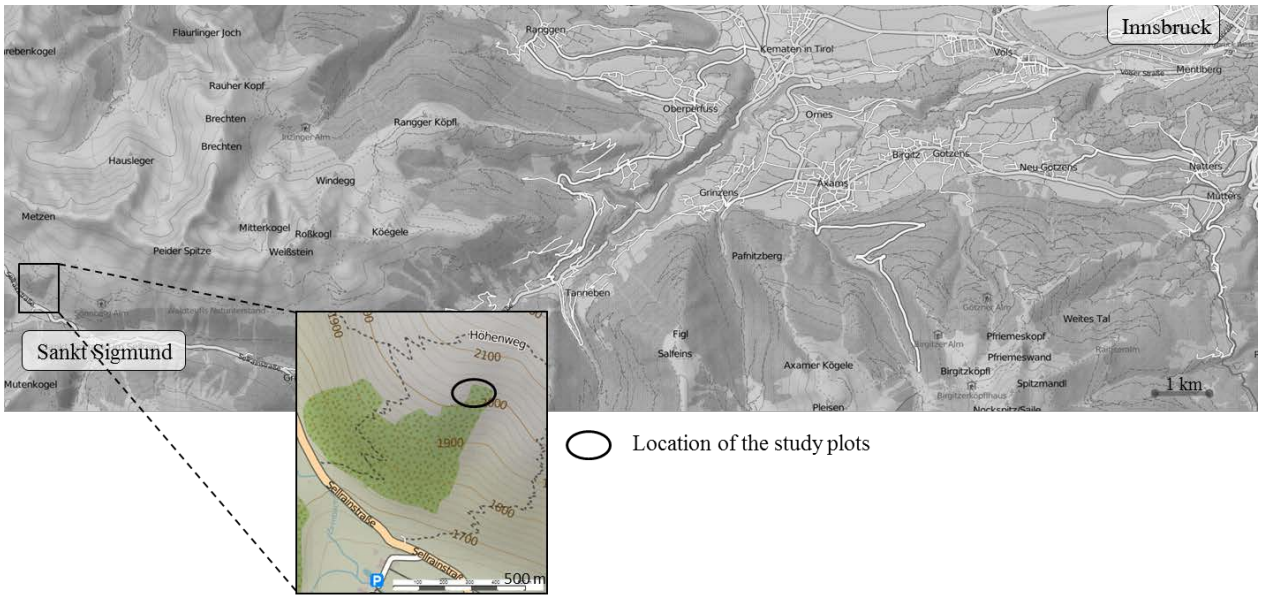

Figure 1.2: Location of the study area and the in the Central Eastern Alps at the alpine treeline and a detail view of the studied forest patch. Source modified from: alpenkarte.eu.

As a pilot project for avalanche protection the area was afforested at lower elevations in the 1970's and in the 1980's afforestation continued up to treeline elevation. A loose native *Pinus cembra* stand was planted at the sunny, south facing slope, which had been clear-cut and used as pasture for cattle and sheep for the last centuries before afforestation. Due to the patchy structure this forest has an important value for treeline research, as it is possible to investigate shade effects and other functional traits of equal aged trees (ca. 35 years in the study period 2011-13) with the same aboveground stature.

The alpine climate is characterized by a short vegetation period and harsh conditions. Temperature is the key variable influencing plant growth and determining the microclimate. The mean annual temperature at a nearby weather station (1975-1994) was 3.2 °C with February being the coldest month (mean temperature= -3.5 °C) and July with 10.7 °C mean temperature being the warmest month. Mean annual precipitation was 909 mm with 610 mm

falling from May to October. In the study year 2012 mean annual precipitation accounted for 882 mm yr^{-1} (Wieser et al. 2015).

The forest grows on an oligotrophic podsolic leptosol (WRB classification) originated from foliated Gneis and Mica schist bedrock (Kronfuss & Havranek 1999). The subsoil consists of loamy sand and is covered by a thin humus layer of around 5 cm thickness (Wieser et al. 2015).

Study objectives and hypotheses

Based on the contradictory findings of former studies about the influence of low soil temperatures on fine roots, the aim of the present study was to look into more details regarding the influence of crown shading on soil temperature and therefore on fine root biomass and morphology in a loose tree stand near the alpine timberline during the vegetation period. We chose suitable tree individuals of Swiss Stone pine (*Pinus cembra*) close to the timberline in the Central Alps. We created a grid experiment with 6 plots in 2 m x 2 m edge length) consisting of 36 uniformly distributed grid points (Figure 1.3) down to 10 cm soil depth. Each plot contained a single pine tree, building up the center of the plot. The diameters at breast height of the sample trees were between 5.5- 7.5 cm with a tree height between 2.8-4.2 m.

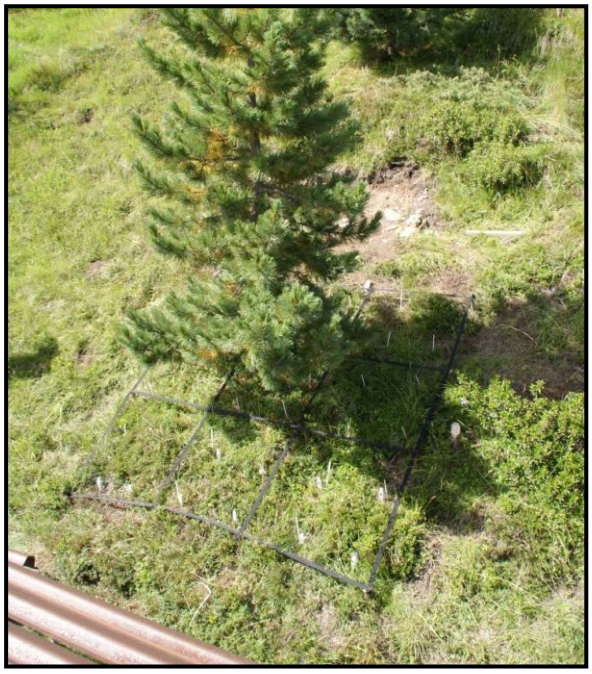

Figure 1.3: Sampling plot with 36 grid points (marked with white plastic sticks) around a pine tree.

Following examinations were conducted to assess the fine root response on soil temperature in the shaded areas around selected tree individuals at a treeline site in the Alps.

CHAPTER 4

(1) Soil temperatures were measured at sun- exposed and shady grid points around mature trees at the treeline three times during a sunny day in the warmest month of the growing season.
(2) Soil samples were taken down to 10 cm depth of every grid point around the tree in the vegetation period (mid-summer).
(3) Fine roots (<2 mm in diameter) were extracted to determine fine root biomass and fine root morphological parameters like fine root length, fine root surface area and mean root diameter as well as SRL, SRA and RTD.
(4) Ingrowth cores were installed northerly (in the shade) and southerly (in the sun) of the stems of adjacent tree individuals within the same tree stand next to the "grid plots" to assess fine root production and turnover.

In Chapter 3 we tested the following hypotheses about the influence of soil temperatures on fine root biomass and morphology.

HYPOTHESES

(i) Tree fine root biomass in sunny and shaded patches of the treeline ecotone is more closely related to the soil thermal regime developed in the afternoon than in the morning hours.
(ii) Tree fine root biomass density is lower in shaded, cooler patches under the canopy than in sunny, warmer areas.
(iii) Tree fine root productivity is considerably lower in shaded, cooler patches under the canopy than in sunny, warmer areas.

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CHAPTER

2

Do ectomycorrhizal and arbuscular mycorrhizal temperate tree species systematically differ in root order related fine root morphology and biomass?

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2.1 Abstract

While most temperate broad-leaved tree species form ectomycorrhizal (EM) symbioses, a few species have arbuscular mycorrhizas (AM). It is not known whether EM and AM tree species differ systematically with respect to fine root morphology, fine root system size and root functioning. In a species-rich temperate mixed forest, we studied the fine root morphology and biomass of three EM and three AM tree species from the genera *Acer*, *Carpinus*, *Fagus*, *Fraxinus* and *Tilia* searching for principal differences between EM and AM trees. We further assessed the evidence of convergence or divergence in root traits among the six co-occurring species. Eight fine root morphological and chemical traits were investigated in root segments of the first to fourth root order in three different soil depths and the relative importance of the factors root order, tree species and soil depth for root morphology was determined. Root order was more influential than tree species while soil depth had only a small effect on root morphology All six species showed similar decreases in specific root length and specific root area from the 1st to the 4th root order, while the species patterns differed considerably in root tissue density, root N concentration, and particularly with respect to root tip abundance. Most root morphological traits were not significantly different between EM and AM species (except for specific root area that was larger in AM species), indicating that mycorrhiza type is not a key factor influencing fine root morphology in these species. The order-based root analysis detected species differences more clearly than the simple analysis of bulked fine root mass. Despite convergence in important root traits among AM and EM species, even congeneric species may differ in certain fine root morphological traits. This suggests that, in general, species identity has a larger influence on fine root morphology than mycorrhiza type.

Keywords: *Acer*, *Carpinus*, *Fraxinus*, *Fagus*, mixed stand, root tips, specific root area, *Tilia*

2.2 Introduction

Trees produce large amounts of woody coarse and large roots, but it is the small amount of fine non-woody roots which provide a large surface area and close contact to the soil enabling the absorption of water and nutrients. Conventionally, the most distal short-lived root segments with diameters < 2 mm ('fine roots') are associated with resource acquisition, while the thicker coarse and large roots are considered as being long-lived with transport, storage and anchorage function (Fitter 1996; Pregitzer 2002).

Recent root morphological research has shown that the distinction between fine and coarse roots with a fixed diameter threshold of 2 mm is not very useful for categorizing the root system of trees with respect to functionality, metabolic activity, and dynamics (Pregitzer et al. 1997, Pregitzer 2002, Pregitzer et al. 2002). It appears that certain root properties such as diameter, specific root surface area or tissue N concentration change more or less continuously with increasing distance from the terminal root tip, while anatomical features as cortex thickness, presence of secondary xylem, and the formation of a continuous cork layer as secondary peripheral tissue change more abruptly, perhaps in conjunction with branching events in the fine root system (Guo et al. 2004, Pregitzer et al. 2002). From the analysis of 23 temperate tree species, Guo et al. (2008) concluded that the shift in root function from resource absorption to transport occurs in the third or fourth root order, with branching events in the root system being counted in proximal direction from the terminal tip. Accordingly, root order was found to be a much better predictor of the functioning of a root segment than its diameter.

It is not well known how fine root morphology varies with the taxonomic position and ecology of trees. Differences in phylogenetic relatedness, mycorrhiza type (ectomycorrhizal vs. arbuscular mycorrhizal), growth rate (fast vs. slow), and successional position (early- vs. late-successional) all could possibly influence fine root morphology and fine root system architecture. Theoretically, the variability in fine root morphology among the 1500 or so temperate tree species could be as large as the variation observed in leaf morphology. Alternatively, coexisting tree species from different genera and families could develop convergent patterns of fine root morphology (Withington et al. 2006), at least when growing in the same stand, because a common dominant selective force controls root development. Root order related analysis of 23 Chinese (Guo et al. 2008) and 9 North American temperate tree species (Pregitzer et al. 2002) showed considerable species differences in fine root

morphological, anatomical and chemical properties, even though some consistent general trends in branching patterns and anatomy along the fine root branches were detected.

The question of convergence or divergence in root system morphology and functionality is particularly interesting with respect to the distinction between ectomycorrhiza-forming (EM) and arbuscular mycorrhiza-forming (AM) trees. In the overwhelming majority of temperate tree species, the finest rootlets are colonized by ectomycorrhizal (EM) fungi. However, a few AM species are also present, coexisting with EM species in broad-leaved temperate mixed forests. Tree species, which mostly or exclusively form arbuscular mycorrhizas, are present, for example, in the temperate genera *Acer, Fraxinus, Prunus* and *Liriodendron*. It is generally believed that AM-forming fungi of the phylum Glomeromycota have a positive effect on their host mainly through enhancement of the uptake of inorganic phosphorus, while EM-forming fungi support their host primarily by accessing organic nitrogen compounds (and other nutrient fractions) (George et al. 1995, Lang et al. 2011, Read & Perez-Moreno 2003, Smith et al. 2003). Because most research on arbuscular mycorrhizas dealt with herbaceous plants, while research on EM primarily focused on trees, a direct functional comparison of these two major types of mycorrhizal association is complicated. We are not aware of a study that systematically searched for principal differences in fine root morphology between temperate EM- and AM-forming trees. Besides species and mycorrhiza type, a third factor with possible influence on fine root morphology is soil depth because soil physics and chemistry are exerting a large influence on root morphogenesis and growth (Wang et al. 2006).

In this study, we examined the variation in fine root morphology and architecture among six co-occurring temperate broad-leaved tree species in a mixed forest, searching for evidence of divergence or convergence in fine root traits under uniform edaphic and climatic conditions. Because root functioning may largely depend on root branching patterns (Guo et al. 2008, Pregitzer et al. 1998, Pregitzer 2002), we adopted a detailed root order-related analysis of fine root morphology. The six species were from five families (Oleaceae, Betulaceae, Tiliaceae, Fagaceae, and two species from Aceraceae), representing considerable phylogenetic and also functional diversity (three EM and three AM species). We investigated eight root morphological and chemical traits and related the observed trait variation across the six species-sample to the possible influence of root order, tree species, mycorrhiza type and soil depth. We also compared the species in terms of the amount of 1^{st} and 2^{nd} order fine root biomass in the topsoil. Main study goals were (1) to examine whether co-occurring species develop similar patterns of fine root system branching irrespective of phylogenetic relatedness, (2) to search for systematic differences in fine root architecture between EM and

AM trees, (3) to compare the species in terms of fine root biomass assigned to root orders, and (4) to assess the advantages of adopting a root order-based analysis over a conventional analysis of bulked fine root material.

2.3 Materials and methods

2.3.1 Study site

The study site is situated in Hainich National Park in Thuringia, Germany, which harbors old-growth beech forests (*Fagus sylvatica* L.) and relatively species-rich broad-leaved mixed forests on calcareous soil (350 m a.s.l.; 51° 04' N, 10° 30' E). Suitable study plots were selected in the 'Thiemsburg area' in the north-eastern part of the national park where at least six tree species co-occur either in quasi-random mixture or in small groups consisting of three to six trees of a species. The species considered were those with highest abundance in this mixed forest (Stellario-Carpinetum association, 'oak-hornbeam forests'): European beech (*Fagus sylvatica* L.), Small-leaved lime *(Tilia cordata* Mill.), European hornbeam (*Carpinus betulus* L.), European ash (*Fraxinus excelsior* L.), Sycamore maple (*Acer pseudoplatanus* L.) and Norway maple (*Acer platanoides* L.). Three of the six selected species have been found to form AM in Hainich forest (*Acer pseudoplatanus, A. platanoides* and *Fraxinus excelsior),* the other three *(Carpinus betulus*, *Fagus sylvatica* and *Tilia cordata*) EM (Lang et al. 2011). The investigated species are well studied with respect to aboveground morphological and functional properties (Köcher et al. 2009, Köcher et al. 2013, Legner et al. 2013, Withington et al. 2006) and also in terms of fine root dynamics and root nitrogen and water uptake capacities (Jacob et al. 2012, Jacob & Leuschner 2014, Korn 2004, Meinen et al. 2009a, Meinen et al. 2009b, see Table A 2.1 in the Appendix). Other forest patches are composed of up to 14 tree species including *Prunus, Ulmus* and *Quercus* species as well (Meinen et al, 2009b). The majority of trees were about 90-150 years old (Schmidt et al.2009) and mean canopy height of the dominant trees was 27-32 m with no larger canopy gaps present (average canopy openness 5.7%, (Seidel et al.2012). The herb layer is patchy with an average cover of ~17 % in the studied stand (Vockenhuber et al.2011). The forest was affected by only minor management activities (selective logging) in the past 50 years because part of the stand was used as military training area and all activities ceased in 1997 with the declaration of a national park.

The region has a semi-humid climate [(mean annual temperature 7.7 °C, mean annual precipitation ~590 mm yr^{-1} (period 1973-2004; Deutscher Wetterdienst, 2005)]. In the study year 2011, a mean annual temperature of 9.5 °C and a precipitation of 470 mm yr^{-1} were

recorded (data of the nearby weather station Weberstedt/Hainich; Deutscher Wetterdienst, 2009).

The calcareous bedrock (Triassic limestone) is overlain by a base-rich Pleistocene loess layer which led to the development of eutrophic Luvisols (FAO taxonomy 2006) with a profile depth of 60 to 70 cm as the most widespread soil type in the study region. The soil texture of the mineral soil (0-30 cm) is characterized by high silt (about 74 %) and low sand (< 5 %) contents (Guckland et al.2009). The soil can dry out strongly in summer and shows partly stagnant properties during spring and winter. Mainly through different foliar nutrient contents, the tree species influence soil chemistry resulting in some variation in topsoil C/N ratio, base saturation and other properties underneath the six tree species (Table 2.1). *Fagus* patches showed accumulation of organic Ol and Of layers with slightly higher C/N ratio of the mineral topsoil.

Table 2.1: Stand and soil properties in the plots of the six species (means ± SE, n=8). The data refer to all trees in a plot of 6 m radius. For pH, the range of values is given.

Parameter	*F. excelsior*	*A. pseudoplatanus*	*A. platanoides*	*C. betulus*	*T. cordata*	*F. sylvatica*
Stand						
Tree height (m)	32.3 ± 1.5	28.6 ± 0.9	23.8 ± 2.0	22.8 ± 1.1	24.2± 1.4	26.4 ± 0.7
dbh (cm)	52.2 ± 3.5	58.1 ± 3.2	51.2 ± 3.6	43.4 ± 3.3	46.4 ± 2.3	43.5 ± 2.2
Basal area (m^2 ha^{-1})	57.1 ± 5.4	47.8 ± 8.8	28.7 ± 2.5	31.6 ± 6.6	50.9 ± 6.0	60.3 ± 7.8
Proportion target species (%)[1]	83.6 ± 3.5	62.7 ± 7.2	77.4 ± 9.9	86.1 ± 8.0	84.8 ± 4.0	90.4 ± 5.7
Soil parameters (mineral topsoil)						
C/N ratio	11.9 ± 0.7	11.6 ± 0.2	12.0 ± 1.0	12.5 ± 0.7	12.0 ± 1.0	12.6 ± 0.4
Base saturation (%)	91.2 ± 4.3	88.8 ± 4.8	87.3 ± 6.8	88.0 ± 0.2	93.4 ± 0.2	78.5 ± 6.8
Water content (%)[2]	37.9 ± 5.5	40.3 ± 1.5	38.8 ± 7.1	37.4 ± 5.0	36.2 ± 3	36.9 ± 2.4
pH (H_2O)	4.65 - 6.30	4.77 - 6.49	4.72 - 6.96	4.87 -6.58	4.81 - 6.70	4.50 - 6.12

[1]of basal area, [2] May 2012

Topsoil base saturation was somewhat lower under *Fagus* (mean: 89 %) than under the other species (range of means: 92-96 %) while only minor pH variation was observed (Table 2.1).

2.3.2 Study design

Root coring was conducted at 150 cm distance to mature trees of the six target species with diameters at breast height (dbh) of 40-60 cm and presence in the upper canopy layer. We selected either two neighboring trees of the target species and cored between them or conducted the coring in vicinity of one dominant tree of the respective species. This plot

selection scheme in the mixed stand minimized possible species effects on soil chemistry (which would have been more pronounced in larger monospecific patches), while it guaranteed that the large majority (typically > 80 %) of the fine roots belonged to the target species. We sampled eight plots per species (i.e. 48 plots (tree clusters) in total) in a stand area of ~15 ha by randomly selecting trees of suitable species and dimension. Edaphic conditions were sufficiently homogenous to exclude soil-borne effects on fine root morphology, as they have been described by Ostonen et al. (2013). Mean distance between the plots was ~50 m (minimum distance: 6 m) which excludes possible root interactions between neighboring plots in nearly all cases. All stems >10 cm dbh in a circle of 6 m radius around the root coring location were examined for their species identity, dbh, basal area and tree height (Table 2.1).

2.3.3 Soil sampling and fine root extraction

Soil samples for root extraction were collected in June 2011 in the upper 30 cm of the soil in all 48 tree clusters using a steel corer of 35 mm diameter. The extracted soil was separated into the 0-10, 10-20 and 20-30 cm layers and stored in plastic bags at 4 °C until final processing was conducted within 3 months. In the laboratory, the soil was gently washed with tap water over a sieve of 0.25 mm mesh width and all fine root branches (diameter < 2 mm) of more than 10 mm length picked out with a pair of tweezers, placed under a microscope (6-40 x magnification), separated into live and dead mass and sorted by species. Criteria to distinguish between biomass (live) and necromass (dead) were root turgor, the elasticity of the stele, and the constitution of root stele and periderm (Leuschner et al. 2004, Meinen et al. 2009a, Meinen et al. 2009b, Rewald and Leuschner 2009). Species identification was conducted with a morphological key based on periderm structure and color, root ramification, root tip morphology and the type of mycorrhiza developed which bases on earlier studies in this forest and elsewhere by lab members (Hölscher et al. 2002, Jacob et al. 2012, Meinen et al. 2009b Meinen et al. 2009c). Characteristic branching features and surface properties of the fine root systems of the six species are displayed in pictures compiled in Figure A 2.1 in the Appendix, where a brief description of fine root morphology is also given.

For determining the fine root biomass of the six species in the topsoil, the following two-step procedure was applied. After having sorted out the longer fine roots, the amount of finest rootlets < 10 mm length was examined in detail under a microscope for half of the samples (4 per species per soil depth). We dispersed the washed sample on filter paper (730 cm^2) with 36 equal squares marked on it. Six of 36 squares were selected by random and the finest rootlets

sorted into living and dead root mass (van Praag et al. 1988, Hertel & Leuschner 2002). The biomass and necromass of those six samples was extrapolated to the whole sample and in the following calculated for all samples. Because species identification was hardly possible in this fraction (which represented about 10 percent or less of the overall fine root mass), the species proportions detected in the >10 mm-samples were applied to this root fraction as well. We considered only the root mass of the tree species (target species and 'other species' in a plot) but discarded the root mass of herbaceous species.

2.3.4 Morphological analysis

All fine root branches of a core were subjected to morphological analysis shortly after collection. This was done separately for the three soil depths. Root segments with diameters > 2 mm were cut off. Specific root length (SRL, m g^{-1}), specific root area (SRA, cm^2 g^{-1}), root tissue density (RTD, g cm^{-3}) and mean root diameter (MD, mm) of the fine root sample were determined for all fine root branches of a sample by placing the roots in Petri dishes filled with purified water for scanning with a flat-bed scanner (EPSON expression 1680, EPSON America Inc.); the scans were analyzed with WinRhizo 2005c software (Régent Instruments Inc., Québec, QC, Canada). The number of root tips was counted in all living root branches under the microscope (6-40 x magnification) and subsequently related to root dry mass. The ectomycorrhizal colonization rate (in %) of the tips was calculated as:

$$\left(\frac{no.\,of\ mycorrhizal\ root\ tips}{no.\,of\ vital\ root\ tips}\right) * 100$$

In the AM species, the root segments were inspected for colonization by hyphae, but quantitative data were not collected. Subsequently, a representative sub-sample of root strands (1 to maximal 6 per sample) was chosen for a root order-based characterization of traits, and the individual segments of the root samples were assigned to root branching orders according to Strahler's stream ordering system (Pregitzer et al. 2002) and dissected into the orders using a razorblade. For all species except *Fraxinus excelsior*, the root tip(s) plus the consecutive first root segment were counted as first-order segments, as it was not possible to clearly identify the transition between the root tip and the subsequent youngest root segment. In addition, root tips colonized by ectomycorrhizal fungi often formed coralloid clusters that were difficult to split into first and second-order segments. Ash root tips were well visible and

were counted as first root order. SRL, SRA, RTD and MD were separately determined for the root orders 1 to 4 using the flat-bed scanner and the Win-Rhizo software as described for the fine root bulk samples. In addition, the relative contribution (in percent) of the four root orders to the total biomass, length or surface area of the investigated root strand was determined in order to quantify biomass partitioning in the terminal part of the fine root system. After morphological investigation, the living root-material (entire root branches and separately analyzed fractions in the root orders) and the root necromass were dried at 70 °C for 48 h, weighed and ground for analysis of C and N concentrations by gas chromatography (Vario EL, elementar, Hanau, Germany). In the analyses, we distinguish between root order-related data and data relating to the bulk sample (all fine root biomass < 2 mm, i.e. all 4 orders combined).

2.3.5 Data analysis

All data sets were tested for normal distribution using a Shapiro-Wilk test. In most cases, normal distribution was not given and the non-parametric Mann-Whitney U-test for pairwise comparisons of means among species, soil depths and root orders was used for all morphological traits. For identifying the principal factors influencing root morphological traits, general linear models (GLM) based on ranks of the independent variables 'species', 'soil depth' and 'root order' were calculated. Species comparisons (based on means of the 0-30 cm profile) were conducted using a general linear model (GLM) followed by a Scheffé-test. All test statistics were conducted with SAS 9.3 Windows software on a significance level of $p < 0.05$. A Principal Components Analysis was conducted in the software CANOCO (biometris, Wageningen, The Netherlands) to analyze relationships between the five investigated root morphological traits and root orders. Linear and non-linear regressions were calculated with the software Xact7 (Sci Lab, Hamburg, Germany).

2.4 **Results**

2.4.1 ***Species differences in fine root morphology: bulk and root order-related*** *analysis*

For comparing the six species, four morphological traits (MD, SRL, SRA, RTD) and root nitrogen concentration were analyzed either for the bulked fine root biomass (all segments < 2 mm in diameter pooled) or separately for the root order classes 1 to 4. In the root order-related analysis, we additionally determined the partitioning of root biomass, root length and surface

area to the four studied root orders (expressed in percent of total biomass, length or surface area in the < 2 mm class) which allows assessing the relative importance of the four root order classes in the fine root system. The photographs Figure A 2.1 in the Appendix display characteristic fine root strands of the six species.

According to a GLM, all examined root morphological and chemical parameters were strongly dependent on species identity (Table 2.2). Nevertheless, the species effect was only secondary to the root order effect in all but one trait (RTD). In the bulked samples without separation of root orders, significant species differences existed for SRL and SRA (relatively high in the *Acer* species, intermediate in *C. betulus*, *F. sylvatica* and *F. excelsior*, and relatively low in *T. cordata*), RTD (lower in *F. excelsior* than in the other five species), MD (higher in *T. cordata* and *F. excelsior*, intermediate in *F. sylvatica*, *A. platanoides* and *C. betulus*, and lower in *A. pseudoplatanus*) and root N concentration (elevated in *F. excelsior*, intermediate in *C. betulus*, the *Acer* species and *F. sylvatica*, relatively low in *T. cordata*; Table 2.3).

