THE EFFECT OF THE MYCORRHIZAL TYPE ON ROOT-RHIZOSPHERE INTERACTIONS IN AM AND ECM TREE SPECIES: FIELD STUDIES AND MESOCOSM EXPERIMENTS

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GENERAL INTRODUCTION

The role of forest soils in carbon storage under global change

A global-scale climate change has been observed over several decades. The global mean of ground-level air temperature has increased, with an accompanied enhanced melting of mountain glaciers and decreasing snow cover. In addition, more frequent extreme weather events, such as heavy rainfall, drought periods and heat waves have been observed. Since 1900 and with progressive industrialization, the increase of global average temperature has exceeded 1°C mainly due to greenhouse gas emissions. Among these gases, rising carbon dioxide (CO_2) concentration represents the largest factor (Hansen *et al.*, 2017). Fifty years ago, atmospheric CO_2 increased by less than 1 ppm per year, while today the increase per year is more than 2 ppm resulting in a global CO_2 concentration of currently *c.* 400 ppm (Betts *et al.*, 2016). The rapid increase of atmospheric CO_2 concentration is mainly due to human activities like fossil fuel combustion, deforestation and land-use change (Raupach & Canadell, 2008). Model calculations assume that about 50% of the total emitted CO_2 remains in the atmosphere, while the other half is absorbed by oceans and terrestrial ecosystems (Bousquet *et al.*, 2000). On global scale, forests store more than half of the organic carbon (C) found in terrestrial ecosystems, whereby European temperate forests represent a strong C sink with 34% of accumulated C in living vegetation, 5% in dead wood, 3% in forest floor and the majority (58%) of C stored in soil organic matter (SOM) (Goodale *et al.*, 2002). Accordingly, soil C storage of temperate forest ecosystems are major players in the global C cycles. To what extent organic C is stored in soil depends on several factors. The sink and strength of soil organic C pools is influenced by biotic factors like tree species and microbial composition (Finzi *et al.*, 1998; Díaz-Pinés *et al.*, 2011) and by abiotic factors like soil temperature (Melillo *et al.*, 2011) and precipitation (Jobbágy & Jackson, 2000). The increase in temperature as a consequence of global change is also accompanied by changes in the world´s hydrological cycle (IPCC, 2013) with consequences for global precipitation regimes. High temperature and reduced precipitation have shown to reduce the content of organic C stored in soils (Jobbágy & Jackson, 2000). In Central Europe, more frequently occurring and more severe droughts and heat waves are predicted (Rowell & Jones, 2006; Fischer & Schär, 2009; Fischer *et al.*, 2012), which can have strong influences for SOC storages and C dynamics in temperate forest ecosystems.

Considering the aboveground C turnover, relevant processes of C cycling in forests comprise C uptake via photosynthesis, C release via leaf respiration, C storage in biomass, and C loss by litter and dead wood. Since forest soil is the major sink of C, belowground processes play a key role in C cycles and dynamics of forest ecosystems. Taking the net photosynthetic carbon fixation as a basis, estimated 30 to 60% are directly allocated to the roots (de Kroon & Visser,

2003), hence roots represent a very important mediator between C uptake via photosynthesis and C storage in forest soils. Kuzyakov & Domanski (2000) estimated - based on a review of published data - that about 50% of the entire C that is allocated to the root is used for root biomass production, about 33% is released via root respiration and microbial utilization and about 17% represent C inputs to soil, which are incorporated in microbial biomass and soil organic matter. The input of root-derived C into soil is also called rhizodeposition, and includes root C release through root turnover and death, exudation, mucilage secretion and border cell loss of living roots (Jones *et al.*, 2009). However, root dynamics and the associated input of root-derived C via rhizodeposition into the soil are still poorly understood aspects of the forest C cycle.

Root influences on carbon and nutrient cycling and the effect of changing climatic conditions

Since roots do not only determine the release of C into soils but also the uptake of resources, they play a decisive role in the biogeochemical cycles of forest ecosystems (Brunner & Godbold, 2017). While roots with larger diameters primarily serve transport and storage functions, fine roots are responsible for nutrient and water acquisition (Fitter, 1996; Pregitzer *et al.*, 2002) and thus determine nutrient cycling and resource uptake from soils. Traditionally, fine and coarse roots have been categorized according to root diameter (<2 mm or >2mm) and more recently according to root order (Pregitzer *et al.*, 2002). The most distal and finest root orders are the most absorptive root parts with high respiration rates (Rewald *et al.*, 2011), high resource uptake efficiency (Guo *et al.*, 2008b; McCormack *et al.*, 2015) and rapid turnover (Joslin *et al.*, 2006). Since fine roots are the most active part in water acquisition, morphological root traits like the proportion of roots with fine root diameter and root order structure (branching patterns) can also influence the productivity of trees under drought (Wasson *et al.*, 2012). Accordingly, fine root morphology and the associated functions play an important role in resource acquisition and SOM accumulation in forest soils especially under changing climatic conditions, for example more frequently occurring droughts.

The input of fine root-derived C into soil and the influences on resource availabilities and acquisition are primarily determined by root functions like fine root turnover and root exudation. An accelerated root turnover increases C fluxes into forest soils and thus influences the transformation into root-derived organic matter (Guo *et al.*, 2004). Since a faster root turnover also implies a shorter root lifespan, which have been linked with high respiration rates (Burton *et al.*, 2002), strong metabolic activities (Comas & Eissenstat, 2004), and increased

resource uptake efficiencies (Volder *et al.*, 2005), root lifespan is not only an important driver in C dynamics but also in water and nutrient uptake capabilities. The effects of changing climatic conditions for example more frequently occurring droughts and increasing temperatures on fine root lifespans still remain poorly understood due to the limited number of respective studies. While shorter root lifespans have been related to increased temperatures (Leppälammi-Kujansuu *et al.*, 2014; Wang *et al.*, 2016), the effect of drought on root longevity is not clarified due to inconsistent results of various studies (Anderson *et al.*, 2003; Bauerle *et al.*, 2008; Meier & Leuschner, 2008). However, changing climate conditions may have important implications for root lifespan and thus for nutrient acquisition of trees and the C input into the soil.

Root C release via rhizodeposition also includes the exudation of organic compounds and mucilage from fine roots (Jones *et al.*, 2009). C release via root exudation ranges between 5 and 10% of the entire photosynthetically fixed C (Jones *et al.*, 2004), representing a considerable source of C entering forest soils. In addition, C-rich exudates have the capability to increase microbial biomass and to stimulate microbial activity via a priming effect (Kuzyakov *et al.*, 2000), which lead to an accelerated microbial decomposition of recalcitrant SOM (Hoosbeek *et al.*, 2004; Joslin *et al.*, 2006; Phillips *et al.*, 2011; Phillips *et al.*, 2012; Meier *et al.*, 2017). This process leads to an exudate driven acceleration of biogeochemical cycles and nutrient mineralization through increased microbial activity (Herman *et al.*, 2006; Finzi *et al.*, 2015). Besides this indirect effect of exudates on resource availability, exudates have also the capability to enhance nutrient availabilities directly by the provision of chelating agents or by alteration of the pH milieu and redox status in the rhizosphere (Grayston *et al.*, 1997; Jones *et al.*, 2004). Root exudation is highly influenced by changing environmental and climatic conditions. Previous studies found that C release through root exudation decreases with increasing root depth (Tückmantel *et al.*, 2017) and increasing N deposition (Phillips *et al.*, 2009). With respect to climate change, root exudation is predicted to increase under elevated CO_2 (Phillips *et al.*, 2009; Phillips *et al.*, 2011) and increasing temperatures (Boone *et al.*, 1998; Yin *et al.*, 2013; Yin *et al.*, 2014; Zhang *et al.*, 2016). Studies on the effect of drought on root exudation are inconclusive: C release via root exudation has been found to increase (Reid & Mexal, 1977; Preece *et al.*, 2018), decrease (Brunner *et al.*, 2015), or to be unaffected by drought (Karst *et al.*, 2017). This represents the uncertainties in predicting exudate-derived C input to the soil and resource acquisition through root exudation under changing precipitation regimes. But not only the magnitude of C release with root exudation, but also the composition and diversity of exudates are assumed to have great influences on the diversity and activity of

soil microbes, biogeochemical processes, and nutrient availability in the rhizosphere (Prescott & Grayston, 2013; Eisenhauer *et al.*, 2017).

As a conclusion, fine root morphology and root functions represent considerable factors that influence the C and nutrient cycle in forest soils and are important factors for the development of forest ecosystems under the impact of the global climate change. Despite root-specific influences, there is increasing evidence that the mycorrhizal symbiont associated to the root has also a significant effect on SOC and nutrient availability in the rhizosphere (e.g. Finlay & Söderström, 1992; Finlay *et al.*, 2006; Jones *et al.*, 2009; Averill *et al.*, 2014; Soudzilovskaia *et al.*, 2015b). Mycorrhizal fungi represent a diverse community of species and strains. The extent of infection of roots in forest ecosystems through the community and its individual members is often largely unknown. In contrast, the relative abundances of the types of mycorrhizal association represent a comparatively easily determinable property of diverse tree species compositions in forest ecosystems. Furthermore, as in most symbiosis, the adaptability of both partner to each other might strongly influence the mutual functioning. However, the extent to which different types of mycorrhizal associations contribute to and influence the C and nutrient dynamics in forest ecosystems remain largely unknown. This 'black box' might turn out to be a key factor in understanding and possibly positively influencing the forest ecosystem dynamics under the global climate changes currently and in the future. The here presented work has the objective to contribute to this understanding.

Mycorrhizal associations in temperate forests

Mycorrhizal fungi are associated with almost all tree species in forests ecosystems, among which the most widespread are arbuscular (AM) and ectomycorrhizal (ECM) associations (Read, 1991). The symbiosis between plants and mycorrhizal fungi is possibly the world´s primarily mutualism and is based on the exchange of photosynthetically fixed C from the host as food source ensuring growth of the fungus, and in turn, enhanced provision of soil-derived nutrients and water to the tree. AM and ECM associations differ in fundamental fungal structures. ECM fungi are characterized by the intercellular Hartig net representing the interface of resource exchange between the host and the fungus and by a thick hyphal mantel and a extraradical mycelium increasing the absorbing area of roots and ensuring nutrient and water uptake (Brundrett *et al.*, 1996). In contrast, AM fungi have intracellular arbuscular structures which are connected with hyphae and vesicles (Brundrett, 2002). In this form of the symbioses, arbuscules ensure nutrient transfer between the host and the fungal symbiont, vesicles provide nutrient storages, and hyphae emanating into the soil extend the absorbing surface (Leake *et*

al., 2004). Beside functional differences in fungal structures, trees associated with AM and ECM fungi in temperate forest ecosystems exhibit different biogeochemical variations, which is due to the characteristic nutrient economy of the mycorrhizal association types (Read & Perez-Moreno, 2003; Phillips *et al.*, 2013). The inorganic nutrient economy of forest ecosystems dominated by AM tree species are characterized through the fact, that the majority of nutrients is not bound organically but in the soil solution or comparatively weakly bound in inorganic forms to the soil particles` surface. Nutrients in this status can mostly be absorbed quickly by the plants, but are also subject to increased leaching. This interconnections apply in particular to growth-limiting nutrients such as N (Phillips *et al.*, 2013; Midgley & Phillips, 2014). Furthermore, AM dominated ecosystems are characterized by a rapid decomposition of high-quality leaf litter of AM tree species (Cornelissen *et al.*, 2001; Hobbie *et al.*, 2006) and by low saprotrophic properties of AM fungi (Read & Perez-Moreno, 2003). In contrast, the organic nutrient economy of ECM dominated ecosystems tend to have higher rates of soil C retention (Vesterdal *et al.*, 2012; Averill *et al.*, 2014), less N leaching losses (Midgley & Phillips, 2014), and a higher proportion of nutrients bound in organic compounds (Phillips *et al.*, 2013). This is a consequence of the more slowly decomposing low-quality leaf litter of ECM trees (Cornelissen *et al.*, 2001; Hobbie *et al.*, 2006). In contrast to AM fungi, ECM fungi have high saprotrophic properties that release oxidative and hydrolytic extracellular enzymes to mine nutrients from SOM (Read & Perez-Moreno, 2003). Based on these systematic differences in nutrient economies of AM and ECM tree species the idea of a mycorrhizal-associated framework for predicting C and nutrient couplings in temperate forests emerged (Phillips *et al.*, 2013). So far, there is increasing evidence that root functions (Phillips & Fahey, 2006; Smith & Read, 2008; Yin *et al.*, 2014), relevant biogeochemical processes in C, N, and P cycling (Phillips & Fahey, 2006; Brzostek *et al.*, 2013; Yin *et al.*, 2014), and root morphology (Brundrett, 2002; Smith & Read, 2008; Comas & Eissenstat, 2009; Comas *et al.*, 2014; Eissenstat *et al.*, 2015) are influenced by the mycorrhizal association. However, to this date, the identification of systematic differences between AM and ECM association on root-rhizosphere interactions is still not far developed.

Root-rhizosphere interactions of AM and ECM trees

The effect of fine roots and their associated mycorrhizal type on biogeochemical cycles starts in the rhizosphere, representing the root surrounding soil, which is directly influenced by rhizodeposition and associated soil microbes. The mycorrhizal colonization with AM or ECM fungi may alter important root functions of the tree that influence biogeochemical processes in

the rhizosphere. Since root lifespan and exudation have strong influences on root-rhizosphere interactions, the effect of the mycorrhizal association type in these root functions is of particular importance. The mycorrhizal status is known to influence root morphology (Smith & Read, 2008), which is linked with root lifespan. However, studies on the effect of the mycorrhizal type on root lifespan are scarce and hitherto there is no evidence that the mycorrhizal association type influences root lifespan (McCormack *et al.*, 2012; Chen & Brassard, 2013). In contrast, root exudation has been related to the associated mycorrhizal type (Langley & Hungate, 2003; Meier *et al.*, 2013) but also to the tree species (Grayston *et al.*, 1997). Since exudation is an important driver that regulates nutrient availability, systematic differences in nutrient economies of AM and ECM tree species may be associated with differences in C release through root exudation. High organic N content in ECM ecosystems may result in a stronger dependency on microbial decomposition of ECM trees and, thus, in an increase in C release via root exudation to prime microorganisms that decompose N containing organic compounds (Brzostek *et al.*, 2013; Yin *et al.*, 2014). The influence of the mycorrhizal association on exudation is also reinforced by the capability of mycorrhizal fungi to release carbohydrates and extracellular enzymes that mineralize C, N and P from SOM (Tawaraya *et al.*, 2006; Meier *et al.*, 2015; Zhang *et al.*, 2016). It is already known that rhizosphere effects (i.e., the relative difference in chemical, physical, and biological properties between rhizosphere and bulk soil) in C, N and P cycling are differently pronounced in AM and ECM dominated stands with higher capabilities of ECM trees to acquire nutrients from SOM (Phillips & Fahey, 2006; Brzostek *et al.*, 2013; Yin *et al.*, 2014). Such effects have often been related to the capability of ECM trees to release extracellular enzymes and to the magnitude of C release via exudation that primes microorganisms that decompose nutrients from SOM. However, not only the magnitude, but also differences in exudate composition between AM and ECM trees may have great influences on nutrient acquisition from SOM, however, there are no direct studies on that subject available. Even though the dominant functional mycorrhizal type could play a key role in rhizosphere processes, there is still a lack of understanding how AM and ECM associations differ in C and nutrient cycles. Furthermore, it remains largely unclear how this is related to mycorrhiza-specific root exudation and root lifespan. This fact has turned out to be an obstacle for the incorporation of belowground processes in ecosystem models.

Linkages of mycorrhiza-specific morphological root traits

Over the last years and with the growing need and interest in identifying indicators for predicting forest ecosystem processes, the number of studies on key above- and belowground

traits in forest ecosystem functioning increased. The identification of plant functional traits represents a useful approach for simplifying complex plant characteristics, which can be implemented in ecosystem and global models. During the last decade, aboveground plant traits have been intensively studied (Poorter & Bongers, 2006; Cornwell *et al.*, 2008; Ordoñez *et al.*, 2009; Díaz-Pinés *et al.*, 2011). Based on these aboveground traits, conceptual frameworks for ecosystem processes like nutrient cycling, decomposition, and resource acquisition were included into modelling efforts, often in relation to their phylogenetic group (evergreen gymnosperm vs. deciduous angiosperm). Due to the elaborated observations of aboveground features, a global leaf economic spectrum has been developed, which describes the nutrient return and biomass investments in leaves of fast, acquisitive or slow, conservative tree species and operates largely independent of plant growth and functional types (Wright *et al.*, 2004). In comparison, less attention has been paid on root specific traits and so far, it remains unclear whether belowground traits correspond to aboveground traits. However, the correlation of morphological and architectural root traits with functional trait syndromes in order to simplify the complexity of ecosystems gained increasing interest. Morphological root traits like specific root length (SRL), specific root area (SRA), root diameter, root order and root branching have already been linked with resource acquisition and foraging strategies (Eissenstat *et al.*, 2015). The association with mycorrhizal fungi directly alter root morphology and chemistry (Smith & Read, 2008) and has the potential to influences plant strategies in resource acquisition (Olsson *et al.*, 2003). ECM root systems are known to have higher branching intensities and thinner root diameters than AM root systems (Eissenstat *et al.*, 2000; Brundrett, 2002; Smith & Read, 2008; Comas & Eissenstat, 2009). Small root diameters with accompanied high specific root length (SRL) and high branching intensities have been linked with root proliferation and high abilities in foraging strategies (Hodge, 2004; Eissenstat *et al.*, 2015). An increase of C release via root exudation in nutrient rich patches is also known to be linked with root morphological traits like increasing root surface are (SRA), high number of root and mycorrhizal tips (Phillips *et al.*, 2008), and intense root branching (Groleau-Renaud *et al.*, 1998; Badri & Vivanco, 2009). Roots with small diameters and strong branching intensity, which are shown to be distinctive for ECM root systems, have been related to decreased root lifespans (Wells & Eissenstat, 2001; Wang & Qiu, 2006; Guo *et al.*, 2008a; Gu *et al.*, 2011; McCormack *et al.*, 2012), high resource uptake capabilities (McCormack *et al.*, 2015) and high respiration rates (Rewald *et al.*, 2011). Fine and strongly branched root systems are also assigned to fast acquisitive growing species (Comas *et al.*, 2002; Comas & Eissenstat, 2004). In contrast, due to the fast decomposition of AM leaf litter and the accompanied accelerated nutrient cycles (Phillips *et al.*, 2013), AM tree species

were proposed to be fast and acquisitive in comparison to ECM tree species. Despite the differences in root morphology of AM and ECM trees, the effect of the mycorrhizal association type on these linkages are hitherto poorly understood and largely not included in belowground trait economies (Weemstra *et al.*, 2016). However, systematic differences in root architecture of AM and ECM associations imply that the mycorrhizal association type should be considered as possible important belowground trait that influences resource acquisition, foraging and thus C and nutrient dynamics in forest ecosystems.

AM and ECM associations under conditions of climate change

Changing climatic conditions will influence important root functions, and C and nutrient dynamics in forest ecosystems, which are relevant for mycorrhizal associations. The most important factor for climate change is the increase in atmospheric CO_2, which is associated with an increase in temperature and changes in rainfall distribution. This will lead to decreased soil water availability in many areas of the world. Additionally, changes in rainfall distribution and temperature are accompanied by a progressive N deposition that increases plant-available N in forest soils, as a consequence of anthropogenic atmospheric N emission (Bobbink *et al.*, 2010). These changes taken together may have a great impact on the function of mycorrhizal associations through an accompanied alteration in resource availabilities, in C allocation to the roots, and in the distribution of mycorrhizal fungi and their hosts (Bellgard & Williams, 2011). While elevated CO_2 seems to stimulate AM and ECM colonization (Treseder, 2004; Alberton *et al.*, 2005; Garcia *et al.*, 2008; O'Neill, 2008; Cheng *et al.*, 2012), an increased N availability has been shown to increase AM (Garcia *et al.*, 2008), but to decrease ECM colonization (Treseder, 2004). While studies on the effect of drought on mycorrhizal colonization are contradictory (Swaty *et al.*, 2004; Clark *et al.*, 2009; Querejeta *et al.*, 2009; Hawkes *et al.*, 2011), Soudzilovskaia *et al.* (2015) showed on a global scale that ECM colonization is highly influenced by seasonal precipitation, while AM colonization is strongly related to seasonal temperature. Despite the influence of drought on the degree of AM and ECM colonization, it remains an open question if the type of mycorrhizal association may improve water uptake of their hosts. Water availabilities have been shown to be enhanced when mycorrhizal fungi form filamentous hyphae, which increase the soil water absorbing surface area, by exploiting micropore water, which is not accessible for roots. Furthermore, an increased production of aquaporin or osmotic metabolites by mycorrhizal fungi are known to decrease the water potential of plants (Lehto & Zwiazek, 2011; Rapparini & Peñuelas, 2014; Phillips *et al.*, 2016). Whether AM or ECM trees are more tolerant to drought is uncertain due to inconsistent findings

(Querejeta *et al.*, 2009; Brzostek *et al.*, 2014; Mohan *et al.*, 2014), underlining that the role of the mycorrhizal type for host plants under drought is not well understood. However, systematic differences in drought tolerance between AM and ECM associations could result in a compositional shift in the dominant mycorrhizal association type under more frequently occurring droughts and may affect important root functions, belowground C allocation, and nutrient dynamics on ecosystem scale.

Objectives of the study

For predicting future developments of forest ecosystems in respect to C dynamics and nutrient cycling under climate change, it is of fundamental importance to understand the extent of effects which arise from the type of mycorrhizal association. The distinction between AM and ECM tree species may provide a powerful tool in framework predictions of global change impacts on temperate forests (Phillips *et al*. 2013). A deeper understanding of functional differences in root-rhizosphere interactions between AM and ECM trees is required to incorporate the type of mycorrhizal association in forest ecosystem models.

The overarching hypothesis of this study was that the type of mycorrhizal association has a strong influence on major root functions like nutrient acquisition, root exudation, and root lifespan and mediates the plant-soil feedback especially under conditions of more frequently occurring droughts.

The objectives of this study were:

- identification of mycorrhiza-specific differences in morphological root traits, functional root traits, and rhizosphere processes.
- evaluating the role of the type of mycorrhizal association in linkages of root functional and morphological traits with nutrient acquisition and microbial activity in the rhizosphere.
- investigation of drought effects on AM and ECM root functions and of the effect of the mycorrhizal association type on drought sensitivity of trees.

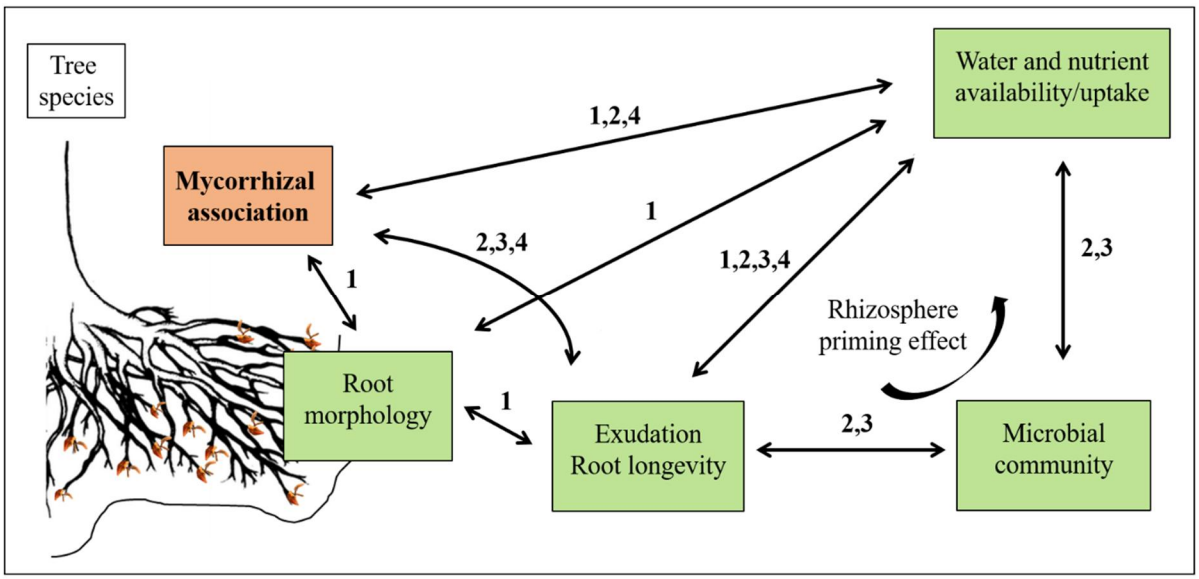

Figure 1 A simplified conceptual causal loop diagram for illustrating the relationships and interactions between the type of mycorrhizal association, important root functions, and rhizosphere processes to elucidate the main hypothesis of this study.

Based on these objectives, the main hypothesis of the study were as follows (see Figure 1):

1. The fine and highly branched root system of ECM trees is linked with decreased root lifespan and related to precision foraging and more acquisitive root traits.
2. The extent of C release through root exudation is higher in ECM than in AM trees, resulting in higher acquisition of organic N as a consequence of the organic nutrient economy of ECM ecosystems.
3. AM and ECM root exudates differ in their composition with strong impacts on microbial activity and biogeochemical processes in the rhizosphere.
4. Drought leads to a stronger reduction in root exudation and root lifespan of ECM than of AM trees, because of a higher drought sensitivity of ECM tree species.

Methodical approach

To answer the question how strong and to what relevance for the ecosystem rhizosphere processes, root functions and root traits are influenced by the type of mycorrhizal association, four tree species per mycorrhizal type were studied in a combination of two main projects:

1. A field study in a temperate broad-leaved mixed forest stand in Central Germany representing a mature forest stand with natural occurrence of several AM and ECM tree species (Chapter 2 and 3).
2. A factorial drought experiment in large-scale mesocosms with young trees of four AM and four ECM tree species. The work was performed in the Göttingen Rhizolab and its associated lysimeters (Chapter 4 and 5).

In both studies, eight tree species were selected which are frequently dominant or subdominant trees of the natural forest vegetation in Central Europe and represent the two mycorrhizal types (*cf*. Wang & Qiu, 2006): common ash (*Fraxinus excelsior* L.), Norway maple (*Acer platanoides* L.), sycamore maple (*Acer pseudoplatanus* L.), and wild cherry (*Prunus avium* L.) are AM tree species. European hornbeam (*Carpinus betulus* L.), European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), and small-leaved lime (*Tilia cordata* MILL.) are ECM tree species.

I. Research project 1: Field study

Study area

The research was conducted in an old-growth mixed forest 'Hainich National Park' in Thuringia in Central Germany (51°08'N, 10°51'E; see Figure 2), which represents with an area of 7,500 ha one of the largest deciduous broadleaf forests in Central Europe. The climate can be characterized as semi-humid with an annual temperature of 7.7°C and a mean annual precipitation of 590 mm (period 1973-2004; Deutscher Wetterdienst, 2005). Mineral soil (0-30 cm) texture of the study site is characterized by a low content of sand (<5%) and a high content (about 74%) of silt (Guckland *et al.*, 2009). From a base-rich Pleistocene loess layer over Triassic limestone (Middle Muschelkalk) a eutrophic Luvisols developed (IUSS, 2006) with a vegetation classified as Stellario-Carpinetum (starwort-oak-hornbeam forest, interfused with elm trees). The study area is a part of a large section of the 'Hainich National Park' that has been unmanaged over the last 40 years and developed basically undisturbed and therefore represents ancient woodland (Wulf, 2003). Soil manipulations like e.g. liming were absent. The forest stand has relatively high tree species richness with a total of up to 14 tree species co-occurring and contains an assemblage of AM and ECM tree species.

Experimental design

Circular plots with a diameter of 8 m were randomly selected and contained mature trees of the eight selected tree species. Two to three neighboring trees or one tree with a dominant position of the targeted tree species formed the center of the circular plots. This cluster scheme ensured that bulk and rhizosphere soil of the fine roots belonged to the targeted tree species (*cf*. Kubisch *et al.*, 2016). To ensure comparability, only mature trees of similar age and crown structure on level to slightly inclined terrain were selected. Each cluster of the studied tree species was replicated three times, resulting in 24 plots in total. All plots were located in an area of approximately 12 ha in the northeast of the national park and in similar landscape positions to

minimize topographic effects. This experiment was sampled over a 12-month period from September 2013 to August 2014.

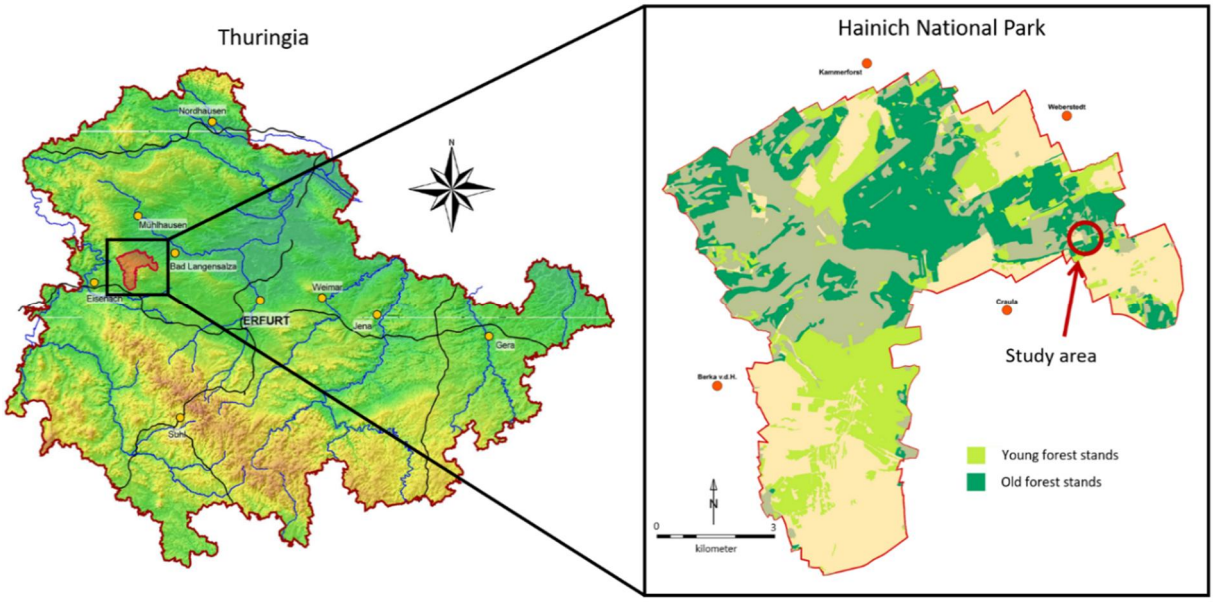

Figure 2 Location of the study area in the Hainich National Park in Thuringia, Germany (National Park Authority Hainich, 2018).

Measured parameters

The focus of research project 1 was on the investigation of differences in root-rhizosphere processes between AM and ECM tree species in order to identify effects of the mycorrhizal type on root functions and biogeochemical dynamics in the rhizosphere. The following parameters were measured during this study:

- Root functions: C release (quantity) and chemical richness (quality) of root exudates
- Root architecture and morphology: fine root diameter, root tissue density, specific root length, specific root area, root branching intensity, degree of mycorrhizal colonization
- Rhizosphere processes: microbial biomass, potential activity of extracellular N and P degrading enzymes, N supply (net N mineralization, free amino acids, C:N ratio), P availability, and water availability

These measurements were accompanied by a parallel study that investigated several leaf traits (specific leaf area, tissue density, N content and C:N ratio) and additional root traits in root order morphology (separation of first and second root from third to fifth root order).

II. Research project 2: factorial drought experiment in large-scale mesocosms

Plant material

About 25 young trees per species of this study were collected from the 'Hainich National Park' in Thuringia in Central Germany in two campaigns in September 2011 (AM: common ash and sycamore maple; ECM: European beech and small-leaved lime) and September 2012 (AM: Norway maple and wild cherry; ECM: pedunculate oak and European hornbeam). The selected individuals were similar in tree height (about 30 cm) and crown dimensions and were colonized by indigenous mycorrhizal communities. Eight young trees per species were planted at the Göttingen Rhizolab and associated lysimeters.

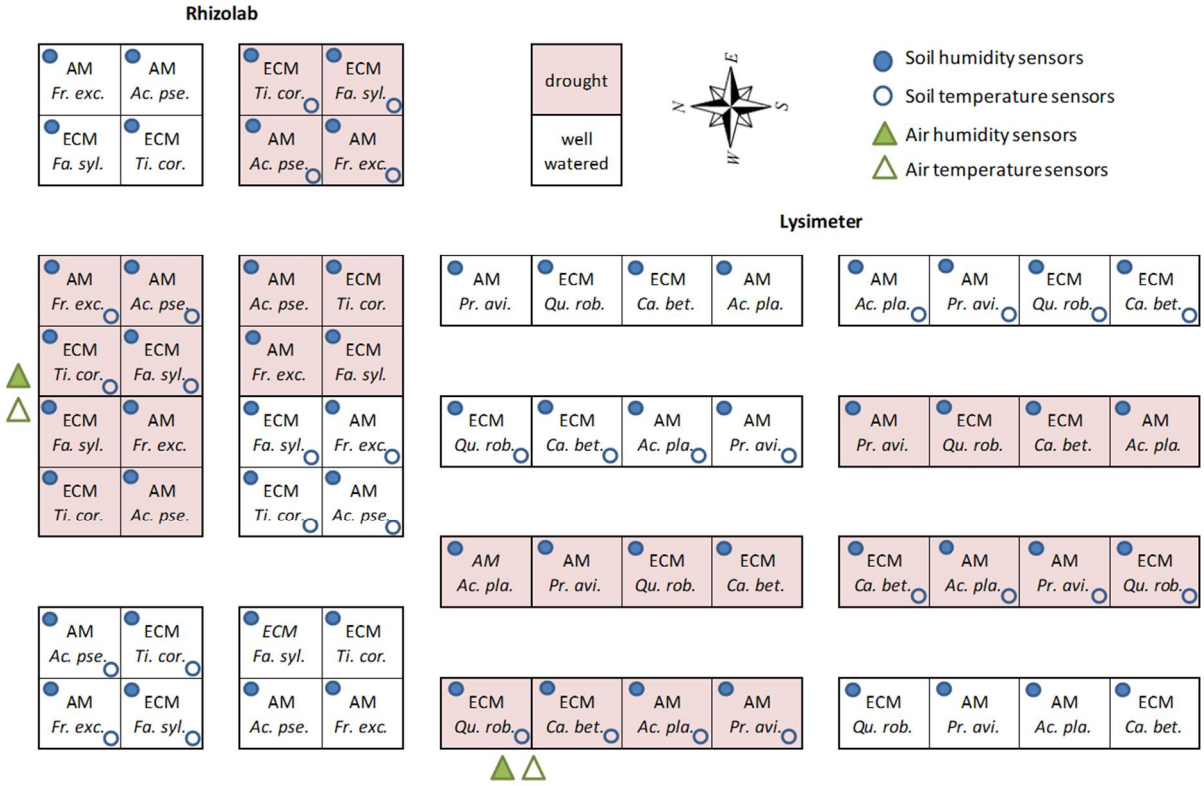

Figure 3 Experimental setup of research project 2.

Experimental design

The Göttingen Rhizolab is an outdoor facility designed for long-term investigations of root growth and dynamics of trees and ensures full control of soil water by an automatic covering of mobile Plexiglas rain shelters during precipitation while glasshouse effect are avoided (*cf.* Meier & Leuschner, 2008). Eight young trees per species were planted in 16 drained large-scale containers with the rims at ground level (according to a randomized block design). Each container was divided by polyethylene plates into four plots, resulting in 64 plots (see Figure 3) in which each mini-rhizotrons were installed. Two soil moisture treatments were initiated

and replicated four times per tree species: a drought treatment (5% SWC, v/v) and a well-watered treatment (10% SWC, v/v). The soil water content was measured continuously and adjusted every other day by homogenous drip irrigation. The drought treatment was paused during the non-growing seasons, where natural precipitation brought the soil water content back to field capacity. The experiment was conducted from spring 2014 to autumn 2015 and thus simulated two consecutive summer droughts of about 24 weeks each. Soil and air temperature and humidity were recorded continuously as microclimatic data.

