Belowground Uptake Strategies

How Fine-root Traits Determine Tree Growth

Monique Weemstra

Thesis committee

Promotors

Prof. Dr G.M.J. Mohren
Professor of Forest Ecology and Forest Management
Wageningen University

Prof. Dr L. Mommer
Personal chair at the Plant Ecology and Nature Conservation Group
Wageningen University

Co-promotor

Dr F.J. Sterck
Associate professor, Forest Ecology and Forest Management Group
Wageningen University

Other members

Prof. Dr E. Hoffland, Wageningen University
Prof. Dr H. de Kroon, Radboud University Nijmegen
Prof. Dr J.H.C. Cornelissen, VU University Amsterdam
Dr A. Weigelt, University of Leipzig, Germany

This research was conducted under the auspices of the C.T. de Wit Graduate School for Production Ecology and Resource Conservation

Belowground Uptake Strategies

How Fine-root Traits Determine Tree Growth

Monique Weemstra

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 28 February 2017
at 1.30 p.m. in the Aula.

Monique Weemstra
Belowground Uptake Strategies. How Fine-root Traits Determine Tree Growth,
168 pages.

PhD thesis, Wageningen University, Wageningen, NL (2017)
With references, with summary in English

ISBN 978-94-6343-039-5
DOI 10.18174/400247

Table of contents

1. General introduction

Tree growth and resource uptake

The growth of trees depends on the availability of light, CO2, water and nutrients, that are acquired by the leaves and fine roots. Aboveground, leaves intercept light and fix CO2 to produce carbohydrates that are prerequisites for growth. Belowground, fine roots (i.e. roots ≤ 2 mm diameter) take up water and different nutrient elements, that are required for photosynthesis in the crown. Because the availability of these resources fluctuates in time and space, trees can adjust their leaves and fine roots to capture them when their availability changes. Consequently, trees of the same and of different species may have very diverse leaves and fine roots, which allows them to grow and survive in different environments.

The variation in leaves that can be observed within and between species is closely related to light availability and interception (Evans and Poorter 2001; Janse-Ten Klooster et al. 2007; Poorter et al. 2012), and to plant growth (Reich et al. 1997; Poorter and Bongers 2006). In contrast, the relationships between variation in fine roots, water and nutrient availability and acquisition, and tree growth, are more ambiguous and less quantified (Mommer and Weemstra 2012). Accordingly, the fine roots of trees and how they fulfil their functional role in tree growth (i.e. acquiring soil resources) are still relatively little understood. As leaf and fine-root functional processes are interdependent and together drive tree growth, more functional insights into the fine roots of trees can enhance our understanding of tree growth in different (resource) environments. **In this thesis, I study the role of fine roots in aboveground functioning and tree growth. I specifically focus on the intra- and interspecific variation in the tree fine roots.**

Leaf and fine-root functional traits

Plant functional traits provide a relevant tool in explaining the plant-ecological processes of interest in this thesis: resource uptake and tree growth. Plant functional traits are defined as plant properties that affect fitness, through their impacts on survival, growth and reproduction (Violle et al. 2007), and can strongly determine the acquisition of resources. They can be categorised into architectural, morphological, chemical and physiological traits, and trait dynamics. Aboveground, crown architectural traits refer to the spatial organization of leaves within the crown (e.g. leaf area index, LAI). Morphological (e.g. area), chemical (e.g. nitrogen content), physiological (e.g. photosynthetic capacity) and dynamic (e.g. lifespan) traits typically refer to the individual leaf. Such leaf traits have been found to control light uptake and tree growth; for instance, a high specific leaf area (SLA, leaf area per leaf mass) has been associated with fast growth across tree species (Poorter and Bongers 2006).

The functional traits of fine roots can be grouped in the same categories (see Table 1.1 for examples of fine-root traits in each category). Architectural traits typically describe the spatial arrangement of the root system as a whole (e.g. fine-root mass and length; Bardgett et al. 2014). Fine-root morphological, chemical and physiological traits represent individual root features related to their form, chemical composition, and uptake and respiration capacities, respectively (Bardgett et al. 2014). Finally, dynamics in fine-root mass or length are also often considered to be functional traits, and include fine-root growth rate and lifespan (e.g. Withington et al. 2006; McCormack et al. 2012). These fine-root traits are also expected to be associated with resource acquisition (Table 1.1). Yet for tree roots, there are less empirical data available to underwrite such expected relationships compared to leaves, so that the functional roles of different fine-root functional traits are still less clear than those of leaf traits.

Intraspecific trait variation and tree growth

Fine-root functional traits can vary *within* species. This trait plasticity allows plants of the same species to grow and survive when resource availability changes (Bradshaw 1965; Sultan 2000; Valladares et al. 2007). Correspondingly, when above- or belowground resource availability decreases, trees are expected to enlarge their leaf or fine-root uptake area, respectively, to enhance the uptake of the most limiting resource. First, they can change their architectural traits, such as total leaf or fine-root mass, to increase resource acquisition (i.e. functional equilibrium, Brouwer 1963). Second, trees can change their leaf and fine-root morphology to enlarge their resource uptake area; an increase in SLA or in specific root length (SRL, fine-root length per fine-root mass) implies a larger uptake area at a given biomass investment.

Aboveground, the functional equilibrium hypothesis has been widely supported. In the shade, plants increase their leaf mass relative to their total plant mass (Poorter and Nagel 2000; Shipley and Meziane 2002). Moreover, when light availability decreases, SLA generally increases in order to intercept more light and enhance carbon gain at an equal biomass investment (Ryser and Eek 2000; Evans and Poorter 2001; Poorter et al. 2012). In fact, the latter (morphological) response is generally stronger than the former (mass) response to light conditions (Poorter et al. 2012), and has been identified as an important driver of carbon gain (Evans and Poorter 2001).

Table 1.1 Fine-root traits and assumed functional roles per trait category. I distinguished two types of functional roles, corresponding to resource economics theory: resource acquisition and resource conservation (Grime 1977; Wright et al. 2004; Reich 2014; further explained in the section 'Interspecific trait variation and tree growth'). The role of a given trait for resource uptake or conservation is often indirect, so in this case, the mechanism(s) through which a trait contributes to either strategy is/are described. Green boxes indicate a positive, and red boxes a negative impact of a trait on resource uptake or conservation. References are indicated by numbers.

Trait category	Trait	Assumed functional role and mechanism	
		Resource acquisition	Resource conservation
Architecture	Fine-root mass	Large uptake area	
	Fine-root length density	Large uptake area	
Morphology	Specific root length	Large uptake area High growth and foraging rates[1-4]	Vulnerability to soil mechanical pressure[5] Fast turnover[6]
	Fine-root tissue density		Resistance to herbivory[3], drought[3]
	Fine-root diameter		Resistance to soil mechanical pressure[5] Slow turnover[6]
Chemistry	Fine-root N content	Fast metabolism High growth and foraging rates[3]	Vulnerability to herbivory
	Fine-root C : N		Resistance to herbivory Slow turnover[7]
Physiology	Fine-root respiration rate	Fast metabolism[4]	High C loss
	Fine-root uptake capacity	Fast resource uptake	
Dynamics	Fine-root growth rate	High foraging and proliferation rates	
	Fine-root lifespan		Low resource losses

References: 1, Eissenstat (1991); 2, Yanai et al. (1995); 3, Roumet et al. (2011); 4, Roumet et al. (2016); 5, Clark et al. (2003); 6, McCormack et al. (2012); 7, Silver and Miya (2001). C, carbon; N, nitrogen.

Belowground plasticity is less consistent and predictable than leaf trait plasticity. The expected increase in fine-root mass om resource-poor soils has been both corroborated (Mokany et al. 2006; Yuan and Chen 2010; Hertel et al. 2013) and refuted (Brunner et al. 2015). Regarding fine-root morphology, trees on poor soils are expected to have a higher SRL than trees on rich soils (Eissenstat 1992; Aerts and Chapin 2000; Holdaway et al. 2011). Yet again, contradictory findings have been reported, demonstrating an increase (Fahey and Hughes 1994; Ostonen et al. 1999), decrease (Ostonen et al. 2007b) or no change (Meier and Leuschner 2008; Brunner et al. 2015) in SRL with soil resource availability. Furthermore, it is so far unclear if one of these two root uptake strategies prevails over the other: according to some studies, fine-root mass is more plastic than morphology (Poorter et al. 2012), whereas others demonstrate the opposite (Freschet et al. 2013; Hertel et al. 2013). Besides these architectural and morphological trait responses, trees can also adjust their fine-root chemical, physiological traits or trait dynamics to their environment, but these plastic responses are far less quantified for large trees. These are beyond the scope of this thesis, which focuses primarily on fine-root mass and morphology.

Thus far, for trees, trait plasticity patterns are less general belowground than aboveground, and cannot always be directly linked to soil resource availability. To gain greater insights into the functional role of fine-root mass and morphology in tree growth on different soils, this thesis studies their plasticity on large trees at contrasting forest soils. We further explore how these traits affect tree growth with a mechanistic whole-tree growth model that simulates fitness under different root-trait combinations and at different nutrient levels.

Interspecific trait variation and tree growth

Across species, functional traits serve as proxies for species fitness (Violle et al. 2007). According to the resource economics hypothesis, functional traits can be coordinated along a spectrum that ranges from an acquisitive to a conservative resource strategy (Grime 1977; Aerts and Chapin 2000; Craine 2009). The trade-off between these two strategies implies that species invest in functional traits that allow *either* fast resource acquisition and therefore fast growth when resources are readily available (i.e. an acquisitive resource strategy), *or* conservation of acquired resources which allows survival under adverse conditions (i.e. a conservative resource strategy). Consequently, resource uptake and use, as well as the potential growth and survival rates of species are expected to be reflected by their functional traits (Reich et al. 1998; Poorter et al. 2006).

Aboveground, a vast number of studies has demonstrated the existence of a worldwide leaf economics spectrum (Reich et al. 1992; Reich et al. 1997; Wright et al. 2004). Species with an acquisitive strategy were found to have a higher SLA and leaf nitrogen contents, and therefore grow faster under high-light conditions. On the other hand, species with a conservative strategy had longer leaf lifespan and lower respiration rates which enabled them to retain plant resources and survive in the shade (Aerts and Chapin 2000; Díaz et al. 2004; Wright et al. 2004; Ordoñez et al. 2009; Kunstler et al. 2016). This resource economics theory has proved highly relevant in linking leaf traits to plant performance (Reich et al. 1998; Poorter and Bongers 2006), species dynamics (Sterck et al. 2006; Kunstler et al. 2016), and ecosystem processes (Reich et al. 1997; Díaz et al. 2004; Díaz et al. 2007; Grigulis et al. 2013).

Belowground, cross-species variation in fine-root traits has been hypothesised to follow the same resource-economics principles in a root economics spectrum (e.g. Comas and Eissenstat 2004; Withington et al. 2006; Comas and Eissenstat 2009; McCormack et al. 2012; Chen and Brassard 2013; Kong et al. 2014; Liu et al. 2015; Valverde-Barrantes et al. 2015). The acquisitive fine-root trait syndrome is expected to consist of roots of high SRL and with high nutrient concentrations and resource uptake capacities (Table 1.1). Such acquisitive roots are assumed to efficiently acquire water and nutrients that are needed to maintain high photosynthetic rates in the crown, and rapid tree growth (Reich 2014). At the other end of the spectrum, conservative species are expected to have thick, long-lived fine roots with dense tissue and low respiration rates. These traits have been associated with lower resource losses (by low root shedding and respiration rates; Table 1.1) and with enhanced survival rates under adverse conditions (Wahl and Ryser 2000; Gu et al. 2011; Reich 2014).

Several studies have tested the existence of a root economics spectrum on forest trees, but with mixed outcomes (e.g. Withington et al. 2006; McCormack et al. 2012; Chen et al. 2013; Hansson et al. 2013; Kong et al. 2014). Establishing a root economics spectrum could contribute to understanding the role of fine-root traits in resource uptake and tree growth across species. Yet at present, it is unclear whether it exists. *Based on a literature review and meta-level analysis, in this thesis, we thus evaluate whether the resource-economics concept presents an equally suitable conceptual framework for linking fine-root traits, resource uptake and growth as it does for leaf traits.*

Mycorrhizal symbiosis

Belowground, a third mechanism exists that enhances a tree's uptake area: mycorrhizal symbiosis. This fungal-plant association implies the colonization of roots by mycorrhizal fungi, and the exchange of nutrients acquired by the fungus and carbon fixed by the host plant. These fungi generally have greater access to soil resources than plants due to their long and thin mycelia that strongly enhance the soil volume available to the host tree (Smith and Read 2008), their capacity to mobilise nutrient elements unavailable to plants (Read and Perez-Moreno 2003), and their ability to transfer resources (e.g. carbon) between trees (Klein et al. 2016). Consequently, this symbiosis forms an important resource-uptake strategy for almost all tree species, and can strongly interact with trees at the whole-plant and fine-root level.

In the first place, mycorrhizal fungi play an essential role in soil resource acquisition and therefore the growth of trees through the mechanisms explained above (Courty et al. 2010). Second, at the fine-root level, mycorrhizal fungi directly influence functional traits, such as diameter (Berta et al. 1995; Eissenstat et al. 2015), length (Pena et al. 2013) and lifespan (King et al. 2002). Therefore, these traits cannot be interpreted in terms of resource uptake or a root economics strategy without accounting for the mycorrhizal fungi present on the root. Third, mycorrhizal fungi may strongly interact with fine-root trait plasticity (Freschet et al. 2015). On nutrient-poor soils, mycorrhizal mycelium abundance is generally larger than on nutrient-rich soils (Nilsson et al. 2005; Kjøller et al. 2012). Such mycorrhizal abundance may reduce the need for morphological plasticity, e.g. increasing SRL. Studying tree growth based on fine-root functional traits and trait-plasticity thus requires considering the mycorrhizal associations involved. *In this thesis, we therefore specifically address mycorrhizal symbiosis when studying intraspecific fine-root trait plasticity, and discuss their role in interspecific resource economic strategies.*

A whole-tree perspective on tree growth

Although this thesis focuses on fine-root traits, tree growth ultimately results from above- and belowground resource uptake and use. These resources need to be functionally balanced, so that neither of them limits growth (Cannell and Dewar 1994). To maintain these functional balances and maximise their growth, trees coordinate their leaf and fine-root traits. For example, crowns with a large LAI, high leaf nitrogen content and high photosynthetic rate, require rapid water and nutrient supply. Therefore, they should be supported by large and dense root systems that can readily capture these resources to maintain tree growth. Such functional relationships between leaves and fine roots are relatively little quantified for large trees. Empirical studies for instance have sought correlations between assumingly parallel leaf and fine-root functional traits associated with resource uptake, such as SRL and SLA; these traits have been found to correlate in some studies (Wright and Westoby 1999; Withington et al. 2006; Freschet et al. 2010), but not in others (Tjoelker et al. 2005; Chen et al. 2013; Valverde-Barrantes et al. 2015).

These mixed results may be partly caused by the different uptake mechanisms through which a tree can maintain or restore its functional balance. High photosynthetic rates in the crown may be met by an increase in fine-root mass rather than in SRL, or by a specific combination of both traits, to enhance water and nutrient uptake. This could explain why SLA and SRL are not correlated across tree species. Moreover, fine-root traits can also be strongly influenced by soil properties other than resource availability. This implies that an increase in SRL may be optimal to acquire soil resources, but may be constrained by e.g. soil compaction (Bejarano et al. 2010; Alameda and Villar 2012). In the field, such confounding variables are difficult to account for, so that the functional role of SRL remains unclear. Therefore, a mechanistic rather than a correlative approach seems more suited for studying the combined effects of fine-root and leaf traits on growth (Mommer and Weemstra 2012).

In this thesis, we applied a mechanistic whole-tree growth model that allows us to simultaneously vary different fine-root traits. This way, their combined impacts on tree growth can be explicitly tested, while excluding the effects of confounding (soil) variables. Furthermore, the model incorporates both above- and belowground traits that are functionally connected; photosynthesis in the crown is thus constrained by belowground resource uptake, and water uptake by the fine roots is driven by evaporation in the crown, and together they drive tree growth. Through this model, we aim to further explore the role of fine-root traits in aboveground functioning, as well as their integrated effects on tree growth.

Rationale and research questions

In sum, understanding tree growth requires a whole-tree approach that integrates above- and belowground traits and resource uptake and loss. However, this whole-tree perspective is currently underexplored because of our relatively poor understanding of 1) fine-root traits, and how and why they vary within and between species, and 2) how fine-root traits relate to aboveground functioning and whole-tree growth. Addressing these issues contributes to obtaining functional insights in the resource uptake and growth rates of different tree species, and on different soils, that are not yet fully understood. Moreover, they are particularly timely and relevant to address in the context of environmental change, as water and nutrients are essential to tree growth but subject to climate change. This thesis seeks to contribute to these two knowledge gaps by addressing the following general research question: **How do fine-root functional traits relate to tree growth?**

To answer this question, this thesis specifically examines across- and within-species variation in tree root functional traits, and relates these fine-root traits to tree growth in four chapters where four corresponding research questions are addressed:

1. How does the growth of trees of different species respond to variation in climate variables and groundwater tables?
2. Can fine-root trait variation across tree species be explained from a resource economics perspective?
3. How do trees of the same species adjust their fine-root functional traits to the soil environment?
4. How do fine-root traits and trade-offs relate to whole-tree fitness under different soil nutrient conditions?

Thesis outline

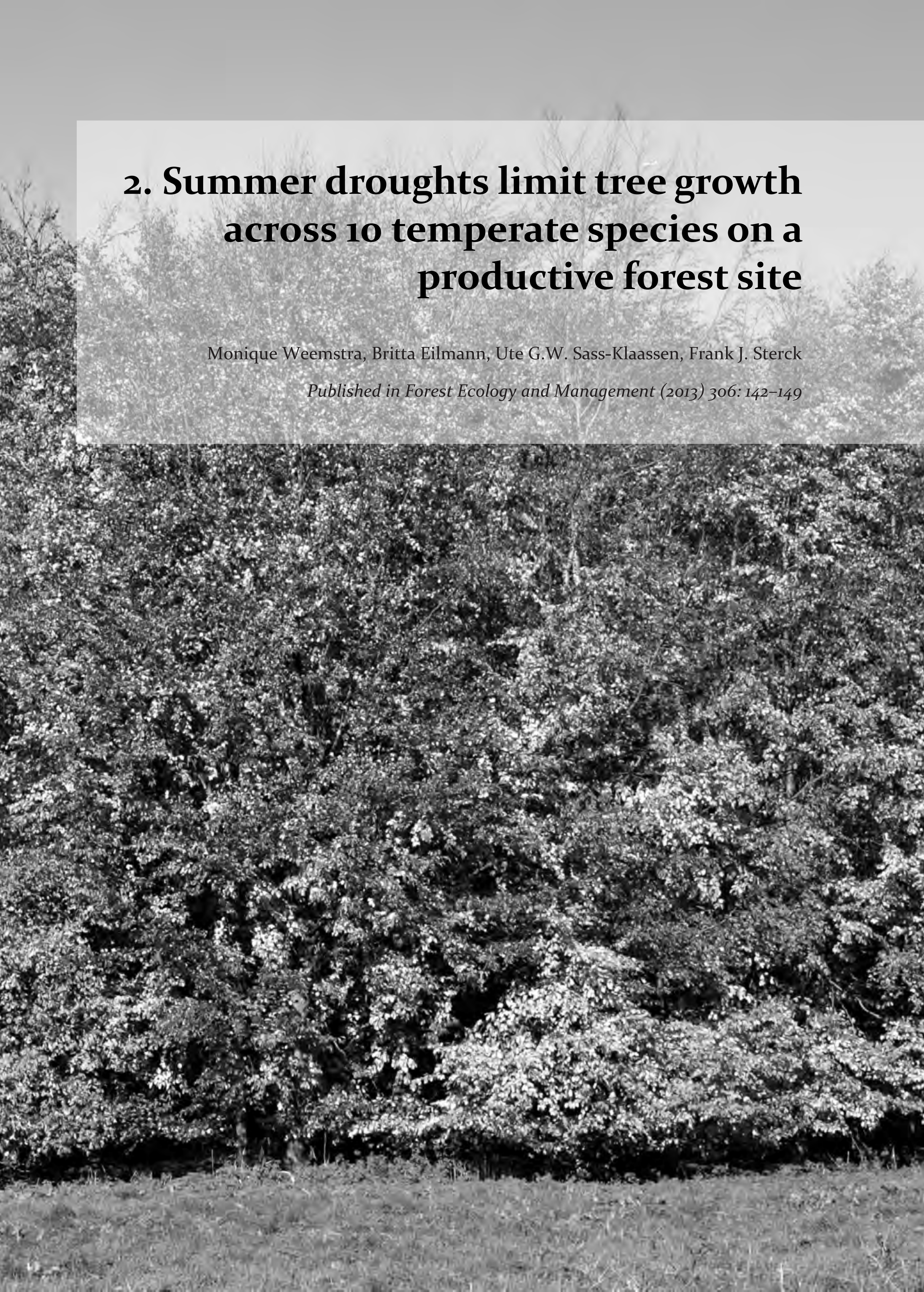
Chapters 2 and 3 focus on cross-species variation in growth (Chapter 2) and fine-root traits (Chapter 3). In the second chapter, we examine the annual growth response of 10 broadleaved tree species to different climate and groundwater variables under uniform soil conditions in the Netherlands. In the third chapter, we expand our cross-species perspective belowground, and present the state-of-the-art regarding a root economics spectrum in a literature review and meta-level analysis. We thus test the root economics spectrum-hypothesis and discuss whether this concept is able to explain interspecific variation in fine-root traits, and the implications of our findings for future root research (Chapter 3).

Chapters 4 and 5 further look into the effects of the soil environment on fine roots and tree fitness. Chapter 4 focuses on fine-root trait plasticity. We examine within-species variation in different fine-root traits and associated mycorrhizal fungi, on beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) Karst.) trees growing on contrasting soils. The fifth chapter builds on the gathered data and insights on within- and across species root-trait variation (from Chapters 2 – 4) to explore how below- and aboveground traits influence tree fitness. We use a whole-tree growth model that integrates different fine-root, stem and crown traits and that simulates tree fitness at different soil nutrient levels. In the general discussion (Chapter 6), I synthesise the results of the previous chapters, and discuss how this thesis contributes to grasping the role of fine-root traits in tree growth.

2. Summer droughts limit tree growth across 10 temperate species on a productive forest site

Monique Weemstra, Britta Eilmann, Ute G.W. Sass-Klaassen, Frank J. Sterck

Published in Forest Ecology and Management (2013) 306: 142–149



Abstract

Studies on climate impacts on tree annual growth are mainly restricted to marginal sites. To date, the climate effects on annual growth of trees in favourable environments remain therefore unclear despite the importance of these sites in terms of forest productivity. Because species respond differently to climate, comparing a multitude of species further enhances our knowledge on climate impacts on tree growth and forest productivity.

We present a first study that reveals to what extent radial growth is limited by climate and the groundwater table across 10 temperate tree species growing on a uniform, productive soil (i.e. high nutrient and water contents) in the Netherlands. We ranked our study species according to their shade tolerance, which is associated with species' resource requirements and growth rates, and examined their annual growth using tree-ring analysis. This allowed us to investigate how these species with diverse ecological backgrounds differ in their growth response to precipitation, temperature, irradiance, potential evapotranspiration (PET), and the groundwater table, when growing under similar and favorable site conditions.

Nine out of 10 species had strikingly similar radial growth rates (on average 3.9 – 4.8 mm yr^{-1}), which contradicts the widely established trade-off between shade tolerance and growth. *Populus trichocarpa*, the least shade-tolerant of our species, however grew much faster (on average 6.8 mm yr^{-1}). Trees of all species reduced their growth significantly during dry summers, driven by low rainfall and high PET. Yet, the magnitude of their growth responses to climate conditions and especially the groundwater table differed across species. Receding groundwater tables significantly and strongly reduced radial growth of the shade-intolerant species, but not of the shade-tolerant species. The climate impacts on growth were not associated with variation in shade tolerance across our species.

Our study demonstrates that even on a productive forest site, summer droughts reduced radial growth across a multitude of common tree species, whereas lower groundwater tables only affected the shade-intolerant species significantly. Thus, the productivity of forests in favourable environments may be seriously affected when summers in north-western Europe become hotter and drier as predicted by climate change models.

Key words

Climate change, dendrochronology, drought, groundwater table, growth conditions, shade tolerance

Introduction

Climate models predict higher yearly mean temperatures and a change in precipitation patterns leading to wetter winters and drier, hotter summers in north-western Europe (Alcamo et al. 2007; Lindner et al. 2010). These forecasts imply that temperate forests will encounter increased water supply in spring, but more frequent and intense droughts in summer (Broadmeadow 2005; Scharnweber et al. 2011) with possibly detrimental effects on tree growth (Bolte et al. 2009; Allen et al. 2010). Tree growth depends on several interrelated climate variables. High evaporative pull from the atmosphere for example, associated with high air temperature and irradiance, can hamper growth when water supply does not meet atmospheric demand (Bréda et al. 2006). Under these conditions, stomatal closure reduces tree water loss but also carbon uptake which limits growth (Bréda et al. 2006; McDowell et al. 2008). At the same time, the effects of temperature on tree growth depend on water availability: temperature can enhance growth (Lindner et al. 2010), but only if water is sufficiently available (Bolte et al. 2009).

Previous dendrochronological studies assessed climate impacts on radial growth, but mainly under extreme site conditions (e.g. van der Werf et al. 2007; Helama et al. 2009; Eilmann et al. 2009; Mérian and Lebourgeois 2011; Scharnweber et al. 2011), or at the limits of a species' natural distribution (e.g. Andreu et al. 2007; Eilmann et al. 2011), where growth-limiting factors are more manifest. These studies are often restricted to a single or a few species (e.g. one to five species in the references given above). So far, it is uncertain if and to what extent trees growing in productive temperate forests with favourable water-holding capacities also suffer from the predicted water deficits and temperature rise, and whether this varies across species. This knowledge is nonetheless essential to predict the productivity of forests, especially because wood production of different species is even more important on productive than on marginal sites. Furthermore, assessing the climate impacts across a large species set could contribute to selecting appropriate species for these economically relevant forests.

In order to expand our insights into climate effects on tree growth beyond marginal sites and few species, this study compares annual growth rates and responses to climatic variation across 10 temperate, deciduous tree species under productive and uniform soil conditions. These beneficial conditions include a calcareous clay soil with high water-holding capacities, high moisture and nutrient contents (Cornelissen et al.

2012), and shallow groundwater tables (Bijlsma and Verkaik 2008). In order to compare our study species from an ecological background, we have ranked them based on a shade tolerance index (Niinemets and Valladares 2006). The shade tolerance of a species is largely associated with its plant strategy (Grime, 1974) where shade-intolerant species typically acquire resources rapidly in order to maintain high growth rates under resource-rich conditions. More shade-tolerant species generally take up resources at a slower pace and retain them longer. This enhances tolerance to and occurrence in resource-poor environments, but at the expense of growth rate (Grime 1974; Aerts and Chapin 2000; Reich et al. 2003; Díaz et al. 2004).

From this species classification, we first test whether more shade-intolerant species indeed grow faster than more shade-tolerant species on this water- and nutrient-rich soil. Second, we hypothesise that shade-intolerant species respond more strongly to climate conditions and to the depth of the groundwater table than shade-tolerant species and that their growth is more limited by low water availability, as they exploit their resources faster. We expect that such growth limitations are stronger in summer when water supply is insufficient to meet the high evaporative demand associated with high temperature and irradiance. We expect less severe water limitations for growth in spring because lower temperatures reduce evaporative water demand and more water will be available from winter rainfall (Dittmar et al. 2003).

To test our hypotheses, we selected an experimental forest setting in the Netherlands that hosts a variety of temperate tree species growing under uniform climate and soil conditions, where the soil is characterised by high nutrient and soil moisture levels compared to other forests in north-western Europe (Cornelissen et al. 2012). This study site provides a unique opportunity to compare growth rates and responses to climate among dominant, mostly 35 – 40-year old trees from 10 different tree species (Table 2.1) in monospecific stands on a homogeneous, favourable soil. We tested the impacts of spring and summer precipitation, temperature, potential evapotranspiration (PET), irradiance, and groundwater depth on tree growth using tree-ring analyses.

Methods

Study site

Field work was conducted at an experimental forest setting in the Hollandse Hout, in the centre of the Netherlands (52º28'N, 5º26'E). The area was claimed from the sea in the 1960s and is situated at approximately 4 m below sea level. It is characterised by a temperate marine climate with mild winters and summers (Figure 2.1) and an annual precipitation of approximately 800 mm (KNMI, 2009). The Hollandse Hout forest comprises approximately 900 ha and was established in the late 1960s and the 1970s, with a variety of deciduous and coniferous species planted in monospecific stands. Its marine clay soil is calcareous and nutrient-rich (see Appendix 2.1 for soil characteristics; Kemmers et al. 2000; Cornelissen et al. 2012) and has a uniform texture across the forest (Alterra, n.d.). Groundwater tables are highly controlled by the regional water board to prevent flooding (P. Ponsteen, personal communication), and range from 1.6 to 1.2 m below the surface averaged over 17 years of available groundwater data (TNO-NITG, 2011).

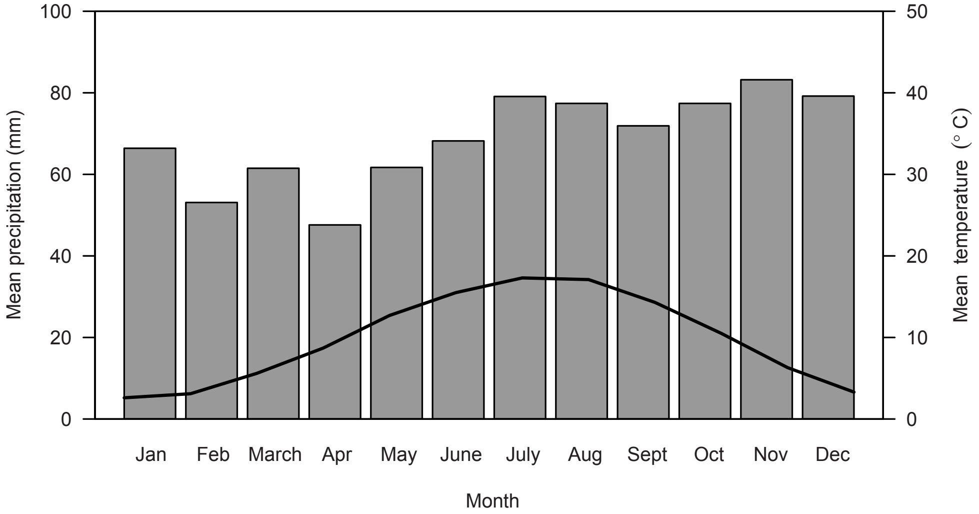


Figure 2.1 Climate diagram of the De Bilt weather station, the Netherlands. Monthly mean precipitation (bars) and temperature (line) over the period 1960 – 2010.

To test growth responses to climate, climate data were retrieved from the De Bilt weather station of the Royal Dutch Meteorological Institute (Klein Tank et al. 2002), at approximately 50 km from the study site. They include daily precipitation (mm), temperature (°C), PET (mm), and irradiance (J cm^{-2}) records. Potential evapotranspiration was calculated according to Makkink's method which integrates temperature and irradiance (Klein Tank et al. 2002). Groundwater depth data (cm below ground level) were available from the Dutch Organization for Applied Scientific Research (TNO-NITG, 2011), and were retrieved from one station located in the centre of the forest, where they were recorded twice per month. The groundwater data covered only a short time span (17 years, 1981 – 1997), with data missing for 23 months that were replaced by data from a nearby station that correlated highly ($r = 0.94$). Climate and groundwater data were grouped into two seasons: spring (1 March – 31 May) and summer (1 June – 31 August), and we calculated seasonal sums (for precipitation and PET) or averages (for temperature, irradiance and groundwater depth).

In this system, spring precipitation and spring groundwater depth were correlated such that high rainfall coincides with shallow groundwater tables (Figure 2.2a, $r = 0.54$, $P < 0.05$, $N = 17$). In summer however, precipitation and groundwater were not significantly correlated (Figure 2.2c, $r = 0.10$. $P = 0.70$, $N = 17$). Precipitation had no significant impact on the summer groundwater table after a one, three and six month time lag either (not shown). Precipitation correlated negatively with irradiance in spring ($r = -0.47$, $P < 0.01$) and in summer ($r = -0.54$, $P < 0.001$), and with PET in summer ($r = -0.43$, $P < 0.01$). Temperature increases evaporative demand in spring (Figure 2.2b, $r = 0.32$, $P < 0.05$, $N = 40$) and summer (Figure 2.2d, $r = 0.87$, $P < 0.001$, $N = 40$). Irradiance (not shown) correlated with PET in summer ($r = 0.97$, $P < 0.001$, $N = 40$), and to a lesser degree in spring ($r = 0.44$, $p < 0.01$, $N = 40$), as well as with temperature in both summer ($r = 0.82$, $P < 0.001$, $N = 40$) and spring ($r = 0.44$, $P < 0.01$, $N = 40$).

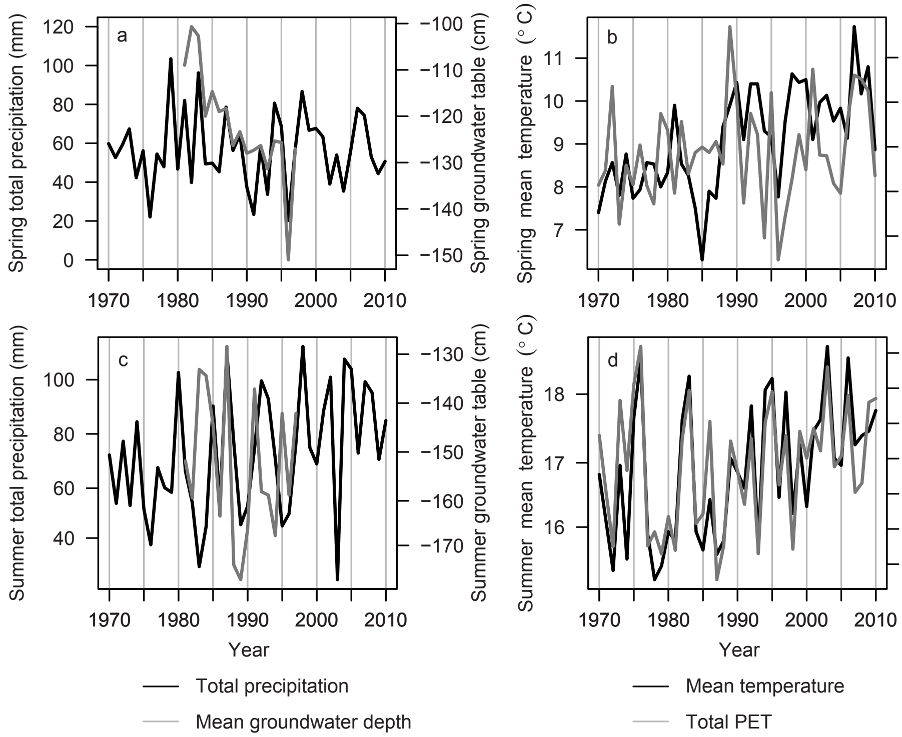


Figure 2.2 Annual variation in spring and summer climate conditions and groundwater depth over the period of the experiment. Spring (a, b) and summer (c, d) total precipitation and mean groundwater table, mean temperature and total PET. Mean values over the time period covered in respectively spring and summer: precipitation 57, 71 mm; temperature 9, 17 °C; PET 65, 89 mm; irradiance 1278, 1659 J cm^{-2}; groundwater depth 122, 154 cm below soil surface. Correlation coefficients and P-values are given in the study site description. Groundwater curves do not cover the entire period due to missing data.

Study species and sampling

We collected increment cores of 10 mostly even-aged (35 – 40 year old) individuals for each of 10 deciduous tree species: *Acer pseudoplatanus* L. (sycamore maple), *Alnus glutinosa* L. Gaertn. (black alder), *Betula pendula* Roth. (silver birch), *Fagus sylvatica* L. (European beech), *Fraxinus excelsior* L. (European ash), *Populus trichocarpa* (black cottonwood), *Prunus avium* L. (wild cherry), *Quercus robur* L. (pedunculate oak), *Salix alba* L. (white willow), *Tilia cordata* Mill. (small-leaved lime) (Table 2.1). Some *P. avium* and *S. alba* trees sampled, were however younger, i.e. 20 – 30 years from the year of establishment.

Table 2.1 Stand and chronology statistics of the study species at the Hollandse Hout forest site. Species are ordered according to their shade tolerance index (ST index; Niinemets and Valladares (2006), from shade-tolerant to shade-intolerant. Data on the years of establishment were retrieved from the State Forestry Service, but were unavailable for *S. alba*, and therefore estimated based on the number of tree rings cored at breast height. Mean DBH (diameter at breast height, cm) and standard deviation (between parentheses) values were derived from field measurements. Mean ring width (mm) and standard deviation (between parentheses) are based on raw data. R_{bar}, species intercorrelation, and MS, mean sensitivity, are explained in the Methods section and calculated based on detrended data.

Species	**ST index**	**Establishment**	**Mean DBH**	**Mean ring width**	**R_{bar}**	**MS**
Fagus sylvatica	4.56	1969-1972	33.6 (2.7)	4.43 (1.52)	0.376	0.213
Tilia cordata	4.18	1969-1979	29.4 (5.7)	3.90 (1.77)	0.306	0.301
Acer pseudoplatanus	3.73	1969-1972	32.9 (2.5)	4.16 (1.41)	0.438	0.239
Prunus avium	3.33	1969-1992	28.7 (3.6)	4.49 (2.58)	0.351	0.255
Alnus glutinosa	2.71	1970-1980	31.3 (3.5)	3.91 (1.52)	0.340	0.332
Fraxinus excelsior	2.66	1969-1971	34.3 (3.7)	4.23 (1.61)	0.275	0.200
Quercus robur	2.45	1970-1983	32.9 (4.5)	4.73 (1.51)	0.470	0.186
Betula pendula	2.03	1970-1975	32.8 (3.4)	4.21 (1.89)	0.440	0.354
Salix alba	1.99	1965-1975	37.0 (3.8)	4.79 (2.21)	0.403	0.355
Populus trichocarpa	1.27	1969-1972	58.8 (5.5)	6.81 (3.68)	0.522	0.456

Two dominant trees were sampled from each of five monospecific stands per species to ensure that our study trees were fully light-exposed and to minimise the effects of competition by other trees on growth. The stands were evenly distributed across the forest to guarantee sufficient replicates. Two increment cores per tree were extracted at breast height, and diameter (DBH) was measured. The study species were categorised according to their shade tolerance index (Table 2.1; Niinemets and Valladares 2006), as an ecological indicator associated with species' resource requirements and growth rates (Grime 1974; Aerts and Chapin 2000; Reich et al. 2003). As in the original data set (Niinemets and Valladares 2006), these indices range between zero and five, where a low index value refers to shade-intolerant species, and a high index value to shade-tolerant species.

Ring-width data

Increment cores were planed with a microtome and sanded to increase the visibility of tree-ring boundaries. Tree-ring width was measured using a LINTAB measuring table (1/100 mm accuracy) and TSAP-Win software (Rinn 2003). The two measurements per tree were cross-dated, verified with Cofecha software (Holmes 1983), and we established species chronologies. The individual tree-ring series were detrended to reduce variation caused by non-climatic (e.g. ontogenetic) trends using the dplR package in R (Bunn 2008; R Development Core Team 2012). A cubic smoothing spline was fit to the individual tree-ring series, because the series did not follow a monotonically increasing or decreasing trend. We set the frequency cut-off at its default (50%), and because series lengths differed within and between species, the bandwidth was set at 0.67 of the series length.

Data analysis

Statistical analyses were carried out in R (R Development Core Team 2012). Mean ring widths and their standard deviation were calculated from the un-detrended ring widths, whereas mean sensitivity and series intercorrelation (R_{bar}) were computed based on the detrended ring widths (dplR package in R; Bunn 2008). The mean sensitivity is a measure of the year-to-year variability in ring-width indices, and indicates the sensitivity of a series to certain environmental conditions. The R_{bar} was calculated as the mean Pearson correlation coefficient between all ring-width series within a species, and indicates to what extent the growth of trees of a single species is limited by the same factors. Impacts on radial growth were assessed with a simple linear regression analysis per species with seasonal climate and groundwater variables as independent, and individual tree-ring width indices (i.e. the detrended ring widths) as dependent variables, using a mixed model with the individual tree as a random factor. The regression coefficients were standardised to allow for further comparison. A one-sample t-test was carried out to detect general trends in the direction (i.e. positive or negative) of the climate and groundwater effects on tree growth across the species set as a whole. We correlated the species' shade tolerance indices (Table 2.1) with the standardised regression coefficients (Table 2.2) to examine patterns in climate and groundwater effects on growth across species (Spearman's rank correlation).