The number of root tips per fine root mass was relatively low in *F. excelsior* and *T. cordata*, intermediate in *A. platanoides*, *C. betulus* and *F. sylvatica*, and highest in *A. pseudoplatanus*. Correspondingly, the number of tips per soil volume was particularly large in *A. pseudoplatanus* and *F. sylvatica* and low in *F. excelsior*, *T. cordata* and *A. platanoides* (
The two congeners *A. pseudoplatanus* and *A. platanoides* had a remarkably different fine root morphology, in particular with respect to SRL and SRA (Figure 2.1 E, F), even though they appeared to be morphologically similar under the microscope (
Figure A 2.1). The first-order rootlets of *A. pseudoplatanus* had a significantly higher SRL and SRA with a tendency for higher N concentration than those of *A. platanoides* in all three soil depths. *A. pseudoplatanus* also produced thinner 4^{th}-order root segments with higher SRA than its congener (Figure 2.1 A, E, F and Figure A 2.1 in the Appendix). Further, *A. pseudoplatanus* forms significantly more fine root tips (per root mass and per soil volume) than *A. platanoides* (Table 2.4).

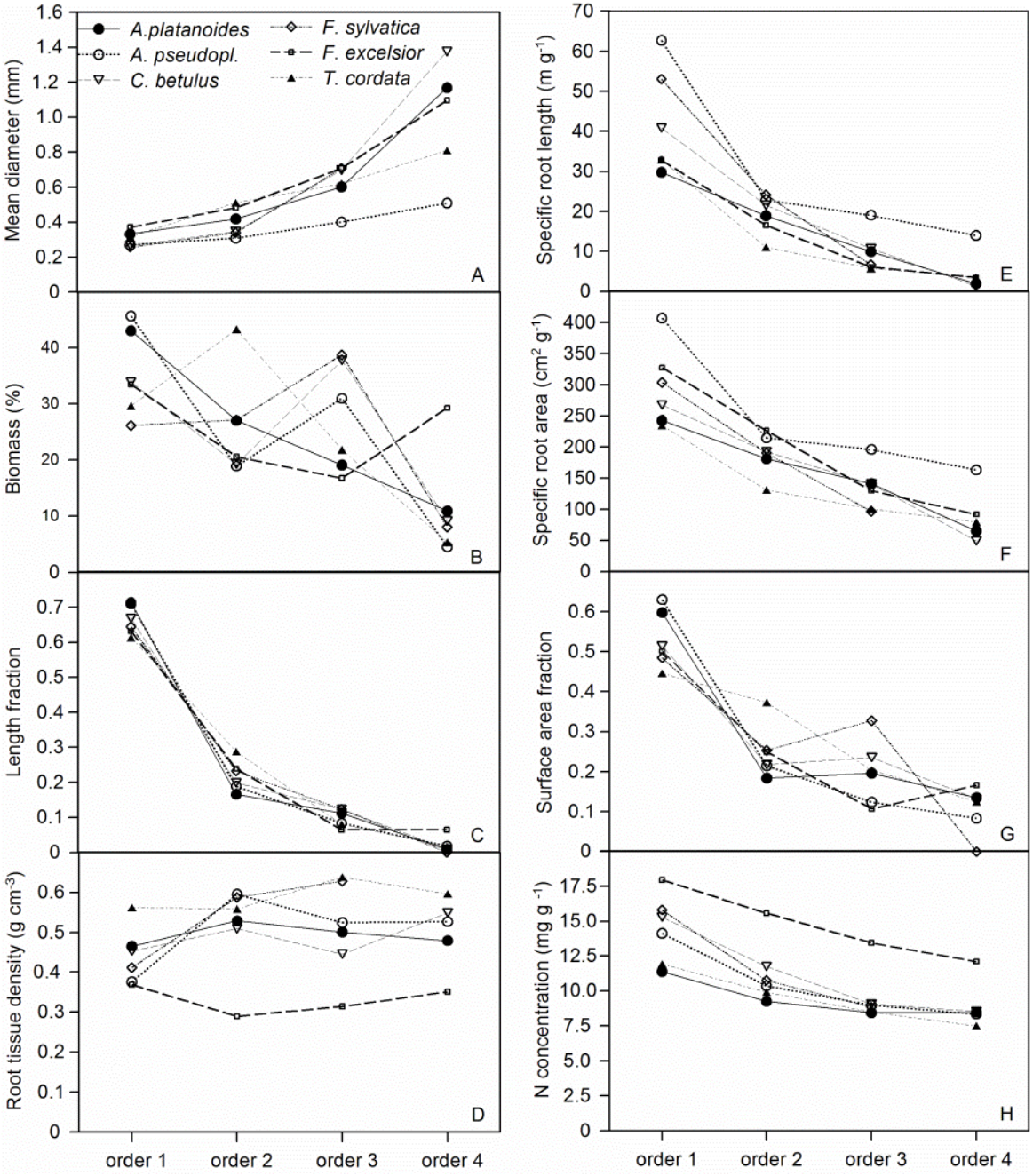

Figure 2.1: Change in eight root morphological or chemical parameters (A-H) along fine root strands from the first to the fourth root order in the six tree species (given are means of 8 replicate plots that were cored; each sample consisted of 1 to 6 roots that were averaged). All root strands had a maximum diameter of 2 mm. The data refer to the 0-10 cm layer.

Table 2.2: General linear models relating the variables 'species', 'soil depth', 'root branching order' and their interactions to the dependent variables root biomass fraction, root surface area fraction, root length fraction, specific root length (SRL), specific root area (SRA), root tissue density (RTD), mean segment diameter (MD) and root N concentration (N) across the sample consisting of six tree species. Given are the F value, the significance level (p) and the R^2 values (only significant factors are presented).

Dependent variable	Model	Species	Depth	Order	Species× depth	Species × order	Depth × order	Species × depth × order
BIOMASS FRACTION								
F		3.15	4.82	8.33	2.52		2.90	
p	0.001	0.01	0.01	0.001	0.01		0.01	
R^2	0.289	0.034	0.021	0.054	0.055		0.038	
SURFACE AREA FRACTION								
F		6.59	4.22	21.98	3.18		2.18	
p	0.001	0.001	0.05	0.001	0.001		0.05	
R^2	0.367	0.064	0.0164	0.128	0.062		0.025	
LENGTH FRACTION								
F		13.66	21.58	141.06	3.16			
p	0.001	0.001	0.001	0.001	0.001			
R^2	0.643	0.074	0.047	0.460	0.034			
SRL								
F		17.08		252.87				
p	0.001	0.001		0.001				
R^2	0.744	0.071		0.628				
SRA								
F		10.68		157.73				
p	0.001	0.001		0.001				
R^2	0.646	0.060		0.535				
RTD								
F		23.36		7.10		2.24		
p	0.001	0.001		0.001		0.01		
R^2	0.393	0.223		0.041		0.060		
MD								
F		31.24		237.06		2.54		
p	0.001	0.001		0.001		0.05		
R^2	0.740	0.124		0.564		0.030		
N CONCENTRATION								
F		40.22	9.09	111.37	2.14			
p	0.001	0.001	0.001	0.001	0.5			
R^2	0.694	0.225	0.020	0.374	0.024			

Table 2.3: Five morphological traits of the fine roots (bulk samples; all segments < 2 mm in diameter) of the five species in three different soil depths and averaged over the 0-30 cm profile (means ± SE). RTD – root tissue density, MD – mean diameter in < 2 mm class, SRA – specific root area, SRL- specific root length, N – root N concentration. Differences between the species in a soil depth are marked by different lower case letters, differences between the soil depths by capital letters. Species differences in the profile average are indicated by different Greek letters.

Trait	Depth (cm)	*F. excelsior*	*A. pseudoplatanus*	*A. platanoides*	*C. betulus*	*T. cordata*	*F. sylvatica*
RTD (g m^{-3})	0-10	0.341 ± 0.054 bA	0.454 ± 0.038 abA	0.556 ± 0.051 acA	0.493 ± 0.018 aA	0.606 ± 0.058 cA	0.620 ± 0.134aA
	10-20	0.371 ± 0.033 cA	0.509 ± 0.057 abAB	0.493 ± 0.015 aA	0.514 ± 0.031 abA	0.563 ± 0.067 bA	0.487 ± 0.041 aA
	20-30	0.378 ± 0.047 cA	0.540 ± 0.031 abB	0.462 ± 0.099 abcA	0.460 ± 0.026 aA	0.478 ± 0.053 abcA	0.536 ± 0.037 bA
	Profile average	**0.340 ± 0.042 α**	**0.491 ± 0.026 α**	**0.576 ± 0.094 α**	**0.488 ± 0.013 α**	**0.525 ± 0.036 α**	**0.521 ± 0.043 α**
MD (mm)	0-10	0.482 ± 0.029 cA	0.317 ± 0.006 bA	0.436 ± 0.070 acA	0.374 ± 0.023 aA	0.468 ± 0.056 acA	0.411 ± 0.103 abcA
	10-20	0.524 ± 0.054 cA	0.318 ± 0.026 bA	0.394 ± 0.019 aA	0.363 ± 0.021 abA	0.567 ± 0.073 cA	0.460 ± 0.053 acA
	20-30	0.587 ± 0.070 bA	0.363 ± 0.034 aA	0.476 ± 0.105 abA	0.511 ± 0.082 abA	0.652 ± 0.093 bA	0.459 ± 0.087 aA
	Profile average	**0.531 ± 0.030 αβ**	**0.333 ± 0.015 α**	**0.416 ± 0.027 αβ**	**0.416 ± 0.028 αβ**	**0.562 ± 0.045 β**	**0.459 ± 0.067 αβ**
SRA (cm^2 g^{-1})	0-10	183.0 ± 13.3 cA	227.1 ± 8.6 bA	150.3 ± 8.6 aA	180.4 ± 28.0 abA	118.0 ± 12.2 cA	191.1 ± 62.6abA
	10-20	154.2 ± 20.2 abA	245.5 ± 76.1 abcAB	176.1 ± 21.0 aA	160.7 ± 0.9 abA	103.7 ± 35.2cA	125.8 ± 17.2 bcA
	20-30	159.5 ± 20.2 aA	163.3 ± 19.4 aB	233.2 ± 72.4 abA	126.2 ± 22.6 abA	107.4 ± 18.4 bA	121.6 ± 19.5abA
	Profile average	**165.1 ± 15.26 αβ**	**183.6 ± 20.0 α**	**145.4 ± 13.2 αβ**	**142.9 ± 19.0 αβ**	**98.7 ± 15.3 β**	**119.5 ± 19.1αβ**
SRL (m g^{-1})	0-10	13.536 ± 1.291 aA	28.038 ± 2.653 bA	15.313 ± 1.922 aA	21.994 ± 5.411 abA	10.911 ± 2.027 aA	29.240 ± 12.629 abA
	10-20	10.921 ± 1.819 bcA	34.769 ± 13.013 aAB	18.612 ± 3.290 abcA	17.615 ± 3.154 abA	7.455 ± 3.408 cA	12.552 ± 2.656 bA
	20-30	10.894 ± 2.130 bcA	19.472 ± 3.310 aB	22.831 ± 7.610 abcA	11.587 ± 3.509 abcA	7.436 ± 1.943 cA	12.797 ± 2.843 abA
	Profile average	**11.717 ± 1.367 αβ**	**22.394 ± 3.151 α**	**13.939 ± 1.396 α β**	**14.967 ± 3.193 αβ**	**7.614 ± 1.698 β**	**12.292 ± 2.935 αβ**
N (mg g^{-1})	0-10	14.267 ± 0.682 cA	11.635 ± 0.551 bA	9.902 ± 0.621 aA	12.196 ± 1.122 abcA	9.953 ± 0.805 abA	10.946 ± 0.943 abA
	10-20	13.440 ± 0.681 cA	9.519 ± 0.522 aB	8.892 ± 0.480 aA	11.362 ± 0.837 bcAB	7.923 ± 0.349 dB	10.315 ± 0.853 abA
	20-30	13.344 ± 0.600 bA	10.076 ± 0.841 aAB	10.469 ± 0.982 aA	9.191 ± 0.817 aB	8.988 ± 0.322 aA	9.102 ± 1.125 aA
	Profile average	**13.601 ± 0.434 β**	**9.442 ± 0.813 α**	**9.221 ± 0.229 α**	**11.110 ± 1.005 αβ**	**9.279 ± 3.030 α**	**9.636 ± 0.708 α**

Table 2.4: Root tips per biomass or soil volume, proportion of root tips colonized by EM fungi, tips per square meter soil and cumulative length of 1st-order root segments per liter soil volume for the six species in the three horizons and the entire profile (0-30 cm). Significant differences between species per soil depth are indicated by different lower case letters, differences for a species between soil depths by capital letters, differences between profile averages by Greek letters.

Species	Soil depth (cm)	Tips per FR biomass ($n\ g^{-1}$)	Tips per soil volume ($n\ L^{-1}$)	Tips per square meter ($n\ m^{-2}$)	Proportion infected (%)	Cumul. length of 1st order roots per soil volume ($m\ L^{-1}$)
F. excelsior	0-10	1807 cA	2092 bA	209151	-	8.13 ± 1.24 dcA
	10-20	1328 bA	1227 aAB	107352	-	5.10 ± 1.15 aAB
	20-30	1654 bcA	878 aB	87799	-	2.89 ± 0.73 abB
Profile average	**0-30**	**1466 γ**	**1169 α**	**331894 αχ**	-	**5.38 ± 0.71 αβ**
A. pseudoplatanus	0-10	11297 bA	7559 cA	755902	-	11.22 ± 0.96 cA
	10-20	9031 aAB	3899 cB	389863	-	6.32 ± 1.30 aB
	20-30	7155 aB	2861 bB	286061	-	4.38 ± 1.09 aB
Profile average	**0-30**	**8557 β**	**4084 β**	**1439768 βδ**	-	**7.31 ± 0.56 β**
A. platanoides	0-10	4948 aA	2349 aA	234907	-	4.63 ± 1.31 abAB
	10-20	5684 aA	2063 aA	206294	-	4.49± 0.98 aA
	20-30	8662 abA	720 aB	72047	-	1.62 ± 0.81 bB
Profile average	**0-30**	**4148 α**	**1178 α**	**509526 αχδ**	-	**2.99 ± 0.71 α**
C. betulus	0-10	8832 abA	4541 aA	454148	85.8 ± 4	5.50 ± 1.24 adAB
	10-20	5034 aA	3799 bA	379855	89.1 ± 3	6.62 ± 0.98 bA
	20-30	3416 abcA	1176 bB	117579	98.1 ± 1	2.93 ± 0.81 abB
Profile average	**0-30**	**4578 α**	**2110 γ**	**956849 βχδ**	**90.0** ± 2	**4.77 ± 0.72 αβ**
T. cordata	0-10	4769 aB	1738 abA	173775	74.8 ± 8	2.25 ± 0.85 bA
	10-20	3789 bA	2773 abA	277295	76.5 ± 8	3.15 ± 1.37 acA
	20-30	1909 cA	1184 abA	118392	77.1 ± 5	3.37 ± 0.39 abA
Profile average	**0-30**	**2888 γδ**	**1354 αγ**	**607816 χδ**	**76.0** ± 4	**2.14 ± 0.63 α**
F. sylvatica	0-10	11427 abcA	3915 abA	395314	88.8 ± 6	4.65 ± 1.74 abdAB
	10-20	4604 abA	5410 bA	540977	86.3 ± 4	8.40 ± 1.34 cbA
	20-30	5323 aA	3686 bA	368591	91.7 ± 4	5.06 ± 1.25 aB
Profile average	**0-30**	**5129 αβδ**	**3251 αβγ**	**1197948 δ**	**89.0** ± 2	**5.30 ± 1.32 αβ**

When comparing the three EM and three AM species, significant differences were only detected for one of the eight traits; SRA was larger in the AM than the EM species (Table 2.5). We did not get hints on systematic differences in root tip frequency between AM and EM species (Table 2.4 and Table 2.5) as it might be expected from the largely different morphology of the two mycorrhiza types. Downward in the soil profile (from 0-10 to 20-30 cm), the AM species showed significant decreases in the number of fine root tips per soil volume and the cumulative length of first-order rootlets (which contain the tips) per volume; in contrast, no such trend was visible in the EM species (Table 2.4). However, AM and EM species did not differ significantly with respect to tip numbers per soil volume.

Table 2.5: Means ± SE of seven root morphological or chemical traits for the each three AM and EM species (data averaged over the 0-30 cm profile). Given is the p value of a comparison of the means (Mann-Whitney U-test) and the significance level (* = $p < 0.05$)

Mycorrhiza type	MD (mm)	SRA ($cm^2\ g^{-1}$)	SRL ($m\ g^{-1}$)	RTD ($g\ cm^{-3}$)	N($mg\ g^{-1}$)	Tips per mass (g^{-1})	Tips per volume(L^{-1})
AM	0.42 ± 0.02	164.64 ± 9.75	16.20 ± 1.56	0.47 ± 0.04	11.77 ± 0.53	4865 ± 821	2186 ± 350
EM	0.48 ± 0.03	120.34 ± 10.54	11.62 ± 1.61	0.51 ± 0.02	10.01 ± 0.46	4198 ± 702	2238 ± 394
p	0.33	0.04*	0.33	0.50	0.50	0.50	0.68

In general, the influence of soil depth on root morphology was relatively small. Only in a few cases, we observed significant directional change in fine root morphological traits from the 0-10 to the 20-30 cm layer. Notable is the increase in RTD with soil depth in *A. pseudoplatanus* and the decrease in root N concentration in *C. betulus* (Table 2.3).

Root order was the key factor influencing fine root traits (Table 2.2). According to the PCA, the morphological traits MD, SRA and SRA showed the closest association with root order, which was located on the first PCA axis. In contrast, the association with order was weaker for the anatomical and chemical parameters N concentration and RTD (Table 2.6). All species showed a similar increase in root diameter and a decrease in SRA, SRL and N concentration from the first to the fourth order, while the RTD pattern along the root was more variable among the species. F. excelsior differed from the other species by particularly low RTD and high N concentrations in all root orders (Figure 2.1 D and H). The root order-based analysis revealed that the species differences were often pronounced in one order but negligible in others (as visible in MD and RTD; see Figure 2.1).

Table 2.6: Principal components for the relatedness of eight root morphological and chemical traits and root order (order 1-3) with the axes 1 to 4 of a PCA covering all species (in brackets cumulative fit values R^2). The eigenvalues of the axes are given in the second row. The closest correlations of the components with the respective axis are given in bold print.

Variables	Axis1	Axis2	Axis3	Axis4
EV	0.738	0.133	0.073	0.039
Root branching order	**-0.944 (0.890)**	0.164 (0.917)	-0.044 (0.919)	0.206 (0.962)
SRL (m g^{-1})	**0.922 (0.850)**	-0.207 (0.894)	-0.118 (0.908)	0.284 (0.988)
SRA (cm^2 g^{-1})	**0.966 (0.933)**	0 (0.933)	-0.041 (0.935)	0.217 (0.982)
RTD (g cm^{-3})	**-0.628 (0.394)**	**-0.697 (0.880)**	0.334 (0.992)	0.090 (1.000)
N concentration (mg g^{-1})	**0.777 (0.604)**	0.310 (0.700)	0.545 (0.997)	-0.015 (0.997)
Mean diameter (mm)	**-0.870 (0.756)**	0.383 (0.903)	0.123 (0.918)	0.241 (0.976)

2.4.2 Species differences in the abundance and distribution of fine root biomass

Total fine root biomass in the 0-30 cm profile (bulked samples) differed up to twofold among the six species with highest plot mean in *F. sylvatica* (301 g m^{-2}) and lowest in *A. platanoides* (142 g m^{-2}, difference significant at $p < 0.05$; Figure A 2.2 in the Appendix). In the profile totals, ~89-95 % of root biomass was contributed by the target species and the remainder (< 25 g m^{-2} in 0-30 cm) by other woody species that grew in the neighborhood. Part of the species differences in fine root biomass seem to be caused by differences in the species' aboveground presence in the plots as indicated by the variable basal areas of the species in the plots (28.7-60.3 m^2 ha^{-1}, Table 2.1). However, the species may also differ inherently in their fine root biomass in the upper soil as indicated by large species differences in the fine root biomass/basal area ratio of the plots (range: 50-124 g fine root biomass per m^2 basal area in the six species; data not shown).

Fine roots of *F. sylvatica* and *T. cordata* seemed to prefer the 10-20 cm layer (47 and 43 % of the biomass profile total) over the top layer (0-10 cm) in the respective plots, while the other species showed similar fine root densities at 0-10, 10-20 and 20-30 cm depth (or a reduced density at 20-30 cm, Figure A 2.2). Correspondingly, GLMs showed that soil depth had a smaller influence on fine root biomass variation across the study plots than species identity (Table 2.2).

2.4.3 Species differences in the abundance of 1st- and 2nd-order fine root biomass

A comparison of the six species with respect to the abundance of root biomass assignable to the 1st or 2nd root orders revealed species differences in root system structure that would not

have been detected by a comparison of bulked fine root biomass totals (Figure 2.2). *F. sylvatica* and *T. cordata* had a significantly smaller 1st- and 2nd-order root biomass in the 0-10 cm layer than the other species. Due to the relatively small biomass proportion of the two species in these root orders, beech and lime differed from the other species more in 1st- and 2nd-order root biomass than in total fine root biomass. *A. pseudoplatanus* had significantly more 1st- and 2nd-order root biomass in the 0-10 cm layer than its congener *A. platanoides*. Highest values in this layer were reached by *F. excelsior*.

In 10-20 cm depth, most species had higher amounts of 3rd- and 4th-order roots than in the top layer. 1st- and 2nd-order fine root biomass decreased toward the 20-30 cm layer and the degree of root branching decreased as well. As a consequence, four of the six species possessed only three root orders in this soil layer until roots exceeded 2 mm in diameter (Figure 2.2); thicker segments were cut off prior to analysis and thus where not investigated here.

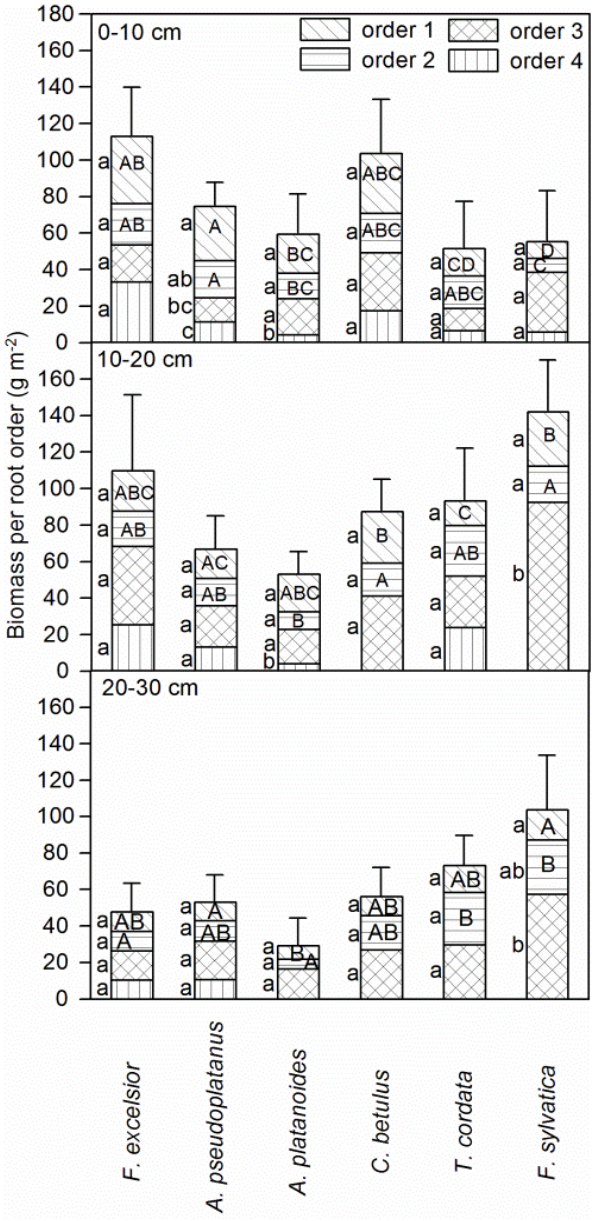

Figure 2.2: Fine root biomass assigned to the root orders 1 to 4 (uppermost to lowermost sections of bars) in three soil depths (0-10, 10-20 and 20-30 cm, in g m^{-2} 10 cm $depth^{-1}$) for the six tree species (means ± SE). Different small letters mark significant differences between root orders for a species, different capital letters significant differences in a given order between the species (only 1st and 2nd order);
Mann Whitney U-test; $p < 0.05$.

2.5 **Discussion**

2.5.1 Interspecific variation in fine root morphology and branching patterns

Our root order-based analysis produced evidence for convergence in important fine root morphological traits and branching patterns across the six investigated species, even though most taxa were not closely related to each other and had a largely different ecology. In particular the functionally important traits SRA, length fraction and surface area fraction, which determine the development of root surface area in the most uptake-active 1st- and 2nd-order root segments, showed coherent patterns of change from the first to the fourth root order in all species. Further, the relative variation among the species was smaller than in several important aboveground traits such as leaf size, the size difference between sun and shade leaves, or mean xylem vessel diameter (Köcher et al. 2013, Legner et al. 2013).

On the other hand, several other root traits differed largely between the species. Most variable was root tip frequency (the number of tips per fine root mass) with ~ 6 fold difference between the species (smallest in *F. excelsior*, largest in *A. pseudoplatanus*) matching results of Ostonen et al. (2007) in three boreal tree species. More relevant for nutrient uptake capacity may be the number of tips per soil volume which still differed about 3.5 fold among the co-occurring species. This variability is also visible in species differences in the cumulative length of 1st-order root segments per soil volume: species means ranged from 2.14 to 7.31 m L^{-1} soil; a substantial part of the first order segment is contributed by the root tip itself. The length of the hyphal net per soil volume is certainly another, probably even more important, morphological factor influencing uptake capacity. Unfortunately, we do not have information on this variable.