Measured parameters

The focus of research project 2 was on the influence of the type of the mycorrhizal association on root functions, C cycling and N uptake under defined drought stress. The following parameters were measured:

- Root functions: C release via root exudation, fine root lifespan (mini-rhizotrons), organic and inorganic N absorption
- Root architecture and morphology: fine root diameter, root tissue density, specific root length, specific root area, root branching intensity, rooting depth, degree of mycorrhizal colonization
- Belowground and aboveground biomass production: total root biomass, coarse root biomass, fine root biomass, total shoot biomass, stem biomass, leave biomass
- Aboveground properties: photosynthesis, leaf respiration, relative growth rates, C:N ratios, foliar ^{13}C and ^{15}N signature

Chapter 2

ROOT BRANCHING IS A LEADING ROOT TRAIT OF THE PLANT ECONOMICS SPECTRUM IN TEMPERATE TREES

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Abstract

Global vegetation models use conceived relationships between functional traits to simulate ecosystem responses to environmental change. In this context, the concept of the leaf economics spectrum (LES) suggests coordinated leaf trait variation, and separates species, which invest resources into short-lived leaves with a high expected energy return rate from species with longer-lived leaves and slower energy return. While it has been assumed that being fast (acquisitive) or slow (conservative) is a general feature for all organ systems, the translation of the LES into a root economics spectrum (RES) for tree species has been hitherto inconclusive. This may be partly due to the assumption that the bulk of tree fine roots have similar uptake functions as leaves, despite the heterogeneity of their environments and resources. In this study we investigated well-established functional leaf and stature traits as well as a high number of fine root traits (14 traits split by different root orders) of thirteen dominant or subdominant temperate tree species of Central Europe, representing two phylogenetic groups (gymnosperms and angiosperms) and two mycorrhizal associations (arbuscular and ectomycorrhizal). We found reflected variation in leaf and lower-order root traits in some (surface areas and C:N) but not all (N content and longevity) traits central to the LES. Accordingly, the LES was not mirrored belowground. We identified significant phylogenetic signal in morphological lower-order root traits, i.e. in root tissue density, root diameter, and specific root length. By contrast, root architecture (root branching) was influenced by the mycorrhizal association type which developed independent from phylogeny of the host tree. In structural equation models we show that root branching significantly influences both belowground (direct influence on root C:N) and aboveground (indirect influences on specific leaf area and leaf longevity) traits which relate to resource investment and lifespan. We conclude that branching of lower order roots can be considered a leading root trait of the plant economics spectrum of temperate trees, since it relates to the mycorrhizal association type and belowground resource exploitation; while the dominance of the phylogenetic signal over environmental filtering makes morphological root traits less central for tree economics spectra across different environments.

Keywords: angiosperm trees, arbuscular mycorrhiza, ectomycorrhiza, fine root traits, gymnosperm trees, precision foraging, root economics spectrum, root order

Introduction

Plant functional trait spectra are valuable tools in simplifying floristic complexity to a level that can be handled in models which scale ecosystem processes to landscape and global scales. Theory on plant growth strategies suggests that plants characteristic of low- and high-resource environments, respectively, evolved a common set of traits linking exploitation (root: shoot, tissue turnover, and concentration of plant defences) with growth (resource uptake and growth rates) (Grime, 1977; Chapin III *et al.*, 1993; Bardgett *et al.*, 2014). In continuation of this theory, the leaf economics spectrum (LES) describes a universal spectrum on the return of nutrient and dry mass investments in leaves (Wright *et al.*, 2004): fast, acquisitive species with high expected rate of energetic return on investment possess relatively large, fast growing leaves with short lifespan, high N content per unit mass, high specific leaf area (SLA), and high instantaneous rates of respiration and photosynthesis in comparison to slow species. This suggests convergence of leaf traits of coexisting species under similar environmental conditions, despite the great genotypic diversity among these species (Reich *et al.*, 2003). The LES seems to operate largely independent of growth form, plant functional type, or biome (Wright *et al.*, 2004), and has been successfully linked to plant performance (Reich *et al.*, 1998; Poorter & Bongers, 2006), species distribution and interactions (Sterck *et al.*, 2006), and ecosystem processes and services (Reich *et al.*, 1997; Díaz *et al.*, 2004; Grigulis *et al.*, 2013; Weemstra *et al.*, 2016).

Despite the successful application of the LES and the translation into a correspondent wood economics spectrum (WES; Chave *et al.*, 2009), its translation into a root economics spectrum (RES) for trees has been inconclusive so far and is still a matter of debate. By theory, being fast or slow should be a general feature of species (Reich, 2014). Consequential, acquisitive species with respect to their leaf traits should possess relatively small-diameter, fast-growing fine roots with short lifespan, high N content, high specific root length (SRL), and high rates of respiration and nutrient acquisition in comparison to slow, conservative species with long-term resource retention. This theoretic RES has been partly confirmed for trees in some studies (Chen *et al.*, 2010; McCormack *et al.*, 2012; Reich, 2014), but scrutinized by others (Comas & Eissenstat, 2004; Withington *et al.*, 2006; Chen *et al.*, 2013; Valverde-Barrantes *et al.*, 2015; Weemstra *et al.*, 2016). Often, not the whole set of traits for a RES for mature trees is covered by single studies using standardized methods, which makes overall conclusions difficult.

The complex architecture of root systems has traditionally been categorized according to root diameter in fine and coarse roots, which may not reflect their functionality, especially among

tree species with systematic differences in mean root diameter. More recent work, which focused on the classification of fine roots according to a stream-based ordering system (Pregitzer *et al.*, 2002), has proved that only the most distal fine root orders serve (primarily) water and nutrient acquisition (Guo *et al.*, 2008b; Rewald *et al.*, 2011; McCormack *et al.*, 2015). These distal fine root orders should have similar functionality across species and be a reflection of the resource acquisition function of leaves, which makes their traits more suitable for an inspection of the RES. However, resource uptake belowground differs vastly from aboveground resource capture: light and CO_2 are predictably available throughout the canopy while nutrients and water are often highly heterogeneously distributed in the soil, which increases the importance of traits related to precision foraging (prolific root branching and mycorrhizal symbioses) over traits which maximize the surface area *per se*. The branching architecture is an expression of the plastic responses of roots to their environment since it seems to be independent from phylogeny, at least in subtropical trees (other than diameter-related root traits; Kong *et al.*, 2014). It has been demonstrated that species with high branching intensity are capable of rapid and extensive proliferation into resource-rich patches (morphological plasticity; reviewed by Hodge, 2004). Traits related to precision foraging of roots are missing in the current version of the RES, though (Weemstra *et al.*, 2016). In particular, the association with mycorrhizal fungi may complement the foraging strategy of roots for limiting nutrients. Trees associated with different mycorrhizal colonization types differ profoundly in root traits related to precision foraging: ECM trees, which mainly occur in ecosystems dominated by organic nutrients, have thinner roots and higher branching intensity than AM trees (Brundrett, 2002; Smith & Read, 2008; Comas & Eissenstat, 2009; Comas *et al.*, 2014; Eissenstat *et al.*, 2015). Yet it is unknown if ECM trees belong systematically to the more acquisitive root spectrum in comparison to AM trees.

In the work presented here, we analyzed sun leaf, stature, and fine root traits of the first to fifth root order of thirteen important temperate tree species of the Central European tree flora, which represented two phylogenetic groups (gymnosperms and angiosperms) and two mycorrhizal association types (AM and ECM). Sun leaf and fine root samples were collected from three mixed forest stands in the center of Germany. For the comparison of fine roots, which serve similar functions among tree species, we separated fine root strands into two root order fractions (first to second and first to fifth root order). We analyzed fine root fractions for nine traits, including specific root area (SRA), SRL, tissue density, branching ratio, branching intensity, root diameter, root N_{mass}, and root C:N, and obtained information on fine root longevity from an accompanying comprehensive literature survey. We hypothesized that (i) fine root

morphology is phylogenetically structured, (ii) the RES is not a mirrored analogy of the LES, but centres around traits related to precision foraging, i.e. around root branching, in which trees with intense root branching belong to the fast, acquisitive spectrum and trees with reduced root branching belong to the slow, conservative spectrum, and (iii) ECM trees have higher branching intensity and more acquisitive root traits in comparison to AM trees.

Materials and Methods

Study sites and tree species

Sampling from thirteen major Central European tree species was conducted in three mixed forest stands in Central Germany, which represented characteristic, mesic mesotroph site conditions for the investigated tree species: two study sites incorporated replicate sites for angiosperm tree species ('Hainich National Park' at 340 m a.s.l., 51°08'N, 10°51'E and 'Experimental Botanical Garden Göttingen' at 200 m a.s.l., 51°55'N, 9°96'E) and one study site covered the gymnosperm tree species ('Moringen City Forest' at 310 m a.s.l., 51°73'N, 9°86'E). Stands were mature and even-aged, and predominately hardwoods and hardwoods interspersed with evergreens, respectively, in the case of the Moringen City Forest. All sites had a mean annual temperature between 7.5 and 9.0°C and mean annual precipitation between 630 and 670 mm. Last forest management activities occurred at least a couple of decades ago and soil manipulation activities such as liming were absent.

The selected major tree species of the Central European forest flora are either dominant species of the natural forest vegetation or are frequently present in forest communities as subdominant or admixed species. The 13 species represent a broad range of taxa, covering eleven genera, eight families, and six orders (Supplementary Table 1). Among the thirteen species are four conifers (family *Pinaceae*) and nine deciduous broad-leaved species from the families *Fagaceae*, *Sapindaceae*, *Malvaceae*, *Betulaceae*, *Oleaceae*, and *Rosaceae*. The species were selected to represent two phylogenetic groups (gymnosperms and angiosperms) and two mycorrhizal association types (AM and ECM; Supplementary Table 1). The association to a mycorrhizal association type was assigned to according to literature (Wang & Qiu, 2006), and was confirmed by measurements of the arbuscular and ectomycorrhizal colonization rates in an accompanying study (Liese, pers. communication).

Leaf and fine root sampling and analyses

Leaf samples of angiosperm tree species were collected from the upper sun canopy with the help of canopy walkways in mid-summer 2014 (*n* = five leaf samples each of five individuals per tree species and study site). Leaf samples were stored at 6°C for no more than a week until processing. All leaves were analyzed for leaf area using a flat-bed scanner and the computer program WinFOLIA (2005b; Régent Instruments Inc., Canada). Subsequently, the total leaf mass was dried (70°C, 48 h) and weighed and the SLA ($cm^2\ g^{-1}$) calculated. Dried leaf samples were ground and total carbon and nitrogen content analyzed using a C:N elemental analyzer (vario EL III, elementar, Hanau, Germany). Sun leaf samples of gymnosperm trees were not easily accessible and trait information was derived from a comprehensive literature survey instead (see below; Supplementary Table 2).

Fine root samples of all tree species were carefully excavated from the uppermost 20 cm of the soil profile in close surroundings (<50 cm) of mature canopy trees of the respective species, which were growing in single-species tree clusters, and were traced towards their mother tree (*n* = ten root samples each of at least five different individuals per tree species and study site). Root samples were immediately transported to the laboratory and stored moist at 6°C for no longer than three weeks until processing. Root strands were cleared from soil particles with tap water and the tree species identity was confirmed a second time under a stereomicroscope (magnification x 40) with a site-specific morphological key based on periderm structure and color, root ramification, and root tip morphology (*cf.* Meinen *et al.*, 2009; Kubisch *et al.*, 2015). All vital, intact root strands were cut at the end of the fifth root order (stream-based ordering system according to Pregitzer *et al.*, 2002, with the most distal root segments being classified as first root order) for comparability between tree species. We selected to cut root systems at the end of the fifth root order, since the sixth and higher order roots occasionally comprised roots with a diameter >2 mm, i.e. could not be classified as fine roots. The first to fifth root orders were constituted of only fine roots (diameter <2 mm) in all investigated tree species. We counted root tips of these intact root systems under a stereomicroscope.

Half of the intact root samples were analyzed for their morphology of the first to fifth root order using a flat-bed scanner and the computer program WinRHIZO (2005c; Régent Instruments Inc., Canada) (200 dpi; *n* = five root samples each of at least five different individuals per tree species and study site) in order to determine root length, surface area, diameter, and volume. Root systems comprising the first to fifth root order were analyzed intact for comparability with other studies that are not separating between different root orders. Subsequently, root strands

were dissected with scalpels under a stereomicroscope to separate the absorbing root orders, i.e. the first and second order (Guo *et al.*, 2008b; Valenzuela-Estrada *et al.*, 2008) from the transport root orders, i.e. third to fifth order. Dissected first and second root orders were scanned again and analyzed for their morphology. The two root order fractions (first and second order and third to fifth order) were dried (70°C, 48 h) and weighed. SRA ($cm^2\ g^{-1}$), SRL ($cm\ g^{-1}$), tissue density ($g\ cm^{-3}$), and mean root diameter were calculated independently for (i) the first and second root order and (ii) the first to fifth root order. The branching ratio was determined from the number of first order roots growing out of second order roots ($n\ n^{-1}$). Branching intensity was calculated from the number of root tips per root length of first and second order roots ($tips\ cm^{-1}$). The absorptive to transport root ratio was calculated by dividing the mass of the first and second root orders by the mass of the third to fifth root orders ($g\ g^{-1}$).

The second half of the intact root samples was dried (70°C, 48 h), ground, and total carbon and nitrogen content analyzed using a C:N elemental analyzer (vario EL III, elementar, Hanau, Germany) (*n* = five root samples each of at least five different individuals per tree species and study site). The analyzed $C:N_{1\text{-}5}$ describes the C:N ratios of a representative fine root population for all tree species, comprising the first to fifth root order.

Additional traits

Based on a comprehensive literature survey and additional data (SLA, leaf N, and maximum tree height) from the TRY Plant Trait Database (Kattge *et al.*, 2011), we assembled a database of about 40 published and unpublished studies that contained information related to SLA and leaf N (for the four gymnosperms of interest to this study), as well as information on leaf longevity, maximum tree height, wood density, maximum tree age, and fine root longevity (for all 13 tree species of interest to this study). Selection criteria for data were (a) study plot located in the cool-temperate zone of Central Europe, (b) measurements taken in mature trees (>40 years old) in monospecific stands with closed canopy, (c) last forest management activities occurred at least a decade ago, and (d) absence of soil manipulation such as liming. All data on SLA referred to sun leaves in the upper sun canopy and mostly were taken using towers or cranes.

Phylogenetic signal

The phylogenetic signal was estimated by the correlation between the phylogenetic distance and trait distance matrices among the investigated tree species. We attached our list of taxa to the master phylogeny presented by Zanne *et al.* (2014) with the help of the software

PHYLOMATIC v3 (a tool associated to PHYLOCOM 4.2; Webb *et al.*, 2008), to generate the initial phylogenetic tree in the Newick format. The simple pairwise matrix of phylogenetic distances was calculated from the Newick code with the 'phydist' phylogeny tool in PHYLOCOM and visualized with the online tool iTOL - Interactive Tree Of Life v3.1 (Ciccarelli *et al.*, 2006; Supplementary Fig. 1).

We identified major trait complexes explaining more than 75% of the variance for leaf, stature, and root traits, respectively, by calculating three independent PCAs, using the package Canoco 5.03 (Biometris, Wageningen University and Research Centre, The Netherlands; Supplementary Table 3). Independent trait distance matrices based on the PCA axes for leaf, stature, and root traits, respectively, were calculated with the package SAS, version 9.3 (Statistical Analyses System, SAS Institute Inc., Cary, NC, USA). For the correlation between the phylogenetic and trait distance matrices, a Mantel permutation test (Mantel, 1967; Mantel & Valand, 1970) was computed with PAST 3.11 (Øyvind Hammer, Natural History Museum, University of Oslo, Norway), and the Pearson correlation coefficient *R* and the one-tailed *P* value from the comparison of the original *R* to the *R* computed in 9999 random permutations were reported. Euclidean similarity indices were used for the Mantel permutation test.

As a second estimate of the phylogenetic signal, we conducted node-level analyses of traits and of trait conservation. We determined the average standard deviation of values at daughter nodes ('divergence') as a measure of trait radiation at this node (conservative: divergence <1, divergent: divergence >1) with the 'aot' phylogenetic trait analysis algorithm in PHYLOCOM (999 randomizations) and calculated the node age as branch length in percent of total phylogenetic distance.

Statistical analyses

All data were tested for probability of fit to normal distribution by a Shapiro-Wilk test (SAS 9.3; SAS Institute Inc., Cary, NC, USA). Leaf and root longevity were log-transformed to correct departures from normality. We tested for multicollinearity between traits by Pearson correlations and identified collinearity for the correlation between leaf C:N and leaf longevity, SRA_{1+2} and tissue $density_{1+2}$, and SRL_{1+2} and tissue $density_{1+2}$ ($R > 0.90$); all three were thus excluded from further analyses. Means of the tree groups (AM angiosperms, ECM angiosperms, and ECM gymnosperms) were compared by one-way analyses of variance (ANOVA) followed by a Scheffé test. Mixed variance-covariance models for fixed and random effects with the variables mycorrhizal association type (AM vs. ECM) and phylogenetic group (gymnosperm vs. angiosperm) were calculated to test for significant effects. Data likelihood

was maximized to estimate the model parameters. A canonical correspondence analysis (CCA) was calculated for the stepwise forward selection of root traits that maximized the centroid distances between ECM gymnosperms, ECM angiosperms, and AM angiosperms, using the package Canoco 5.03 (Biometris, Wageningen University and Research Centre, The Netherlands). A total of 499 random permutations were used.

We used SPSS Amos 24.0.0 software (IBM, Somers, NY, USA) to calculate structural equation models (SEM). SEM was applied for identifying the direct and indirect effects of fine root branching intensity and branching ratio (as indication of the mycorrhizal association type) on leaf, stature, and fine root traits other than root branching intensity and branching ratio in the investigated tree species. We started with an initial model that contained all plausible interactions between root, stature, and leaf traits (Supplementary Fig. 2). Path coefficients were determined as standardized regression weights using the maximum likelihood method. Modification indices were used to evaluate potential modifications of the model, which were plausible and minimized the χ^2. Two goodness-of-fit indices were accounted for [Tucker-Lewis Index TLI (Tucker & Lewis, 1973) and Root Mean Square Error of Approximation RMSEA (Browne & Cudeck, 1993). Insignificant paths were eliminated from the model. The square of the coefficient of multiple correlations R^2 was calculated for all dependent variables.

Results

Above- and belowground trait relations

SLA, leaf C:N, and leaf longevity related to a number of root traits, while leaf N did not relate to any of the investigated root traits (Supplementary Table 4). SLA mainly correlated with the root morphology of the first and second root order (SRL_{1+2} and SRA_{1+2}: positive correlation; diameter and tissue density: negative correlation), as well as with the branching intensity (i.e., the number of root tips per lower order root length; positive correlation) of the root system and its N content (positive correlation). In a direct comparison of the morphology of the absorbing tissues, SLA significantly increased by 12 $cm^2\ g^{-1}$ with an increase in SRL_{1+2} by 10 $m\ g^{-1}$ (Fig. 1a).

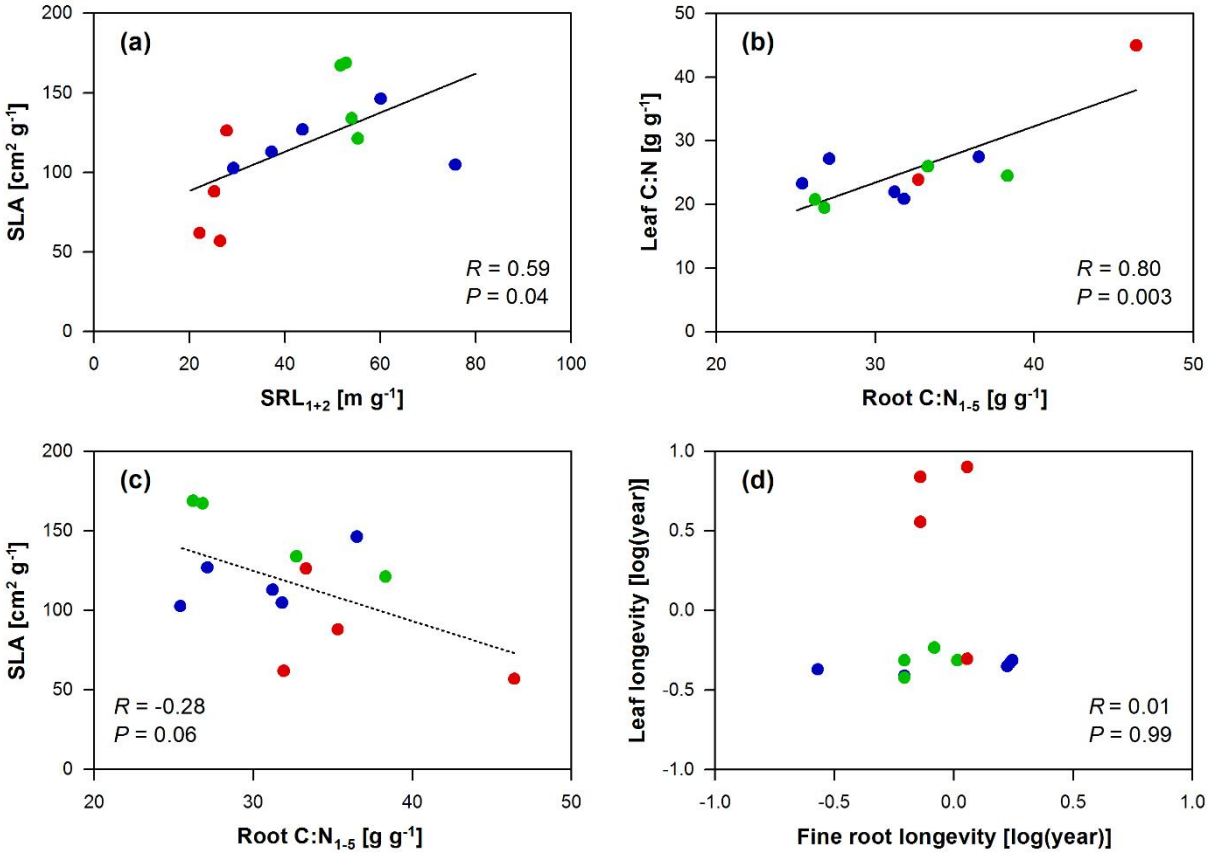

Figure 1 Pearson's correlation analyses between leaf and root traits of the ECM gymnosperm (red), ECM angiosperm (green), and AM angiosperm (blue) tree species analyzed in this study.

In an opposite trend, an increase in the C:N in the root tissue by 10 g g^{-1} correlated to a decrease in SLA by 32 cm^2 g^{-1} (marginal significant; Fig. 1c). Yet the strongest (positive) correlation with the root C:N had the leaf C:N ratio, which may hint to a whole plant trait coordination with respect to C:N variation (Fig. 1b). Surprisingly, leaf longevity did not relate to root longevity neither in the whole tree species data set nor in the subset of angiosperm tree species (P = 0.99 and 0.38, respectively; Fig. 1d). Leaf longevity was strongly positively correlated with root diameter and root tissue density$_{1+2}$ (Supplementary Table 4).

Phylogenetic signal in root and leaf traits

In a comparison of the two investigated phylogenetic groups (gymnosperms and angiosperms) it appears that there was a highly significant influence by phylogenetic group affiliation on the mean root diameters of all roots and on the root tissue densities of lower order roots: gymnosperms had a higher mean root diameter$_{1-5}$ (0.53 vs. 0.39 mm) and a higher root tissue density$_{1+2}$ (0.24 vs. 0.15-0.18 g cm^{-3}) than angiosperm tree species (Table 1).

Table 1 Trait values for AM angiosperm (n = 5), ECM angiosperm (n = 4), and ECM gymnosperm (n = 4) tree species (given are means and standard errors). Absorptive roots are defined as root orders 1+2, transport roots as root orders 3-5. Values in parentheses are SD. Significant differences between the three tree groups are indicated by different upper case letters. The coefficient of variation (CV) describes trait dissimilarity. CVs larger than 50% are written in bold. Asterisks denote a significant effect of the mycorrhizal association type (AM vs. ECM) or phylogenetic group (gymnosperm vs. angiosperm) on the respective trait according to mixed effects models. Significance is indicated as (*) $P \leq 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. [a], literature data.

Traits	AM angiosperm	ECM angiosperm	ECM gymnosperm	CV [%]	Mycorrhizal association	Phylog. group
LEAVES						
SLA [$cm^2\ g^{-1}$]	119 (8) ^{AB}	148 (12) ^{A}	83 (16) ^{B}	30		**
Leaf N_{mass} [$mg\ g^{-1}$]	19 (1)	22 (1)	23 (4)	22		
Leaf C:N [$g\ g^{-1}$]	24 (1)	22 (1)	36 (7)	27		*
Leaf longevity[a] [yr]	0.5 (0.02) ^{B}	0.5 (0.04) ^{B}	4.9 (1.7) ^{A}	**149**		**
STATURE						
Max. tree height[a] [m]	34 (6) ^{B}	48 (7) ^{AB}	60 (6) ^{A}	35		
Wood density[a] [$kg\ m^{-3}$]	598 (16) ^{AB}	653 (62) ^{A}	470 (32) ^{B}	18		**
Max. tree age[a] [yr]	230 (44) ^{B}	400 (54) ^{AB}	413 (38) ^{A}	37	*	
ROOTS						
SRL_{1+2} [$m\ g^{-1}$]	49 (8) ^{A}	53 (1) ^{A}	25 (1) ^{B}	38		**
Tissue $density_{1+2}$ [$g\ cm^{-3}$]	0.18 (0.02) ^{B}	0.15 (0.01) ^{B}	0.24 (0.01) ^{A}	23		***
Branching ratio [$n\ n^{-1}$]	2.8 (0.2)	2.3 (0.2)	2.5 (0.3)	19	(*)	
Branching intensity [$tips\ cm^{-1}$]	5.4 (1.2) ^{AB}	9.6 (1.1) ^{A}	3.3 (0.9) ^{B}	**53**	*	**
Absorptive : transport roots [$g\ g^{-1}$]	1.0 (0.3)	0.5 (0.1)	0.7 (0.2)	**55**	(*)	
$Root\ diameter_{1+2}$ [mm]	0.41 (0.02)	0.42 (0.01)	0.47 (0.01)	9		*
$Root\ diameter_{1-5}$ [mm]	0.39 (0.02) ^{B}	0.39 (0.02) ^{B}	0.53 (0.01) ^{A}	18		***
Root $N_{mass,\ 1-5}$ [$mg\ g^{-1}$]	13 (1)	14 (1)	12 (1)	13		
Root $C:N_{1-5}$ [$g\ g^{-1}$]	30 (2)	31 (3)	37 (3)	18		
Fine root longevity[a] [yr]	1.0 (0.3)	0.8 (0.1)	0.9 (0.1)	**48**		

Consequently, SRL_{1+2} of lower root orders (25 vs. 49-53 $m\ g^{-1}$) and branching intensity (3.3 vs. 5.4-9.6 $tips\ cm^{-1}$) of the gymnosperm root systems were reduced. Our discriminant analysis revealed that mean $root\ diameter_{1-5}$ and root $C:N_{1-5}$ were the most important root traits for the discrimination between gymnosperm and angiosperm tree species, and explained together 45% of the total variation (Fig. 2).

Aboveground, gymnosperms differed by lower SLAs (83 vs. 119-148 $cm^2\ g^{-1}$) and wood densities (470 vs. 598-653 $kg\ m^{-3}$) from the hardwood species (Table 1). As a consequence of the difference in their leaf xeromorphic structure and ecological function, phylogenetic group affiliation had a significant effect on leaf longevity, which distinguished from the other traits by the distinctly highest coefficient of variation (149%). By contrast, despite its moderately high coefficient of variation (48%), mean fine root longevity did not significantly differ between phylogenetic groups. Further, root N_{1-5} and root $C:N_{1-5}$ of the first to fifth root order

varied only little between the investigated tree species (13-18%) and did not significantly differ between phylogenetic groups.

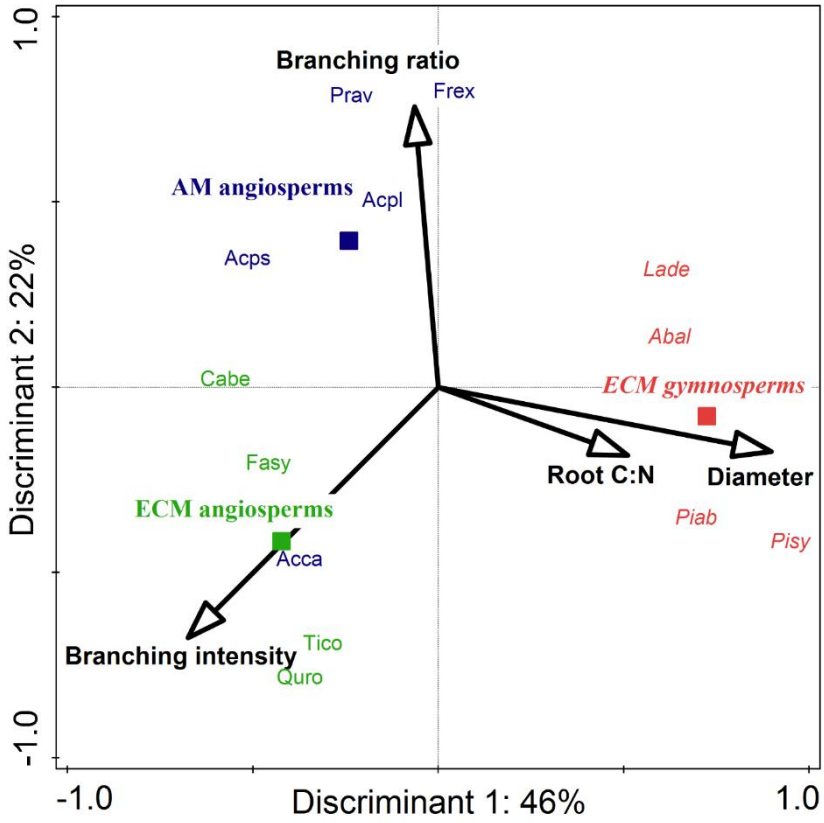

Figure 2 Canonical correspondence analysis (CCA) for the stepwise selection of root traits for the discrimination between ECM gymnosperms (red italic), ECM angiosperms (green), and AM angiosperms (blue) among 13 Central European tree species. Solid squares mark the centroid of each group. Out of a total of eight preselected root traits, four discriminants were needed to explain 65% of the variation, with the highest contribution by root diameter$_{1\text{-}5}$ (P=0.01) and branching intensity (P=0.08). For abbreviations of tree species refer to Supplementary Table 1.

The phylogenetic signal estimated by the correlation between the phylogenetic distance and the trait distance matrices was highly significant for the first PCA axis calculated for root traits (PCA Root 1), which was mainly related to tissue density$_{1+2}$, SRL$_{1+2}$, and root diameter$_{1\text{-}5}$ (Table 2). About 56% of the variation of the trait distance matrix for PCA Root 1 was explained by the relatedness of tree species (R = 0.75), with 6% of the nodes of the phylogenetic tree exhibiting significant trait conservatism towards PCA Root 1 (divergence SD 0.35, mean age 29% branch length of the total phylogenetic distance) and no significant divergence. Another strong phylogenetic signal was detected for the first PCA axis calculated for leaf traits (PCA Leaf 1), which was mainly related to SLA and leaf longevity (explained variation: 37%, R = 0.61), and a slightly weaker signal in the second axis for leaf traits (PCA Leaf 2), which was mainly related to leaf N_{mass} (explained variation: 25%, R = 0.50). Both, the second PCA axis for root traits (PCA Root 2; related to the root branching ratio, root C:N$_{1\text{-}5}$, and root N$_{1\text{-}5}$) and the two PCA axes for stature traits were not significantly influenced by a phylogenetic signal.

Influence of the mycorrhizal association type on root and leaf traits

The mycorrhizal association type (AM and ECM) had a significant effect on the branching intensity of root systems: AM angiosperm root systems had a lower branching intensity than root systems of ECM angiosperms (5 vs. 10 tips cm^{-1}; Table 1). The coefficient of variation between tree species for root branching intensity was moderately high, since it was not only influenced by the mycorrhizal association type but also by the phylogenetic group (the lowest branching intensity was found in ECM gymnosperms: 3 tips cm^{-1}). Trees of differential mycorrhizal association type also differed in their maximum tree age, where AM angiosperms had a significantly lower life expectancy than ECM angiosperm and ECM gymnosperm tree species (230 vs. 390-415 years). The CCA discriminated between AM and ECM tree species mainly by the root traits branching ratio and branching intensity, which explained together 23% of the total variation (Fig. 2). The Mantel permutation test highlighted that there was no phylogenetic signal in the root branching ratio (Table 2).

Table 2 Phylogenetic signal estimated by the correlation between the phylogenetic distance and the trait distance matrices (Mantel permutation test). The trait distance matrices were based on principal components derived for leaf, stature, and fine root traits of 13 Central European tree species (*cf.* Supplementary Table 3). Significant correlations ($P \leq 0.05$) are in bold type.

Trait complex	***R***	***P***
Leaves, PCA axis 1 (SLA and leaf longevity)	**0.61**	**0.003**
Leaves, PCA axis 2 (Leaf N_{mass})	**0.50**	**0.01**
Stature, PCA axis 1 (Max. tree height and age)	-0.07	0.53
Stature, PCA axis 2 (Wood density)	0.27	0.08
Roots, PCA axis 1 (Tissue density$_{1+2}$, SRL_{1+2}, and root diameter$_{1-5}$)	**0.75**	**<0.001**
Roots, PCA axis 2 (Branching ratio, root C:N_{1-5}, and root $N_{mass,\ 1-5}$)	-0.03	0.49

We chose an SEM approach to calculate complex path models of all hypothesized direct as well as indirect effects of root branching on leaf, stature, and root traits (Supplementary Fig. 2). From our previous analyses (see above) we assume that branching ratio and branching intensity can be considered as indication of the mycorrhizal colonization type (*cf.* Brundrett, 2002; Smith & Read, 2008; Comas & Eissenstat, 2009; Comas *et al.*, 2014; Eissenstat *et al.*, 2015). Since leaf C:N and leaf longevity were closely related to each other ($R = 0.92$, $P < 0.001$) and leaf C:N was only little variable, only leaf longevity entered the model. Subsequently, all insignificant paths and variables were eliminated from the primary SEM. The final SEM ($\chi^2 = 44.4$, df = 33, $P = 0.09$) explained approximately 90% of the variation in root diameter$_{1-5}$, 80% of the variation in root C:N, and 45% of the variation in SRL_{1+2} (Fig. 3).

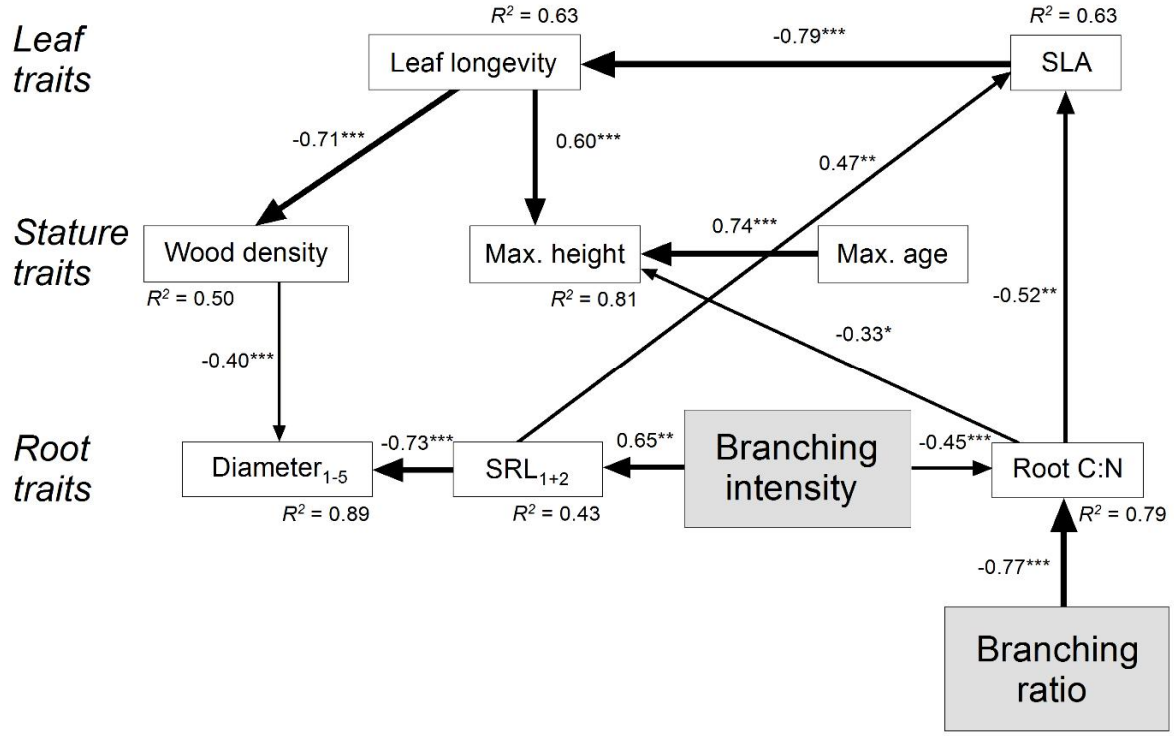

Figure 3 Structural equation model (χ^2 = 44.4, df = 33, P = 0.09) on the effect of fine root branching ratio and branching intensity for leaf, stature, and root traits of major Central European tree species. The direction of the arrows indicates the direction of the influence; the line width illustrates the strength of the path. Path coefficients are standardized regression weights. The square of the coefficient of multiple correlations R^2 is indicated at each variable. Regression weights of latent variables are fixed at unity. Significance is indicated as * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. Insignificant paths and variables (leaf N_{mass}, root $N_{mass, 1-5}$, fine root longevity, and tissue density$_{1+2}$) were eliminated from the final SEM. Data for leaf longevity are log-transformed.