Results

Radial growth rates across species

The mean ring widths suggest that all species grew evenly fast, except for *P. trichocarpa* that had a higher mean ring width and standard deviation (Table 2.1). The mean ring width of *P. avium* and *S. alba* may have been increased by some individuals that were 10 – 20 years younger than the 35 – 40-year old trees of the other species (Table 2.1). When comparing radial growth across species between 5 and 25 years after establishment to avoid such age effects, *P. trichocarpa* still grew the fastest, and the other species again showed similar and almost linearly increasing radial growth patterns over time (Figure 2.3). The DBH in 2011 of the even-aged species (i.e. excluding *P. avium* and *S. alba*) was higher for *P. trichocarpa* than for the other species (Table 2.1), mainly owing to its fast growth between the age of 5 and 15 years (Figure 2.3).

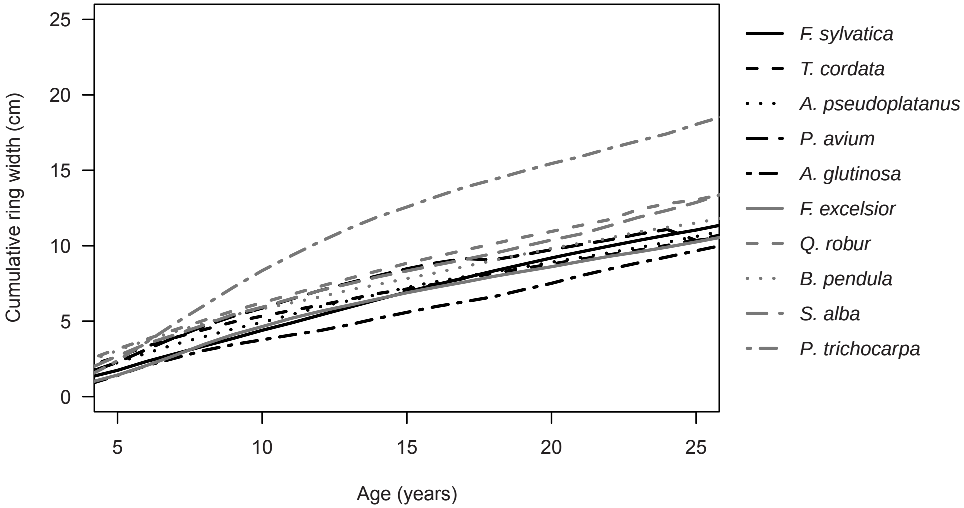


Figure 2.3 Cumulative annual ring width over tree age per species. Data are shown from five years onwards due to insufficient replicates prior to age = 5 years. Age refers to the cambial age of the tree after it reached the coring height of 1.3 m.

Effects of climate and groundwater on tree growth across species

Qualitatively, species responded similarly and most strongly to summer climate (Table 2.2). Summer precipitation had a significant, positive impact on the radial growth of all species, and high summer temperature, irradiance and especially PET caused a reduction in tree-ring width, which was significant for half of the species. The direction (i.e. positive or negative) of these summer impacts was consistent throughout the species set as a whole (Table 2.2). The effect of the summer mean groundwater depth on growth differed across species. A strong negative impact was found for *F. excelsior*, *B. pendula*, *S. alba* and *P. trichocarpa*, suggesting that shallow groundwater tables coincided with fast growth. The remaining species showed a small or no growth response to the depth of the groundwater table (Table 2.2). *T. cordata* however was the only species that showed faster growth in response to deep groundwater tables during summer. Due to this remarkable response of *T. cordata*, the relationship between growth and the groundwater depth across the entire species set was insignificant. Removing *T. cordata* from the analysis did yield a significant, negative effect across the remaining nine species ($t = -3.052$, $P = 0.02$, $N = 9$).

Growth responses to spring climate conditions were weaker and more variable than to summer climate. Spring temperature and PET had a consistently positive impact on growth, but this impact was minor and was significant for only a few species (Table 2.2). The effects of precipitation and irradiance on growth were even smaller and inconsistent across the entire species set and spring groundwater depth had almost no significant influence on growth. The few significant values indicate that *B. pendula* and *P. trichocarpa* were sensitive to deep groundwater tables in spring, and this sensitivity influenced the negative relation between spring groundwater depth and growth observed over the species set as a whole (Table 2.2).

Quantitatively, species differed in their growth response to climate. *Fagus sylvatica* and *P. trichocarpa* for example strongly responded to summer climate, whereas *P. avium* and *F. excelsior* showed only weak growth responses (Table 2.2). We found a trend in the negative correlation between mean sensitivity and species' shade tolerance index ($r = -0.615$, $P = 0.058$, $N = 10$), suggesting that the least shade-tolerant species tended to show greater year-to-year variation in their ring width, possibly pointing at greater sensitivity to climate. The high mean sensitivity of *B. pendula*, *S. alba* and *P. trichocarpa* (Table 2.1) for example, was reflected in their observed and detrended growth curves, which showed the highest growth peaks and lowest depressions (Figure 2.4).

Also, the shade-intolerant species responded more strongly to the depth of the groundwater table than the shade-tolerant species in spring, but especially in summer (Table 2.2). In fact, tree growth of *F. excelsior*, *B. pendula* and *S. alba* was more reduced by summer groundwater levels than by the climate variables. The shade tolerance indices of our species were not significantly related to their growth responses to any of the climate variables (Table 2.2).

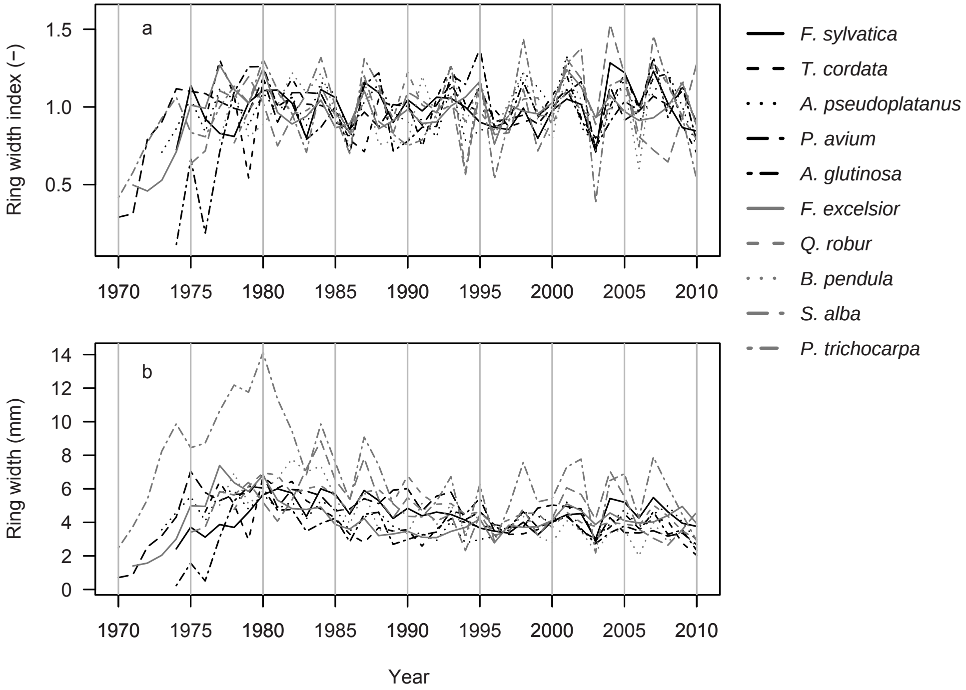


Figure 2.4 Variation in ring width index (a) and annual tree-ring width (b) per species over the period of the experiment. Data points are averaged over 10 individuals per species, except for *S. alba* (N = 6).

Table 2.2 Standardised regression coefficients per species. The regression coefficients are based on a mixed model of annual ring width index as a function of climate variables and groundwater depth for summer and spring (individual tree as random factor). Precip., precipitation; Temp., temperature; PET, potential evapotranspiration; Irr., irradiance; GW, groundwater depth. Summary statistics are provided for the whole species set: Spp. set shows the significance of the direction (positive/negative) of the standardised regression coefficients across the species set as a whole (one-sample t-test, μ = 0, N = 10); Corr. coeff. shows the significance of the correlation (Spearman's rho, N = 10) between the standardised regression coefficients per variable, and the shade tolerance index (see Table 2.1). Species are ordered from shade-tolerant to shade-intolerant.

	Summer					**Spring**				
Species	**Precip.**	**Temp.**	**PET**	**Irr.**	**GW**	**Precip.**	**Temp.**	**PET**	**Irr.**	**GW**
Fagus sylvatica	0.47^{***}	-0.30^{***}	-0.41^{***}	-0.41^{***}	-0.03	-0.01	0.04	0.07	0.059	-0.08
Tilia cordata	0.13^{*}	-0.03	-0.07	-0.08	0.28^{***}	-0.03	0.17^{**}	0.16^{**}	0.096	-0.04
Acer pseudoplatanus	0.31^{***}	-0.30^{***}	-0.35^{***}	-0.32^{***}	-0.08	0.01	0.07	0.18^{***}	-0.025	-0.11
Prunus avium	0.15^{*}	-0.01	-0.04	-0.06	-0.05	-0.04	0.08	0.20^{**}	0.059	-0.07
Alnus glutinosa	0.25^{***}	-0.16^{**}	-0.25^{***}	-0.26^{***}	-0.06	0.20^{***}	0.09	0.09	-0.078	0.01
Fraxinus excelsior	0.14^{*}	-0.00	-0.01	-0.04	-0.20^{**}	0.08	0.06	0.15^{**}	-0.027	-0.08
Quercus robur	0.21^{***}	-0.11	-0.27^{***}	-0.28^{***}	-0.04	0.12	0.28^{***}	0.33^{***}	0.197^{**}	-0.13
Betula pendula	0.23^{***}	-0.10	-0.21^{***}	-0.15^{**}	-0.52^{***}	-0.03	0.06	0.04	-0.054	-0.23^{**}
Salix alba	0.21^{***}	-0.15^{**}	-0.13	-0.11	-0.42^{***}	-0.15^{*}	-0.17^{*}	-0.02	0.001	-0.11
Populus trichocarpa	0.52^{***}	-0.33^{***}	-0.46^{***}	-0.47^{***}	-0.24^{**}	0.11	0.17^{**}	0.24^{***}	-0.029	-0.16^{*}
Spp. set	***	**	**	**	**!**	**ns**	*	**	**ns**	***
Corr. coeff.	**ns**	**ns**	**ns**	**ns**	**	**ns**	**ns**	**ns**	**ns**	*

ns not significant. ! P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001

Discussion

We assessed the impact of several climate variables and the groundwater depth on tree annual growth across 10 species on similar, productive soils to enhance our understanding of these effects across a wider environmental range than currently focused on in research. We tested whether different growth responses across species could be explained from their shade tolerance that is associated with resource uptake and use, growth rates and site preference (Grime 1974; Aerts and Chapin 2000; Reich et al. 2003).

Radial growth rates across species

In contrast to our first hypothesis, the shade-intolerant species did not grow faster than the shade-tolerant species of our species set. Radial growth rates were surprisingly similar among nine out of the 10 species (Figure 2.3) and also higher than generally reported. The DBH measured in 2011 was overall a factor 1.5 – 2 higher than yield table DBH estimates in the highest growth classes (i.e. under the most favourable site conditions) for north-western Europe (Jansen and Faber 1996). *Populus trichocarpa* grew even faster than the other species (Figure 2.3), and had a higher DBH at approximately 40 years old. It was also the most shade-intolerant species studied here (Table 2.1). After the first 15 years, radial growth rates of *P. trichocarpa* converged however to the other species' growth rates (Figure 2.4). This fast radial growth of *P. trichocarpa* may be partly attributed to low investments in wood density. A low wood density is typically found in fast-growing species (Westoby and Wright 2006; Chave et al. 2009), and also in *Populus* species (De Boever et al. 2007; Hacke et al. 2010), but wood density was not measured here.

Our results show that the variation in shade tolerance across our species set does not explain the radial growth rates of our 10 tree species, despite the negative correlation between shade tolerance and growth rates observed in other studies (Kitajima 1994; Reich et al. 2003; Coomes et al. 2009). Studies on this trade-off have been mostly restricted to seedlings or saplings (Kitajima 1994; Kobe et al. 1995; Walters and Reich 1999; Poorter 1999; Sterck et al. 2006), where growth is largely driven by leaf photosynthetic capacities (Walters and Reich 1999; Poorter and Bongers 2006). To explain the similar growth rates across our 35 – 40-year old trees, we speculate that after canopy closure, fast growth is no longer controlled by leaf characteristics such as a high leaf photosynthetic capacity, but can also be achieved by an optimal leaf area display and a large light interceptive area (McMurtrie and Dewar 2011; Sterck and Schieving 2011). Possibly, the shade-tolerant species in our set match the fast growth of the shade-intolerant species by producing large, dense crowns that capture much light to compensate for their low leaf photosynthetic capacities. Although not tested here, we propose that differences in leaf photosynthetic traits related to a plant's shade tolerance are less prominent for dominant canopy trees with fully established crowns.

Effects of climate and groundwater on tree growth across species

Growth responses to climate conditions and groundwater depth were qualitatively similar across species. Even under these favourable soil conditions, characterised by high growth rates across our species, radial growth of all 10 species was reduced by summer water deficits (Table 2.2). This outcome agrees with studies carried out elsewhere in north-western Europe on subsets of our species set (van der Werf et al. 2007; Helama et al. 2009; Mérian and Lebourgeois 2011; Scharnweber et al. 2011), but is nonetheless surprising given the high water-holding capacities and shallow groundwater tables of the Hollandse Hout soils, relative to the poor, dry sandy (van der Werf et al. 2007; Scharnweber et al. 2011), or shallow soils (Helama et al. 2009) covered by other studies. Trees on soils with seemingly sufficient water supply may be as sensitive in stomatal closure responses to local conditions as trees on dry sites in order to maximise carbon gain when water is sufficiently available, but with negative impacts on growth when water becomes limiting. Similarly, trees in very different climates seem to tune their stomatal responses such that they are similarly sensitive to local droughts (Choat et al. 2012).

In summer, water deficits were strongly driven by low precipitation and high air temperature, irradiance and evaporative demand (PET) (Table 2.2). Their impacts were consistently positive (precipitation) and negative (temperature, irradiance and PET) throughout our species set, and significant for most if not all species, indicating a clear summer-climate mechanism affecting tree growth. The depth of the groundwater table had a smaller and overall negative impact on tree growth across our species, implying that deeper groundwater tables reduced growth (Table 2.2). On our study site, groundwater tables are highly artificially controlled, leading to a poor match between groundwater depth and precipitation in summer (Figure 2.2c) suggesting that both variables have largely independent effects on the water availability and growth of trees. Rainwater penetrating the top soil seems a more important water source than groundwater in summer, at least for most species, as the latter significantly affected only four species (Table 2.2).

In spring, climate conditions and the groundwater depth had a weaker and less consistent impact on growth across our species than in summer (Table 2.2), as expected. Precipitation was lower in spring than in summer (Figures 2.1 and 2.2), but the results suggest that trees did not experience water shortage as the evaporative demand was lower (Figure 2.2b,d) and the clay soil was still filled with water reserves from winter precipitation. Consequently, high spring temperature and irradiance enhanced growth as water was sufficiently available to meet evaporative demand and maintain photosynthetic activity (Table 2.2). Yet, although this mechanism was consistent throughout the species set as a whole for spring temperature, PET and the groundwater table, the effects on tree growth of these variables were often significant for only a few species individually. Furthermore, the mechanism is not as straightforward as described here: species that significantly reduced their growth when facing high evaporative demand did not necessarily respond to high spring temperatures (Table 2.2).

Despite qualitative similarities, species differed quantitatively in their growth responses to climate and groundwater depth. We expected shade-intolerant species to suffer more from water deficits than shade-tolerant species. The larger inter-annual variation in the growth rates of these species (i.e. high mean sensitivity, Table 2.1) suggests they are more sensitive to climate variation, possibly because they exploit resources more rapidly. The impacts of the groundwater table and the individual climate variables on growth however only partly support our hypothesis. The shade-intolerant species did indeed respond more strongly to receding groundwater tables than the shade-tolerant species in both spring and summer (Table 2.2), although these results should be treated with caution, because groundwater data were available for only a short time frame (17 years). We cannot provide a mechanistic explanation for these findings, but it may well be found in species' differences in belowground traits, as also suggested by other studies (Sterck et al. 2011), that are beyond the scope of this study, and still poorly understood in general (Bréda et al. 2006; Mommer and Weemstra 2012).

There was however no evidence that shade-intolerant species suffer more from precipitation deficits than shade-tolerant species (Table 2.2). This contrasts with Fekedulegn et al. (2003) who found trees of acquisitive, shade-intolerant species (*Liriodendron tulipifera*) to respond more strongly to droughts than trees of other species, but these drought-sensitive individuals were sampled on a site with a different aspect, which could have confounded this outcome (Fekedulegn et al. 2003). Across the homogeneous soils of our study site, *P. trichocarpa*, our most shade-intolerant species, was little more sensitive to precipitation shortage than *F. sylvatica*, the most shade-tolerant species of our set (Table 2.2).

Conclusions

Our study demonstrates that summer drought, mainly resulting from low precipitation and high evaporative demand, reduces tree growth across a variety of common deciduous tree species, even on soils with favourable water and nutrient conditions. Thus, the dry and hot summers predicted for north-western Europe will also negatively affect forest productivity on such productive soils. The qualitatively similar climate-growth relationships across the species are consistent with their similar radial growth rates, despite differences in shade tolerance across species, which does not agree with the generally observed trade-off between growth and shade tolerance. The negative impact of receding groundwater tables on tree growth among the more light-demanding species indicates that investigating species' differences in fine-root traits may become more important if water becomes increasingly limited. We therefore advocate a whole-plant perspective that links above- and belowground traits and processes to understand the different growth responses to climate and soil conditions across species.

Acknowledgements

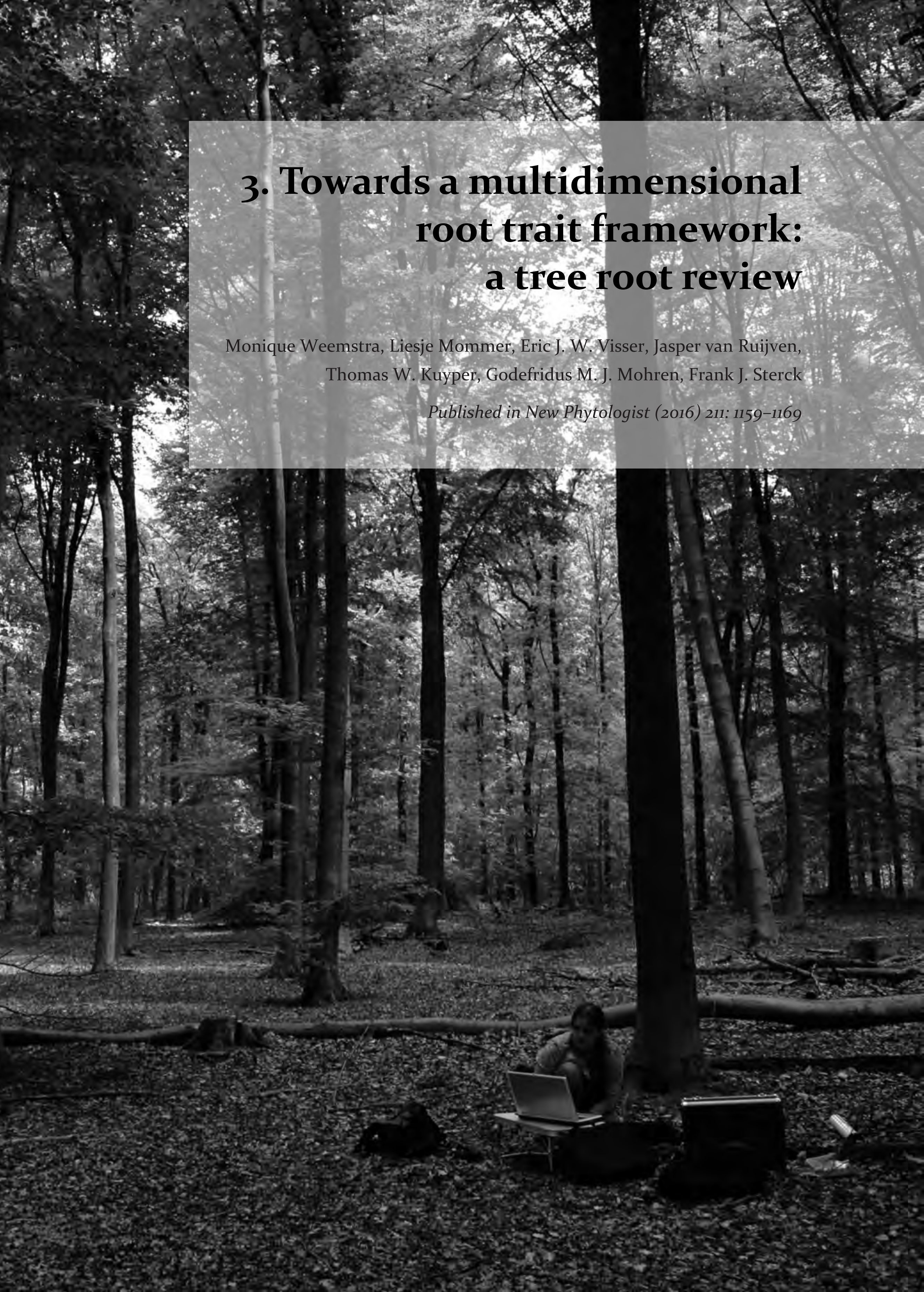
We thank Leo Goudzwaard, Teun Lamers and Merijn Kuiper for field work assistance; Ellen Wilderink for assistance in the lab, and Jos Rutten and Leo Smits (State Forestry Service) for facilitating field work at the Hollandse Hout study site. This study was carried out in the framework of the COST-Action STReESS.

Appendix

Appendix 2.1 Soil characteristics of the Hollandse Hout study site.

Soil variable	Unit	Ah horizont	AC horizont
pH	-	6.8	7.0
Organic matter	$g{\cdot}100\ g^{-1}$	7.9	3.5
Total N	$mg{\cdot}100\ g^{-1}$	445.8	181.0
Total P	$mg{\cdot}100\ g^{-1}$	76.8	57.2
C stock	$g{\cdot}m^{-2}$	1530	2890
N stock	$g{\cdot}m^{-2}$	171.1	299.5
P stock	$g{\cdot}m^{-2}$	29.4	95.6
C / N ratio	-	8.9	9.7
C / P ratio	-	52.0	30.2

Data from Kemmers et al. (2000). C / P ratio deduced by the authors from C and P stocks.



3. Towards a multidimensional root trait framework: a tree root review

Monique Weemstra, Liesje Mommer, Eric J. W. Visser, Jasper van Ruijven,
Thomas W. Kuyper, Godefridus M. J. Mohren, Frank J. Sterck

Published in New Phytologist (2016) 211: 1159–1169

Abstract

The search for a root economics spectrum (RES) has been sparked by recent interest in trait-based plant ecology. By analogy with the one-dimensional leaf economics spectrum (LES), fine-root traits are hypothesised to match leaf traits which are coordinated along one axis from resource acquisitive to conservative traits. However, our literature review and meta-level analysis reveal no consistent evidence of an RES mirroring an LES. Instead the RES appears to be multidimensional. We discuss three fundamental differences contributing to the discrepancy between these spectra. First, root traits are simultaneously constrained by various environmental drivers not necessarily related to resource uptake. Second, above- and belowground traits cannot be considered analogues, because they function differently and might not be related to resource uptake in a similar manner. Third, mycorrhizal interactions may offset selection for an RES.

Understanding and explaining the belowground mechanisms and trade-offs that drive variation in root traits, resource acquisition, and plant performance across species, thus requires a fundamentally different approach than applied aboveground. We therefore call for studies that can functionally incorporate the root traits involved in resource uptake, the complex soil environment, and the various soil resource uptake mechanisms – particularly the mycorrhizal pathway – in a multidimensional root trait framework.

Key words

Functional traits, mycorrhizal symbiosis, resource acquisition, root economics spectrum, trait syndromes

Introduction

One of the basic principles of trait-based plant ecology is the trade-off between plant growth and survival (Grime 1977; Kobe et al. 1995; Craine 2009). This trade-off implies that plants invest in trait attributes that allow either fast resource acquisition and therefore fast growth, or defence and conservation of acquired resources such as carbon (C), nitrogen (N) and phosphorus (P) which allows survival under adverse conditions. The growth–survival trade-off has been demonstrated clearly in leaf traits that span a continuum from acquisitive to conservative leaves (Reich et al. 1992; Wright et al. 2004). On the one hand, the former are characterised by a high specific leaf area (leaf area per leaf mass, SLA), high assimilation and respiration rates, and high nutrient concentrations, which enhance both light interception and C fixation (Figure 3.1). These acquisitive traits come at the expense of large resource losses due to high metabolic rates, increased susceptibility to herbivory, and short lifespan. On the other hand, conservative leaves are equipped for long-term resource retention by having high tissue densities and low respiration rates. These traits enhance their lifespan, but decrease their light interception efficiency and photosynthetic rates (Reich et al. 1992; Reich et al. 1999; Wright et al. 2004). This so-called leaf economics spectrum (LES, Wright *et al.*, 2004) has been successfully linked to plant performance (Reich et al. 1998; Poorter and Bongers 2006), species distribution and interactions (Sterck et al. 2006), and ecosystem processes (Reich et al. 1997; Díaz et al. 2004; Díaz et al. 2007; Grigulis et al. 2013).

Currently, research efforts are directed to test whether the fine-root traits of trees can be positioned within a similar framework, that is, the root economics spectrum (RES) (e.g. Comas & Eissenstat, 2004, 2009; Withington *et al.*, 2006; McCormack *et al.*, 2012; Chen *et al.*, 2013; Kong *et al.*, 2014; Liu *et al.*, 2015; Valverde-Barrantes *et al.*, 2015). Based on assumed trait coordination between above- and belowground organs, it has been hypothesised that root functional traits can also be grouped in trait syndromes associated with fast resource acquisition or enhanced resource conservation. This search for an RES similar to the LES builds on the premise that acquisitive leaves with high evaporative demand and photosynthetic rates require acquisitive roots to ensure sufficient water and nutrient supply to maintain these processes, and ultimately to achieve fast plant growth (Eissenstat 2002; Reich 2014). Conversely, plants that have conservative leaves with lower water and nutrient requirements, but also lower photosynthetic rates, should retain resources longer. They may thus require long-lived roots with lower respiration and uptake rates, resulting in slow plant growth. It is therefore hypothesised that leaf traits are matched by parallel root traits along the acquisitive–conservative resource spectrum (Grime et al. 1997; Reich et al. 2008; Liu et al. 2010; Freschet et al. 2010; Reich 2014; Valverde-Barrantes et al. 2015).

As already demonstrated for leaves in the LES, an RES could offer a relevant framework to provide further insights into plant, vegetation and ecosystem processes and responses to the soil environment and global change. For example, the grouping of species along an RES could help to understand their performance (growth and survival) or distribution across soil resource gradients. However, the existence of an RES analogous to the LES is currently debated, because of contradictory results within and among studies (e.g. Withington *et al.*, 2006; Mommer & Weemstra, 2012; McCormack *et al.*, 2012; Chen *et al.*, 2013; Kong *et al.*, 2014).

This study aims to clarify the uncertainty concerning the existence of an RES by reviewing the available evidence for the RES hypothesis within individual studies, and by performing a meta-level analysis (Smith et al. 2015) to test the RES hypothesis across those studies. In addition to root trait relationships, we also reviewed the literature on correlations between the leaf and root traits of the LES and of the RES. This study focuses on the fine-root traits of trees. This is important as recent papers show that for herbaceous species, root trait correlations provide a better match with the RES than for woody species (Roumet et al. 2016). This suggests that root trait correlations may be fundamentally different for woody species. Our literature review is based on 18 studies that compared the root traits expected to play a role in an RES across more than two tree species (Appendix 3.2). Our meta-level analysis was carried out on a subset of 14 studies (Appendix 3.2), because not all studies provided root trait data at the individual species level, and one study already comprised a meta-analysis. This meta-level analysis was based on raw data and did not include calculating effect sizes, and therefore does not comply with the standards of a meta-analysis (Vetter et al. 2013; Koricheva and Gurevitch 2014).

The root economics spectrum

The root traits expected to feature in the RES are based on a parallel with the key leaf traits in the LES: SLA, leaf N content, maximum photosynthetic rate, respiration rate and leaf lifespan (Figure 3.1). These traits are related to three leaf processes involved in plant growth and survival. First, SLA relates to leaf resource interception at a given biomass investment. Second, leaf N content, photosynthetic rate and respiration rate are related to leaf carbon gain. Third, leaf lifespan refers to the conservation of biomass. Assuming functional similarity between leaves and fine roots, the critical fine-root traits in the RES are therefore expected to be: specific root length (root length per root mass, SRL), root N content, root water and nutrient uptake rate, root respiration rate and root lifespan (Figure 3.1). Similar to leaf traits in the LES, it is expected that SRL reflects the root uptake area at a given biomass cost; root N content, uptake rate and respiration rate are expected to be associated with net soil resource acquisition rate; and root lifespan reflects the degree of biomass conservation. In both the LES and RES it is expected that most traits (SLA/SRL, N content, photosynthetic/resource uptake rates and respiration rates) will decrease from an acquisitive to a conservative strategy, whereas lifespan will increase (Figure 3.1).

Several studies have assessed the support for the RES hypothesis across tree species. In addition to the five key traits, most of these studies measured root diameter and root C : N ratios, and calculated tissue density (from root length, diameter and mass), although their aboveground parallels (leaf thickness, C : N ratio and tissue density) are less explicitly incorporated in the LES. These additional root traits are expected to increase from the acquisitive to the conservative side of the RES (Figure 3.1), because they have been found to contribute to root lifespan and thus to resource conservation (Wahl and Ryser 2000; Gu et al. 2011): thick roots are sometimes assumed to be long-lived due to their relatively large stele cross-sectional area that protects them from mechanical, herbivore and drought stress, and to have low N content and therefore slow metabolism due to their relatively small cortex area (Eissenstat and Achor 1999; Wahl and Ryser 2000; Guo et al. 2008b). However, the exact mechanisms underlying these correlations between these root traits and root lifespan are not fully clear yet: for example, Kong *et al.* (2014) demonstrate that thicker roots have a relatively large cortex area, and other anatomical features such as a well-developed exodermis may also drive the longer lifespan of thicker roots (Withington et al. 2006). In turn, other traits in the RES (e.g. nutrient and water uptake rates, respiration rates, and root lifespan) are measured far less frequently than their aboveground counterparts (i.e. photosynthetic and respiration rates and leaf lifespan).

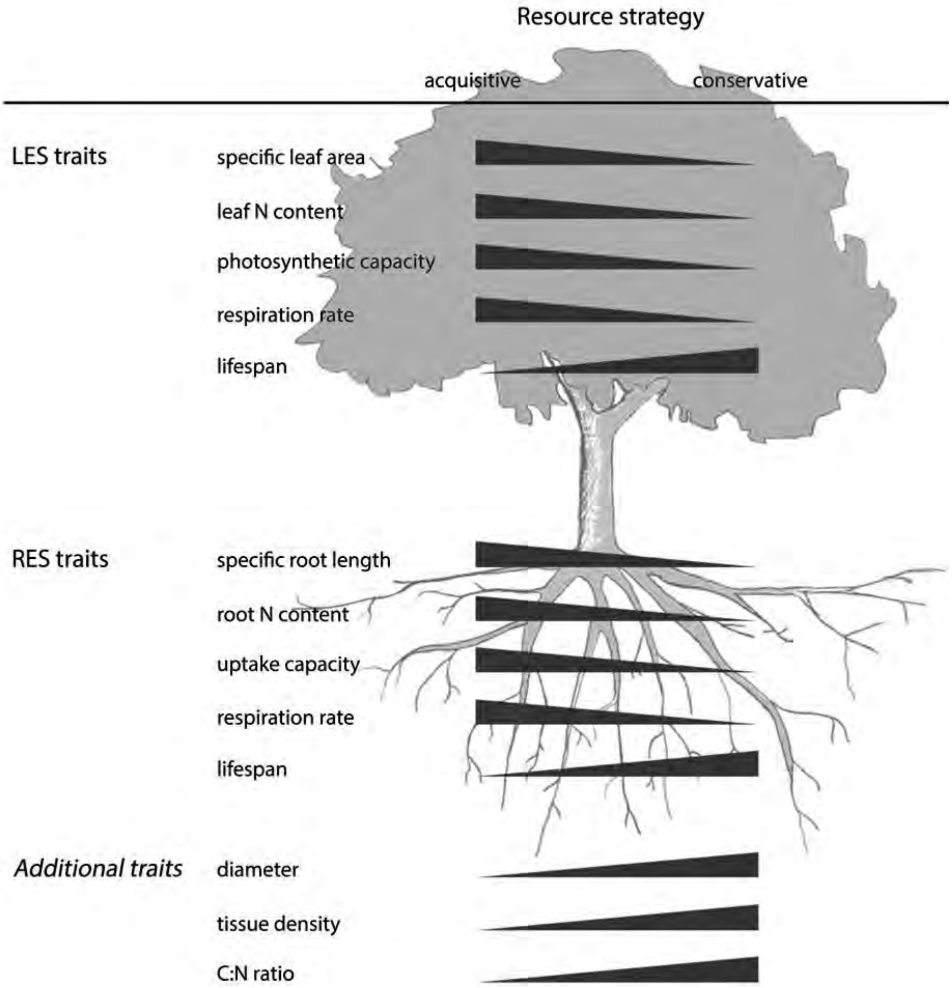


Figure 3.1 Resource economics strategies: the current hypothesis. Leaf and hypothesised root trait attributes associated with an acquisitive or conservative resource strategy in a leaf economics spectrum (LES) and in a root economics spectrum (RES).

In order to maintain the functional parallel with leaves, RES studies have examined absorptive rather than transporting roots. These functional groups were initially separated on the basis of their diameter (e.g. all roots < 1 or 2 mm diameter were considered absorptive), but although both traits may be partially correlated, root order rather than diameter has since proved to be a better proxy for root functioning

(Pregitzer et al. 2002; McCormack et al. 2015). We therefore focus mainly on studies that compared all or some of these RES traits (Appendix 3.2) on first- to third-order roots (first-order roots being the most distal). Because data on root uptake and respiration rate across species are scarce, especially in relation to other root traits, our analysis was restricted to three RES traits: SRL, root N content and root lifespan. As only one study measured both root N content and root lifespan (Valverde-Barrantes et al. 2007), we related root lifespan to root C : N ratios – for which more data were available – instead. We also tested for relationships between these RES traits and root diameter, root tissue density and root C : N ratios.

Correlations between root traits are inconsistent

In terms of correlations between root lifespan and other root traits, the RES is little supported by data. As expected from the RES hypothesis, within the individual studies reviewed, the trait most consistently and positively correlated with root lifespan across species is root diameter (Gu et al. 2011; McCormack et al. 2012; Adams et al. 2013; Hansson et al. 2013), although not in the study by Withington *et al.* (2006) (Table 3.1). Withington *et al.* (2006) attributed their failure to find significant correlations to the limited variation in root diameter across their study species (in their study it ranged between 0.36–0.62 mm across 11 tree species, but in e.g. the study by McCormack *et al.* (2012), it ranged between 0.22–0.64 mm across 12 tree species). Our meta-level analysis also demonstrated that root diameter was the trait most strongly correlated to root lifespan (Appendices 3.1d, 3.3).

In line with the RES hypothesis, the correlation between SRL and root lifespan was negative in the study of McCormack *et al.* (2012) (Table 3.1), as well as in our analysis comprising data from four studies (Figure 3.2a, Appendices 3.1a, 3.3). Similar to the relationship between root diameter and root lifespan, SRL and root lifespan were not significantly correlated in Withington *et al.* (2006) (Table 3.1), because SRL is largely determined by root diameter. In fact, with constant root tissue density, SRL scales inversely with root diameter squared, which has been widely observed across temperate (Withington et al. 2006; Comas and Eissenstat 2009; Holdaway et al. 2011; McCormack et al. 2012; Eissenstat et al. 2015), subtropical (Chen et al. 2013; Kong et al. 2014; Liu et al. 2015) and boreal tree species (Ostonen et al. 2007a), and in our meta-level analysis (Appendix 3.3). As the negative correlations between SRL and diameter largely result from autocorrelation, they may not provide meaningful biological insight into actual RES trade-offs.

Table 3.1 Expected and observed correlations between fine-root lifespan and other fine-root traits.

Root trait	Expected	Observed	Reference
Specific root length	↘	↘	McCormack *et al.* (2012)
		------	Withington *et al.* (2006)
Nitrogen content	↘	------	Valverde-Barrantes *et al.* (2007)
Resource uptake rate	↘	NA	
Respiration rate	↘	------	McCormack *et al.* (2012)
Diameter	↗	↗	Gu *et al.* (2011), McCormack *et al.* (2012), Adams *et al.* (2013), Hansson *et al.* (2013)
		------	Withington *et al.* (2006)
Tissue density	↗	↘	Withington *et al.* (2006)
		------	McCormack *et al.* (2012)
C : N ratio	↗	↗	Withington *et al.* (2006), McCormack *et al.* (2012)

Observed correlations were derived from individual studies that tested the root economics spectrum (RES) hypothesis on more than two tree species. Dashed, horizontal lines represent nonsignificant correlations. NA, no data available across more than two tree species. For only three traits, observed correlations agree with the hypothesis, but in two of those cases, other studies found nonsignificant results as well. Appendix 3.2 presents the number of species and the climate zone studied per study.

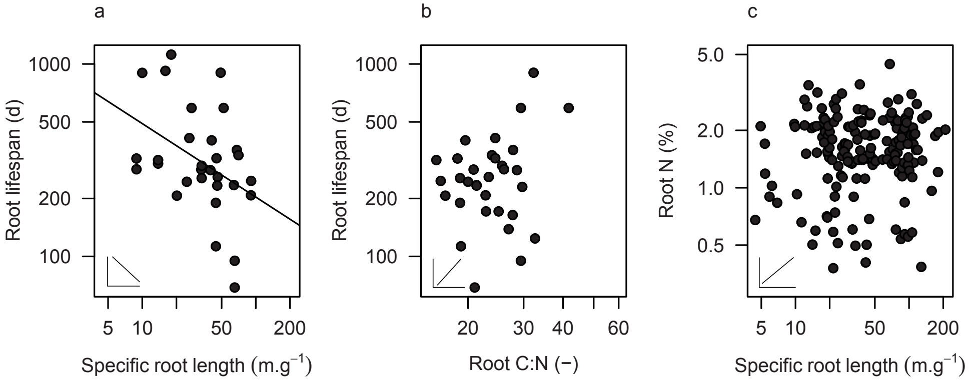


Figure 3.2 Root economics spectrum (RES) trait correlations on tree root data across the literature reviewed. Insets (bottom-left) indicate the expected relationship according to the RES hypothesis. (a) Root lifespan was significantly related to specific root length (SRL; r = -0.4, n = 30, P = 0.03) across data from four references (Withington et al., 2006; McCormack et al., 2012; Adams et al., 2013; Hansson et al., 2013). (b) Root lifespan was not significantly correlated to root carbon : nitrogen (C :N) ratio (r = 0.22, n = 32, P = 0.22), across the four studies included (Withington et al., 2006; Valverde-Barrantes et al., 2007; McCormack et al., 2012; Adams et al., 2013). (c) The correlation between SRL and root N content was not significant (r = 0.1, n = 178, P = 0.37) across the data from five references (Pregitzer et al., 2002; Comas & Eissenstat, 2009; Holdaway et al., 2011; Kong et al., 2014; Liu et al., 2015). Note that data are presented on a logarithmic scale.

We did not find a single study corroborating the positive relationship expected from the RES between the tissue density and lifespan of roots across tree species (Table 3.1). McCormack *et al.* (2012) found no correlation between root tissue density and root lifespan across species, and Withington *et al.* (2006) even reported a negative relationship. Our meta-level analysis also revealed a significant negative correlation between root tissue density and lifespan, but included these two studies only (Appendices 3.1e, 3.3).