A relatively high species variation existed also in the pattern as to how root tissue density and root N concentration changed from the first to the fourth root order. *F. excelsior* differed substantially from the other species with lower RTD and higher N in particular in the second to fourth orders. Thus, apparent convergence in several root traits can go along with markedly diverging patterns in other properties. We reached at similar conclusions when the different soil layers were analyzed separately or the pooled samples of all soil layers were examined. In fact, soil depth exerted only a minor influence on fine root morphology and branching patterns of these six species.

When interpreting the findings from Hainich forest, it is important to recognize that convergence in root traits was detected for the modes of C and N allocation within the fine root system, i.e. the trees' strategy to use plant resources for generating nutrient and water capturing surfaces. We speculate that a relatively compact soil (clay content: 20-30 %) with relatively high bulk density (~ 1.2 g cm^{-3} in the topsoil) and temporal desiccation in dry summer periods may represent conditions favoring convergence in the root traits examined. All species must face similar physical root growth constraints and a comparable carbon-investment-to-nutrient-return ratio of roots exploring the soil. The much larger species variation in root tip numbers than in branching patterns indicates that this trait must be more under genotypic control than others. Observed species differences in root mass- and surface area-specific nutrient uptake capacity (e.g. Jacob & Leuschner 2014) might, in part, be a consequence of differences in root tip numbers, but such dependence has not yet been examined. Alternatively, differences in hyphal length and activity, and in root activity per root surface area, may also be influential factors.

The partly deviating fine root properties of *F. excelsior* (relatively thick, N-rich 1st- and 2nd-order roots with low tissue density and only few root tips), which have already been noted in earlier studies (Jacob et al. 2012, Meinen et al. 2009a), could relate to the ecology of this species. *F. excelsior* differs in important functional traits from the other investigated species, notably in its relatively high growth rate as an early- to mid-successional species, its ring-porous xylem with large vessels in the stem, and a relatively high N demand (Dobrowolska et al. 2011, Ellenberg & Leuschner 2010). *F. excelsior* further deviated from the other species by a particularly low root biomass: necromass ratio which may point to species differences in fine root mortality in this mixed stand. One might assume that ash as a species with preference of base-rich fertile soils does require lower root tip numbers than other species, but all six species of our study grew on similar soil.

Species differences in fine root properties were also notable between the two closely related *Acer* species (particularly high SRL and SRA in 1st-order roots of *A. pseudoplatanus*) which is in agreement with the results of Hölscher et al. (2002).

2.5.2 Root morphological differences between EM and AM tree species

To our surprise, we found significant differences between the three EM and three AM species in only one of the seven root morphological, chemical or branching-related traits. In both groups, considerable among-species variation existed for the variables tip number per root mass and tip number per soil volume. Despite the contrasting modes of interaction between root and fungus in the two mycorrhiza types, the EM species (*C. betulus, T. cordata, F. sylvatica*) on average did not form more root tips per root mass or soil volume than the AM species (*Acer* spp. and *F. excelsior*), where the fungus infects larger sections of the root than in EM trees. In our EM species, we observed insertion of hyphae mainly in the tip with the Hartig net but also in the directly adjacent parts of the 1st and 2nd root orders. While nearly all tips were colonized by fungi in the three EM species (~96 % according to the study of Lang et al. 2011 in Hainich forest), only about 19 % of the roots of *Fraxinus* and *Acer* were found to be infected by AM fungi (Lang et al. 2011). Largely different between the two groups was also the diversity of colonizing fungi in this mixed forest (75, 68 and 43 EM fungal species in *Fagus, Tilia* and *Carpinus*, and 7 different taxa of glomeromycota in the AM species according to ITS sequencing, Lang et al. 2011). It appears that, at least in the studied six species, the type of mycorrhiza is not an important determinant of fine root branching patterns and morphology despite the contrasting patterns of symbiotic interaction. This is somewhat surprising because it is well recognized that colonizing EM fungi have major effects on root

morphology and architecture by inducing the formation of short lateral roots and root tips that become swollen with a coralloid or 'Christmas-tree' like structure (Smith and Read 1997). Infection by AM-forming fungi appears to have more subtle effects on root morphology and architecture with changes observed in branching patterns and in the length of 2nd- and 3rd-order root segments (Hetrick 1991, Hooker et al. 1992). Thus, infections either by glomeromycota (AM) or basidio- or ascomycetes (EM) both tend to alter fine root morphology, but the morphogenetic effect has not yet been compared for AM and EM trees in a shared soil volume. We also found no clear hints for an important influence of mycorrhiza type on root functioning, because aboveground productivity was not systematically different between the AM and EM trees in Hainich forest, and neither foliar nor fine root N concentration showed clear differences between EM and AM species in this forest (Jacob et al. 2010).

At least in the fertile soils of Hainich forest, other factors such as species differences in standing fine root biomass, in fine root turnover, and in local nutrient availability as resulting from tree species effects on soil chemistry (Guckland et al. 2009, Rothe & Binkley 2001) may be more relevant for root functioning than the type of mycorrhiza. The sheer number of root tips also does not seem to be a relevant factor for tree nutrition in this forest, because *A. pseudoplatanus* with highest fine root tip numbers per root mass and soil volume among the six species did not possess higher fine root and foliar N concentrations and was not more productive than the other species.

2.5.3 The importance of 1st- and 2nd-order root segments

The root order-related analysis of fine root biomass showed that only a half to a third of the conventionally sampled fine root biomass (< 2 mm in diameter) referred to 1st- and 2nd-order segments in our study and that this fraction was more variable among the six species than bulk fine root biomass. We also found that the relative proportion of these two root fractions is highest in the topsoil (0-10 cm), while 3rd- and 4th-order segments are more important lower down in the profile where the supply of nitrogen (and other nutrients) is lower and small-diameter roots may primarily have transport functions. Lower root physiological activity deeper in the soil is also suggested by an increasing root C/N ratio with increasing soil depth in our soil profiles (data not shown); this matches results of Gaul et al. (2009) from spruce forest soils.

Our data on order-specific fine root biomass per ground area of mature trees can be compared with only very few other studies (e.g. Guo et al. 2004, Wang et al. 2006, Sun et al. 2011).

These studies are, however, only partly equivalent to our study because they refer to immature stands, sampled only the topsoil (0-10 or 20 cm) and compared only two species with inclusion of conifers. Nevertheless, it appears that different tree species may differ considerably with respect to the proportion of 1^{st}- and 2^{nd}-order roots in fine root biomass. More comparative studies in other forest types are needed for quantifying this root fraction and examining the link to tree resource uptake and productivity.

2.6 Conclusions

Comparative fine root system analysis in the Hainich mixed forest, either by examining bulked fine root samples (< 2 mm in diameter) or through detailed analysis of root orders, revealed that the more precise, but highly labor-intensive, order-based analysis detected several species differences that would have been overlooked in the more rapid analysis of bulked samples. However, species differences in the important traits SRA, root N concentration and MD were also reflected in the bulk analysis. Thus, for many purposes, it may be sufficient to analyze bulked root material (e.g. in the diameter class < 1 or < 2 mm) for characterizing morphological differences between, and similarities among, temperate tree species. Nevertheless, studies in additional tree species have to show, whether the detected convergent patterns in SRA and in the length and surface area fractions along fine root strands are indeed more or less similar among different temperate tree species.

The comparison of AM and EM tree species revealed no systematic fine root morphological differences between the two mycorrhiza types except for SRA. We suggest searching more systematically for different structural and functional consequences of the formation of either AM or EM symbioses in temperate tree species. Our approach of investigating arbuscular and ectomycorrhizal species of the same plant life form in a mixed stand may shed new light on the old discussion about principal functional differences between these two types of plant-fungus interaction.

2.7 Acknowledgments

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2.8 Appendix

Table A 2.1: Some morphological and functional traits of the six studied species according to different sources.

	Fraxinus excelsior	*Acer pseudo-platanus*	*Acer platanoides*	*Carpinus betulus*	*Tilia cordata*	*Fagus sylvatica*
Position in succession [a]	early-mid	mid/late	mid/late	mid/late	mid/late	late
Mycorrhiza type [b]	AM	AM	AM	EM	EM	EM
Wood density (g cm^{-3}) [c]	0.59	0.59	0.62	0.67	0.43	0.65
Sun leaf SLA (cm^2 g^{-1}) [d]	80.1	79.2	n.d.	100.7	102.5	86.9
Below-canopy shade intensity [e]	moderate	high	high	very high	high	very high
Xylem anatomy	ring	diffuse	diffuse	diffuse	diffuse	diffuse
Drought sensitivity [f]	low	moderate-high	moderate	moderate-low	moderate-low	high

[a] according to Ellenberg & Leuschner (2010) and other sources
[b] according to root studies in Hainich forest by Lang et al. (2011)
[c] after different sources
[d] after Legner et al. (2013) (mature trees in Hainich forest)
[e] according to Ellenberg & Leuschner (2010)
[f] after Köcher et al. (2009) and Hölscher et al. (2002)

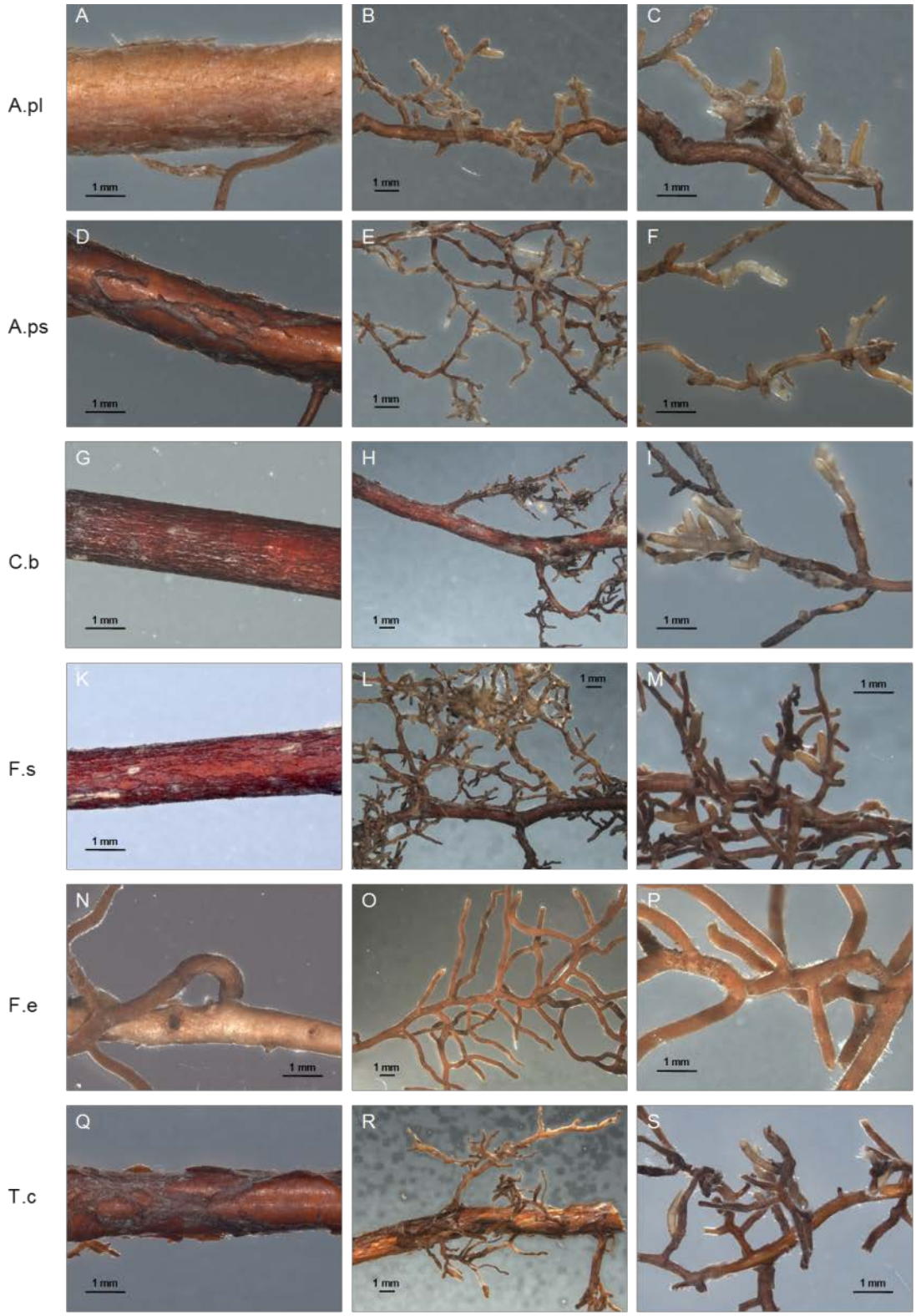

Figure A 2.1: Exemplary pictures of morphological characteristics of fine roots of the six broad-leaved tree species investigated in this study. *Acer platanoides* (A-C); *Acer pseudoplatanus* (D-F) *Carpinus betulus* (G-I); *Fagus sylvatica* (K-M); *Fraxinus excelsior* (N-P); *Tilia cordata* (Q-S).

Species descriptions: *Acer platanoides* (A-C): color of the thicker main axes ochre to dark brown; root tips of very small diameter, typically colonized with arbuscular mycorrhizal (AM) fungi (not ectomycorrhizal EM), often very hairy, not transparent, connection to the

consecutive fine root branch a bit constricted; surface of the thicker main axes with rough longitudinal net structure of older periderm parts, younger periderm parts smooth with shining fine cross-striped structure; second and higher order branches very irregularly ramified and tortuous. *Acer pseudoplatanus* (D-F): color of the thicker main axes beige to dark brown; root tips of very small diameter, typically colonized with AM fungi (not EM), not very hairy, often transparent, connection to the consecutive fine root branch clearly constricted; surface of the thicker main axes with rough dark longitudinal net structure of older periderm parts, younger periderm parts smooth with shining fine cross-striped structure; second and higher order branches very irregularly ramified and tortuous. *Carpinus betulus* (G-I): color of the thicker main axes dark red to dark brown; root tips typically colonized with EM; surface of the thicker main axes with very regular narrow longitudinal furrows, no clear differentiation of older from younger periderm parts; second and higher order branches irregularly ramified and often straight-line structured. *Fagus sylvatica* (K-M): color of the thicker main axes red to reddish brown; root tips typically colonized with EM; surface of the thicker main axes with narrow or wide longitudinal furrows, no clear differentiation of older from younger periderm parts; second and higher order branches irregularly ramified and often tortuous. *Fraxinus excelsior* (N-P): color of the thicker (and also thinner!) main axes beige or light brown to grey brown; root tips typically colonized with AM fungi (not EM); root tips and all other branch orders dense hairy; surface of the thicker main axes as of thinner axes with little or unclear surface structure, mostly no older periderm parts visible; second and higher order branches very regularly ramified and not very tortuous. *Tilia cordata* (Q-S): color of the thicker main axes beige to amber-colored or dark brown; root tips typically colonized with EM; surface of the thicker main axes with rough dark longitudinal slabby net structure of older periderm parts, younger periderm parts smooth with shining fine cross-striped structure; second and higher order branches very irregularly ramified and medium tortuous.

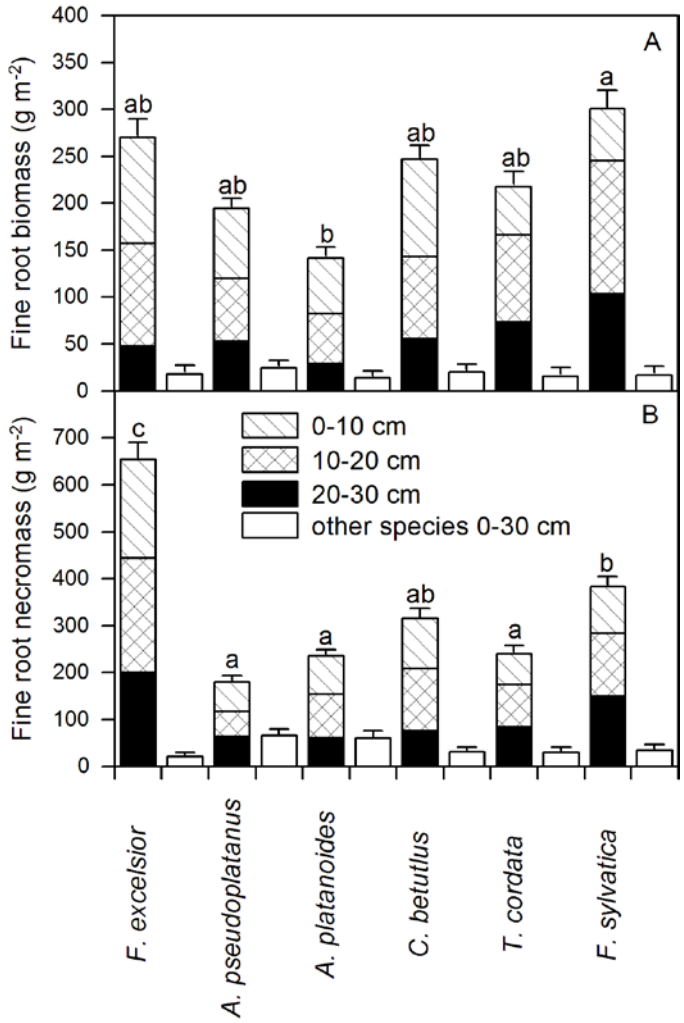

Figure A 2.2: Fine root biomass (A) and necromass (B) in three soil layers in the plots of the six species (n = 8 plots; respective left bar: target species, small right bar: other tree species present in 0-30 cm depth). Given are means ± SE. Significant differences between the species (profile total) are indicated by different letters (Mann-Whitney U test, $p < 0.05$).

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CHAPTER 3

Fine root productivity and turnover of ectomycorrhizal and arbuscular mycorrhizal tree species in a temperate broad-leaved mixed forest

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3.1 Abstract

Advancing our understanding of tree fine root dynamics is of high importance for tree physiology and forest biogeochemistry. In temperate broad-leaved forests, ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) tree species often are coexisting. It is not known whether EM and AM trees differ systematically in fine root dynamics and belowground resource foraging strategies. We measured fine root productivity (FRP) and fine root turnover (and its inverse, root longevity) of three EM and three AM broadleaved tree species in a natural cool-temperate mixed forest using ingrowth cores and combined the productivity data with data on root biomass per root orders. FRP and root turnover were related to root morphological traits and aboveground productivity. FRP differed up to twofold among the six coexisting species with larger species differences in lower horizons than in the topsoil. Root turnover varied up to fivefold among the species with lowest values in *Acer pseudoplatanus* and highest in its congener *Acer platanoides*. Variation in root turnover was larger within the two groups than between EM and AM species. We conclude that the main determinant of FRP and turnover in his mixed forest is species identity, while the influence of mycorrhiza type seems to be less important.

Key words: *Acer, Carpinus, Fagus, Fraxinus*, ingrowth cores, root branching order, root longevity, *Tilia*

3.2 Introduction

Leaves and fine roots are the organs that supply the plant with energy, water and nutrients. Because of their paramount importance for life, trees invest a large part of their annual carbon gain into the formation of new leaves (~30%) and fine roots (~20-40% or more, Keyes & Grier 1981, Vogt et al., 1996, Müller-Haubold et al. 2013). While the annual production of leaf mass and the phenology of leaf formation and abscission are easily measured in temperate deciduous trees, it is much more difficult to investigate the production and turnover of fine roots (conventionally defined as roots < 2 mm in diameter). This is due to the inconspicuous life of roots in the soil, but also because fine roots are not shed synchronously as defined entities at the end of their life like leaves. Rather, fine root death occurs gradually in the more distal root segments (Xia et al. 2010) and new first-, second- and higher-order root segments produced during a subsequent flush of root growth may replace the shed root segments (Fitter 1996). Thus, the most distal root segments of lowest root order generally are more short-lived than more proximate higher-order segments, and root turnover (and its inverse, root lifespan) varies across the fine root system, in marked contrast to foliage (Withington et al. 2006).

Understanding the factors that influence fine root lifespan is important because root growth consumes a substantial amount of the annually produced carbohydrates, thereby lowering timber production (Fogel 1983, Hertel et al. 2013). Moreover, root litter represents an important, if not the largest, source of carbon in forest soils (Fogel 1983, Rumpel et al. 2002, Fan & Guo 2010). Most studies on the fine root dynamics of temperate tree species were conducted with juvenile plants in common garden experiments without interspecific root system interactions (e.g. Withington et al. 2006, McCormack et al. 2014). An alternative approach is the comparison of different forest types (e.g. Guo et al. 2008a, Brunner et al. 2013), where species differences in fine root dynamics may be confounded by different site conditions. A few studies have investigated fine root lifespan and productivity in mature mixed forests (e.g. Tierney & Fahey 2001, Meinen et al. 2009ab), but these studies did not attempt to explain species differences in root dynamics. Eissenstat et al. (2015) were the first to relate fine root productivity and lifespan in a mixed forest to the root morphologies and foraging strategies of the different co-occurring tree species, comparing six arbuscular mycorrhizal (AM) species of the genera *Magnolia, Liriodendron, Juglans, Fraxinus, Acer* and *Ulmus*.

In western Eurasian cool-temperate broad-leaved forests, the majority of tree species are forming ectomycorrhizae (EM) as do, for example, species of the genera *Fagus, Quercus, Tilia, Carpinus* and *Betula*. A few AM species (genera *Acer, Fraxinus, Prunus* and *Ulmus*) co-occur with the dominant EM species in these forests. It is not known whether the two main types of mycorrhizal symbiosis are linked to contrasting fine root traits in terms of root lifespan and growth rate, when the species are co-occurring in the same stand. Different fine root dynamic properties of EM and AM tree species, if existing, could reflect different strategies of belowground resource foraging, given that EM species are thought to be more efficient in terms of nitrogen acquisition and AM species of phosphorus acquisition. Such differences might also explain why EM tree species dominate cool-temperate and boreal forests and AM species are much more abundant in tropical and sub-tropical forests (McGuire et al. 2008, Lang et al. 2011).

In this study, we examined the fine root productivity of each three co-occurring EM and AM tree species in a natural temperate broadleaf mixed forest employing a modified ingrowth core technique according to Meinen et al. (2009b) and Hertel et al. (2013) combined with root coring for biomass determination. This allowed calculating fine root turnover in the < 2 mm-diameter class and obtaining an estimate of the average lifespan of the root mass in this fraction. The six species (*Fraxinus excelsior, Acer pseudoplatanus, A. platanoides, Carpinus betulus, Tilia cordata* and *Fagus sylvatica*) are abundant tree species in central European woodlands and highly (or moderately) important for forestry. They differ not only with respect to mycorrhiza type, but also in terms of canopy architecture, shade tolerance, hydraulic architecture, and their role in forest succession (Köcher et al. 2009, Ellenberg & Leuschner, 2010, Legner et al. 2014). Moreover, fine root morphology differs not only between the genera but also between the closely related *Acer* species (Meinen et al. 2009a, Jacob et al. 2012, Kubisch et al. 2015). The species *A. pseudoplatanus, A. platanoides, T. cordata* and *F. sylvatica* were also studied by Withington et al. (2006) in a common garden experiment, which allows comparison of results, even though tree age, stand density and also methodology (ingrowth core vs. mini-rhizotron approach) differed between the two studies. By investigating the six species' fine root turnover in the same mixed forest in patches with contrasting species dominance; we were able to compare mature trees under natural growing conditions on similar soil. This study builds on an earlier investigation of fine root morphological traits of these species, which showed that root morphology depended mainly on species identity, while mycorrhiza type was of secondary importance (Kubisch et al.

2015). In the present study, we focus on fine root productivity and root lifespan of the six tree species, testing the hypotheses that (i) coexisting AM and EM tree species differ in fine root turnover and root productivity, reflecting different nutrient acquisition strategies, (ii) fine root productivity increases with decreasing mean fine root diameter of the species (Eissenstat 1991), and (iii) fine root productivity is higher, and root lifespan shorter, in tree species with higher aboveground productivity. The latter assumption relates to the generally higher nutrient and water demand of fast-growing species, which might be associated with thinner, more short-lived fine roots (Eissenstat et al. 2015). By comparing the two maple species *Acer pseudoplatanus* and *A. platanoides*, we further tested for congeneric contrasts in fine root dynamics in two closely related tree species.

3.3 Materials and methods

3.3.1 Study site and plot design

The study was conducted in Hainich National Park in Thuringia, central Germany, which protects one of the largest remaining temperate deciduous broadleaf forests in Central Europe (7500 ha). Beside large areas of monospecific European beech (*Fagus sylvatica* L.) forest, the park contains forest stands with relatively high tree species richness. During the past 50 years, this forest was exposed to only minor management activities in form of selective logging. With declaration of a national park in 1997, all activities like logging and military training, practiced in certain areas, were abandoned.

The study site is located on a Triassic limestone plateau (Muschelkalk formation; 308-399 m a.s.l.; 51°04’ N, 10°30’ E) within the 'Thiemsburg area' in the north-east of the national park, where more than six tree species co-occur either in quasi-random mixture or in small groups consisting of three to six trees of a species. The species in this study (European beech (*F. sylvatica*), Small-leaved lime *(Tilia cordata* Mill.), European hornbeam (*Carpinus betulus* L.), European ash (*Fraxinus excelsior* L.), Sycamore maple (*Acer pseudoplatanus* L.) and Norway maple (*Acer platanoides* L.) were the species with highest abundance in this Stellario-Carpinetum community (oak-hornbeam forest). Three of the six species are ectomycorrhizal (EM) (*C. betulus*, *F. sylvatica* and *T. cordata*), while the other species were found to form only arbuscular mycorrhizas (AM) in the study site (*A. pseudoplatanus*, *A. platanoides* and *F. excelsior*); colonization by both AM- and EM-forming fungi as found in some *Acer* species (e.g. Smith & Read 2007) was not observed (see also Meinen et al. 2009, Jacob et al. 2010). Mean annual precipitation in the study region is ~ 590 mm yr^{-1} and mean

annual temperature 7.5 °C (period 1973-2004, Deutscher Wetterdienst 2005). In the study years 2012 and 2013, mean annual air temperatures of 9.7 °C (2012) and 8.5 °C (2013) and precipitation totals of 603 mm (2012) and 598 mm (2013) were recorded at the nearest weather station Weberstedt/Hainich (Deutscher Wetterdienst 2009).

Table 3.1: Aboveground structural characteristic of the target trees and of entire study plots; all species in a plot for the six plot types (species); important soil chemical properties of the mineral topsoil (0-10 cm) are also indicated.