The root branching ratio and intensity directly influenced root C:N (standardized direct negative effects SDE: -0.77 and -0.45). Among the strongest indirect effects of the branching ratio were its effects on SLA (standardized indirect positive effect SIE: 0.40) and leaf longevity (negative SIE: -0.32), i.e. on two aboveground leaf traits. The branching intensity had also a direct influence on SRL$_{1+2}$ (positive SDE: 0.65). Consequently, the strongest indirect effects of the branching intensity were two-directional, on belowground root diameter$_{1-5}$ (negative SIE: -0.59) and on aboveground SLA (positive SIE: 0.54).

Discussion

The LES describes the return on investment in leaves and is thought to better describe the leaf economic variation at the global scale than groupings of plant species into plant functional types (Wright *et al.*, 2004). While the LES has been successfully translated into a wood economics spectrum (WES; Chave *et al.*, 2009), the transfer into a globally consistent RES is still inconsistent. In the current study we could not identify an RES in analogy to the LES. We found two main root trait dimensions that were either influenced by phylogeny (root morphology of

lower order root traits) or by root branching. Root branching was also the leading belowground trait that indirectly influenced (via root C:N) several aboveground traits.

The LES is not mirrored belowground

The physical, chemical, and biological selection pressures for leaves and roots are vastly different. Soil resource uptake, i.e. water and nutrient uptake, is constrained among others by (soil) climatic conditions, diffusion barriers, the soil matrix, bedrock chemistry, pore size, and soil compaction. Yet in a simplification of environmental conditions and constraints acting on leaves and roots, the RES is explored as analog of the aboveground trait axis between SLA, leaf N content per unit mass, rates of respiration and photosynthesis, growth rate, and longevity (Reich *et al.*, 1997). According to this chain of thought, SRL should have a key position in the RES similar to SLA in the LES, and correlate positively with root N and respiration and negatively with root longevity. Empirical evidence for such RES is contradictory, though. In our study some leaf traits of the investigated tree species were reflected by their root counterparts (surface areas and C:N) while others central to the RES were not (N content per unit mass and longevity; Supplementary Table 4). Previously, SLA and SRL as well as leaf and root N and P contents were found positively related across tree species (Reich *et al.*, 1998; Wright *et al.*, 2004; Holdaway *et al.*, 2011), but the generality of the coordinated variation of above- and belowground morphological and chemical traits has been challenged (Valverde-Barrantes *et al.*, 2015; Weemstra *et al.*, 2016). Further, the limited number of studies which have compared the root longevities of different tree species seem to indicate that leaf and root lifespans are generally uncorrelated (Withington *et al.*, 2006; Espeleta *et al.*, 2009; McCormack *et al.*, 2012).

In addition to the missing coordinated variation of above- and belowground traits, we could not identify an RES with respect to relations between SRL or root diameter with root chemistry (root N_{mass}) or function (root longevity). Root N concentration was also not correlated with morphological traits in other studies comparing different temperate tree species (Withington *et al.*, 2006; Comas & Eissenstat, 2009; Chen *et al.*, 2013; Valverde-Barrantes *et al.*, 2015), which was explained by the greater cross-species variation in root morphology than in root N (Comas & Eissenstat, 2009; this study; Chen *et al.*, 2013). Root N content of lower-order roots of temperate trees is generally less variable than its morphology since it is mainly located in cortical tissues which have a relatively constant proportion across roots of different diameter (Guo *et al.*, 2008b). Studies in different biomes have found that root N and SRL of trees correlated with root respiration (Reich *et al.*, 1998; Reich *et al.*, 2003; Chen *et al.*, 2010), and

all correlated with root lifespan (Withington *et al.*, 2006; McCormack *et al.*, 2012; Reich, 2014). However, a literature review and meta-analysis found little evidence for a relationship between root N and N uptake rates, which was explained by the fact that N uptake rates are less limited by the number of nutrient uptake transporters (which contain only a small fraction of N) than by the availability of N in the soil matrix and the extension of the mycorrhizal hyphae (Weemstra *et al.*, 2016). These authors could also not reveal a consistent evidence for an RES mirroring an LES and argued that the reason is that root traits are under multidimensional restrictions: root traits are simultaneously constrained by various environmental drivers not necessarily related to resource uptake, function differently than aboveground traits, and are offset by mycorrhizal interactions (Weemstra *et al.*, 2016). In conclusion, the key functional traits determining uptake acquisition of belowground resources may not be included in the current RES analogy of LES. Conceivable root traits for soil resource acquisition are the number of superficial adventitious roots, length and density of root hairs or hyphae, cluster root formation, and rooting depth, which relate to the branching of the root system and it's rooting density in the soil.

Root morphology is phylogenetically selected

Both, the mixed variance-covariance model and the Mantel permutation test revealed a significant phylogenetic signal (as an indication of selective pressure) for morphological root traits, i.e. for root tissue density$_{1+2}$, root diameter$_{1-5}$, and SRL$_{1+2}$. Root diameter was also the most important root trait discriminating between gymnosperm and angiosperm tree species in the CCA (higher mean root diameter in gymnosperms: 0.53 mm; angiosperms: 0.39 mm). The higher root diameter in gymnosperms than in angiosperms can be explained by anatomical differences in their xylem where more tracheids in gymnosperm roots are needed to achieve a similar transport capacity as in angiosperm vessels (Sperry *et al.*, 2006). But the systematic difference in root diameter between gymnosperms and angiosperms may also give an indication of the divergence time for these morphological root traits and be explanation for the significant conservatism in these traits: the emergence of colder and drier climate during the mid to late Cretaceous has been hypothesized as a cause of adaptation and root trait diversity in angiosperms (Comas *et al.*, 2012; Chen *et al.*, 2013; Zanne *et al.*, 2014); increases in SRL and tissue lignification and decreases in diameter probably increased the efficiency of root systems in an environment with lower N availability, slower decomposition rates, and adverse climatic conditions (Pittermann *et al.*, 2012; Chen *et al.*, 2013). Our study has shown that SRL$_{1+2}$ had comparably high cross-species variability despite the conservatism of the root morphology trait

complex (38%; Table 1), which may be indication of its plasticity towards different environmental conditions.

A high root diameter and a long root lifespan are considered as conservative root traits which are often assigned to conifer trees independent from their leaf habit: in a common garden experiment with different tree species, the deciduous conifer *Larix decidua* had acquisitive leaf traits, i.e., high SLA, high leaf N content, and short lifespan, similar to the deciduous broadleaf trees, but conservative root traits similar to the other evergreen conifers (Withington *et al.*, 2006). Our study only partly confirmed the classification of root traits of *L. decidua* to the conservative trait spectrum, as it resembled the conservative root characteristics of the other conifer species with respect to its root diameter$_{1\text{-}5}$, SRL$_{1+2}$, and tissue density$_{1+2}$, but not with respect to root N content and lifespan. Root N content and lifespan were generally root traits not discriminating between broadleaf trees and conifers.

Earlier studies have also found that common ancestry has strong impact on root traits such as diameter and tissue density (Comas & Eissenstat, 2009; Chen *et al.*, 2013; Kong *et al.*, 2014): it was concluded that ecological filtering acts stronger on leaf than on root traits (Ackerly & Reich, 1999; Reich *et al.*, 2003; Whitman & Aarssen, 2010; Valverde-Barrantes *et al.*, 2015) and that this is the reason why the RES (with SRL and root diameter as the key traits) is stronger supported by data collected from more closely related than from more distant tree species (e.g. Comas & Eissenstat, 2009; McCormack *et al.*, 2012; Weemstra *et al.*, 2016). Our study does not fully support this conclusion since we found (i) no impact of common ancestry on root architecture, but (ii) significant phylogenetic signal in leaf morphology (SLA), longevity, and chemistry (leaf N_{mass}) - yet even though with lower correlation coefficients than for root morphology (R = 0.50-0.61 vs. 0.79). The missing phylogenetic signal in the branching ratio of the root system hints to a stronger impact by the environment and ecological filtering on root branching than by common ancestry.

Increased root branching is a response to the environment

The root branching ratio was not influenced by phylogeny in our study. Root branching patterns are thought to largely affect root functioning (Pregitzer *et al.*, 2002; Guo *et al.*, 2008b): the branching ratio of first to second order roots gives an indication of the plasticity of the absorptive root system to proliferate into locally or temporarily resource-rich patches (Hodge, 2004; Kong *et al.*, 2014). In a study with subtropical forest species, the branching intensity and ratio showed weak phylogenetic conservatism, and were negatively correlated with soil P and N contents, suggesting that higher branching intensity may be required at low-fertility sites

(Kong *et al.*, 2014). Increased root branching is typical for ECM fungal associations (Brundrett, 2002; Smith & Read, 2008; Comas & Eissenstat, 2009; Comas *et al.*, 2014; Eissenstat *et al.*, 2015), which are occurring in ecosystems dominated by organic nutrients and comparably low fertility (Phillips *et al.*, 2013). By contrast, the colonization with AM fungi has only subtle effects on root architecture (Maherali, 2014), even though it can significantly change root diameter (Comas *et al.*, 2012; Kong *et al.*, 2014). This difference in root architecture between ECM and AM roots was confirmed by our study when comparing only angiosperm tree species: ECM trees had a slightly lower branching ratio of first to second order roots than AM trees, but much higher branching intensity (root tips per lower order root length), i.e. ECM root tips were more clustered. However, while the branching intensity of the investigated angiosperm tree species was significantly influenced by the mycorrhizal association type and was next to the branching ratio the key trait discriminating AM from ECM angiosperm tree species, it was also a secondary factor for the discrimination between angiosperm and gymnosperm root traits. Soil nutrients in gymnosperm forests are nearly homogenously distributed due to the accumulation of their recalcitrant leaf litter over many years (Chen *et al.*, 2016), which decreases the importance of root proliferation and leads to the lower branching intensity in gymnosperms as observed in our study.

The influence of the mycorrhizal association type on root branching is also the reason for the missing phylogenetic signal in this root trait: the mycorrhizal association type is not related to the phylogenetic relatedness of the tree host, but in contrast is phylogenetically highly diverse, both with respect to the plant host (particularly AM) and the fungal symbiont (particularly ECM). The ancestral AM symbiosis has been stably inherited since its establishment, but there have been many independent conversions of AM to ECM symbioses (>>12 independent origins) in derived lineages of some major plant clades (Brundrett, 2002; Wang & Qiu, 2006). These independent conversions were probably a consequence of the emergence of new lineages in fungi and plants as an adaptation to a change in the environment to more seasonal and arid climate approximately 135 MYA (Malloch *et al.*, 1980; Moyersoen, 2006) and to nutrient-poorer environments. Due to their saprotrophic capabilities, ECM fungi can access recalcitrant nutrient pools that are inaccessible to AM fungi (Chalot & Brun, 1998; Blum *et al.*, 2002; Courty *et al.*, 2010) and, thus, are better adapted to nutrient deficiency. Increased root branching of ECM trees adds to this by supporting the proliferation and nutrient uptake from locally or temporarily resource-rich patches in the nutrient-poor ECM ecosystems.

Increased branching is a measure for a higher proportion of lower order roots with presumed fast respiration rates (Rewald *et al.*, 2014) and high resource uptake activity (Guo *et al.*, 2008b;

Rewald *et al.*, 2011; McCormack *et al.*, 2015). Our SEM revealed significant negative direct effects of both root branching ratio and intensity on root C:N, and negative indirect effects on SLA and leaf longevity, which may give hint on a whole plant economics spectrum with root branching as the key trait: the fast, acquisitive strategy of nutrient uptake is characterized by intensive root branching in resource-rich patches and corresponds with a tight root C:N (*viz.* relatively low C and high N content, which may be explained by lower suberin content of first order roots and faster N uptake rates), high SLA which is favorable for fast C uptake, and short leaf longevity, while the slow spectrum is characterized by the opposite set of traits. Additionally, intensive root branching also increases SRL of the pool of first and second order roots and decreases the average and lower order root diameter, which are both thought to be essential traits for fast resource acquisition.

In a comparison of the two major mycorrhizal association types in temperate forests, AM tree species have been proposed as fast in comparison to ECM species, due to the more rapid colonization of AM hyphae into N-rich patches (Hodge & Fitter, 2010), the faster turnover and decomposition of AM hyphal, root, and leaf litter (Read & Perez-Moreno, 2003; Hobbie *et al.*, 2006; Anderson & Cairney, 2007), and the quicker soil nutrient cycling rates (Vesterdal *et al.*, 2012; Phillips *et al.*, 2013). The current study makes clear that part of the fast/slow trait difference between AM and ECM tree species is also due to the occurrence of gymnosperms in the ECM association type in temperate regions, which can be assigned to the conservative (slow) trait family. When considering only angiosperms, deciduous ECM trees have to be rather assigned to the acquisitive trait family, since they have significantly higher root branching intensity and higher SLA, but do not differ significantly from AM trees with respect to their root C:N or leaf longevities. This classification to the fast/slow trait spectrum does not relate to the absolute growth rates of trees though, as the majority of the investigated AM species were early-successional, fast-growing species, while the majority of the ECM angiosperms were late-successional, slow-growing species, which become dominant at later stages of ecosystem succession. But the dominance of these latter species, i.e. of European beech, is probably due to better resource exploitation both aboveground (highest SLA) and belowground (comparably high root branching ratio and intensity).

Conclusion

We conclude that root branching relates to the mycorrhizal association type and to precision foraging into resource-rich patches and, thus, is a key belowground trait that influences resource uptake rates and function, which should be central to a revised root or whole plant economics spectrum. The dominating phylogenetic signal in root morphology, i.e. on SRL and root diameter, makes morphological traits less plastic and therefore less central for the description of economics spectra of temperate tree species across different environments - even though they may be useful for the separation of functional groups. Current investigations of the RES may have been inconclusive so far since they focused on those root traits which were in analogy to the LES, but may have disregarded the key functional trait for belowground resource acquisition. Inclusion of root branching as leading root trait of a whole plant economic spectrum may greatly improve modeled growth response of forest communities to environmental change.

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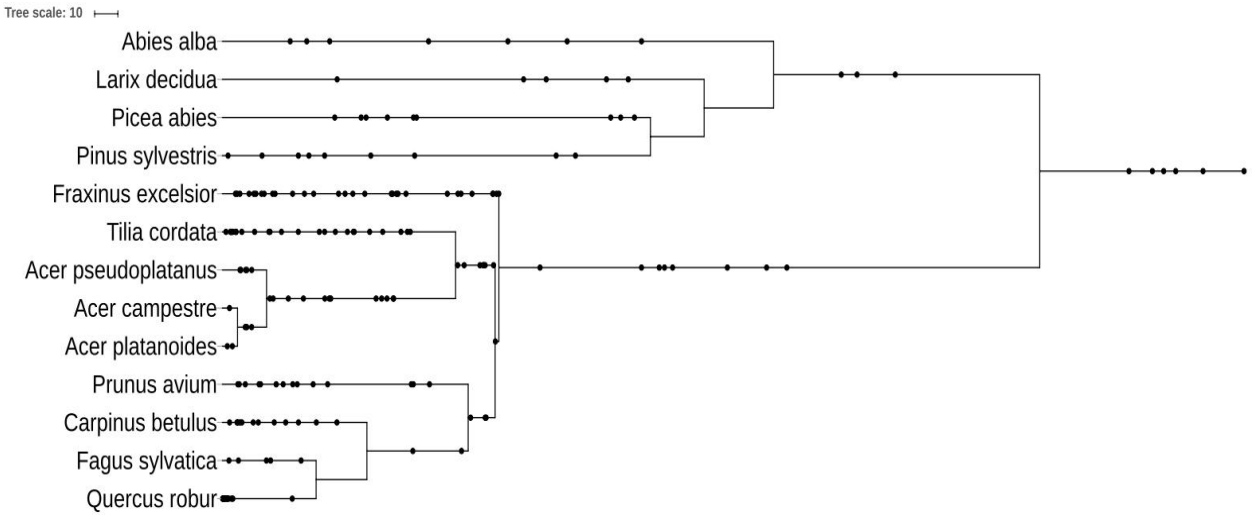
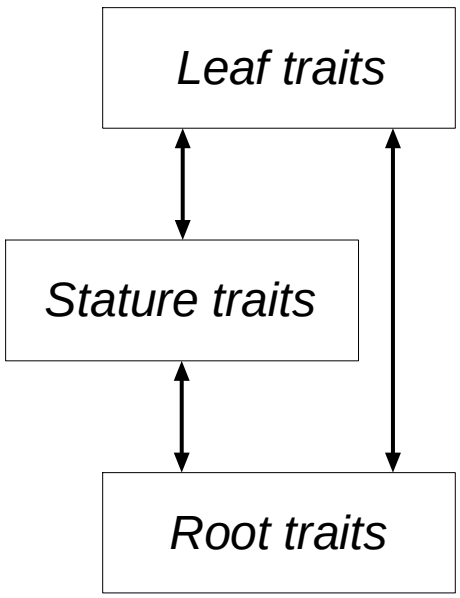
Supplementary Material



Supplementary Figure 1 Phylogenetic tree of 13 Central European tree species based on Zanne *et al.* (2014).



Supplementary Figure 2 Conceptual model on plausible interactions between root, stature, and leaf traits of temperate tree species.

Supplementary Table 1 Taxonomy, phylogenetic group, leaf habit, and mycorrhizal association type of the 13 major Central European tree species of this study.

Species	Code	Common name	Family	Phylogenetic group	Leaf habit	Mycorrhizal association
Pinus sylvestris	*Pisy*	Scots pine	Pinaceae	Gymnosperm	evergreen	ECM
Larix decidua	*Lade*	European larch	Pinaceae	Gymnosperm	summer-green	ECM
Acer campestre	*Acca*	Field maple	Sapindaceae	Angiosperm	summer-green	AM
Prunus avium	*Prav*	Wild cherry	Rosaceae	Angiosperm	summer-green	AM
Fraxinus excelsior	*Frex*	European ash	Oleaceae	Angiosperm	summer-green	AM
Quercus robur	*Quro*	Pedunculate oak	Fagaceae	Angiosperm	summer-green	ECM
Acer platanoides	*Acpl*	Norway maple	Sapindaceae	Angiosperm	summer-green	AM
Acer pseudoplatanus	*Acps*	Sycamore maple	Sapindaceae	Angiosperm	summer-green	AM
Carpinus betulus	*Cabe*	European hornbeam	Betulaceae	Angiosperm	summer-green	ECM
Picea abies	*Piab*	Norway spruce	Pinaceae	Gymnosperm	evergreen	ECM
Abies alba	*Abal*	Silver fir	Pinaceae	Gymnosperm	evergreen	ECM
Tilia cordata	*Tico*	Little-leaved lime	Malvaceae	Angiosperm	summer-green	ECM
Fagus sylvatica	*Fasy*	European beech	Fagaceae	Angiosperm	summer-green	ECM

Supplementary Table 2 Trait values of the 13 major Central European tree species of this study. Succession status: e = early, m = mid, l = late. Mycorrhizal association type: AM, arbuscular mycorrhiza; ECM, ectomycorrhiza. Phylogenetic group: A, angiosperm; G, gymnosperm. [a], literature data. n/d, no data.

	Pisy	*Lade*	*Acca*	*Prav*	*Frex*	*Quro*	*Acpl*	*Acps*	*Cabe*	*Piab*	*Abal*	*Tico*	*Fasy*
Succession status	**e**	**e**	**e**	**e**	**e-m**	**m**	**m-l**	**m-l**	**m-l**	**l**	**l**	**l**	**l**
Mycorrhizal association	**ECM**	**ECM**	**AM**	**AM**	**AM**	**ECM**	**AM**	**AM**	**ECM**	**ECM**	**ECM**	**ECM**	**ECM**
Phylogenetic group	**G**	**G**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**G**	**G**	**A**	**A**
LEAVES													
SLA [$cm^2\ g^{-1}$]	57	126	127	146	103	121	113	105	134	88	62	167	169
Leaf N_{mass} [$mg\ g^{-1}$]	14	31	17	16	20	19	22	22	20	27	18	25	23
Leaf C:N [$g\ g^{-1}$]	45	26	27	28	23	24	22	21	24	n/d	n/d	19	21
Leaf longevity[a] [yr]	3.7	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.6	7.1	8.2	0.4	0.5
STATURE													
Max. tree height[a] [m]	50	50	15	25	49	60	40	40	30	70	68	45	56
Wood density[a] [$kg\ m^{-3}$]	490	550	610	550	650	650	590	590	790	430	410	490	680
Max. tree age[a] [yr]	450	450	100	150	300	500	300	300	250	300	450	400	450
ROOTS													
SRL_{1+2} [$m\ g^{-1}$]	26	28	44	60	29	55	37	76	54	25	22	52	53
Tissue $density_{1+2}$ [$g\ cm^{-3}$]	0.23	0.22	0.17	0.19	0.21	0.16	0.21	0.12	0.17	0.26	0.24	0.15	0.13
Branching ratio [$n\ n^{-1}$]	1.6	2.9	2.4	2.7	3.5	1.9	3.0	2.7	2.3	2.6	2.8	2.2	2.8
Branching intensity [$tips\ cm^{-1}$]	4.3	1.8	8.5	4.5	1.8	12.9	4.9	7.3	8.4	5.5	1.8	8.7	8.6
Absorptive : transport roots [$g\ g^{-1}$]	0.3	1.0	0.7	0.5	2.1	0.4	1.2	0.7	0.6	0.7	0.8	0.4	0.7
Root $diameter_{1+2}$ [mm]	0.46	0.46	0.41	0.36	0.46	0.42	0.42	0.39	0.38	0.44	0.50	0.43	0.44
Root $diameter_{1-5}$ [mm]	0.53	0.52	0.43	0.34	0.46	0.36	0.40	0.34	0.34	0.53	0.54	0.45	0.40
Root $N_{mass1-5}$ [$mg\ g^{-1}$]	10	13	13	12	15	12	13	14	12	12	13	16	16
Root $C:N_{1-5}$ [$g\ g^{-1}$]	46	33	27	36	25	38	31	32	33	35	32	27	26
Fine root longevity[a] [yr]	0.7	1.1	1.7	0.3	0.6	1.0	1.6	1.7	0.8	0.7	1.1	0.6	0.6

Supplementary Table 3 Three individual principal components analyses (PCA) for the identification of major leaf, stature, and root trait gradients, respectively, among the 13 tree species. The most characteristic variables (according to their loading) of each PCA axis are in bold type.

	PCA axis 1	**PCA axis 2**
Leaf PCA	**Leaf 1**	**Leaf 2**
Eigenvalue	0.590	0.325
Explained variation (cumulative)	59.0	91.5
SLA	**-0.933**	-0.132
Leaf N_{mass}	-0.486	**0.868**
Leaf longevity	**0.823**	0.427
Stature PCA	**Crown 1**	**Crown 2**
Eigenvalue	0.880	0.09
Explained variation (cumulative)	88.0	97.0
Max. tree height	**-0.969**	0.170
Wood density	0.331	**-0.880**
Max. tree age	**-0.976**	-0.178
Root PCA	**Root 1**	**Root 2**
Eigenvalue	0.491	0.350
Explained variation (cumulative)	49.1	84.1
SRL_{1+2}	**-0.927**	0.187
Tissue $density_{1+2}$	**0.962**	-0.006
Branching ratio	0.063	**-0.912**
Branching intensity	-0.748	0.459
Root $diameter_{1-5}$	**0.859**	-0.156
Root $N_{mass, 1-5}$	-0.454	**-0.812**
Root $C:N_{1-5}$	0.410	**0.841**
Fine root longevity	-0.042	-0.022

Supplementary Table 4 Pearson's correlation matrix between leaf and fine root traits. Only significant correlations are shown. Significance is indicated as (*) $P \leq 0.1$, * $P \leq 0.05$, and ** $P \leq 0.01$.

Root trait	Root order	SLA	Leaf N_{mass}	Leaf C:N	Log (leaf longevity)
SRL	1+2	0.59*			-0.62*
	1-5			-0.58(*)	-0.66*
SRA	1+2	0.61*		-0.54(*)	-0.63*
	1-5			-0.59*	-0.61*
Diameter	1+2	-0.51(*)			0.55*
	1-5	-0.56*			0.71**
Tissue density	1+2	-0.68*		0.61*	0.70**
	1-5				
Branching ratio	1+2			-0.56(*)	
Branching intensity	1+2	0.49(*)			
Absorptive: transport roots	1-5				
Root N_{mass}	1-5	0.56*		-0.78**	
Root C:N	1-5	-0.53(*)		0.80**	
Log (fine root longevity)					

Chapter 3

EXUDATE RICHNESS OF MYCORRHIZAL TREES DETERMINES SOIL FUNCTIONS OF TEMPERATE FORESTS

Rebecca Liese · Alexander Weinhold · Yvonne Poeschl · Nicole M. van Dam · Ina C. Meier

Abstract

The interaction of plants with soil microbes via root exudation is an important determinant of tree productivity and forest ecosystem function. However, the question whether the quantity or chemical diversity of root exudates is regulating soil functions remains unresolved. In a field study with arbuscular (AM) and ectomycorrhizal (ECM) trees in a mixed forest stand, we show that the type of mycorrhizal association explained a significant part (*c.* 53%) of the variation in exudate-rhizosoil feedbacks. Root exudates of AM trees showed a higher chemical richness than those of ECM trees. Reduced chemical richness of root exudates exerted positive feedback with rhizosoil functions in ECM trees, while the quantity of exuded C displayed a secondary factor for root-rhizosphere relationships across similar abiotic environments. These results suggest that the mycorrhizal type is a crucial factor determining the chemical composition of root exudates. Differences in exudate chemical richness affect soil C and N cycles, which ultimately determines ecosystem productivity of temperate forests.

Introduction

The release of C-rich substrates into the soil via root exudation is a process that is increasingly recognized as a major driver of soil organic matter (SOM) dynamics (Keiluweit *et al.*, 2015) and forest feedbacks to climate change (Phillips *et al.*, 2011; Finzi *et al.*, 2015; Meier *et al.*, 2017). Yet in comparison to other processes of the terrestrial C cycle, root exudation is still poorly understood, and it remains unknown if the mere quantity of soluble organic C or rather the chemical composition and diversity of root exudates is determining the 'rhizosphere effect' (i.e., the relative difference in chemical, physical, and biological properties between rhizosphere and bulk soil) and plant-soil feedbacks. It is assumed that both the quantity and chemical diversity of root exudates have similar function, by increasing the biomass and diversity of soil microbes in the rhizosphere (Eisenhauer *et al.*, 2017). The enhanced release of easily degradable C compounds from roots is thought to stimulate the biomass of rhizosphere microbes and trigger microbes to co-metabolize recalcitrant SOM. This change in native C mineralization in response to increased labile C input is defined as the 'microbial priming effect' (Kuzyakov, 2010). Microbial priming effects can be sufficient in magnitude to delay 'progressive N limitation' (*sensu* PNL hypothesis; Luo *et al.*, 2004) and sustain high rates of net primary productivity under elevated CO_2 (Phillips *et al.*, 2011). With respect to the importance of exudate quantity or quality for the microbial activity in the rhizosphere (*viz.* for rhizosphere and microbial priming effects), two contradictory relations are conceivable. First, the response of soil microbes to the release of organic C by root exudation is independent of

exudate chemical composition. This implies that the majority of the exuded compounds are easily degradable, which makes their chemical identity a subordinate factor for rhizosoil microbes. Alternatively, chemical composition of root exudates may also be major factor since it decreases competition and niche overlap between microbial species leading to microbial diversity and niche complementarity in resource use. This may happen when individual microbes in the rhizobiome respond differentially to compounds in the exudates. Indeed, it has been observed that differences in exudate composition result in differential microbial communities (Steinauer *et al.*, 2016; Pétriacq *et al.*, 2017). Complementarity in microbial use of exudate C may then even lead to retarded SOM decomposition (i.e. to a negative priming effect) if diverse exudates are used as primary C and energy source instead of recalcitrant soil C.

The majority of terrestrial plant species are associated with mycorrhizal fungi. Mycorrhizal symbiosis is an important component of the plant-soil feedback (Bennett *et al.*, 2017): mycorrhizae increase the accessibility and availability of soil nutrients and water to plants in exchange for carbohydrates, which the fungus needs to grow. Mycorrhizae are also known to influence soil carbon storage (Averill *et al.*, 2014) and terrestrial C cycling in forests (Vicca *et al.*, 2012). The two main types of mycorrhizae of temperate trees - AM and ECM - seem to differ in their ability to stimulate microbial decomposition of soil organic C (Terrer *et al.*, 2016). Recently it has been suggested that the type of mycorrhizal symbiosis provides an integrated index of biogeochemical transformations relevant to C cycling and nutrient retention of temperate forests, since AM and ECM trees differ systematically in their nutrient economy (Phillips *et al.*, 2013; Fisher *et al.*, 2016; Liese *et al.*, 2017b). Yet the impact of the mycorrhizal type on the quantity or chemical composition of root exudation in temperate forests has not been tested sufficiently, despite some evidence of enhanced root exudation in ECM trees (Yin *et al.*, 2014; Liese *et al.*, 2017b) and of the influence of mycorrhizal colonization or ECM species identity on exudate composition (Jones *et al.*, 2004). Colonization by mycorrhizal fungi alters the carbohydrate metabolism and root respiration of the host (Bago *et al.*, 2003; Douds *et al.*, 2010), which leads to decreases in the amount of carbohydrates, increases in the amount of phenolics and gibberellins, and changes in the composition of amino acids in the excreted root exudates (Jones *et al.*, 2004; Martin *et al.*, 2008). We assumed that differences in the quantity or composition of root exudates are decisive for rhizosphere effects. Within this broad framework, we tested the specific hypotheses that (i) ECM colonization of roots lead to stronger control of root exudation, that is, the chemical diversity of ECM exudates is decreased in comparison to AM exudates; and (ii) ECM roots increase the microbial activity and biogeochemical cycles in the rhizosphere more than AM roots.

Using a nested design with tree species considered as replicates for mycorrhizal association types (n = 4 tree species per mycorrhizal type; where each tree species was replicated by six samples located in three study plots and was re-sampled in four sampling events), we analyzed the influence of the mycorrhizal type on root exudation and rhizosphere effects in AM and ECM trees in a mixed forest stand, which represents one of the largest old-growth hardwood forests in Central Europe ('Hainich National Park' in Thuringia, Germany, 51°08'N, 10°51'E). For the *in situ* collection of root exudates, we used a culture-based cuvette method (Phillips *et al.*, 2008) and quantified the exuded organic C by combustion catalytic oxidation. The chemical composition of root exudates was analyzed taking an untargeted liquid chromatography quadrupole time-of-flight mass spectrometry (LC-qToF-MS)-based metabolomics approach. As potential rhizosphere responses to exudation, we considered organic C and microbial biomass C as representatives of soil C cycling; amino-acid N, *N*-acetylglucosaminidase (NAG) activity, and net N mineralization as representatives of soil N cycling; and plant-available P and acid phosphatase (AP) activity as representatives of soil P cycling.

Materials and Methods

Study Site

The research was conducted in an old-growth mixed forest stand in Central Germany ('Hainich National Park'; 51°08'N, 10°51'E), which represents one of the largest deciduous forests in Central Europe. The forest is located on eutrophic Luvisols (FAO, 2006), which have developed from a base-rich Pleistocene loess layer over Triassic limestone (Middle Muschelkalk). Soil manipulations such as liming are absent. The mineral soil texture (0-30 cm soil depth) is characterized by low sand (<5%) and high silt contents (Guckland *et al.*, 2009) The climate is semi-humid with mean annual temperature of 7.7°C and mean annual precipitation of 590 mm. During the study period between October 2013 to September 2014, average temperature was 10°C and the sum of precipitation 490 mm, with pronounced summer drought periods being absent. The studied forest is unmanaged and has forest continuity for at least the last 200 years (Schmidt *et al.*, 2009) and therefore represents ancient woodland (Wulf, 2003). The vegetation can be classified as Stellario-Carpinetum (starwort-oak-hornbeam forest, interfused with elm trees) with up to 14 tree species co-occurring in this mixed hardwood stand. We selected eight tree species for our study, which are frequently dominant or subdominant trees of the natural forest vegetation in Central Europe and represent two mycorrhizal types (*cf*. Wang & Qiu, 2006): common ash (*Fraxinus excelsior* L.), Norway maple (*Acer platanoides* L.), sycamore

maple (*Acer pseudoplatanus* L.), and wild cherry (*Prunus avium* L.) are AM tree species. European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), small-leaved lime (*Tilia cordata* MILL.) and European hornbeam (*Carpinus betulus* L.) are ECM tree species. The classification to a mycorrhizal association type was assigned to according to literature (Wang & Qiu, 2006), and was confirmed by measurements of the AM and ECM colonization rates (see below). For each tree species, we randomly selected three single-species circular plots (two to three individuals of the target tree species or one tree with a dominant position; plot diameter 8 m). For comparability, only mature trees of similar age and crown structure on level to slightly inclined terrain were selected.

Root Exudate Collection and Analysis

Root exudates were collected during four sampling campaigns in the growing seasons 2013 and 2014 (October 2013, May 2014, July 2014, and September 2014) from the middle of each single-species plot (n = 24 single-species plots). For the collection of root exudates, a culture-based cuvette method was used (Phillips *et al.*, 2008). For this purpose, terminal fine root strands (<2 mm) attached to a mature target tree were carefully excavated from the upper 10 cm of mineral soil and all adhering soil particles were carefully rinsed off with demineralized water to maintain the integrity of the mycorrhizal root tips (n = 6 fine root strands per tree species, i.e., two root strands per study plot, and sampling campaign). Intact root strands were placed into cuvettes filled with sterile glass beads and moistened with C-free dilute nutrient solution (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.15 mM $MgSO_4$, 0.3 mM $CaCl_2$). Sterile cuvettes with glass beads and nutrient solution were included as controls. After a short equilibration period, fresh nutrient solution was flushed through each cuvette to remove soluble C. After 48 h, trap solution containing exudates were collected from each cuvette, the exact volume determined, the solution filtered through sterile syringe filters (pore size: 0.7 µm; GE Healthcare Life Sciences Whatman, Glass Microfiber Filters, Grade GF/F), and kept frozen at -20°C. Trap solution was immediately analyzed for non-particulate organic C on a total organic carbon (TOC) analyzer (Shimadzu TOC-L CPH/CPN; Shimadzu Scientific Instruments, Germany). Net mass-specific exudation rates (gross root exudation minus reabsorption and microbial consumption) were calculated as the total amount of C flushed from each root system over the incubation period divided by the root mass (mg C g^{-1}_{root} d^{-1}). Annual exudation rates (in g C m^{-2}_{soil} yr^{-1}) were estimated for six tree species by multiplying the average mass-specific exudation rate by the species-specific fine root biomass in the forest stand (*cf.* Kubisch *et al.*, 2015). Photosynthetic C cost of exudation (%) was estimated from the share of

annual C release by root exudation in annual C uptake by photosynthesis in the forest stand (*cf.* Schmidt *et al.*, 2015).