Two studies related root lifespan to root C : N ratio instead of root N content, and found a significant positive relationship, supporting the RES hypothesis (Table 3.1; Withington *et al.* 2006, McCormack *et al.* 2012). Our analysis including data from four studies however, revealed no significant relationship between root C : N ratio and lifespan (Figure 3.2b, Appendices 3.1b, 3.3), and in a study by Valverde-Barrantes *et al.* (2007) root N content was unrelated to root lifespan. Only one of the studies reviewed, correlated root respiration rates and root lifespan across tree species, but found no relationship between the two (McCormack et al. 2012) (Table 3.1). Root uptake rates or uptake capacity are hardly – if ever – measured in the context of an RES across several tree species, so few data are available to confirm or refute the position of this trait in the RES. Wells & Eissenstat (2003) concluded from a study on two species that the roots with high maximal uptake rates had a shorter lifespan, which agrees with the predicted RES.

Some of the expected correlations between other RES traits were confirmed by data. The positive relationship between root N content and respiration rates – which has a clear physiological basis – has been supported by some studies (Pregitzer et al. 1998; Reich et al. 2008), but not by others (McCormack et al. 2012; Adams et al. 2013). Also in line with the RES, a study on 13 tropical tree species found that root respiration decreased with increasing root diameter (and thus increased with increasing SRL) and with increasing root tissue density (Makita et al. 2012). Our analyses also showed that root N content was significantly negatively correlated with root tissue density, as expected from the RES (Appendices 3.1f, 3.3).

Other hypothesised trait correlations of the RES were, however, not supported. For example, when compared with thin roots, thick roots of low SRL did not always have higher tissue densities (Withington et al. 2006; Ostonen et al. 2007a; Comas and Eissenstat 2009; McCormack et al. 2012; Chen et al. 2013; Kong et al. 2014; Eissenstat et al. 2015; Liu et al. 2015). In contrast to the RES hypothesis, our analysis shows that root tissue density is in fact negatively correlated with root diameter, and not significantly correlated with SRL (Appendix 3.3). In addition, neither SRL nor root diameter were related to root N content, both within studies (Withington et al. 2006; Comas and Eissenstat 2009; Holdaway et al. 2011; McCormack et al. 2012; Chen et al. 2013; Kong et al. 2014; Liu et al. 2015; Valverde-Barrantes et al. 2015), and across the studies in our compiled data set (Figure 3.2c, Appendices 3.1c,g, 3.3). Furthermore, neither root diameter nor SRL were correlated to respiration rates per unit root mass in a study by McCormack *et al.* (2012).

Comas and Eissenstat (2004) measured P uptake capacity across 10 tree species, but they correlated this trait to species' growth rates rather than to other root traits. They reported no difference between fast- and slow-growing species, having assumed that fast-growing species have an acquisitive strategy whereas slow-growing species have a conservative strategy (Comas and Eissenstat 2004). Our further analysis of their data (from table 1 in Comas & Eissenstat 2004) revealed no significant correlations between P uptake capacity and root N content (Pearson correlation, r = -0.37, N = 10, *P* = 0.29) or root respiration (Pearson correlation, r = -0.45, N = 10, *P* = 0.19).

Finally, although not an explicit trait in the RES, it is generally expected that a high root growth rate indicates an acquisitive resource strategy. The few data available on root growth rate do not confirm this hypothesis. Withington *et al.* (2006) found that high root growth rates were positively correlated with root C : N ratios and tended to be related to high root lifespan (both presumed conservative trait attributes), and Valverde-Barrantes *et al.* (2007) demonstrated that root growth correlated negatively with root N content. In sum, the reported correlations between root morphological, chemical, physiological traits and root lifespan and growth do not support the RES hypothesis.

Root traits do not necessarily correlate with leaf traits

The hypothesised RES is based on the idea that above- and belowground resource strategies are coordinated (Liu et al. 2010; Reich 2014). If this is so, parallel leaf and root traits should be positively correlated, but here too the available evidence is mixed at best. Specific leaf area and SRL were positively related across species in some studies (Withington et al. 2006; Holdaway et al. 2011) but not in others (Chen et al. 2013; Valverde-Barrantes et al. 2015). The same holds for the correlation between leaf and root N and P content: it was found to be positive in some studies (Kerkhoff et al. 2006; Holdaway et al. 2011; Valverde-Barrantes et al. 2015), but not in Withington *et al.* (2006) and Chen *et al.* (2013). Respiration rates were positively correlated between leaves and roots in tree seedlings in a greenhouse (Reich et al. 1998). Leaf and root lifespan were found to be uncorrelated by Withington et al. (2006) and McCormack et al. (2012), but there are few published data on this correlation. To our knowledge, the correlation between root uptake rates and leaf photosynthetic rates has not yet been tested across tree species.

When explicitly testing above- and belowground trait spectra across species, Withington *et al.* (2006) demonstrated that they are not necessarily correlated. For example, *Larix decidua* – a deciduous conifer – had acquisitive leaf traits (i.e. high SLA, high leaf N content and short leaf lifespan), and in this respect was similar to the other deciduous (but broadleaved) species in the dataset, but it had conservative root traits (i.e. thick roots and long root lifespan) similar to the other (evergreen) conifers

(Withington et al. 2006). A similar outcome was observed in the frankincense tree (*Boswellia papyrifera*). Compared to *Acacia* species, *Boswellia* trees had conservative root traits to retain water, and acquisitive leaf traits that enhanced photosynthesis (Birhane et al. 2015). It can be concluded that leaf resource strategies do not necessarily reflect belowground resource strategies, and that parallel leaf and root traits are not consistently correlated across tree species.

Why the one-dimensional resource economics spectrum does not work for tree roots

Neither the empirical studies reviewed, nor our meta-level analysis provide clear evidence for an RES: tree root traits are neither consistently correlated to each other, nor to parallel leaf traits. In contrast to this study on tree roots, the roots of non-woody plants may follow the patterns expected from the RES. Several studies have demonstrated that across grass and herbaceous species, an acquisitive trait syndrome might exist including roots with a high SRL, N content and respiration rates, versus a conservative trait syndrome that includes thick, long-lived roots with high tissue density and high C : N ratios (Craine et al. 2005; Tjoelker et al. 2005; Roumet et al. 2016). Although these studies acknowledge that tree root trait patterns may differ from the trait correlations observed on herbaceous species (Tjoelker et al. 2005; Roumet et al. 2016), it so far remains unclear why these trait patterns diverge and which are the main differences between tree and herbaceous root traits, and their functioning.

For tree roots, potential causes for this lack of support for an RES are a lack of data or differences in methodology across studies. For instance, compared to aboveground traits, far fewer tree root data are available, and certain traits, such as nutrient uptake rate, have rarely been measured in trees under field conditions (Lucash et al. 2007). To illustrate this difference: the plant trait TRY database includes almost 10,000 observations of leaf photosynthetic rates, covering 1,666 plant species, whereas the same database includes only 24 observations of root N uptake rates of 11 different species (Kattge *et al*., 2012).

In addition, definitions of fine roots differ between studies: they may be based on diameter, order or functioning, which might lead to a bias towards certain categories (e.g. absorptive versus transport fine roots) (Pregitzer et al. 2002; Guo et al. 2008b; Gu et al. 2011; McCormack et al. 2015). Our root trait analysis also covered studies that defined fine roots as roots smaller than 2 mm diameter (Hansson et al. 2013), or included first- to fourth-order fine roots (Holdaway et al. 2011), as well as studies including first-order roots only (e.g. Pregitzer *et al.*, 2002; Adams *et al.*, 2013; Kong *et al.*, 2014; Liu *et al.*, 2015).

Likewise, studies have used different methods to measure root traits: for example, root lifespan has been measured using sequential coring, root ingrowth cores, minirhizotrons, and carbon isotopes, which may lead to different estimates of root lifespan (Majdi 1996; Gaudinski et al. 2001; Guo et al. 2008a; Strand et al. 2008; Gaul et al. 2009; Brunner et al. 2012). Nonetheless, such methodological issues cannot explain why individual studies using large numbers of species (e.g. 65 (Chen et al. 2013) or 96 (Kong et al. 2014) tree species) have also failed to find support for an RES. We will now discuss three fundamental differences between leaf and root traits that explain why the fundamental trade-off between resource acquisition and resource conservation, as implied by the LES, is not suitable for a root trait framework. We argue that we need to work towards a multidimensional framework for a classification of root functional traits.

Roots are subjected to multiple constraints

With regard to resource uptake, leaves are adapted for maximising light capture while reducing resource loss by herbivores (Figure 3.3), whereas differentiation for CO_2 uptake is limited, as this resource is predictably available throughout the canopy. Roots face a more complex optimisation puzzle, in the first place because they need to acquire water and about 15 essential minerals from the soil, and the distribution of these resources can be highly variable within the soil matrix. Depending on their mobility, different nutrients require different traits if their uptake is to be maximised. For example, the acquisition of a mobile nutrient such as nitrate can be optimised by an enhanced SRL or the capacity to proliferate in resource-rich patches, whereas immobile nutrients such as P may require high root hair density, prolific root branching, or mycorrhizal symbiosis (Comas et al. 2012). This implies that traits considered acquisitive for the uptake of one particular resource are not necessarily acquisitive for the uptake of another. Therefore, root trait attributes cannot *a priori* be defined as acquisitive or conservative, because the simultaneous uptake of different resources may be optimised by different traits, depending on the most limiting resource.

In addition to the multiple resources belowground, other environmental components, such as soil texture and chemistry, may present additional limits to root traits that are not present aboveground (Figure 3.3). For example, roots with a small diameter and high SRL are predicted to be efficient at acquiring resources, but may not develop when unfavourable soil structure impedes the formation of thin roots, since thin roots cannot easily penetrate dense soils (Clark et al. 2003). Physical forces may thus constrain resource acquisition traits, a limitation not encountered by leaves. Indeed, soil compaction resulted in larger root diameter and reduced the SRL of tree seedlings (but did not affect root tissue density) (Bejarano et al. 2010; Alameda and Villar 2012). In addition, e.g. free aluminium (Al^{3+}) in the soil solution leads to larger root diameter in tree seedlings (Hirano and Hijii 1998). Such diameter increases are caused by reduced apical growth and associated changes in cell differentiation and growth (Schier 1985; McQuattie and Schier 1990). These additional constraints to root traits do not directly operate in an LES, and imply that root traits result from a variety of trade-offs not present in an RES (Figure 3.3).

Leaf and root traits are not functional analogues

The RES comprises root traits that are considered analogues of the key leaf traits in the LES (see Figure 3.1). However, in contrast to leaves, in roots the links between these traits and resource uptake are not well established. For example, SLA plays an important role in the LES because it directly links photosynthetic rates (by mesophyll packing), to Rubisco content and therefore to leaf N content (Niinemets and Sack 2006). Analogously, SRL should have a similar key position in the RES, but our review and data analysis provide little evidence for relationships between SRL and root N content or nutrient uptake rates.

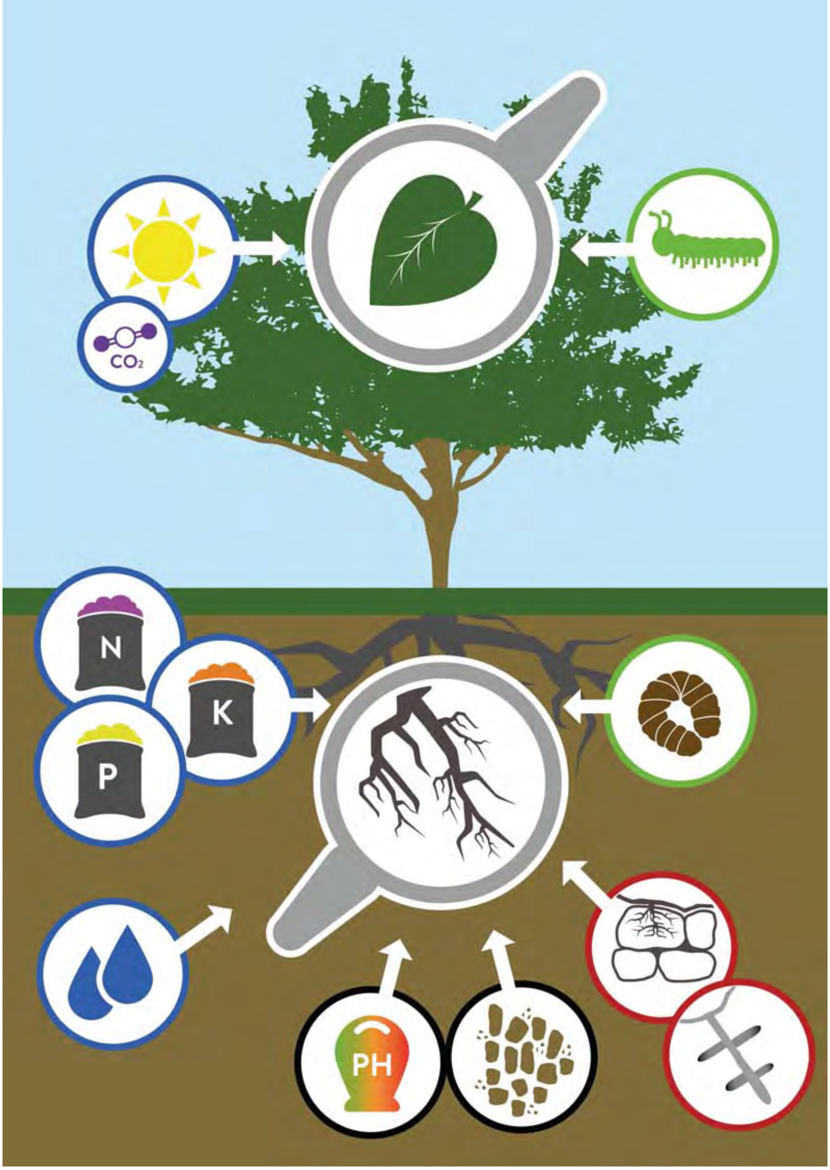


Figure 3.3 Towards a multidimensional root trait framework. Leaf traits are coordinated along a one-dimensional axis, driven by resource availability (light and CO_2) and herbivory. By contrast, root traits are determined by more environmental constraints, including availability of different resources (water and different nutrients represented by blue circles), soil chemistry and structure (brown circles), mycorrhizal fungi (red circles; top-left, arbuscular mycorrhiza; bottom-right, ectomycorrhiza), and herbivory (green circles).

Furthermore, in contrast to photosynthetic rates, root uptake rates are hardly ever measured in trees in forests. As a consequence, we simply do not know whether this trait is as relevant to soil resource acquisition as are photosynthetic rates to light acquisition and carbon fixation. In fact, there are indications that root uptake rates are not as strongly related to root N content as is the case for leaves: the fraction of N represented by the enzymes involved in ion uptake is small compared to the photosynthetic N involved in leaf carbon assimilation. In addition, uptake rates of (immobile) nutrients may not be limited by the number of nutrient uptake transporters, but by the availability of nutrients in the soil matrix. Thus, the relationships between key traits and resource uptake will therefore be different for roots compared to leaves (Chen et al. 2013), and cannot be directly deduced from the tight links between leaf traits and light acquisition as demonstrated in the LES.

At the same time, root traits that are currently *not* included in the RES may turn out to be key for explaining differences in resource uptake and performance across species. Aboveground, light acquisition is clearly associated with one trait syndrome, whereas belowground there are multiple resource uptake strategies, reflected by root traits that do not always have aboveground equivalents (Comas and Eissenstat 2009; Comas et al. 2012; Chen et al. 2013; Kong et al. 2014; Eissenstat et al. 2015; Liu et al. 2015). For example, plants have developed several adaptations to low P availability, including an increased number of superficial adventitious roots, enhanced SRL, greater root biomass and/or root length density, longer and/or denser root hairs, increased exudation of carboxylic acids, and the formation of cluster roots (Lambers et al. 2006; Lynch and Brown 2008). Recent studies (Eissenstat et al. 2015; Liu et al. 2015) also identified the mycorrhizal pathway as a complementary strategy to forage for soil resources, particularly for species with relatively thick absorptive roots (further discussed in the next section). So, plant resource acquisition belowground depends on root traits and associated mycorrhizal fungi that are largely not included in the RES.

Furthermore, phylogenetic conservatism may be stronger for roots than for leaves (Comas and Eissenstat 2009; Chen et al. 2013). Recently, Valverde-Barrantes *et al.* (2015) found little support for an RES, and demonstrated that variation in root traits across 34 tree species was more strongly driven by common ancestry. In contrast, leaf trait variation was not phylogenetically structured, and did match the LES (Valverde-Barrantes et al. 2015). Kong *et al.* (2014) also observed strong phylogenetic conservatism of root traits, particularly those related to root diameter and mycorrhizal symbiosis (e.g. root diameter, cortex area and mycorrhizal colonisation rate). They suggest that species with thicker first-order roots compensate for their relatively low absorptive area by hosting more (arbuscular) mycorrhizal fungi to enhance the soil volume available (Kong et al. 2014).

Such strong phylogenetic signals acting on root traits may explain why the RES is more strongly supported by data collected from more closely related than distant species (e.g. Comas & Eissenstat, 2009; McCormack *et al.*, 2012). The study by Withington *et al.* (2006) for example, included largely distant species (i.e. conifers and hardwood species) and the authors did not correct for phylogenetic background in their root trait correlations. Controlling for phylogenetic structure may thus be important to identify the drivers of root trait distributions, but it should also be noted that two studies that explicitly took phylogenetic structure into account found only little support for the RES (Chen *et al.*, 2013; Valverde-Barrantes *et al.*, 2015).

Mycorrhizal interactions offset selection for an RES

Belowground resource uptake is not solely determined by root traits, because almost all tree species rely heavily on the mycorrhizal pathway. The mycorrhizal uptake mechanism has no parallel aboveground, and is not incorporated in the RES hypothesis. Reich (2014) proposed that a larger dependency on (or responsiveness to) mycorrhizal symbiosis represents a conservative strategy, because it correlates with conservative root traits (e.g. large diameter, long lifespan and low SRL). Furthermore, within the mycorrhizal plants, ectomycorrhizal plant species are considered more conservative than arbuscular mycorrhizal plant species, because they predominantly occur in nutrient-poor ecosystems that select for conservative strategies (Read 1991; Cornelissen et al. 2001). However, Koele *et al.* (2012) observed that ectomycorrhizal and arbuscular mycorrhizal plant species had similar leaf nutrient concentrations (a key trait in the LES), when comparing sister clades with arbuscular mycorrhiza and ectomycorrhiza. Furthermore, Comas *et al.* (2014) reported smaller root diameters for ectomycorrhizal trees than for arbuscular mycorrhizal trees, whereas Kubisch *et al.* (2015) observed no differences in root morphological traits between arbuscular mycorrhizal and ectomycorrhizal tree species. These studies thus contradict the assumption that mycorrhizal or ectomycorrhizal dependency represents a conservative uptake strategy.

Despite the potential confounding effects of mycorrhizal type, the evidence for an RES within mycorrhizal types is also unclear. For example, species with thicker absorptive roots have been suggested to be more dependent on and responsive to arbuscular mycorrhizal symbiosis than species with thinner roots, because these roots are less efficient in nutrient uptake (Brundrett 2002; Smith and Read 2008; Kong et al. 2014; Eissenstat et al. 2015; Liu et al. 2015; Valverde-Barrantes et al. 2016b). However, a recent meta-analysis by Maherali (2014) showed that in terms of growth, plants with relatively thin roots benefited as much from mycorrhizal colonisation as plants with thick roots. These findings suggest that, even across species within a mycorrhizal type, root traits and their mycorrhizal dependency may not be consistently coordinated along an RES.

Mycorrhizal interactions partly explain why parallel trait syndromes are not coordinated between plant organs. As illustrated above by *Larix* and *Boswellia* trees, plants can have an acquisitive strategy aboveground and what is generally considered a conservative strategy belowground (Withington et al. 2006; Birhane et al. 2015). The latter trait syndrome (i.e. thick and long-lived roots) may stimulate mycorrhizal symbiosis by supporting more mycorrhizal fungal colonisation per unit root length (Comas et al. 2002), and by sustaining these fungi for longer (Bauhus and Messier 1999). Consequently, these root traits may also be associated with high soil resource acquisition via mycorrhizal extraradical hyphae that can efficiently exploit the soil (Smith and Read 2008), in order to maintain high photosynthetic rates. Therefore, mycorrhizal root trait attributes cannot necessarily be categorised as an acquisitive or conservative resource strategy, nor be deduced from aboveground traits.

Finally, it should be noted that the potentially large impacts of mycorrhizal fungi on root traits (e.g. increasing root lifespan (King *et al.*, 2002)), may further confound root trait data and correlations. Moreover, these effects differ between ectomycorrhizal and arbuscular mycorrhizal roots, and between fungal species. For example, Ouimette *et al.* (2013) reported that 36–54 % of the total root N content of ectomycorrhizal *Larix* trees was in fact attributable to fungal tissue, compared to only 5–10% of arbuscular mycorrhizal *Fraxinus* trees. Also, different mycorrhizal fungal species were observed to have different effects on root length and diameter in both ectomycorrhizal and arbuscular mycorrhizal trees (Berta et al. 1995; Van der Heijden and Kuyper 2003; Ostonen et al. 2009). This implies that root trait data cannot be interpreted without considering their mycorrhizal associations, possibly even at the fungal species level. To conclude, the mycorrhizal symbiosis cannot be accurately incorporated in the RES, but rather calls for a different and multidimensional framework that allows the diversity of belowground uptake mechanisms to be recognised (Eissenstat et al. 2015; Liu et al. 2015).

Outlook

Based on our analysis of the literature and their data, we argue that an RES likely does not exist parallel to an LES: a single acquisition–conservation axis cannot capture the variety of belowground mechanisms and trade-offs that drive differences in resource acquisition and plant performance across species (Figure 3.3). Alternatively, a multidimensional root trait framework may better accommodate and explain the variation in root traits observed across species (see also McCormack *et al.*, 2012; Kong *et al.*, 2014; Eissenstat *et al.*, 2015; Liu *et al.*, 2015). Below, we will discuss three objectives that can contribute to establishing such a framework: 1) capturing the complexity of the soil environment, 2) linking root form and function, and 3) incorporating the diversity and trade-offs in belowground resource uptake strategies, including the mycorrhizal pathway. Finally, compiling large root trait data sets are important in developing a multidimensional root trait spectrum covering a wide range of species and biomes.

Firstly, the complexity of the soil environment, which presents a variety of constraints to root trait variation, is a clear argument to move towards a new multidimensional root trait framework. This complexity largely results from the multiple critical soil resources (i.e. water, macro- and micronutrients) that affect plant performance. There are fundamental differences between the availability and uptake of water and of nutrients, and also between nutrients of different mobility. Therefore, root traits cannot be positioned along one single axis of resource availability, calling for a multidimensional framework instead. In addition, structural (e.g. soil compaction) and chemical (e.g. soil pH) soil properties further confound root trait variation, and may further explain why root traits are not always optimally adjusted to soil resource acquisition alone, and therefore deviate from the RES hypothesis. We argue that in order to understand divergent uptake strategies across species and environments, root trait variation should be studied along multiple soil resource axes and in relation to soil structural and chemical characteristics.

Secondly, an alternative root trait framework should be established from a mechanistic perspective to identify functional root traits in relation to plant resource uptake. We stress that studying the anatomy of fine roots can strongly contribute to establishing such a mechanistic link between root form and function. For example, determining whether roots consist of a relatively large cortex or stele area may reveal the metabolic functioning and structural composition of such roots (Guo et al. 2008a), and can help explaining why traits such as SRL and root N content are not correlated as expected from the RES hypothesis (Kong et al. 2014). Moreover, root anatomical properties have been found to relate to root lifespan (e.g. a thick exodermis associated with long-lived roots; Withington *et al.*, 2006), and to (arbuscular) mycorrhizal colonisation (e.g. a high stele : root diameter ratio indicating low fungal colonisation; Valverde-Barrantes *et al.*, 2016). Finally, root anatomical features have proven indicative of the functioning of a fine root (i.e. absorptive or transporting) and can be used to ensure that functionally similar roots are compared across species (McCormack et al. 2015).

Furthermore, although challenging, more data are required to test the functionality of individual root traits and their relationships with other root traits. We realise that particularly root uptake rates, respiration and lifespan are difficult to measure, especially on large trees in forests. Using relatively easy-to-measure proxies could be useful in linking root traits to each other, and ultimately to plant performance and ecosystem processes. Indeed, both the individual studies reviewed and our analysis suggest that root diameter may be a relatively reliable proxy of root lifespan (Table 3.1; Appendices 3.1d, 3.3), although it should also be considered that traits can be better explained by a *combination* of root traits, than by a single one (McCormack et al. 2012). For root respiration and uptake capacity however, it is too early to agree on using a general proxy such as root N content, because data are few and inconsistent. Consequently, more mechanistic studies are needed to clarify the functionality and relevance of specific root traits in resource uptake, and to determine whether reliable proxies can be used instead.

Thirdly, to incorporate the various belowground uptake strategies adopted by different species, the current RES traits set is incomplete. For example, root architectural traits commonly associated with resource uptake, such as root length density, root hair length and density (e.g. Holdaway *et al.*, 2011) and branching (e.g. Chen *et al.*, 2013; Comas *et al.*, 2014; Kong *et al.*, 2014; Liu *et al.*, 2015) are lacking. The importance of including them is illustrated by Kong *et al.* (2014), who demonstrated that root branching traits (e.g. branching ratio, the number of first-order roots relative to the number of second-order roots) represent an additional dimension of root trait variation, independent from the acquisition-conservation spectrum. Species can enhance root resource uptake not only by producing thinner roots of high SRL as predicted by the RES, but also by enhancing root length or root branching to rapidly exploit resource-rich patches (Kong et al. 2014). Therefore, it is important to include these additional traits simultaneously in order to explain root trait variation across species.

In particular, mycorrhizal interactions present an additional and complex but crucial uptake strategy for most plant species (Comas et al. 2014; Eissenstat et al. 2015; Liu et al. 2015). Mycorrhizal fungi affect the availability of soil resources and interfere with root traits, and may contribute to differential resource uptake and performance across species. Identifying the mycorrhizal type is an important first step, but this classification does not necessarily reflect a particular resource strategy, nor does it consistently explain root trait attributes. Determining fungal exploration types based on their morphology (e.g. extraradical hyphae and rhizomorphs; Agerer, 2001), and quantifying mycelium abundance (Wallander et al. 2001; Wallander et al. 2013; Ekblad et al. 2013) may further reveal the role of mycorrhizal fungi in soil exploration and exploitation capacities of different tree species. Recent studies have extended the functional trait approach to mycorrhizal fungi (Koide et al. 2013; Aguilar-Trigueros et al. 2015; Fernandez and Kennedy 2015), for example by measuring the fungal enzyme spectrum – related to plant resource availability – and linking this to resource acquisition (Aguilar-Trigueros et al. 2015). Such steps have the potential to identify the relevant traits that reflect how mycorrhizal fungi interact with tree roots and resource uptake for different species, and across multiple soil resource gradients.

Large, worldwide root trait data sets are needed to detect generic root trait patterns across a variety of species and genera, and biomes (e.g. Chen *et al.*, 2013; Roumet *et al.*, 2016), as they have proved for leaves (e.g. the TRY database, Kattge *et al.* 2012). Compiled data sets from different studies should however preferably maintain the same definition of fine roots, apply consistent and comparative methods of sampling and analyses, quantify their soil environment and measure functionally relevant root traits. Such large-scale root trait data sets allow us to simultaneously test phylogenetic effects and apply phylogenetic corrections, and (soil) environmental and climate effects on root traits, which may explain a large part of their variation.

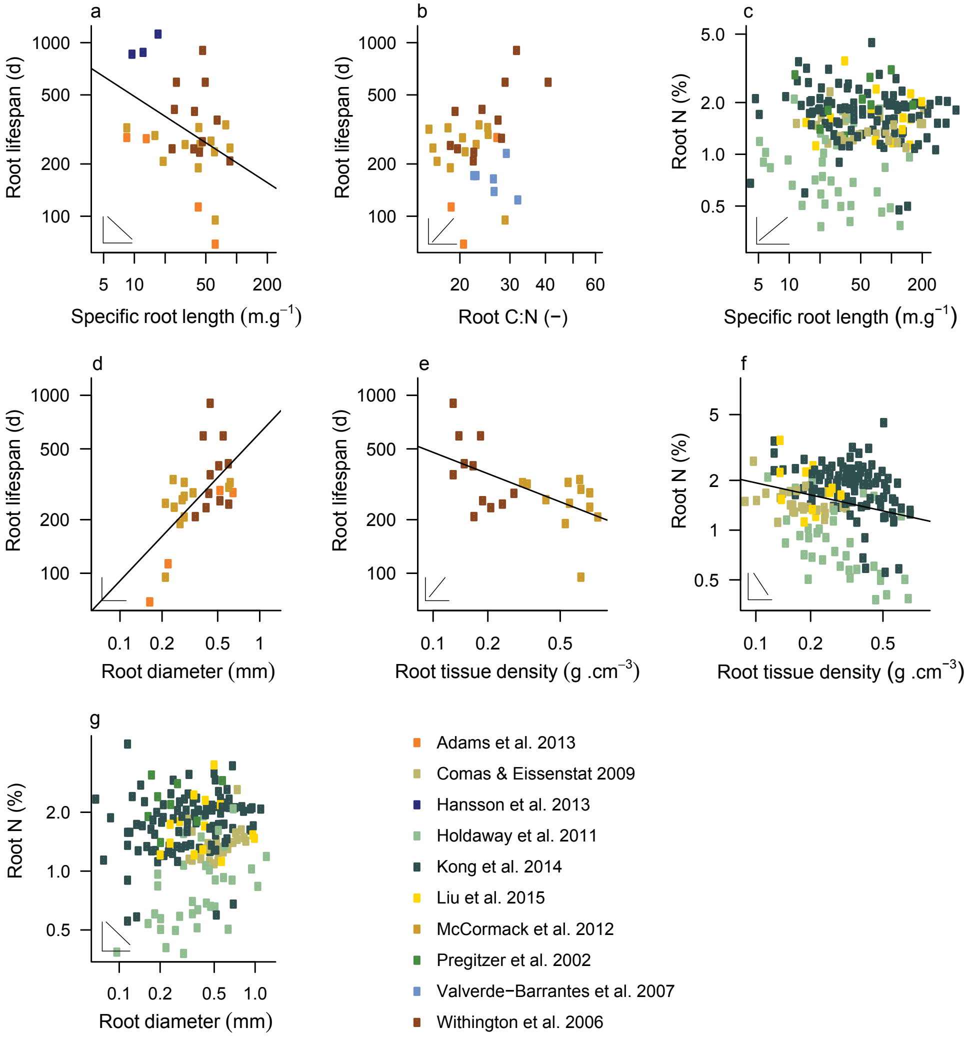
Consequently, their establishment offers a crucial step in examining and establishing the different axes that together comprise a multidimensional root trait spectrum, and potentially link these axes to plant performance and ecosystem functioning.

Acknowledgements

MW was supported by the Netherlands Organisation for Scientific Research (NWO) as part of a graduate programme grant to the Graduate School Production Ecology and Resource Conservation (PE&RC). LM was supported by a NWO-VIDI grant (864.14.006). Joy Burrough advised on the English. We thank two anonymous reviewers for their constructive comments.

Appendices

Appendix 3.1 Correlations between key root traits. Root economics spectrum (RES) traits (root nitrogen content, specific root length, root lifespan) and additional root traits (diameter, tissue density, C : N ratio) were correlated across studies reviewed. Insets (bottom-left) indicate the expected relationship according to the RES hypothesis. Black lines represent a significant correlation between the two traits ($P < 0.05$). Colours refer to different references from which data were retrieved. Note that data are presented on a logarithmic scale. Correlation statistics are presented in Appendix 3.3.



Appendix 3.2 Reviewed references and the number of species, climate zone, and the fine-root traits studied.

Reference	**No. of species**	**Climate zone**	**RES traits measured**
Adams *et al.* (2013)*	4	Temperate	SRL, respiration, RL, diameter, C : N ratio
Chen *et al.* (2013)	65	Subtropical, temperate	SRL, N content, diameter, RTD
Comas & Eissenstat (2009)*	25	Temperate	SRL, N content, diameter, RTD
Comas *et al.* (2014)*	33	Temperate	SRL, diameter, RTD
Eissenstat *et al.* (2015)*	6	Temperate	SRL, diameter, RTD
Gu *et al.* (2011) (meta-analysis)	18	Boreal, temperate, subtropical	RL, diameter
Hansson *et al.* (2013)*	3	Temperate	SRL, RL
Holdaway *et al.* (2011)*	34 woody spp. (out of 50 spp.)	Temperate	SRL, N content, diameter, RTD
Kong *et al.* (2014)*	96	Subtropical	SRL, N content, diameter, RTD, C content
Liu *et al.* (2015)*	14	Subtropical	SRL, N content, diameter, RTD, C content
Makita *et al.* (2012)	13	Tropical	SRL, respiration, diameter, RTD
McCormack *et al.* (2012)*	12	Temperate	SRL, respiration, RL, diameter, RTD, C : N ratio
Ostonen *et al.* (2007)*	3	Boreal	SRL, diameter, RTD
Pregitzer *et al.* (2002)*	9	Temperate	SRL, N content, diameter, C content, C : N ratio
Valverde-Barrantes *et al.* (2007)*	6	Tropical	N content, RL, C content
Valverde-Barrantes *et al.* (2013)*	14	Temperate	SRL, diameter, RTD
Valverde-Barrantes *et al.* (2015)	34	Temperate	SRL, N content, diameter, RTD, C content
Withington *et al.* (2006)*	11	Temperate	SRL, RL, diameter, RTD, C : N ratio

SRL, specific root length; RL, root lifespan; N content, root nitrogen content; RTD, root tissue density; C content, root carbon content; C : N ratio, root C : N ratio. Some studies have measured additional traits, e.g. branching intensity, which we do not refer to here. References with an asterisk were included in the meta-level analysis.

Appendix 3.3 Pearson correlation statistics between root traits, the number of studies and the number of data points included in the analysis.

Correlated root traits		**No. of studies**	***N***	**Pearson *r***	***P***
SRL	N content	5	178	0.07	0.37
	Root lifespan	4	30	-0.4	**0.03**
	Diameter	12	268	-0.75	**< 0.001**
	RTD	10	255	0.07	0.23
Root lifespan	C : N ratio	4	32	0.22	0.22
	Diameter	3	27	0.62	**< 0.001**
	RTD	2	23	-0.58	**< 0.01**
N content	Diameter	5	178	0.09	0.24
	RTD	4	169	-0.23	**< 0.01**
Diameter	RTD	10	255	-0.56	**< 0.001**

SRL, specific root length; N content, root nitrogen content; RTD, root tissue density. Bold P-values represent significant correlations ($P < 0.05$).

4. Root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on contrasting soils

Monique Weemstra, Frank J. Sterck, Eric J. W. Visser, Thomas W. Kuyper,
Leo Goudzwaard, Liesje Mommer

Accepted by Plant and Soil (in press)

Abstract

Tree roots show plastic responses to their resource environment. Several, contrasting hypotheses exist on this plasticity, but empirical evidence for these hypotheses is scattered. This study aims to enhance our understanding of tree root plasticity by examining intra-specific variation in fine-root mass and morphology, fine-root growth and decomposition, and associated mycorrhizal interactions in beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) Karst.) forests on soils that differ in resource availability.

We measured the mass and morphological traits of fine roots (i.e. ≤ 2 mm diameter) sampled to 50 cm depth. Fine-root growth was measured with ingrowth cores, and fine-root decomposition with litter bags. Mycorrhizal fungal biomass was determined using ingrowth mesh bags.

Both tree species showed more than three times more fine-root mass, and a ten-fold higher fine-root growth rate on sand than on clay, but no or marginal differences in overall fine-root morphology. *Within* the fine-root category however, beech stands had relatively more root length of their finest roots on clay than on sand. In the spruce stands, ectomycorrhizal mycelium biomass was larger on sand than on clay.

In temperate beech and spruce forests, fine-root mass and mycorrhizal fungal biomass, rather than fine-root morphology, are changed to ensure uptake under different soil resource conditions. Yet enhancing our mechanistic understanding of root trait plasticity and how it affects tree growth requires more attention to fine-root dynamics, the functional diversity within the fine roots, and mycorrhizal symbiosis as an important belowground uptake strategy.

Key words

Fagus sylvatica, ectomycorrhizal fungi, *Picea abies*, plasticity, root functional traits

Introduction

The expression of plant functional traits can be adjusted to the environment, allowing plants of the same species to grow and survive under various environmental conditions (Bradshaw 1965; Valladares et al. 2007). Such intraspecific plastic responses to resource availability have been widely observed on leaves (e.g. Ryser & Eek, 2000; Poorter *et al.*, 2012; Sterck *et al.*, 2013), but are far less studied on roots (Bardgett et al. 2014), especially of mature trees growing in forest environments (but see e.g., Ostonen *et al.*, 1999; Leuschner *et al.*, 2004; Valverde-Barrantes *et al.*, 2007; Hertel *et al.*, 2013). Plastic responses of roots can be grouped into different root trait categories, being architectural, morphological, chemical and biotic traits (sensu Bardgett et al. 2014). Here, we focus on root architectural traits that describe the spatial arrangement of the root system as a whole (e.g. fine-root mass and length over depth); root morphological traits that represent properties at the individual fine-root level (e.g. fine-root diameter, specific root length (SRL), and root tissue density (RTD)); and their relation with biotic traits (e.g. mycorrhizal abundance).

Fine-root *architectural* traits are hypothesised to change with the availability of soil resources (water and nutrients), following the functional equilibrium hypothesis (Brouwer 1963). This hypothesis predicts that fine-root mass increases relative to total plant biomass when soil resources are more limiting than light. However, some studies have confirmed this hypothesis (Yuan and Chen 2010; Hertel et al. 2013), while others have not (Leuschner and Hertel 2003; Finér et al. 2007; Meier and Leuschner 2008). Different drivers may underlie such architectural responses; a high fine-root biomass can be achieved by rapid fine-root growth, but also by low turnover rates, or both. These underlying drivers of tree root biomass are however difficult to quantify in forest environments (but see e.g. Withington et al. 2006, Gaul et al. 2008, McCormack et al. 2012).

Root *morphological* traits also respond to soil resource availability. On the one hand, following the resource economics hypothesis (Grime 1977; Craine 2009), poor soils select for species with thick fine roots that live long, so that valuable plant resources are conserved (Eissenstat and Yanai 1997; Aerts and Chapin 2000; Wahl and Ryser 2000; Pérez-Ramos et al. 2012; Reich 2014); such relationships between fine-root traits and the soil environment might also exist within species. A contrasting hypothesis, however, predicts thinner fine roots on poor soils, because these roots grow fast and can more efficiently exploit the soil for resources (Eissenstat 1992; Ryser and Lambers 1995; Eissenstat and Yanai 1997; Ostonen et al. 2007b; Holdaway et al. 2011; Prieto et al. 2015). Both hypotheses have been corroborated and refuted with empirical data (e.g. Fahey & Hughes, 1994; Leuschner *et al.*, 2004; Ostonen *et al.*, 2007a,b; Hertel *et al.*, 2013).

The extent to which these different root trait categories are plastic in relation to each other, is currently subject of debate. On the one hand, it has been suggested that fine-root morphological traits are more plastic than architectural traits (Poorter et al. 2012), but other studies have demonstrated the opposite (Ostonen et al. 2007a; Hertel et al. 2013; Freschet et al. 2015). Furthermore, both fine-root architectural and morphological traits may vary as a consequence of interactions with soil biota, such as ectomycorrhizal fungi, which may confound plastic root responses to resource availability (Freschet et al. 2015). On poor soils for example, ectomycorrhizal mycelium biomass increases (Nilsson et al. 2005; Kjøller et al. 2012; Bahr et al. 2013), which enlarges the soil volume available to the plant (Smith and Read 2008), and therefore possibly reduces the need for tree root architectural or morphological adjustments.

Because of these complex interactions between various fine-root traits and soil biota, understanding fine-root trait plasticity requires studying fine-root dynamics (e.g. root growth), as well as mycorrhizal interactions. Therefore, we studied the plasticity of several fine-root (i.e. ≤ 2 mm diameter) architectural and morphological traits, fine-root growth and decomposition, and mycorrhizal fungal biomass in forests on soils of different resource availability. We hypothesised that – within a species – forests on resource-poor soils are characterised by 1) greater (relative) fine-root mass and length, 2) faster fine-root growth and/or slower decomposition to achieve such a high fine-root mass, 3) higher SRL and lower RTD and fine-root diameter, and 4) larger ectomycorrhizal fungal biomass, compared to forests on resource-rich soils. We tested these hypotheses by comparing fine-root traits of two temperate ectomycorrhizal tree species (European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.)) growing in forests on contrasting soils, i.e. a resource-poor, sandy soil, and a resource-rich clay soil, in the Netherlands.