Variable	Species					
	F. excelsior	*A. pseudoplatanus*	*A. platanoides*	*C. betulus*	*T. cordata*	*F. sylvatica*
Target species						
Tree height (m)	32.3±1.5	28.6±0.9	23.8±2.0	22.8±1.1	24.2±1.40	26.4±0.7
ABW (Mg ha^{-1})	313.2±47.1	182.8±30.7	136.1±21.8	168.7±34.6	169.1±24.9	207.0±24.2
ABW (Mg Ind.$^{-}$$tree^{-1}$)	1.9±0.3	1.8±0.1	1.4±0.2	1.1±0.2	0.8±0.1	1.2±0.1
Dbh (cm)	52.2±3.5	58.1±3.2	51.2±3.6	43.4±3.3	46.4±2.3	43.5±2.2
BA (m^2 ha^{-1})	47.82±4.69	27±4.01	22.07±3.24	26.29±5.16	43.60±6.12	52.32±5.04
All species per plot						
Total BA (m^2 ha^{-1})	57.1±5.4	47.8±8.8	28.7±2.5	31.6±6.6	50.9±6.0	60.3±7.8
Proportion of target species (%)[1]	83.6±3.5	62.7±7.2	77.4±9.9	86.1±8.0	84.8±4.0	90.4±5.7
Stem density (no. trees ha^{-1})	409±71	376±64	232±23	232±41	365±35	696±98
Soil chemical properties (0-10 cm)						
C/N (g/g)	11.9±0.2	11.6±0.4	12.0±0.4	12.5±0.2	12.0±0.3	12.6±0.3
CEC ($\mu mol_c/g$)	213.5±75.5	195.8±69.2	206.5±73.0	178.5±63.1	192.2±67.9	139.2±49.2
Base saturation (%)	91.2±5.8	88.8±4.8	87.3±5.4	88.0±6.8	93.4±4.2	78.5±8.2
pH (H_2O/KCl)	5.29/4.19	5.07/4.10	5.20/4.11	5.29/4.15	5.41/4.31	5.02/3.94

[1] % of BA

The soil of the study area is a base-rich Eutric Luvisol (FAO taxonomy 2006) with a profile depth of 60-70 cm developed in clay-rich Pleistocene loess that covers the limestone bedrock. Due to high clay content, the soil can dry out strongly in summer, while it may show partly stagnant properties during spring and winter. The plots were established in patches with dominance of each one of the six species which were characterized by similar soil properties. Marginal differences in soil conditions between the plots of the species were primarily caused by the specific litter properties of the species (Rothe & Binkley, 2001, Guckland et al. 2009). For example, the base saturation at the cation exchangers in the topsoil and lower mineral soil was marginally lower under *Fagus* (78.5%) than under the other species (Kubisch et al. 2015; Table 3.1). However, none of the measured properties differed significantly between the plots. Circular plots (diameter 12 m; area 113 m^2) containing mature trees of one of the six target species ('tree clusters') were randomly selected in the area. For our analysis, two neighboring trees of the target species with dominant position in the upper canopy layer, or one dominant tree of the respective species, were chosen; they formed the center of the plots. The selected trees had diameters at breast height (dbh) of 40-60 cm (Table 3.1). This plot scheme was

chosen to minimize possible species effects on soil chemistry in the mixed forest, which would have been more pronounced in larger monospecific stands. The scheme ensures that the bulk of fine roots in the soil belonged to the target species (typically >80%). We sampled eight plots per species resulting in 48 plots in total. All stems >10 cm dbh in a 'tree cluster' were investigated for their species identity, dbh, basal area and tree height (Table 3.1).

3.3.2 Fine root productivity and root dynamics

For quantifying fine root productivity (FRP; in g m^{-2} yr^{-1}), we applied a modified ingrowth core approach. To achieve more natural root growth conditions in the cores, we modified the conventional ingrowth core technique (Persson, 1980, Powell & Day 1991, Majdi 1996) and refrained from enclosing the core in a net in order to minimize soil disturbance. Compared to other techniques, the ingrowth core method has been found to produce rather conservative figures of fine root production in temperate forests (e.g. Hertel & Leuschner 2002, Hendricks et al. 2006). The ingrowth cores were installed in June 2011 immediately after an inventory of standing fine root biomass (FRB; diameter < 2 mm) which was conducted in the same 48 plots by coring the topsoil to 30 cm depth at 150 cm distance to the stem of the central tree in the plot using a steel corer of 35 mm diameter (Kubisch et al. 2015). The sampling holes were refilled with root-free soil from a nearby place (distance ca. 30-50 cm) and used as ingrowth cores for a period of two years. Each refilled coring site was precisely marked with three plastic sticks inserted down to 30 cm soil depth which allowed a resampling of the core at exactly the same place after 24 months. Earlier investigations at the same forest sites had shown that re-colonization of the cores by fine roots started typically 12 months after their installation (Meinen et al. 2009b). We thus assumed that fine root growth in the cores took place from May 2012 to May 2013, i.e. over 365 d, while the period of core exposure lasted from July 1, 2011 to May 16, 2013. The cores were resampled on May 16, 2013 in the same manner as done in the initial biomass inventory in 2011 (Kubisch et al. 2015). Upon harvest, the extracted soil cores were divided into the 0-10, 10-20 and 20-30 cm soil layers and stored in plastic bags at 4 °C. The root samples were subsequently analyzed in the lab within 3 months by carefully rinsing the soil cores with tap water over a sieve of 0.25 mm mesh size and extracting all fine root branches >10 mm length. Assignment of root mass to tree species was done with a morphological key that has been developed earlier in this stand using periderm properties such as color and surface structure, the mode of root branching, and mycorrhiza type as criteria (Hölscher et al. 2002, Meinen et al. 2009a, Kubisch et al. 2015). Properties like elasticity of the stele, and the cohesion of periderm and stele were used for

distinguishing live from dead root mass (Persson, 1978, Hertel & Leuschner 2002). The turnover of fine root mass (unit: yr^{-1}; i.e. the inverse of root longevity) was calculated by dividing annual FRP by standing FRB (inventory data). Turnover data are bulk values for the entire fine root biomass < 2 mm in diameter, thus averaging root lifespan over all root orders. At the date of harvest, no fine root necromass was observed in the ingrowth cores and this component was thus not considered in the analysis of fine root production and turnover.

All extracted live fine roots from the ingrowth cores were scanned and analyzed for their specific surface area (SRA, in $cm^2\ g^{-1}$), specific length (SRL, in $m\ g^{-1}$), and tissue density (RTD, in $g\ cm^{-3}$) using a flat-bed scanner and WinRhizo software (Régent Instruments Inc., Quebec, Canada). The annual production of fine root length (m per m^2 ground area and year) and fine root surface area ($m^2\ m^{-2}\ yr^{-1}$) was calculated using the measured morphological characteristics of the fine roots collected in the ingrowth cores.

To obtain a rough estimate of the fine root biomass produced annually in the different root orders, we used the percent distribution of fine root biomass to the root orders #1 to #4 which was determined in a detailed root order analysis of the standing FRB inventory done by Kubisch et al. (2015) according to the ordering system proposed by Pregitzer et al. 2002. This analysis was conducted in a representative sub-sample of the FRB material of every soil sample and soil depth (>10 mm length) which had been subjected to a fractionation into root orders. The individual segments of a root branch were assigned to the first four branching orders according to the ordering system proposed by Fitter (1987) and Pregitzer et al. (2002) and dissected into the orders using a razorblade

For all species except *F. excelsior*, the root tip(s) was counted together with the adjacent root segment as first-order segment, as it was often not possible to clearly recognize the transition between tip and subsequent root segment. The separation was especially problematic in case of the ectomycorrhizal tree species which often formed coralloid cluster-like structures that could not be split into first and second-order segments (Valtanen et al. 2014). In contrast, in *F. excelsior* with arbuscular mycorrhiza, the individual tips were clearly recognizable and hence were counted as first root order. Root segments of the 5^{th} or higher orders contributed with less than 5% to the fine root biomass < 2 mm and were lacking in various samples; they were not considered in the subsequent analysis. The proportion of the 1^{st} to 4^{th} root orders in the total fine root mass of a root branch was detected and the ratios were applied to estimate the production of root mass in the four orders in the ingrowth cores. This extrapolation can give only a very rough number as it is based on the assumption that fine roots growing into the ingrowth cores do not differ in their branching structure from the fine roots collected in

the inventory (Figure 3.1), and root longevity is similar in the root orders. The latter assumption is probably not valid (McCormack et al. 2015) suggesting that our calculation can only indicate the magnitude of root biomass produced in the different orders. We thus use these data only as an estimate of the production of absorptive roots (1st- and 2nd-order roots) per ground area of the six species.

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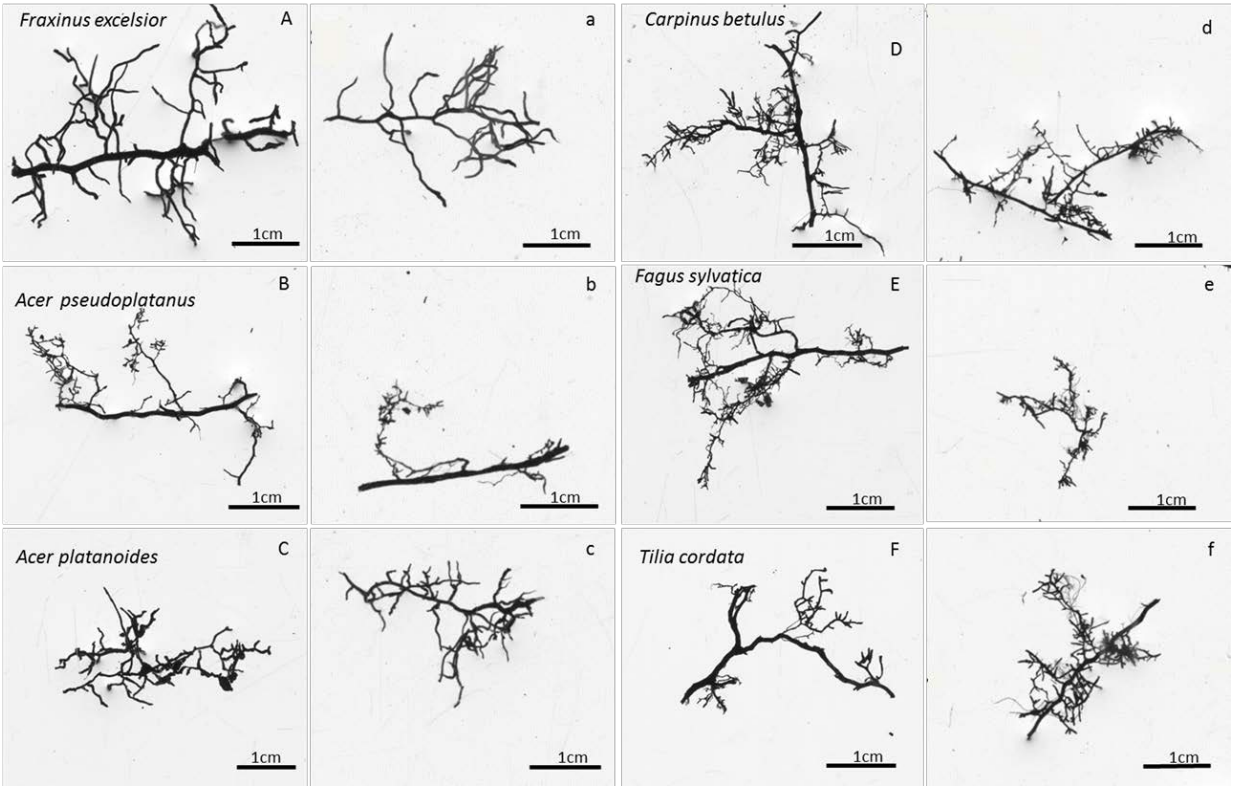

Figure 3.1: Photographs of typical terminal fine root branches of the six species (A–F) as collected in soil cores of the inventory (respective left columns, marked with capital letters) or in the ingrowth cores (respective right columns, marked with small letters). Images were taken with WinRhizo software.

After the detailed analysis, all extracted fine root material was dried at 70 °C for 48 h until constant weight. The carbon and nitrogen concentrations were analyzed by gas chromatography (vario EL, elementar, Hanau, Germany) in the ground root material of the initial FRB inventory.

3.3.3 Aboveground woody biomass production

The annual production of above-ground woody biomass (ABWP in Mg ha^{-1} yr^{-1}; including stem and larger branches) was calculated from stem increment data obtained for every target tree using permanently installed dendrometer tapes (UMS, München, Germany) mounted at

1.50 m stem height. The height of the target trees was measured at the beginning of the study using a Vertex IV ultrasonic height meter (Haglöf, Langsele, Sweden). To calculate the total aboveground woody biomass (ABW) of the trees, we used the following allometric equations given for the respective species in Zianis et al. (2005):

Fagus sylvatica and *Carpinus betulus:*

$ABW = 0.04736 \cdot dbh^{1.80521} \cdot h^{0.99603}$

Tilia cordata:

$\ln (ABW) = -2.6788 + 2.4542 \cdot \ln (dbh)$

Acer pseudoplatanus and *A. platanoides:*

$\ln (ABW) = -2.7606 + 2.5189 \cdot \ln (dbh)$

Fraxinus excelsior:

$\ln (ABW) = -2.4598 + 2.4882 \cdot \ln (dbh)$,

with ABW being total aboveground woody biomass including branches (in kg per tree), dbh diameter at breast height (in cm), and h tree height (in m).

For *C. betulus*, no specific allometric equation was found in the literature. We thus used the equation for *F. sylvatica* as an approximation. For *A. platanoides*, we used the same equation as for *A. pseudoplatanus* because specific equations seem to lack for this species as well. We assumed that height growth during the relatively short (1 year) measuring period was negligible and excluded it from the calculations. Annual woody biomass production (ABWP) of the target trees in the plots was then calculated as the change in aboveground woody biomass of each tree from spring 2012 to spring 2013.

3.3.4 Statistical analyses

All data sets were tested for normal distribution using a Shapiro-Wilk test. As normal distribution was often not given and the data sets could not sufficiently be transformed, non-parametric statistics were applied in these cases. For most variables, a Kruskal-Wallis H-test followed by a Mann-Whitney U-test for pairwise comparison between the species was conducted ($p < 0.05$). The relationship between ABWP, or root morphological traits, and the fine root productivity or root turnover of the six species was explored with Spearman rank correlation analysis. ANCOVA was employed to separate between effects of mycorrhiza type and effects of various root morphological traits on fine root turnover. These tests were conducted with SAS 9.3 software. In order to analyze the inter-relationships between fine root

biomass, root morphological properties, fine root productivity, root turnover, and aboveground tree structure, biomass and wood production, we conducted a Principal Components Analysis (PCA) using the package CANOCO, version 4.5 (Biometris, Wageningen, The Netherlands).

3.4 **Results**

3.4.1 Fine root productivity and turnover

Fine root biomass per ground area (0-30 cm profile) ranged between 140 and 300 g m^{-2} among the six tree species in the plots with dominance of the respective species (Table A 3.1. in the Appendix). As for biomass, annual fine root productivity in the soil profile varied by a factor up to two among the species. Highest productivity was measured for *C. betulus, F. sylvatica,* and *F. excelsior* (~ 150-170 g m^{-2} yr^{-1}), intermediate values for *A. platanoides* and *T. cordata,* and lowest for *A. pseudoplatanus* (~ 80 g m^{-2} yr^{-1}, Figure 3.2). Interestingly, the six species differed not significantly in FRP in the uppermost soil layer (0-10 cm), while marked differences existed in the two deeper soil layers. Particularly high productivity was measured in the 20-30 and 10-20 cm layers for *F. sylvatica,* while *A. pseudoplatanus* reached only low values in these depths (Figure 3.2).

Table 3.2: Median of fine root turnover (yr^{-1}) of the six species in the three different soil depths. Significant differences (pairwise comparison; Mann-Whitney U-test; $p < 0.05$) between the soil depths for a species are marked with different lower case letters, those for two species at a given soil depth are marked with different capital letters.

	Soil depth		
	0-10 cm	10-20 cm	20-30 cm
Fraxinus excelsior	0.42 aAB	0.16 aAB	0.44 aA
Acer pseudoplatanus	0.22 aA	0.21 aAB	0.44 aA
Acer platanoides	1.14 aB	1.18 aA	1.60 aB
Carpinus betulus	0.70 aAB	0.60 aAB	0.22 aA
Tilia cordata	0.50 aAB	0.17 aAB	0.36 aA
Fagus sylvatica	0.42 aAB	0.33 aB	0.70 aAB

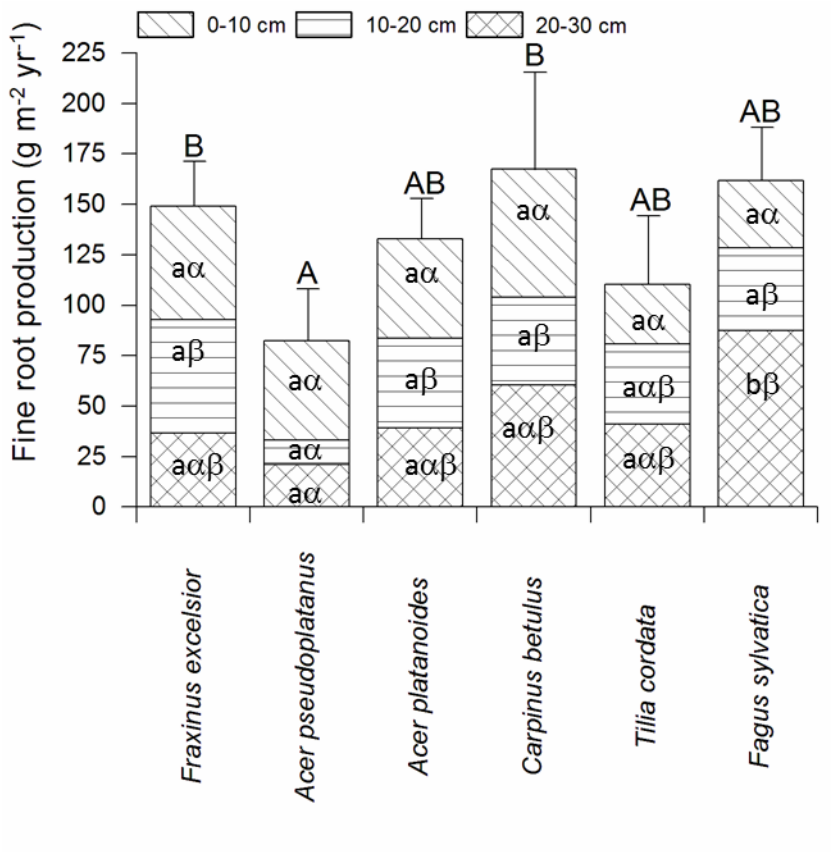

Figure 3.2: Fine root productivity (FRP) of the six tree species in the three soil depths according to the ingrowth core study (mean ± SE; n = 8 plots). Different capital letters indicate significant differences ($p < 0.05$) between the species in the soil profile (0–30 cm); significant differences between the soil depths for a given species are indicated by different lower case Latin letters, differences between tree species within a given soil depth by lower case Greek letters.

When FRP was related to aboveground woody biomass production (ABWP, measured as kg biomass increment per target tree(s) per m^2), *C. betulus* and *A. pseudoplatanus* reached highest ratios (>1), *T. cordata*, *A. platanoides* and *F. sylvatica* intermediate ratios (0.6 – 0.8), and *F. excelsior* a very low ratio (< 0.2; Figure 3.3).

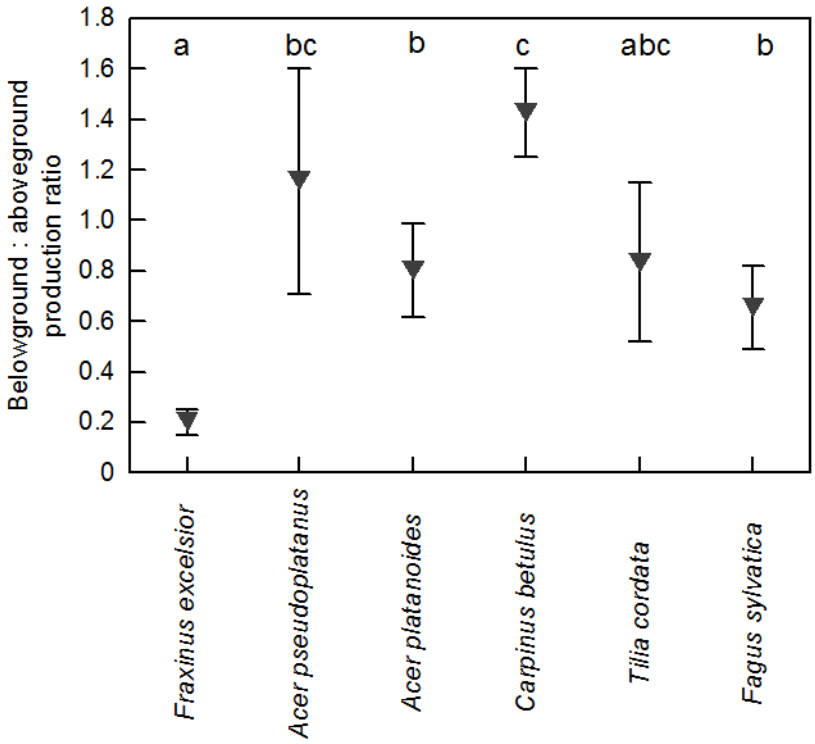

Figure 3.3: Ratio of annual belowground (fine root) to aboveground (woody biomass) production in the six species. FRP was expressed per m^2 ground area; woody biomass production is the growth of the target trees. Statistically significant differences between the species are indicated by different letters.

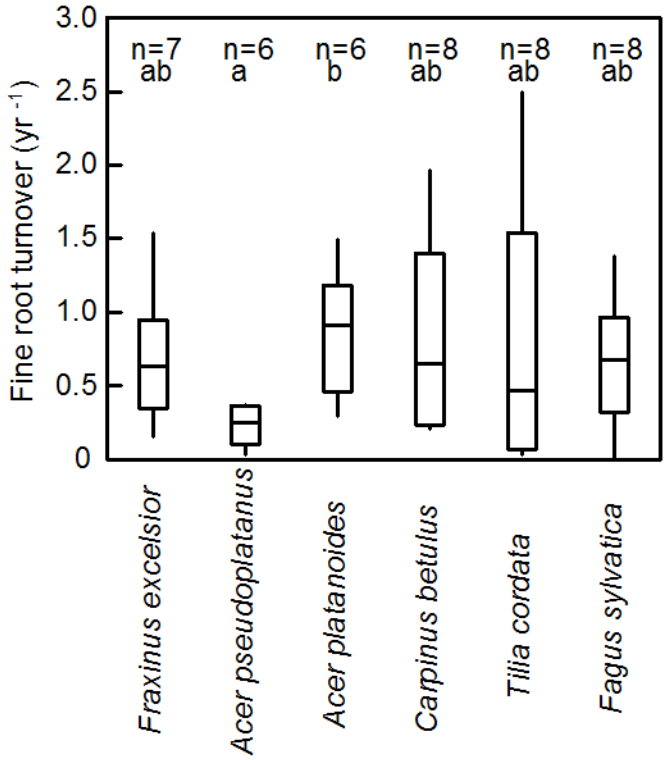

Figure 3.4: Median fine root turnover ($year^{-1}$) of the six tree species according to ingrowth core data for the 0–30 cm profile. Given are the median, the 25- and 75- percentiles and the minima and maxima. Significant differences ($p < 0.05$) between the species are indicated by different letters.

Fine root turnover (productivity per standing biomass) in the 0-30 cm profile was highest in *A. platanoides* and lowest in *A. pseudoplatanus* (difference significant; Figure 3.4).
However, the variation in turnover values within a species was generally large (ranging from 0.1 or 0.2 to 2.0 yr^{-1} or higher). Consequently, in most cases, species differences in root turnover were not significant. Moreover, there was no uniform trend of fine root turnover change with soil depth across the six species (Table 3.2). *C. betulus* and *T. cordata* showed a decrease in turnover with depth, while *F. sylvatica*, *F. excelsior* and the two *Acer* species had the highest fine root turnover in the deepest (20-30 cm) layer (Table 3.2). However, the variation in turnover figures within a species and soil depth was also large.

Table 3.3: Comparison between the EM and AM tree species in terms of fine root turnover and fine root productivity (FRP). Given are the group means ± SE, based on the species' median turnover and mean FRP. Both differences were not significant at $p < 0.05$ according to a Mann-Whitney U-test.

Mycorrhiza type	No. of species	Turnover (yr^{-1})	FRP ($g\ m^{-2}\ yr^{-1}$)
AM	3	0.72 ± 0.22	121.51 ± 20.07
EM	3	0.60 ± 0.07	146.49 ± 18.15

Neither fine root turnover nor fine root productivity differed significantly between the EM and AM species groups (Table 3.3). Analysis of covariance with mycorrhiza type as independent and fine root turnover as dependent variable revealed that among several root morphological traits introduced as covariates, only SRL had a significant influence ($P = 0.008$; results not shown).

3.4.2 Annual production of fine root length and surface area

Fine root length growth in the 0-30 cm soil profile ranged from 1359 m $m^{-2}\ yr^{-1}$ in *T. cordata* to 3303 m $m^{-2}\ yr^{-1}$ in *F. excelsior* (difference significant between the low values in *T. cordata* and *A. pseudoplatanus*, and the high values in *F. excelsior* and *C. betulus*). Most species had the highest fine root elongation rate in the upper 10 cm and a decrease to lower layers (Table 4). However, *T. cordata* and *A. platanoides* had the highest fine root length production in 10-20 cm depth and *F. sylvatica* showed a significant increase in fine root length production from 0-10 to 20-30 cm depth (Table 3.4). As expected, annual fine root surface area production revealed a similar pattern as length production; *F. excelsior* produced the largest root surface area per year (4.1 $m^2\ m^{-2}\ yr^{-1}$), *T. cordata* the lowest (1.7 $m^2\ m^{-2}\ yr^{-1}$). Fine root biomass production was positively related to root length growth in *A. pseudoplatanus*, *F. excelsior*, *A.*

platanoides and *T. cordata* as expected, while this relation was not significant in *C. betulus* and *F. sylvatica* (data not shown).

Table 3.4: Annual fine root length and surface area production (SA) per square meter ground area in 0–10 cm, 10–20 cm, 20–30 cm soil depth and for the profile (0–30 cm). Given are means ±SE for 8 plots. Statistically significant differences between the soil depths are indicated by different lower case letters, significant differences between the species in a soil depth by different capital letters; differences between the species in the 0-30 cm profile are indicated by bold capital letters (Mann-Whitney U Test; $p < 0.05$).

