

# ***Tree diversity effects on fine-root soil exploitation and decomposition in European forests***

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## **Statement of originality**

I hereby declare that this thesis has never been submitted, in the same or similar form, to another examination commission in Germany or another country for a degree. This thesis contains no material previously published or written by another person except where proper acknowledgement is made.

Janna Wambsganß

Freiburg, January 2021



“Maybe you are searching among the branches, for what only appears in the roots”

جلال الدين محمد رومی (Jalal ad-Din Muhammad Rumi)



## Statement of contributions and manuscripts

This doctoral research was conducted between February 2017 and December 2020. I carried out the data collection, the data analyses and the writing of manuscripts, the introduction, the discussion and the synthesis of this dissertation.

The manuscripts included in this thesis are:

**Chapter two: Wambsganss, J., Beyer, F., Freschet, G. T., Scherer-Lorenzen, M., Bauhus, J. (2021): *Tree species mixing reduces biomass but increases length of absorptive fine roots in European forests*, **Journal of Ecology**. DOI: <https://doi.org/10.1111/1365-2745.13675>**

**Chapter three: Wambsganss, J., Freschet, G. T., Beyer, F., Goldmann, K., Prada-Salcedo, L. D., Scherer-Lorenzen, M., Bauhus, J. (2021): *Tree species mixing causes a shift in fine-root soil exploitation strategies across European forests*, **Functional Ecology**. DOI: <https://doi.org/10.1111/1365-2435.13856>**

**Chapter four: Wambsganss, J., Freschet, G. T., Beyer, F., Bauhus, J., Scherer-Lorenzen, M.: *Tree diversity, initial litter quality, and macroclimate drive early-stage fine-root decomposition in European forests*, submitted to **Ecosystems**.**

In addition to the three first-author papers of my dissertation, I contributed to the following publications as results of interdisciplinary scientific collaboration with research partners in the SoilForEUROPE project. These additional papers and manuscripts are not included in this dissertation.

Prada-Salcedo, L. D., **Wambsganss, J.**, Bauhus, J., Buscot, F. and Goldmann, K. (2021): *Low root functional dispersion enhances functionality of plant growth by influencing bacteria activities in European forest soils*, **Environmental Microbiology**. DOI: 10.1111/1462-2920.15244.

Prada-Salcedo, L. D., Goldmann, K., Heintz-Buschart, A., Reitz, T., **Wambsganss, J.**, Bauhus, J., Buscot, F. (2021): *Fungal guilds and soil functionality respond to tree community traits rather than to tree diversity in European forests*, **Molecular Ecology** 30 (1): 527-591. DOI: <https://doi.org/10.1111/mec.15749>.

Gillespie, L., Hättenschwiler, S., Milcu, A., **Wambsganss, J.**, Ammar, S., Fromin, N. (2021): *Tree species mixing affects soil microbial functioning indirectly via root and litter traits in European forests*, **Functional Ecology**, accepted.

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Chapter	Tasks	<i>Janna Wambsganß</i>	<i>Jürgen Bauhus</i>	<i>Michael Scherer- Lorenzen</i>	<i>Friderike Beyer</i>	<i>Grégoire T. Freschet</i>	<i>Luis D. Prada- Salcedo</i>	<i>Kezia Gold- mann</i>
two	Conceptualisa- tion of the study	X	X	X	X	X		
	Data collection	X	X	X			X	
	Data processing	X						
	Manuscript preparation	X	X	X	X	X		
three	Conceptualisa- tion of the study	X	X	X	X			
	Data collection	X	X	X			X	
	Data processing	X					X	X
	Manuscript preparation	X	X	X	X	X	X	X
four	Conceptualisa- tion of the study	X	X	X	X	X		
	Data collection	X						
	Data processing	X						
	Manuscript preparation	X	X	X	X	X		

*Diese Dissertation ist meiner Familie gewidmet*

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## List of abbreviations

AM	arbuscular mycorrhiza
BEF	biodiversity-ecosystem functioning
C	carbon
CE	complementarity effect
DOM	dissolved organic matter
EcM	ectomycorrhiza
FDis	functional dispersion
FRB	fine-root biomass
LMM	linear mixed-effect model
MAP	mean annual precipitation
MAT	mean annual temperature
N	nitrogen
NE	net diversity effect
OM	organic matter
PCA	principal component analysis
RLD	root length density
RTD	root tissue density
SE	selection effect
SOC	soil organic carbon
SOM	soil organic matter
SRA	specific root area
SRL	specific root length