Methods

Study sites and design

To assess fine-root plastic responses to soil conditions, we selected forest stands on a clay and on a sandy soil located in the centre of the Netherlands. These forests are subjected to similar climate conditions, i.e. a temperate maritime climate with mild winters and summers, and an annual precipitation of approximately 800 mm (KNMI, 2009). The clay and sandy soils largely differ in resource availability (Table 4.1; Appendices 4.1, 4.2). The sandy soils had lower pH, lower CEC, higher C : N ratios of soil organic matter, and a lower ratio of nitrate over ammonium. Furthermore, plant available water was lower, and groundwater levels were generally deeper at the sandy soils (Table 4.1; see Appendix 4.1 for a description of the soil sampling and analyses). For these reasons, we refer to the sandy soil as resource-poor, and the clay soil as resource-rich (Högberg et al. 2003).

On these contrasting soils, we compared fine-root traits of two common, temperate tree species: European beech and Norway spruce. At each of the two soil types, we selected three monospecific stands per species. We aimed to minimise age effects by selecting even-aged stands, but could not avoid some age differences (Table 4.1), although all stands had a closed canopy. Fine-root architectural and morphological data were collected on roots sampled randomly throughout each stand. Data on fine-root growth and decomposition, and on mycorrhizal fungal biomass were gathered by conducting experiments at six selected individual and dominant trees randomly distributed within each stand in order to maintain a fixed distance between our experiments and the base of a tree stem.

Root architectural and morphological traits

We collected eight soil samples randomly distributed throughout each stand in August 2013, using a 42 mm root auger. We sampled up to 50 cm depth and separated six layers: 0 – 5, 5 – 10, 10 – 20, 20 – 30, 30 – 40, 40 – 50 cm below the soil surface. Roots were extracted from the soil cores by washing away the soil with a fine-meshed (1 mm) sieve and by picking the roots from the remaining organic matter and soil particles. Microscopic inspection of a subset of roots indicated the common occurrence of dark brown to blackish ectomycorrhizal root tips, colonised by *Cenococcum geophilum*.

Discriminating live and dead roots colonised by *Cenococcum* is not easy, the more so as turnover rates of these roots are much lower than that of other roots (Fernandez et al. 2013). In some samples, black rhizomorphs of *Armillaria* species were common; they could usually be distinguished from roots by observing frequent anastomoses, i.e. fusing of different rhizomorphs (Lamour et al. 2007), but smaller fragments could not always be separated visually from dead roots. Samples where these rhizomorphs were frequent were not further studied, so that sample sizes for fine-root length density, SRL, RTD and fine- root diameter varied between four and eight per stand.

Table 4.1 Mean stand and soil variables per species and soil type (± 1 standard deviation).

Species	**Beech**		**Spruce**	
Soil type	**Clay**	**Sand**	**Clay**	**Sand**
Stand properties				
Year of establishment	1970s	1938 – 1949	1970s	1974 – 1981
Stand density (trees ha^{-1})	1071 (92.0)	224 (36.6)	917 (438.6)	1553 (446.8)
Basal area (m^{2} ha^{-1})	37.1 (4.3)	21.8 (4.1)	43.3 (11.4)	28.2 (3.6)
Mean DBH (cm)	19.6 (1.6)	34.6 (6.3)	24.6 (3.5)	14.5 (1.1)
Mean dominant height (m)	25.0 (1.4)	25.8 (1.5)	22.5 (1.2)	17.5 (1.7)
Top soil fine-root mass (g m^{-2})	55.5 (39)	322.1 (113)	158.6 (62)	684.0 (181)
Soil properties				
Soil pH (-)	6.8 (2.0)	3.4 (0.9)	6.6 (2.1)	3.4 (0.9)
Soil C : N ratio (-)	11.5 (3.6)	14.5 (6.3)	11.3 (2.7)	14.9 (6.2)
Soil organic matter content (%)	6.6 (1.8)	9.7 (11.4)	8.3 (4.2)	10.4 (14.1)
Soil N-NO$_3^-$ (mg kg^{-1})	9.0 (5.4)	1.0 (1.7)	10.1 (5.4)	1.7 (3.6)
Soil N-NH$_4^+$ (mg kg^{-1})	5.8 (6.9)	12.7 (12.6)	4.6 (2.9)	17.6 (13.3)
Soil P-P$_2$O$_5$ (mg kg^{-1})	0.5 (0.3)	1.2 (2.2)	0.5 (0.4)	0.6 (1.2)
Plant available water (mm)	239.8 (33.0)	126.0 (23.6)	141.1 (42.1)	122.6 (9.4)
Groundwater level* (m below soil surface, range)	0.7 – 1.9	16 – 22	0.7 – 1.9	14 – 32 (0.3 – 2.4)

* Groundwater data represent the range observed across plots, and were less deep at one of the spruce plots than at the other spruce plots on the sandy soils (between parentheses). Top soil fine-root mass represents the fine-root mass in the top 10 cm of the soil. Soil variables are represented by the weighted mean over three soil layers: 0 – 5, 10 – 20, 30 – 40 cm soil depth (their data collection methods and depth distribution are presented in Appendices 4.1 and 4.2). DBH, diameter at breast height; dominant height, mean height of six dominant trees per plot; C, carbon; N, nitrogen.

Fine-root samples were scanned and the scans analysed using WinRhizo (Regent Instruments, Canada), which measures the total root length and volume per diameter class and average diameter per soil layer. Fine (≤ 2 mm diameter) and coarse (> 2 mm diameter) roots were separated, oven-dried (48h at 70°C) and weighed to determine root mass, and functional traits were only determined for the fine roots. Fine-root architectural traits (i.e. fine-root mass and length) were ultimately expressed on a soil area (i.e. fine-root mass, g m^{-2}) and volume (i.e. fine-root length density, cm cm^{-3}) basis to allow comparison with other studies.

Regarding fine-root morphological traits, we calculated SRL (fine-root length / fine-root dry mass) and RTD (fine-root dry mass / fine-root volume) per soil layer. Because the WinRhizo software measures the mean diameter of *all* roots present in one sample (that is, including the roots > 2 mm diameter), we could not use the software output on mean root diameter directly for fine roots. Therefore, we calculated the length-weighted mean diameter of only the *fine* roots separately; we calculated the mean root diameter for each diameter class from their mean root volume and length measured by WinRhizo, and averaged this for all diameter classes ≤ 2 mm diameter based on the proportion of the total fine-root length covered by each diameter class. Within this fine-root category, we also calculated the mean root length for different diameter classes, relative to the total fine-root length.

Root growth

Fine-root growth rates were measured using ingrowth cores. In April 2013, two root ingrowth cores were installed at each of six study trees per stand (see study sites and design section). At 1.2 m (spruce) or 1.5 m (beech) from the stem base, we replaced a block of forest soil covered mainly with litter (20 x 20 x 20 cm) by root-free sand. After 18 months, roots were collected using a 12 cm diameter cylinder from the top 10 cm at the centre of the ingrowth core. We separated fine and coarse roots and oven-dried and weighed them as described previously. Fine-root mass data were averaged per stand as an estimate of fine-root growth rate over the incubation period. Ingrowth data were expressed per soil area, per growing season (i.e. we divided the root mass after two growing seasons by two to compare our data with other studies).

Fine-root decomposition

Fine-root decomposition rates were measured using root litter bags. In March 2014, roots were sampled from one stand per species from both soil types, and fine roots were selected and air-dried. Polyester mesh bags (10 x 10 cm, 1 mm mesh size) were filled with 0.5 g of these fine roots. We buried litter bags in proximity to three of the six study trees in each of the twelve stands at 10 – 15 cm depth, and at 1.2 m (spruce) or 1.5 m (beech) from their stem base, each bag containing the native roots only (i.e. the roots collected at the respective soil type). Litter bags were collected after seven months. Four out of the 36 litter bags buried could not be retrieved. The remaining roots were carefully rinsed, dried and weighed to determine mass loss (% of initial fine-root mass) as an estimate of fine-root decomposition rate over the incubation period.

Mycorrhizal fungal biomass

Mycelium biomass was determined as a measure of mycorrhizal abundance because mycelia are the main fungal structure enhancing or even substituting the root absorptive area (Smith and Read 2008). We used polyester mycorrhizal mesh bags (10 x 6 x 2 cm, 38 µm mesh size) that allow the ingrowth of EM mycelia but not of roots (Wallander et al. 2001; Wallander et al. 2013), which were filled with 145 g of quartz sand. Due to the low nutritional value of this substrate, mycorrhizal mesh bags are assumed to predominantly contain mycorrhizal fungi (Wallander et al. 2001). In April 2013, four mesh bags were buried at each of the six study trees per stand between the organic and mineral soil at a fixed distance to the stem base (1.1 m for spruce and 1.4 m for beech). Most but not all mesh bags were retrieved in November 2013: for almost all 72 trees, 3 – 4 mesh bags were retrieved; for one tree, only one bag was found. The contents of the mesh bags were pooled per tree. From this pooled sample, two subsamples of 5 g sand each were analysed as ecological replicates for their ergosterol content following the protocol described by Bahr *et al.* (2013). Ergosterol mass per tree was then averaged per stand, and served as a measure of mycorrhizal fungal biomass (Nylund and Wallander 1992).

Data analyses

Data analyses were carried out *within* the species (see Appendix 4.3 for full model statistics including both species). Data were log- or square-root transformed to improve homogeneity of variance. Statistical analyses were carried out in R (R Core Team 2014, packages lme4 and nlme). Fine-root architectural (i.e. root mass and length) and morphological traits (i.e. SRL, RTD and mean fine-root diameter) and their distribution throughout the soil profile were compared between soil types using mixed models that corrected for our nested design and potential dependencies between root traits from the same stand or soil core (Zuur et al. 2009). Soil type (clay vs. sand), soil depth and their interaction were included as fixed factors. Stand (nested within the soil types) and soil core sample (nested within the stand) were added as random factors. Because fine-root growth and decomposition. and mycelium biomass were measured at one soil depth only, they were analysed in a mixed model with soil type as the fixed factor, and stand as random factor. We compared the difference in relative root length per fine-root diameter class between the two soil types with a similar mixed-effects model, as these data were analysed for the top soil only.

Results

Intraspecific differences in fine-root architectural traits

In the stands of both species, fine-root architectural traits significantly differed between soil types. Fine-root mass and length were more than three times higher on the sandy soil than on the clay soil over the 50 cm soil depth sampled (Figure 4.1a,b, Appendix 4.4). For both species, basal area was larger, reflecting greater aboveground biomass at the stand level, on clay than on sand (Table 4.1). Accounting for these differences in aboveground biomass shows that in the beech forests, fine-root mass per basal area was 5.8 times higher ($N = 12$, $F_{1,4} = 63.33$, $P = 0.001$), and in spruce forests 5.1 times higher on sand than on clay ($N = 12$, $F_{1,4} = 78.59$, $P < 0.001$). Soil depth had a significant effect on fine-root mass and length, and the significant interaction between soil type and soil depth indicates that the distribution of fine-root mass throughout the soil profile differed between sandy and clay soils (Figure 4.2, Appendix 4.4). On the sandy soils, fine-root mass decreased with greater soil depth. On the clay soils, this vertical decrease in fine-root mass was less strong for spruce, and for beech, fine-root mass was even slightly higher at 10 – 30 cm soil depth.

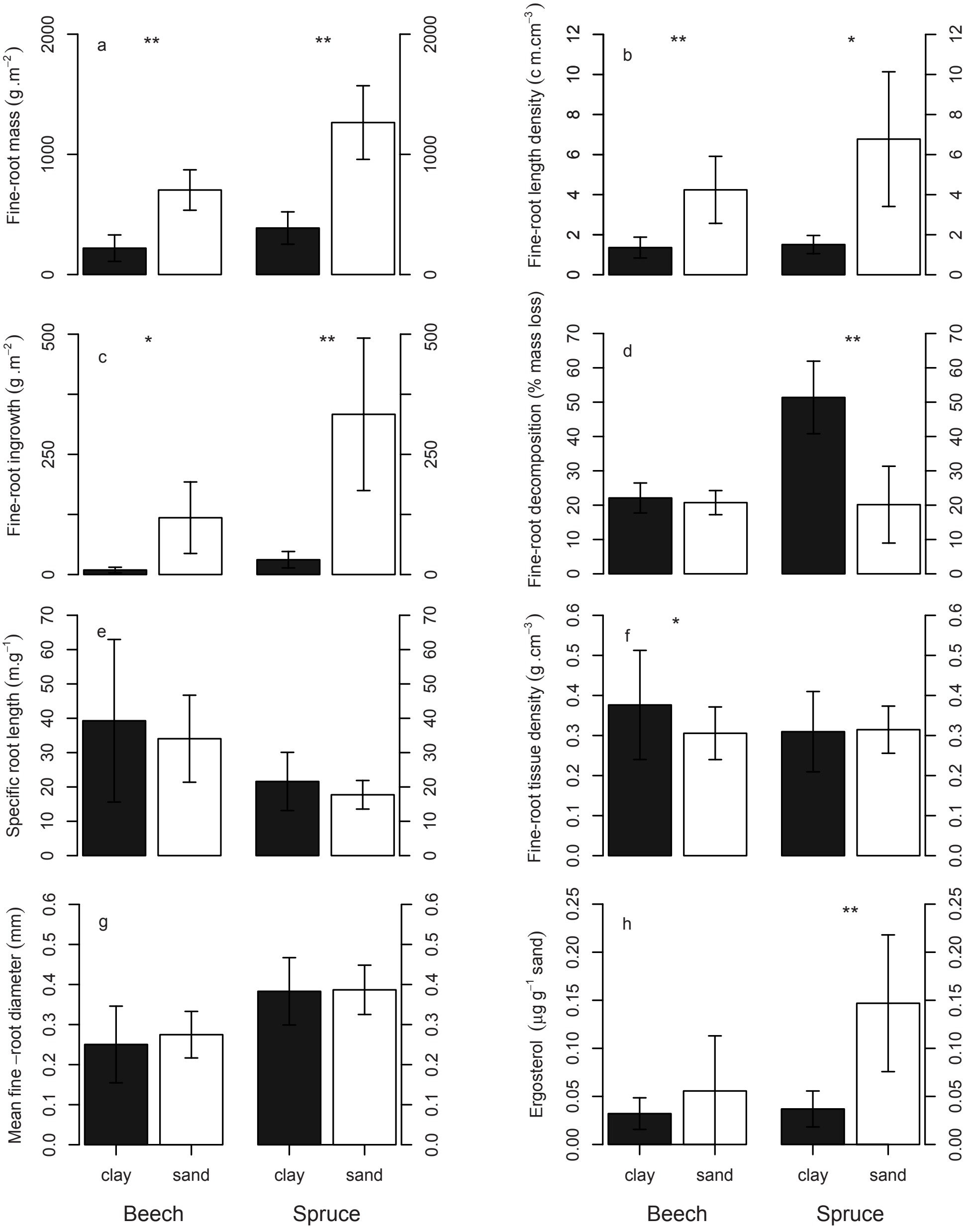


Figure 4.1 Fine-root traits and ergosterol mass for beech and spruce forest stands on clay and sandy soils (means ± 1 standard deviation). Asterisks mark significant differences between soil types within species, with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Fine-root mass and length were summed over 50 cm soil depth, and then averaged per site and species. Fine-root SRL, RTD and mean fine-root diameter were averaged

over the soil depth per site and species. Ergosterol mass and fine-root decomposition rate were determined over one growing season. Fine-root ingrowth was measured over two growing seasons but divided by two to present an annual fine-root growth rate (see Methods section). Ergosterol mass, fine-root decomposition and fine-root growth rate were measured at one soil depth. Graphs represent raw data, whereas mixed model outputs are based on log- or square-root transformed data (Appendix 4.4).

Differences in fine-root growth and decomposition

Fine-root growth rates in the ingrowth cores were more than 10 times higher on the sandy soils than on the clay soils for both species (Figure 4.1c, Appendix 4.4). This difference may have been caused by the higher initial root mass density at the sandy soils. However, after one growing season, fine-root mass in the ingrowth cores relative to the fine-root mass in the bulk soil was also higher at the sandy soils than at the clay soils. For beech, on the clay soils, the fine-root mass in the ingrowth cores was on average 17% of the fine-root mass in the bulk soil summed over the top 10 cm (similar to the soil depth covered by the ingrowth cores; Table 4.1), whereas on the sandy soils, this percentage was 37%. Spruce fine-root mass in the ingrowth cores was 19% of the mass in the top 10 cm of bulk soil on the clay soils, and 49% on the sandy soils.

Fine-root decomposition in the beech stands did not differ between soil types, and after seven months, approximately 20% of the initial fine-root mass in the litter bags was lost (Figure 4.1d, Appendix 4.4). In the spruce stands, fine-root decomposition rates were significantly lower at the sandy soils than at the clay soils, with approximately 20% on the sandy soils and 50% on the clay soils of the initial fine-root mass lost after seven months.

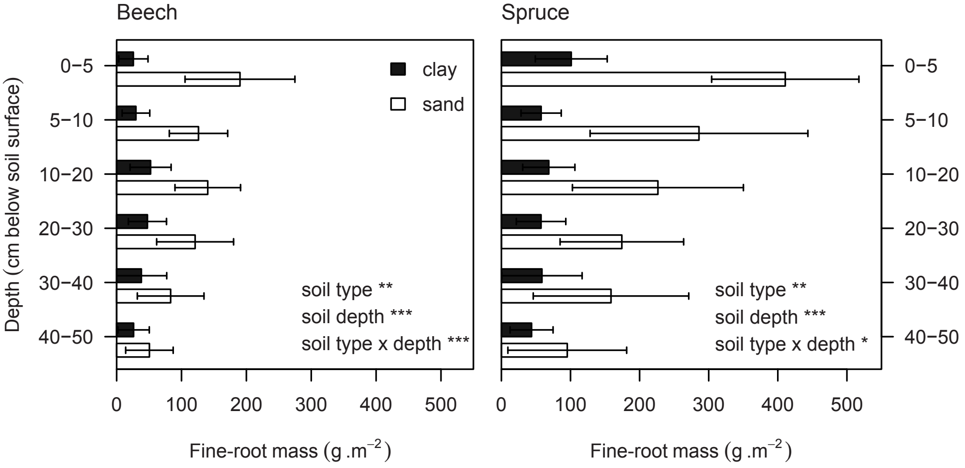


Figure 4.2 Fine-root mass per soil layer of beech and spruce forest stands on clay and sandy soils (means ± 1 standard deviation). Asterisks mark significant differences between soil types within species, with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Graphs represent raw data, whereas mixed model outputs are based on log- or square-root transformed data (Appendix 4.4).

Intraspecific differences in fine-root morphological traits

Fine-root morphological traits differed little between soil types. Specific root length and fine-root diameter did not differ between the clay and sandy soils for either species (Figure 4.1e,g, Appendix 4.4). Fine-root tissue density was significantly higher at the clay soil than at the sandy soil for beech, but did not differ between soil types for spruce (Figure 4.1f, Appendix 4.4). *Within* the fine-root category (i.e. the roots ≤ 2 mm diameter), shifts occurred in the root length – diameter distribution between the two soil types for beech (Figure 4.3). At the clay soil, the relative root length of the smallest roots (0.1 – 0.3 mm diameter) was higher than on the sandy soil. At the sandy soil, we observed relatively more root length of the thicker beech roots (0.4 – 1 mm diameter). For spruce, fine-root length distribution per diameter class did not differ between the sandy and clay soils (Figure 4.3).

Soil depth did not affect the morphology of beech roots, as SRL, RTD and mean fine-root diameter did not change significantly with soil depth (Appendices 4.4, 4.5). For spruce, fine-root morphology did differ across the soil layers (Appendix 4.4). However, the variation in SRL showed no clear vertical pattern throughout the soil profile, whereas RTD slightly decreased, and mean fine-root diameter slightly increased with greater depth (Appendix 4.5).

Differences in mycorrhizal mycelium biomass

In the beech stands, mycelium biomass (represented by ergosterol mass) was on average almost two times higher in the ingrowth bags at the sandy soil than at the clay soil, but this difference was not significant (Figure 4.1h, Appendix 4.4). In the spruce stands, mycelium biomass did differ significantly between soil types, and was four times higher at the sandy soil than at the clay soils.

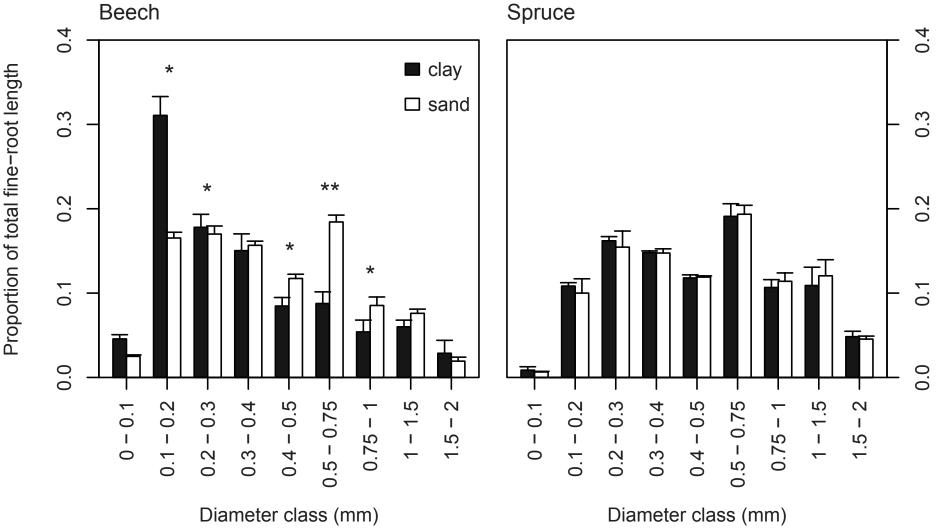


Figure 4.3 Proportion of the fine-root length per diameter class relative to the total fine-root length (i.e. roots ≤ 2 mm diameter) for the top soil (0 – 5 cm depth) of beech and spruce trees on clay and sandy soils (means ± 1 standard deviation). Asterisks mark significant differences between soil types within a diameter class: * P < 0.05, ** P < 0.01. Diameter classes of roots larger than 0.5 mm diameter vary in size.

Discussion

Our work demonstrates strong fine-root architectural and biotic but not morphological plasticity in beech and spruce forests on contrasting soils. Regarding architectural plasticity (first hypothesis), fine-root mass and length were higher on the sandy soils than on the clay soils in both beech and spruce forests. This finding may be related to the higher fine-root growth rate on the sandy soils compared to clay soils (second hypothesis). Fine-root morphological traits (i.e. SRL, RTD and diameter) did not or only marginally differ between the two soil types for either species, refuting our third hypothesis. More important than plasticity in fine-root morphology, may be the mycorrhizal response to the different soil types. Indeed, mycorrhizal biomass was higher on the sandy soils than on the clay soils, but these differences were only significant for spruce (fourth hypothesis). Trees on poor soils may thus rely more on changes in fine-root mass and mycorrhizal abundance than in fine-root morphology to acquire soil resources, although this dependence may differ between species.

Fine-root mass is larger on sandy soils

In line with our first hypothesis, fine-root mass and length were more than three times greater on our poor, sandy soils than on the rich, clay soils for both species, as also observed in other studies (Espeleta and Donovan 2002; Yuan and Chen 2010; Hertel et al. 2013). It should be noted that we cannot exclude the possibility that fine-root trait differences also partly resulted from genetic differences (e.g. planting different beech clones on sand and clay). Furthermore, this result needs to be interpreted cautiously as differences in aboveground stand variables may confound differences in fine-root mass between soil types (Finér et al. 2011). Stand basal area (i.e. the total cross-sectional area of the stems) takes both the mean stem diameter (DBH) as well as the stem density (i.e. number of trees per hectare) into account, and therefore serves as a proxy of aboveground biomass at the stand level. Correcting for the smaller aboveground biomass on sand than on clay resulted in five to six times larger fine-root mass on sand than on clay, indicating that fine-root mass even more strongly increased relative to the total plant biomass.

Particularly on the sandy soils, fine-root mass and length decreased with greater soil depth, measured to 50 cm soil depth. This vertical root distribution follows the distribution of nitrogen, phosphorus, and soil organic matter throughout the soil profile (Appendix 4.2). At the clay soils, fine-root mass and length differed across the soil layers as well, but did not show the strong exponential decrease that was observed on the sandy soils. At these soils, nutrients and soil organic matter were also more equally distributed between soil layers than on the sandy soils (Appendix 4.2). For both species, approximately 75% of the fine-root mass was located in the top 30 cm on the clay soils, versus 82% on the sandy soils. These values are higher than those reported in a meta-analysis by Jackson *et al.* (1996), i.e. 52% for temperate coniferous forests, and 65% for temperate deciduous forests, possibly partly because of the greater soil depth covered by Jackson *et al.* (1996, i.e. more than 2 m soil depth in some of the studies included in the analysis).

When we compare our fine-root mass data to previous work in beech forests, the patterns are as expected from our soil properties. In a European-wide root-mass review, an average of 389 g fine-root biomass m^{-2} was observed for beech (Finér et al. 2007); in our study, beech fine-root mass on the clay soils was lower (i.e. 56% of their average) and on our sandy soils almost two times higher than the average reported by Finér et al. (2007). More surprisingly, in our spruce stands, mean fine-root mass was 1.4 times higher on our clay soils, and 4.5 times higher on our sandy soils, than the average for spruce documented by Finér et al. (2007), i.e. 281 g m^{-2}, and we are unaware of the cause of this discrepancy. Our results thus support our hypothesis that (relative) fine-root mass increases under limiting soil resource conditions, but without additional information on the underlying root dynamics, data may be difficult to interpret and to compare across studies.

Differences in fine-root dynamics driving fine-root biomass

Consistent with our second hypothesis, fine-root mass in the ingrowth cores was higher in the sandy soils than in the clay soils for both species. This ten-fold difference cannot merely be attributed to the differences in initial fine-root mass on the different soil types. On the clay soils, fine-root mass in the ingrowth cores was 15 – 20 % of the fine-root mass in the top 10 cm of the bulk soils, versus 35 – 50 % on the sandy soils, suggesting faster fine-root growth on the sandy soils for both species. The large fine-root mass found on the sandy soils may thus be at least partly driven by faster fine-root growth.

The fine-root growth rates we observed differed from those documented in other studies. Fine-root growth rates in our beech stands on the clay soils were only 10 % of the mean annual fine-root production rate reported in a meta-analysis by Brunner *et al.* (2012): 115 g m^{-2} y^{-1}, and were comparable on our sandy soils. In our spruce forests, fine-root ingrowth on the clay soils was approximately half of the mean fine-root production rate reported by Brunner et al. (2012): 73 g m^{-2} y^{-1}, whereas on the sandy soils it was almost five times higher. Root productivity estimates may be difficult to compare across studies due to methodological differences involved (e.g. in ingrowth core substrate, incubation time). Nevertheless, the differences between fine-root growth rates in our work and in other studies are roughly in line with the discrepancies in fine-root mass between our and other European studies, as here too, fine-root growth was much faster than predicted from earlier studies, especially for spruce.

The decomposition pattern was partly in line with our second hypothesis, where we predicted a slower fine-root decomposition on our sandy soils than on our clay soils. Beech root decomposition rates did not differ between the two sites, whereas spruce fine-root decomposition was faster on the clay soils than on the sandy soil, as expected. Higher soil moisture content and soil pH have been found to stimulate microbial activity and litter decomposition, whereas higher soil C : N ratios generally have negative effects (Solly et al. 2014). The faster decomposition of spruce roots on the clay soils can thus be (partly) explained from the differences between our clay and sandy soils (Table 4.1), but the similar decomposition rates between soil types of beech roots cannot. Only few studies examined the decomposition rates of beech and spruce fine roots. We observed approximately 20% mass loss over one growing season, with the exception of spruce roots on our clay soil (approximately 50% mass loss). Hobbie *et al.* (2010) observed 30% mass loss for spruce and 40% for beech fine roots after two years of soil incubation.

Finally, it should be noted that fine-root growth and decomposition rates were measured in an experimental setting (ingrowth cores and litter bags, respectively), and will likely not represent actual root dynamics in the bulk soil. For example, the low fine-root mass at the clay bulk soil may also (partly) result from slow fine-root growth due to e.g. soil compaction, that may not occur in our ingrowth cores (Kozlowski 1999). Furthermore, fine-root turnover *during* the 18-month incubation may also partly explain differences in fine-root growth between sand and clay. As spruce decomposition in our litter bags was for example faster on clay than on sand, the actual difference in fine-root growth rate between the two soil types may be smaller than reported. However, because both fine-root growth and decomposition were measured in different experiments, they cannot be directly related to each other. Nonetheless, as relative measures comparing the two soil types, these experimental data suggest that the differences in fine-root mass at the two soil types were to a large extent driven by differences in growth rate, and not (beech), or to a lesser degree (spruce), by differences in decomposition rates.

Fine-root morphological traits do no differ between soil types

We further hypothesised that on poor soils, SRL is higher and RTD and fine-root diameter are lower than on resource-rich soils, in order to increase resource uptake at relatively low biomass investments (Eissenstat 1992; Ryser and Lambers 1995; Eissenstat and Yanai 1997; Ostonen et al. 2007b). An opposite hypothesis follows from resource economics theory that predicts that poor soils select for species with thick roots of low SRL in order to retain the scarce resources acquired (i.e. a conservative strategy), whereas rich soils select for species with thin roots of high SRL to efficiently and rapidly acquire soil resources (i.e. an acquisitive strategy) (Eissenstat and Yanai 1997; Aerts and Chapin 2000; Reich 2014). This trade-off between fine-root traits aimed at resource conservation and fine-root traits equipped for resource acquisition might also act within species.

We found no support for either hypothesis. Specific root length, RTD and diameter did not or only marginally differ between the sandy and clay soils. In the beech stands, the constant SRL, RTD and mean fine-root diameter throughout the soil profile also suggest that fine-root morphological traits are little affected by soil resource availability. For spruce, SRL, RTD and mean diameter differed across the soil layers sampled, but did not gradually change with increasing soil depth as could be expected based on decreasing resource availability with greater depth. These results indicate that fine-root morphology at our sites is not or hardly determined by soil resource availability. This contrasts with the large number of studies that have reported consistent increases in specific leaf area (SLA) when light availability diminishes (e.g. Evans & Poorter, 2001; Poorter *et al.*, 2012; Freschet *et al.*, 2015). Belowground, other variables such as the anchorage and transport functions of roots (Poorter and Ryser 2015), or mycorrhizal interactions (Ostonen et al. 2011; Freschet et al. 2015) may constrain the plastic response of fine-root morphology to soil resource availability only. These constraints may also explain why our overall results do not match the resource economics theory that has been generally observed across species on leaf functional traits, but not on fine-root traits (Weemstra et al. 2016).

Similar to our results, several studies did not find plasticity in tree fine-root morphological traits (George et al. 1997; Espeleta and Donovan 2002; Leuschner et al. 2004; Meier and Leuschner 2008; Hertel et al. 2013), whereas others did. For example, Fahey and Hughes (1994) and Ostonen *et al.* (2007a) found lower SRL on resource-poor compared to resource-rich soils, whereas Ostonen *et al.* (2007b) observed the opposite response. These results may be partly caused by the different definitions of fine roots applied across studies. For example, Fahey & Hughes (1994) focused on fine roots < 1 mm diameter, Ostonen *et al.* (2007a) studied short, mycorrhizal roots, and Ostonen *et al.* (2007b) reported a stronger plastic response in the < 0.5 mm diameter roots than in the thicker fine roots, whereas we included all roots ≤ 2 mm diameter. These different fine-root classifications may confound the degree of plasticity generally observed for root morphology.

Indeed, recent studies suggest that differences in root morphological plasticity may occur *within* the fine roots (≤ 2 mm diameter) (Poorter and Ryser 2015), because even within this category, root functions may vary (Pregitzer et al. 2002; Gu et al. 2011; McCormack et al. 2015). Our results agree with these observations. In contrast to our hypothesis but in line with resource economics theory, beech may exhibit a more acquisitive resource strategy on rich soils, by producing relatively more length of its finest roots (0.1 – 0.3 mm diameter) that are responsible for resource acquisition. On poor soils, the species may adopt a more conservative strategy by producing bigger (fine) roots (0.4 – 1 mm diameter) to enhance fine-root lifespan and retain resources (Bardgett et al. 2014; Reich 2014). Possibly, fine-root morphological plasticity is most relevant for the finest roots, which are assumingly most active in nutrient uptake (McCormack et al. 2015). However, these morphological adjustments may be species-specific, as for spruce, the fine-root length distribution per diameter class was strikingly similar between sandy and clay soils. To conclude, overall fine-root morphology did not differ between our two contrasting soil types; it did change, however, within the beech fine-root category and these shifts were in line with resource economics theory.

Ectomycorrhizal fungal biomass is higher on sandy soils

As expected, mycelium biomass was higher on the sandy soils than on the clay soils, although this was statistically only significant for spruce. Compared to other studies on spruce, ergosterol mass on our clay soils was low, but on the sandy soils within the range of values reported in previous studies. Hansson *et al.* (2013) for example found a mean ergosterol mass of 0.13 µg g^{-1} sand in their 55 – 65 year old spruce stand in southwest Sweden, and Bahr *et al.* (2013) found highly variable values between 0.03 – 0.2 µg ergosterol g^{-1} sand across 50 – 109 year old spruce stands in south Sweden. Other studies, however, reported much higher values, e.g. 0.25 – 0.5 µg ergosterol g^{-1} sand (Wallander *et al.* 2001), up to 1 µg g^{-1} sand found by Wallander *et al.* (2011) in southern Sweden. These high values could be attributed to nitrogen deposition that is more than three times higher in the Netherlands than in south Sweden (Waldner et al. 2014) and which has a negative effect on mycelium productivity (Bahr et al. 2013). We did not find comparable studies that measured mycelium biomass based on ergosterol content in mycorrhizal mesh bags in beech forests.

In addition to our mesh bag results, we also observed more mycorrhizal morphotypes that produce mycelia on the root tips collected at our sandy soils than on our clay soil. On the clay soils, we only encountered root tips colonised by mycorrhizal types that do not produce extraradical mycelia (Appendix 4.6). Together, our results thus imply that at the sandy soils, particularly in the spruce forests, more carbon was invested in mycorrhizal fungi that produce extraradical mycelia and are therefore better equipped for soil exploration and resource uptake than at the clay soils (Bahr et al. 2013). This strategy also agrees with the thicker fine roots observed on spruce, compared to beech (Appendix 4.3), which may be beneficial to sustain more mycorrhizal symbioses (Comas et al. 2002), and over a longer term (Bauhus and Messier 1999).

Concluding remarks

In temperate beech and spruce forests, fine-root architecture and interactions with mycorrhizal fungi, rather than fine-root morphology, are plastic in response to soil resource availability. The lack of a morphological plasticity suggests that SRL is more constrained by interacting plant and soil properties than assumed. Possibly, unexplored trade-offs with other root traits (e.g. the trade-off between SRL and fine-root lifespan; Weemstra et al. 2016), root functions (e.g. resource transport that may also occur within the < 2-mm diameter class; Guo et al. 2008b) or alternative belowground uptake mechanisms (e.g. the impact of ectomycorrhizal fungi on fine-root diameter; Van der Heijden and Kuyper 2003) control the degree of morphological plasticity. Furthermore, fine-root plasticity may be species-specific as suggested by the morphological shifts within the fine roots observed for beech only, and the stronger mycorrhizal response observed for spruce on the two contrasting soils.

Understanding why certain fine-root traits are (not) adjusted to the soil environment requires more attention to the underlying drivers of root mass and morphology, and to other mechanisms that are involved in soil resource uptake. As plasticity in fine-root mass may be more important than in morphology, further study on its underlying drivers (e.g. root growth and turnover) is needed to explain these patterns and test how general they are. Second, mycorrhizal interactions cannot be ignored in root plasticity studies as they serve as an important alternative uptake strategy, especially on poor soils, and may compensate for, and interact with, fine-root morphological responses to resource-limitations. Third, plastic responses may occur within the fine-root category, thus calling for greater insights in the functional diversity of this root class. Including these insights in root research is important to enhance our understanding of tree root trait plasticity, and ultimately to determine its impacts on tree performance under different soil conditions.

Acknowledgements

We are grateful to Verónica Fernández Belmonte, Purificación Gómez Ordóñez, Cristina Bernal Girona, Hans Reijnen, Jan van Walsem, Frans Möller, Jan Willem van der Paauw, Natasa Kiorapostolou, Lan Zhang, Henk van Roekel, Gerard Mekking, Ellen Wilderink, Mathieu Decuyper, Lisanne van Willigen and Carlette Nieland for their help with field and lab work. We also thank Dr. Simon Egli, Dr. Martina Peter, Prof. Håkan Wallander and Dr. Adam Bahr for their advice and support in mycorrhizal analyses, and Dr. Jos van Dam for his contribution on quantifying soil water availability. Jos Rutten, Leo Smits, Frank Klinge, Theo Gokke, Jan Floor, Rein Berends kindly facilitated and hosted our field work at the different forest stands. Finally, we thank two anonymous reviewers for their useful comments on an earlier version of this manuscript. This research was funded by the Netherlands Organisation for Scientific Research (NWO) as part of a graduate programme grant to the Graduate School Production Ecology and Resource Conservation (to M.W.), the COST Action STReESS (FP1106 to M.W.), and NWO-VIDI (grant 864.14.006 to L.M.).

Appendix

Appendix 4.1. Full mixed model statistics on soil differences between soil types (i.e. clay vs. sand), species, soil layers, and their interactions. Bold *P*-values are significant ($P < 0.05$); df_{num}, numerator degrees of freedom, df_{den}, denominator degrees of freedom.

	df_{num}	df_{den}	*F*	*P*
Soil pH				
Soil type	1	8	3445.82	**< 0.001**
Species	1	8	1.52	0.25
Soil depth	1	106	30.37	**<0.001**
Species x soil type	1	8	0.61	0.46
Soil type x soil depth	1	106	4.63	**0.03**
Species x soil depth	1	106	1.21	0.27
Species x soil type x soil depth	1	106	13.09	**<0.001**
Soil C : N ratio				
Soil type	1	8	21.72	**0.002**
Species	1	8	0.00	0.95
Soil depth	1	116	7.75	**0.006**
Species x soil type	1	8	0.05	0.83
Soil type x soil depth	1	116	2.46	0.12
Species x soil depth	1	116	1.42	0.24
Species x soil type x soil depth	1	116	10.13	**0.002**
Soil organic matter content				
Soil type	1	7	1.66	0.24
Species	1	7	0.45	0.53
Soil depth	1	88	244.57	**<0.001**
Species x soil type	1	7	0.04	0.84
Soil type x soil depth	1	88	29.50	**<0.001**
Species x soil depth	1	88	0.05	0.83
Species x soil type x soil depth	1	88	1.31	0.26
Available NO_3^-				
Soil type	1	8	52.38	**<0.001**
Species	1	8	0.05	0.84
Soil depth	1	110	145.28	**<0.001**
Species x soil type	1	8	0.01	0.93
Soil type x soil depth	1	110	1.02	0.31

Species x soil depth	1	110	0.11	0.72
Species x soil type x soil depth	1	110	2.51	0.12
Available NH_4^+				
Soil type	1	8	19.64	**<0.001**
Species	1	8	0.44	0.53
Soil depth	1	110	77.51	**<0.001**
Species x soil type	1	8	1.74	0.22
Soil type x soil depth	1	110	84.57	**<0.001**
Species x soil depth	1	110	0.08	0.77
Species x soil type x soil depth	1	110	7.11	**0.009**
Available P-P_2O_5				
Soil type	1	8	1.09	0.33
Species	1	8	2.66	0.15
Soil depth	1	110	162.33	**<0.001**
Species x soil type	1	8	1.36	0.28
Soil type x soil depth	1	110	19.72	**<0.001**
Species x soil depth	1	110	5.67	**0.02**
Species x soil type x soil depth	1	110	5.37	**0.02**
Plant available water*		*df*	*F*	*P*
Soil type		1	9.82	**0.01**
Species		1	5.65	**0.04**
Species x soil type		1	4.99	0.06

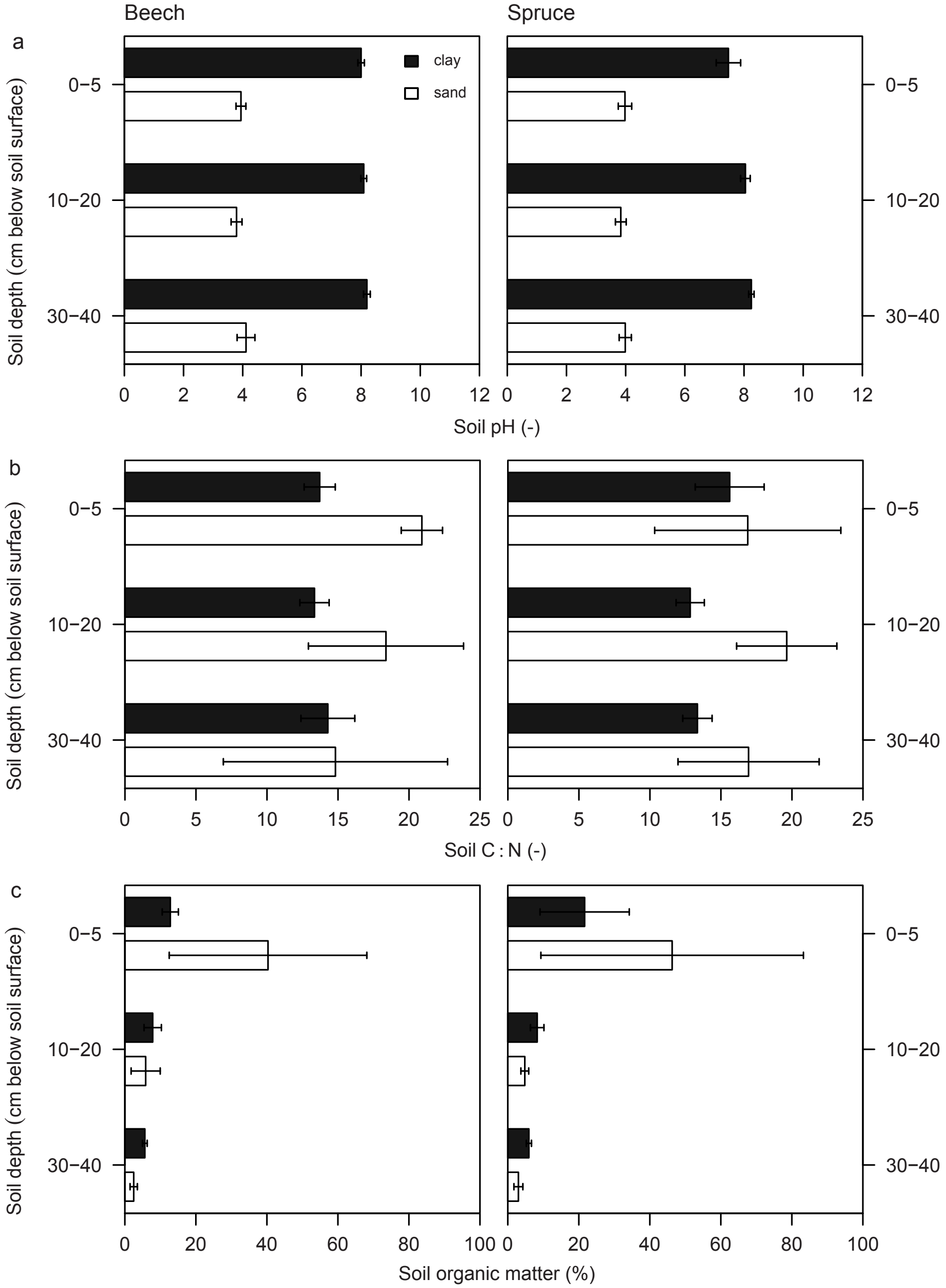
* Plant available water is an integrated variable based on the effective rooting zone (estimated at 120 cm below the soil surface) and covers the entire soil profile sampled (0 – 40 cm) separated over three layers as described below. Because this variable was already averaged per stand, it was analysed in a linear model including the soil type, species and their interaction.