Species	Depth (cm)	Length (m m^{-2} yr^{-1})	S.A. (cm^2 m^{-2} yr^{-1})
Fraxinus excelsior	0-10	1455 ± 479 aA	17504 ± 5604aA
	10-20	1210 ± 231aA	14984 ± 2976aA
	20-30	638 ± 202 bABC	8660 ± 2350bABC
	Profile	**3303 ± 639 A**	**41148 ± 7345A**
Acer pseudoplatanus	0-10	903 ± 269 aA	11617 ± 4004aAB
	10-20	340 ± 73 aBC	3473 ± 945aB
	20-30	333 ± 72aABC	4376 ± 978aABC
	Profile	**1576 ± 300B**	**19465 ± 4759B**
Acer platanoides	0-10	907 ± 248aA	11030 ± 3140aAB
	10-20	933 ± 209aABC	10644 ± 2504aAB
	20-30	695 ± 188aAC	7584 ± 1927aAC
	Profile	**2535 ± 343AB**	**29259 ± 4235AB**
Carpinus betulus	0-10	1519 ± 567abA	13877 ± 4402aAB
	10-20	1089 ± 268aAC	10213 ± 2191aA
	20-30	553 ± 171bAB	5528 ± 2093bAB
	Profile	**3161 ± 796A**	**29618 ± 6408AB**
Tilia cordata	0-10	545 ± 179aA	6524 ± 2263aB
	10-20	615 ± 214aC	7665 ± 2666aAB
	20-30	275 ± 102aB	3918 ± 1331aA
	Profile	**1359 ± 395B**	**17149 ± 5376B**
Fagus sylvatica	0-10	560 ± 186aA	8036 ± 2109aAB
	10-20	808 ± 216abABC	10074 ± 2622abABC
	20-30	1095 ± 202bC	13589 ± 3052aC
	Profile	**2462 ± 417AB**	**31699 ± 5106B**

3.4.3 Fine root dynamics in relation to root properties and aboveground productivity

To explore relationships between fine root morphology, fine root productivity, and aboveground structure and productivity (ABWP) among the six tree species, a PCA with four axes was conducted. Fine root biomass production as well as fine root length and surface area growth were positively related to the first axis together with root nitrogen concentration and FRB. Aboveground woody biomass production and tree height were also positively associated with axis 1, while RTD was negatively related to this axis (Table 3.5). The SRA and SRL values of the produced fine root biomass in the ingrowth cores correlated closest with axis 2. Fine root turnover was the only variable not being associated with the other variables; it correlated with axis 3 (Table 3.5). The PCA plot in Figure 3.5 indicates that the three EM species resemble each other in terms of root morphology and root productivity, while the two *Acer* species (AM) group separately, and *F. excelsior* (AM) seems to differ from the other five species in most tested influential parameters.

Contrary to expectation, species differences in fine root productivity could not be explained by species differences in root morphological properties such as different mean fine root diameters, SRL, SRA, root tissue densities or root N contents (Table 3.6). Neither root N concentration nor root tissue density correlated with FRP in any of the species (except for a negative relation between root N and FRP in *A. platanoides*). In *A. pseudoplatanus*, FRP was negatively related to SRA and SRL indicating that *Acer platanoides* produced more fine root biomass, when the newly grown fine root branches were shorter and thicker. Similarly, species differences in root turnover could be explained neither by mean fine root diameter nor by other root morphological traits (Table A 3.2 in the Appendix).

Aboveground productivity (ABWP) was not related to FRP in the 0-30 cm profile, neither in the sample of all species (Table 3.6) nor in separate correlation analyses at the species level, except for *C. betulus* (Table 3.7).

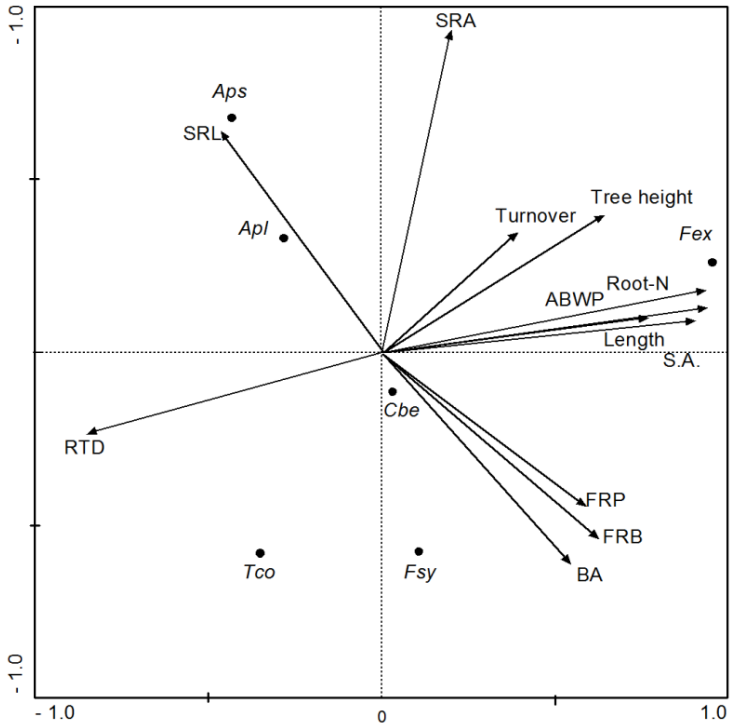

Figure 3.5: Results of a Principal Components Analysis regarding the parameters fine root biomass (FRB), root morphological properties (RTD, SRA, SRL), annual production of fine root biomass (FRP), length (Length) and surface area (SA), root turnover, and tree basal area (BA) and aboveground woody biomass production (ABWP). Shown are the inter-relationships along the first two axes (axis 1 = x axis; axis 2 = y axis). Species: Fex, *Fraxinus excelsior*; Aps, *Acer pseudoplatanus*; Apl, *Acer platanoides*; Cbe, *Carpinus betulus*; Tco, *Tilia cordata*; Fsy, *Fagus sylvatica*.

Table 3.5: Results of a principal components analysis (PCA) regarding the variables fine root biomass of the plots (FRB), root morphological properties, annual FRP and length and surface area production, fine root turnover, and aboveground tree structure, biomass and wood production (ABWP).

Variables	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalues	0.4809	0.2134	0.166	0.1023
FRP	**0.590**	-0.446	0.566	-0.359
Length production	**0.771**	0.099	0.555	-0.294
Surface area production	**0.900**	0.091	0.334	-0.121
Fine root turnover	0.398	0.351	**0.586**	**0.585**
FRB	**0.627**	-0.538	-0.344	-0.445
RTD	**-0.862**	-0.233	0.350	-0.006
SRA	0.202	**0.936**	-0.122	-0.260
SRL	-0.469	**0.644**	-0.111	-0.535
Root N concentration	**0.935**	0.181	-0.003	-0.011
BA of target species	0.542	**-0.614**	-0.499	0.108
Tree height	**0.645**	0.399	-0.607	0.137
ABWP	**0.939**	0.129	-0.151	0.270

Table 3.6: Spearman rank correlation coefficients (rs) for the relationship between aboveground productivity and morphological properties with FRP for the pooled data set (all six species) based on species means; ABWP, aboveground woody biomass production; SRL, specific root length; SRA, specific root area; RTD, root tissue density; MD, mean diameter; root N, fine root nitrogen concentration.

	r_s	p
ABWP	0.371	0.469
SRL	-0.257	0.623
SRA	-0.314	0.544
RTD	-0.371	0.469
MD	-0.143	0.787
Root N	0.600	0.208

Table 3.7: Spearman rank correlation coefficients (r_s) for the relationship between aboveground productivity and morphological properties with fine root productivity conducted separately for the six species. Significant correlations at $p < 0.05$ are marked with an asterisk. ABWP = aboveground woody biomass production; SRL = specific root length; SRA = specific root area; RTD = root tissue density; Root N = fine root N concentration.

	Species					
	Fraxinus excelsior	*Acer pseudoplatanus*	*Acer platanoides*	*Carpinus betulus*	*Tilia cordata*	*Fagus sylvatica*
ABWP	0.024	0.190	-0.476	0.881*	0.429	-0.262
SRL	-0.167	-0.929*	-0.190	-0.143	-0.595	-0.524
SRA	-0.286	-0.810*	-0.238	-0.071	-0.524	-0.524
RTD	0.571	0.333	0.048	0.238	-0.238	0.429
MD	-0.429	0.738*	0.048	0.119	0.524	0.167
Root N	0.429	0.143	-0.491	-0.381	-0.167	-0.357

Relating ABWP to root traits across the six species revealed a significant negative relation to root tissue density; this relation disappeared, when *F. excelsior* with particularly thick and N-rich fine roots was excluded (data not shown). The species means of ABWP were not related to the species SRL, SRA or root N concentration means (Table A 3.3 in the Appendix). When this analysis was conducted at the species level, none of the ABWP – root trait relations were significant at $p < 0.05$ (in *F. sylvatica*, a marginally significant relation at $0.05 < p < 0.1$ between ABWP and root N appeared; Table A 3.4 in the Appendix).

3.5 **Discussion**

3.5.1 Factors influencing fine root longevity

We obtained mean fine root turnover rates between 0.16 and 1.60 yr^{-1} in the six species and three horizons for the bulked fine root biomass < 2 mm, equivalent to a mean root lifespan of 0.6 yr (*Acer platanoides*) to 6.3 yr (*Fraxinus excelsior*); this is a 10 fold difference between the species. The majority of turnover figures, however, ranged between 0.3 and 0.7 yr^{-1} (i.e. lifespans of 1.4 - 3.3 yr). With minirhizotron observation, Withington et al. (2006) found median fine root lifespans between 0.6 and 2.5 yr for *A. pseudoplatanus, A. platanoides, T. cordata* and *F. sylvatica,* which matches well with our ingrowth core-based estimates, given that Withington et al. (2006) considered only 1st-order roots, while our data include also the higher root orders with longer life spans in the bulked root mass < 2 mm diameter, and Whithington et al. (2006) did not investigate *F. excelsior*. In agreement with Withington et al. (2006), we found a relatively high lifespan in *A. pseudoplatanus*, while our data indicate a short mean lifespan of *A. platanoides* roots, in contrast to their results. It has to be kept in mind that the results of minirhizotron and ingrowth core studies on fine root turnover in forests are often poorly comparable (Burke & Raynal, 1994), and turnover rates derived from minirhizotron observation typically are higher than ingrowth core estimates (e.g. Finér et al. 2011). This offers another explanation of the higher lifespan values found in our study compared to the figures of Withington et al. (2006). Further, the trees in the common garden study of Withington et al. (2006) likely were exposed to lower root competition intensity than the trees in the mixed forest of our study, which could also have influenced root longevity. Finally, climatic differences between the studies of Withington et al. (2006) and our study (Poland vs. Germany) could partly explain differences in longevity values (cf. Leppalammi-Kujansuu et al. 2014).

Comparing fine root turnover among different species based on the bulked <2 mm fine root biomass instead of focusing on root orders might introduce errors, if the species differ largely in their branching patterns and the functionality of the respective segments is different (McCormack et al. 2015). Earlier root order-based morphological analyses of the six species by Kubisch et al. (2015) showed that the branching patterns of the species were relatively similar despite belonging to different families and mycorrhiza types. For example, the fine root mass <2 mm in diameter of all species consisted to about 95 % of the root orders 1 to 4, while higher order-segments contributed always with less than 5 % to fine root biomass. Further, mean diameter, fine root length fraction and root tissue density as important morphological traits influencing nutrient acquisition followed in all species a remarkably

similar trend from the 1st to the 4th order, suggesting that root segments with either absorptive functions or storage and conductance functions occupied rather similar proportions of fine root biomass in these species. McCormack et al. (2015) assume that the 4th root order should in temperate tree species mainly be responsible for nutrient and water transport, while nutrient and water acquisition is located in the 1st to 3rd root orders. Even though we do not have information on the longevity of individual root segments and orders, we assume that the observed species differences in fine root turnover of the <2 mm-class should not result from different branching patterns and contrasting proportions of 1st- and 2nd-order segments in the species, but rather reflect species differences in overall fine root longevity, as the species were relatively similar with respect to fine root length fractions. Clearly, a detailed root order-based analysis of root lifespan would give a more reliable picture of species differences in the dynamic properties of the fine root system and of possible contrasts between EM and AM species.

We could not detect relationships between root longevity and root morphological and chemical properties in our six-species sample. This is unexpected and does contrast with the findings of McCormack et al. (2012, 2015) who reported a positive relation between root lifespan and fine root diameter, root C/N ratio and root Ca concentration, and a negative one between lifespan and SRL for North-American tree species. However, analysis of covariance showed for our data a positive effect of SRL on fine root turnover, when separated from the influence of mycorrhiza type. This is a hint that thinner roots were shorter lived, even though mean fine root diameter was not an influential factor in our analysis. In a literature review of fine root lifespan in temperate tree species, Guo et al. (2008b) found that lifespan generally increased with root diameter. Similarly, Eissenstat et al. (2015) measured a higher median fine root lifespan in thick-rooted AM tree species. These results refer to the first two root orders. For grasses, Ryser (1996) reported higher root longevity when root tissue density was higher, while a relation to diameter did not appear. These observations indicate that root production is indeed behaving in a manner, which fits to the resource optimization concept proposed by Eissenstat & Yanai (1997). Large-diameter roots require higher investment of carbon and nutrients per unit root length or surface area, which should be coupled with greater root lifespan in order to ensure a favorable nutrient and water return on the amount of carbon and nutrients invested.

In our sample, mean fine root diameter differed only moderately among the six species (means of 0.33 – 0.59 mm) and greater root lifespan was found not only in the species with

largest diameters (*F. excelsior* and *T. cordata*) but also in *A. pseudoplatanus* with the thinnest fine roots. Large spatial and also temporal variation in fine root turnover (McCormack et al. 2014) together with only limited species differences in root diameter may explain our failure to detect relationships between fine root morphology and lifespan in this mixed forest. The species sample of Eissenstat et al. (2015) covered a much greater range of root diameters (0.2 – 1.3 mm) and referred only to AM species.

3.5.2 Are fine root productivity and root lifespan different between EM and AM tree species?

In contradiction to our first hypothesis, the three ectomycorrhizal tree species of our sample differed not significantly from the three arbuscular mycorrhizal species in terms of FRP and fine root turnover when comparing the group means (see Table 3). The two groups were also similar with respect to the belowground : aboveground production ratio. We had expected that *F. excelsior* and the two *Acer* species as AM species would have longer-lived fine roots because fine root longevity has been found to increase with root diameter (McCormack et al. 2012), and *F. excelsior* had the thickest fine roots of the six species. Further, the minirhizotron data of Withington et al. (2006) indicate that the two *Acer* species have particularly long fine root lifespans (median lifespan of 1st and 2nd order roots: 1.5 and 2.6 yr in *A. pseudoplatanus* and *A. platanoides*, respectively). This result was only in part confirmed by our ingrowth core study which showed a very high longevity of the *A. pseudoplatanus* roots (means of 2.3 - 4.8 yr for roots <2 mm in diameter), but not of the *A. platanoides* roots (0.6 – 0.9 yr). In fact, mean fine root diameter was not different between AM and EM species in our sample, and *A. pseudoplatanus* had particularly thin roots in the first four root orders (Kubisch et al., 2015). The morphological comparison showed that systematic differences between the two mycorrhiza types did only exist with respect to SRA (higher values in AM species), but not for root diameter, RTD, SRL or root N concentration in our sample. In an analysis of 25 North-American woody species, Comas & Eissenstat (2009) reported for the EM species a higher branching intensity (number of root tips per total root length) than for the AM species; this was not visible in our six-species sample (Kubisch et al. 2015).

The long lifespan of the two *Acer* species observed by Withington et al. (2006) was explained with a very thick exodermis in the fine roots of the two *Acer* species. Our results with the diverging *Acer* species suggest that this explanation may not be generally valid.

A shortcoming of our approach is that the root morphological and productivity measurements refer to the standard size class of fine roots < 2 mm in diameter in all species, thus including a substantial fraction of non-mycorrhizal root mass in the analysis. This could have masked potential effects of mycorrhizal type on root morphology and dynamics. Experimental duration may also have influenced the results. For example, Ostonen et al. (2005) observed different contributions of roots < 1 mm and roots >1 mm diameter to FRP over the course of a 3 yr-long root production study.

Despite these possible sources of bias, it appears that the type of mycorrhiza is a less important factor influencing fine root lifespan than other possibly relevant factors. Previous research has shown that plant-internal resource allocation rules (Eissenstat & Duncan, 1992) and external abiotic and biotic factors act as the main determinants of fine root lifespan, among the latter nutrient availability, drought stress, temperature extremes and the activity of root herbivores, pathogens and fungal symbionts (Wells et al., 2002; Guo et al., 2008a; Rasmann & Agrawal, 2008; Adams & Eissenstat, 2015). Most of the abiotic factors should have been more or less similar among the study plots of the six species in Hainich forest, while differences in herbivore and pathogen activity may vary with the specific chemical and biological conditions in the rhizosphere of the species (Guckland et al. 2009, Cesarz et al. 2013, Scheibe et al. 2015).

Interesting is the direct comparison of fine root dynamics in the two coexisting congeners *Acer pseudoplatanus* and *A. platanoides,* which may reveal the development of different strategies of belowground resource foraging in closely related tree species. The two congeners showed marked morphological differences (more tips per root mass, a higher fine root surface area and thinner 2^{nd} and 4^{th} order root segments in *A. pseudoplatanus* than in *A. platanoides*) and a higher overall fine root biomass in A. *pseudoplatanus*. Thus, it appears that tree species with similar fine root diameters as in the Hainich mixed forest can achieve elevated resource uptake rates either through maintaining a large surface area of 1^{st}-order roots (*A. pseudoplatanus*) or by frequently turning over the existing fine root mass (*A. platanoides*) which should increase mean root uptake capacity by reducing mean root age (Eissenstat & Yanai 1997). In our species sample, species differences in root tip frequency (tips per root mass) were the most influential fine root morphological traits (Kubisch et al. 2015).

In tree species assemblages with higher phylogenetic diversity as in tropical or subtropical moist forests, fine root morphology often is more variable among different species than in the temperate mixed forest of our study. Under these conditions, nutrient foraging strategies may

largely depend on fine root diameter, with thin-root species often showing greater fine root growth rates, whereas thick-root species are apparently relying more on mycorrhizal fungi with respect to nutrient acquisition. Across 14 evergreen or deciduous broad-leaf or coniferous AM trees in subtropical China, Liu et al. (2015) found much larger fine root diameter variation (0.19 – 0.86 mm) than in our study (0.33 – 0.59 mm), which was associated with differences in root growth rate and the degree of AM colonization.

3.5.3 Aboveground – belowground linkages

While leaf lifespan was more or less similar among the six species (6-7 months), the lifespan of fine roots (averaged over all fine root mass < 2 mm) varied up to fivefold among the species (ca. 11 to 54 months) and up to threefold between the horizons within a species. This suggests that fine root and leaf lifespan are only poorly related to each other in this species sample, and fine root longevity is controlled by other factors than aboveground phenology. A similar conclusion was drawn by Withington et al. (2006) for their five-species broadleaf tree sample, which included four of our species. In a global literature survey, Finér et al. (2011) detected no significant influence of stand basal area or stem density on fine root turnover. We also found no relation between the species' aboveground woody biomass production and fine root productivity and root turnover, disproving our third hypothesis. However, across all species, wood production increased with mean fine root diameter and decreased with increased root tissue density. We speculate that, in a fertile soil with more or less stable nutrient-rich patches as in Hainich forest, species with thicker fine roots may achieve a greater nutrient return on resource investment in root mass than thin-root species; this could promote aboveground productivity.

3.5.4 Relative importance of 1st- and 2nd-order roots in fine root dynamics

The detailed root order-related biomass analysis showed that about 30-50% of total fine root biomass (< 2 mm) referred to the first and second orders which are assumed to conduct most of nutrient and water uptake (Guo et al. 2008a) and are termed absorptive roots (McCormack et al. 2015). These segments contain the fine root tips and the directly adjacent finest root segments with generally lowest degree of suberization. Assessment of the resource foraging strategies of the six species should therefore primarily consider the amount of carbon invested into the production of 1st- and 2nd-order roots. We multiplied total fine root production as measured in the ingrowth cores with the biomass fractions of the 1st to 4th root orders as found in the FRB inventory of Kubisch et al. (2015), assuming similar root longevity in the different

root orders. Extrapolating the biomass distribution key from the inventory to the ingrowth cores may be justified in our study, because we found similar branching patterns of the fine root strands in the inventory and the ingrowth core analysis for all six species (Figure 3.1). According to this very rough calculation, the species produced between 50 and 90 g root biomass per m^2 yr^{-1} in 0-30 cm soil depth in the 1st and 2nd root orders, which is much less than the global fine root production mean (337 g m^{-2} yr^{-1}, mean sampling depth 37 cm, total root biomass < 2 mm) given by Finér et al. (2011) for temperate forests. To our knowledge, there are only two other studies that attempted to quantify the root order-related root production on a plot level basis (Xia et al. 2010; *Fraxinus mandshurica*; Sun et al. 2011: *Fraxinus mandshurica* and *Larix gmelinii*, both roughly 25 year-old plantations). No other study seems to exist, where such an approach has been applied to mature (>100 year-old) forest stands. In these two East Asian tree plantations, much lower FRP values (42 and 27 g m^{-2} yr^{-1} in 0-10 cm soil depth (Xia et al., 2010) or 0-20 cm soil depth (Sun et al. 2011) were reported than in our mature stands, even though the same methodology (ingrowth cores) was used at least in the latter study. Given that temperate trees invest about 300 g m^{-2} yr^{-1} in leaf biomass, and coarse and large root production has its equivalent in twig and branch production, we draw the conclusion that temperate trees achieve nutrient and water uptake at lower cost for absorbing structures than is needed for carbon assimilation. Nevertheless, the total length of absorbing roots produced annually in the topsoil is enormous, exceeding 1 km per m^2 ground area in the 0-30 cm profile in the six species investigated here.

3.6 **Conclusions**

The co-existence of ectomycorrhizal and arbuscular mycorrhizal tree species in cool-temperate mixed forests raises the question about possible differences in belowground resource foraging strategies between these two tree groups. In our sample of six relatively wide-spread species, variation in root dynamics occurred mainly within the two groups and not between them, contradicting our main hypothesis. Investigation of a larger number of tree species might reveal significant group differences in fine root lifespan and root productivity, but there exist only few other AM tree species in Central Europe with wider distribution (e.g. *Acer campestre* and *Ulmus glabra*). Since many of the common species were included in our sample, our results and those of Kubisch et al. (2015) on root morphological differences suggest that species differences in fine root morphology, lifespan and growth rate in Central European broadleaved mixed forests are primarily determined by species identity, while the

influence of mycorrhiza type is only of secondary importance. Species differences manifested primarily in differences in root tip frequency, while variation in root diameter was of minor importance (Kubisch et al. 2015).

Possible differences between ECM and AM species in root morphology and turnover in many cases will be overlain by effects of environmental and stand structural variation (Finér et al. 2011, Leppalammi-Kujansuu et al. 2014). In correspondence, a global analysis of measured fine root turnover rates in forests (Finér et al. 2011) seems to suggest that temperature (or growing season length), and not mycorrhiza type, is a main determinant of tree fine root lifespan and fine root productivity, because mean turnover rate continuously increased from 0.77 to 1.21 and 1.44 yr^{-1} and FRP from 311 to 428 and 596 g m^{-2} yr^{-1} from boreal to temperate and tropical forests. Thus, a root productivity increase and root lifespan decrease is occurring with both biome transitions, i.e. from the boreal to the temperate forest and from the temperate to the tropical forest, even though a shift in predominant mycorrhiza type (from EM to AM) does occur only between temperate and tropical forests, but not between boreal and (cool) temperate forests. More data from species-rich mixed forests is needed to understand the influence of mycorrhiza type on tree fine root morphology, dynamics and functioning.

3.7 Acknowledgements

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3.8 **Appendix**

Table A 3.1: Fine root biomass (in g m^{-2}) in the six plot types at the beginning of the study in June 2011 (only fine root biomass of target species). Significant differences ($p < 0.05$) between the species within a soil depth are marked with different capital letters.

	Soil depth			
Species	0-10 cm	10-20 cm	20-30 cm	Profile
Fraxinus excelsior	113.0 ± 26.2 B	109.7 ± 51.2 AB	47.5 ± 15.7ABC	270.2 ± 17.5 AB
Acer pseudoplatanus	74.5 ± 12.4 AB	66.8 ± 17.8 A	53.1 ± 14.7 B	194.3 ± 8.5 AB
Acer platanoides	59.5 ± 21.1 AB	53.1 ± 11.8 A	29.1 ± 15.3 A	141.6 ± 9.5 A
Carpinus betulus	103.5 ± 29.0 AB	87.4 ± 17.1AB	55.9 ± 15.9 ABC	246.8 ± 12.6 AB
Tilia cordata	51.5 ± 25.1 A	93.3 ± 28.3 AB	73.1 ± 16.4 BC	217.9 ± 13.6 AB
Fagus sylvatica	55.2 ± 27.2 A	142.0 ± 27.8 B	103.4 ± 30.2 C	300.6 ± 17.4 B

Table A 3.2: Pearson correlation coefficients (r) for the relation between fine root turnover (unit: yr^{-1}) and five root traits based on species means. None of the relationships was significant at $p < 0.05$. FRP = fine root productivity, ABWP = aboveground woody biomass production.

	r	*p*
SRL	-0.29	0.29
SRA	0.18	0.37
RTD	-0.17	0.37
MD	-0.04	0.46
Root N	0.37	0.24
FRP	0.21	0.35
ABWP	0.50	0.16

Table A 3.3: Pearson correlation coefficients (r) and probability of error (p) for the relationship between aboveground woody biomass production (ABWP) and fine root traits in the sample of six species based on species means. Significant relations ($p < 0.05$) are printed in bold.

	r	*p*
SRL	-0.464	0.178
SRA	0.258	0.313
RTD	**-0.870**	**0.013**
MD	0.363	0,240
Root N	0.763	0.137

Table A 3.4: Pearson correlation coefficients (r) for the relation between aboveground woody biomass production (ABWP, in g m^{-2} yr^{-1}) and five root traits in the six species. None of the relationships was significant at $p < 0.05$; relationships with $0.05 < p < 0.01$ are marked with ° SRL= specific root length; SRA= specific root area; RTD= root tissue density; Root N= root nitrogen concentration.