Metabolomics of Root Exudates

Root exudates from the summer sampling campaign (July 2014) were kept frozen at -20°C until further processing. Samples were enriched according to a method modified from (Strehmel *et al.*, 2014). Approximately 30 ml of the root exudate solution were evaporated in falcon tubes until dryness using a freeze dryer. The residue was then suspended in 2 ml water/methanol (95%/5% (v/v%)). The samples were sonicated at ambient temperature for 10 min and the supernatant was transferred to a 2 ml Safe-Lock tube. After 10 min of centrifugation at 6000 g, 1.5 ml of the sample solution was loaded on a SPE cartridge (Chromabond Hydra C18 -200 mg/3 ml for SPE (Marchery and Nagel) that was previously conditioned with 1 ml pure methanol and 1 ml water/formic acid (98%/2% (v/v%)). The cartridge was washed with 1 ml pure water and the sample was eluted with 1 ml methanol/formic acid (98%/2% (v/v%)) into 2 ml Safe-Lock tubes. The samples were reduced to dryness in vacuum centrifuge at 40°C and reconstituted in 150 µl methanol/water (70%/30% (v/v%)). After sonication for 10 min at ambient temperature and centrifugation for 10 min at 6000 g, the supernatant was transferred to a glass vial and subjected to LC-qToFMS analysis.

Enriched root exudate samples were run twice on LC-qToFMS and were analyzed with an Ultimate 3000 UHPLC system (Thermo Scientific Dionex) equipped with an Acclaim RSLC 120 column (150×2.1 mm, particle size 2.2 µm; Thermo Fischer Scientific) using the following gradient at a flow rate of 0.5 ml/min: 0-2 min isocratic 95% A (water/formic acid 99.95/0.05 (v/v%)), 5% B (acetonitrile/formic acid 99.95/0.05 (v/v%)); 2-25 min, linear from 5% to 95% B; 25-30 min, isocratic 95% B; 30-34 min, linear from 95% to 5% B; 35-42 min, isocratic 5% B. Compounds were detected with a maXis impact – quadrupole time-of-flight (qToF) mass spectrometer (Bruker Daltonics, Bremen, Germany) applying the following conditions in negative mode: scan range 50-1400 mz; acquisition rate 3 Hz; end plate offset 500 V; capillary voltage 2500 V; nebulizer pressure 3 bar; dry gas 11 L min^{-1}; dry temperature 240°C. Mass calibration was performed using sodium formate clusters (10 mM solution of NaOH in 50/50 (v/v%) isopropanol water containing 0.2 % formic acid. Every ten samples a mixture of all the samples was injected as a quality control sample.

Phylogenetic signal

The phylogenetic signal in root exudation was estimated by the correlation between the phylogenetic distance and trait distance matrices among the investigated tree species. We attached our list of taxa to the master phylogeny presented by (Zanne *et al.*, 2014) with the help of the software PHYLOMATIC v3 (a tool associated to PHYLOCOM 4.2; Webb *et al.*, 2008), to generate the initial phylogenetic tree in the Newick format. The simple pairwise matrix of phylogenetic distances was calculated from the Newick code with the 'phydist' phylogeny tool in PHYLOCOM and visualized with the online tool iTOL - Interactive Tree Of Life v3.1 (Ciccarelli *et al.*, 2006). Independent trait distance matrices based on root exudation rates, chemical richness of exudation, and chemical diversity of exudation, respectively, were calculated with the package SAS, version 9.3 (Statistical Analyses System, SAS Institute Inc., Cary, NC, USA). For the correlation between the phylogenetic and trait distance matrices, a Mantel permutation test (Mantel, 1967; Mantel & Valand, 1970) was computed with PAST 3.11 (Øyvind Hammer, Natural History Museum, University of Oslo, Norway), and the Pearson correlation coefficient *R* and the one-tailed *P* value from the comparison of the original *R* to the *R* computed in 9999 random permutations were reported. Euclidean similarity indices were used for the Mantel permutation test.

Mycorrhizal Colonization and Root Morphology

After exudate collection, root strands were cut off the tree and stored at 6°C for no longer than one week until processing. Tree species identity was confirmed a second time under a stereomicroscope (magnification ×40) with a site-specific morphological key based on periderm structure and color, root ramification, and root tip morphology (*cf*. Meinen *et al.*, 2009; Kubisch *et al.*, 2015). We analyzed root morphology by optical surface area measurements with a flatbed scanner and the software WinRHIZO 2013e (Régent Instruments Inc., Canada). The degree of AM colonization was quantified with the gridline-intersect method (Giovanetti & Mosse, 1980) after bleaching the root strands in 10% KOH (24 h, 80°C) and staining in an ink-based solution (5% ink in 5% acetic acid; Vierheilig *et al.*, 2005). The degree of ECM colonization of root tips was determined from unstained roots by examining the size, color, and morphology of fungal structures (Agerer, 1991, Goodman *et al.*, 1996).

Soil Sampling and Chemical Analyses

Parallel to the root exudate collections, soils were sampled in the upper 10 cm of the mineral soil. We used a steel corer (35-mm diameter) and combined each three replicate cores per study

plot to a mixed sample to ensure that fine roots had sufficient mass of adhering rhizosphere soil (n = 3 mixed soil samples per tree species and sampling campaign). Soil samples were stored at 4°C for no longer than 1 week until fine roots with adhering soils were separated from non-adhering soil. Soil adhering to fine roots was dislodged with fine forceps and was operationally defined as rhizosphere soil (*cf*. Weaver *et al.*, 1994), while non-adhering soil was considered bulk soil. All soil was sieved to 2 mm. Soil subsamples for chemical analyses were stored at 4°C. The fraction of plant available P was determined by resin bag extraction (anion exchange gel: Dowex 1 x 8-50; Dow Water & Process Solutions, USA). The resin was placed for 16 h in a solution of 1 g field-moist soil suspended in 30 ml water (Sibbesen, 1978). P was re-exchanged by 10% NaCl and 2% NaOH solutions and analyzed by color reaction with 5 mM hexaammonium heptamolybdate (Murphy & Riley, 1962) and photometric measurement at 712 nm against water (spectrophotometer; Libra S 21, Biochrom, UK). The concentration of free amino acids was analyzed according to the ninhydrin colorimetric analysis by (Rosen, 1957) following K_2SO_4 extraction (see below). Amino acid concentrations were measured spectrophotometrically at 570 nm (GENESYS 20 Visible Spectrophotometer; Thermo Fisher Scientific, USA) and compared with a glycine standard curve (0 to 0.75 mM). Total C and N were determined in samples dried at 70°C (48 h) with an elemental analyzer (Vario EL III; Elementar, Germany). Rhizosphere effects (RE, in %) for all variables were calculated from the difference between bulk and rhizosoil pools or fluxes, and were standardized by the respective bulk soil pool or flux. Accordingly, a positive RE illustrates a greater pool or flux in the rhizosphere soil and negative RE a greater pool or flux in bulk soil (Phillips & Fahey, 2006).

Microbial Activities

Soil subsamples for the analyses of microbial activities were stored at -20°C. Soil microbial biomass C was determined by the chloroform fumigation extraction method (Vance *et al.*, 1987). The extracted organic C was analyzed on a TOC analyzer. We calculated microbial biomass C from the difference between fumigated and non-fumigated samples by using a k_{EC} factor of 0.45 (Joergensen, 1996). The potential activities of two extracellular enzymes involved in the depolymerization of N and P from soil were assayed. The two enzymes – β-1,4-*N*-acetyl-glucosaminidase (NAG) and acid phosphatase (AP) – were analyzed according to the approaches by (Eivazi & Tabatabai, 1977) and (Verchot & Borelli, 2005). The activities of NAG and AP were measured spectrophotometrically as absorbance at 400 nm (AP) and 410 nm (NAG), respectively, using 4-nitrophenyl phosphate (NPP) as the substrate. Enzyme activities were expressed in units of substrate cleaved g^{-1} d^{-1}. The rate of potential net N

mineralization was measured under field-moist conditions: one 5 g-subsample was extracted immediately in 20 mL 0.5 M K_2SO_4, while the second 5 g-subsample was incubated for seven days in the dark before K_2SO_4 extraction. The amount of NH_4^+ (Berthelot reaction) and NO_3^- (copper-cadmium reduction method) was measured by flow injection analysis (Cenco/Skalar Instruments, Breda, Netherlands) and the net N mineralization potential calculated as the difference in the amount of ammonium and nitrate between day one and seven.

Statistical Analyses

Statistical analyses were conducted with the package SAS, version 9.4 (Statistical Analyses System, SAS Institute Inc., USA). Significance was determined at $P \leq 0.05$. Means and standard errors were calculated from the mean of four tree species per mycorrhizal association type and sampling campaign, while the replicate plots for each tree species were considered as pseudo-replication. The probability of fit to a normal distribution was tested using a Shapiro–Wilk test. Means were compared by one-way Kruskal-Wallis single factor analyses of variance and non-parametric multiple comparison tests after Wilcoxon to analyze the differences between tree species, mycorrhizal types, and soil types. Additionally, data were analyzed by applying two-factorial nested ANOVAs with sampling date as random effect to test for significant effects of the mycorrhizal type (main effect) and tree species identity (nested effect) in root exudation and rhizosphere effects in N, C and P dynamics (see Table S6). We summarized the rhizosphere effects by calculating a small-sample bias corrected response ratio based on the 'Linearity of Expectation' rule (*cf.* Lajeunesse, 2015).

The LC-qToFMS raw data were converted to the mzXML format by using the CompassXport utility of the DataAnalysis software (Bruker Daltonic). Peak picking, feature alignment and feature grouping was done in R v3.4.0 (R Core Team) using the Bioconductor (Huber *et al.*, 2015) packages 'xcms' v1.52.0 (Smith *et al.*, 2006; Tautenhahn *et al.*, 2008; Benton *et al.*, 2010) and 'CAMERA' v1.32.0 (Kuhl *et al.*, 2012). For preprocessing with 'xcms' and 'CAMERA' all samples were sorted into tree species-specific folders and were then loaded and processed at once. The following 'xcms' parameters were applied: peak picking method "centWave" (snthr = 10; ppm = 10; peakwidth = 4, 15); peak grouping method "density" (minfrac = 0.7; bw = 3; mzwid = 0.05); retention time correction method "peakgroups" (family = symmetric). 'CAMERA' was used to annotate adducts, fragments, and isotope peaks with the following parameters: extended rule set (www.github.com/stanstrup/chemhelper/tree/master /inst/extdata); perfwhm = 0.6; calcIso = TRUE; calcCaS = TRUE. CAMERA additionally sorts these adducts/fragments into pseudo compound (PC) groups whereas each group potentially

represents a metabolite. Lastly, we collapsed each PC group (hereafter referred to as 'metabolite') using an in-house "maximum heuristic" approach, i.e., the intensity values of the fragment, which most often displayed the highest intensity across all respective samples represents the PC group. We visualized the differences in metabolome between tree species and between different mycorrhiza by computing partial least squares-discriminant analyses (PLS-DA) using the r package 'mixOmics' v6.2.0 (Le Cao *et al.*, 2017). The r package 'vegan' v2.4-4 (Oksanen *et al.*, 2017) was used to test for significant differences in the metabolome composition between different mycorrhiza types by using multi-response permutation procedures (MRPP) on log +1-transformed data. The MRPP dissimilarity matrix was Euclidian and each analysis was permuted 10,000 times. The 'vegan' package was additionally used to compute the chemical richness (CR) (per sample), which is the number (n) of metabolites with abundances > 0 and the chemical diversity (CD) (per sample), which is implemented as Shannon diversity (H´).

After the determination of the chemical composition of root exudates, we tested for the relationship between root exudation and rhizosphere effects. We conducted linear regressions of root exudation rates, chemical richness of root exudates, and chemical diversity of root exudates with rhizosphere effects, and adjusted *P* values by the Benjamini-Hochberg procedure to correct for multiple comparisons.

Results

Partial least squares-discriminant analysis (PLS-DA) revealed that the chemical richness of root exudates significantly differed between the mycorrhizal association types ($P < 0.03$ multi-response permutation procedure; Fig. 1b). The difference between the mycorrhizal types was mainly due to chemical richness of root exudates (α diversity): chemical richness of root exudates was larger in AM trees (as many as 390 operational chemical units) than in ECM trees (343 operational chemical units, $P = 0.02$; Fig. 1c). When the operational chemical units of AM and ECM exudates were compared, an intersection of 507 chemical units was found (Fig. 1d), which corresponded to 53% of all operational chemical units. This difference in chemical richness of root exudates had no phylogenetic signal (Table S4), since the ECM association type developed independent from the phylogenetic relatedness of the host tree and, thus, is phylogenetically highly diverse (Fig. S1). Larger chemical richness of AM root exudates did not result in significantly higher chemical diversity, though (Fig. S2), since the relative

frequency of individual operational chemical units was reduced with an increase in chemical richness in AM trees.

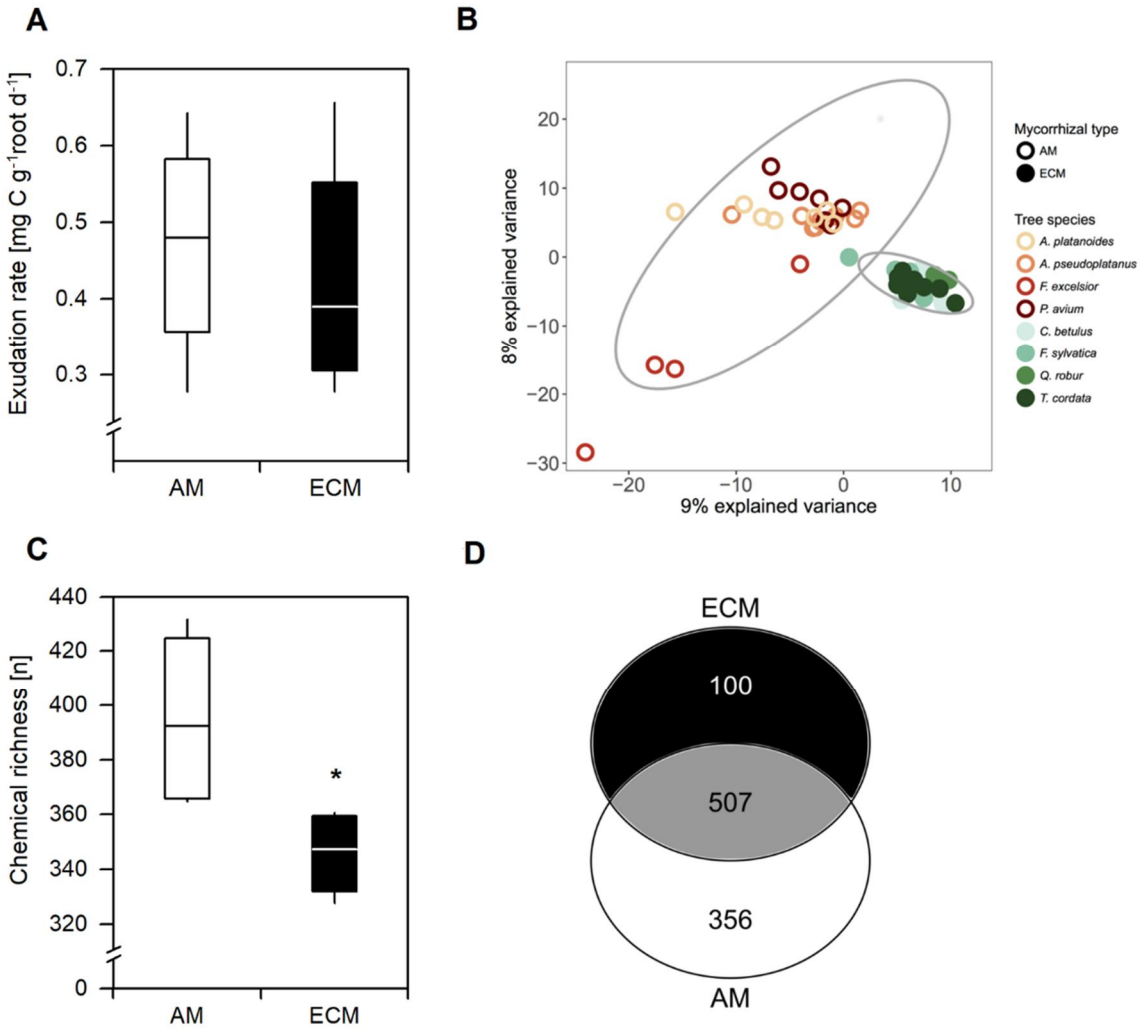


Figure 1 Mycorrhizal control of root exudation. (A to D) Control of (A) the quantity and (B, C, D) the chemical richness of root exudation of arbuscular mycorrhizal (AM, white) and ectomycorrhizal (ECM, black) trees in a mixed forest stand. Exudation rate values represent means of AM and ECM trees (n = 4 tree species per mycorrhiza type; each tree species is represented as an average of four sampling campaigns, during which six replicate samples from three study plots were sampled; significant differences between mycorrhiza types are indicated by asterisks). Mycorrhizal effects for chemical richness were identified by (B) a multi-response permutation procedure following a partial least squares-discriminant analysis (PLS-DA), by (C) a comparison of means between AM and ECM trees (n = 4 tree species per mycorrhiza type; each tree species is represented as an average of six replicate samples from three study plots) and by (D) a Venn diagram showing the number of equal and differentiated operational chemical units in AM and ECM exudates.

By contrast, the quantity of root exudation was influenced neither by the mycorrhizal association type (Fig. 1a) nor by tree species identity (Table S3). Root exudation rates varied considerably between individual trees of a species (average coefficients of variation 52-85% across sampling events), much more than between tree species (21%) and mycorrhizal types (10%). It is surprising that root exudation rates did not differ between mycorrhizal association types, since both root morphology (specific root area, $P = 0.02$) and root architecture (branching

intensity, P = 0.004) differentiated the mycorrhizal types (Table S1) – both of which were shown to correlate with root exudation in other studies (Tückmantel *et al.*, 2017). In this study, ECM trees had significantly higher branching intensity (4 tips cm^{-1}) and lower specific root area (SRA; 254 cm^2 g^{-1}) than AM trees (2.5 tips cm^{-1} and 300 cm^2 g^{-1}). In addition, ECM roots were almost completely colonized by mycorrhizal fungi (99%), while root colonization was less complete in AM roots (84%).

ECM symbioses exerted a positive influence on the rhizosphere effects on C, N, and P cycling, while the influence of AM symbioses was either less strong or even negative in some cases (for the rhizosphere effect on amino-acid N, plant-available P, and microbial C; Fig. 2). Accordingly, the mean rhizosphere effect (average of all rhizosphere effects on C, N, and P cycling) was much stronger in ECM trees (77 %) than in AM trees (38 %, P = 0.02; Table S3).

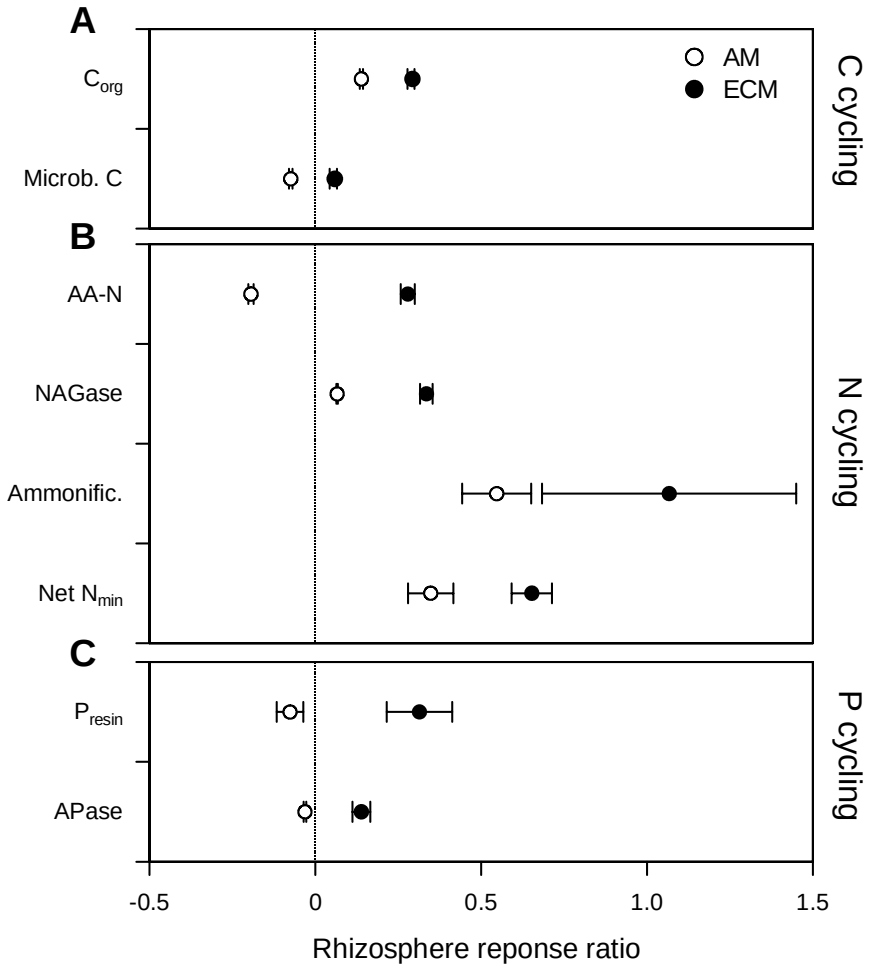


Figure 2 Mycorrhizal control of the rhizosphere response. (A to C) Control of (A) the rhizosphere C cycle, (B) N cycle, and (C) P cycle of arbuscular mycorrhizal (AM, white) and ectomycorrhizal (ECM, black) trees in a mixed forest stand. Values represent means of four sampling campaigns (n = 4 tree species per mycorrhiza type; each tree species is represented as an average of four sampling campaigns, during which six replicate samples from three study plots were sampled). Meta-analysis was conducted to summarize rhizosphere effects on biogeochemical cycles, where the biogeochemical cycles in the rhizosphere were considered as 'treatment group' and the biogeochemical cycles in bulk soil as 'control group'. We calculated mean small-sample bias corrected response ratios based on the 'Linearity of Expectation' rule (*cf.* Lajeunesse, 2015) and the approximate variances of the bias corrected response ratios.

The strongest positive rhizosphere influence had ECM trees on net ammonification and net mineralization rates (that is on N cycling), which had response ratios of +1.1 and +0.6, respectively: net ammonification increased threefold from 0.8 µg N in bulk soil to 2.4 µg N g^{-1} d^{-1} in ECM rhizosoil (Table S2). The increase in net nitrification related negatively to the chemical diversity of root exudates (Table S5, $P = 0.02$). Generally, the chemical richness of exudates related negatively to the rhizosphere effects. Both mean and the C_{org} rhizosphere effects levelled off with an increase in chemical richness to more than 500 operational chemical units in AM trees (Fig. 3a, b). Surprisingly, chemical richness had the strongest negative relationship with the rhizosphere effect for soil moisture: the chemical richness increased beyond 370 operational chemical units (mostly) in AM trees when the soil moisture in rhizosoil decreased relative to bulk soil (Fig. 3c), while the quantity of root exudation decreased concurrently. The chemical diversity of exudates related negatively to the rhizosphere effects for pH(H_2O) and net nitrification (Table S5).

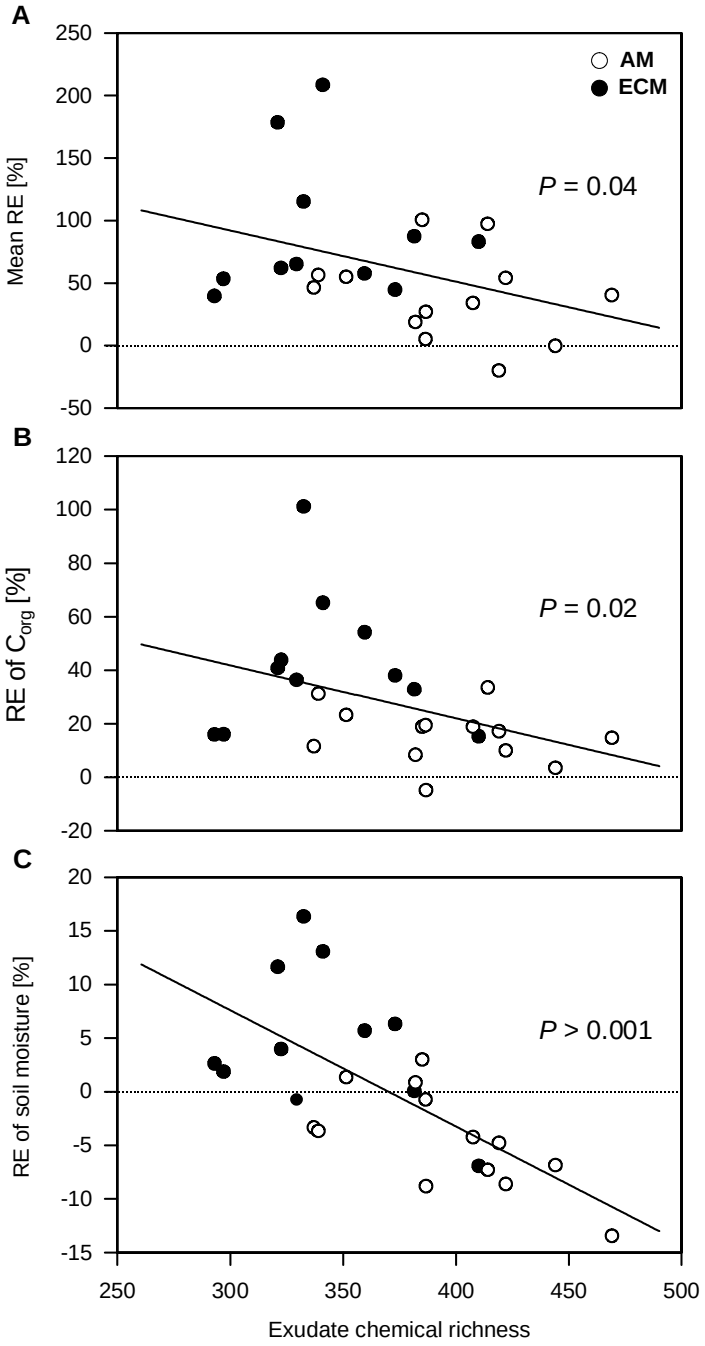


Figure 3 Rhizosphere effects (RE) as a function of root exudates. (A to C) Linear regressions between the chemical richness of root exudates with (A) the mean rhizosphere effect, (B) the rhizosphere effect for organic C, and (C) the rhizosphere effect for soil moisture of arbuscular mycorrhizal (AM, white) and ectomycorrhizal (ECM, black) trees in a mixed forest stand (n = 24 plots; each plot is represented as an average of four sampling campaigns). RE were calculated from the differences between bulk and rhizosoil pools or fluxes, and were standardized by the respective bulk soil pool or flux. P values were adjusted by the Benjamini-Hochberg procedure to correct for multiple comparisons.

Discussion

Despite the accepted importance of root exudation for ecosystem-scale processes, the understanding of the chemical communication between tree roots and rhizosoil functions is still in its infancy: our study is the first to identify systematic differences in root exudation patterns between trees colonized by different mycorrhizal types by a combining *in situ* exudate collection from mature forest trees with untargeted ecometabolomics. By taking the unique approach, we show for the first time that the chemical composition, i.e. the chemical richness, of root exudates coherently differs between AM and ECM forest trees, while tree species identity has no significant effect in the nested ANOVAs. It is generally expected that the mycorrhizal symbiosis influences root exudation patterns since the fungal symbiont is rewarded by photosynthates from the plant, which alters the carbohydrate metabolism and root respiration of the host (Bago *et al.*, 2003; Douds *et al.*, 2010). Following mycorrhizal colonization, some (inconsistent) reductions in the release of carbohydrates, alterations in the composition of amino acids, and increases in the release of phenolics and gibberellins have been observed so far (Jones *et al.*, 2004; Martin *et al.*, 2008). It appears that the chemical composition of root exudates is also regulated to communicate with mycorrhizal fungi and other soil microbes. In other investigations it has been shown that roots less completely colonized by mycorrhizal fungi increased the chemical richness of their exudates as a consequence of the production of signal molecules for the attraction of propagules and defense substances against pathogens (Meier *et al.*, 2015). Accordingly, the chemical composition of AM root exudates can be expected to differ from ECM exudates, since AM trees are frequently less completely colonized - as was the case in our study.

Root exudate diversity has been shown to influence the abundance of specific microbes and community shifts in microbes (Steinauer *et al.*, 2016; Pétriacq *et al.*, 2017). Diverse microbial communities are assumed to be functionally complementary in their ability to acquire and utilize labile exudate C, i.e. in their ecological niche (*sensu* niche complementarity hypothesis, Fargione *et al.*, 2007). Higher chemical richness of AM root exudates should accordingly lead to more complete resource exploitation of the exuded C and reduced niche overlap among rhizosoil microbes. If diverse rhizosoil microbes of AM trees are less dependent on recalcitrant SOM decomposition and mainly meet their C needs from root exudates, this explains the reduced rhizosphere effects in AM soils.

Differences in the chemical composition of rhizodeposits between AM and ECM trees have been invoked to affect root-rhizosphere couplings and ecosystem nutrient economy. Higher

chemical quality of AM litter and root exudates is thought to accelerate mineralization of plant-derived C, causing inorganic 'nutrient economy' (i.e., the primary forms of nutrients utilized by plants and microbes are inorganic) in AM forests (Smith, 1976; Phillips *et al.*, 2013). By contrast, ECM fungi are a greater C cost to the host plant (Smith & Read, 2008) and ECM tree litter is of lower quality, which enhances the importance of soil mining for N-bearing compounds and tight root/rhizosphere couplings. As a consequence, ECM fungi have the ability to exude significant amounts of extracellular enzymes to degrade SOM (Phillips *et al.*, 2013; Averill *et al.*, 2014). Enhanced rhizosphere effects in ECM rhizosoils of our study may thus be explained by a combination of (i) accumulation of soil N in organic forms as a result of slow bulk SOM decomposition and nitrification rates, (ii) increased capability to produce hydrolytic enzymes that degrade fast-cycling N pools (amino sugars), and (iii) reduced chemical richness in root exudates. The latter can be speculated to lead to niche overlap and enhanced competition between rhizosphere microbes for the exuded labile C. Increased exudate niche overlap among rhizosphere microbes may then enforce the co-metabolism of less bioavailable SOM, which should be at the attention of future research. From this, we conclude that the exudate richness – rhizosphere function relationship may be considered as negative feedback between mycorrhizal trees and soil microbes, whereas the quantity of exuded C is a secondary factor across similar environments.

Acknowledgements

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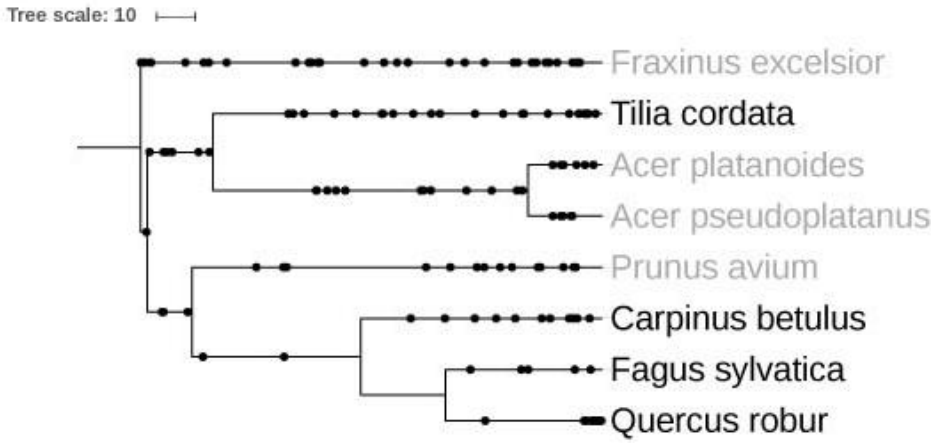
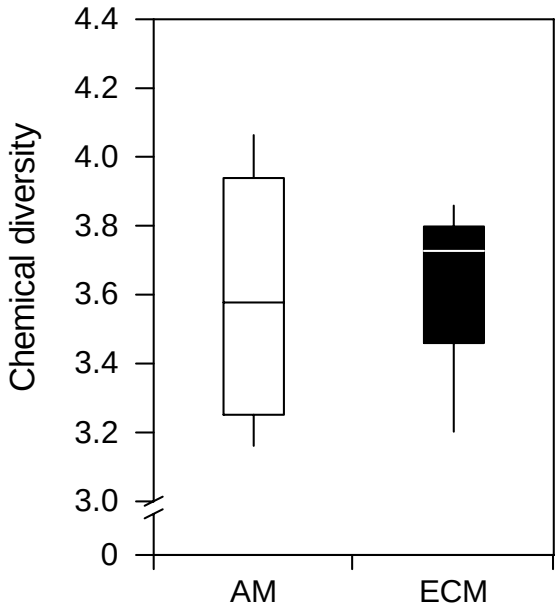
Supplementary Material



Supplementary Figure 1 Phylogenetic relatedness. Phylogenetic tree of four arbuscular mycorrhizal (grey letters) and four ectomycorrhizal (black letters) tree species based on (Zanne *et al.*, 2014). The tree scale represents 10 nucleotide substitutions per 100 residues. Points illustrate nodes where at least one leaf branches off. The root of the displayed phylogenetic tree originates in the Eudicotyledonae.



Supplementary Figure 2 Mycorrhizal influence of root exudation**.** Influence on the chemical diversity of root exudates of arbuscular mycorrhizal (AM; white) and ectomycorrhizal (ECM; black) trees in a mixed forest stand (n = 4 tree species per mycorrhiza type; each tree species is represented as an average six replicate samples from three study plots were sampled).

Supplementary Table 1 Mycorrhizal control of root exudation and root morphology. Control of root exudation, root morphology, and mycorrhizal colonization of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) trees in a mixed forest stand (means and standard errors in parentheses of $n = 4$ tree species per mycorrhizal type (or $n = 3$ tree species per mycorrhizal type for the annual exudation flux); each tree species is represented as an average of four sampling campaigns, during which six replicate samples from three study plots were sampled). Exudation data represent the average values of four sampling campaigns between October 2013 and September 2014. Significant differences between the mycorrhizal types are indicated by asterisks (***, **, and * for $P \leq 0.001$, 0.01 and 0.05; n.s., not significant). n/a, not applicable.

	AM	**ECM**	***P***
Exudation rate [mg C g^{-1}_{root} yr^{-1}]	171 (25)	156 (27)	n.s.
Annual exudation flux [g C m^{-2}_{soil} yr^{-1}]	32.6 (5.9)	44.8 (10.7)	n.s.
Photosynthetic C cost of root exudation [%]	4.1 (0.8)	5.7 (1.4)	n/a
Fine root diameter [mm]	0.55 (0.03)	0.54 (0.02)	n.s.
Tissue density [g cm^{-3}]	0.31 (0.03)	0.34 (0.02)	n.s.
SRL [m g^{-1}]	19 (1)	15 (1)	n.s.
SRA [cm^2 g^{-1}]	300 (10)	254 (13)	*
Branching intensity [tips cm^{-1}]	2.6 (0.4)	4.1 (0.7)	**
Mycorrhizal colonization [%]	84 (3)	99 (1)	***

SRL, specific root length; SRA, specific root area

Supplementary Table 2 Rhizosphere effects. Nutrient cycling in the rhizosoil and bulk soil of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) trees in a mixed forest stand (means and standard errors in parentheses of $n = 4$ tree species per mycorrhizal type; each tree species is represented as an average of four sampling campaigns, during which six replicate samples from three study plots were sampled). Significant differences ($P \leq 0.05$) of a mixed effect model with sampling campaign as random effect across soil types are indicated by different superscript letters and significant differences between the mycorrhizal types by bold letters.