# Summary

## Background

Mixed-species forests have often been shown to enhance above-ground ecosystem properties and functions compared to their mono-specific counterparts. For example, they are often more productive than pure stands. However, the underlying mechanisms of positive diversity-ecosystem functioning relationships have been analysed mainly for above-ground processes, with less attention paid to the role of below-ground interactions. Consequently, our understanding of the functioning of mixed forests is still largely incomplete. To promote diverse, productive, and resilient forests capable of adapting to the impacts of climate change, a comprehensive understanding of the functioning of mixed-species forest is indispensable. Fine roots generally play a fundamental role for plant growth and fitness, but also in carbon and nutrient cycling. Nevertheless, as to how species diversity affects below-ground functions driven by fine roots, including soil resource exploitation, remains largely unknown. Methodological constraints related to root research and inconsistent root classification bear major challenges for analysing the role of the below-ground ecosystem component. Consequently, contradictory results of previous studies do not allow broad conclusions to be drawn about the role of fine roots for positive biodiversity-ecosystem functioning relationships.

## Overall aim

The overarching goal of this thesis was to assess the effect of tree diversity on fine-root soil exploitation and decomposition in four wide-spread European forest types.

## Research objectives

The main research objectives were:

- (1) To assess the soil space occupation by tree fine roots in response to tree species mixing
- (2) To examine soil exploitation strategies by tree fine roots and mycorrhizal partners in response to tree species mixing
- (3) To investigate tree fine-root litter decomposition rates in response to tree species mixing

## Study sites and sampling design

In total, 63 mostly mature forest plots distributed across four sites across Europe were selected from an existing exploratory plot network (FunDivEUROPE) in semi-natural forests. The sites were located in four countries and representative of boreal (Finland), hemiboreal (Poland), mountainous beech (Romania), and thermophilous deciduous forests (Italy). The plots either

represented tree species mixtures with three target species or mono-specific stands. Within each plot, five tree neighbourhoods (*triplets*) were selected for soil sampling and subsequent incubation of root litter samples. In the centre of each of these neighbourhoods, soil cores at three depth increments (0-10, 10-20, 20-30 cm) were taken in spring 2017. The following year, in spring 2018, 1,330 litter bags with fine-root material were incubated near the soil sampling spots for one year.

In total, 928 soil samples were processed in the laboratory, and morphological, chemical, and microbial fine-root traits were measured. The vertical distribution of fine roots across soil depths was examined. Roots were sorted by species, and the functional classification approach was applied to distinguish absorptive, i.e., the first three most distal root orders, from transport fine roots, i.e., fourth or fifth-order roots with a diameter  $\leq 2$  mm. Moreover, ectomycorrhizal diversity and abundance data from nearby soil samples were integrated into subsequent analyses.

Fine-root decomposition rates were determined via mass loss after one year of incubation. Initial fine-root traits of tree species that were incubated were measured to determine initial litter quality.

## **Result and discussion**

Across all sites, tree species mixing significantly affected tree fine-root traits and decomposition rates. Tree species mixtures supported on average less biomass of absorptive fine roots than corresponding mono-specific stands. This underyielding was mainly reflected in negative complementarity effects, and to a lesser extent, in negative selection effects. The species-specific and overall rooting patterns across the three soil depth layers did not provide evidence for vertical root stratification in mixtures. Nevertheless, as total length density of absorptive fine roots (i.e., across the entire soil profile) did not significantly differ between mixtures and mono-specific stands, overall soil space occupation by tree fine roots and thereby the trees' resource uptake capacity did not change in response to mixing. Instead, an increased root length density in mixtures in the most nutrient-rich soil depth (0-10 cm) indicates an enhanced soil resource uptake capacity compared to pure stands.

The second analysis suggested that the observed underyielding of biomass of absorptive roots in response to tree species mixing was related to changes in fine-root traits. Fine roots in mixtures were characterised by higher specific root lengths, lower diameters, lower root tissue densities, and higher root nitrogen concentrations than trees in pure stands. Overall, these changes at the community level suggest a shift in soil resource acquisition strategies by trees in mixtures

compared to mono-specific stands towards a *faster* resource foraging. A higher ectomycorrhizal colonisation intensity of roots and, at the same time, higher diversity and abundance of ectomycorrhizae in soil samples in mixtures compared to mono-specific stands suggest positive biotic feedbacks from mycorrhizae likely enhancing soil resource capture by trees in mixtures. An important finding was that thin-rooted broadleaved tree species showed stronger responses to mixing than thick-rooted conifer tree species, particularly in terms of root morphology and ectomycorrhizal colonisation.