	Species					
	Fraxinus excelsior	*Acer pseudoplatanus*	*Acer platanoides*	*Carpinus betulus*	*Tilia cordata*	*Fagus sylvatica*
SRL	-0.254	-0.223	-0.044	-0.269	-0.225	0.186
SRA	-0.247	-0.117	0.312	-0.133	-0.231	0.498
RTD	0.215	-0.021	-0.436	0.119	-0.028	-0.614
MD	0.160	0.429	0.268	0.456	0.377	0.451
Root N	0.088	0.021	-0.033	-0.519	-0.358	0.662°

Table A 3.5: Five morphological traits of the fine roots (bulk samples; all segments < 2 mm in diameter) of the five species in three different soil layers and averaged over the o-30 cm profile (means ± SE). RTD = root tissue density, MD = mean diameter in < 2 mm class, SRA = specific root surface area, SRL = specific root length, N – root = nitrogen concentration. Differences ($p < 0.05$) between the species in a soil layer are marked by different lower case letters, differences between the soil depths by capital letters. Species differences in the profile average are also indicated by different capital letters.

Trait	Depth (cm)	*F. excelsior*	*A. pseudoplatanus*	*A. platanoides*	*C. betulus*	*T. cordata*	*F. sylvatica*
RTD (g m^{-3})	0-10	0.341 ± 0.054 bA	0.454 ± 0.038 abA	0.556 ± 0.051 acA	0.493 ± 0.018 aA	0.606 ± 0.058 cA	0.620 ± 0.134aA
	10-20	0.371 ± 0.033 cA	0.509 ± 0.057 abAB	0.493 ± 0.015 aA	0.514 ± 0.031 abA	0.563 ± 0.067 bA	0.487 ± 0.041 aA
	20-30	0.378 ± 0.047 cA	0.540 ± 0.031 abB	0.462 ± 0.099 abcA	0.460 ± 0.026 aA	0.478 ± 0.053 abcA	0.536 ± 0.037 bA
	Profile aver.	**0.356 ± 0.040 B**	**0.501 ± 0.029 A**	**0.491 ± 0.037 A**	**0.494 ± 0.014 A**	**0.558 ± 0.033 A**	**0.530 ± 0.044 A**
MD (mm)	0-10	0.482±0.029 cA	0.317±0.006 bA	0.436 ± 0.070 acA	0.374±0.023 aA	0.468±0.056 acA	0.411±0.103 abcA
	10-20	0.524±0.054 cA	0.318±0.026 bA	0.394 ± 0.019 aA	0.363±0.021 abA	0.567±0.073 cA	0.460±0.053 acA
	20-30	0.587±0.070 bA	0.363±0.034 aA	0.476 ± 0.105 abA	0.511±0.082 abA	0.652±0.093 bA	0.459±0.087 aA
	Profile aver.	**0.526 ± 0.042 C**	**0.333 ± 0.018 A**	**0.416 ± 0.027 B**	**0.408 ± 0.025 B**	**0.563 ± 0.056 C**	**0.459 ± 0.067 B**
SRA (cm^2 g^{-1})	0-10	182.993 ± 13.262 cA	227.135 ±8.627 bA	150.302 ±8.601 aA	180.432 ± 28.027 abA	117.973 ± 12.228 cA	191.089 ± 62.552 abA
	10-20	154.241 ± 20.154 abA	245.491 ± 76.058 abcAB	176.058 ± 20.949 aA	160.680 ± 0.852 abA	103.731 ± 35.174 cA	125.755 ± 17.214 bcA
	20-30	159.455 ± 20.201 aA	163.301 ± 19.398 aB	233.232 ± 72.350 abA	126.169 ± 22.632 abA	107.379 ± 18.400 bA	121.628 ± 19.472 abA
	Profile aver.	**169.339 ± 13.380 A**	**211.976 ± 32.309 A**	**194.024 ± 21.864 AB**	**160.760 ± 16.324 B**	**104.589 ± 14.621 C**	**138.094 ± 19.844 AC**
SRL (m g^{-1}]	0-10	13.536 ± 1.291 aA	28.038 ± 2.653 bA	15.313 ± 1.922 aA	21.994 ± 5.411 abA	10.911 ± 2.027 aA	29.240 ± 12.629 abA
	10-20	10.921 ± 1.819 bcA	34.769 ± 13.013 aAB	18.612 ± 3.290 abcA	17.615 ± 3.154 abA	7.455 ± 3.408 cA	12.552 ± 2.656 bA
	20-30	10.894 ± 2.130 bcA	19.472 ± 3.310 aB	22.831 ± 7.610 abcA	11.587 ± 3.509 abcA	7.436 ± 1.943cA	12.797 ± 2.843 abA
	Profile aver.	**12.104 ± 1.276 C**	**27.426 ± 5.551 A**	**20.088 ± 2.771 AB**	**18.064 ± 2.942 B**	**8.164 ± 1.727 D**	**16.018 ± 3.410 A**
N (mg g^{-1})	0-10	14.267 ± 0.682 cA	11.635 ± 0.551 bA	9.902 ± 0.621 aA	12.196 ± 1.122 abcA	9.953 ± 0.805 abA	10.946 ± 0.943 abA
	10-20	13.440 ± 0.681 cA	9.519 ± 0.522 aB	8.892 ± 0.480 aA	11.362 ± 0.837 bcAB	7.923 ± 0.349 dB	10.315 ± 0.853 abA
	20-30	13.344 ± 0.600 bA	10.076 ± 0.841 aAB	10.469 ± 0.982 aA	9.191 ± 0.817 aB	8.988 ± 0.322aA	9.102 ± 1.125 aA
	Profile aver.	**13.646 ± 0.460 C**	**10.204 ± 0.493 AB**	**9.655 ± 0.311 AB**	**11.041 ± 0.900 A**	**9.263 ± 0.537 B**	**10.107 ± 0.732 AB**

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CHAPTER 4

Is fine root abundance and dynamics of Stone pine (*Pinus cembra*) at the alpine treeline impaired by self-shading?

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4.1 Abstract

Low temperatures are crucial for the formation of the alpine treeline worldwide. Since soil temperature in the shade of tree canopies is lower than in open sites, it was assumed that self-shading may impair the trees' root growth performance.

While experiments with tree saplings demonstrate root growth impairment at soil temperatures below 5-7 °C, field studies exploring the soil temperature – root growth relationship at the treeline are missing. We recorded soil temperature and fine root abundance and dynamics in shaded and sun-exposed areas under canopies of isolated Pinus cembra trees at the alpine treeline.

In contrast to the mentioned assumption, we found more fine root biomass and higher fine root growth in colder than in warmer soil areas. Moreover, colder areas showed higher fine root turnover and thus lower root lifespan than warmer places.

We conclude that Pinus cembra balances enhanced fine root mortality in cold soils with higher fine root activity and by maintaining higher fine root biomass, most likely as a response to shortage in soil resource supply.

The results from our study highlight the importance of in situ measurements on mature trees to understand the fine root response and carbon allocation pattern to the thermal growth conditions at the alpine treeline.

Key words: Austrian Alps, ectomycorrhiza, fine root biomass, fine root mortality, fine root morphology, fine root turnover, soil temperature

4.2 Introduction

The alpine treeline is one of the most conspicuous vegetation boundaries on earth, which has been studied by ecologists and geographers for decades. However, the causes for the formation of this steep ecotone are still intensively debated. The majority of scientists concerned with the topic may agree that adverse thermal conditions at (and above) the alpine treeline ecotone are crucial for explaining treeline formation. While a 'classic' explanation assumes that insufficient photosynthetic carbon gain is limiting tree growth at the treeline (‘carbon-source limitation hypothesis’; see reviews by Troll 1973, Tranquillini 1979, Stevens & Fox 1991, Körner 1998, 2012a, Holtmeier 2009), it has been proposed more recently that cold temperatures directly are limiting metabolic activity and hence cell division and cell growth in meristematic tissues (referred to as 'carbon-sink limitation hypothesis'; Körner 1998, 2012a, see also Hoch et al. 2002, Hoch & Körner 2003). Microclimatic measurements at various tree lines indicate that the elevational position of the alpine treeline apparently is more closely related to soil than to air temperature (e.g. Sveinbjörnsson 2000, Körner & Hoch 2006, Körner 2012a). In a global survey of soil temperature data from treeline habitats, Körner & Paulsen (2004) found the treeline position to coincide with a 6.7 °C growing season mean in 10 cm soil depth. According to Körner (2012a), this correlation does not necessarily show a more prominent soil temperature than air temperature effect on tree growth, but may simply reflect lower diurnal and seasonal variation in soil than air temperature at the treeline (Körner & Paulson 2004). Moreover, the apparently critical temperature of ~ 6.7 °C experienced by treeline trees may not directly relate to a low temperature threshold of biological processes at this temperature (Körner 1998, Körner 2012a).

For most processes of carbon turnover, the temperature dependence of metabolic activity in treeline trees is not precisely known. If empirical data are available, they often were obtained from sapling studies in greenhouses, such as the experiments on low-temperature limits of root growth in temperate tree species conducted by Alvarez-Uria & Körner (2007), Hoch & Körner (2009), Körner (2012b) and Schenker et al. (2014). Accordingly, the root growth of tree saplings is greatly reduced below ~ 5 °C, which may represent a signal to slow down shoot growth (see literature review in Körner 2003). These findings were applied to the treeline question by postulating that self-shading by a closed canopy in the treeline forest impairs the aboveground and belowground growth of the trees more than under isolated tree individuals above the tree line, where more light penetrates to the ground and soil temperatures may be more favorable (Körner 1998, Li et al. 2003, Körner 2012b). The self-

shading hypothesis predicts that the upper limit of closed forest is partly a consequence of an unfavorable soil thermal regime caused by the trees themselves. However, convincing evidence from field studies in support of this hypothesis does not yet exist.

In apparent contrast to the experimental results on root growth impairment at low temperatures in tree saplings, findings from field studies in mature forests along elevation transects show a pronounced increase in fine root biomass with decreasing temperature towards the alpine treeline (Kitayama & Aiba 2002, Leuschner et al. 2007, Hertel & Wesche 2008, Hertel et al. 2008, Hertel & Schöling 2011a). Furthermore, several authors detected no root productivity differences, or even higher fine root growth rates, at the treeline compared to stands at lower elevation (Graefe et al. 2008, Hertel & Schöling 2011b, Mao et al. 2013). This apparent contradiction calls for a detailed study of root zone temperatures and tree root growth at the alpine treeline.

We examined the temperature dependence of fine root biomass and fine root productivity in isolated Stone pine trees (Pinus cembra L.) in the alpine treeline ecotone of the Alps by synchronously mapping soil temperature and fine root biomass at high resolution in six plots and comparing fine root productivity between sunny and shaded soil patches in the surroundings of 12 trees. Study aim was to search for evidence in support of the postulated self-shading effect of tree crowns on the soil thermal regime and related negative effects on root biomass and productivity in the treeline ecotone. We conducted root coring for fine root biomass at 216 locations with variable irradiance and soil thermal conditions and measured root productivity and fine root turnover with the ingrowth core technique at 24 sunny or shaded locations.

With reference to the assumptions made by Körner (1998, 2012b), we tested the hypotheses that a) tree fine root biomass in sunny and shaded patches of the treeline ecotone is more closely related to the soil thermal regime developed in the afternoon than in the morning hours, b) tree fine root biomass density is lower in shaded, cooler patches under the canopy than in sunny, warmer areas, and c) tree fine root productivity is considerably lower in shaded, cooler patches under the canopy than in sunny, warmer areas. The study was conducted in mid-summer, when thermal differences between sunny and shady areas in the treeline ecotone should be greatest.

4.3 Materials and Methods

4.3.1 Study site

The study was conducted in the treeline ecotone of the central Eastern Alps (Stubai Alps, Tyrol, Austria) with isolated Stone pine trees (Pinus cembra L.) representing the uppermost occurrence of trees. The study site belongs to a large former afforestation area on the 'Haggener Sonnberg' (47°12'42" N, 11°5'04" E) north of the upper Sellrain valley near St. Sigmund on a south-facing slope (25 °) at ca. 2025 m a.s.l.. In the 1970s, this area was afforested with native Stone pine to provide protection against avalanches. It started as a pilot project at lower elevation and was continued in 1980 up to the treeline elevation, where the study site is located. Prior to afforestation, the south-facing slopes had been clear-cut and used for grazing by cattle and sheep for centuries. The pine trees in our study therefore all had roughly the same age (ca. 30 years) and similar aboveground stature. The soil of the study site is an oligotrophic podzolic leptosol (WRB classification) originating from gneiss and micaschist bedrock (Kronfuss & Havranek 1999). The sandy-loamy mineral soil is covered by a humus layer of ca. 5 cm thickness (Wieser et al. 2015).

Mean annual air temperature at a nearby weather station at 1800 m a.s.l. (Kronfuss 1997) was 3.2 °C in the years 1975-1994 with February being the coldest month (mean temperature –3.5 °C) and July the warmest (10.7 °C). The mean annual precipitation is 909 mm with highest precipitation occurring from May to October (610 mm). During the growing season (May - October) in the study year 2012, daily mean air temperature averaged 9.2 °C and varied between –4.2 °C on May 16th and 18.4 °C on August 20th; during this period, 889 mm of precipitation was recorded (Wieser et al. 2015).

4.3.2 Study design and soil temperature measurements

For our investigation, we selected six Stone pine tree individuals which were representative for the whole afforestation site in terms of aboveground stature, slope position and soil conditions. The six trees were between 2.8 (plot 2) and 4.2 m (plot 3) high with a stem diameter at breast height (130 cm) of 5.5 to 7.5 cm (Table 4.1). For each tree, we established a measuring and sampling plot of 2 m x 2 m size with the stem in the center of the plot. Each plot contained a systematic grid of 6 x 6 = 36 points (with 40 cm distance between two points) where soil temperature measurements and soil sampling for the quantitative analysis of living and dead Pinus fine roots were conducted (Figure 4.1).

A spatially detailed mapping of soil temperatures under the canopy of the six Pinus tree plots was conducted on August 1, 2012, a completely cloudless day in the summer period of the year, when most pronounced thermal differences between sunny and shaded soil areas can be expected. Soil temperature was measured at 10 cm depth with a mobile thermometer at the each 36 grid points of the six study plots. Measurements were conducted in three periods of the day: The first measurement campaign was conducted at dawn (7:26 solar time at the 1st plot until 8:48 in the 6th plot) reflecting the soil conditions after maximum night cooling; the second campaign took place at noon (11:14 solar time in the 1st plot until 12:33 in the 6th plot), i.e. the period of highest solar radiation input, and the third campaign was conducted in the early afternoon between 14:42 solar time in the 1st plot until 16:00 in the 6th plot, representing the period of the daily maximum thermal differences in 10 cm soil depth through surface warming. All measurements were taken manually with the same soil thermometer (DET3R penetration thermometer, Voltcraft, Germany) at each grid point in ascending plot order (starting with plot 1 and continuing to plot 6) until a constant temperature reading was achieved at the respective location.

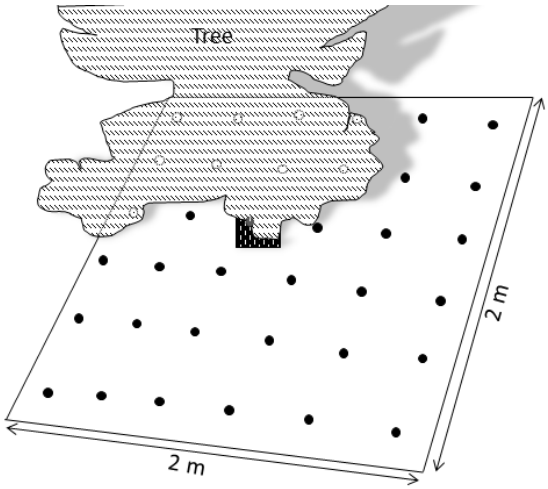

Figure 4.1: Sampling grid at the slope of our study area. The dots illustrate the soil sampling points around the tree individuals.

A continuous long-term recording of soil temperature in 10 cm depth in shaded area and sunny areas of Pinus individuals was done under 12 trees in the vicinity of the six plots mentioned above that were selected to investigate fine root dynamics (see below).For our investigation, we selected six Stone pine tree individuals representative for the whole afforestation site in terms of aboveground stature, site slope and soil conditions. All six trees were between 2.8 (plot 2) and 4.2 meters (plot 3) high with a stem diameter at breast height (130 cm) of 5.5 cm to 7.5 cm (Table 4.1).

Table 4.1: Plot descriptions showing mean diameter at breast height (dbh), tree height and exposition (aspect).

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6
Dbh (cm)	7	5.5	7	7.5	6	6
Tree height (m)	3.6	2.8	4.2	3.3	3.2	3.0
Exposition	south	south	south	south	south	south

4.3.3 Analysis of living and dead Pinus fine root mass

Soil samples for root abundance analysis were taken in 0-10 cm depth at the same grid points on the day after measuring soil temperature using a steel corer of 35 mm in diameter. The soil samples were stored in plastic bags at 4 °C until further processing within six weeks. The fine roots (roots <2 mm in diameter) were washed from the soil by rinsing the soil sample over a sieve with 0.25 mm mesh size. All fine roots longer than 10 mm were picked out with a pair of tweezers and root segments of Pinus cembra were separated under the stereomicroscope from other species (e.g. *Calluna vulgaris*, *Rhododendron ferrugineum*, *Arctostaphylos uva-ursi* and herbaceous species) based on morphological characteristics. In this study, only *Pinus* fine roots were considered. The fine roots were sorted into biomass (living) and necromass (dead) under a stereomicroscope (6-40 x magnification) using the criteria root turgescence, the constitution of periderm and stele as well as elasticity of the stele (Hertel & Leuschner 2002, Hertel et al. 2013). All living fine roots of Pinus were scanned under water using an EPSON expression 1680 scanner (EPSON America Inc.), and a morphological analysis was conducted using WinRhizo 2005c software (Regent Instruments Inc. Quebéc, Canada) to determine average fine root diameter, fine root length, surface area and root tissue density. Living and dead fine root mass was weighed after drying the material at 70 °C for 48 hours to constant weight. For a subsample of living root strands from sampling points that were assigned to the 25 % coldest or 25 % warmest locations in the plots, the number of root tips and their ectomycorrhizal colonization status were analyzed under the stereomicroscope.

4.3.4 Analysis of annual fine root production and turnover in their dependence on soil temperature

For measuring annual fine root productivity in the study area, we selected 12 Pinus cembra trees adjacent to the six grid-sampling plots described above and installed ingrowth cores (24 in total) in sunny patches (ca. 40 cm south of the tree stem) and shaded patches (ca. 40 cm north of the same stem). In comparison with other techniques, the ingrowth core approach has

been found to deliver relatively conservative fine root production estimates in temperate forests (e.g. Hertel & Leuschner 2002, Hendricks et al. 2006, Finér et al. 2011a). Cores of 35 mm diameter and 10 cm length (which covered the mineral topsoil and the organic layer) were installed in April 2011 and re-sampled after 24 months in April 2013. For installing the ingrowth cores, the upper 10 cm of the soil was extracted with the soil corer. Bulk soil of the same soil depth from adjacent places was cleaned by hand from all macroscopically visible live and dead rootlets in the field, and the soil material was subsequently refilled into the hole by conserving the natural sequence of horizons. Care was taken to maintain the natural structure and density of the soil as accurately as possible. The exact position of each ingrowth core was marked with 3 plastic sticks to allow re-sampling. Field studies in temperate forests have shown that tree fine roots typically start recolonizing root-free soil after a time lag of about one year (Hertel & Leuschner 2002, Meinen et al. 2009, Hertel et al. 2013). We therefore assumed that the ingrowth period started in spring 2012. In April 2013, the cores were re-sampled, taken to the laboratory and processed as described above. Annual fine root production (in g m-2 yr^{-1}) was estimated by relating the total mass of living and dead Pinus roots in the ingrowth core samples to one year and to one square meter ground area.

To calculate fine root turnover (and its inverse, mean fine root lifespan), we analyzed the mass of living and dead Pinus fine roots in the soil samples that were initially extracted from the ingrowth core holes, applying the same procedure as described above. Fine root turnover was calculated by dividing annual fine root production in the ingrowth cores by the initial fine root biomass at the ingrowth core locations.

To compare the soil temperature of the sunny and shaded ingrowth core locations (sunny: ca. 40 cm south of the tree stem, i.e. downslope; shaded: ca. 40 cm north of the same tree stem, i.e. upslope), miniature temperature sensors and data loggers ('iButtons', Maxim Itgr., San Jose, USA) were installed in direct vicinity of each ingrowth core in 10 cm soil depth to record soil temperature continuously in bi-hourly intervals over the whole ingrowth period.

4.3.5 Statistical data analysis

All data sets were tested for normal distribution using a Shapiro-Wilk test. Since in most cases normal distribution was not given, we applied a one-way Kruskal-Wallis single factor analysis of variance and, subsequently, a non-parametric Mann-Whitney two-sample test (U test) to locate significant differences. A significance level of $p < 0.05$ was used in all statistical tests. To analyze the shading effect under the isolated Pinus trees on soil temperature and fine root abundance in the six plots, we divided the 36 grid points in each

plot into four temperature categories (#1 to #4) which represented the four quartiles (n=9 temperature values per quartile and plot) of the 36 ranked temperature values measured in each plot; category #1 represented the 9 grid points with the lowest recorded temperature values, category 2 those with the 9 next-higher temperature values, category #3 the 9 points the second-highest values, and category #4 contained the 9 grid points with the highest soil temperatures.

The distribution of soil temperatures in the plots during the third (afternoon) measurement campaign was visualized in maps by interpolating between the data points using Xact8 software (SciLab, Hamburg, Germany). A regression analysis for the dependence of fine root biomass and necromass on soil temperature was conducted using the same software. Temperature effects on fine root biomass, and cumulative fine root length and area per unit soil volume were tested with a correlation analysis using the PROC CORR routine in SAS 9.3 software. In the correlation analyses, either absolute soil temperatures or standardized temperature values in form of the relative deviation of temperature values at each grid point from the temperature maximum recorded in a plot and measurement period were used. Relative soil temperature values were calculated in order to compare the thermal variation in the plots independently of absolute deviations in the temperature regime of the six plots.

4.4 **Results**

4.4.1 Soil temperature patterns under the Pinus *crowns*

On the cloudless day August 1, 2012, temperature mapping showed a soil temperature average of 10.1 °C for the dawn measuring period (07:26 - 08:48 solar time) and of 13.0 °C for the afternoon period (14:42 - 16:00 solar time) in the 6 study plots (6 x 36 measuring points) around the *Pinus cembra* trees (Table 4.2).

Table 4.2: Temperature regime (°C) at 10 cm soil depth in the six study plots during the measuring period between 07:26 to 16:00 solar time on the cloudless measuring day 1st of August 2012. Values are given ± SE for all 36 grid points of the six study plots.

Time	Mean	Mean minimum	Mean maximum	Absolute minimum	Absolute maximum
Dawn	10.1±0.1	9.0±0.1	11.0±0.2	8.6	11.4
Noon	11.0±0.1	9.8±0.1	13.3±0.6	9.6	16.1
Afternoon	13.0±0.2	10.6±0.1	17.5±1.0	10.3	20.4

The absolute recorded maximum temperature was 20.4 °C during the afternoon measurement, the absolute minimum temperature 8.6 °C during the dawn measurement. While the mean minimum soil temperature varied only slightly over the day in the shaded areas (9.0 - 10.6 °C), patches that were warmed by direct solar irradiance showed a much larger variability of mean maximum soil temperature (11.0 - 17.5 °C; Table 4.2). Correspondingly, the diurnal variation in absolute minimum temperatures was low (ranging between 8.6 °C at dawn to 10.3 °C in the afternoon), while the absolute maxima varied from 11.4 °C (dawn) to 20.4 °C (afternoon) (Table 4.2, Figure A 4.1, Appendix). Categorizing the measuring points by means of to their median temperature into the four quartiles produced the temperature categories #1 to #4 which differed significantly from each other (Figure 4.2).

Continuous soil temperature measurement in 10 cm depth in July and August 2012 in the sunny and shaded patches adjacent to the 12 *Pinus* trees of the ingrowth core study revealed a by 1.8 K higher mean afternoon temperature in the sunny plots (difference significant; Figure 4.3).

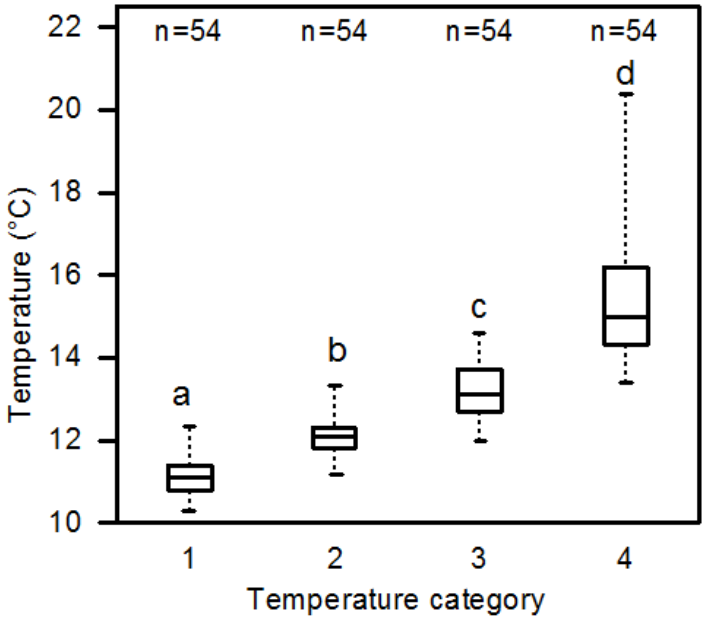

Figure 4.2: Median temperatures of the four temperature categories of the afternoon measurement representing the 4 quartiles of 36 soil temperature measurements in each of the six *Pinus* plots. Values increased significantly for each category ($p < 0.05$; Mann Whitney U-test).