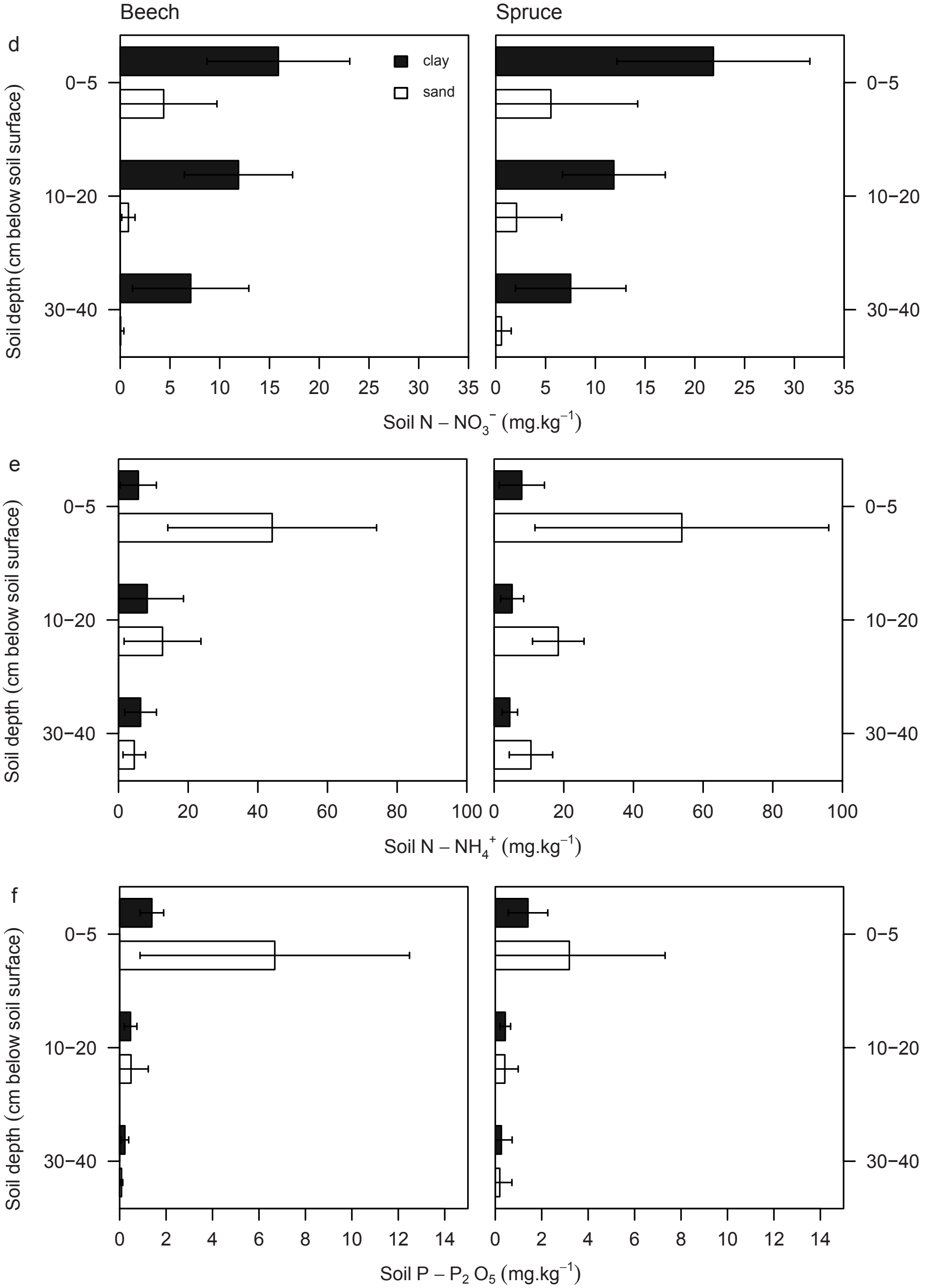
Soil data methods and analyses

Soil data were collected at the plot-level and analysed at three different depths: 0 – 5, 10 – 20 and 30 – 40 cm depth below the soil surface. Between late August and early September 2013, we collected five soil samples per plot, which were analysed for available pH, C : N ratios, organic matter content, nitrate, ammonium, and phosphorus. Plant available water was measured from water retention curves, based on two soil samples per plot at the same three soil depths. We measured the soil volumetric water content at different soil water potentials, and deduced the soil water content at field capacity (VWC_{FC}, soil water potential = -0.01 MPa) and at wilting point (VWC_{WP}, soil water potential = -1.5 MPa). The differences between VWC_{FC} and VWC_{WP} per plot and soil layer were used as a measure of plant available water, and integrated throughout the soil profile assuming an effective rooting depth of 120 cm.

Differences in soil variables between the two soil types (clay vs. sandy soils) were tested using a mixed model similar to the model used to compare fine-root traits between soil types (see also the Methods section). Soil data were log- or square-root transformed to improve homogeneity of variance, and statistical analyses were carried out in R (R Core Team 2014, packages lme4 and nlme). All soil variables except for plant water availability were compared between soil types using mixed models that can correct for our nested design and potential dependencies between soil data from the same plot or soil core (Zuur et al. 2009). Soil type (clay vs. sand), species (beech vs. spruce) soil depth and their interaction were included as fixed factors. Plot (nested within the soil types) and soil core sample (nested within the plot) were added as random factors.

Appendix 4.2 Soil characteristics throughout the soil profile in beech and spruce stand on clay and sandy soils (means ± 1 standard deviation). Appendix 4.1 describes the data collection and analyses).




Beech
Spruce
d
e
f
clay
sand
Soil depth (cm below soil surface)
0−5
10−20
30−40
0
5
10
15
20
25
30
35
Soil N – NO_3^- (mg.kg^{-1})
0
20
40
60
80
100
Soil N – NH_4^+ (mg.kg^{-1})
0
2
4
6
8
10
12
14
Soil P – P_2O_5 (mg.kg^{-1})

Appendix 4.3 Full mixed model statistics on fine-root trait differences between species, soil types (i.e. clay vs. sand), soil layers and their interactions. Bold *P*-values are significant ($P < 0.05$); df_{num}, numerator degrees of freedom, df_{den}, denominator degrees of freedom (see model description in the Methods section).

	N	df_{num}	df_{den}	*F*	*P*
Fine-root mass	459				
Species		1	8	28.253	**<0.001**
Soil type		1	8	122.992	**<0.001**
Soil depth		5	362	38.692	**<0.001**
Species x soil type		1	8	2.176	0.178
Species x soil depth		5	362	8.073	**<0.001**
Soil type x soil depth		5	362	19.795	**<0.001**
Species x soil type x soil depth		5	362	0.598	0.702
Fine-root length density	430				
Species		1	8	0.098	0.762
Soil type		1	8	59.797	**<0.001**
Soil depth		5	334	84.447	**<0.001**
Species x soil type		1	8	0.362	0.564
Species x soil depth		5	334	6.360	**<0.001**
Soil type x soil depth		5	334	3.102	**0.009**
Species x soil type x soil depth		5	334	0.631	0.676
Fine-root ingrowth	72				
Species		1	8	11.678	**0.009**
Soil type		1	8	55.760	**<0.001**
Species x soil type		1	8	0.000	0.993
Fine-root decomposition	32				
Species		1	8	4.811	0.060
Soil type		1	8	10.647	**0.012**
Species x soil type		1	8	9.151	**0.016**
Specific root length	429				
Species		1	8	54.339	**<0.001**
Soil type		1	8	2.238	0.173
Soil depth		5	333	0.767	0.574
Species x soil type		1	8	0.940	0.361
Species x soil depth		5	333	2.552	**0.028**
Soil type x soil depth		5	333	1.605	0.158

Species x soil type x soil depth		5	333	1.637	0.150
Fine-root tissue density	429				
Species		1	8	5.578	**0.046**
Soil type		1	8	3.904	0.084
Soil depth		5	333	5.287	**< 0.001**
Species x soil type		1	8	8.896	**0.018**
Species x soil depth		5	333	0.601	0.670
Soil type x soil depth		5	333	1.917	0.091
Species x soil type x soil depth		5	333	0.652	0.660
Mean fine-root diameter	428				
Species		1	8	95.309	**<0.001**
Soil type		1	8	3.014	0.12
Soil depth		5	332	7.556	**<0.001**
Species x soil type		1	8	0.545	0.482
Species x soil depth		5	332	6.138	**<0.001**
Soil type x soil depth		5	332	8.301	**<0.001**
Species x soil type x soil depth		5	332	2.042	0.072
Mycelium mass	72				
Species		1	8	14.400	**0.005**
Soil type		1	8	33.080	**<0.001**
Species x soil type		1	8	11.137	**0.010**

Fine-root trait differences between species

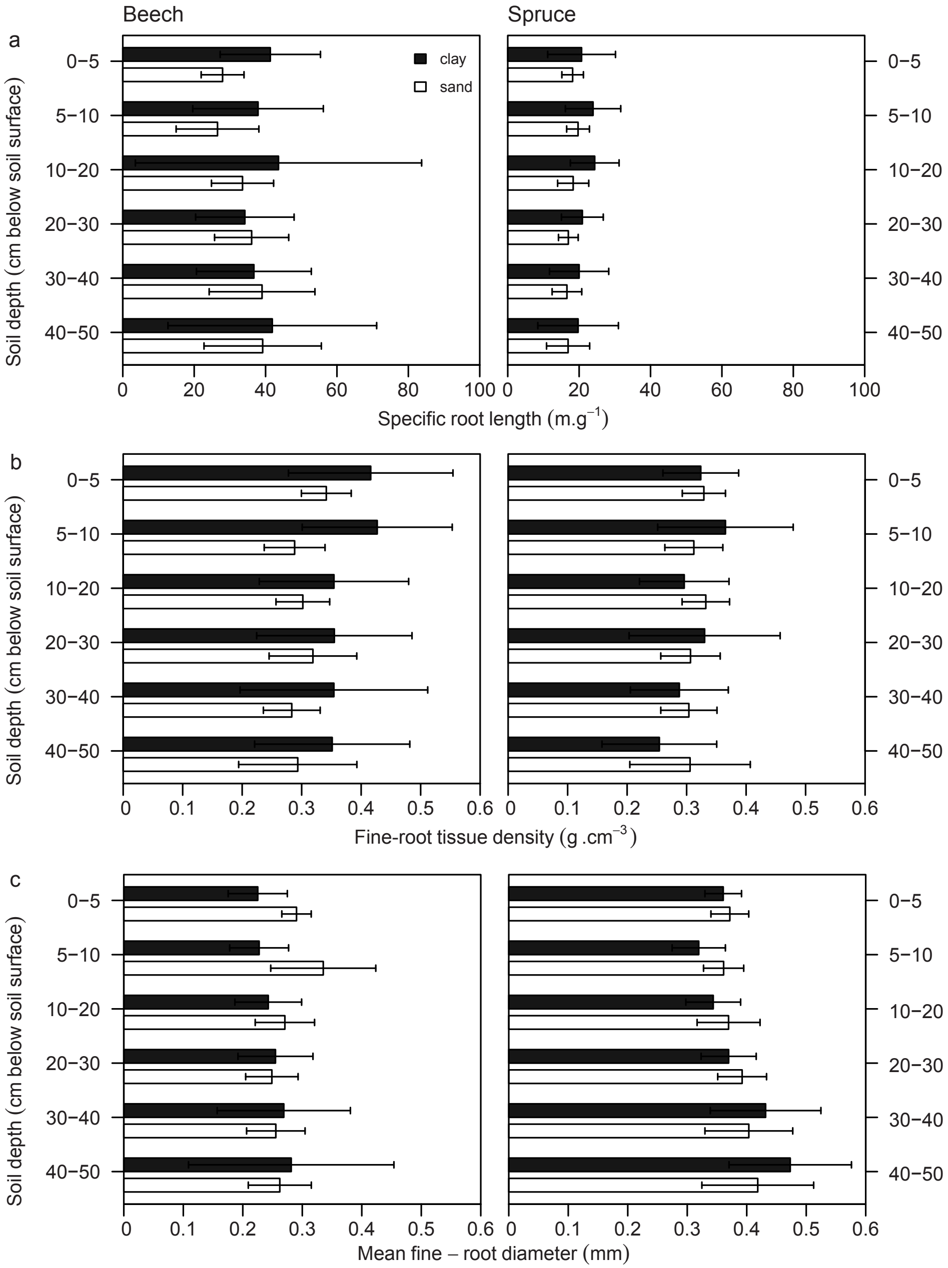
Overall, spruce forests had significantly (c. 1.8 times) more fine-root mass than beech forests, although fine-root length density did not differ between the two species (Figure 4.1a,b). Furthermore, fine-root growth in the ingrowth cores was significantly and almost three times higher for spruce than for beech, but fine-root decomposition rates did not differ between the species (Figure 4.1c,d). Moreover, spruce also had thicker fine roots (i.e. a greater mean fine-root diameter and lower SRL) and higher RTD (Figure 4.1e-g). Mycelium biomass was also significantly (c. 2 times) greater in the spruce than in the beech plots (Figure 4.1h). Interaction effects of species and soil type on fine-root traits measured were not significant with the exception of RTD and fine-root decomposition.

Appendix 4.4 Mixed model statistics on fine-root trait differences within species between soil types (i.e. clay vs. sand), soil layers, and their interactions. Bold *P*-values are significant ($P < 0.05$); $\mathrm{df}_{\mathrm{num}}$, numerator degrees of freedom, $\mathrm{df}_{\mathrm{den}}$, denominator degrees of freedom. Because the use of degrees of freedom and *P*-values in mixed effects models is debated, we also selected the best model based on Akaike information criterion (AIC). The *P*-values of the models reported here agreed with model selection based on AIC and are therefore considered robust.

Species	**Beech**					**Spruce**				
	N	df_{num}	df_{den}	*F*	*P*	*N*	df_{num}	df_{den}	*F*	*P*
Fine-root mass	221					238				
Soil type		1	4	62.0	**0.001**		1	4	50.2	**0.002**
Soil depth		5	174	13.5	**<0.001**		5	188	22.8	**<0.001**
Soil type x depth		5	174	10.3	**<0.001**		5	188	2.3	**0.049**
Fine-root length density	210					220				
Soil type		1	4	46.3	**0.002**		1	4	13.9	**0.020**
Soil depth		5	163	24.1	**<0.001**		5	171	73.6	**<0.001**
Soil type x depth		5	163	2.0	0.077		5	171	1.6	0.164
Fine-root ingrowth	36					36				
Soil type		1	4	20.5	**0.011**		1	4	43.6	**0.003**
Fine-root decomposition	17					15				
Soil type		1	4	0.4	0.543		1	4	23.4	**0.008**
Specific root length	210					219				
Soil type		1	4	0.1	0.767		1	4	6.5	0.063
Soil depth		5	163	0.5	0.766		5	170	4.1	**0.002**
Soil type x depth		5	163	2.2	0.057		5	170	0.4	0.862
Fine-root tissue density	210					219				
Soil type		1	4	8.6	**0.043**		1	4	0.6	0.476
Soil depth		5	163	2.2	0.060		5	170	4.3	**0.001**
Soil type x depth		5	163	0.9	0.455		5	170	1.9	0.097
Fine-root diameter	**209**					**219**				
Soil type		1	4	2.4	0.196		1	4	0.66	0.463
Soil depth		5	162	0.2	0.940		5	170	22.5	**<0.001**

Soil type x depth		5	162	5.1	**<0.001**		5	170	4.4	**<0.001**
Mycelium biomass	**36**					**36**				
Soil type		1	4	2.3	0.206		1	4	68.0	**0.001**

Appendix 4.5 Fine-root morphological trait distribution over the soil profile for beech and spruce stands on clay and sandy soils (means ± 1 standard deviation). Soil depth effects on fine-root traits are described in Appendix 4.3 (between species) and Appendix 4.4 (within-species).



Appendix 4.6 Ectomycorrhizal exploration types.

In addition to our mesh bag analyses, we categorised mycorrhizal root tips into different exploration types (Agerer 2001). Therefore, we sampled fine roots from six soil cores per plot and from three different soil layers (0 – 5, 10 – 20 and 30 – 40 cm depth) in March (spruce) and May (beech) 2014. After carefully washing out and rinsing the fine roots, they were microscopically classified into three exploration types based on fungal morphological properties. Using the identification criteria of Agerer (2001), contact exploration types were characterised by a smooth hyphal mantle with few or no extraradical mycelium or rhizomorphs; short-distance exploration types were identified based on having many emanating hyphae but no rhizomorphs; medium- and long-distance exploration types were characterised by long emanating hyphae and, in some cases, by the presence of rhizomorphs. We estimated the number of root tips that were colonised by these exploration types, the uncolonised root tips and the dead root tips, and calculated their relative abundance on the roots sampled.

For both tree species, we observed only contact exploration types on the roots collected at the clay soil. Due to their smooth hyphal mantle with few extraradical mycelium, they present relatively low carbon costs, but they are also less efficient in terms of nutrient foraging and transfer to the host tree (Agerer 2001). In contrast, on the sandy soils, we also observed short- and medium distance exploration types. With their extraradical mycelia and/or rhizomorphs, they efficiently acquire soil resources, but at higher carbon costs to the host tree (Hobbie and Agerer 2009). Consistent with our mesh bag results and other studies, at more fertile soils the abundance of contact exploration types increased, whereas medium- and long-distance exploration type abundance decreased (Kjøller et al. 2012), and mycelium productivity was reduced (Nilsson and Wallander 2003; Bahr et al. 2013).

5. Understanding fine-root trait effects on tree fitness based on above-belowground trait interactions

Monique Weemstra, Liesje Mommer, Jorad de Vries, Jasper van Ruijven,
Frits Mohren, Frank Sterck

Abstract

Tree fitness is optimised by a functional balance in above- and belowground resource uptake. To this end, trees can adjust their leaf and fine-root traits and regulate resource acquisition. For leaves, such adjustments and their impacts on fitness are consistent across species and environments, but for roots these are still largely uncertain. This study explores the impacts of variation in fine-root mass and specific root length (SRL, fine-root length per fine-root mass) on nutrient acquisition and tree fitness in different nutrient environments.

We used a whole-tree model that simulates tree fitness for different fine-root trait combinations and at different nutrient levels. Through above- and belowground interactions, the model simulates photosynthetic carbon gain, and carbon losses via tissue respiration and turnover at the whole-tree level. The resulting net carbon gain is used as a proxy for fitness. We first varied fine-root mass and SRL at a constant fine-root lifespan and tested their combined effects on tree fitness. Next, we included a trade-off between fine-root lifespan and SRL in the model to examine its consequences for tree fitness. This trade-off implies that high-SRL roots have high turnover, which present carbon replacement costs to the tree. Finally, we lowered the soil nutrient concentrations to study how fine-root traits influenced fitness in a different resource environment.

According to the model, tree fitness increased with fine-root mass. This increase was particularly strong when SRL increased with constant fine-root lifespan. Incorporating the trade-off between SRL and fine-root lifespan showed that a high fine-root mass in combination with low SRL also provides a viable uptake strategy, resulting in similar fitness for different fine-root trait combinations. On nutrient-poor soils, the optimal fitness could be achieved by both an increase in fine-root mass and in SRL. Yet the SRL values required to optimise fitness according to the model, are high compared to previous empirical work. This suggests that the forest soil presents important constraints to, and opportunities for, tree resource uptake, that were absent in the model. This study is a first exploration of how whole-tree models can yield qualitative insights in above- and belowground interactions, but quantitative parameterization and validation of such models require more data.

Key words

Above-belowground interactions, fine-root mass, whole-tree model, fine-root lifespan, specific root length

Introduction

Tree fitness depends on the availability and uptake of light, CO_2, water and nutrients. To optimise fitness, i.e. growth, survival and reproduction, trees maintain a functional balance in the uptake of these different resources, i.e. carbon, water and nutrient acquisition need to be matched in such a way that neither of these resources limits growth (Cannell and Dewar 1994). This implies that trees change their leaf and fine-root traits in order to acquire the resource that is most limiting. Consequently, when light availability decreases, trees generally increase their aboveground uptake area by an increase in total leaf area, or in specific leaf area (SLA, leaf area per leaf mass) to enhance their carbon gain and hence, fitness (Poorter and Nagel 2000; Janse-Ten Klooster et al. 2007; Poorter et al. 2012). Similarly, when soil resources are limiting, trees may increase their fine-root mass and/or specific root length (SRL, fine-root length per fine-root mass) to enhance water and nutrient uptake (Ostonen et al. 2011; Hertel et al. 2013). However, if and how these fine-root traits are adjusted and how this affects fitness is still largely unclear.

The relationships between soil resource availability, fine-root traits and tree fitness have been studied within and across species. Within species, these relationships have often been studied in terms of plasticity, i.e. individuals of the same species adjusting their phenotype to enhance fitness in different environments (Bradshaw 1965; Sultan 2000; Valladares et al. 2007). On poor soils, the expected increase in fine-root mass (Brouwer 1963) and/or SRL (Eissenstat and Yanai 1997) relative to conspecific on rich soils has not been unambiguously confirmed by data (Chapter 4; Fahey and Hughes 1994; Leuschner and Hertel 2003; Finér et al. 2007; Meier and Leuschner 2008; Yuan and Chen 2010; Hertel et al. 2013; Brunner et al. 2015). Across species, the resource economics hypothesis predicts that species with a high SRL acquire resources more rapidly, and therefore grow faster, than species with a low SRL (Reich 2014), but this hypothesis is also not consistently supported (Chapter 3). Neither the plasticity, nor the resource economics approach have thus yielded general insights regarding the role of fine-root traits in tree fitness in different resource environments.

Several issues may contribute to these inconsistent relationships between resource availability, fine-root traits and fitness. First, fine-root traits are not always optimised for resource acquisition alone, because they are also influenced by soil properties not directly related to resource availability, such as mycorrhizal fungi or soil compaction (Chapters 3 and 4). Therefore, variation in e.g. SRL may not directly reflect a plastic response to the soil environment or species' fitness, but a mere response to e.g. soil density. Second, various uptake strategies exist belowground (Chapters 3 and 4). Therefore, fast-growing species do not necessarily require a high SRL to ensure resource supply to the crown. They may also increase their fine-root mass, a trait rarely considered in the context of resource economics (Chapter 3). Third, the impacts of changing fine-root mass or SRL on tree fitness do not only depend on their uptake benefits, but also on their carbon costs which may come at the expense of tree fitness. An increase in fine-root mass requires carbon for the production and respiration of fine roots, and an increase in SRL may shorten fine-root lifespan (Chapter 3; McCormack et al. 2012) so that carbon is more rapidly needed to replace shed roots.

This study aims to unravel the impacts of fine-root traits on nutrient acquisition, aboveground functioning and tree fitness in different nutrient environments. More specifically, we test which combination of fine-root mass and SRL leads to optimal fitness under different soil nutrient conditions. We address our research objective with a mechanistic growth model that integrates below- and aboveground traits and resource uptake, and simulates tree fitness based on their interactions. This model permits us to test fine-root trait effects on fitness at different nutrient levels, while keeping other soil parameters constant, premises difficult to realise in the forest. Furthermore, the model allows us to not only include how these fine-root traits together contribute to uptake, but also to quantify the carbon costs involved in changing these two traits, and determine under which conditions one strategy may be more advantageous to fitness than another.

We therefore coupled roots and nutrient uptake mechanisms to an existing aboveground growth model (Sterck and Schieving 2011; Sterck et al. 2014; Sterck et al. 2016). The model now integrates above- and belowground traits to simulate the net carbon gain, which equals the carbon fixed by photosynthesis minus the carbon that is lost by whole-tree respiration and tissue turnover (i.e. via shedding plant biomass; Figure 5.1). In our simulations, these carbon processes are controlled by fine-root mass, SRL and indirectly fine-root lifespan, and leaf area index (LAI). Fine-root mass and SRL determine water and nutrient uptake, and LAI determines crown photosynthetic carbon gain. These below- and aboveground traits are connected as water and nutrient uptake by fine roots limit LAI and crown photosynthesis, whereas LAI drives the water uptake by the roots by evaporative demand. At the same time, changes in fine-root mass, SRL and LAI incur carbon losses at the whole-tree level. A high fine-root mass and high LAI lead to carbon losses by tissue respiration and turnover; a high SRL may incur carbon losses due to the short lifespan of high-SRL roots.

Here, we explore how trees optimise their fine-root and crown traits such that they achieve maximum net carbon gain, which is considered a proxy for plant fitness. With this model, we test three hypotheses to explore the role of fine-root mass and SRL in tree fitness. First, we expect that the net carbon gain increases with fine-root mass and SRL, as the availability of more water and nutrients will allow higher crown photosynthetic rates. We also expect that this positive effect is stronger for SRL than fine-root mass, because the latter involves greater carbon costs for a given belowground uptake area (due to higher fine-root respiration and turnover costs) as illustrated by Figure 5.1. This first hypothesis assumes a fixed fine-root lifespan, i.e. independent from SRL. This assumption is however challenged in the second hypothesis, where fine-root lifespan varies with SRL. We expect that the trade-off between fine-root lifespan and SRL reduces the net carbon gain for high-SRL roots, because such roots need to be replaced more rapidly and therefore imply additional carbon costs. Third, we hypothesise that trees on nutrient-poor soils will invest in a larger absorptive area by increasing their fine-root mass, SRL, or both.

Model

Our model is based on an existing tree-growth model that calculates daily carbon gain based on aboveground (crown and stem) structure and physiology (Sterck and Schieving 2011; Sterck et al. 2014; Sterck et al. 2016). In this model, crown photosynthesis is calculated from a biochemical photosynthesis model (Farquhar et al. 1980), a stomatal conductance model and water transport model (Tuzet et al. 2003), and scaling procedures from leaf to the whole-tree level (Sterck and Schieving 2011). This model has been previously applied to examine plant aboveground traits, structure and mechanisms along environmental and climatic gradients (Sterck and Schieving 2011; Sterck et al. 2014; Sterck et al. 2016). Here, we focus on incorporating a belowground component into the model that is linked to aboveground functioning, and which together determine tree fitness.

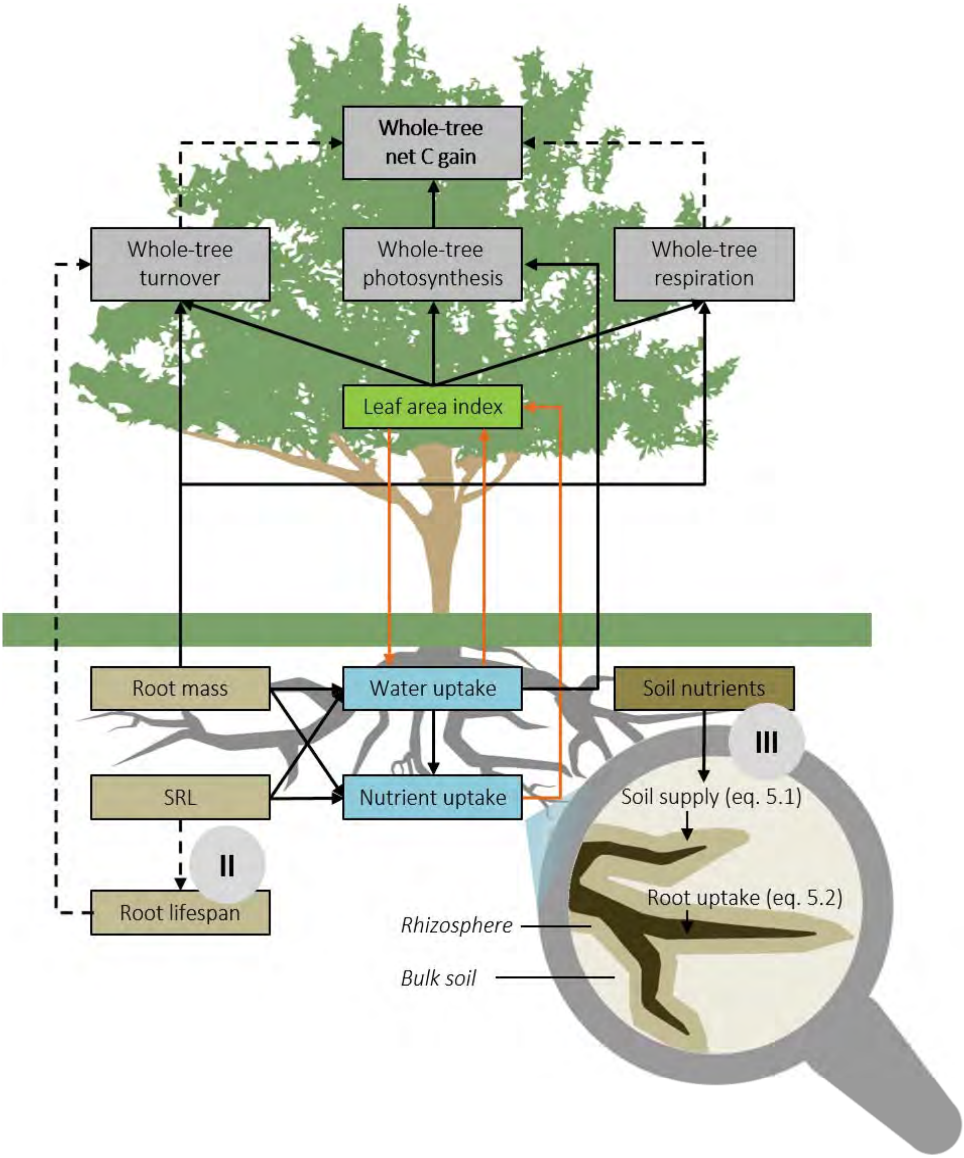


Figure 5.1 Conceptual whole-tree growth model. The whole-tree net carbon gain is calculated based on underlying whole-tree carbon dynamics (i.e. tissue turnover, photosynthesis and respiration; grey boxes) that are in turn determined by interacting crown (i.e. LAI; green box) and fine-root (i.e. mass, SRL, lifespan; light-brown boxes) traits and uptake rates. Uptake of water and nutrients (blue boxes) is determined by the root-absorptive area (reflected by fine-root mass and SRL), and drives the LAI a tree can maintain. Leaf area index determines the amount of carbon gained via photosynthesis. Carbon

losses are driven by fine-root mass, LAI and sapwood mass in stem and coarse roots (via tissue turnover and respiration), and indirectly by SRL when it is negatively correlated to fine-root lifespan (via tissue turnover). Scenario I serves as a base line simulation and builds on the model as a whole, but excludes the negative correlation between SRL and fine-root lifespan; this trade-off is added to the model in scenario II (grey circle); in scenario III, the soil nutrient level in the bulk soil was halved (grey circle). Water availability did not differ in this modelling study. Solid lines indicate positive relationships; dashed arrows represent negative relationships; orange arrows emphasise the above-belowground interactions that are key to the model. The inset shows how fine-root nutrient uptake is modelled (explained in Methods section) as a function of the bulk soil nutrient concentration (soil nutrients, brown box), among others. Note that in the model, the actual shapes of the crown and root system are considered to be cylinders in which leaves and fine roots, respectively, are uniformly distributed.

Whole-tree structure and resource fluxes

Aboveground, the model describes a simplified, three-dimensional tree structure, which consists of a cylindrical crown of given dimensions with leaves distributed homogeneously, and a stem of a given length and sapwood area connected with the leaves. Within the crown, leaves are equally distributed, and their photosynthetic capacities are characterised by an average leaf nitrogen content and SLA. Leaf nitrogen is optimally distributed along the vertical light intensity gradient within the crown, following a big-leaf model approach (Sellers et al. 1992). This simulation model, including the aboveground traits and mechanisms and their effect on the whole-tree carbon balance, has been described in greater detail by Sterck and Schieving (2011), and Sterck et al. (2014, 2016).

Belowground, the fine-root system is considered a cylindrical shape of given dimensions parallel to the crown. In addition, a coarse-root system of given length and sapwood area is connected with the stem base and the fine roots. Analogous to the leaves in the crown, the fine roots are evenly distributed within this root system cylinder. This uniform fine-root distribution is not conform reality where fine roots are heterogeneously distributed over the soil (Jackson et al. 1996), but our simplified system allows an explicit study of the impacts of fine-root traits on resource uptake, the carbon balance, and fitness. Three crucial components have been added to the existent model: 1) resource supply from the bulk soil to the fine roots, 2) resource uptake by the fine roots, and 3) transport of resources from the fine roots to the stem base and ultimately to the leaves.

At the fine-root level, we simulated the supply and uptake of nutrients by separating the soil into two components: the bulk soil and the rhizosphere. Soil nutrient supply is represented by solutes moving from the bulk soil to the rhizosphere, and root nutrient uptake is represented by nutrients moving from the rhizosphere into the fine root (Figure 5.1). The rhizosphere is here represented by a depletion zone set at 1-mm length surrounding the fine-root surface area. In this model, we assume a steady-state in the nutrient concentration in the rhizosphere, so that the nutrient supply rates from the bulk soil equals the nutrient uptake rates by the fine root. We used nitrogen as the model nutrient that is a key component in tree functional processes, e.g. photosynthetic capacity in the leaves and nutrient acquisition capacity in the fine roots, that were both fixed in the simulations.

First, the nutrient flux from the bulk soil to the rhizosphere (that is, soil supply) is determined by diffusion and water mass flow. Diffusion is determined by the area of the rhizosphere ($area_{rh}$), a diffusion coefficient (D_N), the difference between the solute concentration in the bulk soil (S_b) and the rhizosphere (S_{rh}), and the length of the diffusional pathway (L). Mass flow is determined by the water influx (v) and the solute concentration in the bulk soil. Both processes thus drive the nutrient influx rate into the rhizosphere, which was calculated with the following equation:

$$Soil\ supply = area_{rh} * D_N - \frac{(S_b - S_{rh})}{L} + v * S_b \qquad \text{(eq. 5.1)}$$

where the first term describes nutrient influx by diffusion, and the second term describes nutrient influx by mass flow.

Second, the solute flux from the rhizosphere to the fine root (that is, uptake), is described by an adjusted Michaelis-Menten equation. As such, uptake is determined by the root area ($area_r$), the root uptake capacity (V_{max}), the solute concentration in the rhizosphere, and the Michaelis constant (K_m, i.e. the substrate concentration required to achieve 50% of V_{max}). The root area in this equation is described by fine-root mass and SRL that characterise the root absorptive area, and hence, regulate nutrient uptake. Root uptake was calculated with the following equation:

$$Uptake = area_r * \frac{V_{max} * S_{rh}}{S_{rh} + K_m} \qquad \text{(eq. 5.2)}$$

Third, nutrients and water are transferred from the fine roots through the coarse roots and stem to the crown where they drive photosynthetic carbon gain. The above- and belowground plant parts are thus connected via the fluxes of resources. As explained above, the uptake of water and nutrients partly depends on the belowground uptake area of the tree, which is determined by fine-root mass and SRL. These acquired soil resources constrain the LAI and hence, photosynthetic carbon gain, but up to a predefined maximum LAI of 5 (Bréda 2003); greater values were assumed ecologically unviable as too little additional light is intercepted with a greater LAI due to self-shading within the crown (Horn 1971). In turn, the carbon acquired in the crown allows for paying the respiration and turnover costs of different plant parts, and the greater the surplus of acquired carbon, the greater the expected fitness of the tree.

Whole-tree carbon balance

Together, fine-root traits and LAI drive the different components of the whole-tree carbon budget, i.e. photosynthesis, respiration and tissue turnover, in different but interrelated ways (Figure 5.1). First, photosynthesis is directly constrained by LAI: a larger LAI allows greater photosynthetic carbon gain as more light is intercepted in the crown. In turn, the LAI may be limited by nutrient uptake, as leaves require relatively high nutrient levels to maintain photosynthetic capacity. Second, whole-tree respiration is driven by the respiration costs of leaves, sapwood (in the stem and coarse roots) and fine roots. The sapwood respiration was kept constant, and is based on a constant mass-based respiration rate. Fine-root and leaf respiration rate are driven by their respective nutrient contents, which are supposed parts of active proteins involved in nutrient uptake and photosynthesis, respectively. Third, whole-tree tissue turnover represents the total carbon loss per day via shedding of leaves, sapwood of the coarse roots and stem, and fine roots. It is directly determined by tissue mass and tissue lifespan (as explicitly illustrated for fine roots in Figure 5.1). From these integrated carbon-processes based on crown and fine-root properties, the net carbon gain is calculated as photosynthesis – respiration – turnover (kg carbon day^{-1}).

Model parameterization

The key aboveground parameters describing our model tree are presented in Table 5.1. For other aboveground trait values, the model was parameterised as described in Sterck et al. (2016). The belowground structure is defined by the root system radius that is set equal to that of the crown, that is, at 5 m, and a rooting depth fixed at 2 m (Canadell et al. 1996). Within this fine-root system cylinder, we modelled a range of fine-root mass (i.e. 0 – 1 kg fine-root mass m^{-3} soil) and SRL (i.e. 0 – 200 m g^{-1}) values. These values are based on previous work (Chapters 3 and 4), and extended to better demonstrate and understand the underlying mechanisms of the whole-tree carbon balance. The belowground processes that directly contribute to this carbon balance are fine-root respiration and turnover (Figure 5.1). Their respiration and turnover parameters (and those of other plant organs) were obtained from the literature (Appendix 5.3), and summed for all plant organs as whole-tree measures of respiration and turnover. Fine-root respiration rates were based on Reich et al. (2008), but were lower in our model simulations and used to balance the carbon budget and arrive at more realistic carbon balance predictions. Soil resource supply and fine-root uptake parameters are obtained from the literature, but were not completely available for tree species specifically (Table 5.1).

Table 5.1 Key model parameters of above- and belowground traits and soil nutrient availability.