	Rhizosoil		**Bulk soil**	
	AM	**ECM**	**AM**	**ECM**
C CYCLING				
C_{org} [mg C g^{-1}]	41.5 (2.1) ^{a}	45.7 (2.5) ^{a}	36.1 (1.6) ^{b}	34.0 (2.9) ^{b}
Microbial biomass C [mg C g^{-1}]	0.57 (0.03)	0.60 (0.05)	0.61 (0.03)	0.57 (0.04)
N CYCLING				
N_{total} [mg N g^{-1}]	33.3 (1.0) ^{a}	34.6 (1.8) ^{a}	30.5 (0.8) ^{b}	28.9 (3.4) ^{b}
$N_{amino\ acid}$ [µg N g^{-1}]	**16.0 (0.3)** $^{\mathbf{b}}$	**24.1 (2.9)** $^{\mathbf{a}}$	19.3 (1.7) ^{ab}	18.3 (1.3) ^{ab}
C_{org}/N_t [mol C mol N^{-1}]	**14.2 (0.2)** $^{\mathbf{b}}$	**15.2 (0.3)** $^{\mathbf{a}}$	**13.9 (0.1)** $^{\mathbf{a}}$	**14.7 (0.3)** $^{\mathbf{b}}$
NAG activity [mg N g^{-1} d^{-1}]	**12.6 (0.4)** $^{\mathbf{bc}}$	**19.7 (2.4)** $^{\mathbf{a}}$	**11.4 (0.4)** $^{\mathbf{b}}$	**14.0 (0.8)** $^{\mathbf{c}}$
Net ammonification [µg N g^{-1} d^{-1}]	1.5 (0.2) ^{ab}	2.4 (0.9) ^{a}	0.8 (0.2) ^{b}	0.8 (0.4) ^{b}
Net nitrification [µg N g^{-1} d^{-1}]	**2.1 (0.2)** $^{\mathbf{a}}$	**1.0 (0.4)** $^{\mathbf{b}}$	1.6 (0.4) ^{ab}	1.0 (0.5) ^{b}
Net N mineralization [µg N g^{-1} d^{-1}]	3.5 (0.2) $^{\mathbf{a}}$	3.4 (0.7) ^{a}	2.4 (0.6) ^{ab}	1.8 (0.3) ^{b}
P CYCLING				
P_{resin} [µg P g^{-1}]	5.1 (0.7)	7.0 (1.0)	5.9 (0.8)	5.0 (1.4)
AP activity [mg P g^{-1} d^{-1}]	12.9 (0.1)	14.6 (2.2)	13.3 (0.8)	12.9 (0.9)
OTHER SOIL PROPERTIES				
Soil moisture content [%]	32.2 (0.7) ^{b}	32.1 (1.3) ^{b}	37.3 (0.5) ^{a}	35.8 (1.3) ^{a}
pH(KCl)	4.5 (0.1)	4.4 (0.3)	4.4 (0.1)	4.3 (0.2)
Base saturation [%]	94.9 (0.6) ^{a}	90.3 (4.2) ^{ab}	92.5 (0.7) ^{b}	82.5 (6.8) ^{b}

Supplementary Table 3 Mycorrhizal control of root exudation and rhizosphere effects. Results of two-factorial nested ANOVAs on the significance of the effects of mycorrhizal type (n = 2, df = 1) and tree species identity (n = 8, df = 6) on the variance of root exudation and rhizosphere effects of eight tree species from a mixed forest stand. Given are results of F tests of a mixed effects model, with mycorrhizal type as main effect, tree species identity as nested effect, and sampling date as random effect (***, **, and * for $P \leq 0.001$, 0.01 and 0.05). RE were calculated from the differences between bulk and rhizosoil pools or fluxes, and were standardized by the respective bulk soil pool or flux.

		Mycorrhizal type	**Tree species**
ROOT EXUDATION	Exudation rate	0.3	2.0
	Chemical richness (exudates)	**20.6 *** **	**3.4** **
	Chemical diversity (exudates)	0.03	**2.5***
RHIZOSPHERE EFFECTS	Mean rhizosphere effect	**8.1 ***	1.7
C cycling	C_{org}	**8.1 ***	1.3
	Microbial biomass C	**8.6 ****	1.9
N cycling	N_{total}	**64.3 *****	**11.7 ****
	$N_{amino\ acid}$	0.06	0.3
	NAG activity	**5.4 ***	1.6
	Net ammonification	**11.6 ****	2.4
	Net nitrification	**5.5 ***	0.8
	Net N mineralization	0.3	0.2
P cycling	P_{resin}	2.0	0.7
	AP activity	0.8	0.6

Supplementary Table 4 No phylogenetic signal in root exudation. Phylogenetic signal estimated by a permutation test for the correlation between two distance matrices (Mantel test): here we show Pearson's correlation coefficient R and the probability of error P for the correlation between the phylogenetic distance matrix (on the basis of the relative nucleotide substitutions) and each one of three independent trait distance matrices (on the basis of the root exudation rate, chemical richness of root exudates, and chemical diversity of root exudates) of eight tree species from a mixed forest stand.

Trait	***R***	***P***
Exudation rate	-0.07	0.72
Chemical richness (exudates)	0.06	0.33
Chemical diversity (exudates)	0.20	0.12

Supplementary Table 5 Rhizosphere effects as a function of root exudates. Linear regressions between rhizosphere effects and root exudation rates, chemical richness of root exudates, and chemical diversity of root exudates (n = 24). Given are correlation coefficients of simple linear regressions with single explanatory variables (***, **, and * for $P \leq 0.001$, 0.01 and 0.05. P values were adjusted by the Benjamini-Hochberg procedure to correct for multiple comparisons). RE were calculated from the differences between bulk and rhizosoil pools or fluxes, and were standardized by the respective bulk soil pool or flux.

		Exudation rate	**Chemical richness**	**Chemical diversity**
RHIZOSPHERE EFFECTS	Mean rhizosphere effect	**0.49 ****	**-0.37 ***	-0.07
C cycling	C_{org}	0.35	**-0.40 ***	-0.007
	Microbial biomass C	0.27	-0.38	-0.18
N cycling	$N_{amino\ acid}$	-0.07	-0.04	0.30
	NAG activity	0.33	-0.19	0.07
	Net ammonification	0.42	-0.24	0.003
	Net nitrification	-0.17	0.09	**-0.49 ***
	Net N mineralization	-0.22	0.41	-0.45
P cycling	P_{resin}	**0.60 ****	-0.14	0.32
	AP activity	-0.23	**-0.47 ****	-0.27
Other soil properties	Soil moisture	0.44	**-0.68 *****	-0.05
	pH	-0.30	-0.21	**-0.62 *****
	Base saturation	-0.18	-0.40	-0.10

Supplementary Table 6 Traits used in the study for statistical analyses.

Trait	Description	Unit
ROOT EXUDATION		
Exudation rate	Rate of C release via root exudation	mg C g^{-1}_{root} yr^{-1}
Annual exudation flux	Estimated annual rate of C release via exudation	g C m^{-2}_{soil} yr^{-1}
Photosynthetic C cost of root exudation	Estimated percentage of C exudation from NPP	%
Chemical richness (exudation)	Total number of different exudate compounds	n
Chemical diversity (exudation)	Diversity of exudate compounds	*H´*
ROOT MORPHOLOGY		
Fine root diameter	Average diameter of fine roots	mm
Tissue density	Root mass per unit root volume	g cm^{-3}
Specific root length (SRL)	Root length per unit root mass	m g^{-1}
Specific root area (SRA)	Root area per unit root mass	cm^2 g^{-1}
Branching intensity	Root tips per unit root length	tips cm^{-1}
Mycorrhizal colonization	Degree of mycorrhizal colonized roots	%
RHIZOSPHERE PROPERTIES		
C_{org}	Mass of organic C per unit soil mass	mg C g^{-1}
Microbial biomass C	Mass of microbial C per unit soil mass	mg C g^{-1}
N_{total}	Mass of total N per unit soil mass	mg N g^{-1}
$N_{amino\ acid}$	Mass of Glycine N per unit soil mass	µg N g^{-1}
C_{org}/N_{total}	Ratio of organic C and total N in soil	mol C mol N^{-1}
NAG activity	Potential activity of NAGase per unit soil mass	mg N g^{-1} d^{-1}
Net ammonification	Changes of NH_4^+-N pool sizes over time per unit soil mass	µg N g^{-1} d^{-1}
Net nitrification	Changes of NO_3^--N pool sizes over time per unit soil mass	µg N g^{-1} d^{-1}
Net N mineralization	Net release of inorganic N over time per unit soil mass	µg N g^{-1} d^{-1}
P_{resin}	Mass of plant available P per unit soil mass	µg P g^{-1}
AP activity	Potential activity of APase per unit soil mass	mg P g^{-1} d^{-1}
Soil moisture content	Percentage of water contained in soil mass	%
pH(KCl)	Negative decimal logarithm of hydrogen ions	-lg $c(H^+)$
Base saturation	Percentage of potential cation exchange capacity occupied by cations	%

Chapter 4

THE MYCORRHIZAL TYPE GOVERNS ROOT EXUDATION AND N UPTAKE OF TEMPERATE TREE SPECIES

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Abstract

Even though the two dominant mycorrhizal associations of temperate tree species differentially couple carbon (C) and nitrogen (N) cycles in temperate forests, systematic differences between the biogeochemical cycles of arbuscular (AM) and ectomycorrhizal (ECM) tree species remain poorly described. A classification according to the mycorrhizal type offers the chance, though, to develop a global frame concept for the prediction of temperate ecosystem responses to environmental change. Focusing on the influence of mycorrhizal types on two key plant processes of biogeochemical cycling (root exudation and N acquisition), we investigated four temperate deciduous tree species per mycorrhizal type in a drought experiment in large mesocosms. We hypothesized that (H1) C loss by root exudation is higher in ECM than in AM trees, (H2) drought leads to higher reductions in root exudation of drought-sensitive ECM trees, and (H3) inorganic N uptake is higher in AM than in ECM trees. In contradiction to H2, we found no systematic difference in root exudation between the mycorrhizal types at ample soil moisture, but almost two-fold higher exudation in ECM trees when exposed to soil drought. In addition, photosynthetic C cost of root exudation strongly increased by approximately ten-fold in drought-treated ECM trees, while it only doubled in AM trees, which confirms H1. With respect to H3, we corroborated that AM trees had both higher absolute and relative inorganic N acquisition rates than ECM trees, while the organic N uptake did not differ between mycorrhizal types. We conclude that ECM trees are less efficient in inorganic N uptake than AM trees, but increase root C release in dry soil potentially as an adaptive response to increase hydraulic conductivity and/or nutrient availability. These systematic differences in key biogeochemical processes supports hints on the key role of the mycorrhizal types in coupling C and N cycles in temperate forests.

Keywords: arbuscular mycorrhiza, carbon cycling, deciduous tree species, drought, ectomycorrhiza, inorganic nitrogen uptake, organic nitrogen uptake, rhizodeposition

Introduction

A fundamental question for the development of global change models is to what degree shifts in tree species and their associated microbes influence biogeochemical cycles (Johnson *et al.*, 2013; Phillips *et al.*, 2013; Terrer *et al.*, 2016). The increasing evidence that tree species identity is critical for important biogeochemical processes like nutrient cycling (e.g. Binkley & Giardina, 1998; Phillips & Fahey, 2006; Hobbie, 2015) and soil carbon (C) sequestration from root inputs (Eviner & Chapin III, 2003; van der Heijden *et al.*, 2015) calls for an integrated framework for different tree species, which can be implemented into Earth system models. It has been recently suggested that the relative abundance of AM and ECM trees may provide an integrated index of biogeochemical transformations relevant to C cycling and nutrient retention in temperate forests, since the two types of mycorrhizal fungi differ in their mode of nutrient acquisition (Phillips *et al.*, 2013; Fisher *et al.*, 2016).

The majority of tree species are associated with mycorrhizal fungi, among which symbiotic associations with either AM or ECM fungi are the most widespread. The association with mycorrhizal fungi influences plant growth (Vicca *et al.*, 2012), nutrient cycling (Johnson *et al.*, 2013; Terrer *et al.*, 2016), and soil C storage (Averill *et al.*, 2014). So far, some systematic differences in important ecosystem processes between the two major mycorrhizal types have already been revealed: ECM root associations are linked with low and AM associations with high ecosystem C turnover due to differences in key C cycling traits, i.e. differences in relative growth rate of plants and in litter decomposability (Cornelissen *et al.*, 2001; Read & Perez-Moreno, 2003). The soil C storage in ecosystems dominated by ECM associations (i.e., cold coniferous and many temperate forests) is consequently 70% greater than in ecosystems where AM associations dominate (i.e., in tropical forests and in grassland) (Averill *et al.*, 2014). By implication, the distribution and evolutionary development of AM and ECM dominated systems is associated with the quantity and quality of soil C and nutrients (Read & Perez-Moreno, 2003).

In terrestrial ecosystems, soil organic matter (SOM) is the largest C pool (Fontaine *et al.*, 2007). The size and sink strength of SOM is determined by rhizodeposition, since the release of easily degradable C-rich substrates from roots drive microbial decomposition processes and stimulates microbes via a priming effect (Kuzyakov *et al.*, 2000) to decompose less bioavailable soil organic C (SOC; Hoosbeek *et al.*, 2004; Joslin *et al.*, 2006; Phillips *et al.*, 2011; Phillips *et al.*, 2012; Meier *et al.*, 2017). Greater C inputs by root exudation, e.g. as consequence of elevated CO_2 (Phillips *et al.*, 2011) or warming (Boone *et al.*, 1998; Yin *et al.*, 2013; Zhang *et*

al., 2016), do not only increase the decomposition of recalcitrant C, but may also enhance soil N-cycling (Phillips *et al.*, 2011; Phillips *et al.*, 2012; Meier *et al.*, 2017). By contrast, N enrichment from N deposition decreases belowground C allocation and root exudation (Phillips *et al.*, 2009), and consequently reduces the priming effects (Phillips & Fahey, 2006). Next to priming effects, root exudates also enhance nutrient availabilities directly by alteration of pH milieus or provision of chelating agents (Grayston *et al.*, 1997; Jones *et al.*, 2004). The amount and composition of root exudates also depends on plant species (Grayston *et al.*, 1997), and on the type of fungi colonizing the roots (Langley & Hungate, 2003; Meier *et al.*, 2013). In addition, it is known that fungal hyphae also exude carbohydrates and extracellular enzymes into the hyphosphere (Tawaraya *et al.*, 2006; Meier *et al.*, 2015; Zhang *et al.*, 2016). In a pulse labeling experiment with potted saplings, Phillips & Fahey (2006) found that roots of ECM yellow birch released more C than roots of AM sugar maple. In two New England forests, Brzostek *et al.* (2013) could not prove any systematic difference in root exudation between two AM and two ECM tree species, even though ECM beech had the highest and AM ash the lowest rhizosphere effect. Reinforcing this result, a study in a deciduous hardwood forest in the US Midwest showed that exudation rates and rhizosphere effects on nutrient cycling were nearly two times higher in two ECM than in two AM tree species (Yin *et al.*, 2014). While these studies conform in their general tendency, it remains unclear whether trait variations of specific tree species (and of specific mycorrhizal species, respectively) are driving these differences, or if they can be generalized to other mycorrhizal tree species, ecosystems, and larger scales.

The two major mycorrhizal types seem to differ in their nutrient acquisition strategies and nutrient economy (Phillips *et al.*, 2013; Averill *et al.*, 2014): N acquisition of ECM tree species, which occur mainly in habitats with high organic N content (Smith & Smith, 2011), seems to be dominated by organic N acquisition, at least at high elevation (Averill & Finzi, 2011). Organic N acquisition increases the C cost of N uptake, since C is invested not only in the production of extracellular enzymes to decompose polymeric N_{org} molecules rendering monomeric forms of organic N for direct absorption by the tree (Read & Perez-Moreno, 2003), but also in enhanced root C exudation to induce microbial priming effects. AM tree species also can access both inorganic (Govindarajulu *et al.*, 2005) and organic N forms (Whiteside *et al.*, 2012). Yet given the limited saprotrophic capabilities of AM fungi and the dominance of inorganic N forms in the habitats in which they occur, it is believed that AM tree species primarily utilize inorganic N forms (Gallet-Budynek *et al.*, 2009; Smith & Smith, 2011). However, even though the dominant functional mycorrhizal type could play a key role in N

dynamics in forest ecosystems, systematic differences between mycorrhizal types in organic and inorganic N acquisition remain poorly understood so far.

Apart from their potential role in C and N cycling, mycorrhizal associations may also diminish the sensitivity of plants to drought (Lehto & Zwiazek, 2011; Kivlin *et al.*, 2013; Mohan *et al.*, 2014), mainly due to physiological and morphological properties of the fungi (Phillips *et al.*, 2016), but also due to physiological changes in the host. Drought tolerance mediated by AM fungi can be fostered by enhanced accumulation of osmotic metabolites, which lowers the water potential of the host plant (Rapparini & Peñuelas, 2014; Latef *et al.*, 2016). In case of ECM fungi, modified aquaporin expression and melanin concentration in cell walls under reduced water supply may help to ensure higher tolerance of plants to soil desiccation (Lehto & Zwiazek, 2011; Groppa *et al.*, 2012; Fernandez & Koide, 2013; Brunner *et al.*, 2015; Phillips *et al.*, 2016). However, whether AM or ECM associations provide higher drought resistance for their host trees appears to be uncertain due to contrasting findings (Querejeta *et al.*, 2009; Brzostek *et al.*, 2014; Mohan *et al.*, 2014). Hypothetically, the higher drought sensitivity of ECM trees (Querejeta *et al.*, 2009) could result in stronger down-regulation of photosynthetic C gain and root growth, thus, leading to stronger reductions in the C loss via root exudation than in AM trees (Brunner *et al.*, 2015). However, the response of root exudation of forest trees to soil drought is unknown so far and evidence for this line of thought remains absent.

In the current study, we investigated systematic differences in C and N turnover between AM and ECM trees in a factorial drought experiment with four AM and four ECM tree species in large-scale mesocosms. Among the major biogeochemical processes, we focused on the C flux via root exudation, photosynthesis, and leaf respiration, as well as on N acquisition rates from organic and inorganic N sources. Tree saplings of the experiment originated from ancient woodland and were colonized by indigenous mycorrhizal communities. We assumed that the mycorrhizal type has greater influence on key processes of biogeochemical cycling (root exudation and N acquisition strategies) than differences in tree species identity. We hypothesized that (H1) C loss by root exudation is higher in ECM than in AM tree species as a consequence of the organic nutrient economy in ECM ecosystems and enhanced root/rhizosphere couplings, (H2) drought leads to higher reductions in root exudation of drought-sensitive ECM tree species, and (H3) inorganic N uptake is higher in AM than in ECM tree species, whereas organic N acquisition is greater in ECM tree species.

Materials and Methods

Plant material

Saplings of eight major Central European deciduous tree species were collected from an old-growth mixed forest ('Hainich National Park' in Thuringia, Germany, 51°08'N, 10°51'E). The forest is located at sub-montane elevation (340 m a.s.l.) on eutrophic Luvisols (IUSS, 2006), which have developed from a base-rich Pleistocene loess layer over Triassic limestone (Middle Muschelkalk). Soil manipulation activities such as liming were absent. The climate is characterized by a mean annual temperature of 7.7°C and mean annual precipitation of 590 mm. During summer, irregular drought periods of two to three weeks are the rule. Over the last 40 years, few individuals have been extracted from the stand, which has continuity as a forest for at least the last 200 years (Schmidt *et al.*, 2009) and therefore represents ancient woodland (Wulf, 2003). The forest stand is a mature mixed hardwood stand with up to 14 tree species co-occurring. The vegetation is classified as Stellario-Carpinetum (starwort-oak-hornbeam forest, interfused with elm trees). The selected tree species for this study are frequently dominant or subdominant trees of the natural forest vegetation in Central Europe and represent two mycorrhizal types (*cf*. Wang & Qiu, 2006): common ash (*Fraxinus excelsior* L.), sycamore maple (*Acer pseudoplatanus* L.), Norway maple (*Acer platanoides* L.), and wild cherry (*Prunus avium* L.) are AM tree species. European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), small-leaved lime (*Tilia cordata* MILL.), and hornbeam (*Carpinus betulus* L.) are ECM tree species. About 25 saplings per tree species were collected in two campaigns in September 2011 (AM: ash and sycamore; ECM: beech and lime) and September 2012 (AM: Norway maple and cherry; ECM: oak and hornbeam). The selected individuals were similar in tree height (about 30 cm) and crown dimensions. Saplings were excavated and adherent soil material was carefully removed from the roots. Saplings were stored in moist plastic bags to minimize transpiration, kept cool, and transported to the greenhouse immediately. Saplings were re-planted in 5 L plastic pots filled with sterilized sand, placed in a randomized array in the Experimental Botanical Garden Göttingen, and kept well-watered.

Experimental design

The eight saplings for each tree species were planted into the Göttingen Rhizolab, which is an outdoor facility designed for the investigation of root growth dynamics of woody plants (*cf*. Meier & Leuschner, 2008), as well as into neighboring lysimeters. In total, sixteen drained, large-scale containers (volume 7 m^3) arranged in four rows in belowground facilities, with the

rims of the containers at ground level, were included in the experiment. Each container was divided by polyethylene plates into four plots, resulting in 64 plots in total. These facilities were automatically covered by a mobile Plexiglas rain shelter during precipitation events, thus allowing full control of the soil water while glasshouse artifacts were avoided. Tree saplings were fertilized during the growing seasons (June 2012 to August 2015) every second week with 2 L of a 0.1% NPK fertilizer solution containing trace elements (concentrations of undiluted NPK fertilizer: 2.5 M NH_4, 0.5 M NO_3, 0.4 M CH_4N_2O, 0.7 M P_2O_5, 0.8 M K_2O, 11.5 mM B, 0.8 mM Cu, 4.4 mM Fe, 2.7 mM Mn, 0.13 mM Mo, 0.8 mM Zn).

Tree saplings were arranged in a randomized block design, with two AM tree species and two ECM tree species planted together into one container, at a spacing of 1 m between individuals. Planting occurred in two subsequent campaigns for the Rhizolab (May 2012; AM: ash and sycamore; ECM: beech and lime) and the lysimeters (June 2013; AM: Norway maple and cherry; ECM: oak and hornbeam). In our experiment, saplings were grown in mineral sand with a particle size of ≥ 2 mm. We maintained two soil water contents (SWC) among the containers: a drought treatment (5% SWC, v/v) and a well-watered treatment (10% SWC, v/v), each treatment replicated four times per tree species. Soil water content was measured throughout the profile to a depth of 1.1 m: one access tube was inserted vertically in each plot and the volumetric soil water content measured every second day by frequency domain reflectometry (FDR; Diviner2000, Sentek Sensor Technologies, Australia). Water loss by transpiration or evaporation was quantified based on soil water measurements to a depth of 40 cm and was replaced every day by irrigating the soil surface homogeneously and at a slow rate (drip irrigation). The drought treatment was initiated in May 2014 after complete leaf expansion and was paused during the non-growing season to allow natural precipitation to bring the soil back to field capacity. The experiment simulated two summer droughts of about 24 weeks each (May to September in 2014 and 2015, respectively).

Air temperature and air humidity were recorded continuously at 10-min intervals with a Hobo Pro RH/Temp data logger (Onset Computer, USA). Soil temperature was measured with several negative temperature coefficient (NTC) thermistors arranged in 16 horizontal lines at 10-cm soil depth and in four vertical profiles to a depth of 1 m.

Photosynthesis and leaf respiration

Leaf gas exchange measurements were conducted during mid and late season 2015 on one canopy leaf per plant with an infrared CO_2 analyzer (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) during the middle of an overcast day. We measured leaf photosynthesis

(A; µmol m^{-2} s^{-1}) at ambient photosynthetically active radiation (PAR: 873 ± 40 µmol m^{-2} s^{-1}) and leaf respiration in the dark (PAR: 0.7 ± 0.03 µmol m^{-2} s^{-1}). For each light level, the leaves were allowed to equilibrate for three minutes before data were logged. While leaf photosynthesis was already in equilibrium with the respective ambient PAR, the measurement of leaf respiration included the acclimation of the leaf to a change in light, i.e. to zero PAR. Even though we did not observe any further increases in leaf respiration rates after the equilibration time, we cannot completely rule out that further slight increases in leaf respiration may have had occurred after this equilibration time and that leaf respiratory rates are underestimated to a certain extent. During the measurement in July 2015, measurement conditions were slightly warmer and drier (average leaf temperature 32 ± 0.2ºC, relative humidity 33 ± 1%, vapor pressure deficit 33 ± 1 hPa), while in September conditions were slightly cooler and moister (average leaf temperature 22 ± 0.3ºC, relative humidity 44 ± 1%, vapor pressure deficit 15 ± 0.3hPa). CO_2 concentrations were ambient [CO_2] during both measurement periods (400 and 393 µmol CO_2 mol^{-1} air, respectively). Absolute leaf photosynthesis and respiration of the whole plant (in mol CO_2 h^{-1}) were calculated by multiplying the specific photosynthetic and leaf respiration rate, respectively, by the total leaf area.

Root exudate collection

In five sampling campaigns during the growing seasons 2014 and 2015 (i.e., May 2014, August 2014, May 2015, July 2015, and September 2015), intact fine root strands still attached to a tree sapling were carefully extracted from the soil and sand adhering to the root system was cautiously removed with deionized water and fine forceps to maintain the integrity of the root. Living root systems were then placed into root cuvettes filled with sterile 2-mm diameter glass beads (*cf*. Phillips *et al.*, 2008), which were moistened with C-free nutrient solution (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.15 mM $MgSO_4$, and 0.3 mM $CaCl_2$). Sterile cuvettes with glass beads and nutrient solution were included as controls. Roots were allowed to equilibrate in the cuvette environment for 24 h before being flushed with dilute nutrient solution using a low-pressure vacuum. New nutrient solution was added and the root was allowed to equilibrate for another 48 h. We collected these trap solutions containing exudates from each cuvette, determined their exact volume by high-precision weighing, and filtered the solution through sterile syringe filters (pore size: 0.7 µm; GE Healthcare Life Sciences Whatman, Glass Microfiber Filters, Grade GF/F). The trap solution was stored at -20°C until analysis. The solutions were analyzed for dissolved organic C on a total organic carbon analyzer

(Shimadzu TOC-L CPH/CPN; Shimadzu Scientific Instruments, Duisburg, Germany). Root area-specific exudation rates (gross root exudation minus reabsorption and microbial consumption; in µmol C m^{-2} h^{-1}) were calculated as the total amount of C flushed from each root system over the incubation period divided by the root surface of the investigated root strand, and hereafter referred to as specific exudation rate. Absolute exudation C flux of the whole plant (in mmol C h^{-1}) was estimated by multiplying the root area-specific exudation rate by the total fine root surface, which was determined at the end of the experiment. Photosynthetic C cost of root exudation (%) was calculated by the share of absolute C loss by root exudation in absolute C uptake by photosynthesis.

Root morphology

After root exudate collection, root strands were clipped off the tree and stored at 6°C for no longer than one week until processing. Fine root morphology (length, surface area, and diameter) was analyzed for all fine root samples by optical surface area measurement with a flatbed scanner and the program WinRHIZO 2013e (Régent Instruments Inc., Québec, Canada). Subsequently, root biomass was determined by drying (48 h, 70°C) and weighing. Specific root area (SRA, in cm^2 g^{-1}), specific root length (SRL, in m g^{-1}), and root tissue density (RTD, in mg cm^{-3}) were calculated from these measurements.

The degree of AM colonization was investigated by bleaching the root strands in 10% KOH (24 h, 80°C) and staining in an ink-based solution (5% ink in 5% acetic acid; Vierheilig *et al.*, 2005). Stained root samples were stored in 50% glycerol solution (Brundrett *et al.*, 1996) for no longer than one week until processing. The degree of AM colonization of roots was quantified by examining vesicles, arbuscules, and internal hyphae with the gridline-intersect method (Giovanetti & Mosse, 1980). The degree of ECM colonization of root tips was determined from unstained roots according to differences in their color, thickness, texture, and branching patterns.

Inorganic and organic ^{15}N uptake

In early September 2015, towards the end of the study, we conducted an isotope labeling experiment in the containers of the well-watered treatment. Before the application of the tracer solution, topsoil samples were collected with a soil corer for determination of the background pool of inorganic and organic N sources (n = 4 soil samples per species): the contents of NH_4^+ and NO_3^- were measured by extraction with 0.5 M K_2SO_4 and analysis with a continuous flow injection colorimetry auto-analyzer (Cenco/Skalar Instruments, Breda, Netherlands).

Subsequently, the concentration of free amino acids in the K_2SO_4 extracts were determined by a colorimetric approach (*cf*. Rosen, 1957): amino acids were reduced with 3% ninhydrin solution and the optical absorbance at 570 nm (GENESYS 20 Visible Spectrophotometer; Thermo Fisher Scientific, Waltham, MA, USA) was compared to a glycine standard curve.

Within each container quadrant, four randomly selected sub-quadrants (0.25 x 0.25m) around each tree received equal volumes of either 98% atom-enriched $^{15}NH_4Cl$, 98% atom-enriched $K^{15}NO_3$, 98% atom-enriched $C_2H_5{}^{15}NO_2$, or water (control) (225 ml aliquots of the labeling solution or water). Tracer solutions were distributed evenly across the soil surface of the sub-quadrant. The leaching depth of these tracer solutions was experimentally determined previously by the application of an ink solution and measurement of the leaching front line, which was found at a soil depth of at most 10 cm. We added ^{15}N at tracer level and increased the background N pool by no more than 15% to avoid fertilization effects and the switch of roots to low-affinity transporters (Näsholm *et al.*, 2009). Given the rapid turnover time of amino acids in soil (Finzi & Berthrong, 2005) we sampled fine root strands 1 h following isotope addition, to observe intact uptake of amino acids, and to avoid $^{15}NH_4^+$ losses by nitrification and axial efflux of ^{15}N with the xylem flow out of the investigated fine root segment. Fine root strands were carefully extracted from the topsoil, rinsed in 0.5 M $CaCl_2$ to remove remaining ^{15}N adsorbed to the root cortex, followed by a rinse with water, and were then immediately frozen at -20°C to prevent further metabolism. The frozen samples were dried (48 h, 70°C) and ground. The nitrogen (well-watered treatment: tracer ^{15}N; drought treatment: natural-abundance ^{15}N) and carbon (natural-abundance ^{13}C) isotope signatures as well as the N and C contents of fine roots (< 2 mm) were determined by elemental analysis (NA 1108; Fisons-Instruments, Rodano, Milano, Italy) coupled with isotope mass ratio spectroscopy (Delta plus, ThermoFinnigan, USA) at the Centre for Stable Isotope Research and Analysis (KOSI) of the University of Göttingen.

The mass-specific rate of ^{15}N uptake after 1 h was calculated as the product of the N content of fine roots and the atom% ^{15}N excess of the bulk fine root sample (U_{15N}; in µg ^{15}N g root^{-1} h^{-1}; *cf*. Gallet-Budynek *et al.*, 2009). The isotope dilution of each N form was determined by dividing the concentration of each N form in the soil ($C_{available\ N}$; in µmol N g soil^{-1}) by the concentration of applied ^{15}N label ($C_{15N\ label}$; in µmol^{15}N g soil^{-1}). The root area-specific rate of N uptake (U_N; in µmol N m^{-2} root h^{-1}) for each N form was computed by dividing $C_{available\ N}$ with $C_{15N\ label}$ and multiplying the quotient with U_{15N} (*viz.* specific N uptake). Under the assumption that the uptake of different N forms was unaffected by the label, total inorganic N uptake was calculated as the sum of the specific $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake rates, while organic

N uptake equaled the specific ^{15}N-glycine uptake rates. Ratios of organic to inorganic N uptake (µmol m^{-2} h^{-1}) were calculated by dividing specific rates of organic N with specific rates of inorganic N. The absolute N uptake rate of the whole plant (in mmol N h^{-1}) was estimated by multiplying the specific N uptake rate by the total fine root surface.

Despite the limited volume of the labeling solution, the addition of the liquid may have imposed a wetting event in dry soil and, thus, we refrained from conducting a labeling experiment in the drought treatment of our experiment. To reveal differences in (inorganic) N uptake by the trees in the drought treatment (*cf*. Gallet-Budynek *et al.*, 2009; Averill & Finzi, 2011), we analyzed the natural-abundance δ^{15}N signature of their sun leaf dry mass by isotope mass ratio spectroscopy. In addition, differences in photosynthetic C uptake between tree species and treatments were analyzed from the natural-abundance δ^{13}C signature of leaves. Natural-abundances of ^{15}N and ^{13}C, respectively, are reported in ‰ and are calculated by dividing the heavy to light isotope abundance ratios of samples by the respective isotope ratio of a reference standard.

Biomass production

In September 2015, all tree saplings were harvested within a two-week period following a rotating harvesting scheme. The trees were divided into aboveground and belowground biomass and carefully extracted from the soil, while the root system was divided into topsoil (0-40 cm) and subsoil (> 40 cm) segments. Additionally, the maximum depth of the root system was determined. To remove all soil particles, the roots were carefully washed under tap water and subsequently sorted by diameter (fine roots ≤ 2 mm, coarse roots > 2 mm). SRA was determined for all trees from each one randomly selected, intact branch root system (15 cm in length) per soil layer, and was multiplied by fine root mass to compute total fine root surface area. Leaf size and specific leaf area (SLA, in cm^2 g^{-1}) were determined for all leaves of each tree. Root and leaf area measurements were conducted by optical surface measurement with a flatbed scanner and the programs WinRHIZO and WinFOLIA, respectively (Régent Instruments). After analysis, leaves, shoots, coarse roots, and fine roots were dried (72 h, 70°C) and weighed. Dried subsamples were ground and analyzed for total C and N using an elemental analyzer (vario EL III; elementar, Hanau, Germany).

Statistical analyses

Statistical analyses were conducted with the package SAS, version 9.3 (Statistical Analyses System, SAS Institute Inc., Cary, NC, USA). Significance was determined at $P \leq 0.05$. Means

and standard errors were calculated from the mean of four individuals per tree species and soil moisture treatment. The probability of fit to a normal distribution was tested using a Shapiro–Wilk test ($P \leq 0.05$). Non-normally distributed data were log-transformed to resemble normality. Means were compared by one-way Kruskal-Wallis single factor analyses of variance and non-parametric multiple comparison tests after Wilcoxon to analyze the differences between tree species and soil moisture treatments. Normally distributed data were analyzed by applying two-factorial nested ANOVAs to test for significant effects of mycorrhizal type (main effect) and tree species identity (nested effect) on a selection of the investigated variables (i.e., specific photosynthetic and leaf respiration rates, natural-abundance foliar ^{13}C and ^{15}N signatures, specific root exudation rates, specific uptake rates for ammonium, nitrate, and organic N, and the ratios of organic to inorganic N uptake rates). We calculated linear correlations of inorganic and organic N uptake rates and tree biomass for the eight tree species.

Results

Roots traits, plant morphology and biomass production of AM and ECM tree species

Root morphology and the degree of mycorrhizal colonization were significantly different between the two mycorrhizal types: AM trees had a significantly higher average root diameter (2.3 vs. 0.8 mm), lower SRA (297 vs. 391 $cm^2\ g^{-1}$), lower SRL (18 vs. 38 $m\ g^{-1}$), lower branching intensity (1.4 vs. 3.2 tips cm^{-1}), and lower degree of mycorrhizal colonization (85 vs. 99%) than ECM trees (Table 1). The two mycorrhizal types also differed significantly in their aboveground and belowground size and biomass: AM trees were bigger-sized and had more biomass than ECM trees (Table S1). AM trees also had a higher fine root to leaf biomass ratio, but smaller root C to leaf C ratio than ECM trees under ambient water supply. AM trees responded to drought by significantly reduced total shoot and stem biomass, tree height, and coarse root diameter, and increases in the taproot to shoot length ratio and in root tissue density, while there was no significant difference in leaf and fine root biomass. By contrast, ECM trees did not respond to drought with significant reductions in stem, leaf, and fine root biomass, decreases in coarse root diameter, or changes in root morphology. The only drought response of ECM trees was a significant reduction of tree height and an increase in the fine root to leaf biomass ratio.

Table 1 Root morphology and architecture of well-watered and drought-treated arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species (four tree species per mycorrhizal type and soil moisture treatment with standard errors in parentheses. Significant differences between the mycorrhizal types and water treatments are indicated by different lower case letters.

ROOT TRAIT	**AM**		**ECM**	
	well-watered	**drought**	**well-watered**	**drought**
Root diameter [mm]	2.3 (0.2) a	2.2 (0.2) a	0.8 (0.1) b	1.0 (0.1) b
RTD [g cm^{-3}]	0.9 (0.1) a	1.2 (0.08) b	1.0 (0.09) ab	1.1 (0.25) ab
SRA [$cm^2 g^{-1}$]	297 (21) a	265 (10) a	391 (17) b	368 (25) b
SRL [m g^{-1}]	18 (2) a	17 (1) a	38 (4) b	34 (2) b
Branching intensity [tips cm^{-1}]	1.4 (0.3) a	1.3 (0.1) a	3.2 (0.5) b	3.8 (0.6) b
Mycorrhizal colonization [%]	83 (5) a	74 (5) a	99 (1) b	98 (1) b

The effect of the mycorrhizal type on the leaf and root C flux

During the early growing season, we found significant influences by the mycorrhizal type on specific photosynthetic and leaf respiration rates in both the well-watered and drought treatment (highly significant for the mycorrhizal type; Table 2). Similarly, specific root exudation was significantly influenced by the mycorrhizal associates (and not by tree species identity), but only at reduced soil moisture conditions. During the late growing season, tree species identity lost its influence on aboveground C fluxes, while the significant influence of the mycorrhizal type on both above- and belowground C fluxes remained. However, the influence of the mycorrhizal type occurred for different physiological processes in different soil moisture conditions in the late growing season: the mycorrhizal type influenced leaf respiration and root exudation at ample soil moisture conditions, but had no effect on leaf and root C loss in dry soil. By contrast, C uptake by photosynthesis was only in dry soil influenced by the mycorrhizal type and not in well-watered soil. Long-term reductions in stomatal conductivity, i.e. the leaf foliar $\delta^{13}C$ signature, were in both soil moisture conditions influenced by both, the mycorrhizal type and tree species identity.