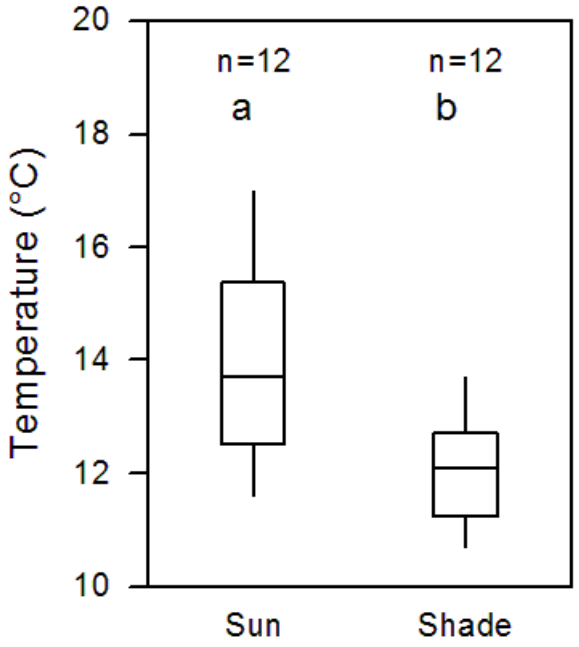

Figure 4.3: Temperature differences between the shaded (north of the stem) and more sunny (south of the stem) side under 12 tree canopy of Pinus cembra trees representing median and four quartiles of the afternoon temperatures in July and August 2012 (14:00-16:00 solar time) in 10 cm soil depth. Values are significantly different ($p < 0.001$; Mann Whitney U-test).

4.4.2 Fine root morphological traits

For most traits, the fine root morphology of *Pinus cembra* did not differ between warmer and colder soil patches. Mean root diameter was almost identical in soil samples of the four temperature categories, while specific root length (SRL), specific root surface area (SRA) and root tissue density (RTD) showed only moderate variation across this thermal gradient (Figure 4.3). Similarly, *Pinus* root samples from the coldest (#1) and warmest temperature categories (#4) did not differ in the degree of ectomycorrhizal colonization of root tips (84 vs. 82 %; 216 samples analyzed in total; data not shown). However, the fine roots in the coldest plots had more than twice the number of root tips per sample as compared to the roots of the warmest plots (categories #1 and 4: 24 and 11 tips per sample; $p < 0.01$).

Table 4.3: Mean diameter, specific root length (SRL), specific root surface area (SRA), and root tissue density (RTD) of fine roots of the four different soil temperature categories (n=6 plots); category #1 represents the coldest soil areas, category #4 the warmest ones. In total, 216 root samples were analyzed. No statistically significant differences between the four temperature categories regarding the four morphological root traits were detected ($P < 0.05$).

Category	Mean diameter (mm)	SRL (m g^{-1})	SRA (cm^2 g^{-1})	RTD (g cm^{-3})
1	0.612 ± 0.016	11.632 ± 0.328	210.4 ± 6.5	0.34 ± 0.022
2	0.635 ± 0.012	10.013 ± 0.853	187.9 ± 15.2	0.53 ± 0.169
3	0.629 ± 0.025	11.942 ± 1.008	211.1 ± 12.3	0.36 ± 0.021
4	0.645 ± 0.019	11.458 ± 0.874	212.1 ± 14.4	0.38 ± 0.049

4.4.3 Standing fine root biomass and necromass

Fine root biomass (or cumulative fine root length and surface area) in the plots showed a significant negative relation to soil temperature (absolute values or deviation from the absolute maximum per plot) in case of the afternoon temperature measurement. This relationship was less clear for the noon temperature data (only significant for root length and area but not for biomass) and disappeared for the temperature measurements at dawn (Table 4.4).

Table 4.4: Correlation between absolute soil temperature or normalized temperature (i.e. the relative deviation at each grid point from soil temperature maximum) per plot and measurement period and fine root biomass, total fine root length and surface area per unit soil volume at the three different time periods of the day. Pearson correlation coefficients (r) and significances (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) are shown.

Time	Variable	Absolute temperature Correlation coefficient	Normalized temperature Correlation coefficient
Dawn	Biomass (g L^{-1})	-0.003	-0.071
	Length (m L^{-1})	-0.029	-0.036
	Surface area (m^2 L^{-1})	-0.031	-0.049
Noon	Biomass (g L^{-1})	-0.102	-0.109
	Length (m L^{-1})	-0.146*	-0.194**
	Surface area (m^2 L^{-1})	-0.139*	-0.206**
Afternoon	Biomass (g L^{-1})	-0.164*	-0.297***
	Length (m L^{-1})	-0.178**	-0.327***
	Surface area (m^2 L^{-1})	-0.159*	-0.308***

Similarly, when the plots were assigned to the four temperature classes, linear or non-linear negative relationships between fine root biomass (or cumulative fine root length or surface area) and median afternoon temperature in the temperature classes #1 to #4 were detected in the range from 11.0 to 15.5 °C (Figure 4.4A-C). A similar relation existed for fine root necromass. These findings match the observation that both fine root biomass and necromass were significantly lower in the southeastern quarters of the plots with highest solar irradiance as compared to the northeastern and northwestern quarters, where shading by the tree canopy was most effective (data not shown).

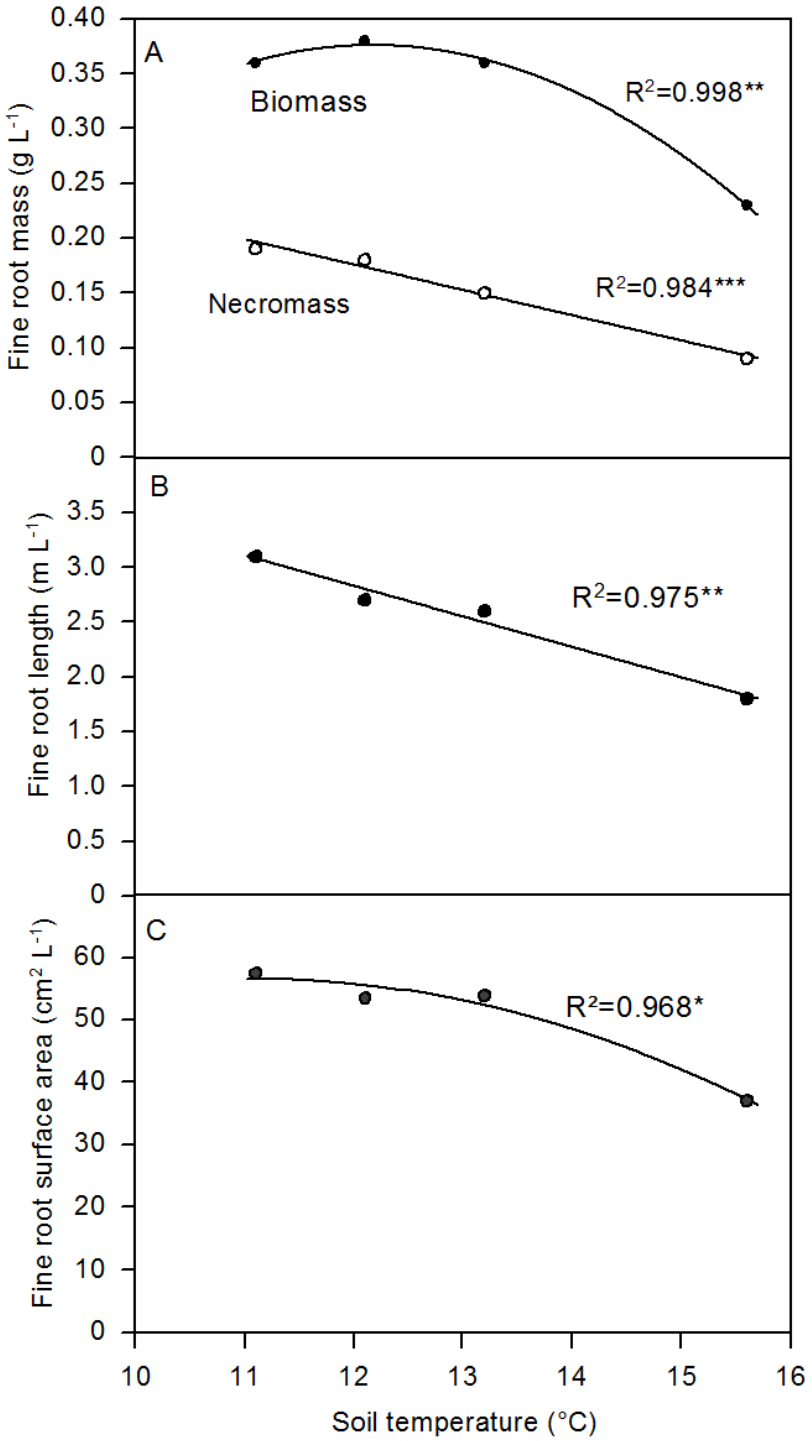

Figure 4.4: Dependence of mean fine root biomass and necromass density of Pinus cembra from mean soil temperature of the four temperature categories 1 4 representing the quartiles of soil temperature values measured in the six plots at the early afternoon measurement; *p< 0.05, **p< 0.01.

4.4.4 Fine root growth and turnover

Annual fine root growth into the ingrowth cores that were placed in vicinity of the 12 isolated *Pinus cembra* trees outside of the grid-sampling plots, was ~50 % higher in the shaded areas under the tree canopies than in the sunny areas, but this difference was only significant at a

marginal probability level (Figure 4.5A). An even larger and significant difference was found for fine root turnover: in shaded patches, mean turnover was 2.1 yr^{-1} while it was 0.5 yr^{-1} in the sunny areas ($p < 0.05$) (Figure 4.5B). These figures are equivalent to a mean fine root lifespan < 0.5 yr in the shaded areas and of ~ 2 yr in the sunny areas.

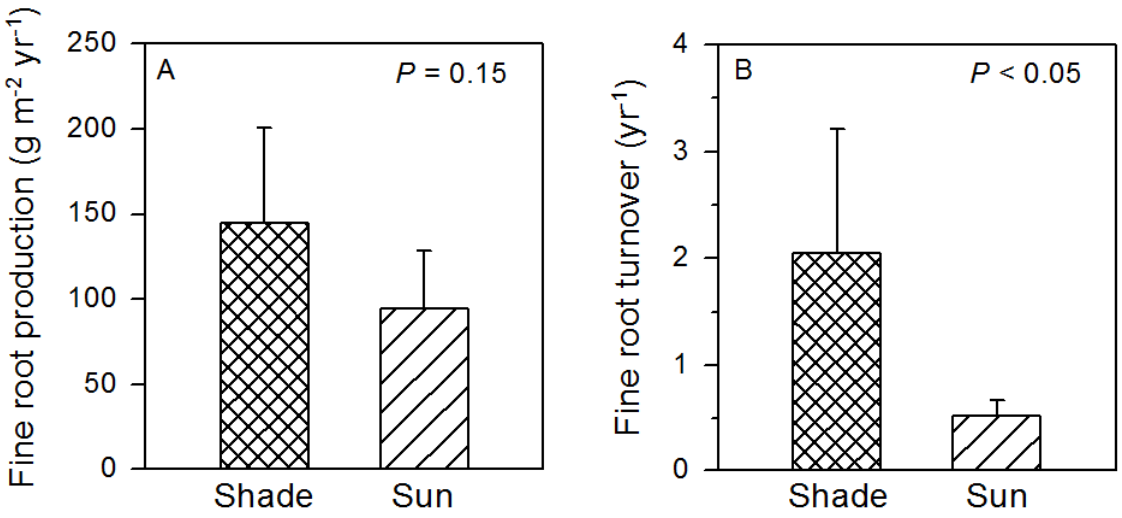

Figure 4.5: Annual fine root production and turnover in the shaded area (i.e. ca. 40 cm upslope of the tree stem in a northerly direction) and in sunny area (ca. 40 cm downslope of the tree stem in a southerly direction) of 12 Pinus cembra trees in the vicinity of the six study plots in the year 2012. The figure shows means + SE. The difference is statistically significant at p = 0.15.

4.5 Discussion

The results confirm our first hypothesis on the biological significance of the afternoon temperature regime, but they do not support the second and third hypothesis concerning the temperature influence on fine root biomass and productivity. The size of the soil temperature differences between shaded and sunny patches in the plots is displayed by the temperature maps produced on August 1, and it is also evident from the long-term soil temperature measurements in July/August 2012 which show a nearly 2 K warmer soil in mid-summer in sunny plot areas. As postulated, the soil temperature regime recorded at dawn in the differently illuminated patches around the *Pinus cembra* trees did not explain the observed variation in fine root density or fine root length and surface area per soil volume in the six plots. The explained proportion of root biomass variation increased over the day with increasing heating of the sun-exposed areas in the plots, i.e. with growing soil temperature differences between shaded and sunny areas. Variation in fine root biomass density and fine root length and surface area per soil volume between the grid points is thus best explained by the afternoon temperature measurements.

More surprising is the finding that fine root biomass (and fine root length and surface area per soil volume) was not higher, but significantly lower, in plot areas with higher soil temperatures as compared to colder soil patches. In fact, the highest standing fine root biomass was found in the coldest, most shaded areas under the trees. These temperature differences also seem to affect root morphology. While most examined root traits and the degree of ectomycorrhizal colonization did not vary across locations differing in soil temperature, we found a significantly higher fine root tip frequency on the roots of colder patches: fine root strands from the 25 % coldest sampling points had more than two times higher root tip frequencies than roots from the 25 % warmest soil locations. It appears that *Pinus* roots in particularly cold soil zones tend to increase their nutrient uptake capacity by forming more root tips which are thought to be the root segments with highest uptake activity. Equally important is our result that fine root productivity as estimated by the ingrowth core experiment was stimulated by colder temperatures in summer. Cores installed in the shaded, colder patches north of the pine trees showed by 50 % higher fine root growth than cores placed in more sunny areas south of the stems, contradicting our third hypothesis. This finding is important with respect to the assumption that self-shading is reducing tree productivity at the alpine treeline.

These results clearly point to a higher carbon allocation of *Pinus* to the fine root system in shaded, colder soil areas than in less shaded, warmer patches. This finding contrasts with the assumption that colder temperatures under the crown of single trees or under the canopy of closed stands in the treeline ecotone would impair root growth activity and result in lower fine root biomass, as it has been concluded from ex-situ experiments with tree saplings (Häsler *et al.* 1999, Alvarez-Uria & Körner 2007, Hoch & Körner 2009, Schenker *et al.* 2014). Our findings are matching earlier observations that stand-level fine root biomass is often high at the alpine treeline compared to lower elevation stands (e.g. Helmisaari *et al.* 2007, Leuschner *et al.* 2007, Hertel & Wesche 2008, Hertel *et al.* 2008, Hertel & Schöling 2011a). Our results further confirm reports that fine root turnover is relatively high at the low soil temperatures which prevail at the alpine treeline (Graefe *et al.* 2008, Moser *et al.* 2011, Hertel & Schöling 2011b, Mao *et al.* 2013). As our results were obtained from isolated trees, it is confirmed that the relatively high root biomass in the cold soil zones must be caused by the low soil temperatures or a factor related to them rather than being a consequence of high stem densities which typically increase in mountain forests towards the alpine treeline (e.g. Hertel & Wesche 2008).

While it is well established that herbaceous plants increase their root :shoot biomass ratio with increasing elevation toward the alpine treeline (e.g. Körner & Renhardt 1987), elevational patterns in belowground/aboveground biomass partitioning are barely understood in adult trees (Körner 2012a). Our findings of a higher fine root biomass in shaded, colder patches at the treeline would be plausible, if trees were responding in a similar manner to reduced temperatures at higher elevations as herbs. To close the knowledge gap on C partitioning patterns of mountain forest trees, we compiled own, mostly unpublished data from four elevation transect studies on the fine root biomass of mature trees in temperate and subtropical mountains (

Table **4.5**). Apart from the well-known phenomenon that tree density generally increases towards the alpine treeline, while tree height and aboveground tree biomass decrease, comparison of montane and treeline forests shows that the amount of fine root biomass per tree either remains constant (Mt. Brocken) or slightly decreases with elevation (Mt. Ventoux and Patagonian sites), reflecting the marked decrease in aboveground tree biomass with elevation. Physiologically more important is the result that the fine root-to-aboveground biomass ratio is much higher at the alpine treeline than in the montane forests. In the four transects, this ratio was 2 to 2.7 fold (Mt. Brocken, Mt. Ventoux, Mt. Tronador) or even 11 times higher at the treeline (El Chalten). The fine root-to-aboveground biomass ratios measured are also impressive in absolute terms: While fine root biomass typically represents only a few percent of total tree biomass in mature trees (e.g. Vogt *et al.* 1996, Finér *et al.* 2011b), this component accounts for about 10-15 % of total biomass in the treeline stands of Table **4.5** (an exception is Mt. Ventoux with only ca. 1 %). These results confirm that trees at the treeline in temperate and subtropical mountains invest heavily in the root system, which is difficult to explain by the carbon sink limitation hypothesis.

The published information on the temperature dependence of tree root growth activity and the buildup of fine root biomass at treeline sites contains partly conflicting evidence. On the one hand, *ex-situ* experiments with tree saplings or young trees in laboratory or garden environments in most cases revealed a linear (or non-linear) decrease of root growth rate with decreasing soil temperature, or they indicated low-temperature thresholds of root growth between 2 and ~7 °C (Bilan 1967, Lopushinsky & Max 1990, Häsler *et al.* 1999, Lahti *et al.* 2005, Alvarez-Uria & Körner 2007, Hoch & Körner 2009, Schenker *et al.* 2014). On the other hand, field studies of adult trees and forest stands indicated much more dynamic fine root growth in cold soils near the treeline than would be expected from the seedling or sapling experiments mentioned above. For example, Benecke *et al.* (1978) found that *Pinus contorta*

and *Nothofagus solandri* at the treeline in New Zealand maintained notable fine root growth activity for at least 9 months of the year, although the conventionally defined growing season is much shorter. For *Picea abies* near the treeline in the European Alps, Sandhage-Hofmann & Zech (1993) observed large seasonal changes in fine root biomass and necromass (2.5 fold variation during 1.2 months) indicating remarkable fine root dynamics despite unfavourable cold growing conditions. Similarly, Hertel & Schöling (2011b) found in ingrowth cores relatively high fine root productivity in a Norway spruce stand at the alpine treeline on Mt. Brocken (Central Germany) with a growing season soil temperature of 6.7 °C and a mean annual soil temperature of 3.8 °C. Based on direct observation in rhizoskopes, Mao *et al.* (2013) reported notable fine root elongation of *Abies alba* and *Picea abies* trees near the treeline in the French Alps at very cold soil temperature conditions. Using a minirhizotron approach, Sullivan *et al.* (2015) recorded fine root growth activity of adult *Picea glauca* trees even at soil temperatures of ca. 2.0 °C; these authors found no difference in annual fine root productivity between two treeline forest stands in Alaska with mean growing season soil temperatures of 8.9 and 4.9 °C. Using minirhizotrons, Gaul *et al.* (2008a) observed fine root growth in mature *Picea abies* trees in southern Germany at soil temperatures around the freezing point.

By comparing the temperature response of root growth of tree seedlings with the root dynamics of mature trees of the same species in cold soils in Alaska, Tryon & Chapin (1983) recognized a principle disagreement between ex-situ and field data, as it was recognized above. In that study, mature trees in the field showed notable fine root growth activity even under very cold soil temperatures (< 5 °C), while sapling root growth was negatively influenced by low soil temperatures. Ruess *et al.* (2006) concluded from a review of fine root dynamics data from boreal forests that the fine roots of boreal conifers must have specific adaptations to function at low soil temperatures.

Greenhouse experiments with potted tree seedlings or saplings and field studies on mature trees may lead to different results because the soil constraints for root growth (soil volume, mycorrhization, root competition intensity) and the biological controls of belowground C allocation in the plants (carbohydrate storage, hormonal regulation of growth) may differ largely. Moreover, the root growth of potted tree seedlings or saplings is generally explorative during the first months or even years of the experiment. Most of the fine roots of the young trees are built to access new soil volume and therefore increase the root biomass of the young tree, while root mortality is generally low in these early stages of a pot experiment (pers. observ., see also Aspelmeier 2001, Beyer *et al.* 2013, Hajek *et al.* 2014). At field sites with

mature long-established trees, the upper soil layers commonly are completely occupied by the fine root system of the trees, as long as no larger canopy gaps are present. Exploratory fine root growth at such sites normally is only of minor importance, while the major trigger for root growth activity is compensatory replacement of died fine roots. The magnitude of root mortality is thus an important factor influencing the growth activity of fine roots in established forests, and fine root growth and turnover will largely depend on variables affecting the lifespan of fine roots (Eissenstat & Yanai 1997, Leuschner *et al.* 2001, Hertel & Leuschner 2002, Gaul *et al.* 2008b, Hertel et a. 2013). This is also visible from the results of our study, where not only fine root biomass, but also fine root necromass was markedly higher in shaded, colder patches than in the sunny, warmer areas. Our finding that not only necromass but also fine root turnover was much higher in the colder soil patches in the shade compared to warmer areas (mean fine root longevity: ca. 0.5 vs. 2.0 years), confirms that the elevated fine root necromass values are not primarily a consequence of slower root decomposition due to hampered microbial activity, but rather are the result of higher fine root mortality at low temperatures. Similar results were obtained by Ruess *et al.* (2003) in a cold boreal forest site, where mature *Picea mariana* trees showed a high fine root turnover of 3.4 yr^{-1} (i.e. a lifespan of only 108 days) despite a growing season soil temperature of only 3.0-5.6 °C. In an experiment with artificial frost application, Gaul *et al.* (2008a) observed that fine root mortality due to winter frost stimulated compensatory fine root growth even under soil temperatures around the freezing point. Evidence for a stimulating effect of root mortality in cold soils to promote compensatory fine root growth is also presented in the studies of Ruess *et al.* (1998), Weih & Karlsson (2002), and Ruess *et al.* (2003). Interestingly, our results clearly demonstrate that a stimulation of compensatory root growth (and hence a modification in belowground carbon allocation patterns) must result from autonomous stress sensing and signaling in individual fine root strands and not from a response of the whole tree individual. This is shown by the different responses of shaded colder and sun-exposed warmer root system components, which are part of the same *Pinus cembra* individual.

While the evidence for a negative relation between root zone temperature, and root biomass and root turnover at the studied treeline is striking, attempts to explain this phenomenon must remain speculative. According to the optimal resource partitioning theory, enhanced carbon allocation to the fine root system should indicate low availability of an essential soil resource (Bloom *et al.* 1985, Poorter & Nagel 2000, Reich 2002). This assumption would also explain why fine root mortality is higher under more stressful colder than under warmer soil conditions leading to higher fine root turnover. Since it is unlikely that soil water is limited at

this site with > 600 mm growing season precipitation, one or more nutrient elements may be short in supply. The temperature dependence of the processes controlling nutrient supply, including the activity of mineralizing microorganisms, nutrient diffusion in the soil, and the uptake kinetics of carriers in the root membranes, can result in reduced nutrient availability to plants in cold soils (e.g. Meentemeyer 1977, Sveinbjörnsson *et al.* 1995, Timoney 1995, Sveinbjörnsson 2000, Müller *et al.* 2016). For example, the tree size and productivity decrease of *Picea abies* from submontane elevation to the alpine treeline on Mt. Brocken (Germany) was related not only to the temperature decrease but also to a reduction in net nitrogen mineralization rate (Plapp *et al.*, unpublished data). In tropical montane forests in Ecuador, the aboveground productivity decrease toward the alpine treeline was associated with a fine root biomass and productivity increase, and decreases in soil pH, decomposition rate and mineral N supply (Moser *et al.* 2011).

Trees in the treeline ecotone could compensate for reduced nutrient supply by increasing their absorbing fine root surface area and producing higher fine root tip frequencies at the cost of aboveground productivity. Such compensation should increase fitness particularly in the shaded colder patches of the ecotone. Soil acidity and low N availability may also reduce fine root longevity (e.g. Eissenstat & Yanai 1997, Eissenstat *et al.* 2000) and thus could be among the causes leading to increased fine root mortality, as was observed in the colder patches of our study site. According to Sullivan *et al.* (2015), low nutrient availability is among the factors causing the alpine treeline in Alaska by mediating the effects of low temperature on above- and belowground productivity. This contradicts the statement of Körner (2003, 2012ab) that nutrient deficiency should never dominate over unfavourable thermal growth conditions at the alpine treeline. However, the latter perception is not consistent with the reports of a marked C allocation shift toward the root system in trees near the treeline. Low soil temperatures are also likely to impair root water uptake through a higher viscosity of water and reduced aquaporine-mediated water transport into the root xylem, but this has not been studied in the field so far.

Table 4.5: Compilation of fine root biomass data from four mountain ranges in Central Germany (Mt. Brocken), southern France (Mt. Ventoux) and two sites in the Patagonian (Argentinian) Andes (El Chalten, Mt. Tronador) comparing montane and treeline forests. Given are the elevation of the sites, mean annual air temperature inside the stands, the tree species building the stand, mean tree height, tree density, mean aboveground biomass (AGB) per tree, mean fine root biomass (FRB) per tree, and the FRB:AGB ratio. AGB data are based on calculations using allometric equations from the literature for the respective species or (if not available) for a related species with similar structural characteristics. Fine root biomass data were obtained from fine root inventory campaigns similar to that conducted in this study. Most root data are unpublished so far except for part of the data from Mt. Brocken (Hertel & Schöling 2011ab)

Site	Elevation (m asl.)	Mean annual stand air temperature (°C)	Species	Tree density (no. ha^{-1})	Mean tree height (m)	AGB (kg tree^{-1})	FRB (kg tree^{-1})	FRB/AGB (kg kg^{-1})
Mt. Brocken (Germany, 51.5°N)								
Montane forest	990	2.9	*Picea abies*	888	11.7	49.0	3.18	0.065
Treeline forest	1100	2.1	*Picea abies*	1406	5.9	17.4	3.06	0.177
Mt. Ventoux (France, 44°N)								
Montane forest	1510	n.a.	*Fagus sylvatica*	1800	10.9	226.6	1.34	0.006
Treeline forest	1760	n.a.	*Pinus uncinata*	3911	3.3	25.2	0.31	0.012
Patagonian Andes (Argentina)								
El Chalten (49.3°S)								
Montane forest	920	3.9	*Nothofagus pumilio*	800	25.8	297.5	3.06	0.010
Treeline forest	1050	2.8	*Nothofagus pumilio*	5625	3.6	9.9	1.06	0.107
Mt. Tronador (41°S)								
Montane forest	1410	4.9	*Nothofagus pumilio*	600	15.1	81.5	6.26	0.077
Treeline forest	1690	4.0	*Nothofagus pumilio*	8800	3.9	4.3	0.68	0.158

4.6 Conclusions

The results of this systematic study on the temperature dependence of fine root biomass and fine root turnover in differently illuminated areas under the crown of isolated *Pinus cembra* trees in the treeline ecotone clearly show that self-shading does not impair fine root growth activity and the development of a large fine root system, despite lower temperatures in the shade. Rather, we found a higher root biomass density, higher root growth activity and accelerated root turnover in the shade, which can only be interpreted as a compensatory response of the tree to reduced soil resource availability in a colder soil. Neither the carbon source limitation hypothesis nor the carbon sink limitation hypothesis, which propose

explanations for the halting of tree growth at the alpine treeline, can convincingly explain this phenomenon. This suggests that future efforts to achieve a causal explanation of the alpine treeline should explicitly consider plant-internal C allocation shifts and their possible abiotic causes. The role of nutrient deficiency as a possible factor contributing to alpine and arctic treelines deserves further study in this context. Our results on tree root biomass and dynamics at the treeline confirm the well-recognized fact that results from ex-situ experiments with tree seedlings and saplings can rarely been transferred to the field. This calls for well-designed field studies on the C economy of trees below and at the treeline, which must include root dynamics.