	Value	**Unit**	**Source**
Aboveground traits			
Specific leaf area	200	m^{2} kg^{-1}	Lambers et al. (1998)
Leaf nitrogen content	2	%	Lambers et al. (1998)
Wood density	500	kg m^{-3}	Niklas (1992); van Gelder et al. (2006)
Fine-root traits			
Diameter	0.5	mm	Chapter 4, this thesis
Tissue density	0.3	g cm^{-3}	Chapter 4, this thesis
Fine-root uptake parameters			
Michealis-Menten constant (K_m)	0.2	mol nutrient m^{-3} soil water	Siddiqi et al. (1990)
Fine-root uptake capacity (V_{max})	$10*10^{-6}$	mol nutrient m^{-2} fine-root area s^{-1}	Itoh and Barber (1983)
Soil nutrient parameters			
Diffusion coefficient (D_N)	$10*10^{-10}$	m^{2} s^{-1}	Nielsen (2006)
Soil nutrient content (S_b)	0.5 (I, II) 0.25 (III)	mol nutrient m^{-3} soil	Leeters and Vries (2001)

Latin numbers refer to scenarios when parameters differed between scenarios.

Model simulations

We decided to simulate trees with a steady state in their water and nutrient uptake and loss. This implies that the simulated trees maintain their initial structure. Trees with a negative carbon balance are expected to die, and those with a positive carbon balance are expected to use their carbon for storage, growth or reproduction, which are associated with fitness.

Consequently, transpiration rates in the crown equal the water flow through the stem and the uptake rate of water by the fine roots. In this model, water uptake is regulated by the LAI that represents the evaporative pull, and by the leaf water potential, as stomata are being closed when insufficient water is available to maintain transpiration rates. At the same time, fine-root mass and SRL determine water uptake as a large root-absorptive area allows more rapid water uptake. Earlier work studies the tree water relations with this model (e.g. Sterck et al. 2016), whereas this study focuses explicitly on nutrient uptake and how it constrains plant structure and functioning.

Furthermore, nutrient uptake rates by the fine roots are assumed equal to the nutrient losses via whole-tree tissue turnover. This steady-state in whole-tree nutrients indicates that nutrient uptake by fine roots constrains the LAI, as leaves require nutrients and need to be replaced. We chose to relate root nutrient uptake to the LAI of a tree, representing its *crown* photosynthesis, and not to its photosynthetic capacity at the leaf (mass or area) level.

Hypothesis-testing

First, a sensitivity analysis of our model demonstrates to what extent the whole-tree net carbon gain and its three underlying components (photosynthesis, respiration, turnover) respond to variation in root traits (SRL, fine-root mass, fine-root lifespan, total root system radius, and fine-root tissue density). Next, we determine the (carbon) costs and (uptake) benefits of adjusting SRL and fine-root mass to the soil environment, and explore their impacts on the whole-tree net carbon gain by running three model scenarios corresponding to our three hypotheses: I) fixed fine-root turnover, II) the trade-off between SRL and fine-root turnover, and III) changes in nutrient availability. In scenario I, we examine the combined effects of fine-root mass and SRL on resource uptake and LAI, and ultimately on the net carbon gain and its underlying carbon processes (photosynthesis, respiration and turnover). In this scenario, fine-root lifespan is fixed at 200 days, based on field observations on tree roots reported in the literature (Chapter 3). This scenario enables us to test our first hypothesis that both an increase in fine-root mass and in SRL lead to a higher net carbon gain, but that the impact of increasing SRL would be larger than that of fine-root mass because its carbon costs per absorptive area are lower.

The second scenario tests the effects of the assumed trade-off between SRL and fine-root lifespan on tree fitness by running the model with a variable fine-root lifespan as a function of SRL based on Chapter 3 (Figure 5.1). Comparing the outcomes of scenario I (fixed fine-root lifespan) and II (variable fine-root lifespan) thus demonstrates its impacts on whole-tree performance. Scenario II allows us to test our second hypothesis, where we expect that – compared to the first scenario – the net carbon gain is suppressed by a high SRL due to larger carbon costs via faster fine-root turnover.

In the third scenario, we run the same model as in the second scenario, but reduce the nutrient concentration in the bulk soil with 50% (Table 5.1). Similar to scenario II, fine-root lifespan still varies with SRL. We then compare the outcomes of scenario III (nutrient-poor soil) to those of scenario II (fertile soil) to determine whether fine-root trait effects on fitness change in a different nutrient environment. We test the hypothesis that on a nutrient-poor soil the optimal net carbon gain is achieved by an increase in fine-root mass, SRL, or both.

Results

Sensitivity analysis

The whole-tree net carbon gain was most sensitive to the root system radius (i.e. the radius of the cylinder describing the size of the root system; Table 5.2). Next, it responded strongly to SRL and fine-root mass. These three root traits all positively affected the net carbon gain, whereas fine-root turnover – and to a lesser extent fine-root tissue density – had a negative impact. Whole-tree photosynthesis, respiration and turnover responded similarly (Table 5.2). They showed strongest and positive effects of root system radius, SRL and fine-root mass, and small and negative effects of fine-root tissue density. None of these underlying components responded to fine-root turnover (except for whole-tree turnover rates) because these components were not related in our model structure (Figure 5.1). Overall, this analysis suggests that the fine-root traits considered in our study can influence the whole-tree carbon balance substantially.

Table 5.2 Fine-root trait effects on the whole-tree net carbon carbon balance. Sensitivity values (%) reflect the relative change in the response of the carbon balance components when the explanatory variable (i.e. root trait) changes with 10% and other variables remain constant.

	Root traits (units)				
Sensitivities (%)	**Specific root length ($m\ g^{-1}$)**	**Fine-root mass (kg root m^{-3} soil)**	**Root turnover (d^{-1})**	**Root tissue density (kg root m^{-3} root)**	**Root system radius (m)**
Net carbon gain	**3.69**	**3.08**	**-2.13**	**-0.78**	**6.39**
Photosynthesis	0.70	0.99	0	-0.15	2.10
Respiration	0.45	0.64	0	-0.09	1.35
Turnover	0.10	0.74	0.60	-0.02	1.52

Fine-root trait effects on the whole-tree carbon budget

Scenario I simulated whole-tree carbon processes (i.e. turnover, respiration, photosynthesis and net carbon gain) from the defined range of fine-root mass and SRL values and a constant fine-root lifespan fixed at 200 days. The whole-tree net carbon gain ranged between -0.4 and 0.4 kg carbon d^{-1} (Figure 5.2a). According to our model, it was optimised at relatively high SRL (i.e. more than 100 m g^{-1}) and low fine-root mass (i.e. less than 0.3 kg m^{-3}). Furthermore, the 0-isocline indicated a negative carbon balance for high fine-root mass values (i.e. more than ~0.6 kg m^{-3}), almost regardless of variation in SRL. Whole-tree photosynthesis and respiration increased with fine-root mass and SRL (Figure 5.2d,g), whereas whole-tree turnover increased predominantly with fine-root mass (Figure 5.2j). These impacts of fine-root mass and SRL on the carbon balance were explained by the patterns in LAI (Appendix 5.1a). Increasing SRL and fine-root mass led to higher LAI due to increased soil resource uptake up to a predefined maximum LAI of 5 (see Model section), so that a higher SRL and/or fine-root mass also hardly influenced photosynthesis and respiration beyond this value.

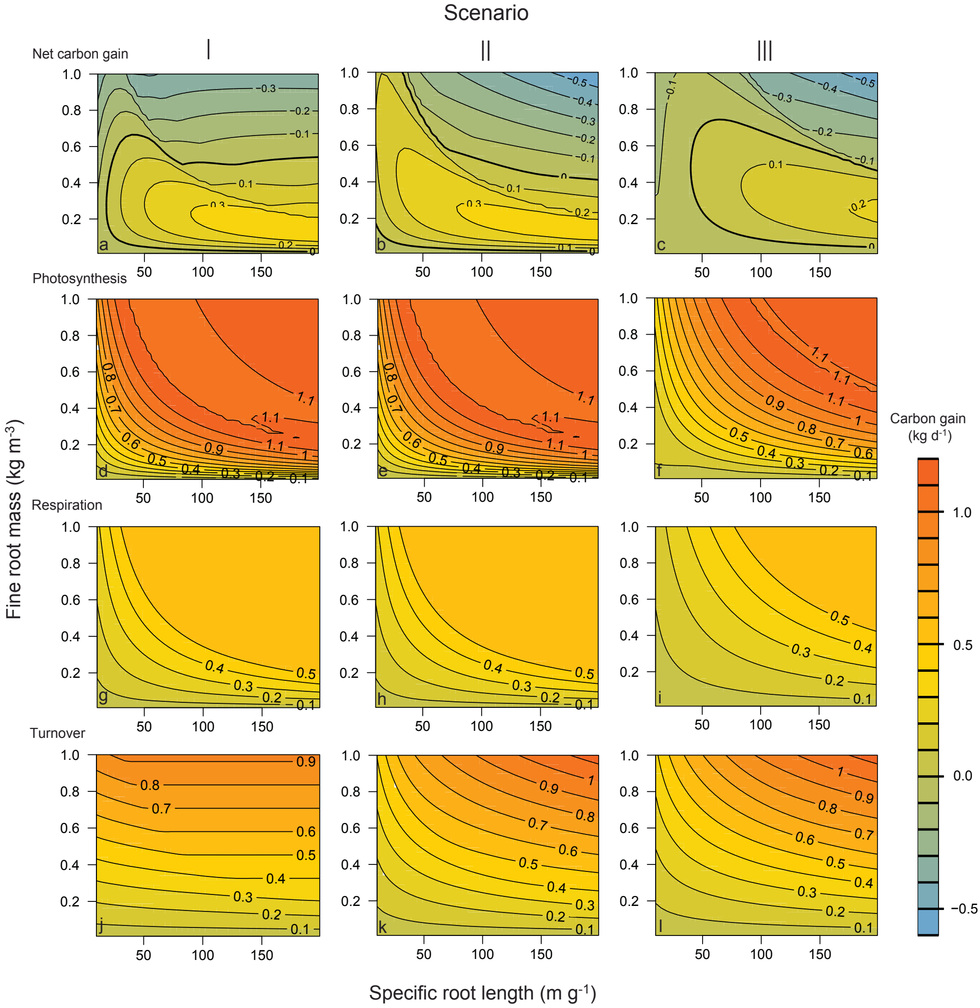


Figure 5.2 Simulated whole-tree responses to fine-root trait variation. Net carbon gain (a – c) and underlying components (d – f, photosynthesis, g – i, respiration, and j – l, turnover) under given ranges of SRL (x-axes) and fine-root mass density (y-axes) for three model scenarios (columns: I, fixed fine-root lifespan of 200 days; II, fine-root lifespan as a function of SRL; III, reduced soil nutrient concentrations). Bold lines (a – c) mark the 0-isoclines where whole-tree carbon gain = carbon loss.

Scenario II equalled scenario I, except that fine-root lifespan was not fixed but depended on SRL. Here, the modelled net carbon gain ranged between -0.6 and 0.4 kg carbon d^{-1} (Figure 5.2b). Similar to the first scenario, the optimal net carbon balance was still achieved at high SRL and low fine-root mass. Compared to scenario I (Figure

5.2a), the optimum moved slightly to the left (i.e. towards lower SRL values, Figure 5.2b) due to the higher turnover rates and associated carbon costs of high SRL-roots. Furthermore, the 0-isocline indicated an additional uptake strategy where trees with a fine-root mass of more than 0.6 kg m^{-3} and a SRL below 70 m g^{-1} also had a positive carbon balance, as opposed to scenario I, where the same root-trait combination resulted in a negative carbon balance. This viable fine-root trait combination resulted from the changes in whole-tree turnover, where trees with low-SRL roots had lower turnover carbon costs (compare Figure 5.2j and k). The trade-off between SRL and fine-root lifespan did not influence whole-tree photosynthesis and respiration (compare Figure 5.2d and e, and 5.2g and h, respectively).

In scenario III, we tested the impacts of soil nutrient availability on the tree carbon balance. We reduced the nutrient concentration in the bulk soil by 50%, and ran the same model as in scenario II. On this nutrient-poor soil, the net carbon balance ranged between -0.5 and 0.2 kg carbon d^{-1}, and was lower than on the more fertile soil of scenario II for given fine-root trait combinations (compare Figure 5.2b and c). The 0-isocline further showed that on the nutrient-poor soil, a SRL lower than 50 m g^{-1} resulted in a negative carbon balance (Figure 5.2c), where the more fertile soil still allowed a positive carbon balance (Figure 5.2b). Furthermore, the optimal net carbon gain shifted further to the right and upwards, indicating that a higher SRL and larger fine-root mass were required to maximise the whole-tree net carbon balance on poorer soils. These patterns resulted from the lower whole-tree photosynthesis and respiration rates owing to a lower LAI that could be supported by the same combination of fine-root mass and SRL on the nutrient-poor compared to the more fertile soil (compare Figure 5.2e and f, and 5.2h and i). Lowering the bulk soil nutrient concentration had a marginal impact on whole-tree turnover (compare Figure 5.2k and l).

Discussion

This study explores the consequences of variation in fine-root mass and SRL for nutrient acquisition, crown structure and the net carbon gain of trees at different nutrient availabilities. The simulated tree fitness could be qualitatively explained by above- and belowground trait interactions and resource loss and uptake within our model. Furthermore, our model simulations demonstrate how the trade-off between SRL and fine-root lifespan gives rise to alternative uptake strategies; trees that invest in fine-root mass have a similar fitness as trees that increase their SRL to enhance nutrient uptake. On nutrient-poor soils, tree fitness benefited from an increase in SRL and in fine-root mass, but for the same root-trait combinations, tree fitness was lower than on the more fertile soils. Here, we discuss our model outcomes and compare with empirical data from Chapters 3 and 4 in order to interpret them in the context of tree fitness. These empirical results represent data on interspecific root-trait variation, resource acquisition and tree growth from a literature review (Chapter 3), as well as data from our field study on intraspecific differences in fine-root traits from a resource-poor sandy soil and a resource-rich clay soil (Chapter 4).

Fine-root trait effects on tree fitness

We first hypothesised that the net carbon gain would increase with both fine-root mass and especially SRL when fine-root lifespan was fixed at 200 days (scenario I). In line with this expectation, the highest net carbon gain was realised at relatively high SRL (higher than 100 m g^{-1}) and low fine-root mass (less than 0.3 kg m^{-3}). High-SRL roots greatly increased the belowground uptake area and therefore led to higher carbon gain in the crown, which is also predicted by the root economics spectrum hypothesis (Reich 2014). At the same time, the carbon costs of high-SRL roots were relatively low and indirectly occurred through a high LAI, which coincided with high leaf respiratory and turnover costs. An increase in fine-root mass also enlarged the belowground uptake area but at a substantial carbon cost to the tree due to higher whole-tree respiration and especially due to higher turnover rates: fine-root turnover accounted for 60% of the whole-tree turnover (Table 5.2). In our model, increasing fine-root mass beyond 0.6 kg m^{-3} was no viable strategy to enhance fitness. From this point onward, the higher photosynthetic carbon gain did not compensate for the increased carbon costs involved in producing and maintaining more fine roots.

Compared to empirical data, the SRL-values leading to maximum fitness we found, are high. In fact, only eight out of 94 temperate tree species included in our literature review (Chapter 3) had a SRL larger than 100 m g^{-1}. Across all these species, the mean SRL was 45 m g^{-1}. In Chapter 4, we found SRL-values below 50 m g^{-1} for *Fagus sylvatica* (L.) and *Picea abies* ((L.) Karst.) (see Figure 5.3a) and so did several other studies for temperate tree species (Bauhus and Messier 1999; Makita et al. 2010; Hertel et al. 2013). In contrast, the fine-root mass values we observed for our two study species

(Chapter 4) did approximately agree with the range of fine-root mass values that would lead to a positive carbon balance in the model (Figure 5.3a). In the field, especially the SRL of temperate tree species may thus be limiting tree fitness as expected from this first model scenario.

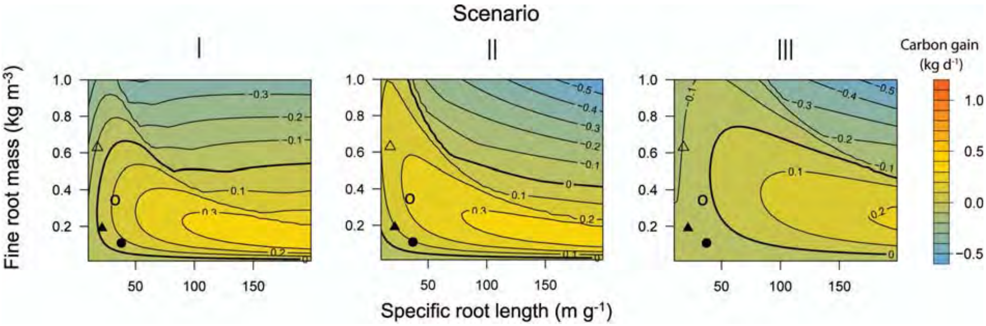


Figure 5.3 *Fagus sylvatica* and *Picea abies* trees in our modelled fitness landscape for three model scenarios. Model scenarios: I, fixed fine-root lifespan of 200 days; II, fine-root lifespan as a function of SRL; III, reduced soil nutrient concentrations). Bold lines mark the 0-isoclines where whole-tree carbon gain = carbon loss. Species' positions are based on empirical fine-root mass and SRL data measured on a resource-rich clay soil and a resource-poor sandy soil in the Netherlands (Chapter 4). Triangles represent *P. abies* trees, circles represent *F. sylvatica* trees, open symbols refer to the sandy soils, black symbols refer to the clay soils.

In fact, the model suggests that especially our study species *P. abies* (Chapter 4) had a negative or neutral carbon balance based on its fine-root mass and SRL on both study sites (Figure 5.3a). In the field though, these studies trees grow and survive. This discrepancy between empirical and model outcomes could point at the importance of mycorrhizal symbiosis for tree nutrition (Smith and Read 2008), as almost all tree species are mycorrhizal. Through their thin hyphae, mycorrhizal fungi strongly enhance the belowground uptake area and therefore nutrient uptake of the tree in exchange for carbon. By comparison, specific hyphal length (i.e. hyphal length per unit hyphal mass, the fungal equivalent of SRL) may range between 20,000 and 40,000 m g^{-1} for ectomycorrhizal fungi associated with temperate tree species (Bakker et al. 2009; Bakker et al. 2015). Investing in mycorrhizal symbiosis may thus reduce the necessity to produce thin, high-SRL roots, and efficiently increase nutrient acquisition, and explain how forest trees grow and survive with thick, less efficient fine roots than expected.

Furthermore, high-SRL roots are in the forest likely constrained by other variables that are absent in the model. Soil properties, such as soil compaction (Clark et al. 2003; Bejarano et al. 2010; Alameda and Villar 2012), drought and herbivory (Pagès 2011) also limit SRL. These factors may thus prevent trees from increasing their SRL, but instead stimulate trees to increase their fine-root mass to enhance resource uptake, despite the higher carbon costs involved. The low SRL that we observed empirically compared to the model, may imply that soil (a)biotic conditions present considerable limits, as well as alternative uptake mechanisms (i.e. mycorrhiza), to SRL and therefore affect nutrient acquisition and tree fitness.

Implications of the trade-off between SRL and fine-root lifespan for tree fitness

Fine-root lifespan has been identified as one of the constraints to SRL. Evidence suggests that carbon costs are involved in producing high-SRL roots because of their shorter lifespan (Chapter 3; McCormack et al. 2012). This trade-off is based on resource optimization which predicts that thick, low-SRL fine roots need to be long-lived in order to balance their uptake benefits and carbon costs (Eissenstat and Yanai 1997). Such low-SRL roots are generally better protected against drought and mechanical pressure and consequently live long (Wahl and Ryser 2000; Clark et al. 2003). In the second scenario, we thus incorporated this trade-off such that fine-root turnover increased with SRL, to test its impacts on the whole-tree carbon balance (Figure 5.1). We expected that the net carbon gain observed in scenario I would be restricted by SRL due to the faster turnover rates of high-SRL roots implemented in scenario II.

Indeed, the trade-off between SRL and fine-root lifespan shifted the net carbon optimum to the left but this shift was only minor. So, even when high-SRL roots had shorter lifespans than low-SRL roots, they were still beneficial in terms of uptake capacities per biomass investment and led to the highest net carbon gain. Because fine-root lifespan thus seemed to pose only a minor constraint to SRL, the optimal SRL in terms of plant fitness (i.e. > 80 m g^{-1}) was still high compared to the empirical data presented earlier; only 18 out of the 94 temperate tree species had a mean SRL higher than 80 m g^{-1} (Chapter 3). This implies that the other (soil) environmental constraints as well as the mycorrhizal alternative to resource acquisition discussed earlier may present stronger limits to SRL than fine-root lifespan does.

The trade-off between SRL and fine-root lifespan revealed an additional root trait niche where different combinations of fine-root mass and SRL resulted in similar fitness (i.e. at the top left of Figure 5.2b). In contrast to scenario I, scenario II shows that trees with a fine-root mass of more than 0.6 kg m^{-3} can also achieve a positive carbon balance when their fine roots are relatively thick (i.e. SRL less than 70 m g^{-1}). These trees have a net carbon gain comparable to trees with higher SRL (i.e. more than 70 m g^{-1}) and lower fine-root mass (i.e. approximately between 0.3 and 0.4 kg m^{-3}). To illustrate, on our clay soils, SRL of *F. sylvatica* was twice as high (40 versus 20 m g^{-1}) and fine-root mass was only 50% (0.1 versus 0.2 kg m^{-3}) compared to *P. abies* (Chapter 4), but in our model, they had a similar net carbon gain of 0.09 kg carbon d^{-1} (Figure 5.3b). These outcomes evidently result from the indirect effect of SRL on whole-tree turnover: as low-SRL roots are long-lived, the replacement costs of the fine-roots are lower, which constitutes a considerable reduction in whole-tree turnover. In line with previous work (Marks and Lechowicz 2006), our study demonstrates that under the same soil nutrient conditions, species with inherently different fine-root traits (e.g. SRL) may perform equally well.

Soil nutrient effects on fine-root traits and tree fitness

Plasticity in above- and belowground functional traits allows plants of the same species to grow, survive and reproduce in different resource environments (Bradshaw 1965; Valladares et al. 2007). Through such plasticity, the most limiting resource can be more rapidly acquired, so that functional balances are restored and plant fitness enhanced. For leaves, common plastic responses have been widely observed (Ryser and Eek 2000; Poorter et al. 2012). Fine-root trait plasticity however is far less consistent, and its impacts on plant fitness in different environments are still uncertain (Sultan 2000; Nicotra et al. 2010). In the third scenario, we simulated a lower soil nutrient concentration compared to the second scenario to test if trees increased their fine-root mass and/or modified their SRL to maintain their fitness on nutrient-poor soils.

Our model suggests that both a high SRL and a high fine-root mass increased fitness on a nutrient-poor soil. In the first place, fitness was optimised by doubling SRL, but at a similar fine-root mass on the poor (scenario III) compared to the more fertile soil (scenario II). So, the minimum SRL needed to optimise fitness increased from 80 to 175 m g^{-1}, corresponding to a fine-root mass of 0.24 and 0.28 kg m^{-3} on the nutrient-poor versus the more fertile soil. As a second strategy, optimal fitness could be realised by doubling fine-root mass at a constant SRL; on the poor soil, a minimum fine-root mass of 0.24 kg m^{-3} was required for an optimal fitness compared to 0.13 kg m^{-3} on the fertile soil. On both soils, however, this required a SRL of 200 m g^{-1} which is very high compared to empirical observations on forest trees. Qualitatively, our model thus identified two alternative strategies that led to equal fitness under nutrient limitations: via an increase in SRL or in fine-root mass. Quantitatively however, especially the limits to SRL under field conditions may need further study to better understand how SRL drives tree uptake and fitness.

The importance of a high SRL on poor soils for plant fitness is in line with our hypothesis and previous work (Ostonen et al. 2007b). However, other studies demonstrated a negative or no response of SRL to the soil resource environment (e.g. Fahey and Hughes 1994; George et al. 1997; Espeleta and Donovan 2002; Leuschner et al. 2004; Ostonen et al. 2007a; Meier and Leuschner 2008). In our empirical plasticity study, SRL also did not differ between *F. sylvatica* and *P. abies* (Chapter 4). Instead, both species strongly increased their (relative) fine-root mass on the poor, sandy soil compared to the rich clay soil (Chapter 4), which corresponds to the second strategy derived from our model.

The model also shows that the fine-root : leaf mass ratio was higher on the poor (scenario III) than on the more fertile soil (scenario II), particularly in the net carbon gain optimum, where it doubled from 2 to 4 (Appendix 5.2b,c). This outcome qualitatively agrees with the functional equilibrium hypothesis (Brouwer 1963). Quantitatively, these modelled fine-root : leaf mass ratios (mostly between 0 and 15) had the same order of magnitude as reported for the total (i.e. including coarse roots) fine-root : leaf mass ratios in temperate forests (that is, 4 – 7; Poorter and Nagel 2000; Poorter et al. 2012). However, our predicted fine-root : leaf mass ratios widely varied for different combinations of fine-root mass and SRL, and were considerably larger on the poor soil at low SRL-values than empirically observed.

Nonetheless, for our study trees, this increase in fine-root mass still resulted in a negative carbon balance according to our model (Figure 5.3c). In this third scenario too, mycorrhizal fungi may explain the differences between model and empirical outcomes. We experimentally measured a strong increase in mycorrhizal mycelium abundance on the sandy soils compared to the clay soils (Chapter 4). Possibly, adding the thin, efficient fungal hyphae we found in the field to the model may shift our study trees towards a positive carbon balance in our fitness landscape.

Whereas the trade-off between SRL and fine-root lifespan introduced a new root-trait niche for optimal growth (compare scenarios I and II), this niche is less pronounced when nutrient levels are reduced (scenario III). Trees with a high fine-root mass and low SRL did not acquire sufficient nutrients from these poor soils to maintain photosynthetic rates, so that the respiration and turnover costs of a large fine-root mass exceeded carbon gain. In fact, the absolute net carbon gain as well as the area that marks a positive carbon balance are reduced compared to scenario II. For example, on the fertile soil (scenario II), a fine-root mass of 0.25 kg m^{-3} and a SRL of 50 m g^{-1} resulted in a net carbon gain of 0.25 kg d^{-1}. On the poor soil (scenario III), the same root-trait combination resulted in a net carbon gain of 0 kg d^{-1}. Similar fine-root trait combinations thus resulted in a lower net carbon gain on the nutrient-poor compared to the more fertile soil, simply because nutrient uptake per root uptake-area is lower.

Perspectives on whole-tree modelling

Our study shows how whole-tree models may yield qualitative, mechanistic insights in tree fitness, but also highlights several focal points for future whole-tree modelling work. In the first place, quantitative tests of these models are still largely constrained by the availability of belowground data. In this study, model parameterization was particularly difficult for root uptake capacity, which is notoriously little quantified, especially for forest trees. More extensive quantification of basic fine-root uptake parameters is paramount. Such quantitative data combined with whole-tree growth models will offer more mechanistic insights in the drivers of belowground traits and their effects at the whole-tree level.

Secondly, the question is which soil (a)biotic properties need to be included in a most parsimonious whole-tree growth model. Forest soils are characterised by large spatial and temporal variation in e.g. the availability of different nutrient elements, soil density, pH, organic matter content, and mycorrhizal fungi. Consequently, roots are heterogeneously distributed throughout the soil too. To specifically study root trait effects on growth, we chose to exclude such soil properties in our model, and assumed a homogeneous distribution of fine roots and soil resources instead. Furthermore, we only studied changes in nutrient availability but not water availability, which may have differential effects on fine-root traits and tree fitness, and may interact with nutrient availability. This way, our model system offers a simplified but more mechanistic perspective on above- and belowground resource uptake and use, and their integrated impact on tree growth. Nevertheless, the discrepancies between modelling and empirical tree root trait expression may partly result from the substantial impact of different soil characteristics on fine-root traits and their distribution throughout the soil.

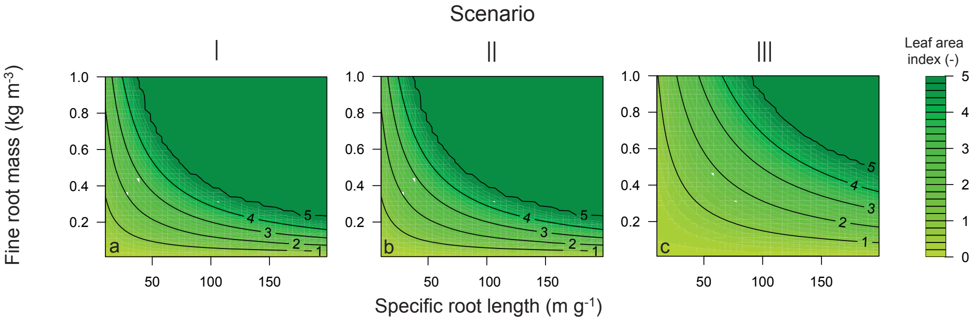
This study particularly identifies mycorrhizal fungi as an important parameter in whole-tree growth models. Brzostek et al. (2014) for instance also demonstrated how including mycorrhizal interactions strongly improved model predictions of the carbon costs of nitrogen uptake across forest sites. Mycorrhizal hyphae could be modelled analogously to fine roots, i.e. with a certain mass density, specific hyphal length, turnover rates, uptake capacity and respiration cost. It should however be considered that the nutritional benefits and carbon costs of mycorrhizal symbiosis to the host tree are still partly uncertain. The construction costs of hyphae have been assumed to be approximately 10% higher than those of roots (Eissenstat 1992), but this strongly differs between fungal species (Agerer 2001; Bidartondo et al. 2001). At the whole-tree level, Hobbie and Hobbie (2008) demonstrated that trees may allocate around 20 % of their net primary productivity to mycorrhizal fungi. However, to what extent these mycorrhizal carbon investments influence the tree's carbon balance is unclear as the carbon-sink strength and therefore photosynthetic rates, may also increase with mycorrhizal carbon requirements (Dosskey et al. 1990; Corrêa et al. 2012). Incorporating mycorrhizal fungi in whole/tree models thus not only requires more data on mycorrhizal traits, but also more functional insights in how they interact with the tree in terms of uptake and carbon costs.

Conclusions

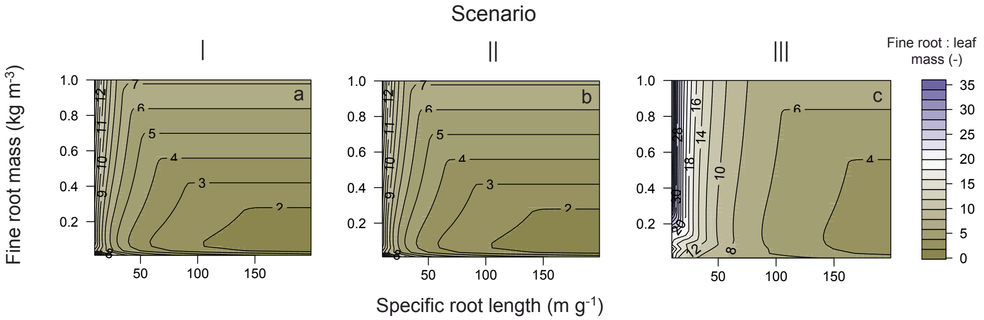
This study explores how nutrient acquisition and tree fitness can be explained by belowground traits using a mechanistic whole-tree model. We examined the interactions between fine-root mass, SRL and fine-root lifespan and determined their integrated impact on fitness. At a first glance, SRL seems a main driver of tree resource acquisition and fitness, even when considering the short lifespan of high-SRL roots. Empirically though, the modelled optimal SRL in terms of fitness seems too high to be realised for many temperate tree species, possibly due to (soil) environmental constraints to SRL. As an alternative strategy, trees may therefore increase their fine-root mass rather than their SRL to enhance fitness, in spite of the (assumed) higher carbon costs involved. Moreover, our model indirectly highlights the importance of mycorrhizal symbiosis for understanding and explaining the nutrition and fitness of large forest trees. In order to grasp inter- and intraspecific variation in tree fitness and environmental site preferences, it is important to recognise that these different belowground uptake strategies can lead to similar performance.

Appendix

Appendix 5.1 Simulated responses in LAI for given ranges of SRL and fine-root mass for three model scenarios. Model scenarios: I, fixed fine-root lifespan of 200 days; II, fine-root lifespan as a function of SRL; III, reduced soil nutrient concentrations).



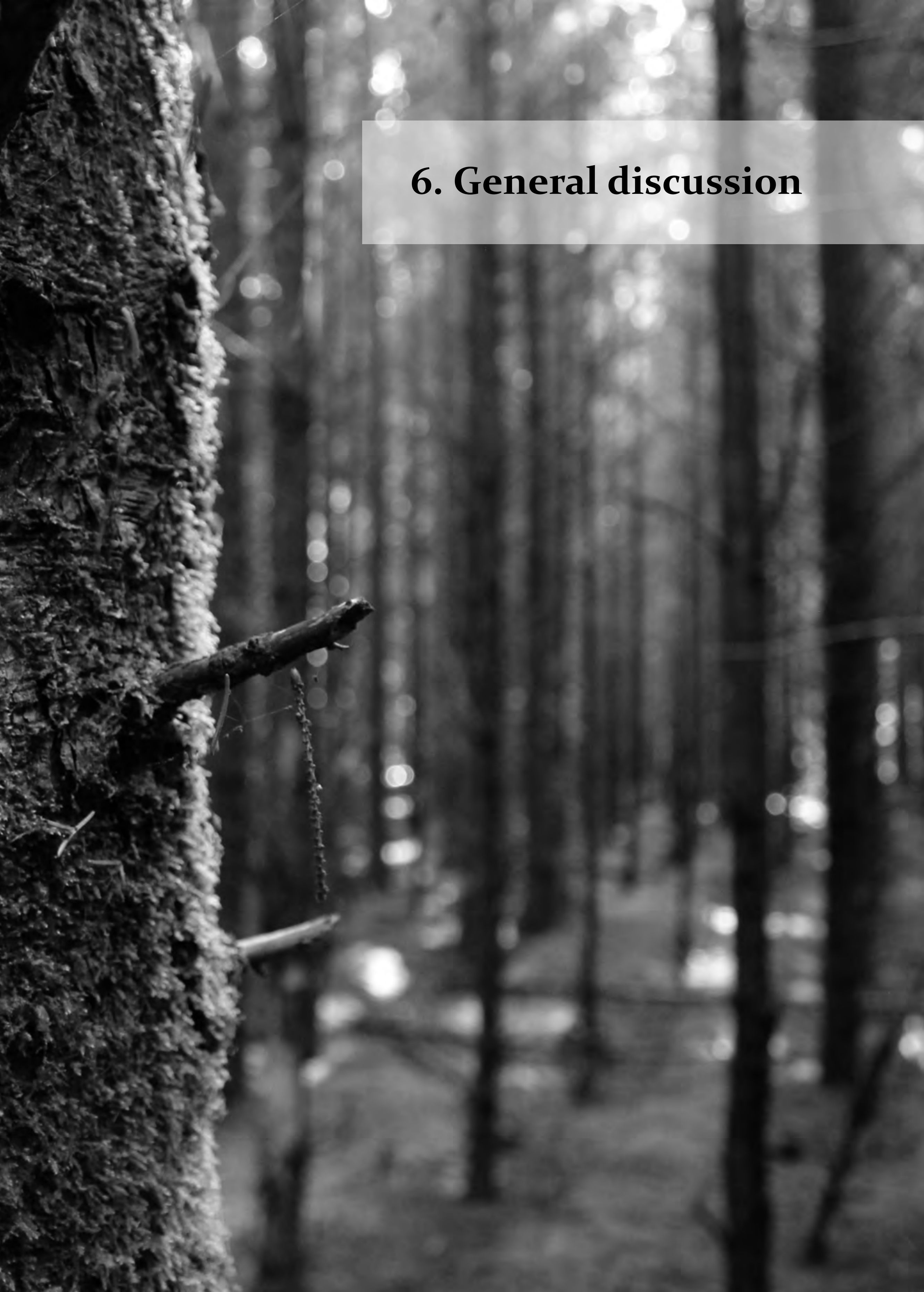
Appendix 5.2 Simulated responses in fine-root : leaf mass ratio for given ranges of SRL and fine-root mass for three model scenarios. Model scenarios: I, fixed fine-root lifespan of 200 days; II, fine-root lifespan as a function of SRL; III, reduced soil nutrient concentrations).



Appendix 5.3 Carbon cost model parameters for different plant organs and sources.

	Value	Source
Turnover rate		
Leaves	0.005 d^{-1}	Sterck and Schieving (2011)
Wood / coarse roots	0.0003 d^{-1}	Sterck and Schieving (2011)
Fine roots	0.005 d^{-1}	Chapter 3, this thesis
Respiration rate		
Leaves	1000 μmol CO_2 kg N^{-1} s^{-1}	Lambers et al. (1998); Sterck and Schieving (2011)
Wood / coarse roots	0.4 μmol CO_2 kg^{-1} s^{-1}	Sterck and Schieving (2011)
Fine roots	100 μmol CO_2 kg N^{-1} s^{-1}	Reich et al. (2008)[1]

[1] Fine-root respiration rates were based on Reich et al. (2008), but were lower in our model simulations and used to balance the carbon budget and arrive at realistic carbon balance predictions.



6. General discussion

Tree growth and resource acquisition

Forests offer a range of ecosystem functions that include the provision of timber and other products, regulation of nutrient, water and carbon cycles, and climate change mitigation by sequestering approximately 45% of globe's terrestrial carbon pool (Bonan 2008). At the same time, climate models predict more frequent, intense and prolonged droughts in Europe that will have negative effects on tree growth and forest productivity (Allen et al. 2010; Lindner et al. 2010; Lindner et al. 2014). These climate effects on tree growth will vary between species as each copes differently with, for example, drought (Bréda et al. 2006). Predicting the climate effects on trees and selecting appropriate species for specific environments could benefit from categorizing species based on their resource use and their growth in different resource environments.

Tree growth results from photosynthetic carbon gain by the leaves, which is enabled by simultaneous uptake of water and nutrients by the tree's fine roots. To ensure sufficient resource acquisition, trees can regulate their above- and belowground resource uptake by adjusting their leaf and fine-root functional traits, respectively. Functional traits are defined as plant properties that determine plant fitness through their impacts on growth, reproduction and survival (Violle et al. 2007). For leaves, functional traits have been successfully linked to light acquisition and tree growth across sites and species (Reich et al. 1997; Wright et al. 2004; Onoda et al. 2011). In contrast, the role of fine-root traits in resource acquisition and tree growth is far less clear and consistent (Mommer and Weemstra 2012). As above- and belowground functional processes (such as resource uptake) are interdependent and together drive tree growth, understanding tree growth requires a whole-tree approach that links these leaf and fine-root interactions. This thesis explores the role of fine-root functional traits in resource uptake, aboveground functioning and, ultimately, tree growth.

Variation in growth and fine-root traits *across* tree species

Tree growth rates vary strongly across tree species, depending on species properties and site conditions. Part of this variation can be explained by the plant strategies of species (Grime 1974). According to this theory, species can be broadly categorised based on their resource use, growth capacities and environmental site preferences. On the one hand, acquisitive species rapidly acquire resources to maintain high growth rates when resources are readily available, but with high mortality rates when resources are scarce. On the other hand, conservative species take up resources at a slower pace but retain them longer within their plant tissue. This enhances their tolerance to low resource levels but comes at the expense of their growth rate (Grime 1974; Aerts and Chapin 2000; Reich et al. 2003; Díaz et al. 2004). This species classification along an acquisitive – conservative axis has proved highly valuable in explaining and understanding plant fitness (Reich et al. 1998; Poorter and Bongers 2006), species' distribution and dynamics (Sterck et al. 2006; Kunstler et al. 2016), and ecosystem processes (Reich et al. 1997; Díaz et al. 2004; Díaz et al. 2007; Grigulis et al. 2013).

In Chapter 2, we first applied the plant strategy theory to explain variation in growth rates and drought sensitivity across 10 temperate broad-leaved tree species (listed in Figure 6.1). We expected that more acquisitive species would grow faster but also suffer more from drought than more conservative species, due to their greater need for water to maintain their high rates of photosynthesis. However, more acquisitive species did not grow faster than conservative species: in fact, nine out of 10 species had remarkably similar growth rates, with only *Populus trichocarpa* growing considerably faster. Moreover, they did not show a stronger growth response to rainwater availability than conservative species. Consistent with our hypothesis, acquisitive species were more sensitive to the depth of the groundwater table than conservative species. When the groundwater table receded, acquisitive species such as *Betula pendula* and *Salix alba* reduced their growth more strongly than conservative species. In addition, some species showed differential growth responses to declines in rainwater and groundwater availability. The growth of *Fagus sylvatica*, for instance, strongly decreased during summers with low rainfall, but was not affected by receding groundwater tables. Drought sensitivity thus differed between species, but only sensitivity to groundwater depth was related to their plant strategy.