Table 2 Significance of the effects of tree species identity (SPEC) and mycorrhizal type (MYC) on leaf and root C and N uptake rates of well-watered and drought-treated arbuscular mycorrhizal and ectomycorrhizal tree species during the early (June) and late (September) growing season 2015. Values given are F-values of two-factorial nested ANOVAs with mycorrhizal type as main effect and tree species identity as nested effect (significance: *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$).

		Early season				Late season			
		Well-watered		Drought		Well-watered		Drought	
		SPEC	MYC	SPEC	MYC	SPEC	MYC	SPEC	MYC
Photo-synthesis	mol C-CO_2 m^{-2} h^{-1}	1.6	14.8***	3.5*	10.3**	0.9	1.7	0.9	9.5**
Leaf respiration	mol C-CO_2 m^{-2} h^{-1}	3.2*	15.9***	1.6	16.3***	2.4	4.7*	1.2	2.7
Leaf $\delta^{13}C$	‰					3.9**	11.3**	3.9**	5.8*
Leaf $\delta^{15}N$	‰							1.5	1.2
Root exudation	mol C_{org} m^{-2} h^{-1}	0.9	1.2	1.2	7.6**	1.2	3.6*	1.8	0.3
NH_4^+ uptake	mol NH_4^+-N m^{-2} h^{-1}					37.5***	1.9		
NO_3^- uptake	mol NO_3^--N m^{-2} h^{-1}					10.2***	4.4		
N_{org} uptake	mol AA-N m^{-2} h^{-1}					22.6***	0.8		
N_{org}: N_{inorg}	mol mol^{-1}					4.6**	6.1*		

Across spring and fall, we found a significant seasonal decrease in root C loss by specific exudation in both well-watered AM and ECM trees by 37 and 61%, respectively (AM: reduction from 57 to 36 µmol C m^{-2} h^{-1}; ECM: 54 to 21 µmol C m^{-2} h^{-1}, Fig. 1). Similarly, absolute C loss by root exudation and leaf respiration decreased seasonally by 60 and 70% in AM and ECM trees, respectively (Fig. 2a). The photosynthetic C cost of root exudation at ample soil moisture was similar between AM and ECM tree species in the early season, but decreased in ECM trees in the late season (1.6 vs. 0.6%; Fig. 2b).

In both the early and late growing seasons, drought increased specific root exudation by 1.7 times in ECM trees (early: significant increase; late: not significant), while it had no

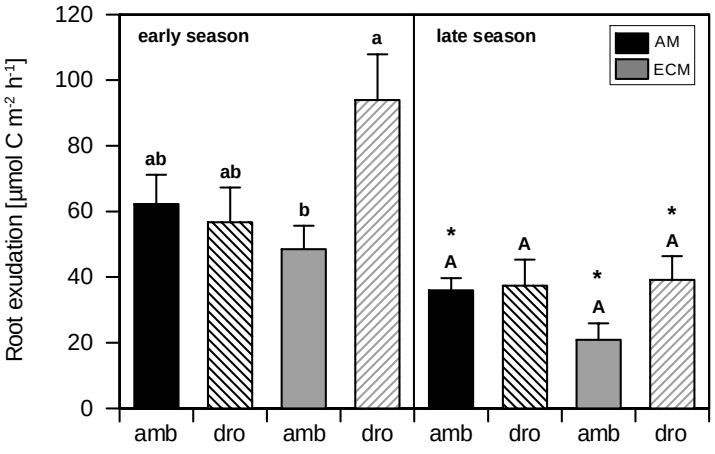

Figure 1 Root area-specific exudation rates of well-watered watered (amb) and drought-treated (dro) arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species during the early (June) and late (September) growing season 2015 (n = 4 tree species per mycorrhizal type and soil moisture treatment). Significant differences between the mycorrhizal types and water treatments are indicated by different lower (early season) and upper case letters (late season). Significant differences between the early and late season are indicated by asterisks.

significant effect in AM trees (Fig. 1). In both seasons, soil drought caused a reduction in absolute C uptake and absolute C release of the two mycorrhizal types (difference not significant; Fig. 2a). With drought, both mycorrhizal types increased the percentage of photosynthates invested into root exudation (Fig. 2b). The increased photosynthetic C cost of root exudation due to drought was significant during the early growing season in AM trees (significant increase from 2.2 to 5.0%), and occurred in both the early (increase from 3.0 to 28.5%, not significant) and late growing season (significant increase from 0.6 to 6.8%) in ECM tree species.

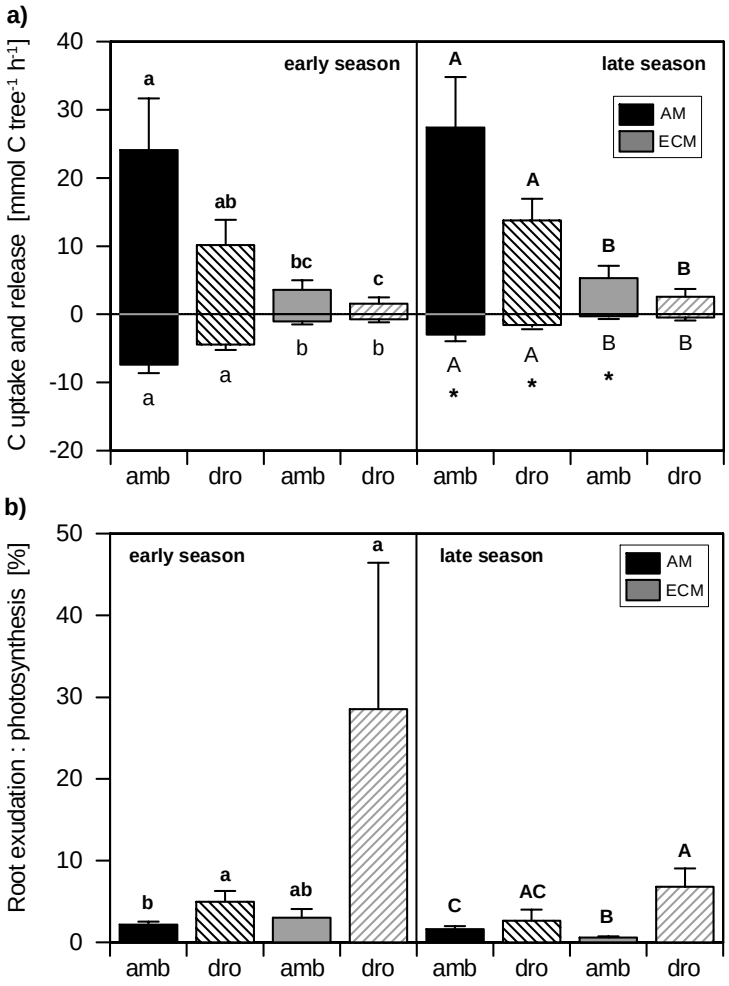

Figure 2 Carbon flux by leaf photosynthesis, leaf respiration, and root exudation of well-watered (amb) and drought-treated (dro) arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species during the early (June) and late (September) growing season 2015 (n = 4 tree species per mycorrhizal type and soil moisture treatment). Given are **(a)** C uptake by photosynthesis and C release by leaf respiration and root exudation, and **(b)** the portion of root C exudation in photosynthetic C uptake (C cost of root exudation). Significant difference between the mycorrhizal types and water treatments are indicated by different lower (early season) and upper (late season) and Greek letters. Significant differences between the early and late season are indicated by asterisks.

Nitrogen uptake of AM and ECM tree species

Specific ammonia, nitrate, or organic N uptake rates were not significantly different between the two mycorrhizal types (Fig. 3a), but were influenced by tree species identity (Table 2).

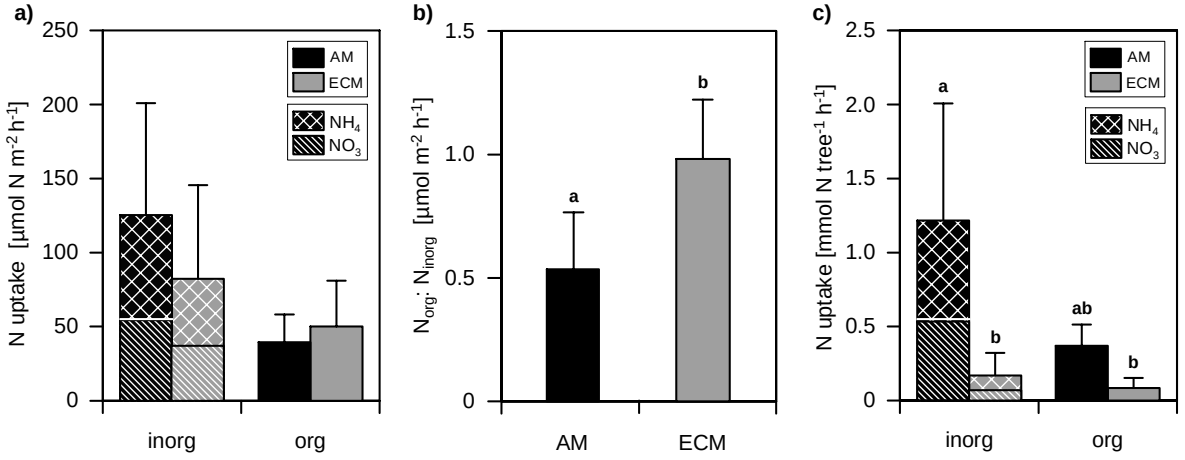

Figure 3 Rate of inorganic (ammonia and nitrate) and organic (glycine) N uptake (U_N) of well-watered arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species in September 2015 (n = 4 tree species per mycorrhizal type). Given are **(a)** root mass-specific N uptake rates, **(b)** ratios of organic: inorganic N uptake and **(c)** tree-specific N uptake rates of organic and inorganic N. Significant difference between the mycorrhizal types are indicated by different lower case letters.

However, the ratio of specific organic to inorganic N uptake rates was influenced by both the mycorrhizal type and tree species identity (Table 2) and significantly reduced in AM trees (0.5 mol mol^{-1}) in comparison to ECM trees (1.0 mol mol^{-1}, Fig. 3b), i.e. AM trees had a higher relative inorganic N uptake. AM trees also had significantly higher absolute inorganic N uptake rates than ECM trees (AM: 1.2 mmol N $tree^{-1}$ h^{-1}; ECM: 0.2 mmol N $tree^{-1}$ h^{-1}; Fig. 3c), while organic N uptake rates on a per tree basis were not significantly different between mycorrhizal types.

Table 3 Correlation of root exudation with nitrogen uptake and total biomass of each four well-watered arbuscular mycorrhizal and ectomycorrhizal tree. Given are Pearson's correlation coefficients R and probabilities of error P. Significant correlations are shown in bold.

VARIABLE	NO_3^-uptake		NH_4^+uptake		N_{INORG} uptake		N_{ORG} uptake		Tree biomass [g]	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*
Exudation [µmol C g^{-1} h^{-1}]	**-0.44**	**0.03**	-0.29	0.14	**-0.42**	**0.04**	**-0.45**	**0.02**	**0.4**	**0.03**
NO_3^-uptake [µmol NO_3^--N g^{-1} h^{-1}]			**0.81**	**<0.001**	**0.96**	**<0.001**	**0.9**	**<0.001**	-0.11	0.59
NH_4^+uptake [µmol NH_4^+-N g^{-1} h^{-1}]					**0.94**	**<0.001**	**0.83**	**<0.001**	-0.05	0.79
N_{INORG} uptake [µmol N_{INORG} g^{-1} h^{-1}]							**0.91**	**<0.001**	-0.13	0.52
N_{ORG} uptake [µmol AA-N g^{-1} h^{-1}]									-0.14	0.48

Tree biomass did not correlate with specific N uptake rates, which remained comparably constant despite large differences in final tree biomass (Table 3). Surprisingly, specific root exudation rates were significantly negatively correlated with specific inorganic N uptake rates (R = -0.42) as well as individually with NO_3-N and glycine-N uptake rates (nitrate: R = -0.44; glycine: R = -0.45), but did not correlate with NH_4-N uptake rates (Table 3). All specific N uptake rates of the three different N forms were highly positively correlated ($P < 0.001$).

Discussion

The distinction between AM and ECM root associations may provide a powerful tool in framework predictions of global change impacts on temperate forests (Phillips *et al.*, 2013). In this study, we found evidence that summer droughts, which are predicted to increase in frequency and duration in Central Europe, lead to higher photosynthetic C costs of root exudation in ECM tree species than in AM tree species. ECM trees also differed by higher organic to inorganic N acquisition ratios than in AM tree species, which suggests that the mycorrhizal associates have the capability to influence the C and N economies of their host trees.

Carbon release by root exudation of AM and ECM tree species

Photosynthetic C cost of root exudation of well-watered saplings of this study was 1-3%, which is comparable to the C cost of root exudation in a Midwest hardwood forests (3%; Yin *et al.*, 2014), and marginally surpassed by estimations from labeling studies (5-12%; Jones *et al.*, 2004; Phillips & Fahey, 2006). Root mass-specific exudation rates measured in this study were approximately 10-23 µg C g^{-1} h^{-1} in well-watered trees, which is similar to those reported for black locust (10-22 µg C g^{-1} h^{-1}; (Uselman *et al.*, 2000), loblolly pine saplings (12-26 µg C g^{-1} h^{-1}; Meier *et al.*, 2013), and a temperate hardwood forests (8-20 µg C g^{-1} h^{-1}; Yin *et al.*, 2014). Up to four times higher root exudation was reported for mature European beech trees (33-82 µg C g^{-1} h^{-1}; Tückmantel *et al.*, 2017) and a mixed hardwood forest (29-100 µg C g^{-1} h^{-1}; Brzostek *et al.*, 2013), which may be a consequence of differences in plant age and/or differences in soil organic matter (SOM) content. Topsoils which have developed from glacial deposits are SOM-dominated with comparably low N availability mainly from organic N forms (Brzostek *et al.*, 2013; Tückmantel *et al.*, 2017), which may explain enhanced topsoil exudation rates in these studies (Tückmantel *et al.*, 2017).

Contrary to our first hypothesis, specific root exudation rates of well-watered AM and ECM tree species did not differ significantly, which might be due to the homogeneity of their soil

environment composed of fertilized mineral sand. However, several studies suggested that ECM trees have higher specific exudation rates than AM tree species (e.g. Phillips & Fahey, 2006; Yin *et al.*, 2014), which is interpreted to reflect differences in N availability between these two major mycorrhizal types, with the majority of soil N contained in SOM rather than in mineral-associated C forms in ECM forests (Brzostek *et al.*, 2014; Yin *et al.*, 2014). Differences in the specific root exudation rates between AM and ECM trees could be a result of either physiological acclimation or genotypic adaptation to their contrasting natural environments (Yin *et al.*, 2014).

The missing differences in the quantity of specific root exudation between well-watered AM and ECM trees reported in this study hint to phenotypic acclimation to the amount of organic matter driving the rate of root exudation of (at least) ECM tree species. Such acclimation is in accordance with a strategy to maximize whole-tree carbon-use efficiency, as C loss by exudation is reduced in soil spots where positive priming effects are unlikely. Moreover, C exudation is enhanced where microbes can mine less bioavailable SOM (Tückmantel *et al.*, 2017). This assumption is further supported by the fact that ECM trees reduced their investment of photosynthates into root exudation from the early to the late growing season significantly more than AM trees, which may indicate a potential of short- to mid-term acclimation to seasonal differences in growth demands, nutrient availability, and climate. Some authors suggested that seasonal patterns in soil temperature influence root exudation rates (Phillips *et al.*, 2011; Yin *et al.*, 2013) due to temperature dependent changes in the speed of metabolic processes (Neumann & Römheld, 2007), while others contradicted a dominant temperature effect on root exudation (Tückmantel *et al.*, 2017). Future studies should focus on the potential role of soil temperature and organic matter content in increasing root exudation of ECM trees.

Elevated C cost of root exudation in drought-treated ECM trees

Root exudation of ECM trees increased with drought both in terms of specific rates (increase by 1.7 times) and in terms of photosynthetic C costs (increase by ten times), while exudation of AM trees did not, which is in contrast to hypothesis (2). This difference has important implications for the C cycle in AM and ECM ecosystems exposed to increasing intensity and frequency of summer droughts under climate change as predicted for many parts of Europe and eastern North America, since ECM trees will probably have increasing investments into root exudation and soil C inputs. The acclimation of root exudation of ECM trees to dry soil could be interpreted (i) as a stress response due to increased friction in dry soil (Boeuf-Tremblay *et al.*, 1995; Walker *et al.*, 2003), which could lead to damage of the root cortex and leakier roots

(Phillips & Fahey, 2006; Neumann & Römheld, 2007). Increased root exudation may also be (ii) an adaptive response to low water availability via the active secretion of mucilage to increase hydraulic conductivity of the rhizosphere (Carminati, 2013) or accompany hydraulic lift (Kroon *et al.*, 1998; Querejeta *et al.*, 2007). Finally, increased root exudation could also be (iii) a response to low nutrient availability in dry soils and the implementation of a priming effect. Results on the effect of drought on root exudation in the literature are contradictory so far: some studies found an increase in root exudation in wheatgrass (Henry *et al.*, 2007) and ECM pine seedlings (Reid & Mexal, 1977), while others found no drought effect in non-mycorrhizal aspen seedlings (Karst *et al.*, 2017), which is generally in line with the results of our study. However, in reviews it is assumed that the amount of photosynthates and, thus, root exudation decrease with drought (Lehto & Zwiazek, 2011; Brunner *et al.*, 2015). Here we show that it is not a fixed portion of photosynthates that is invested into root exudation, but a variable amount that changes with season, soil moisture conditions, and mycorrhizal type.

In AM trees, drought did not significantly influence root exudation but had a distinct negative effect on growth-related traits of AM trees. Limited soil water supply significantly reduced stem biomass production and tree height (reductions by 50 and 48%, respectively, in AM trees and by 15 and 25% in ECM trees), as well as fine root biomass production and mycorrhizal colonization rates (reductions by 32 and 10%, respectively, in AM vs. no reduction in ECM). These results imply that elevated root exudation in ECM trees exposed to drought can be interpreted as an adaptive response to alleviate drought-induced reductions in the productivity of ECM trees. Elevated root exudation under drought may significantly add to the effect by the extension of the absorbing root surface area (as suggested by Lehto & Zwiazek, 2011) in enhancing the water and nutrient status of ECM trees. This adaptability of root exudation in ECM tree species is further supported by the increased branching intensity of ECM root systems, which is linked to the fast, acquisitive spectrum of functional traits (Liese *et al.*, 2017a): high branching intensities have been related to high root respiration rates (Rewald *et al.*, 2014) and high resource uptake activities (Guo *et al.*, 2008b; Rewald *et al.*, 2011; McCormack *et al.*, 2015), but may also support enhanced root exudation of ECM trees in dry soil. It remains an open question, though, whether the main function of increased root exudation in dry soil is to increase hydraulic conductivity or the nutrient availability in the ECM rhizosphere.

In addition to greater root exudation, the investigated ECM root systems had greater C sink strength in their biomass, potentially due to the higher mycorrhizal colonization and the generally higher C costs of the ECM fungi (Smith & Read, 2008). Both mycorrhizal

colonization and association type are thought to have cascading effects on C cycling processes like litter decomposition, soil respiration, soil C:N ratio (Cornelissen *et al.*, 2001; Soudzilovskaia *et al.*, 2015a, b), and ecosystems C storage (Averill *et al.*, 2014). Our results further indicate that C fluxes via specific root exudation are influenced by the mycorrhizal type and not by the tree species identity. Altogether, these results highlight the importance of distinguishing between the two major mycorrhizal types when predicting root C cycles.

Higher inorganic N uptake in AM than in ECM trees

Specific rates of inorganic N uptake were not different between the two mycorrhizal types, but absolute and relative inorganic N uptake were higher in AM than in ECM trees, which may reflect the inorganic environment in which they naturally occur. It has been suggested that the mycorrhizal type is an important factor in predicting forest N fluxes (Midgley & Phillips, 2014). In contrast, other studies demonstrated a greater effect on N dynamics by tree species identity than by the mycorrhizal type (Templer & Dawson, 2004; Jacob & Leuschner, 2014). AM hyphae can take up and transport both inorganic (Govindarajulu *et al.*, 2005) and organic N forms (Hodge *et al.*, 2001; Whiteside *et al.*, 2012). Yet given the limited saprotrophic capabilities of most AM fungi and the high availability of inorganic N in AM ecosystems, it is assumed that AM trees are specialized in inorganic N uptake (Smith & Read, 2008), which is supported by the results of our study. In conclusion, a higher absolute inorganic N uptake and lower organic to inorganic N uptake rate ratio of AM tree species in our study are in accordance with hypothesis (H3). This specialization could be caused by a genetic adaption to their natural habitats e.g. by the production of a higher density or affinity of membrane transporters for ammonium and nitrate in AM root systems (see Guether *et al.*, 2009; Pérez-Tienda *et al.*, 2011).

Several studies suggest a link between N availability or uptake and exudate release (Phillips *et al.*, 2009; Fransson & Johansson, 2010; Yin *et al.*, 2013; Yin *et al.*, 2014). For example, Yin *et al.* (2014) found that in natural habitats AM tree species had lower organic to inorganic N ratios in rhizosphere soil than ECM tree species, and lower root exudation. These authors assumed that increased root exudation in ECM trees is linked with high organic N contents in the soil. In addition, Fransson & Johansson (2010) found that low-molecular-weight organic compound exudation was negatively affected by inorganic N in ECM trees. However, to our knowledge, specific root exudation rates have not been directly correlated with specific N uptake rates so far. In our study with SOM poor soil conditions, correlation analyses showed that specific exudation rates were negatively correlated with specific rates of nitrate and glycine uptake, whereas the correlation with ammonium was also negative but not significant. Wojtaszek *et al.*

(1993) have previously demonstrated in a greenhouse experiment that root exudation of phenolic compounds in white lupine is negatively related to the concentration of inorganic N in the growing medium, and that nitrate had a greater effect on exudation than ammonium. The negative correlation might possibly indicate that roots (i) react to low uptake rates of N with higher exudation rates in order to optimize nitrogen acquisition, and/or (ii) decrease exudation during high N uptake rates to prevent unproductive C losses. Since we measured net specific exudation it is also possible that low contents of exudates must not be considered as a general reduction in exudation, but can also indicate effects of bidirectional C fluxes. In this context, it is conceivable that (iii) low specific exudation rates with simultaneously high specific N uptake rates may represent high C investments of the tree in the release of chelators which mobilize soil N followed by a combined (re)absorption of both, N and C, and thus a low net C release by root exudation. While all three processes seem plausible, it warrants further investigations to decide on their actual (independent or concurrent) contribution to the observed negative relationship between root exudation and N uptake.

Conclusion

This study contributes to the key challenge of a mycorrhiza-based framework in order to predict ecosystem processes under global change. Here we present evidence of systematic differences between AM and ECM trees in some key biogeochemical processes like root exudation and the organic to inorganic acquisition ratio, and the lesser role of tree species identity in determining root C release. Photosynthetic C cost of root exudation in ECM trees is increased with soil drought, despite their limited biomass response, which may hint at an adaptive increase of root exudation in dry soil to increase either hydraulic conductivity (via the secretion of mucilage or as a consequence of hydraulic lift) or nutrient availability (via a priming effect). We further prove that AM trees are specialized in inorganic N uptake, which does not align to their root exudation. While our study has demonstrated some process-based evidence, it remains a challenge to translate these important root functions into the context of their natural habitats in mature forest stands. Despite this open challenge, our results suggest that the mycorrhizal type can be a key trait for predictions of biogeochemical cycles that warrants further investigations across different ecosystems.

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Supplementary Material

Supplemental Table 1 Aboveground and belowground size, biomass, and relative growth rates (RGR) of well-watered and drought-treated arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species (4 tree species per mycorrhizal colonization type and soil moisture treatment with standard errors in parentheses). Significant differences between the mycorrhizal association types and water treatments are indicated by different lower case letters.

	AM		**ECM**	
	Well-watered	**Drought**	**Well-watered**	**Drought**
ABOVEGROUND				
Total shoot [g]	545 (179) a	294 (79) b	76 (26) c	61 (16) c
Leaves [g]	151 (46) a	98 (24) a	23 (6) b	16 (7) b
SLA [$cm^2\ g^{-1}$]	123 (7) a	122 (7) a	162 (13) b	163 (12) b
Leaf C:N [$g\ g^{-1}$]	24 (2) a	21 (1) a	20 (1) a	19 (2) a
Leaf $\delta^{13}C$ [‰]	-30.2 (0.2) b	-29.6 (0.7) b	-31.2 (0.5) a	-30.7 (0.6) ab
Leaf $\delta^{15}N$ [‰]	n/a	6 (5) a	n/a	11 (4) a
Stem [g]	395 (133) a	196 (58) b	53 (20) c	45 (12) c
Tree height [cm]	227 (27) a	152 (31) b	86 (11) c	65 (9) d
RGR tree height [$mm\ d^{-1}$]	0.04 (0.01) ab	0.02 (0.01) b	0.06 (0.01) a	0.03 (0.01) b
BELOWGROUND				
Total root [g]	812 (285) a	543 (188) a	93 (38) b	71 (32) b
Coarse roots [g]	473 (181) a	313 (106) a	63 (29) b	39 (17) b
Coarse root diameter [mm]	36 (5) a	27 (4) b	17 (1) c	14 (2) c
RGR coarse root diameter [$mm\ d^{-1}$]	0.006 (0.002) a	0.004 (0.001) b	0.005 (0.001) ab	0.003 (0.001) c
Max. rooting depth [cm]	128 (28) a	123 (27) a	78 (18) b	71 (11) b
Fine roots [g]	343 (105) a	234 (80) a	33 (11) b	34 (17) b
Root C:N [$g\ g^{-1}$]	32 (2) a	35 (3) a	37 (5) a	37 (2) a
RATIOS				
Fine root: leaf biomass [$g\ g^{-1}$]	2.09 (0.28) a	2.15 (0.20) a	1.40 (0.16) b	3.41 (1.16) a
Root: shoot length [$m\ m^{-1}$]	0.61 (0.09) b	1.19 (0.31) a	0.93 (0.10) a	1.13 (0.10) a
Root C: leaf C [$g\ g^{-1}$]	1.4 (0.1) b	1.7 (0.2) ab	1.9 (0.2) a	2.1 (0.3) a

Chapter 5

THE EFFECT OF DROUGHT AND SEASON ON ROOT LIFESPAN IN TEMPERATE AM AND ECM TREE SPECIES

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Abstract

- Fine roots play a key role in carbon (C), nutrient, and water cycling, with fine root lifespan controlling a major source of soil organic C and regulating plant resource acquisition. Yet, measuring root lifespan remains a technical challenge, which impedes predictions of root lifespan across plant functional types.
- We explored differences in fine root lifespan between four arbuscular mycorrhizal (AM) and four ectomycorrhizal (ECM) trees using mini-rhizotrons in a factorial drought experiment in large mesocosms.
- Median root lifespan of AM and ECM trees differed fundamentally in its response to soil moisture and seasonality: ECM root lifespan was reduced by half in dry soil (from 176 to 81 d), independent of season. By contrast, AM root lifespan was less responsive to drought, but decreased by a third from early to mid-season (from 185 to 127d). In both mycorrhiza types, root lifespan was positively related to root diameter and negatively to the proportion of lower-order roots.
- While our results indicate morphological and architectural traits that predict root lifespan across tree species, they also indicate principal differences in the environmental response of root lifespan in AM and ECM trees, which may reduce uncertainties in global predictions of root lifespan.

Keywords: arbuscular mycorrhiza, deciduous tree species, drought, ectomycorrhiza, fine root longevity, mini-rhizotrons, root morphology, season

Introduction

The root system of forest ecosystems represents a major sink for C fixed by photosynthesis. It has been estimated that up to 30% of the annual C gain of forests can be consumed by root growth and root respiration, especially by the highly dynamic fine root fraction (Scarascia-Mugnozza *et al.*, 2000; Leuschner & Ellenberg, 2017). Fine root turnover, i.e. the growth of new roots and the shedding of senesced roots, represents a major component of net primary production and an important source of soil organic C (Eissenstat & Yanai, 2002; Matamala *et al.*, 2003). Fine root longevity, which is the inverse of the fine root turnover rate, determines how rapidly newly formed roots are transferred to necromass and thus enter the soil as organic matter. This root trait therefore partly controls the amount of root-borne C which is entering the soil (Guo *et al.*, 2004), with consequences for microbial activity in the rhizosphere through priming effects and total soil C storage. Root longevity may also influence the fluxes of water and nutrients in forest ecosystems, as uptake rates have been found to decrease with root ageing (Volder *et al.*, 2005) and root systems with a larger proportion of young roots may be more active. Despite the assumed key role of fine root lifespan in the C, nutrient and water cycles of forest ecosystems, studies investigating the survival of tree fine roots are still rare, which limits our ability to integrate this important root trait into models predicting the functioning of trees and forests under global change. Owing to the difficulty in accessing and observing root longevity directly, various researchers have attempted to identify root morphological proxies of lifespan. In several studies, a positive relation between root diameter and root lifespan has been reported (Wells & Eissenstat, 2001; Anderson *et al.*, 2003; Joslin *et al.*, 2006; McCormack *et al.*, 2012). Lifespan also increases with increasing root order (Gu *et al.*, 2011). However, it must be kept in mind that both fine root diameter and branching patterns are influenced by root colonization with mycorrhizal fungi (Smith & Read, 2008).

In temperate forests, the majority of trees is associated with ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) fungi (Read, 1991). It has been suggested that ecosystems dominated either by AM or by ECM tree species are characterized by different nutrient economies. In systems with abundant AM species, inorganic nutrients should be more readily available than in systems dominated by ECM species, where nutrients are predominantly bound in organic form (Phillips *et al.*, 2013). Furthermore, there is evidence that root morphology (Brundrett, 2002; Comas & Eissenstat, 2009; Comas *et al.*, 2014; Eissenstat *et al.*, 2015), root architecture (Liese *et al.*, 2017a), root functioning (Phillips & Fahey, 2006; Smith & Read, 2008; Yin *et al.*, 2014; Liese *et al.*, 2017b), and biogeochemical fluxes in the rhizosphere (Phillips & Fahey, 2006; Brzostek *et al.*, 2013; Yin *et al.*, 2014) differ between the two

mycorrhizal associations. Several studies reported smaller fine root diameters (Guo *et al.*, 2008b; Smith & Read, 2008; Comas *et al.*, 2014) and higher branching intensity (Liese *et al.*, 2017a) in ECM trees as compared to AM trees, and this could affect root lifespan. According to the described differences in root diameter and branching intensity, one would expect a generally higher fine root lifespan in AM trees than in ECM trees. However, no conclusive evidence of principal differences in the lifespan of AM and ECM tree roots does yet exist (McCormack *et al.*, 2012; Chen & Brassard, 2013).

Apart from root morphology and architecture, environmental conditions have been found to influence fine root lifespan (Comas *et al.*, 2005; Brunner *et al.*, 2015). Various studies have reported a change in root survivorship with season (Johnson *et al.*, 2000; Anderson *et al.*, 2003; Wang *et al.*, 2016), which might in many cases be caused by temperature variation. Not only cold temperatures in winter, but also warm summer temperatures may reduce root longevity, as was observed in trembling aspen and Norway spruce (King *et al.*, 1999; Leppälammi-Kujansuu *et al.*, 2014). Root survivorship may also decrease in periods with lowered photosynthetic C gain, when C assimilation is limited by reduced photosynthetic active radiation (PAR) flux densities or drought, and less carbohydrates are available for root growth (Reich *et al.*, 1998). Soil desiccation can influence root longevity also directly through turgor loss and dehydration of root cells and subsequent dieback of root segments (Brunner *et al.*, 2015) or hydraulic failure in the root xylem and the shedding of fine roots (Chenlemuge *et al.*, 2013; Kotowska *et al.*, 2015). As a consequence, various studies reported a reduction in root lifespan in dry soil (Mainiero & Kazda, 2006; Peek *et al.*, 2006; Meier & Leuschner, 2008; Leppälammi-Kujansuu *et al.*, 2014; McCormack & Guo, 2014). By contrast, other studies reported no change in root lifespan with drought (Anderson *et al.*, 2003; Bauerle *et al.*, 2008), which has been suggested to depend on the degree of soil desiccation (McCormack & Guo, 2014). In severely dry soil, the reduction of root longevity through the shedding of roots or root segments may represent a strategy to save resources under conditions when costs of root maintenance are not covered by the amount of water and nutrients that can be taken up (Eissenstat *et al.*, 2000). Investment into new root growth when resources become available again may then even increase resource use intensity, as young roots generally have higher resource uptake rates than older ones (Volder *et al.*, 2005). However, it is not known if the response of root lifespan to temperature and soil moisture differs between AM and ECM trees, even though some studies reported temperature sensitivity in AM fungi (Lingfei *et al.*, 2005; Soudzilovskaia *et al.*, 2015) and drought sensitivity in ECM fungi (Soudzilovskaia *et al.*, 2015).

In this study, we investigated systematic differences in root lifespan between four temperate AM and four ECM tree species that were colonized by indigenous mycorrhizal communities and were cultivated under controlled soil conditions. Trees were grown for two seasons in large outdoor containers, in which two soil moisture treatments were established (moist and dry) to explore the effect of soil desiccation on root survival. We used the mini-rhizotron technique for directly observing and comparing fine root lifespan between AM and ECM trees. For identifying possible morphological determinants of root lifespan in the two mycorrhizal association types, several root morphological and architectural traits were investigated. Based on the existing information about morphological and functional differences between the root systems of AM and ECM trees, we hypothesized that (H1) AM tree species have on average larger fine root diameters and longer fine root lifespan than ECM trees; (H2) AM root lifespan responds stronger to seasonal changes than ECM root lifespan; and (H3) the drought-induced decrease in root lifespan is greater in ECM than AM trees (H3).

Materials and Methods

Plant material

Tree saplings of eight major Central European deciduous tree species were collected from an old-growth mixed forest stand in Central Germany ('Hainich National Park' in Thuringia, , 51°08'N, 10°51'E). The forest is located at sub-montane elevation (340 m a.s.l.) on eutrophic Luvisols (IUSS, 2006), which have developed from a base-rich Pleistocene loess layer over Triassic limestone (Middle Muschelkalk). Soil manipulation activities such as liming have not been conducted in the past. The climate is semi-humid with mean annual temperature of 7.7°C and mean annual precipitation of 590 mm. The stand is a mature mixed hardwood forest with up to 14 co-occurring tree species. The eight tree species selected for this study represent dominant or subdominant trees of the natural forest vegetation of Central Europe (Leuschner & Ellenberg, 2017) and belong to two different mycorrhiza types (*cf*. Wang & Qiu, 2006): common ash (*Fraxinus excelsior* L.), sycamore maple (*Acer pseudoplatanus* L.), Norway maple (*Acer platanoides* L.), and wild cherry (*Prunus avium* L.) are AM tree species. European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), small-leaved lime (*Tilia cordata* MILL.), and hornbeam (*Carpinus betulus* L.) are ECM tree species.

In two campaigns in September 2011 (AM: ash and sycamore; ECM: beech and lime) and September 2012 (AM: Norway maple and cherry; ECM: oak and hornbeam), we collected about 25 young trees per species with similar tree height (about 30 cm) and crown dimensions.

Saplings were excavated with their entire mycorrhizal root system, stored in moist plastic bags to minimize transpiration, kept cool, and transported to the glasshouse immediately. The young trees were re-planted in 5 L plastic pots filled with sterilized sand, placed in a randomized array in the Experimental Botanical Garden Göttingen, and kept well-watered.