4.7 Acknowledgements

This work was supported by the Austrian Science Fund (FWF Project No. P22836-B16, 'Growth response of *Pinus cembra* to experimentally modified soil temperatures at the treeline').

4.8 Appendix

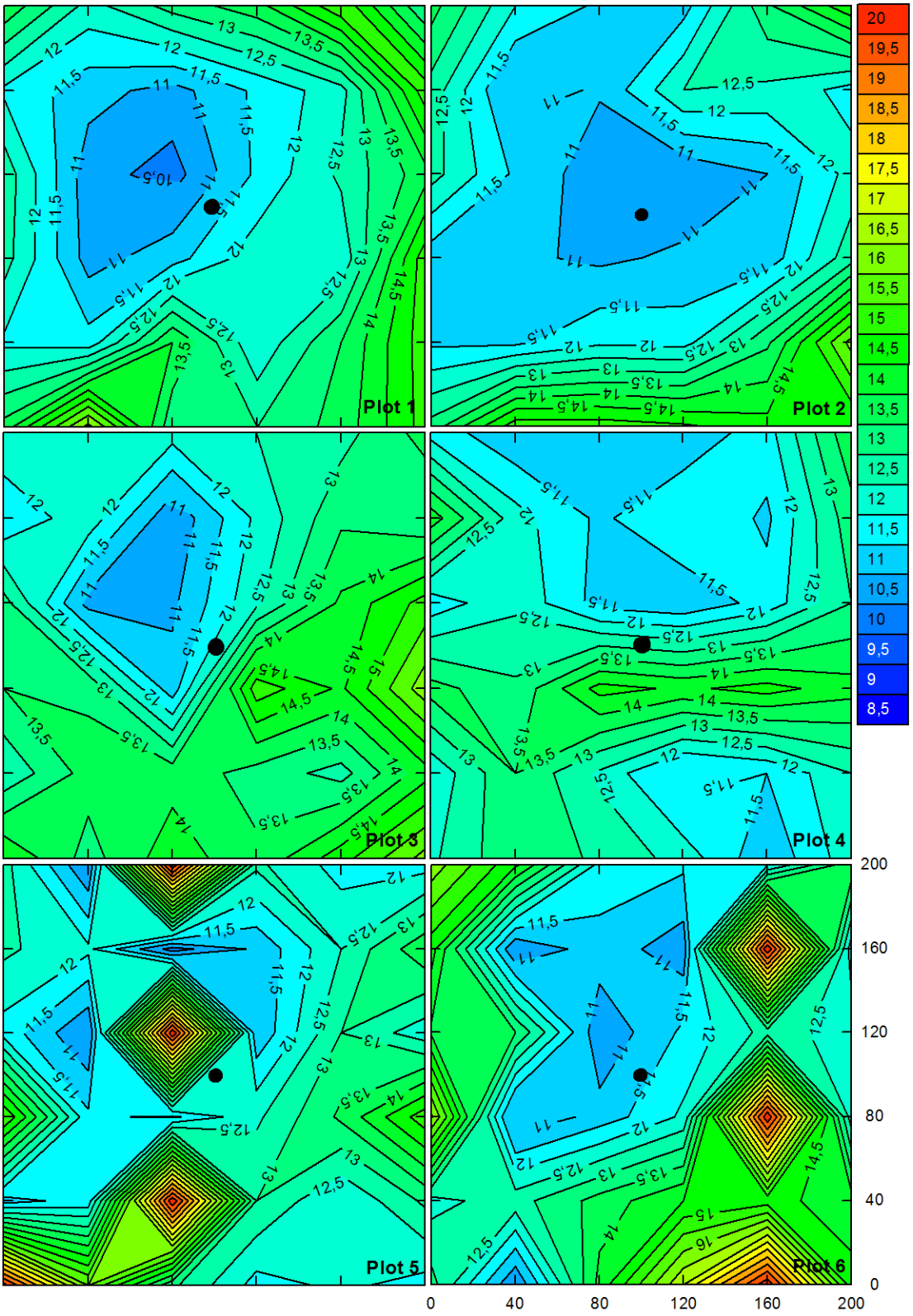

Figure A 4.1: Soil temperatures at 10 cm depth in the 6 pine plots interpolated from 36 temperature measurements of the afternoon period. Black dots mark the position of the tree stem in the plots. All graphs are oriented to north.

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CHAPTER 5

Synopsis

Background

The following chapter integrates the main results of this thesis and a final interpretation of the results of the Chapters two to four. The first two studies were conducted in the Hainich National Park and the third study in the Central Eastern Alps in Austria.

While biodiversity did not show large influences on fine roots and their morphological traits in former studies in Hainich National Park, it is mostly unknown, which influence species identity and mycorrhization type of mature trees has in detail on fine root morphology and dynamics. In the first part of this thesis, we assessed the influence of species identity and the effects of mycorrhization type on fine root traits like biomass, root order related morphology, and dynamics of six temperate broad leaved tree species (Chapters 2 and 3).

The other part of the thesis addressed the influence of soil temperature on fine root mass distribution, morphology and fine root dynamics of a coniferous tree species (*Pinus cembra* L.) at the alpine timberline (Chapter 4).

5.1 Fine root morphology, biomass and dynamics of six co-occurring temperate broad leaved species

5.1.1 Species-specific differences in fine root morphology

When we compared the morphology of the fine root individuals we detected differences in specific root length (SRL), specific root area (SRA) and mean diameter (MD) between the six investigated species. *Acer pseudoplatanus* was found to have the lowest mean diameter and the highest SRL and SRA in the whole soil profile and differed significantly from *Tilia cordata.* The other four investigated species had intermediate values and did not differ markedly. Root nitrogen concentration was elevated in *F. excelsior* compared to the other species and lower in *T. cordata* (Chapter 2). Nevertheless, as hypothesized, root order had in general a higher influence on fine root morphological traits than the species regarding the whole root branches. This can be explained by differing functional areas along the fine root branches independent of species identity (McCormack 2015).

The hypothesis that co-occurring species show similar patterns of fine root system branching was proved. There was evidence for convergence of root order related fine root traits across the six species of different taxa and mycorrhiza type (like a decrease of SRL, SRA, root nitrogen and a general increase of MD from root order 1-4). Although we found this convergence across the root orders, we found a strong dependency of root morphology and

chemistry on tree species identity (Chapter 2) and unexpectedly marked differences even between the two congeners *Acer pseudoplatanus* and *A. platanoides* in the respective root orders.

In comparison to the mentioned root morphological traits, root tip frequency (tips per fine root biomass) showed the highest variations between the species (Chapter 2) matching the results of a study by Ostonen et al. (2007) for three boreal tree species. The differences in root tips per fine root biomass accounted up to 6-fold between *F. excelsior* (1466 tips/g) and *A. pseudoplatanus* 8577 tips/g) respectively. The number of root tips per soil volume still reached differences of up to 3.5-fold between the species and might be an indicator for differing nutrient and water uptake capacity and soil colonization strategies. Because of the much higher species variability of root tip patterns in comparison to root branching properties, we assume a higher genotypic control of this root trait according to Jacob & Leuschner (2014). They concluded that species differences in nutrient uptake capacity (regarding root surface and root biomass) depends mostly on differences in root tip numbers.

Still, the sheer number of root tips however does not seem to be a relevant factor for tree nutrition in this forest, because *A. pseudoplatanus*, with highest fine root tip numbers per root mass and soil volume among the six species, did not possess higher fine root and foliar N concentrations and was not more productive than the other species (Chapter 3). Species functional variability was rather visible in the cumulative length of 1^{st} order root segments per volume where species means ranged from 2.14 m liter^{-1} up to 7.31 m liter $^{-1}$ soil volume.

Within a study on different AM species Eissenstat et al. (2015) assumed that higher diameter roots produce a higher amount of external hyphae and that roots of low diameter have more profit in resource uptake by root proliferation.

The direct comparison of the two congeneric species *A. platanoides* and *A. pseudoplatanus* also showed marked differences in fine root traits, which may reveal the development of different resource acquisition strategies even within closely related species of the same mycorrhiza type in the same stand (see Chapter 3).

Besides general variation of fine root properties between the species, *Fraxinus excelsior*, which has relatively thick fine roots, high nitrogen content and low tissue density, showed substantial deviations from the other species (Chapter 2) as well as for aboveground traits (Chapter 3). This phenomenon was already observed in studies of Meinen et al. (2009a) and Jacob et al. (2012) and might be related to the ecology of this species as *Fraxinus* was the only ring-porous species with a relatively high growth rate, large stem vessels and a relatively high nitrogen demand (Ellenberg & Leuschner 2010, Dobrowolska et al. 2011).

5.1.2 The influence of mycorrhiza type on fine root morphology

Arbuscular (AM) and ectomycorrhizal (EM) fungi differ strongly in their functionality and their mode of interaction with their host trees (George et al. 1995, Read and Perez-Moreno 2003, Smith et al. 2003, Lang et al. 2011). According to Lang et al. (2011), the microbial biodiversity within the northern part of the Hainich National park, was with up to 75 fungal species in beech, much higher in EM tree species than in AM tree species (only around 7 different taxa of glomeromycota). Strong variations were also found in colonization rates by mycorrhizal fungi. In EM tree species nearly all root tips (~96 %) were infected and only about 19 % of the root tips in AM tree species (*Acer* and *Fraxinus*, Lang et al. 2011) showed mycorrhization.

Contrary to the hypothesis that EM and AM tree species might show similar trends in fine root morphology within the same mycorrhization types and vary between them, we found only one of seven fine root traits (SRA) to show differences between AM and EM tree species. We could not even detect an influence of mycorrhiza type on root proliferation (root tips per biomass). It seems that despite contrasting symbiotic interactions and a formerly found influence of mycorrhiza type on fine root architecture (e.g. swollen root tips in EM species Smith & Read 1997, changed branching patterns in AM species Hetrick 1991, Hooker et al. 1992), at least the type of mycorrhiza was not an important determinant for fine root branching patterns and morphology in our six studied species (Chapter 2).

5.1.3 Root order related biomass and vertical distribution of the six tree species

Fine root biomass distribution in mixed forests was often investigated in relation to diversity effects and complementary of different tree species. In fact species mixtures were found to have lower influences on fine root biomass than species identity (Meinen 2009ab, Jacob 2012).

In this study, we found biomass to differ up to twofold among the six species in the whole soil profile (0-30 cm) with the highest biomass in *F. sylvatica* plots (301 g m^{-2}). When we compared different soil layers, fine roots of *F. sylvatica* and *T. cordata* were preferably in 10-20 cm soil depth instead of the top layer (0-10 cm). The other species showed similar fine root densities at 0-10, 10-20 and 20-30 cm depth or a decline of fine root biomass in deeper soil layers. This decline towards deeper soil was in accordance to the results of Meinen et al.

(2009 ab) within the same forest. In general we found species identity to be the more important factor for differences in fine root biomass than soil depth.

The root order proportions among the species give us information about fine root functions in differing soil depth. In the topsoil (0-10 cm) where most nitrogen is present we found the highest relative biomass proportion of the highly active first two root order fractions. Deeper in the profile, with less nitrogen supply, 3rd- and 4th-order segments are more abundant. This functional shift between the respective orders was suggested by Guo et al. (2008) in a study on 23 temperate tree species and fits well to our results.

5.1.4 Fine root dynamics of the six species

Fine root dynamics in terms of fine root production, fine root lifespan, or fine root turnover (fine root production per standing fine root biomass) are quite difficult to observe in the field but important to assess as they have a strong influence on belowground processes and nutrient cycling. Direct observation of fine root dynamics in the soil is often conducted via mini-rhizotron technique while for example ingrowth cores, as used in our study, are indirect measurements of fine root production in soil samples. It has to be kept in mind that results of those methods on fine root lifespan and turnover can differ widely and are often not comparable (Burke & Raynal 1994).

Plant-internal resource allocation rules (Eissenstat & Duncan 1992) and external abiotic and biotic factors like nutrient availability, drought stress, temperature extremes and the activity of root herbivores, pathogens and fungal symbionts (Wells & Eissenstat. 2002, Guo et al. 2008, Rasmann & Agrawal 2008, Adams & Eissenstat 2015) are the main determinants for fine root dynamics. The plots within our study had similar chemical conditions and were comparable. The conditions which are directly influenced by species specific deviations in soil biology (pathogens, herbivory, microbial activity; Guckland et al. 2009, Cesarz et al. 2013, Scheibe et al. 2015) might differ but were not investigated in this study.

Median fine root turnover rates of our investigated species in the whole soil profile (0-30 cm) ranged between 0.16 (*Fraxinus excelsior*) and 1.6 (*Acer platanoides*) which is equivalent to a lifespan of 6.3 and 0.6 years, respectively. In comparison, Withington et al. (2006), found lifespans between 0.6 to 2.5 years in *Acer, Tilia* and *Fagus* in mini-rhizotron observation but addressed only first two root orders which have in general a shorter lifespan than the whole root individual.

When we linked fine root morphological traits with fine root longevity and turnover, we could not detect any coincidence of fine root morphology and fine root lifespan so we have to reject

our hypothesis of an interrelation between fine root morphology and lifespan. This is contradictory to former studies who showed strong positive correlations between fine root diameter and fine root longevity (Guo et al. 2008, Eissenstat et al. 2015), but most of them addressed only the first two root orders while we included higher root orders as well. In studies of McCormack et al. (2012, 2015) fine root lifespan in North American trees correlated positively with mean root diameter, root C/N ratio and Ca concentration and negatively with SRL. We expected that in our study fine roots of the three AM species would have longer fine root lifespans than EM species as the data of Withington et al. (2006) indicate that *A. pseudoplatanus* and *A. platanoides* had particular long fine root lifespans in the first two root orders. In our study *Fraxinus excelsior* had the highest diameter, the highest N-content and AM thus it was expected to have the highest root lifespan. *Fraxinus excelsior* and *Acer pseudoplatanus* indeed had quite high lifespans, but this was not systematically valid for all AM species (Chapter 3). Nevertheless, in our investigated species the span of mean root diameters was comparable small and differed only moderately which might be the reason for the similarities in fine root lifespans. The hypothesis of an interrelation between fine root morphology and fine root lifespan as well as between mycorrhiza type and fine root dynamics must be rejected.

To reveal possible differences in resource foraging strategies of the six species we considered the investment of carbon into the production of 1st and 2nd root order branches which are supposed to conduct most of the nutrient and water uptake (Guo et al. 2008). According to our calculations, first and second root order branches accounted for 30-50 % of total fine root biomass resulting in a relatively high production of 50 to 90 g of the first two root orders per square meter in the whole soil profile (0-30 cm depth). The 2nd and 3rd root order biomass did not differ significantly between the species, while the amount of 3rd and 4th root order proportions differed strongly between the species.

Only two other studies (Xia et al. 2010, Sun et al. 2011) exist to our knowledge, who conducted investigations on root order production at plot level. In East Asian trees they found much lower fine root production values of 27 to 42 g m^{-2} yr^{-1} in the first two root orders than in the present study but tree age was only 25 years in average though much younger than in our stands.

5.1.5 Interrelation between fine root-and aboveground properties and ecology

Our investigation showed several differences in fine root morphology between the six broad leaved species in the same stand. Considering each tree as one individual organism, we

expected aboveground traits and ecology to interrelate with fine root properties in some way. We suggested in our hypotheses that fast growing species might have a higher demand on nutrients which might be associated with a higher fine root biomass or thinner and short lived fine roots (Eissenstat et al. 2015). We cannot confirm these hypotheses within our study. There was no interrelation between species' aboveground woody biomass production and fine root productivity or root turnover. The same tendency was observed by Finér et al. (2011) in a global literature review on the influence of stand basal area or stem density on fine root turnover. However, across all species, there were interrelations between aboveground and belowground traits. Wood production was higher with higher mean fine root diameter and decreased with increasing root tissue density for all species though we assume that an increase of root tissue density however may point out a higher investment of carbohydrates into fine root tissue resulting in lower timber production (Fogel 1983, Hertel et al. 2013).

Fine root turnover and production were similar for the species of both mycorrhiza types (Chapter 3) neither differed aboveground productivity between AM and EM species (Jacob et al. 2010).

On the fertile soils of the Hainich forests one or the other mycorrhization type might not provide any advantages or disadvantages for trees. Other influences might be more relevant for fine root efficiency and function than the type of mycorrhization such as species differences in fine root traits and local, species induced nutrient availability and soil chemistry (Rothe & Binkley 2001, Guckland et al. 2009).

The lifespan of fine roots (all fine roots < 2 mm) was strongly attached to species identity and varied strongly among the species. Regarding the results of Withington et al. (2006) who found a similar leaf lifespan across four of our studied species, fine-root and leaf lifespan seem to be only poorly related. Fine root longevity seems to be controlled by other factors than aboveground phenology and biomass production. We must reject our hypotheses that species with higher aboveground productivity have shorter lived fine roots.

A closer view on fine root dynamics and morphology of *A. pseudoplatanus* and *A. platanoides* indicated differences in ecology and foraging strategies between those congeners. It seems that resource uptake can be augmented by either a high root surface area (*A. pseudoplatanus*) or by a high root turnover rate (*A. platanoides*) (Eissenstat & Yanai 1997). A higher turnover is suggested to support a higher root uptake capacity caused by reduced root age and a faster return of nutrients to the soil by the decay of nitrogen rich short living root orders.

5.1.6 Conclusion and outlook

An investigation of more species within the mycorrhiza groups might reveal group differences between mycorrhization types in fine root traits but there are only a few more widespread AM species in Central Europe. Since many of the common species are already included in our study, our results of Chapter 2 and Chapter 3 suggest that fine root morphology, lifespan and productivity are mostly determined by genotype and biotic and abiotic environment. In this study mycorrhiza type seems to be only of secondary importance among the influencing variables on fine root traits. Still more data on root order production, turnover the and hyphal net are needed to understand the influence of mycorrhiza type on tree fine root morphology, dynamics and functioning, in particular from species-rich mixed forests.

5.2 Fine root response of *Pinus cembra* to soil temperature at the alpine treeline

5.2.1 Soil thermal conditions under the canopy of Pinus cembra trees

Given that temperature and especially soil temperature is crucial for fine root and aboveground tree growth at the treeline, the shade under the trees seems to play a key role for the limit of closed forests at the treeline (Hoch & Körner 2003, Dang & Cheng 2004).

We expected strong differences between shaded and sun exposed soil areas under the *Pinus cembra* trees at the treeline. Especially during summer, the season with the highest thermal deviations between night and day and the most pronounced temperature difference between sunny and shaded soil. Morning and noon temperatures are expected to show fewer differences between sunny and shaded areas than afternoon soil temperatures, due to the delay of soil heating compared to the more rapid temperature increase of air temperatures.

Holtmeier & Broll (1992) found closed tree canopies lowering soil temperatures markedly compared to not shaded areas. In the afternoon (maximal soil heating) our results showed high soil temperature differences (in 10 cm soil depth) between shaded and sunny areas below the canopies of more or less isolated trees at the treeline. Rooting zone temperatures in the shade below the crowns were in average 1.4 times lower than in sun exposed soil. Absolute minimum soil temperatures did not differ markedly across the day and ranged between 8.6 °C and 10.3 °C, while absolute maxima in contrast changed nearly by a factor 2 between 11.4 (dawn) and 20.4 °C (afternoon). When we subdivided the soil temperatures of 36 grid points into 4 categories of each 9 ranked temperature subsamples and generated a graph with

temperature isotherms around the trees, the influences of the crown on the afternoon soil temperatures was clearly visible. Shaded (category 1) and sun exposed zones (category 4) around the trees as well as the transition zones (categories 2 and 3) differed significantly to each other.

5.2.2 Fine root mass distributions and morphology in different soil temperatures under the tree crown

The combination of soil temperature data and fine root traits around treeline trees showed interesting results. In contrast to findings of other authors who found low soil temperatures to hamper growth and activity of fine roots in tree seedlings (Holtmeier & Broll 1992, Häsler et al. 1999, Alvarez-Uria & Körner 2007), our study (Chapter 4) revealed a higher fine root biomass and necromass density in shaded, cooler areas under the tree canopy. Biomass density values varied between 0.23 g L^{-1} and 0.36 g L^{-1} while fine root necromass density ranged between 0.09 and 0.19 g L^{-1}. Our results showed a closer association of fine root abundance and the afternoon soil temperatures and confirmed our first hypothesis, but the second hypotheses must be rejected, as fine root biomass density was not lower but significantly higher in shaded, cooler areas under the tree canopy, than in sunny warmer ones. Our results lead to the assumption of a higher carbon allocation to the fine root system with decreasing soil temperatures at the treeline. The results of this study fit to the results of other investigations, who found an increasing fine root biomass with increasing elevation (Helmisaari et al. 2007, Leuschner et al. 2007, Hertel & Wesche 2008, Hertel et al. 2008, Hertel & Schöling 2011ab). Our study confirms that the higher fine root mass must depend on soil temperatures rather than on an elevational factor, as it increases even around trees at the same elevation with differing soil temperatures and not only in transects with an elevation gradient as found in previous studies. We can clearly contradict the assumptions of ex-situ experiments on tree saplings, who found colder temperatures to impair root growth and activity hence leading to lower fine root biomass at cold sites (Häsler et al. 1999, Alvarez-Uria & Körner 2007, Hoch & Körner 2009, Schenker et al. 2014).

Another explanation for a higher fine root mass in cooler tree surroundings of mature trees can be found in a higher growth activity of fine roots in shaded areas, which is necessary to compensate difficulties in water and nutrient uptake. It is known, that nutrient mineralization is often hampered in cold soil conditions (Meentemeier 1977, Sveinbjörnsson et al. 1995, Timoney 1995, Sveinbjörnsson 2000) and water owns a higher viscosity at lower temperatures. Thus the water conductivity in the xylem vessels decreases with decreasing

temperatures. Compared to warmer environments trees of cool environments require a higher fine root density and carbon allocation into fine root production, for long distance transport of water and nutrients (Stephens & Fox 1991, Sveinbjörnsson 2000). To maintain the supply of resources and to compensate the high fine root mortality in cool soils, our results lead to the assumption that trees in cooler soils need a higher amount of fine roots and a higher root length and root surface area while the proportion of root tips with mycorrhization did not differ between cool and warm soil temperatures (80% mycorrhization rate). Additionally fine root individuals of cold areas around the trees had around two times more root tips per root sample, than those of warmer areas, which is a sign for a higher "uptake" activity and effort in water and nutrient acquisition in cooler areas.

Fine root production differed marginally significant ($p=0.15$) between the sampling points northerly and southerly next to the tree stems and ranged between ~56 g m^{-2} yr^{-1} in the sun exposed and 145 g m^{-2} yr^{-1} in the shaded areas under the trees.

Fine root turnover and its inverse fine root lifespan varied also between northerly and southerly points and fine root lifespan was shorter in northerly situated sampling points next to the trees. Instead of a hampered fine root production in cool soils, we rather assume a compensatory root production due to a lower fine root lifespan and a higher fine root dieback and lower fine root longevity with decreasing soil temperatures. We assume that elevated necromass values in cooler soil of our study are rather caused by a higher mortality than by lower decomposition rates, as the fine root turnover was much higher in those areas than in warmer ones (2.0 northerly versus 0.5 southerly) and fine root lifespan was much shorter.

Investigations by Gaul et al. 2008 observed a higher fine root growth after frost events even at temperatures around 0 °C but a shorter fine root lifespan with decreasing soil temperatures. Soil frost enhanced the fine root dieback and a higher compensation of the fine root loss though a higher carbon allocation to the fine roots was necessary. Trees growing at low temperatures need larger absorbing organs to cover the demand of resources (Gaul et al. 2008). This is also reflected in the larger fine root surface area and length in shaded areas under the tree canopy in our study.

We believe that a higher fine root turnover and a shorter fine root lifespan guarantee a higher availability of young and more effective root branches for resource acquisition as more distal root branches are "cheap" in their construction, but expensive to maintain (Eissenstat &Yanai 1997). Based on the findings of Wieser et al. (2015) who found an increased sap flow in *Pinus cembra* in warm soils, we suppose a higher activity of fine roots in warmer soils and irrespective to fine root biomass a lower activity of the single rootlets (but in sum more root

tips!) in cold soil as well as smaller vessels with lower hydraulic conductivity at lower temperature (Van den Oever et al. 1982, Baas 1982, Noshiro et al. 1994, 1995).

It might be, that in open patches, soils warm up rapidly and exhibit higher soil temperatures than occur under compact tree stands (Holtmeier & Broll 1992) and thus tree growth and nutrient supply might be influenced more positively by less dense crown density. However, the decrease of dense forests close to the upper climatic timberline should maybe not be taken as an adaptation to cold soil temperatures as isolated trees might have a more suitable soil temperature regime on the one hand, but on the other hand they are more exposed to climatic injury than in dense stands (Holtmeier 2009). In general we conclude that in fact low soil temperature is one key influencing factor for tree growth at the timberline, but it is rather a higher carbon investment to belowground biomass compared to aboveground biomass with decreasing temperatures, than a hampered tree growth in general, as often hypothesized. In addition the fine root reactions on cold soil temperatures cannot be compared between tree saplings and mature trees at the treeline. Our findings point out, that compensatory fine root growth and carbon allocation must be triggered by the fine root individuals and their surrounding rhizosphere, as fine roots grew differently within shaded and sunny areas under one and the same isolated Pine tree.

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Declaration of originality and certificate of ownership

I, Petra Kubisch, hereby declare that I am the author of the present dissertation entitled 'Species-specific fine root biomass, morphology and dynamics of six co-occurring deciduous tree species in the Hainich National Park and a conifer tree species at the alpine treeline'. All references and sources that were used in the dissertation have been appropriately acknowledged and cited. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure.

Göttingen, August 2016

(Petra Kubisch)