We speculated that belowground differences between species might explain the different growth responses to rainwater and groundwater availability. Species exhibit large variation in their fine-root traits, even under homogeneous soil conditions (Withington et al. 2006), and in their rooting depth (Gale and Grigal 1987). This may determine their water (and nutrient) uptake capacity from different soil layers and, hence, their sensitivity to rainwater or groundwater availability. Typical root traits associated with drought tolerance include a high specific root length (SRL, fine-root length per unit of fine-root mass), high fine-root mass and increased rooting depth

(Comas et al. 2014; Brunner et al. 2015). The more drought-sensitive species of our set may have had a low fine-root mass, a low SRL or insufficient rooting depth, which would prevent them from acquiring sufficient water during dry summers.

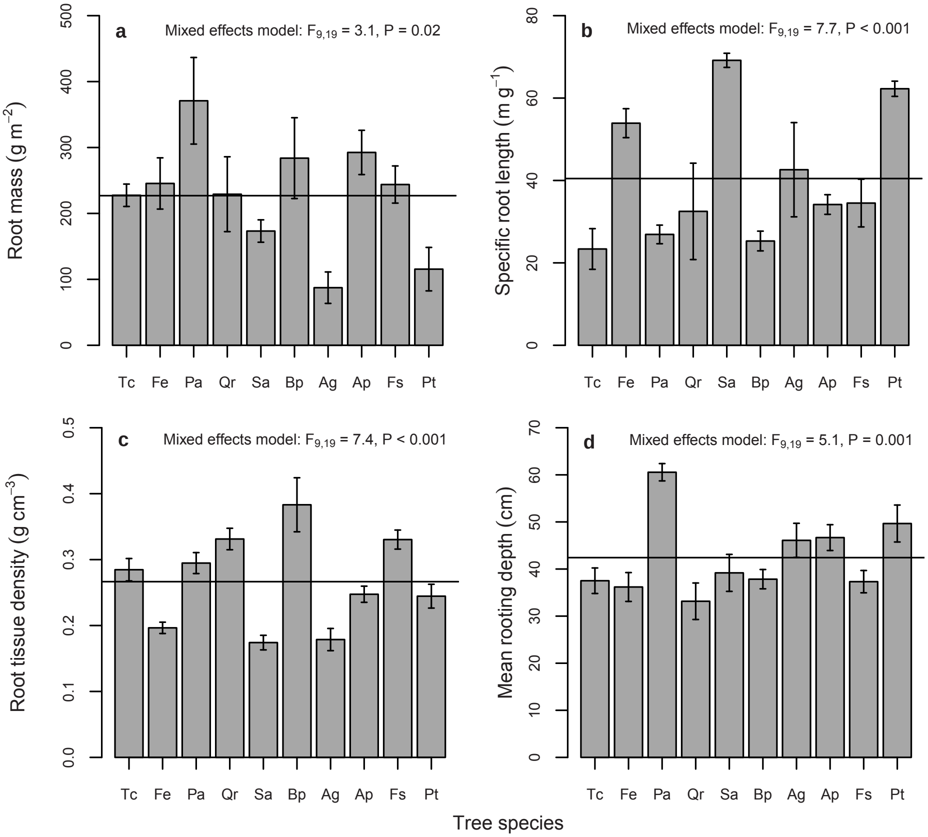


Figure 6.1 Fine-root properties across tree species. Mean ± 1 se (a) total fine-root mass, (b) SRL, (c) tissue density, and (d) mean rooting depth, across 10 tree species: Tc, *Tilia cordata*; Fe, *Fraxinus excelsior*; Pa, *Prunus avium*; Qr, *Quercus robur*; Sa, *Salix alba*; Bp, *Betula pendula*; Ag, *Alnus glutinosa*; Ap, *Acer pseudoplatanus*; Fs, *Fagus sylvatica*; Pt, *Populus trichocarpa*. Species are ordered from least (left) to most (right) sensitive to summer precipitation (Chapter 2). Horizontal lines represent means across all species. Mean rooting depth was calculated as the weighted mean of fine-root biomass in all soil layers (Ravenek et al. 2014). Output of the mixed-effects model represents differences between species, accounting for dependencies between samples collected from the same plot (fixed factor: species, random factor: plot; see also Chapter 4).

To test this hypothesis, we collected, in addition to the results presented in the individual chapters of this thesis, fine-root trait data of the same 10 tree species (see Figure 6.1) on the same site. In short, we collected the fine roots (≤ 2 mm diameter) from three soil cores sampled up to 1 m depth in each of three plots per species on the Hollandse Hout forest site. We compared the fine-root mass, SRL, tissue density and mean rooting depth across the 10 species (see Chapters 2 and 4, and Figure 6.1 for methodological and statistical details). These additional results show that the 10 species differed significantly in fine-root mass, SRL, tissue density and mean rooting depth (Figure 6.1). Across species, fine-root mass was also differently distributed throughout the soil profile. Some species showed a constant proportional fine-root mass over the soil depth measured, whereas others showed an increase or decrease in their proportional fine-root mass with greater soil depth (Figure 6.2).

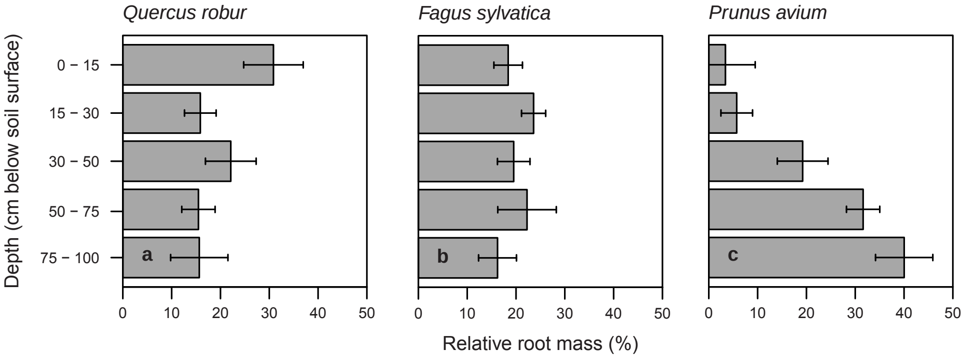


Figure 6.2 Examples of three fine-root mass distribution patterns. Mean fine-root relative mass ± 1 se, (a) decreasing, (b) remaining constant and (c) increasing over 100 cm soil depth. Relative fine-root mass is expressed per soil layer as the percentage of the total fine-root mass per species. Species could be qualitatively grouped in either pattern: *Q. robur* for decreasing proportions of fine-root mass; *F. sylvatica, B. pendula, T. cordata and F. excelsior* for similar proportions of fine-root mass; and *A. glutinosa*, *A. pseudoplatanus, P. avium, P. trichocarpa, S. alba* for increasing proportions of fine-root mass. Note that the depth of the soil layers may differ.

This variation in fine-root mass, SRL and mean rooting depth was, however, not correlated to species' growth rates (Table 6.1). In addition, species with high values of fine-root mass tended to have a lower SRL, and vice versa ($N = 10$, $P = 0.06$, $r = -0.61$). Fine-root mass is often strongly determined by the soil environment (Yuan and Chen 2010) and is, therefore, difficult to compare across species. The soil on the study site at the Hollandse Hout is, however, relatively uniform throughout the forest (Chapter 2), so that overriding soil effects on fine-root mass are minimal. This indicates species may have different strategies to enhance their belowground uptake area (i.e. by either a high fine-root mass or a high SRL), but which resulted in similar growth rates as we observed in Chapter 2.

Table 6.1 Pearson correlations coefficients between fine-root traits and mean rooting depth, on the one hand, and, on the other, tree growth rates, drought sensitivity and shade-tolerance index across the 10 tree species listed in Figures 6.1 and 6.2. Tree growth data are represented by observed mean tree-ring width (Chapter 2); drought sensitivity is represented by the regression coefficients describing the responses of tree-ring width to summer precipitation and groundwater level. More negative regression coefficients indicated greater drought sensitivity (Chapter 2); species' plant strategies are derived from shade-tolerance indices (obtained from Niinemets and Valladares 2006), where light-demanding species have acquisitive strategies and shade-tolerant species conservative ones. Higher plant strategy values represent a more conservative plant strategy and vice versa. For all correlations, $p > 0.05$ and $N = 10$.

	Pearson r			
	Tree growth rate	Drought sensitivity		Plant strategy
		precipitation	groundwater	
Fine-root mass	-0.37	-0.37	0.07	0.44
Fine-root SRL	0.57	0.29	-0.47	-0.60
Fine-root tissue density	-0.06	0.10	0.03	0.22
Mean rooting depth	0.26	0.11	0.06	-0.04

None of these fine-root traits were related to species' drought sensitivity either (Table 6.1). The species most sensitive to summers with low rainfall (*F. sylvatica* and *P. trichocarpa*) or deep groundwater tables (*S. alba* and *B. pendula*) did not have a fine-root mass or SRL lower than those of less-sensitive species. Furthermore, acquisitive species that were severely affected by receding groundwater tables did not have deeper fine roots than more conservative species, which did not respond, or only marginally responded, to groundwater depth. Moreover, *F. sylvatica* (Figure 6.2b) and *S. alba* were sensitive to rainwater deficits but not to groundwater depth; however, they did not have less deep roots than other species. So, unexpectedly, differences in fine-root traits did not explain the variation in growth sensitivity to drought across these 10 species.

These findings suggest that differences in growth rates and drought sensitivity across tree species may not be explained by their plant strategy nor their fine-root traits alone. Instead, they appear to result from both above- and belowground interactions, as trees may not only regulate water supply via their fine roots, but also water loss in the canopy, for example via stomatal control (Bréda et al. 2006), to cope with limited water availability. Moreover, our work shows that trees of different species can realise similar growth rates with considerable variation in fine-root mass, morphology and depth. The aboveground demand for soil resources may thus be met through different uptake strategies belowground. Understanding tree growth seems, therefore, to require a whole-tree perspective, albeit with particular attention being paid to the various strategies available for acquiring soil resources.

A root economics spectrum to explain interspecific variation in fine-root traits?

From a literature review and through meta-level analysis, we also explored whether plant strategies could explain fine-root trait variation across species (Chapter 3). Based on the plant strategy theory, previous studies have established a 'worldwide leaf economics spectrum' (LES; Wright et al. 2004). Acquisitive and conservative strategies are tightly reflected by syndromes of correlated leaf traits (Reich et al. 1992; Reich et al. 1999; Wright et al. 2004). Acquisitive species are generally characterised by leaves with a high specific leaf area (SLA, leaf area per leaf mass), high nitrogen contents and high photosynthetic capacities, which allow fast plant growth. Conservative species, on the other hand, typically have long-lived, sturdy leaves of dense tissue that enhance resource retention and survival rates (Reich et al. 1992; Reich et al. 1997; Wright et al. 2004).

Based on the LES, it is increasingly assumed that a parallel root economics spectrum (RES) links fine-root trait variation to the LES and, ultimately, to plant growth (Reich 2014). The RES is thus expected to consist of correlated fine-root traits associated with either rapid resource acquisition or enhanced resource conservation. The acquisitive syndrome is expected to comprise roots of high SRL with high nutrient concentrations and resource uptake capacities – required to maintain acquisitive leaves with high photosynthetic rates, and rapid tree growth (Reich 2014). Conservative species on the other hand are expected to feature thick, long-lived roots of dense tissue and low respiration rates – to minimise resource losses and enhance tree survival under adverse conditions.

Contrary to our expectations, this thesis shows that an RES probably does not exist. In Chapter 3, we evaluated the assumed correlations among fine-root traits in trait syndromes, and the expected correlations between seemingly parallel fine-root and leaf traits, that have been reported in the literature. Our analysis only confirmed that across species, fine-root lifespan consistently decreased with higher SRL. This trade-off can be explained from a resource optimization perspective: this predicts that fine roots of low SRL need to be long-lived to balance their uptake benefits and carbon costs (Eissenstat and Yanai 1997). We found, however, no consistent support for any of the other hypothesised correlations. Furthermore, for the 10 temperate tree species in our additional data set, fine-root SRL and tissue density were neither related to observed growth rates in the field, nor to their assumed plant strategy (Table 6.1).

We argued (Chapter 3) that a resource economics spectrum does not apply below ground, owing to the fundamental differences between fine roots and leaves. In the first place, fine roots are subject to several environmental drivers, resulting in a large variety of belowground uptake strategies. For example, the uptake of a mobile nutrient such as nitrate may be enhanced by a high SRL, while immobile nutrients such as phosphorous may be more efficiently acquired via mycorrhizal symbiosis (Comas et al. 2012). Second, similar leaf and fine-root traits, such as nitrogen content, function differently. For example, whereas leaf nitrogen content is directly related to photosynthetic capacity, the assumed functional link between fine-root nitrogen content and fine-root uptake capacity has yet to be confirmed. Third, for almost all tree species, mycorrhizal symbiosis in particular presents a crucial strategy for acquiring belowground resources, yet it is not included in the RES. Capturing cross-species variation in fine-root traits and linking it to resource uptake and tree growth thus requires a multidimensional framework that includes additional root traits related to the various uptake mechanisms, and soil environmental drivers.

Recent studies arrived at similar conclusions regarding this multidimensionality, and highlight the additional axes relevant to explaining root-trait variation. Kramer-Walter et al. (2016) concluded that ‘root traits are multidimensional’. Whereas SLA holds a key position in the LES, they demonstrate that variation in SRL is independent of RES and more strongly determined by the soil environment, thus adding an extra dimension to the RES (Valverde-Barrantes et al. 2016a). Incorporating this soil dimension is particularly important for roots because it may influence their traits and resource uptake in different ways. Whereas leaves are mainly adapted to the acquisition of light, roots need to acquire water and minerals from the soil, which may require different root traits (Chapter 3). Soil environment is, thus, an additional, key explanatory dimension in the root-trait framework we proposed in Chapter 3 (Holdaway et al. 2011; Laliberté 2016; Valverde-Barrantes and Blackwood 2016).

Our work further implies that a mere correlative approach is insufficient for understanding above-belowground interactions. Functional balances may be maintained by a multitude of belowground uptake strategies that may be equally successful in terms of resource acquisition (Valverde-Barrantes and Blackwood 2016), but which are not embedded in the RES (Chapter 3; Laliberté 2016). For instance, our earlier analysis of the fine-roots of the 10 tree species at the Hollandse Hout suggests that some species may satisfy a high water and/or nutrient demand in the crown by an increase in fine-root mass rather than SRL; yet fine-root mass is not considered in the RES. The premise that acquisitive leaves require acquisitive roots forms the basis of the RES hypothesis (Reich 2014), and this may still hold. Yet the mechanisms through which soil resources are acquired by fine roots and supplied to the canopy are many and vary across species and sites. Consequently, understanding above-belowground interactions and their impact on tree growth requires a functional, mechanistic approach, rather than one correlating the current set of fine-root and leaf traits.

Variation in fine-root traits and growth *within* species

This thesis examines the impacts of the soil environment on several fine-root traits and associated mycorrhizal fungi *within* tree species. Chapter 4 demonstrates that trees of the same species (i.e. *F. sylvatica* and *Picea abies*) strongly increased their fine-root mass, but did not change their overall fine-root morphology (i.e. SRL, tissue density and mean diameter) when growing on a poor, sandy soil rather than a rich clay soil. These results support the functional equilibrium theory (Brouwer 1963) that predicts a (relative) increase in fine-root mass when soil resource availability is low. Ectomycorrhizal symbiosis also proved an important strategy for enhancing resource uptake, especially for *P. abies*. At the same time, *F. sylvatica* trees did show a morphological shift, but *within* their fine roots: they produced relatively more root length of their finest roots on clay rather than on sandy soils. Our work thus shows the importance of fine-root mass and mycorrhizal fungi, but also the uncertain role of fine-root morphology for acquiring soil resources when these are limiting.

A higher SRL may be more efficient in terms of resource uptake per unit biomass than an increase in carbon allocation to fine-root mass or mycorrhizal fungi. Yet, trees may still favour strategies of the latter type over the first to enhance their belowground uptake area (Chapter 4; Ostonen et al. 2007; Hertel et al. 2013; Freschet et al. 2015 on herbaceous species). Possibly, changes in fine-root SRL are constrained by soil variables not related to resource availability, such as soil compaction (Clark et al. 2003; Bejarano et al. 2010; Alameda and Villar 2012), drought or herbivory (Pagès 2011). Furthermore, roots of high SRL may have a shorter lifespan (Chapter 3), which can induce resource losses via root shedding. Due to such plant or soil constraints to SRL, trees may rely more heavily on other strategies to ensure soil-resource uptake.

That the plasticity of some fine-root traits and the uptake strategies adopted on different soils are species-specific needs to be taken into consideration. This is demonstrated by the greater dependence on mycorrhizal fungi of spruce trees than beech trees on poor soils (Chapter 4), and by the many belowground uptake strategies that exist across species (Chapter 3), and has also been suggested by other studies (George et al. 1997; Eissenstat et al. 2015; Chen et al. 2016). Beech trees adjusted their morphology within the fine-root category, whereas spruce trees did not (Chapter 4). Consequently, for some species, morphological plasticity may occur, but then only for the finest roots, whereas other species invest more in mycorrhizal fungi.

To summarise, our work on root-trait variation *within* species (Chapter 4) reiterates what has been discussed earlier in relation to *across* species (Chapter 3). First, changing fine-root mass and mycorrhizal investment represent important alternative strategies for altering fine-root morphology when soil resource availability is reduced; other strategies are, however, also available and which strategy prevails over another may be species-specific. Second, the functional role and drivers of SRL and other fine-root morphological traits (i.e. tissue density and diameter) are largely unknown, but may not always be related to resource acquisition and availability alone. Third, across- and within-species variation in fine-root traits is not as generic as leaf-trait variation. Belowground functional mechanisms underlying either the resource economics spectrum or trait plasticity can therefore not be deduced from the aboveground mechanisms.

Integrating above- and belowground traits and resource uptake into a whole-tree approach

Exploring the role of fine-root traits in tree growth thus requires a whole-tree approach in which above- and belowground traits and resource exchange are functionally linked and balanced. Both fine-root mass and SRL can play important roles in resource acquisition and, therefore, growth, because they determine the belowground uptake area. Yet changing these traits creates different carbon costs for the tree, which may result in different net impacts on growth. This thesis explores the simultaneous effects of these fine-root traits – weighing their carbon costs and uptake benefits – on resource uptake in the soil and resource supply to the crown, and on tree fitness.

To this end, an aboveground tree growth model (Sterck and Schieving 2011; Sterck et al. 2014; Sterck et al. 2016) was extended with a belowground component (Chapter 5). This extended model integrates above- and belowground traits to simulate whole-tree carbon balance under varying combinations of fine-root mass and SRL. This carbon balance incorporates photosynthesis, tissue respiration and turnover at the whole-tree level. Photosynthetic carbon gain is determined by the leaf area index (LAI) of a tree, and is constrained by water and nutrient uptake by its fine roots. Tissue respiration and turnover result in carbon losses for the tree: a higher fine-root mass and LAI lead to an increase in whole-tree respiration and turnover (i.e. tissue replacement) costs; a high SRL may increase turnover costs because it decreases fine-root lifespan (Chapter 3; McCormack et al. 2012). From these carbon processes, the tree's net carbon gain is calculated as the photosynthetic carbon gain minus carbon losses due to respiration and turnover. The net carbon gain can be used as a proxy for tree fitness: high net carbon gain allows a tree to store more carbon, grow more rapidly, or produce more fruits, which are all considered components of fitness.

According to the model, trees or species with a relatively low fine-root mass and a high SRL have the highest net carbon gain (Chapter 5). Under these trait combinations, the turnover and respiration costs of fine-root mass are kept within viable limits, whereas belowground resource uptake is increased due to a high SRL. Consequently, soil resources can be efficiently transported to the crown, where they support high photosynthetic rates. Qualitatively, the expected relationships between fine-root mass, SRL and tree fitness can thus be mechanistically explained with our model. Quantitatively, high values of SRL that result in the greatest fitness are rarely observed in temperate forests: for most temperate tree species, SRL is lower than 100 m g^{-1}, and for many even lower than 50 m g^{-1} (Chapters 3 and 4). For the trees we studied in Chapter 4, i.e. *P. abies and F. sylvatica*, SRL ranged between 18 and 22 m g^{-1} and 35 and 40 m g^{-1}, respectively. The constraints to SRL previously discussed may explain the discrepancy between the optimal SRL predicted by the model and the SRL observed in the field.

Our model outcomes are in line with the RES hypothesis, which predicts high growth rates for species with a high SRL (Reich 2014). Nevertheless, we found no consistent empirical support for this relationship between SRL and growth rate across species (Chapter 3). In the field, water and nutrient supply for photosynthetic gain may be enhanced by an increase in fine-root mass when SRL is otherwise constrained, for instance by fine-root lifespan. In fact, after incorporating the trade-off between SRL and fine-root lifespan, our model revealed two strategies that led to similar fitness under the same site conditions: (1) a high SRL (and low fine-root lifespan) combined with a low fine-root mass; and (2) a low SRL (and a high fine-root lifespan) combined with a high fine-root mass (Chapter 5). Based on 34 leaf, root and stem functional traits, Marks and Lechowicz (2006) have shown that many alternative functional designs exist among species, and that these ultimately lead to similar levels of fitness under the same environment. In a similar vein, we suggest in this thesis that under the same soil nutrient environment, species exhibiting different fine-root trait combinations may perform equally well in terms of fitness.

Moreover, the root-trait strategy applied may be species-dependent, This is suggested by the trade-off between fine-root mass and morphology we found across the 10 tree species studied in the forest at the Hollandse Hout: some species had a high fine-root mass and others had fine roots with high SRL values (Figure 6.1a,b), but they grew equally fast under similar soil conditions (Chapter 2). Our findings imply that, for example, fine-root morphology alone does not indicate an acquisitive or conservative resource strategy, nor does it explain species' differences in growth rates. Various uptake strategies and corresponding root traits, such as fine-root mass, need to be considered when linking fine-root traits to uptake and tree growth or fitness.

These alternative root-trait strategies could also be observed when soil nutrient availability was lowered in our model. On nutrient-poor soil, fitness was optimised by doubling SRL for similar values of fine-root mass, or by doubling fine-root mass in combination with constant SRL, when compared with more fertile soil (Chapter 5). Both strategies are expected to increase soil resource uptake on poor soils (Brouwer 1963; Eissenstat 1992; Eissenstat and Yanai 1997). However, our plasticity study shows that *P. abies* or *F. sylvatica* trees on poor soils increased their fine-root mass but did not change their SRL relative to their SRL on a rich soil (Chapter 4). Mycorrhizal symbiosis may explain why trees do not produce the thin roots that our model predicts: for both species, mycorrhizal mycelia were more abundant on the poor rather than the rich soils. Due to their great length for a given biomass (e.g. 20,000–40,000 m g^{-1} reported by Bakker et al. (2009) and Bakker et al. (2015)), mycorrhizal mycelia are highly efficient in the uptake of soil resources. As such, they may compensate for the relatively low SRL observed in the field (compared to our model) and supply ample resources to trees with thick fine roots and high fine-root mass.

This thesis shows how a modelling approach for studying tree fitness can contribute to understanding the above- and belowground interactions that underpin fitness. We demonstrate how different fine-root trait combinations lead to similar degrees of tree fitness and suggest that these strategies may be species-specific. Yet this thesis also stresses the importance of plant (e.g. fine-root lifespan) and soil biotic (e.g. mycorrhizal symbiosis) and abiotic (e.g. soil density) constraints for expressing fine-root traits that may be optimal for resource uptake and growth but unattainable in the field. Quantitatively, there is, however, a great need for more fine-root and mycorrhizal data, especially their uptake capacities to further parameterise and improve such models.

General conclusion: The role of fine-root traits in tree growth

This thesis addresses the question of how fine-root functional traits relate to tree growth from several approaches: within and across tree species, based on empirical field work, a literature review and a model, and at different scales: from the fine root to the whole tree.

Through these approaches, this thesis first shows that across and within species, SRL does not play the key role in belowground resource uptake and tree growth that SLA does above ground. The forest soil may present such strong constraints to SRL that trees on a resource-poor soil do not necessarily have a higher SRL than conspecifics on a resource-rich soils. Nor do species with a high SRL necessarily acquire resources or grow faster than species with a low SRL. These environmental constraints are much stronger for roots than for leaves, because fine-root traits are optimised to function within a far more diverse environment, where they have to acquire various resources, interact with different soil biota, and are subject to soil physical and chemical properties (Fig 3.3). As a result, trees and species rely on various belowground uptake strategies to ensure resource uptake and growth in different environments. Conceptually, this implies that a given fine-root trait, such as SRL, cannot be *a priori* interpreted as a proxy of either an acquisitive or conservative plant strategy. Rather, its function may be a mere consequence of the site conditions.

Fine-root mass is a highly important trait to consider when studying the plasticity of trees, but even more so when investigating the ecological strategies across species, because this trait is not often considered in this context. An increase in fine-root mass may be more carbon-expensive than an increase in SRL in terms of uptake area. However, it can still be the most viable uptake strategy in given environments (e.g. with compact soils where an increase in SRL may not be possible) or for certain species, as I have shown in this synthesis across our 10 temperate tree species. In fact, I speculate that these two strategies – i.e. mass versus morphology – may actually be traded off, with certain species favoring the first strategy, and others the latter. Although this hypothesis requires further testing, this species' separation could lead to selecting species that contribute to belowground carbon sequestration based on their root-trait strategy. Our model further suggested that such different root-trait strategies can result in equal fitness, which could explain the large divergence in fine-root traits across species under similar site conditions.

Besides fine-root mass and morphology, this thesis identifies mycorrhizal fungi as key players in the relationships between soil resources, roots and tree growth, both within and across species. Mycorrhizal symbiosis proved to be a highly relevant mechanism for trees having to cope with low soil-resource availability, although its importance may differ between species and sites. It provides an explanation as to why trees in the forest rarely produce the highly efficient, high-SRL roots through which their resource uptake and fitness could be theoretically optimised. Besides serving as an essential uptake mechanism for most tree species, they also directly influence fine-root functional traits such that root traits *alone* cannot be classified as acquisitive or conservative. This thesis thus stresses that mycorrhizal symbiosis should be explicitly included in studies that examine variation in tree roots, resource uptake and tree growth across and within species.

Selecting species that, based on their ecological strategies, will be appropriate for our future forests is a challenging undertaking. As this thesis shows, fine roots play a pivotal role in determining the fitness of species under different environmental conditions, but still in concert with aboveground traits and functioning. Understanding the fitness of species in a changing world therefore requires **a whole-tree approach;** here, I stress that this warrants a specific focus on belowground functioning, as a whole-tree approach is particularly limited by our functional understanding of and availability of data on fine roots. Furthermore, species have adopted various **belowground uptake strategies that may lead to equal fitness**. This prevents us from classifying them along a resource economics spectrum, and instead, calls for a **multidimensional framework** for exploring the links between belowground resource availability, traits and tree growth (Figure 3.3).

In this respect, **fine-root mass** and **mycorrhizal symbiosis** seem critical traits for further study *across* species. These traits may be considered to be alternative resource-acquisition strategies for SRL in certain species. A better understanding of their role in resource uptake and tree growth across species would be particularly relevant in the light of climate change, which may lead to more frequent and intense droughts. Such alternative root-trait strategies can also contribute to characterising species based on their belowground carbon storage potentials, which may be higher for species that allocate carbon to fine-root or mycorrhizal biomass, than for species that increase their SRL instead. Yet although the role of fine-root traits in tree growth is complex and context-dependent, recent interest in the functional traits of roots offers an encouraging prospect for the further unravelling of these relationships.

Acknowledgements

I gratefully acknowledge the contribution of Natasa Kiorapostolou and Leo Goudzwaard to collecting the fine-root trait data for our 10 study species that were used in this synthesis. Jos Rutten and Leo Smits of the Dutch State Forestry Service kindly allowed us to collect these data in the Hollandse Hout.

Literature

Adams TS, McCormack ML, Eissenstat DM (2013) Foraging strategies in trees of different root morphology: The role of root lifespan. Tree Physiol 33:940–948.

Aerts R, Chapin F (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67.

Agerer R (2001) Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114.

Aguilar-Trigueros CA, Hempel S, Powell JR, et al (2015) Branching out: Towards a trait-based understanding of fungal ecology. Fungal Biol Rev 29:34–41.

Alameda D, Villar R (2012) Linking root traits to plant physiology and growth in Fraxinus angustifolia Vahl. seedlings under soil compaction conditions. Environ Exp Bot 79:49–57.

Alcamo J, Moreno JM, Nováky B, et al (2007) Europe. In: Parry ML, Canziani OF, Palutikof JP, et al. (eds) Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, pp 541–580

Allen CD, Macalady AK, Chenchouni H, et al (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684.

Alterra (n.d.) Bodemdata [online]. http://www.bodemdata.nl. Accessed 15 Jan 2012

Andreu L, Gutiérrez E, Macias M, et al (2007) Climate increases regional tree-growth variability in Iberian pine forests. Glob Chang Biol 13:804–815.

Bahr A, Ellström M, Akselsson C, et al (2013) Growth of ectomycorrhizal fungal mycelium along a Norway spruce forest nitrogen deposition gradient and its effect on nitrogen leakage. Soil Biol Biochem 59:38–48.

Bakker MR, Delerue F, Andreasson F, et al (2015) Hyphal growth in ingrowth mesh bags in Fagus sylvatica, Quercus petraea and Pinus pinaster stands in France. Eur J Soil Biol 70:111–117.

Bakker MR, Jolicoeur E, Trichet P, et al (2009) Adaptation of fine roots to annual fertilization and irrigation in a 13-year-old Pinus pinaster stand. Tree Physiol 29:229–238.

Bardgett RD, Mommer L, de Vries FT (2014) Going underground : root traits as drivers of ecosystem processes. Trends Ecol Evol 29:692–699.

Bauhus J, Messier C (1999) Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. Can J For Res 29:260–273.

Bejarano MD, Villar R, Murillo AM, Quero JL (2010) Effects of soil compaction and light on growth of Quercus pyrenaica Willd. (Fagaceae) seedlings. Soil Tillage Res 110:108–114.

Berta G, Trotta A, Fusconi A, et al (1995) Arbuscular mycorrhizal induced changes to plant growth and root system morphology in Prunus cerasifera. Tree Physiol 15:281–293.

Bidartondo MI, Ek H, Wallander H, Söderström B (2001) Do nutrient additions alter carbon sink ectomycorrhizal fungi? New Phytol 151:543–550.

Bijlsma RJ, Verkaik E (2008) De spontane ontwikkeling van een jong bos op zeeklei (bosreservaat Hollandse Hout, Oostelijk Flevoland); een analyse voorafgaand aan de aantakking van de Hollandse Hout op de Oostvaardersplassen. Alterra, Wageningen, The Netherlands

Birhane E, Kuyper TW, Sterck FJ, et al (2015) Arbuscular mycorrhiza and water and nutrient supply differently impact seedling performance of dry woodland species with different acquisition strategies. Plant Ecol Divers 8:387–399.

Bolte A, Ammer C, Löf M, et al (2009) Adaptive forest management in central Europe: Climate change impacts, strategies and integrative concept. Scand J For Res 24:473–482.

Bonan GB (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320:1444–9.

Bradshaw A (1965) Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13:115–155.

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann For Sci 63:625–644.

Bréda NJJ (2003) Ground-based measurements of leaf area index: a review of methods, instruments and current controversies. J Exp Bot 54:2403–17.

Broadmeadow MSJ (2005) Climate change and the future for broadleaved tree species in Britain. Forestry 78:145–161.

Brouwer R (1963) Some aspects of the equilibrium between overground and underground plant parts. Jaarb van het Inst Voor Biol en Scheikd Onderz aan Landbouwgewassen 31–39.

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304.

Brunner I, Bakker MR, Björk RG, et al (2012) Fine-root turnover rates of European forests revisited: an analysis of data from sequential coring and ingrowth cores. Plant Soil 362:357–372.

Brunner I, Herzog C, Dawes MA, et al (2015) How tree roots respond to drought. Front Plant Sci 6:547.

Brzostek E, Fisher J, Phillips R (2014) Modeling the carbon cost of plant nitrogen acquisition: Mycorrhizal trade-offs and multipath resistance uptake improve predictions of retranslocation. J Geophys Res Biogeosciences 119:1684–1697.

Bunn AG (2008) A dendrochronology program library in R (dplR). Dendrochronologia 26:115–124.

Canadell J, Jackson RB, Ehleringer JB, et al (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia 108:583–595.

Cannell M, Dewar R (1994) Carbon allocation in trees: a review of concepts for modelling. Adv Ecol Res 25:59–104.

Chave J, Coomes D, Jansen S, et al (2009) Towards a worldwide wood economics spectrum. Ecol Lett 12:351–66.

Chen HYH, Brassard BW (2013) Intrinsic and Extrinsic Controls of Fine Root Life Span. CRC Crit Rev Plant Sci 32:151–161.

Chen W, Koide RT, Adams TS, et al (2016) Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. Proc Natl Acad Sci 113:8741–8746.

Chen W, Zeng H, Eissenstat DM, Guo D (2013) Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. Glob Ecol Biogeogr 22:846–856.

Choat B, Jansen S, Brodribb TJ, et al (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–5.

Clark LJ, Whalley WR, Barraclough PB (2003) How do roots penetrate strong soil? Plant Soil 255:93–104.

Comas LH, Bouma TJ, Eissenstat DM (2002) Linking root traits to potential growth rate in six temperate tree species. Oecologia 132:34–43.

Comas LH, Callahan HS, Midford PE (2014) Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. Ecol Evol 4:2979– 2990.

Comas LH, Eissenstat DM (2004) Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. Funct Ecol 18:388–397.

Comas LH, Eissenstat DM (2009) Patterns in root trait variation among 25 co-existing North American forest species. New Phytol 182:919–28.

Comas LH, Mueller KE, Taylor LL, et al (2012) Evolutionary Patterns and Biogeochemical Significance of Angiosperm Root Traits. Int J Plant Sci 173:584–595.

Coomes DA, Kunstler G, Canham CD, Wright E (2009) A greater range of shade-tolerance niches in nutrient-rich forests: an explanation for positive richness-productivity relationships? J Ecol 97:705–717.

Cornelissen J, Aerts R, Cerabolini B, et al (2001) Carbon cycling traits of plant species are linked with mycorrhizal strategy. Oecologia 129:611–9.

Cornelissen JHC, Sass-Klaassen U, Poorter L, et al (2012) Controls on coarse wood decay in temperate tree species: birth of the LOGLIFE experiment. Ambio 41 Suppl 3:231–45.

Corrêa A, Gurevitch J, Martins-Loucao MA, Cruz C (2012) C allocation to the fungus is not a cost to the plant in ectomycorrhizae. Oikos 121:449–463.

Courty P-E, Buée M, Diedhiou AG, et al (2010) The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biol Biochem 42:679–698.

Craine J (2009) Resource strategies of wild plants. Princeton University Press, Princeton

Craine J, Lee W, Bond W (2005) Environmental constraints on a global relationship among leaf and root traits of grasses. Ecology 86:12–19.

De Boever L, Vansteenkiste D, Van Acker J, Stevens M (2007) End-use related physical and mechanical properties of selected fast-growing poplar hybrids (Populus trichocarpa× P. deltoides). Ann For Sci 64:621–630.

Díaz S, Hodgson JG, Thompson K, et al (2004) The plant traits that drive ecosystems: Evidence from three continents. J Veg Sci 15:295.

Díaz S, Lavorel S, de Bello F, et al (2007) Incorporating plant functional diversity effects in ecosystem service assessments. Proc Natl Acad Sci U S A 104:20684–20689.

Dittmar C, Zech W, Elling W (2003) Growth variations of Common beech (Fagus sylvatica L.) under different climatic and environmental conditions in Europe—a dendroecological study. For Ecol Manage 173:63-78.

Dosskey M, Linderman R, Boersma L (1990) Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. New Phytol 115:269–274.

Eilmann B, Zweifel R, Buchmann N, et al (2009) Drought-induced adaptation of the xylem in Scots pine and pubescent oak. Tree Physiol 29:1011–1020.

Eilmann B, Zweifel R, Buchmann N, et al (2011) Drought alters timing, quantity, and quality of wood formation in Scots pine. J Exp Bot 62:2763–2771.

Eissenstat D (2002) Root structure and function in an ecological context. New Phytol 148:353–354.

Eissenstat D, Yanai R (1997) The ecology of root lifespan. Adv Ecol Res 27:1–59.

Eissenstat DM (1991) On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. New Phytol 118:63–68.

Eissenstat DM (1992) Costs and benefits of constructing roots of small diameter. J Plant Nutr 15:763–782.

Eissenstat DM, Achor DS (1999) Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. New Phytol 141:309–321.

Eissenstat DM, Kucharski JM, Zadworny M, et al (2015) Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytol 208:114–124.

Ekblad A, Wallander H, Godbold DL, et al (2013) The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. Plant Soil 1–27.

Espeleta JF, Donovan LA (2002) Fine root demography and morphology in response to soil resources availability among xeric and mesic sandhill tree species. Funct Ecol 16:113–121.

Evans J, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24:755–767.

Fahey TJ, Hughes JW (1994) Fine Root Dynamics in a Northern Hardwood Forest Ecosystem, Hubbard Brook Experimental Forest, NH. J Ecol 82:533–548.

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149:78–90.

Fekedulegn D, Hicks RR, Colbert J. (2003) Influence of topographic aspect, precipitation and drought on radial growth of four major tree species in an Appalachian watershed. For Ecol Manage 177:409–425.

Fernandez CW, Kennedy PG (2015) Moving beyond the black-box: fungal traits, community structure, and carbon sequestration in forest soils. New Phytol 205:1378–1380.

Fernandez CW, McCormack ML, Hill JM, et al (2013) On the persistence of Cenococcum geophilum ectomycorrhizas and its implications for forest carbon and nutrient cycles. Soil Biol Biochem 65:141–143.

Finér L, Helmisaari H-S, Lõhmus K, et al (2007) Variation in fine root biomass of three European tree species : Beech (Fagus sylvatica L.), Norway spruce (Picea abies L. Karst.), and Scots pine (Pinus sylvestris L.). Plant Biosyst - An Int J Deal with all Asp Plant Biol 141:394–405.

Finér L, Ohashi M, Noguchi K, Hirano Y (2011) Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics. For Ecol Manage 262:2008–2023.

Freschet GT, Bellingham PJ, Lyver PO, et al (2013) Plasticity in above- and belowground resource acquisition traits in response to single and multiple environmental factors in three tree species. Ecol Evol 3:1065–1078.

Freschet GT, Cornelissen JHC, van Logtestijn RSP, Aerts R (2010) Evidence of the “plant economics spectrum” in a subarctic flora. J Ecol 98:362–373.

Freschet GT, Swart EM, Cornelissen JHC (2015) Integrated plant phenotypic responses to contrasting above- and below-ground resources : key roles of specific leaf area and root mass fraction. New Phytol 206:1247–1260.

Gale MR, Grigal DF (1987) Vertical root distributions of northern tree species in relation to successional status. Can J For Res 17:829–834.

Gaudinski AJB, Trumbore SE, Davidson EA, et al (2001) The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. Oecologia 129:420–429.

Gaul D, Hertel D, Borken W, et al (2008) Effects of experimental drought on the fine root system of mature Norway spruce. For Ecol Manage 256:1151–1159.

Gaul D, Hertel D, Leuschner C (2009) Estimating fine root longevity in a temperate Norway spruce forest using three independent methods. Funct Plant Biol 36:11–19.

George E, Seith B, Schaeffer C, Marschner H (1997) Responses of Picea, Pinus and Pseudotsuga roots to heterogeneous nutrient distribution in soil. Tree Physiol 17:39–45.

Grigulis K, Lavorel S, Krainer U, et al (2013) Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. J Ecol 101:47–57.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169–1194.

Grime JP (1974) Vegetation classification by reference to strategies. Nature 250:26–31.

Grime JP, Thompson K, Hunt R, et al (1997) Integrated screening validates primary axes of specialisation in plants. Oikos 79:259–281.

Gu J, Yu S, Sun Y, et al (2011) Influence of root structure on root survivorship: an analysis of 18 tree species using a minirhizotron method. Ecol Res 26:755–762.

Guo D, Li H, Mitchell RJ, et al (2008a) Fine root heterogeneity by branch order: exploring the discrepancy in root turnover estimates between minirhizotron and carbon isotopic methods. New Phytol 177:443–56.

Guo D, Xia M, Wei X, et al (2008b) Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. New Phytol 180:673–83.