Experimental design

Eight young trees of each species were planted into the Göttingen Rhizolab, an outdoor facility designed for studies of root growth dynamics of woody plants (*cf*. Meier & Leuschner, 2008), as well as in nearby tanks filled with soil (lysimeters). The facility included in total 16 large-scale, drained containers (volume 7 m^3 each) that were arranged in four rows with the rims of the containers at ground level. Each container was divided by polyethylene plates into four plots, resulting in 64 plots in total. Saplings were grown in mineral sand with a particle size of ≥ 2 mm; a soil texture which facilitates root studies. To establish different soil moisture treatments, the container facilities were automatically covered by mobile Plexiglas rain shelters during precipitation events, thus allowing full control of soil moisture while glasshouse microclimate artifacts were avoided. During the growing seasons, tree saplings were fertilized every second week with 2 L of a 0.1% NPK fertilizer solution containing trace elements (concentrations of undiluted NPK fertilizer: 2.5 M NH_4, 0.5 M NO_3, 0.4 M CH_4N_2O, 0.7 M P_2O_5, 0.8 M K_2O, 11.5 mM B, 0.8 mM Cu, 4.4 mM Fe, 2.7 mM Mn, 0.13 mM Mo, 0.8 mM Zn).

In a randomized block design, each two AM and two ECM tree species were planted together into one container at a spacing of 1 m between individual trees. Planting occurred in two subsequent campaigns in the Rhizolab (May 2012; AM: ash and sycamore; ECM: beech and lime) and the lysimeters (June 2013; AM: Norway maple and cherry; ECM: oak and hornbeam). In April 2014, we established two soil water contents (SWC) in the containers: a dry treatment (5% SWC, v/v) and a well-watered treatment (10% SWC, v/v), with each treatment being replicated four times for each tree species. One access tube was inserted vertically in each plot and the volumetric soil water content measured every second day by frequency domain reflectometry (FDR; Diviner2000, Sentek Sensor Technologies, Stepney, Australia). Soil water contents were quantified based on soil moisture measurements to a depth of 40 cm and adjusted for plant water consumption by irrigating the soil surface homogeneously. The drought experiment was started in April 2014 and 2015, respectively, but was paused during the non-growing season 2014/2015 to allow natural precipitation to bring the soil back to field capacity. Consequently, the experiment simulated two consecutive summer droughts of ~24 weeks each.

Air temperature and air humidity were recorded continuously at 10-min intervals with a Hobo Pro RH/Temp data logger (Onset Computer, Bourne, MA, USA). Soil temperature was measured with NTC thermistors arranged in 16 horizontal lines at 10 cm soil depth. During the period of fine root observations (April to September 2015), the early season was colder and less humid (air temperature: 13°C, soil temperature: 15°C, air humidity: 62%) than the mid-season (air temperature: 18°C, soil temperature: 19°C, air humidity: 74%). Photosynthetically active radiation was measured during the early (mean: 884 µmol m^{-2} s^{-1}) and the mid-season (630 µmol m^{-2} s^{-1}) with a PAR sensor of the LI-6400 system (LI-COR Biosciences, Lincoln, NE, USA) (Table S1).

Mini-rhizotron imaging and root growth analysis

In the Rhizolab, twelve Plexiglas mini-rhizotron tubes (length 2.05 m, diameter 7 cm) were installed horizontally in each container at 15.0, 30.5, and 46.0 cm soil depth. In the lysimeters, four mini-rhizotron tubes were installed vertically in each tank to a depth of 40 cm. The protruding part of all mini-rhizotron tubes (128 tubes in total) was covered by light-impermeable foil and sealed with a removable plastic cover.

Fine root observations started in April 2015 (in the second year of the drought experiment) and were continued until September 2015. Images were recorded every fourth week over the entire surface of the mini-rhizotron tubes with a mobile scanner system (CI-600, CID Inc., Camas, WA). To determine temporal changes in root diameter and root architecture (branching patterns), image sequences where analyzed with the program WinRHIZOTron (Régent Instruments, Quebec City, Canada). Root order, as defined by Pregitzer *et al.* (2002), was determined visually and was used for the calculation of branching ratios (number of first order roots growing out of second order roots; n n^{-1}) and the proportion of lower order roots. We used the date of disappearance as the date of assumed root death. To obtain precise dates, root birth and death events were assumed to have occurred midway between two successive imaging dates. Individual root lifespan was calculated as the number of days from root birth to root death. Using Weibull distribution for right-censored data, root survivorship curves were calculated from the recorded birth and death events or from birth events and the time until the end of the experiment. We differentiated between root cohorts born in the early (April to June) and mid (July to September) growing season 2015.

Root morphology and biomass production

In September 2015, all tree saplings were harvested and the roots were carefully washed under tap water and subsequently sorted by diameter (fine roots ≤ 2 mm, coarse roots > 2 mm). Root

architecture, morphology, and the degree of mycorrhizal colonization were analyzed for all trees from each one randomly selected, intact fine root system of 15 cm length. From these subsamples, branching intensity was determined as the number of tips per total root length. Root morphology was analyzed by optical surface area measurement with a flatbed scanner and the program WinRHIZO (Régent Instruments Inc., Quebec, Canada). The degree of AM colonization was investigated by bleaching and staining the root strands in an ink-based solution (*cf.* Vierheilig *et al.*, 2005) and quantifying AM colonization by examining vesicles, arbuscules, and internal hyphae with the gridline-intersect method (Giovanetti & Mosse, 1980). The degree of ECM colonization of root tips was determined from unstained roots according to differences in their color, thickness, texture, and branching patterns. After analysis, fine and coarse roots were dried (72 h, 70°C) and weighed. Root diameter, specific root length (SRL), specific root area (SRA), and root tissue density (RTD) were calculated from these measurements.

Statistical analyses

Statistical analyses were conducted with the package SAS, version 9.3 (Statistical Analyses System, SAS Institute Inc., Cary, NC, USA). Significance was determined at $P \leq 0.05$. Means and standard errors were calculated from the mean of four tree species per mycorrhizal type and soil moisture treatment, while the replicates for each tree species were considered as pseudo-replications. Means were compared by one-way Kruskal-Wallis single factor analyses of variance and non-parametric multiple comparison tests after Wilcoxon to analyze the differences between mycorrhiza types and soil moisture treatments. To test for significant effects of mycorrhizal type (main effect) and tree species identity (nested effect), the data were analyzed by applying a mixed model with the soil moisture treatment as random effect.

Root survivorship curves were calculated by using Weibull distribution for right-censored data (n = 4 tree species per mycorrhizal type). For identifying root traits with significant influence on individual root lifespan, we used the Cox proportional hazard regression, allowing the evaluation of the effects of each covariate, while controlling for the effects of other covariates (Cox, 1972). Tested covariates in the stepwise regression model included the mycorrhizal type, drought treatment, season, portion of lower-order roots, branching ratio, root order, and root diameter. The hazard risk ratio of categorical covariates, which were coded as 0 and 1, can be interpreted as the estimated hazard for roots with a code of 1 in comparison to those for roots coded 0 (Table S2). We tested the relationship between abiotic conditions (i.e., soil temperature, soil humidity, and PAR) and root lifespan by conducting linear regressions.

Results

The effect of the mycorrhizal type on root traits

Despite considerable variation among the four AM and four ECM species (Table 1), the influence of the mycorrhizal type on mean fine root diameter, SRL, SRA, and root branching intensity was stronger (significant) than the influence by tree species identity (not significant) in the mixed model (Table 2).

Table 1 Mean fine root diameter (fraction <2 mm in diameter), specific root length (SRL), specific root area (SRA), root tissue density, branching intensity (fine root tips per cm root length), mycorrhizal colonization, and fine root lifespan in early and mid-season for the eight studied tree species in the moist and dry treatments (means ± SE of four trees per species and treatment).

TREE SPECIES	Fine root diameter [mm]	SRL [m g^{-1}]	SRA [cm^2 g^{-1}]	Tissue density [g m^{-3}]	Branching intensity [tips cm^{-1}]	Mycorrhizal colonization [%]	Early season median lifespan [d]	Mid-season median lifespan [d]
AM tree species								
MOIST								
Fraxinus excelsior	0.55 (0.03)	13.3 (1.1)	241 (13)	1.18 (0.15)	0.90 (0.07)	96 (2)	268	257
Acer pseudoplatanus	0.46 (0.05)	23.0 (1.5)	335 (28)	0.75 (0.07)	1.69 (0.05)	84 (5)	214	162
Acer platanoides	0.52 (0.01)	19.4 (2.6)	320 (40)	0.84 (0.19)	2.05 (0.77)	80 (7)	92	48
Prunus avium	0.49 (0.03)	17.2 (1.3)	291 (17)	0.79 (0.08)	0.95 (0.04)	73 (11)	157	94
DRY								
Fraxinus excelsior	0.55 (0.01)	13.2 (1.0)	237 (23)	1.26 (0.16)	1.09 (0.05)	90 (2)	192	391
Acer pseudoplatanus	0.39 (0.03)	19.9 (3.3)	266 (21)	1.29 (0.31)	1.60 (0.18)	68 (9)	175	171
Acer platanoides	0.56 (0.03)	16.7 (1.9)	285 (29)	1.11 (0.25)	1.33 (0.17)	67 (6)	213	50
Prunus avium	0.47 (0.02)	17.0 (1.9)	271 (11)	0.96 (0.11)	1.02 (0.06)	69 (16)	107	43
ECM tree species								
MOIST								
Fagus sylvatica	0.30 (0.03)	45.0 (6.8)	403 (40)	0.99 (0.16)	2.79 (0.65)	100 (0)	172	391
Tilia cordata	0.36 (0.03)	27.5 (2.5)	340 (36)	0.97 (0.11)	5.98 (1.28)	100 (0)	181	149
Quercus robur	0.33 (0.02)	40.3 (4.2)	413 (37)	0.90 (0.05)	2.17 (0.63)	99 (1)	229	43
Carpinus betulus	0.35 (0.02)	37.8 (3.8)	340 (36)	0.87 (0.24)	3.27 (0.15)	100 (0)	86	215
DRY								
Fagus sylvatica	0.31 (0.03)	32.4 (6.9)	301 (34)	1.88 (0.24)	3.99 (0.45)	100 (0)	222	139
Tilia cordata	0.38 (0.02)	37.7 (4.8)	434 (31)	0.87 (0.24)	2.49 (0.77)	100 (0)	127	81
Quercus robur	0.36 (0.06)	37.1 (9.1)	381 (69)	1.00 (0.25)	5.25 (1.27)	99 (1)	83	48
Carpinus betulus	0.37 (0.06)	37.7 (4.8)	423 (31)	0.81 (0.15)	3.39 (0.24)	94 (6)	36	82

The AM species had on average higher fine root diameters (0.50 vs. 0.33 mm), lower SRL (18 vs. 38 m g^{-1}), lower SRA (297 vs. 391 cm^2 g^{-1}), lower branching intensity (1.4 vs. 3.2 tips cm^{-1}), and a lower degree of mycorrhizal colonization (85 vs. 99%) than the ECM species (Table 2).

Table 2 Morphology and biomass of the entire root system of well-watered (moist) and drought-treated (dry) arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species (n=4 tree species per mycorrhizal colonization type and soil moisture treatment with standard errors in parentheses). Significant differences between the mycorrhizal association types and soil moisture treatments are indicated by different lower case letters. Significance of the effects of tree species identity (SPEC) and mycorrhizal type (MYC) on fine root traits of the entire root system of arbuscular mycorrhizal and ectomycorrhizal tree species. Given are the *F*-values of a mixed model with mycorrhizal type (n = 2) as main effect, tree species identity (n = 8) as nested effect, and drought as random effect (significance: *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$).

	AM		**ECM**			
	moist	**dry**	**moist**	**dry**	**SPEC**	**MYC**
ROOT MORPHOLOGY						
Fine root diameter [mm]	0.50 (0.02) a	0.49 (0.04) a	0.33 (0.01) b	0.36 (0.02) b	1.8	75.1***
SRL [m g^{-1}]	18 (2) b	17 (1) b	38 (4) a	34 (2) a	1.4	79.1***
SRA [cm^2g^{-1}]	297 (21) b	265 (10) b	391 (17) a	368 (25) a	0.9	27.6***
Root tissue density [g cm^{-3}]	0.9 (0.1) b	1.2 (0.08) a	1.0 (0.09) ab	1.1 (0.25) ab	1.4	0.2
Branching intensity [tips cm^{-1}]	1.4 (0.3) b	1.3 (0.1) b	3.2 (0.5) a	3.8 (0.6) a	0.3	34.9***
Mycorrhizal colonization [%]	83 (5) b	74 (5) b	99 (1) a	98 (1) a	2.9*	48.1***
ROOT BIOMASS						
Fine root biomass [g]	343 (105) a	234 (80) a	33 (11) b	34 (17) b	6.63***	52.2***
Coarse root biomass [g]	473 (181) a	313 (106) a	63 (29) b	39 (17) b		
Total root biomass [g]	812 (285) a	543 (188) a	93 (38) b	71 (32) b		

Total fine root biomass was much larger in the AM species, while the degree of mycorrhizal infection was higher in the ECM species. The influence of soil moisture (well-watered vs. dry

treatment) on root morphology, architecture, and biomass was weaker than the effect of the mycorrhizal type (Table 2). In contrast, root tissue density was only weakly influenced by the mycorrhizal type and tree species identity, but increased significantly upon soil drought in the AM species.

The effect of seasonality and soil moisture on root lifespan in AM and ECM species

According to the Cox proportional hazards regression analysis of individual roots, the mycorrhizal association type had a significant influence on root survivorship in the eight species in both seasons and both soil moisture treatments: the mortality risk of roots of ECM species strongly deceased upon drought, while the lifespan of AM species varied primarily with season (Table 3).

Table 3 Summary of proportional hazard fits for the effects of mycorrhizal type, soil drought and season of root birth on root lifespan. Values given are hazard ratios and the percentage change in the risk of root mortality of proportional hazards regression analyses for the individual root lifespan of four tree species per mycorrhiza type (significance: *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$). Only significant hazard ratios are presented.

	All tree species		
	Hazard risk	Risk of mortality	
EFFECT OF MYCORRHIZAL TYPE			
mid-season	1.45***	+45%	Mortality risk increased in AM
soil moisture	0.68***	-32%	Mortality risk increased in ECM

	AM		ECM	
	Hazard risk	Risk of mortality	Hazard risk	Risk of mortality
EFFECT OF SOIL MOISTURE				
early season			1.88***	+88%
mid-season	2.49***	+149%	3.59***	+259%
EFFECT OF SEASON				
moist	2.51***	+151%	0.41***	-59%
dry	8.06***	+706%	5.55***	+455%

In well-watered soil, the roots of AM species born early in the season had a somewhat longer median lifespan than ECM species roots (185 d in AM vs. 176 d in ECM), while roots born in mid-season lived longer in the ECM species (127 vs. 182 d; Fig. 1). In dry soil, the roots of AM species lived longer than ECM species roots in the early (184 vs. 105 days) and the mid-season (111 vs. 81 days; Fig. 1), and the mortality risk increased stronger in ECM roots than in AM

roots (P>0.0001; Table 3). Drought reduced the root lifespan of the AM tree species slightly by 0.5% in the early and by 13% in mid-season, but strongly affected ECM root lifespan in both seasons (reduction by 40% and 56%; Fig. 1), with a mortality risk increase by 88% in the early and by 259% in the mid-season (Table 3).

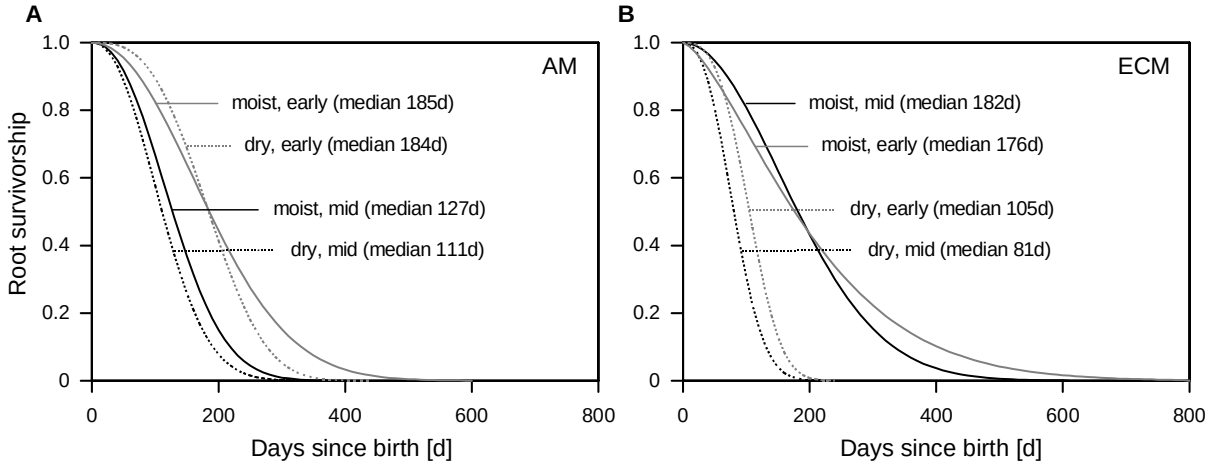

Figure 1 Root survivorship curves derived from mini-rhizotron data of moist (solid line) and drought-treated (dashed line) roots of AM (A) and ECM (B) tree species (n=4 tree species per mycorrhizal association, soil moisture treatment and season). Roots born in the early season are depicted by grey, and roots born in mid-season by black lines. Root initiation and death events were assumed to have occurred midway between successive sampling dates. Root survivorship curves were calculated from presence/absence data using the Weibull distribution for right-censored data.

When comparing the two seasons with respect to root lifespan under well-watered conditions, lifespan strongly decreased from early to mid-season in the AM species (from 185 to 127 d), while it slightly increased in the ECM species (from 176 to 182 d). According to the Cox proportional hazard regression analysis, the mortality risk of well-watered trees increased by 151% AM roots and decreased by 59% in ECM roots compared to the early season (Table 3), resulting in a shorter mean root lifespan in AM than in ECM trees in the late season. In contrast, both mycorrhiza types reduced root lifespan in the dry treatment toward mid-season (from 184 to 111 d in AM, from 105 to 81 d in ECM), in which the mortality risk of roots increased by 706% in AM roots and by 455% in ECM roots. Accordingly, the effect of season on the risk of mortality was always higher in the dry than in the well-watered soil treatment.

According to the linear regression analyses, the lifespan of ECM roots was positively related to soil moisture (P=0.001; Fig. 2a), while the lifespan of AM roots increased with decreasing soil temperature (P=0.002) and increasing PAR flux density (P=0.006; Fig. 2b, c).

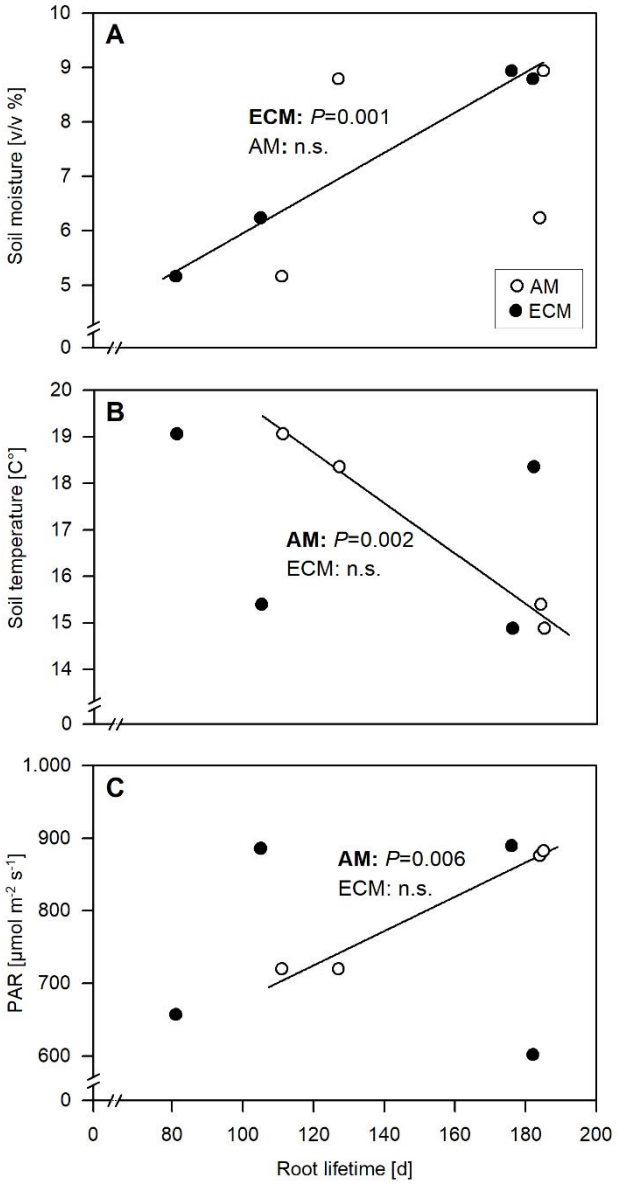

Figure 2 Linear regressions between root lifetime and (A) soil moisture, (B) soil temperature, and (C) photosynthetically active radiation (PAR) for arbuscular mycorrhizal (AM, white) and ectomycorrhizal (ECM, black) tree species (n=4 tree species per mycorrhizal type). Given are means per season (early and mid) and soil moisture treatment (moist and dry).

Relationship between root structural traits and the root lifespan of AM and ECM species

Root lifespan increased with root diameter in both AM and ECM species. The risk of root mortality decreased in AM species by 94% and in ECM species by 62% with a 1 mm increase in root diameter (P<0.001; Table 4). Lifespan was in both groups also negatively correlated with the proportion of lower order roots (mortality risk increase with 1% increase in the portion of lower order roots by 16 % in AM and by 15% in ECM trees; P<0.001). In the AM roots, the

mortality risk increased also with an increasing branching ratio (+2% with a one unit increase in the ratio of root branching; *P*=0.003), resulting in a shorter lifespan with higher branching. Higher root orders had a significant longer lifespan in AM trees, but not in ECM trees.

Table 4 Summary of proportional hazard fits for the effects of AM and ECM root traits on root survivorship. Values given are hazard ratios and the percentage change in the risk of root mortality of proportional hazards regression analyses for the individual root lifespan of four tree species per mycorrhiza type (significance: *, P ≤ 0.05, **, P ≤ 0.01, ***, P ≤ 0.001). Only significant hazard ratios are presented. The percentage mortality risk gives the change with an increase of one unit of the quantitative covariate (see Table S2).

	AM		ECM	
	Hazard risk	Risk of mortality	Hazard risk	Risk of mortality
EFFECT OF ROOT TRAITS				
Root diameter	0.06***	-94%	0.38***	-62%
Portion of lower order roots	1.16***	16%	1.15***	15%
Branching ratio	1.02**	2%		
Root order	0.77*	-23%		

Discussion

Root longevity is a key functional trait with large influence on the root-borne C flux to the soil (Eissenstat & Yanai, 2002) and probably also the nutrient and water cycles of ecosystems. However, information on the root longevity of different tree species is still scarce (e.g. Withington *et al.*, 2006), and systematic data on putative root lifespan differences between AM and ECM trees are virtually non-existent. In this study, we explored the response of fine root lifespan of AM and ECM tree species to seasonality and variation in soil moisture and found largely different responses between the two mycorrhiza types. In a comparison of the average root lifespan of each four AM and four ECM trees during the early season and in the well-watered treatment, it appears that median lifespan of AM trees was only about 5% higher than in ECM trees. However, larger differences appeared between AM and ECM root lifespan as a consequence of soil drought and season: while root lifespan of ECM trees was mainly determined by soil moisture conditions (decrease by 48% in dry soil), root lifespan of AM trees was mainly a function of season (decrease by 36% from early to mid-season). In addition, root architecture (proportion of lower-order roots, negative effect) and root morphology (root diameter, positive effect) influenced root lifespan independent of the mycorrhizal types.

The influence of mycorrhiza type on root morphological traits and root survivorship

Several studies indicate that AM and ECM tree species differ in various root morphological properties, when co-occurring in the same habitat (Brundrett, 2002; Comas & Eissenstat, 2009; Comas *et al.*, 2014; Eissenstat *et al.*, 2015; Liese *et al.*, 2017a; but see Kubisch *et al.* 2015). Our results from saplings grown under defined soil conditions indicate that AM trees developed fine root systems with higher average root diameters, but lower SRL, SRA, and branching intensity than ECM trees. These differences in root morphology can be due to systematic differences between morphological root traits between AM and ECM trees, but may also be partly consequence of different growth rates between the investigated AM and ECM trees. In our species sample, AM species (genera *Acer, Fraxinus, Prunus*) were characterized by faster tree growth than ECM species (*Fagus, Quercus, Tilia, Carpinus*).

Root morphology is a key to understand root lifespan. Numerous studies across a wide range of species have revealed a linkage between various root traits and root survivorship. A central role is likely played by root diameter (Wells & Eissenstat, 2001; Anderson *et al.*, 2003; Joslin *et al.*, 2006; McCormack *et al.*, 2012), with thicker roots typically having a longer lifespan. This is in line with resource optimization theory, which postulates that higher C and nutrient investment required to build thicker roots should be compensated by a greater root lifespan, so that the additional resource consumption for root construction is balanced by a longer period of resource capture (Eissenstat & Yanai, 1997; McCormack *et al.*, 2012). Our mini-rhizotron observations show a significant effect of the mycorrhizal type on fine root survival in the eight-species sample, which is linked to the significantly smaller mean fine root diameter of the ECM species. AM fine roots lived significantly longer than ECM roots in the early season in both the moist and dry treatment, which is in accordance with the greater diameter of AM roots. In contrast, ECM roots born in mid-season were in the moist treatment longer-lived than AM tree roots. This suggests that the root diameter influence is overlain by other, under certain conditions more influential, factors. Cox proportional hazard regressions showed that the root diameter effect on root lifespan was stronger in the AM species than in the ECM species. The AM trees developed in the experimental period larger root systems with in most cases thicker and longer-lived roots, which indicates that the smaller root systems of the ECM species are not only a result of slower root growth, but also of higher fine root turnover. Our data further show that the lifespan of AM and ECM roots is not only influenced by root diameter, but also by the proportion of 1st and 2nd order roots. While this is expected from the generally negative relationship between root diameter and the proportion of lower-order roots in fine root mass (Fitter *et al.*, 1991; Pregitzer *et al.*, 1997; Wells *et al.*, 2002), the influence of the proportion of

lower-order roots on root lifespan has not yet been reported. Since the change in root mortality risk was greater for a root diameter change than for a change in the proportion of lower-order roots, we assume that root diameter is a more important determinant of root lifespan. A possible explanation is that root diameter may be more directly related to a root's sensitivity to physical and biological hazards that threaten its integrity. Our results suggest that the thinner ECM tree roots generally were suffering more from live-reducing hazards than the AM roots.

While root diameter and the portion of lower root orders influenced the lifespan of both AM and ECM roots, branching ratio and the individual root order were determinants of root lifespan only in AM species. Although branching ratio was significantly negatively related to AM root lifetime, this effect was negligible with a 2% change in the risk of mortality. In contrast, the root order to which a segment was assignable, showed a stronger and positive relation to root lifespan. This relation might be due to order-specific root functions, where low-order roots have their main function in resource uptake and high-order roots in storage and transport (Pregitzer *et al.*, 2002). Longer root lifespan in higher root orders have been reported for other AM plant species as well, e.g. for sugar maple (Eissenstat *et al.*, 2000), peach trees (Wells *et al.*, 2002), and alpine meadow grasses (Wang *et al.*, 2016). This seems to support the influence of root order on root lifespan found in the AM trees of our study. The non-existent root order effect in ECM trees of our study may have been consequence of the Hartig net produced in lower-order ECM roots, which protects against physical hazards and pathogen attack.

Decreased lifespan of drought-exposed ECM roots

Root systems may adopt two different strategies in response to drought, (i) producing longer-lived, more robust roots, which are often thicker and better protected against desiccation, or (ii) increasing the production of tender, short-lived roots with high turnover. In the first strategy, the plant uses resources primarily for building and maintaining durable belowground structures, while more C and nutrients will be lost with the acceleration of fine root turnover in the second strategy (Eissenstat *et al.*, 2000; Brunner *et al.*, 2015).

Since new short-lived roots have been shown to have higher resource uptake efficiencies (Volder *et al.*, 2005) and hydraulic conductivities, roots with short lifespan should be more effective in absorbing water and nutrients, which may maximize total plant productivity under drought (Eissenstat & Yanai, 2002) and compensate for the higher resource consumption of short-lived roots. Our results show a marked reduction in the lifespan of ECM tree roots in the dry treatment and an associated close positive relation between soil water content and lifespan, which is not found in the AM species. This is in support of our second hypothesis and matches

with observations in several ECM trees in the field, even though comparison to AM trees is lacking. With decreasing water availability in drought periods or along precipitation gradients, a decreasing root lifespan was found in European beech (Mainiero & Kazda, 2006; Meier & Leuschner, 2008), Douglas fir (Marshall, 1986) and in trees of a mixed North American hardwood forest (Pregitzer *et al.*, 1993). In contrast, two studies with woody AM species did not detect a significant change in root longevity with decreasing water availability (grape: Anderson *et al.*, 2003; grapevine: Bauerle *et al.*, 2008). Espeleta *et al.* (1999) found under drought a lower fine root mortality in AM-colonized than in uncolonized roots of red grapevine, which may point at a drought sensitivity reduction with the infection by AM-forming fungi. Our results may be the first to show a greater reduction in root longevity in ECM than AM trees upon drought. If this difference is of more general validity for temperate ECM and AM trees, it would indicate contrasting drought response strategies of the two mycorrhizal association types. While ECM trees may lean stronger towards a strategy, which increases fine root turnover upon drought, the strategy of AM trees may rely more on the formation of more durable root structures. Since shorter root lifespan has been related to higher metabolic activity and faster resource acquisition (Comas & Eissenstat, 2004; Volder *et al.*, 2005), the shorter-lived roots of ECM tree species under drought may possibly represent a response to alleviate drought stress by improving root water uptake efficiencies. Such a strategy could increase root water uptake by expanding the active surface area and increasing resource acquisition. It will also influence C cycling in the soil, as higher root turnover in ECM species under drought will increase the root-borne C input to the soil. The AM species in our experiment seemed to respond differently by avoiding increased C investment into root turnover. Their total biomass production showed a stronger reduction upon drought than in the ECM species, either due to a higher overall drought sensitivity of the plant or as a consequence of a different root system response to drought.

The effect of season on AM root lifespan

Seasonal differences in fine root lifespan have been observed in various studies investigating trees or herbaceous plants (Johnson *et al.*, 2000; Anderson *et al.*, 2003; Kern *et al.*, 2004; Gu *et al.*, 2011; Wang *et al.*, 2016). Our results show that the root lifespan of AM and ECM tree species is differently influenced by season. While ECM root lifespan only slightly increased toward mid-season, AM trees responded stronger and decreased root lifespan by 31%. In accordance, Gu *et al.* (2011) found a stronger effect of season on root lifespan in the AM species Manchurian ash (102% change) than in the ECM species Dahurian larch (52%). By contrast,

studies on root dynamics in AM concord grape and alpine meadow grasses found a longer lifespan in roots borne later in the season (Anderson *et al.*, 2003; Wang *et al.*, 2016).

We speculate that the marked root lifespan decrease in AM trees later in the season could be related to higher temperatures, even though the ECM species did not respond in a similar way. Effects of season on root survivorship have been linked to assumed temperature effects by several authors (King *et al.*, 1999; Johnson *et al.*, 2000; Leppälammi-Kujansuu *et al.*, 2014; Wang *et al.*, 2016). In the AM tree *Acer saccharum*, Hendrick & Pregitzer (1993) found that warmer temperatures are indeed linked to higher root mortality. Among the possible causes are carbohydrate shortage as a consequence of elevated respiration rates and accelerated root senescence due to the formation of free oxygen radicals (Burton *et al.*, 2002; Wang *et al.*, 2016). In our study, the strong decrease in AM root lifespan was accompanied by a soil temperature increase during mid-season by 4°C, resulting in a negative relation between root lifespan and temperature in the AM species, which was not detected in the ECM species. Whether this difference is caused by differences in the temperature response of root physiology in ECM and AM species, must remain open.

Seasonal variation in carbohydrate supply may also have influenced root lifespan. In their review, Norby & Jackson (2000) found a close dependence of root longevity on photosynthetic activity and thus carbohydrate supply to the roots. The positive relation between PAR and root lifespan observed for the AM species in our experiment points to an important influence of carbohydrate supply in this group. A possible explanation could be the generally higher above- and belowground growth rates of the AM trees compared to the ECM species in the experiment, which must have caused a higher carbohydrate demand of the AM tree root systems. Our results confirm the idea that root longevity is determined by various abiotic as well as biotic factors, among them plant age, carbohydrate supply and growth rate, and root symbioses.

Conclusion

Our experiment with saplings of eight temperate tree species belongs to the very few studies, which investigated systematic differences in root longevity between AM and ECM tree species growing under the same environmental conditions. Knowledge about the determinants of tree root longevity is needed to increase our capability of predicting the effects of climate change and management alteration on forest ecosystem functioning and related biogeochemical fluxes. Root lifespan of ECM tree species was strongly reduced by drought, while the lifespan of AM roots was found to be less drought-sensitive, but showed a strong seasonality, which was related to changes in temperature and PAR across the seasons. Root diameter and the proportion of lower-order roots in fine root biomass were identified as determinants of root lifespan in both mycorrhiza types, while branching ratio and root order were only related to the root lifespan in AM species. When interpreting these results in a wider context, two facts have to be taken into account: First, since the study was conducted with young trees, the findings can be transferred to adult trees only with great care. Second, it is likely that part of the differences found between AM and ECM tree species in terms of fine root morphology and dynamics are caused by the different growth rates of the investigated AM and ECM trees. Yet, our study has produced first evidence that AM and ECM trees may differ systematically in root traits that determine fine root dynamics. Thus, the mycorrhizal type could be of high relevance when predicting the fate of temperate forests under changing climate. Further studies on root longevity with other tree species and conducted under different environmental conditions are needed to deepen our understanding of possible systematic differences between the lifespan of AM and ECM tree roots and the abiotic and biotic determinants of root longevity in the two groups.

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Supplementary Material

Table S1 Seasonal means of air an soil temperature (ambient water supply, 10% SWC, v/v; drought, 5% SWC, v/v), relative air humidity and photosynthetic active radiation in the Göttingen Rhizolab facility for the year 2014 and the year 2015.

	2014				2015 (mini-rhizotron observations)			
	Early season		Mid-season		Early season		Mid-season	
	Apr - June	Min - Max	July - Sep	Min - Max	Apr - June	Min - Max	July - Sep	Min - Max
Air temperature [°C]	15.0	12.5 – 16.8	17.6	15.3 – 20.6	13.0	9.2 – 20.6	18.4	14.3 – 20.7
Soil temperature of well-watered plots [°C]	18.2	14.0 – 21.2	19.7	16.4 – 23.2	14.9	11.7 – 17.8	18.4	14.2 – 20.8
Soil temperature of drought-treated plots [°C]	18.4	14.5 – 21.5	20.2	16.5 – 23.7	15.4	11.8 – 18.7	19.1	14.8 – 21.6
Relative air humidity [%]	70.4	68.0 – 72.3	78.5	73.2 – 86.1	62.3	67.2 – 54.8	74.0	69.2 – 81.9
PAR [µmol m-2 s-1]					884	649 - 1133	630	406 - 977

PAR, photosynthetically active radiation

Table S2 Variables tested in proportional hazards stepwise regression analyses of individual root lifetimes.

Variable	Coding and description
Mycorrhizal type	0 = ECM, 1 = AM
Drought	0 = ambient water supply, 1 = drought
Cohort	0 = cohort 1 (early season), 1 = cohort 2 (mid-season)
Portion of lower order roots	Portion of 1st and 2nd order roots in all root orders
Branching ratio	Number of first order roots growing out of second order roots
Root order	Number of root order, as defined by Pregitzer *et al.* (2002)
Root diameter	Average diameter (mm)

Chapter 6

SYNOPSIS

Differences in morphological root traits of AM and ECM trees and the relationship to nutrient acquisition

The association with mycorrhizal fungi influences the morphology of tree roots and complements the resource acquisition strategies of trees. Trees that belong to the fast and acquisitive strategy have a high capability in acquiring resources and therefore high growth potentials. In contrast trees that are assigned to the slow and conservative strategy have long-term resource retentions and slow growth rates. ECM root systems are often described as fine and strongly branched (e.g. Hodge, 2004; Smith & Read, 2008), which is assigned to root traits of fast and acquisitive species (Comas *et al.*, 2002; Comas & Eissenstat, 2004).