Hacke UG, Plavcová L, Almeida-Rodriguez A, et al (2010) Influence of nitrogen fertilization on xylem traits and aquaporin expression in stems of hybrid poplar. Tree Physiol 30:1016–25.

Hansson K, Helmisaari H-S, Sah SP, Lange H (2013) Fine root production and turnover of tree and understorey vegetation in Scots pine, silver birch and Norway spruce stands in SW Sweden. For Ecol Manage 309:58–65.

Helama S, Läänelaid A, Raisio J, Tuomenvirta H (2009) Oak decline in Helsinki portrayed by tree-rings, climate and soil data. Plant Soil 319:163–174.

Hertel D, Strecker T, Mueller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient – is optimal resource partitioning theory applicable to water-limited mature trees? J Ecol 101:1–18.

Hirano Y, Hijii N (1998) Effects of low pH and aluminum on root morphology of Japanese red cedar saplings. Environ Pollut 101:339–347.

Hobbie EA, Agerer R (2009) Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant Soil 327:71–83.

Hobbie EA, Hobbie JE (2008) Natural abundance of 15N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: A review. Ecosystems 11:815–830.

Hobbie SE, Oleksyn J, Eissenstat DM, Reich PB (2010) Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. Oecologia 162:505–13.

Högberg MN, Baath E, Nordgren A, et al (2003) Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs – A hypothesis based on field observations in boreal forest. New Phytol 160:225–238.

Holdaway RJ, Richardson SJ, Dickie IA, et al (2011) Species- and community-level patterns in fine root traits along a 120 000-year soil chronosequence in temperate rain forest. J Ecol 99:954–963.

Holmes R (1983) Computer-assisted quality control in tree-ring dating and measurement. Tree-Ring Bull 43:69–78.

Horn HS (1971) The Adaptive Geometry of Trees. Princeton University Press, Princeton.

Itoh S, Barber SA (1983) A numerical solution of whole plant nutrient uptake for soil-root systems with root hairs. Plant Soil 70:403–413.

Jackson RB, Canadell J, Ehleringer JR, et al (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411.

Janse-Ten Klooster SH, Thomas EJP, Sterck FJ (2007) Explaining interspecific differences in sapling growth and shade tolerance in temperate forests. J Ecol 95:1250–1260.

Jansen JJ, Faber PJ (1996) Deel 3: Opbrengsttabellen. In: Jansen JJ, Sevenster J, Faber PJ (eds) Opbrengst Tabellen voor Belangrijke Boomsoorten in Nederland. Instituut voor Bos-en Natuuronderzoek, Wageningen, the Netherlands, pp 41–144

Kattge J, Bönisch G, Günther A, Wright I, Zanne A, Wirth C, Reich PB, TRY Consortium. (2012). TRY - Categorical Traits Dataset. Data from: TRY - a global database of plant traits. TRY File Archive. URL https://www.try-db.org/TryWeb Accessed 28 January 2016

Kemmers RH, Mekkink P, De Waal RW (2000) Humusprofielen in de bosreservaten Hollandse Hout en Houtribbos; Basisprogramma bosreservaten. Wageningen, The Netherlands

Kerkhoff AJ, Fagan WF, Elser JJ, Enquist BJ (2006) Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. Am Nat 168:E103–E122.

King JS, Albaugh TJ, Allen HL, et al (2002) Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytol 154:389–398.

Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. Oecologia 98:419–428.

Kjøller R, Nilsson L-O, Hansen K, et al (2012) Dramatic changes in ectomycorrhizal community composition, root tip abundance and mycelial production along a stand-scale nitrogen deposition gradient. New Phytol 194:278–86.

Klein T, Siegwolf RTW, Körner C (2016) Belowground carbon trade among tall trees in a temperate forest. Science 3520:342–344.

Klein Tank AMG, Wijngaard JB, Können GP, et al (2002) Daily dataset of 20th-century surface air temperature and precipitation series for the European Climate Assessment. Int J Climatol 22:1441– 1453.

Kobe R, Pacala S, Silander J, Canham C (1995) Juvenile tree survivorship as a component of shade tolerance. Ecol Appl 5:517–532.

Koele N, Dickie IA, Oleksyn J, et al (2012) No globally consistent effect of ectomycorrhizal status on foliar traits. New Phytol 196:845–852.

Koide RT, Fernandez C, Malcolm G (2013) Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. New Phytol 201:433–439.

Kong D, Ma C, Zhang Q, et al (2014) Leading dimensions in absorptive root trait variation across 96 subtropical forest species. New Phytol 203:863–872.

Koricheva J, Gurevitch J (2014) Uses and misuses of meta-analysis in plant ecology. J Ecol 102:828–844.

Kozlowski TT (1999) Soil Compaction and Growth of Woody Plants. Scand J For Res 14:596–619.

Kubisch P, Hertel D, Leuschner C (2015) Do ectomycorrhizal and arbuscular mycorrhizal temperate tree species systematically differ in root order-related fine root morphology and biomass? Front Plant Sci 6:64.

Kunstler G, Falster D, Coomes DA, et al (2016) Plant functional traits have globally consistent effects on competition. Nature 529:1–15.

Laliberté E (2016) Below-ground frontiers in trait-based plant ecology. New Phytol. doi: 10.1111/nph.14247 [Epub ahead of print].

Lambers H, Chapin III F, Pons TL (1998) Plant physiological ecology. Springer, New York

Lambers H, Shane MW, Cramer MD, et al (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Ann Bot 98:693–713.

Lamour A, Termorshuizen AJ, Volker D, Jeger MJ (2007) Network formation by rhizomorphs of Armillaria lutea in natural soil: Their description and ecological significance. FEMS Microbiol Ecol 62:222–232.

Leeters EEJM, Vries W De (2001) Chemical composition of the humus layer, mineral soil and soil solution of 200 forest stands in the Netherlands in 1995. Wageningen, The Netherlands

Leuschner C, Hertel D (2003) Fine root biomass of temperate forests in relation to soil acidity and fertility, climate, age and species. Prog Bot 64:405–438.

Leuschner C, Hertel D, Schmid I, et al (2004) Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant Soil 258:43–56.

Lindner M, Fitzgerald JB, Zimmermann NE, et al (2014) Climate change and European forests: What do we know, what are the uncertainties, and what are the implications for forest management? J Environ Manage 146:69–83.

Lindner M, Maroschek M, Netherer S, et al (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manage 259:698–709.

Liu B, Li H, Zhu B, et al (2015) Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species. New Phytol 208:125–136.

Liu G, Freschet GT, Pan X, et al (2010) Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. New Phytol 188:543–553.

Lucash MS, Eissenstat DM, Joslin JD, et al (2007) Estimating nutrient uptake by mature tree roots under field conditions: challenges and opportunities. Trees 21:593–603.

Lynch JP, Brown KM (2008) Root strategies for phosphorus acquisition. In: White P, Hammond J (eds) The Ecophysiology of Plant-Phosphorus Interactions. Springer Netherlands, pp 83–116.

Maherali H (2014) Is there an association between root architecture and mycorrhizal growth response? New Phytol 204:192–200.

Majdi H (1996) Root sampling methods – applications and limitations of the minirhizotron technique. Plant Soil 185:255–258.

Makita N, Hirano Y, Mizoguchi T, et al (2010) Very fine roots respond to soil depth: biomass allocation, morphology, and physiology in a broad-leaved temperate forest. Ecol Res 26:95–104.

Makita N, Kosugi Y, Dannoura M, et al (2012) Patterns of root respiration rates and morphological traits in 13 tree species in a tropical forest. Tree Physiol 32:303–12.

Marks CO, Lechowicz MJ (2006) Alternative designs and the evolution of functional diversity. Am Nat 167:55–66.

McCormack ML, Adams TS, Smithwick EH, Eissenstat DM (2012) Predicting fine root lifespan from plant functional traits in temperate trees. New Phytol 195:823–31.

McCormack ML, Dickie IA, Eissenstat DM, et al (2015) Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytol 207:505–518.

McDowell N, Pockman WT, Allen CD, et al (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–39.

McMurtrie RE, Dewar RC (2011) Leaf-trait variation explained by the hypothesis that plants maximize their canopy carbon export over the lifespan of leaves. Tree Physiol 31:1007–23.

McQuattie C, Schier G (1990) Response of red spruce seedlings to aluminum toxicity in nutrient solution: alterations in root anatomy. Can J For Res 20:1001–1011.

Meier IC, Leuschner C (2008) Genotypic variation and phenotypic plasticity in the drought response of fine roots of European beech. Tree Physiol 28:297–309.

Mérian P, Lebourgeois F (2011) Size-mediated climate–growth relationships in temperate forests: A multi-species analysis. For Ecol Manage 261:1382–1391.

Mokany K, Raison RJ, Prokushkin AS (2006) Critical analysis of root : shoot ratios in terrestrial biomes. Glob Chang Biol 12:84–96.

Mommer L, Weemstra M (2012) The role of roots in the resource economics spectrum. New Phytol 195:725–727.

Nicotra AB, Atkin OK, Bonser SP, et al (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15:684–692.

Nielsen NE (2006) Nutrient diffusion, bioavailability and plant uptake. In: Lal R (ed) Encyclopedia of Soil Science. Taylor & Francis Group, New York, NY, pp 1147–1152

Niinemets Ü, Sack L (2006) Structural determinants of leaf light-harvesting capacity and photosynthetic potentials. Prog Bot 385–419.

Niinemets Ü, Valladares F (2006) Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. Ecol Monogr 75:521–547.

Niklas KJ (1992) Plant biomechanics. University of Chicago Press, Chicago.

Nilsson LO, Giesler R, Bååth E, Wallander H (2005) Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. New Phytol 165:613–22.

Nilsson LO, Wallander H (2003) Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. New Phytol 158:409–416.

Nylund J-E, Wallander H (1992) Ergosterol analysis as a means of quantifying mycorrhizal biomass. In: Norris JR, Read DJ, Varma AK (eds) Methods in Microbiology. Academic Press, London, pp 77–88.

Onoda Y, Westoby M, Adler PB, et al (2011) Global patterns of leaf mechanical properties. Ecol Lett 14:301–312.

Ordoñez JC, Van Bodegom PM, Witte J-PM, et al (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Glob Ecol Biogeogr 18:137–149.

Ostonen I, Helmisaari H-S, Borken W, et al (2011) Fine root foraging strategies in Norway spruce forests across a European climate gradient. Glob Chang Biol 17:3620–3632.

Ostonen I, Lõhmus K, Helmisaari H, et al (2007a) Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. Tree Physiol 27:1627–1634.

Ostonen I, Lõhmus K, Lasn R (1999) The role of soil conditions in fine root ecomorphology in Norway spruce (Picea abies (L .) Karst .). Plant Soil 208:283–292.

Ostonen I, Püttsepp Ü, Biel C, et al (2007b) Specific root length as an indicator of environmental change. Plant Biosyst - An Int J Deal with all Asp Plant Biol 141:426–442.

Ostonen I, Tedersoo L, Suvi T, Lõhmus K (2009) Does a fungal species drive ectomycorrhizal root traits in Alnus spp.? Can J For Res 39:1787–1796.

Ouimette A, Guo D, Hobbie E, Gu J (2013) Insights into root growth, function, and mycorrhizal abundance from chemical and isotopic data across root orders. Plant Soil 367:313–326.

Pagès L (2011) Links between root developmental traits and foraging performance. Plant Cell Environ 34:1749–60.

Pena R, Simon J, Rennenberg H, Polle A (2013) Ectomycorrhiza affect architecture and nitrogen partitioning of beech (Fagus sylvatica L.) seedlings under shade and drought. Environ Exp Bot 87:207–217.

Pérez-Ramos IM, Roumet C, Cruz P, et al (2012) Evidence for a “plant community economics spectrum” driven by nutrient and water limitations in a Mediterranean rangeland of southern France. J Ecol 100:1315–1327.

Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. Funct Plant Biol 27:595–607.

Poorter H, Niklas KJ, Reich PB, et al (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol 193:30–50.

Poorter H, Ryser P (2015) The limits to leaf and root plasticity: what is so special about specific root length ? New Phytol 206:1188–1190.

Poorter L (1999) Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. Funct Ecol 13:396–410.

Poorter L, Bongers F (2006) Leaf traits are good predictors of plant performance across 53 rain forest species. Ecology 87:1733–1743.

Poorter L, Bongers L, Bongers F (2006) Architecture of 54 moist-forest tree species: traits, trade-offs, and functional groups. Ecology 87:1289–301.

Pregitzer KS, DeForest JL, Burton AJ, et al (2002) Fine root architecture of nine North American trees. Ecol Monogr 72:293–309.

Pregitzer KS, Laskowski MJ, Burton AJ, et al (1998) Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiol 18:665–670.

Prieto I, Roumet C, Cardinael R, et al (2015) Root functional parameters along a land-use gradient: Evidence of a community-level economics spectrum. J Ecol 103:361–373.

R Development Core Team (2012) R: A Language and Environment For Statistical Computing.

Ravenek JM, Bessler H, Engels C, et al (2014) Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. Oikos 1528–1536.

Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–391.

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems[mdash]a journey towards relevance? New Phytol 157:475–492.

Reich P (2014) The world-wide "fast-slow" plant economics spectrum: a traits manifesto. J Ecol 102:275–301.

Reich PB, Ellsworth DS, Walters MB, et al (1999) Generality of leaf trait relationships: A test across six biomes. Ecology 80:1955–1969.

Reich PB, Tjoelker MG, Pregitzer KS, et al (2008) Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. Ecol Lett 11:793–801.

Reich PB, Walters M, Tjoelker M, et al (1998) Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. Funct Ecol 12:395–405.

Reich PB, Walters MB, Ellsworth DS (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecol Monogr 62:365–392.

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. Proc Natl Acad Sci U S A 94:13730–4.

Reich PB, Wright IJ, Cavender-Bares J, et al (2003) The Evolution of Plant Functional Variation: Traits, Spectra, and Strategies. Int J Plant Sci 164:S143-164.

Rinn F (2003) TSAP-Win. Time Series Analysis and Presentation for Dendrochronology and Related Applications. Version 0.59 for Microsoft Windows [software]. Rinntech, Heidelberg, Germany.

Roumet C, Birouste M, Picon-Cochard C, et al (2016) Root structure – function relationships in 74 species : evidence of a root economics spectrum related to carbon economy. New Phytol 210:815-26.

Roumet C, Urcelay C, Díaz S (2011) Suites of root traits differ between annual and perennial species growing in the field. New Phytol 170:357–367.

Ryser P, Eek L (2000) Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. Am J Bot 87:402–411.

Ryser P, Lambers H (1995) Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. Plant Soil 170:251–265.

Scharnweber T, Manthey M, Criegee C, et al (2011) Drought matters – Declining precipitation influences growth of Fagus sylvatica L. and Quercus robur L. in north-eastern Germany. For Ecol Manage 262:947–961.

Schier G (1985) Response of red spruce and balsam fir seedlings to aluminum toxicity in nutrient solutions. Can J For Res 15:29–33.

Shipley B, Meziane D (2002) The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. Funct Ecol 16:326–331.

Siddiqi MY, Glass AD, Ruth TJ, Rufty Jr TW (1990) Studies of the Uptake of Nitrate in Barley. Plant Physiol 93:1426–1432.

Silver WL, Miya RK (2001) Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407–419.

Smith MD, La Pierre KJ, Collins SL, et al (2015) Global environmental change and the nature of aboveground net primary productivity responses: insights from long-term experiments. Oecologia 177:935–947.

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Academic Press, London, UK

Solly EF, Schöning I, Boch S, et al (2014) Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. Plant Soil 382:203–218.

Sterck F, Anten NPR, Schieving F, Zuidema PA (2016) Trait Acclimation Mitigates Mortality Risks of Tropical Canopy Trees under Global Warming. Front Plant Sci 7:1–10.

Sterck F, Markesteijn L, Schieving F, Poorter L (2011) Functional traits determine trade-offs and niches in a tropical forest community. Proc Natl Acad Sci U S A 108:20627–32.

Sterck F, Markesteijn L, Toledo M, et al (2014) Sapling performance along resource gradients drives tree species distributions within and across tropical forests. Ecology 95:2514–2525.

Sterck F, Schieving F (2011) Modelling functional trait acclimation for trees of different height in a forest light gradient: emergent patterns driven by carbon gain maximization. Tree Physiol 31:1024–37.

Sterck FJ, Duursma RA, Pearcy RW, et al (2013) Plasticity influencing the light compensation point offsets the specialization for light niches across shrub species in a tropical forest understorey. J Ecol 101:971–980.

Sterck FJ, Poorter L, Schieving F (2006) Leaf traits determine the growth-survival trade-off across rain forest tree species. Am Nat 167:758–765.

Strand AE, Pritchard SG, McCormack ML, et al (2008) Irreconcilable differences: fine-root life spans and soil carbon persistence. Science 319:456–458.

Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. Trends Plant Sci 5:537–542.

Tjoelker MG, Craine JM, Wedin D, et al (2005) Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytol 167:493–508.

TNO-NITG (n.d.) DINOLoket [online]. http://www.dinoloket.nl. Accessed 15 Dec 2011

Tuzet A, Perrier A, Leuning R (2003) A coupled model of stomatal conductance, photosynthesis and transpiration. Plant, Cell Environ 26:1097–1116.

Valladares F, Gianoli E, Gómez JM (2007) Ecological limits to plant phenotypic plasticity. New Phytol 176:749–763.

Valverde-Barrantes OJ, Blackwood CB (2016) Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. J Ecol 104:1299–1310.

Valverde-Barrantes OJ, Blackwood CB, Kramer-Walter KR, et al (2016a) Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. J Ecol 104:1299–1310.

Valverde-Barrantes OJ, Horning AL, Smemo KA, Blackwood CB (2016b) Phylogenetically structured traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms. Plant Soil 404:1-12

Valverde-Barrantes OJ, Raich JW, Russell AE (2007) Fine-root mass, growth and nitrogen content for six tropical tree species. Plant Soil 290:357–370.

Valverde-Barrantes OJ, Smemo KA, Blackwood CB (2015) Fine root morphology is phylogenetically structured, but nitrogen is related to the plant economics spectrum in temperate trees. Funct Ecol 29:796–807.

Valverde-Barrantes OJ, Smemo KA, Feinstein LM, et al (2013) The distribution of below-ground traits is explained by intrinsic species differences and intraspecific plasticity in response to root neighbours. J Ecol 101:933–942.

Van der Heijden EW, Kuyper TW (2003) Ecological strategies of ectomycorrhizal fungi of Salix repens: root manipulation versus root replacement. Oikos 103:668–680.

van der Werf GW, Sass-Klaassen UGW, Mohren GMJ (2007) The impact of the 2003 summer drought on the intra-annual growth pattern of beech (Fagus sylvatica L.) and oak (Quercus robur L.) on a dry site in the Netherlands. Dendrochronologia 25:103–112.

van Gelder HA, Poorter L, Sterck FJ (2006) Wood mechanics, allometry, and life-history variation in a tropical rain forest tree community. New Phytol 171:367–78.

Vetter D, Rücker G, Storch I (2013) Meta-analysis: A need for well-defined usage in ecology and conservation biology. Ecosphere 4:1–24.

Violle C, Navas M-L, Vile D, et al (2007) Let the concept of trait be functional! Oikos 116:882–892.

Wahl S, Ryser P (2000) Root tissue structure is linked to ecological strategies of grasses. New Phytol 148:459–471.

Waldner P, Marchetto A, Thimonier A, et al (2014) Detection of temporal trends in atmospheric deposition of inorganic nitrogen and sulphate to forests in Europe. Atmos Environ 95:363–374.

Wallander H, Ekblad A, Bergh J (2011) Growth and carbon sequestration by ectomycorrhizal fungi in intensively fertilized Norway spruce forests. For Ecol Manage 262:999–1007.

Wallander H, Ekblad A, Godbold DL, et al (2013) Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils – A review. Soil Biol Biochem 57:1034–1047.

Wallander H, Nilsson L, Hagerberg D, Baath E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytol 151:753–760.

Walters M, Reich P (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? New Phytol 143:143–154.

Weemstra M, Mommer L, Visser EJW, et al (2016) Towards a multidimensional root trait framework: a tree root review. New Phytol 211:1159–1169.

Wells CE, Eissenstat DM (2003) Beyond the roots of young seedlings: The influence of age and order on fine root physiology. J Plant Growth Regul 21:324–334.

Westoby M, Wright IJ (2006) Land-plant ecology on the basis of functional traits. Trends Ecol Evol 21:261–8.

Withington J, Reich P, Oleksyn J, Eissenstat D (2006) Comparisons of structure and life span in roots and leaves among temperate trees. Ecol Monogr 76:381–397.

Wright IJ, Reich PB, Westoby M, et al (2004) The worldwide leaf economics spectrum. Nature 428:821–827.

Wright IJ, Westoby M (1999) Differences in seedling growth behaviour among species : shifts along trait correlations across species , and trait shifts along nutrient compared to rainfall gradients. J Ecol 87:85–97.

Yanai RD, Fahey TJ, Miller SL (1995) Efficiency of nutrient acquisition by fine roots and mycorrhizae. In: Smith WK, Hinckley TM (eds) Resource physiology of conifers. Acquisition, allocation and utilization. Academic Press, San Diego, pp 75–103

Yuan ZY, Chen H (2010) Fine Root Biomass, Production, Turnover Rates, and Nutrient Contents in Boreal Forest Ecosystems in Relation to Species, Climate, Fertility, and Stand Age: Literature Review and Meta-Analyses. CRC Crit Rev Plant Sci 29:204–221.

Zuur AF, Ieno EN, Walker N, et al (2009) Mixed effects models and extensions in ecology with R. Springer New York, New York, NY.

Summary

The growth of trees depends on photosynthetic carbon gain by the leaves, which in turn relies on water and nutrient acquisition by the fine roots. Because the availability of carbon, water and nutrients fluctuates, trees can adjust their leaf and fine-root functional traits to maintain their resource uptake and growth rates. These functional traits refer to plant properties that determine plant fitness, and are associated with plant resource uptake and use. Because such traits can be expressed differently, trees of the same, and of different species can grow and survive in various environments.

Aboveground, the variation in leaf traits is closely related to light availability, light uptake and tree growth. *Within* species, leaves show general, plastic responses to their light environment, so that trees can still intercept light when its availability changes. This plasticity allows trees of the same species to grow and survive under different light conditions. *Across* species, leaf traits are coordinated along a leaf economics spectrum (LES), which reflects species resource strategies. On the one end of this spectrum, species with an acquisitive strategy have leaves that allow fast resource uptake and therefore fast tree growth in resource-rich environments, but that lead to low survival rates when resources are less available. On the other end, species with a conservative strategy acquire resources more slowly, but retain them longer, so they can tolerate low resource availability, but at the expense of their growth rate.

Belowground, the relationships between fine-root functional traits, water and nutrient availability and acquisition, and tree growth are expected to be similar to those aboveground. However, *within* species, fine-root plastic responses to the soil environment are far less general than the plasticity of leaves. *Across* species, it is assumed that fine-root traits can be coordinated along a root economics spectrum (RES) parallel to an LES, that ranges from fine-root traits associated with high soil resource uptake rates, to fine-root traits that allow resource conservation. Yet at present there is only mixed evidence for this root-trait spectrum.

Consequently, both within and across species, the relationships between fine-root traits, resource availability and tree growth are ambiguous. Understanding these relationships is essential as tree growth results from the simultaneous uptake of both above- and belowground resources. Therefore, in this thesis, I examine how fine-root functional traits relate to tree growth, and specifically focus on across- and within-species variation in tree root traits.

To first test whether plant resource strategies can explain drought effects on tree growth, we compared the growth-sensitivity to climate variables and the groundwater table across 10 common tree species that ranged from acquisitive to conservative species (Chapter 2). We analysed tree-ring widths for these 10 species growing on a productive forest site in the Netherlands. The growth rates of all species were significantly lower in years with dry summers. The strength of these growth responses differed, but these differences were not related to species resource strategies. The growth-responses to the depth of the groundwater table, however, were: acquisitive species grew slower when groundwater levels receded, but conservative species did not, which suggests root trait differences across these species. Even on productive sites, tree growth may thus be seriously affected by the more frequent and intense droughts that are predicted by climate models, but these effects may not always be explained from an acquisitive or conservative resource strategy.

We further evaluated whether resource strategies explain variation in fine-root functional traits across species, and tested whether fine-root traits can be coordinated along an RES parallel to an LES based on a literature review and meta-level analysis (Chapter 3). We show that there is no consistent evidence for an RES, due to three fundamental differences between fine roots and leaves. First, fine-root trait expression is not only aimed at increased resource uptake or conservation, but is also constrained by several other (soil) environmental variables. Second, the relationships between traits and function differ for leaves and roots, and are far less clear for roots. Therefore, fine-root functions cannot yet be unambiguously deduced from their traits. Third, the expected relationships between fine-root traits and resource uptake are strongly obscured by mycorrhizal fungi that interact with both fine-root traits and functioning (i.e. resource uptake). Revealing the links between fine-root traits, resource acquisition, and growth across species, therefore requires a multidimensional approach that incorporates the diversity of fine-root traits involved in resource uptake; the complexity of the soil environment; and the various belowground uptake mechanisms that exist across species – in particular the mycorrhizal pathway.

In Chapter 4, we assessed the plasticity of fine-root traits in different resource environments. To this end, we examined the intraspecific variation in several fine-root traits and mycorrhizal biomass in beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) forests on a resource-poor, sandy soil and resource-rich clay soil in the Netherlands. Both species increased their fine-root mass and fine-root growth rates on the sandy soils compared to the clay soils. In contrast, specific root length (SRL), diameter and tissue density did not differ between the soil types. Especially in the *P. abies* stands, ectomycorrhizal biomass was larger on sand than on clay. In the *F. sylvatica* stands, significant morphological responses were observed *within* the fine-root category: the relative root length of its smallest roots (0.1 – 0.3 mm diameter) was higher than on the clay soil than on the sandy soil, and at the sandy soil, we observed relatively more length of its thicker fine roots (0.4 – 1 mm diameter). Besides the strong increase in fine-root mass observed for *both* species, species may thus also *differ* in their fine-root plasticity strategies to cope with various soil environments.

To understand tree growth from below- and aboveground trait integration, we used an integrated whole-tree growth model to explore fine-root impacts on nutrient acquisition, aboveground functioning and tree fitness (Chapter 5). More specifically, we tested which combination(s) of fine-root mass and SRL led to optimal fitness, based on the uptake benefits (i.e. increasing the belowground uptake area) and carbon costs (i.e. turnover and respiration) of these traits. Our results show that tree fitness increased with fine-root mass but especially through an increase in SRL. Furthermore, both a combination of high fine-root mass and low SRL, and of low fine-root mass and high SRL, resulted in similar net carbon gain. Likewise, on a poor soil, trees could optimise their fitness by increasing their SRL at constant fine-root mass, or vice versa. These outcomes indicate two alternative strategies that may lead to similar fitness. It is important to note that fine roots of most temperate tree species do not show the high SRL values our model predicted for optimising growth, which may suggest that mycorrhizal symbiosis (not yet implemented in our model) is needed to enhance the belowground uptake area. Our model offers qualitative insights into the role of fine roots in tree fitness, but also calls for more fine-root and mycorrhizal data to parameterise whole-tree models.

To conclude, trees rely on various uptake strategies to ensure belowground resource uptake and tree growth in different environments. Specific root length is often expected to be tightly linked to tree growth, but this thesis shows that there is little support for this hypothesised relationship. Consequently, the functional meaning of SRL requires further study. Instead, fine-root mass and mycorrhizal symbiosis may present more important alternatives to enhance water and nutrient uptake, both across and within species. Moreover, to cope with the highly complex soil (resource) environment, species have adopted various other uptake strategies besides fine-root mass, morphology and mycorrhiza. This thesis stresses that a multidimensional root-trait framework is needed to link fine-root traits to tree growth, that can accommodate this variety of fine-root traits and the diversity of the soil environment.

Acknowledgements

Acknowledgements

My PhD-project was an amazing time that brought me scientific and personal lessons, skills and insights, old and new lovely friends and colleagues, and lots of fun; everyone I acknowledge here contributed to that in various ways.

My supervisors, Frank Sterck, Liesje Mommer and Frits Mohren, made up a great team of scientists with overlapping, and more importantly, very complementary skills. What I appreciated most during my PhD project was the freedom, trust and encouragement I received from them in following my own scientific interests and curiosity. In terms of research, my plans were not always as successful as I had hoped for; but my supervisors always pointed out some valuable lesson to learn – either scientifically or personally – from both the successes and the not so successful attempts I undertook.

Frank, you were the first to spark my interest in a PhD-position, and you motivated me to write a research proposal. You have supervised me as an MSc and PhD student, and during those six years your enthusiasm and encouragement never failed. You were always readily available to provide me with ambitious plans, feedback, advice or solutions, and were equally open to mine. This way, I felt your true interest in, and appreciation for my work. Liesje, throughout my PhD project, your proved not only a good supervisor, but also a great coach at both a scientific and personal level. I'm not sure whether I learnt more about roots or about myself during my PhD, but you played an important role in both processes. You also showed that team work not only increases productivity or success, but also brings more fun; a lesson I aim to apply wherever I am going next. My promotor, Frits, pretty much always had an open door to give me scientific, but especially strategic and personal advice on how to proceed with my research; at the same time, he kept an eye on my well-being. Frits, your pep talks and sense of perspective truly helped me during this PhD project, but in particular to get me through the last couple of weeks.

No thesis without roots, and no roots without Leo Goudzwaard. Dear Leo: thank you very much for your help and company in the forest, in the lab and in the root-washing room of Unifarm. Your good spirit, enthusiasm and tireless efforts during my field work were priceless. Furthermore, Eric, Jasper and Thom played an important role in my project with great ideas and knowledge, as well as pragmatism and positive, insightful and constructive ways of giving feedback.

Dear Hans, it feels weird to even try to write down how grateful I am for your love and support during these last four years (and then some…). Fortunately, you already know this, but still: thank you for your encouragement, reassurance and care in my undertakings. More importantly but very typical of you, you were there to console me and pick me up in challenging times, when you proved more important to me than ever. I cannot describe how much I have learnt from you, but I am sure *you* see the change in me.

Mijn ouders hebben een grote rol gespeeld in mijn promotie-onderzoek, met name in de dertig jaar daaraan voorafgaand. Andries en Riky, jullie hebben me altijd aangemoedigd om mijn nieuwsgierigheid en ambities te volgen. Doordat ik weet dat ik *altijd* en overal op jullie steun en hulp kan vertrouwen, is het niet erg als ik soms faal, want dan zijn jullie daar! Ik hoor nog steeds '*Niet van dat benauwde!*' in mijn hoofd als ik voor eender welke uitdaging sta. Lieve Esther: jouw pragmatische en praktische visie hebben me regelmatig weer even met beide benen op de grond gezet, en ervoor gezorgd dat ik soms even de tijd nam mezelf te herpakken alvorens weer verder te gaan. Dit bleek een zeer waardevolle les tijdens een promotie-traject dat soms bijna het uiterste van je vraagt.

The great time (Hans and) I had during my PhD can be largely attributed to my colleagues at the FEM-group. Our many, many coffee breaks and cakes, beers, dinners, movies and parties together made the past four years a wonderful time. Hosting diverse people from all over the world has clearly contributed to the warm, supportive and welcoming atmosphere that describes our group. As I have learnt how much this improved not only our social but also scientific environment, I aim to take some of this to wherever I will end up next; hopefully to the same avail. Therefore, *many* thanks and all the best to all of you: Alan, Alejandra, Carolina B., Carolina L., Catarina, Ellen, Federico, Frank, Frans, Frits, Gert-Jan, Jan, Joke, Jose, Juan Ignacio, Kathelyn, Koen, Lan, Leo, Linar, Lourens, Lu, Madelon, Marielos, Marlene, Mart, Meike, Pieter, Surya and Ute. The same applies to my ex-FEM-ily members and the other friends I made in Wageningen: André, Arildo, Bas, Bernardo, Danaë, Estela, Jamir, Mandy, Masha, Mathieu, Merel, Paul, and Sarah.

Being a part of a second chair group (Plant Ecology and Nature Conservation) gave me additional insights to, and new perspectives on my PhD research, and meant more parties. I specifically want to thank my fellow 'rooties' for sharing lunch, home-made cookies, field and lab work, interesting papers, useful R scripts, and what not: Dina, Lisette, Natalie, Peng, Robert, and Wei. Jan, Frans and Jan Willem helped me a lot out in the field and in the lab, offered great practical advice, and made tedious lab work easier and more fun: thanks! I am also grateful to my other colleagues at the PEN-group: David, Elmar, Frank, Juul, Marinka, Monique, Philippine, and Thijs.

Of course, a very special thanks to my dear paranymphs from both chair groups, Lu and Lisette, who have greatly contributed to preparing this thesis, my defense, and myself *for* my defense by their practical and moral support, and good ideas!

I also want to thank several students for helping me out and for the fun we had collecting data in the field and in the lab: Véronica, Natasa, Íngrid, Puri, Cristina, Lisanne and Carlette. Moreover, I really welcomed the help of Hannie and Annemiek at the Experimental Plant Ecology group at the Radboud University in Nijmegen for facilitating, and advising me on, my work with the minirhizotrons, and other root trait analyses I carried out in their lab. Our colleagues at Unifarm also kindly hosted me and facilitated my root-washing and lab work, and – particularly Henk, Wim and Gerard - helped me out in the field on numerous occasions, for instance when roots needed to be dug up to 1 m deep on heavy clay soils.

Last, but not least, I want to thank my (or better yet, our) new and old friends. Lena, Marije and Mirjam, we met at the start of our studies in Wageningen during and after which we spent (and are going to spend) great times together: in the forest, at home (somewhere between Groningen and Germany), during lectures, in labs. We've moved different ways, but have always stayed connected, and let's keep it this way!

To my dear friends since way back when: we got to know each other about twenty years ago, and have done many great, fun and foolish things ever since. Being such old friends, it does not really matter anymore what I do, whether a manuscript gets rejected or whether an R code fails; hanging out and having fun with you guys and your loving partners always offered a great and healthy opportunity to forget about all this, and put things in perspective. Dearest Arjan, Daniel, Gerbrand, Gieneke, Marloes, and Willem, and your lovely partners that joined us along the way: I am grateful that we're still all together as the close group of friends we've always been; up to the next 20 years!

Biography

Monique Weemstra was born in 1981 in Meppel, the Netherlands. She grew up on the countryside, and spent much of her leisure time playing in the forest; her interest in trees was however still mainly restricted to building tree houses.

After receiving her high school diploma at the Linde College in Wolvega, she travelled to Australia where she was fascinated by indigenous cultures, and decided to study Cultural Anthropology and Development Studies at the Radboud University in Nijmegen. For her MA thesis, she returned to Australia to collect data on customary activities in indigenous remote communities and their economic value. As she noticed to what extent remote economies and personal well-being of the indigenous people there depend on the natural environment, she became more and more interested in ecology. After receiving her MSc diploma she worked for a few years at a company advising people about travelling, and working abroad.

Yet as her interest in ecology increased over time, she quit this job to study Forest and Nature Conservation at the Wageningen University. She carried out her MSc thesis in Australia again, this time using a whole-plant model to determine how plant architecture determines shade tolerance, under the supervision of dr. Frank Sterck and dr. Remko Duursma at the Hawkesbury Institute for the Environment. For her MSc research internship, she studied climate – growth relationships based on tree ring analysis, being supervised by dr. Frank Sterck, dr. Ute Sass-Klaassen and dr. Britta Eilmann at Wageningen University.

The latter work pointed at the important but still largely unexplained role of tree roots in growth, which motivated her to write a research proposal for a PhD project focusing on tree roots. This proposal was granted by the Netherlands Organisation for Scientific Research (NWO) as part of a graduate programme grant to the Graduate School Production Ecology and Resource Conservation (PE&RC); it started in 2012 and resulted in, but did not completely end with this thesis. Monique aims to continue studying the relationships between soils and plants, and the role of roots herein.

Publications

Weemstra M, Sterck, FJ, Visser EJW, Kuyper TW, Goudzwaard L, Mommer L (2016) Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. Plant Soil DOI 10.1007/s11104-016-3148-y. [Epub ahead of print].

Weemstra M, Mommer L, Visser EJW, van Ruijven J, Kuyper TW, Mohren GMJ, Sterck FJ (2016) Towards a multidimensional root trait framework: a tree root review. New Phytol 211(4):1159–1169.

Zhang L, Copini P, Weemstra M, Sterck F (2015) Functional ratios among leaf, xylem and phloem areas in branches change with shade tolerance, but not with local light conditions, across temperate tree species. New Phytol 209:1566-1575.

Sterck, FJ, Duursma RA, Pearcy RW, Valladares F. Cieslak M, Weemstra M (2013) Plasticity influencing the light compensation point offsets the specialization for light niches across shrub species in a tropical forest understorey. J Ecol 101(4):971–980.

Weemstra M, Eilmann B, Sass-Klaassen UGW, Sterck FJ (2013) Summer droughts limit tree growth across 10 temperate species on a productive forest site. For Ecol Manage 306:142–149.

Mommer L, & Weemstra M (2012) The role of roots in the resource economics spectrum. New Phytol 195:725–727.

Cornelissen JHC, Sass-Klaassen U, Poorter L, van Geffen K, van Logtestijn RSP, van Hal J, Goudzwaard L, Sterck FJ, Klaassen RKWM, Freschet GT, van der Wal A, Eshuis H, Zuo J, de Boer W, Lamers T, Weemstra M, Crétin V, Martin R, den Ouden J, Berg MP, Aerts R, Mohren GMJ, Hefting MM (2012) Controls on coarse wood decay in temperate tree species: birth of the LOGLIFE experiment. Ambio 41:231–45.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Towards a multidimensional root trait framework: a tree root review (2016)

Writing of project proposal (4.5 ECTS)

- Beyond leaf economics: integrating root, stem and crown traits to examine resource acquisition and tree growth (2012)

Post-graduate courses (3.3 ECTS)

- Root ecology: drivers of foraging and interactions in a spatial context; PE&RC, KU-Science, University of Copenhagen and NUE-CROPS (2012)
- Linear models; PE&RC (2014)
- Mixed models; PE&RC (2014)
- Climate models revisited: the biogeochemical consequences of Mycorrhizal dynamics; Royal Netherlands Academy of Arts and Sciences, KNAW (2015)

Laboratory training and working visits (9 ECTS)

- Mycorrhizal mesh bag and ergosterol analysis; Microbial Ecology group, Lund University (2013)
- Mycorrhizal morphotyping; Forest Dynamics group, WSL, Birmensdorf, Switzerland (2014)

Invited review of (unpublished) journal manuscript (3 ECTS)

- Functional Ecology: above- and belowground resource acquisition (2012)
- Journal of Ecology: fine root production and phenology (2015)

- New Phytologist: belowground frontiers in trait-based plant ecology (2016)

Competence strengthening / skills courses (2.5 ECTS)

- Data management; Wageningen UR Library Courses (2012)
- PhD Competence assessment; WGS (2013)
- Jena PhD writing workshop; Jena Project (2015)
- Orientation on teaching for PhD candidates; University & Research Centre (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC Weekend (2012)
- PE&RC Day (2012, 2015)

Discussion groups / local seminars / other scientific meetings (4.6 ECTS)

- Ecological theory and application; organizer (2012-2016)
- International scientific symposium: vegetation – soil interactions: from rhizosphere to ecosystem; organizer and chair of the day (2014)
- Current themes in ecology (2014)
- Wageningen PhD symposium; convener (2015)

International symposia, workshops and conferences (8.8 ECTS)

- Clim Tree: international conference on climate change and tree responses in Central European forests; poster presentation; Zürich, Switzerland (2013)
- Netherlands annual ecology meeting; poster presentation; Lunteren, the Netherlands (2013)
- Rhizoshere4; poster presentation; Maastricht, the Netherlands (2015)
- COST Action meeting BioLink: linking belowground biodiversity and ecosystem function in European forests; Krakow, Poland (2015)
- EcoSummit: ecological sustainability: engineering change; oral presentation; Montpellier, France (2016)

Lecturing / supervision of practicals / tutorials (2.1 ECTS)

- Forest resources and sustainable management systems (2013-2016)

Supervision of MSc students

- Leaf and root trait effects on climate sensitivity
- Nutrient balances of two contrasting Dutch soils and the effect of harvesting on soil nutrient availability

The research described in this thesis was financially supported by the Netherlands Organisation for Scientific Research (NWO) as part of a graduate programme grant (project number 022.002.004) provided to the Graduate School Production Ecology & Resource Conservation (PE&RC), and by Wageningen University.

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design

Ferdinand van Nispen tot Pannerden, Citroenvlinder DTP & Vormgeving, Ede, the Netherlands

Photography

Leo Goudzwaard, Hans Reijnen

Printed by

GVO Drukkers & Vormgevers B.V. Ede, the Netherlands