The results of the present study confirm a higher branching of the ECM root system, which was independent from phylogeny, while root diameter had a significant phylogenetic signal and was not different between the mycorrhizal types in their natural habitat (Chapter 2). Several studies have suggested that root diameter is one of the key morphological traits that is closely related to root lifespan (e.g. Joslin *et al.*, 2006; McCormack *et al.*, 2012). The results of the mini-rhizotron observations (Chapter 5) are consistent with these studies and showed that root diameter is a good indicator that can be used to predict root lifespan across tree species of the two mycorrhizal association types. However, this study also revealed that the relation of root diameter with root lifespan can be overlain by other influential factors like drought.

In contrast to root diameter, root branching was constantly increased in ECM trees in this study and a key feature for discriminating AM from ECM tree species, but not related to individual ECM root lifespans (Chapter 5). In principle, a decreased root lifespan might be expectable to occur with increased root branching. Since increased root branching is a measure for a high proportion of lower order roots, the death of a higher-order root would entail the death of its branches. In accordance, the proportion of higher order roots was significantly and negatively correlated with root lifespan in this study. However, both root branching and root lifespan can vary strongly under the influence of several factors like nutrient status, the type and degree of mycorrhizal colonization. Nevertheless, root branching and root lifespan not necessarily are concurrently and evenly strong affected what might hide an actually existing interdependence.

However, root branching was directly or indirectly (via root C:N) related to acquisitive aboveground traits (high SLA, short leaf lifespan) and belowground traits (high SRL of 1st and 2nd root order and small root diameter; Chapter 2). In addition, high root branching intensity has also been related to high resource uptake capabilities (McCormack *et al.*, 2015) and high capabilities of proliferation into resource-rich patches (Hodge, 2004). The latter in combination

with the missing phylogenetic signal in root branching indicates the plasticity of this root trait, which is further supported by increased branching of ECM roots under drought (Chapter 4). Due to the relation of root branching with nutrient acquisition and proliferation capabilities reported in the literature and due to the relation to other acquisitive traits described in this study (Chapter 2), ECM trees should in fact rather be assigned to the acquisitive trait family. In contrast, ECM trees are often suggested to assign to the slow/conservative family due to the dominance of ECM trees in nutrient-poorer ecosystems, while AM tree species have been proposed to be fast in terms of resource acquisition and thus also in plant growth because of the more rapid decomposition of AM leaf litter and accelerated soil nutrient cycling (Cornelissen *et al.*, 2001; Read & Perez-Moreno, 2003; Hobbie *et al.*, 2006; Vesterdal *et al.*, 2012; Phillips *et al.*, 2013).

In general, being fast or having acquisitive traits can only be advantageous if the investment of resources that is needed to build and deploy such traits is returned by high resource acquisition (Reich, 2014; Weemstra *et al.*, 2016). This interdependence also implies that under nutrient scarcity in soils, acquisitive root traits may not be viable. In this sense, the soil environment of ECM trees, where nutrients are primarily bound in organic forms, is rather represented by soils with high nutrient mineralization potential or soils with nutrient-rich patches than nutrient-poor soils. Keeping this in mind, less root branching of AM trees would not lead to a slower growth potential, but simply indicates less need for an adaptation in this trait to a more inorganic soil environment, where nutrients can be absorbed quickly by the plant. It is reasonable to assume that an optimized uptake of different nutrients (organic and immobile vs. inorganic and mobile nutrients) requires different root traits. For example, the uptake of N in the AM rhizosphere with high nitrate content and the uptake of N in ECM rhizosphere with high amino acid content (Chapter 3, Table S2) may not be equally increased by a higher branching of roots, even when both N sources being exploited. The acquisition of more homogenously distributed, mobile, and plant-available nutrients such as nitrate may not require an increased root branching, whereas a high branching intensity can be profitable in the often patchy-distribution of immobile and organic nutrients. In accordance, root branching has been negatively related to the availability of plant-available nutrients in soil (Holdaway *et al.*, 2011). In this sense, AM trees would be less dependent on increased root branching as an acquisitive root trait, since their high quality and fast decomposing leaf litter provides higher amounts of plant available nutrients in comparison to the low quality and decomposition-recalcitrant nature of ECM leaf litter. Based on these facts, it is reasonable to assume that the higher branching in ECM root systems represents an adaption that improves the acquisition of organic nutrients via

exploitation of resource-rich patches in ECM ecosystems. Accordingly, AM and ECM trees seem to differ in the trade-off between resource investment for building and using acquisitive traits and resource return by an improved resource uptake. The investment of resources for an acquisitive root trait is beneficial for ECM trees through an improved exploitation of less-available nutrients. In contrast, such a costly adaption is less profitable in the mainly inorganic AM soil environment, where AM trees grow rapidly and acquire nutrients efficiently, despite their less acquisitive root traits.

Even though root branching has been assigned to the acquisitive trait spectrum, nutrient availability in the soil environment (organic and immobile vs. inorganic and mobile) must be considered as an important factor that may be highly important for a correct interpretation of the acquisition potential of a root trait and the incorporation in a whole plant economic spectrum. In this sense, morphological root traits should (i) not be strictly categorized into fast/acquisitive or slow/conservative spectra because an improved acquisition of different resources can be differently manifested and (ii) also be related to precision foraging strategies (Weemstra *et al.*, 2016). However, the study revealed that root branching is related to the mycorrhizal type and gives information about the strategies of resource exploitation and precision foraging (e.g. into resource rich patches) and, thus, represents an important belowground trait. To transfer these findings to a global scale, more detailed knowledge of differences in morphological root traits between AM and ECM trees roots across different biomes, different soil conditions, and changing climatic gradients are necessary.

Root-rhizosphere interactions of AM and ECM trees

Based on the differences in the nutrient economy between AM and ECM tree species as suggested by Phillips *et al.* (2013), several studies focused on possible differences in rhizosphere processes between the mycorrhizal association types. In the study of Yin *et al.* (2014), ECM trees had a stronger rhizosphere effect (i.e., the relative difference in chemical, physical, and biological properties between rhizosphere and bulk soil) than AM trees. It was furthermore shown that the higher C release through root exudation of ECM trees and a consequently induced priming effect is closely linked with this phenomenon. The present study partly confirms these results since ECM trees showed an in general stronger rhizosphere effect and a higher amount of microbial C in the rhizosphere. However, AM and ECM trees released comparable amounts of C through root exudation, consequently this cannot be the reason for the observed results.

In theory, due to the high proportion of nutrients bound in SOM in ECM ecosystems, a greater need to exude organic C compounds of ECM trees to prime microorganisms that accelerate nutrient transformation from SOM is reasonable to expect. In this sense, a high amount of exuded C of ECM trees would deliver the benefit of an increased nutrient availability in the rhizosphere due to accelerated microbial SOM decomposition. In contrast, a high C release through AM root exudates would be less beneficial, since AM ecosystems are dominated by inorganic nutrients that can be absorbed directly by the plant. However, this relationship would also imply that the majority of all exuded compounds are equally easy degradable, independent of the exudate composition. In contrast, this study observed a significant difference in the chemical richness (i.e. number of different compounds found in exudates) between AM and ECM exudates, which was strongly correlated to the rhizosphere effect (Chapter 3). In conclusion, the results of this study suggest that not only the amount of C that is released by root exudation, but also the composition of the exudates can cause different rhizosphere processes, as it occurs in AM and ECM trees. Since the mycorrhizal association types exuded equal amounts of C, but AM exudation had a greater chemical richness, it is logical that ECM associations exude higher amounts of the individual compounds found in ECM exudates. Such a mycorrhiza-specific release of a lower number but a higher individual amount of exudate compounds exerted a positive effect on rhizosphere processes.

Based on these outlined evidence, there is a direct and indirect way of how the composition of root exudates of ECM trees can drive positive effects in the rhizosphere. It is already known that ECM fungi have a great capability in directly releasing extracellular enzymes that accelerate SOM decomposition (Read & Perez-Moreno, 2003). In accordance, a greater activity of enzymes that degrade N and P from SOM were found in ECM rhizosoil of this study. However, not only mycorrhizal fungi, but also rhizosphere microbes have the capability of releasing extracellular enzymes (Kuzyakov, 2010). Based on a higher amount of microbial C and considering the multifaceted effects on C, N, and P cycling in ECM rhizosoil, it is reasonable to assume that enhanced microbial activity in the ECM rhizosphere is driving the positive effects (indirect way), despite the equal amount of provided C through root exudation of AM and ECM trees. In this sense, the lower chemical richness in ECM exudates must be related to enhanced microbial activity and to the priming of microbes. Thus, the reduced chemical richness in ECM root exudates may lead to an enhanced niche overlap and thus to enhanced competition between the microbes for the exuded C as a food source. This would result in a higher need of microbes to decompose SOM and thus in a generally enhanced rhizosphere effect. In contrast, the higher chemical richness of AM exudates may lead to a

reduced niche overlap and thus to reduced competition among rhizosphere microbes. In such a situation the exuded C might cover the microbes demand. This would result in a lower need to decompose recalcitrant SOM and would explain a less rhizosphere effect in AM soils (see Figure 1).

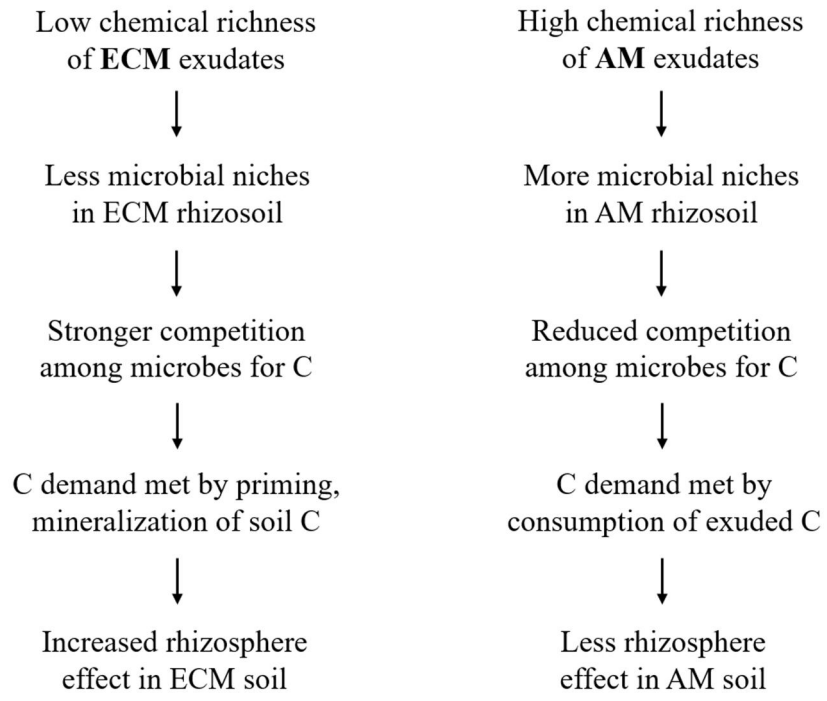

Figure 1 Schematic relationship between chemical composition of exudates, microbial decomposition, and rhizosphere effects in AM and ECM soils.

An adaptation of exudate composition in ECM trees to the conditions of a dominantly organic nutrient economy of ECM ecosystems provides a strategy, best suited to the prevailing conditions of the soil environment of their natural habitats. Not only the composition of root exudates, but also the high capability to exude extracellular enzymes and the observed higher branching intensities of ECM root systems are factors contributing to an adapted strategy. These factors represent additive traits that have evolved for a best possible adaptation to the greater proportion of nutrients bound in SOM. In contrast, in AM ecosystems less need for such adaptations exists due to the high content of plant available nutrients. This is consistent with the higher inorganic N uptake of AM tree species when compared to ECM tree species observed in research project 2 of this study (Chapter 4). The preference in the uptake of inorganic N of AM trees may reflect the limited capabilities of exuding extracellular enzymes of AM fungi and could be caused by a genetic adaptation to the inorganic environment in which AM trees occur naturally. In conclusion, AM trees seem to be specialized in the uptake of inorganic nutrients while ECM in the uptake of organic nutrients with a high dependency on microbial decomposition.

The results of this study show that AM and ECM trees have distinct strategies adapted to their respective nutrient economies, representing an optimal exploitation of the available resources. Root exudate composition seem to play a key role in mediating rhizosphere processes, where mycorrhiza-specific differences in the chemical richness of exudates affect microbial activity, enzyme activity and nutrient availability in the rhizosphere. Such adaptations to the prevailing form of nutrients in the respective ecosystems can have important implications for the mycorrhizal distribution under N deposition. It is reasonable to assume that with increasing contents of inorganic N in forest ecosystems due to deposition of ammonium and nitrate, AM associations with high capabilities in the uptake of inorganic N have advantages in comparison to ECM associations, which are more adapted to an environment with higher contents of organic nutrients. This can lead to shifts in species composition and changes in mycorrhizal distribution in temperate forest ecosystems. The results of this study emphasize the importance of considering the mycorrhizal type when predicting biogeochemical processes of temperate forests. Furthermore, the findings show that beyond the effect of the mycorrhiza type, root rhizosphere interactions are determined by a complex relationship of soil properties, microbial activity, microbial competition and root exudate composition.

The effect of drought on root functions of AM and ECM trees

Roots evolved several strategies to avoid or tolerate drought stress, including adjustments in root biomass production, morphological root traits, functional root traits, and rooting strategies (Brunner *et al.*, 2015). How tree roots respond to drought can be profoundly affected by the associated mycorrhizal type. A study that focused on the tree growth response showed that ECM trees species had a lower sensitivity to drought than AM trees (Brzostek *et al.*, 2014). One hypothesis to explain such findings has been that differences between the mycorrhizal type responses to drought are possibly caused by differences in root functions and belowground C allocation of AM and ECM tree species (Phillips *et al.*, 2013). The results of this study confirmed that AM and ECM trees differ in root functions and belowground C allocations under drought (Chapter 4 and 5; main results of the effect of drought summarized in Figure 2).

Since biomass production and the degree of mycorrhizal colonization were significantly less reduced in ECM than in AM trees under drought, it is reasonable to assume that higher root exudation, a decreased root lifespan, and increased root branching of ECM associations are adaptive responses that alleviate drought effects in dry soils. In the line of this thought, high C release through root exudation of ECM trees under drought might be explained by an active secretion of mucilage that increases hydraulic conductivity or accompany hydraulic lift (Kroon

et al., 1998; Querejeta *et al.*, 2007; Carminati, 2013), which would result in an improved water uptake capability. The role of exudates in water acquisition was further supported by the results of project 1 of this study. A strong relationship between exudate composition and soil moisture in the rhizosphere of the natural habitats of AM and ECM trees could be shown (Chapter 3). ECM tree species had a strong positive effect on the moisture content of rhizosphere soil, which was highly correlated with the chemical richness of the exudates. The fact that drought induced a decrease in ECM fine root lifespan with a simultaneously unaffected fine root biomass may also increase water absorption potential. Young roots with high turnover rates have a higher resource the uptake capability than older roots (Volder *et al.*, 2005) and are thus more efficient in water and nutrient acquisition. These adaptive responses in root functions might strongly support total plant productivity of ECM trees under drought.

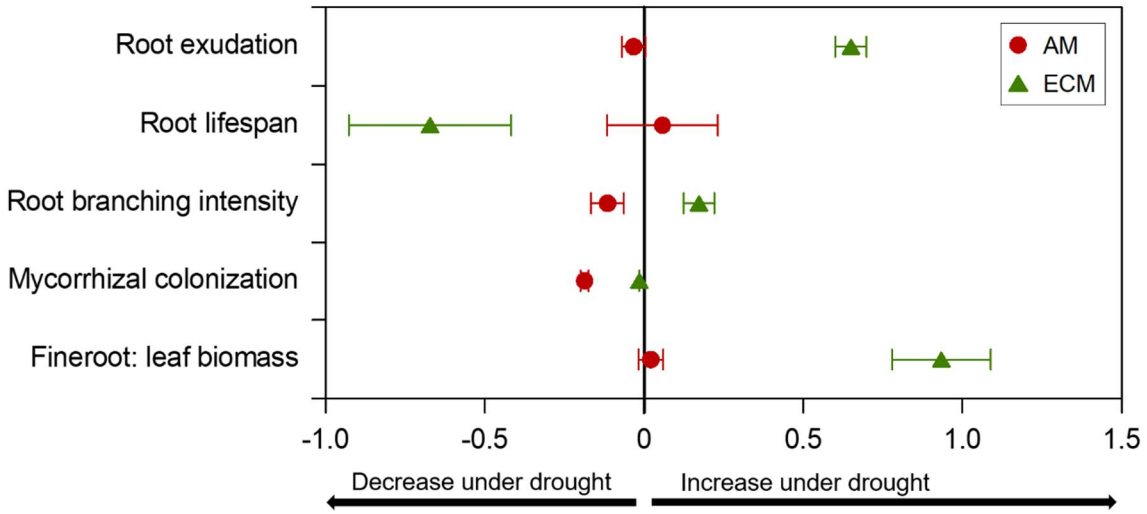

Figure 2 Drought response ratio of AM (red) and ECM (green) associations. Values are the means of two seasons (n = 4 tree species per mycorrhiza type). A meta-analysis was conducted summarizing drought effects on root exudation, root lifespan, root branching intensity, and on fine root: leaf biomass ratio. The drought treatment was considered as 'treatment group' and the treatment with ambient water supply as 'control group'.

These outlined evidences combined suggest that the ECM trees allocate their carbohydrate resources in dry soils to support both, increased exudation and increased production of short-lived fine roots. In contrast, AM trees allocate less C belowground when soil moisture decreases and keep the C investment moderate by maintaining durable and robust roots that are better protected against desiccation. Furthermore, the increased fine root: leaf ratio of ECM trees is in accordance with an optimal C partitioning strategy, where plants allocate more C to roots under drought and nutrient shortages in the soil (Bloom *et al.*, 1985). According to Eissenstat *et al.* (2000), C allocation to roots under drought is dependent on the cost: benefit ratio of C investment and resource acquisition, where C is invested until the efficiency of resource acquisition is maximized. In this sense, higher C allocation to roots of ECM trees is increasing

resource acquisition. In doing so, ECM trees seem to invest C for root functions (e.g. in young acquisitive roots) that increase uptake capabilities up to the point when the optimal acquisition potential is surpassed. In conclusion, ECM trees seem to respond to drought by investing high amounts of C (increasing costs) to improve resource uptake (increasing benefits), while AM trees seem to avoid C investments (decreasing costs) and accept as a consequence lower resource uptake (decreasing benefits) with simultaneously decreasing biomass and accompanied reduced demand. Accordingly, the root systems of AM and ECM trees may adopt two contrasting strategies to balance C investment and resource acquisition in response to drought. AM trees tend more to the strategy that tolerate drought by avoiding C and resource losses and by biomass reduction, while ECM tree species adapt a strategy that improve resource uptake under drought conditions by investing C and other resources into belowground parts (see Figure 3). However, the strategy of ECM trees under drought does not necessarily lead to an improved drought tolerance under all conditions. A high C investment with a concurrently reduced C assimilation in ECM trees under drought might lead to a negative C balance in ECM trees, especially during long lasting drought periods, which could make them more vulnerable for tree mortality. Accordingly, the strategy of AM trees during long periods of drought might increase the survival propability by saving C and by a decreased resource demand due to biomass reduction.

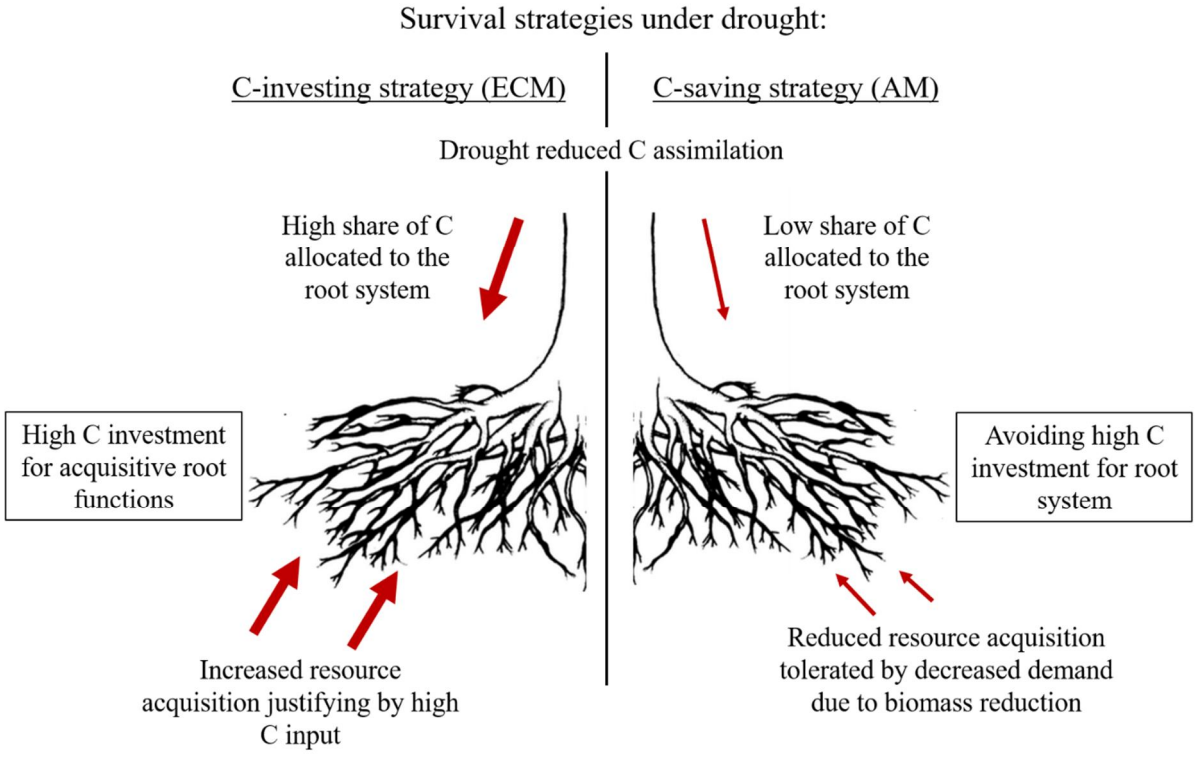

Figure 3 Illustration of two different survival strategies under drought.

Different strategies under drought of AM and ECM tree species that affect belowground C allocation to roots have important implications for root derived C input into the soil. According to the results of this study, one can generally expect higher input of C into soils through increased root exudation and accelerated root turnover of ECM trees under drought. Dead fine roots represent an input of organic material to soil which is not rapidly decomposed in dry soils. Since drought-treated ECM trees show a simultaneous increased release in root exudates which can drive microbial decomposition processes of SOM and stimulate microbes via a priming effect (Kuzyakov *et al.*, 2000), less-bioavailable SOM might be decomposed, even under drought conditions. Since in dry soils, not only water but also nutrients can be a limiting factor, an accelerated decomposition of dead root material through enhanced provision of exudates as food source for microbes could increase nutrient acquisition and thus productivity of ECM trees under drought.

The results of research project 2 support earlier studies that attributed a key role in maintaining tree productivity under drought to mycorrhizal associations. Furthermore, the study revealed new findings that indicate that AM and ECM roots have evolved different strategies to maintain tree productivity and survival under drought by adapting physiological root functions and C distribution. The differences in root functions of the two mycorrhizal types under drought found in this study may further explain the inconsistent findings of root exudation and root lifespan of a variety of tree species under drought as reported in the literature (e.g. Brunner *et al.*, 2015). Systematic differences in AM and ECM trees under more frequent and intense drought stress in temperate forests would have important implications for nutrient and C cycling in forest ecosystems under global change. Furthermore, differences in drought sensitivity of AM and ECM trees would also influence the trees' mortality and thus affect shifts in tree species and fungal composition in temperate forests. However, for a reliable knowledge whether AM or ECM trees have a better chance of survival under drought, a detailed study on the effect of intensity and duration of drought periods on the two mycorrhizal associations in their natural habitats is necessary.

Conclusions

From the present study on the effect of the mycorrhizal type on root-rhizosphere processes the following conclusions can be drawn:

I. Root branching is influenced by the mycorrhiza association type and represents a key discriminant between AM and ECM trees. Furthermore, increased root branching in ECM trees may reflect a beneficial trade-off between resource investment for a higher branching intensity and resource return by an improved resource uptake in the organic ECM soil environment.

II. AM trees have high capabilities in the uptake of inorganic N, while ECM trees enhance the exploitation of organic nutrients through high enzyme activities and microbial decomposition. These differences in resource acquisition may represent an adaptation to the respective nutrient economies of AM and ECM trees.

III. Rhizosphere processes are distinctly influenced by the mycorrhiza-specific composition of exudates even under equal C release through root exudation. The lower chemical richness of ECM exudates exerts strong effects in the rhizosphere through high microbe activities.

IV. Under drought, ECM trees invest a high amount of C to optimize acquisitive root functions (i.e. increased root exudation and decreased root lifespan), while AM trees avoid high C investment in roots and reduce the entire biomass production to tolerate limited resource uptake by low input and a reduced demand. These processes represent two different strategies in the trees' response to drought.

V. Differences in C partitioning and acquisitive root traits and functions between AM and ECM trees under changing environmental conditions are crucial for predicting biogeochemical processes and compositional shifts of temperate forests under global change. Accordingly, the type of mycorrhizal association should be considered as important belowground trait for trees in temperate forests.

Summary

Based on the two main types of mycorrhizal associations in temperate forests, which are arbuscular (AM) and ectomycorrhiza (ECM), the idea of a mycorrhizal-associated nutrient economy framework developed (Phillips *et al.*, 2013). This framework predicts that AM dominated forests, with fast decomposition of high chemical quality litter, have an inorganic nutrient economy. In contrast, forests dominated by ECM trees have low chemical quality litter and slow decomposition rates, resulting in a dominantly organic nutrient economy. The acquisition of nutrients from soil and as a result also tree productivity, is distinctly determined by fine roots and the associated mycorrhizal fungi, which concertedly play not only a key role in resource acquisition but also in C and nutrient dynamics of forest ecosystems under global change. However, only few studies addressed a direct comparison of several AM and ECM trees in morphological and functional root traits. Accordingly, information about mycorrhiza based differences in root functions and how they relate to resource acquisition and biogeochemical processes in the rhizosphere are scarce.

In the present study, two research projects were conducted that had the objective to answer the question how root morphological and functional traits of eight different tree species (four per mycorrhizal type; AM: *Acer platanoides* L., *Acer pseudoplatanus* L., *Fraxinus excelsior* L., and *Prunus avium* L.; ECM: *Fagus sylvatica* L., *Quercus robur* L., *Tilia cordata* MILL., and *Carpinus betulus* L.) are influenced by the mycorrhizal association type and to what extent this influences rhizosphere processes. The first research project was conducted in the natural habitats of the tree species, an old mixed broad-leaved forest, and focused on mycorrhiza-specific differences in root morphology, root exudation, and rhizosphere processes. The second research project, a factorial drought experiment in large-mesocosms in the Göttingen Rhizolab Facility, aimed to study root morphology, root functions (i.e. root exudation, root longevity, and N absorption), biomass production and aboveground properties like photosynthetic rates of the studied four AM and four ECM tree species under drought conditions.

Consistent with the mycorrhiza-associated framework that suggest a classification of temperate forests according to the two mycorrhizal association types, the present study revealed significant influences of the mycorrhizal association type on root-rhizosphere interactions (i.e. in some morphological and architectural root traits, at least under drought in the majority of root functional traits, and in the majority of rhizosphere processes).

When transferring the mycorrhizal-associated nutrient economy framework to the revealed differences in root-rhizosphere interactions of AM and ECM trees of this study, the respective

nutrient economy of AM and ECM trees is reflected in root properties, root functions and also in the rhizosphere, and led to different acquisition strategies between the mycorrhizal associations types: AM trees adapt to the inorganic nutrient economy by high capability and efficiency in the uptake of inorganic N. In contrast, ECM trees adapt to the organic nutrient economy by several traits (i.e. by strongly branched roots, stronger mycorrhizal colonization, and lower chemical richness of root exudates), that increase their acquisition potential and prime microbial activities in the rhizosphere (as proven by accelerated enzyme activity, high amounts of microbial C, and strong positive rhizosphere effects on C, N, and P cycles). Even though roots of AM and ECM trees released equal amounts of C by exudation, the reduced chemical richness of ECM exudates distinctly accelerated rhizosphere processes and microbial SOM decomposition. These facts underline that the two mycorrhizal types differ in their strategy for resource exploitation.

Under drought, root functions of AM and ECM trees were differently affected, representing two different strategies in root functioning under soil desiccation: ECM trees invested a high amount of C to optimize acquisitive root functions (i.e. increased root exudation and decreased root lifespan) under drought, while AM trees avoided high C investment in roots and reduced the biomass production to tolerate limited resource uptake by low investments and a reduced demand.

The results of the present study suggest that differences in C partitioning and acquisitive root traits and root functions between AM and ECM trees are crucial for biogeochemical processes and possible compositional shifts in tree species and their associated microbes in temperate forests under global change. In accordance with the mycorrhiza-associated framework, a classification of temperate forests according to the mycorrhizal association type enables more precise predictions of present and future developments of forest ecosystems in response to climate change. Consequently, the mycorrhizal association type should be considered as an important belowground trait for trees in temperate forests.

Zusammenfassung

Die zwei dominierenden Mykorrhizierungstypen in gemäßigten Wäldern sind die arbuskuläre Mykorrhiza (AM) und die Ektomykorrhiza (ECM). Basierend auf den jeweilig dominierenden Mykorrhizierungstyp in gemäßigten Wäldern, wurde die Idee eines Mykorrhiza-basierten Nährstoffökonomiekonzepts entwickelt (Phillips *et al.*, 2013). Dieses Rahmenkonzept besagt, dass AM-dominierte Wälder durch eine schnelle Zersetzung von nährstoffreichem Laub eine anorganische Nährstoffökonomie aufweisen. Im Gegensatz dazu haben Wälder, die von ECM-Bäumen dominiert werden, eine geringe Streuqualität mit langsamer Zersetzungsrate, was zu einer organischen Nährstoffökonomie führt. Die Aufnahme von Nährstoffen aus dem Boden und damit auch die Produktivität der Bäume werden maßgeblich von den Feinwurzeln und deren assoziierten Mykorrhizapilze bestimmt. Gemeinsam spielen diese nicht nur eine Schlüsselrolle beim Ressourcenerwerb, sondern auch bei der C- und Nährstoffdynamik von Waldökosystemen, insbesondere unter den Bedingungen des globalen Klimawandels. Dennoch haben sich bisher nur wenige Studien mit einem direkten Vergleich von morphologischen und funktionellen Wurzelmerkmalen zwischen mehreren AM- und ECM-Baumarten befasst. Dementsprechend liegen nur begrenzt Informationen über Mykorrhiza-spezifische Unterschiede in den Wurzelfunktionen vor und es ist noch unklar, wie diese mit dem Ressourcenerwerb und den biogeochemischen Prozessen in der Rhizosphäre im Zusammenhang stehen.

In der vorliegenden Studie wurden zwei Forschungsprojekte durchgeführt, um die Frage zu beantworten, wie morphologische und funktionelle Wurzelmerkmale von acht verschiedenen Baumarten (vier pro Mykorrhizatyp; AM: *Acer platanoides* L., *Acer pseudoplatanus* L., *Fraxinus excelsior* L., and *Prunus avium* L.; ECM: *Fagus sylvatica* L., *Quercus robur* L., *Tilia cordata* MILL., and *Carpinus betulus* L.) durch den Mykorrhizatyp beeinflusst werden und in welchem Ausmaß dies die Rhizosphärenprozesse bestimmt. Das erste Forschungsprojekt wurde in dem natürlichen Lebensraum der Baumarten, einem alten gemischten Laubwald, durchgeführt und untersuchte Mykorrhiza-spezifische Unterschiede in der Wurzelmorphologie, Wurzelexsudation und den Rhizosphärenprozessen. Das zweite Forschungsprojekt, stellte ein Trockenheitsexperiment mit faktoriellem Design in großen Mesokosmen des Göttinger Wurzellabors dar. Im Rahmen dieses Experiments wurde die Wurzelmorphologie, Wurzelfunktionen (i.e. Wurzelexsudation, Wurzellanglebigkeit und N-Absorption), Biomasse-produktion und oberirdische Eigenschaften wie die Fotosyntheserate der vier AM- und vier ECM-Baumarten unter Trockenheit untersucht.

Übereinstimmend mit dem Mykorrhiza-basierten Rahmenkonzept, das eine Klassifikation gemäßigter Wälder in die beiden Mykorrhizatypen nahelegt, zeigte die vorliegende Studie signifikante Einflüsse des Mykorrhizierungstyps auf Wurzel-Rhizosphären-Interaktionen (i.e. auf einige morphologische und strukturelle Wurzelmerkmale, auf die Mehrheit der funktionellen Merkmale der Wurzel, zumindest unter Trockenheit, und auf die meisten Rhizosphären-prozesse).

Bei der Übertragung des Mykorrhiza-basierenden Nährstoffkonzeptes auf die in dieser Studie aufgedeckten Unterschiede in der Wurzel-Rhizosphären-Interaktionen von AM- und ECM-Bäumen konnte festgestellt werden, dass sich die jeweilige Nährstoffökonomie darin widerspiegeln. Die unterschiedlichen Wurzeleigenschaften, Wurzelfunktionen und Rhizosphärenprozesse zwischen AM- und ECM-Bäumen stellen dabei verschiedene Akquisitionsstrategien zwischen den Mykorrhizatypen dar: AM-Bäume sind durch großes Potential in der anorganischen N-Aufnahme an die anorganische Nährstoffökonomie angepasst. Im Gegensatz dazu passen sich ECM-Bäume durch verschiedene Merkmale (durch stark verzweigte Wurzeln, stärkere Mykorrhiza-Besiedlung, geringere chemische Vielfalt der Exsudate) an die organische Nährstoffökonomie an. Diese Anpassungen erhöhen Aufnahme-potentiale und fördern die mikrobiellen Aktivitäten in der Rhizosphäre (gezeigt durch eine verstärkte Enzymaktivität, hohe Mengen an mikrobiellem C und starke Ankurbelung der Rhizosphärenprozesse in C-, N- und P-Kreisläufen). Selbst bei gleicher C-Freisetzung durch Wurzelexsudation zwischen AM- und ECM-Bäumen beschleunigte die stoffliche Zusammen-setzung der Exsudate (i.e. geringere chemische Vielfalt) von ECM-Bäumen deutlich die Rhizosphärenprozesse und die mikrobielle Zersetzung. Dies unterstreicht, dass sich die beiden Mykorrhizatypen in ihren Strategien der Ressourcenausnutzung unterscheiden.

Die Wurzelfunktionen von AM- und ECM-Bäumen wurden zudem in unterschiedlicher Weise durch Trockenheit beeinflusst und stellen zwei verschiedene Strategien bei der Anpassung an Bodenaustrocknung da: ECM-Baumarten investieren eine große Menge an C, um die Ressourcen-erschließenden Wurzelfunktionen unter Trockenheit zu optimieren (i.e. erhöhte Wurzelexsudation und verringerte Wurzellebensdauer), während hingegen AM-Bäume hohe C-Investitionen in Wurzeln vermeiden und gleichzeitig ihre Biomasseproduktion reduzieren, um eine begrenzte Ressourcenaufnahme durch niedrigeren Aufwand und einen verringerten Bedarf zu tolerieren.

Die Ergebnisse der vorliegenden Studie zeigen, dass Unterschiede in der Partitionierung von C und in den Ressourcen-erschließenden Wurzelmerkmalen und -funktionen zwischen AM- und

ECM-Bäumen ausschlaggebend für biogeochemische Prozesse sind. Darüber hinaus können die Mykorrhiza-spezifischen Unterschiede in der Nährstofferschließung und der Reaktion auf Trockenheit zu einer Veränderung der Zusammensetzung von Baumarten und deren assoziierter Mikroben in gemäßigten Wäldern im Zuge des globalen Klimawandels führen. In Übereinstimmung mit dem Mykorrhiza-basierten Rahmenkonzept ermöglicht eine Klassifizierung gemäßigter Wälder anhand des Mykorrhizierungstyps genauere Vorhersagen der gegenwärtigen und zukünftigen Entwicklung von Waldökosystemen. Daher sollte der Mykorrhizatyp als wichtiges unterirdisches Merkmal für Bäume in gemäßigten Wäldern in Betracht gezogen werden.

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Publications (related to dissertation)

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