The decomposition study suggested that decomposition rates of mixed-species fine-root litter in mixed tree neighbourhoods can differ from component single-species litter in mono-specific neighbourhoods. As such, mixed-species litter decomposed faster than single-species litter across the four study sites. Differences in micro-environmental conditions between mixed and mono-specific tree neighbourhoods rather than interactions among litter species in mixed-species litter likely caused these non-additive effects. Nevertheless, the analyses further showed that initial chemical traits explained a greater proportion of the variability in the data than tree diversity. The additional incubation of standard root litter species across the plot network further suggests that macro-climate and regional-scale differences, as well as litter species identity, may be more important predictors of fine-root litter decomposition than tree diversity.

## **Conclusions**

This thesis enhances our understanding of overall tree diversity effects on ecosystem functioning by shedding more light on the role of the *hidden half*, i.e., the below-ground component of forest ecosystems. The obtained results provide evidence for positive below-ground species interactions in mixtures, possibly enhancing soil resource acquisition by trees. Hence, these findings contribute to a better mechanistic understanding of positive diversity-productivity relationships in forest ecosystems. Overall relatively consistent tree species mixing effects on fine-root soil exploitation and decomposition across a broad range of environmental conditions and different species compositions in four wide-spread European forest types demonstrate the generality of the results.

# **Zusammenfassung**

## **Hintergrund**

Zahlreiche Untersuchungen belegen, dass Mischwälder Ökosystemleistungen häufig in einem höheren Maße erfüllen als Reinbestände. Zum Beispiel sind Mischungen oft produktiver und weisen höhere Bodenkohlenstoffvorräte als Reinbestände auf. Die zugrundeliegenden Mechanismen der positiven Zusammenhänge zwischen Baumartendiversität und Ökosystemfunktionen wurden bisher jedoch vorwiegend für oberirdische Prozesse analysiert, wobei die Rolle der unterirdischen Funktionen weniger Beachtung fand. Daher ist unser Wissen über Mischwälder und deren Funktionsweise noch weitgehend unvollständig. Um zukünftig diverse und produktive Wälder, die an die Auswirkungen des Klimawandels und anderer globaler Veränderungen angepasst sind bzw. eine hohe Resilienz aufweisen, fördern zu können, ist ein umfassendes grundlegendes Verständnis von der Funktionsweise von Mischwäldern jedoch unabdingbar.

Insbesondere Feinwurzeln könnten hier eine bedeutende Rolle spielen, da sie zusammen mit Mykorrhizapilzen durch die Aufnahme von essenziellen Bodenressourcen wesentlich das Wachstum und die Fitness von Bäumen bestimmen. Außerdem haben Feinwurzeln eine große Bedeutung für Kohlenstoff- und Nährstoffzyklen und könnten somit auch das Potential für Kohlenstoffspeicherung im Boden beeinflussen. Allerdings ist nicht bekannt, ob und wie Artenvielfalt ihre Funktionalität, wie z. B. die Erschließung und Nutzung von Bodenressourcen, beeinflusst. Methodische Schwierigkeiten und eine inkonsistente Klassifizierung von Feinwurzeln stellen eine große Herausforderung für die Analyse der unterirdischen Ökosystemkomponente dar. Die relativ wenigen bisherigen Studien wiesen weitgehend widersprüchliche Ergebnisse auf und erlaubten daher keine umfassenden Schlussfolgerungen zu der Rolle von Feinwurzeln für positive Biodiversitäts-Ökosystem-Funktionsbeziehungen.

## **Zielsetzung**

Das Ziel dieser Arbeit war es, den Einfluss der Baumartenvielfalt auf die Bodenerschließung durch Feinwurzeln und den Verlauf ihrer Zersetzung in vier weit verbreiteten europäischen Waldtypen zu untersuchen.

Die übergeordneten Forschungsziele waren:

- (1) Erfassung der Bodenraumbesetzung durch Feinwurzeln in Abhängigkeit von Baumartendiversität

- (2) Untersuchung der Bodenerschließungsstrategie von Feinwurzeln und Mykorrhizapilzen in Abhängigkeit von Baumartendiversität
- (3) Quantifizierung der Zersetzungsraten von Feinwurzelstreu in Abhängigkeit von Baumartendiversität

## **Methodik**

Insgesamt wurden 63 Bestände an vier Standorten aus einem bestehenden paneuropäischen Netzwerk von Untersuchungsflächen unterschiedlicher Baumartendiversität (FunDivEU-ROPE) ausgewählt. Die Standorte repräsentierten boreale (Finnland), hemiboreale (Polen) und montane Buchenwälder (Rumänien) sowie thermophile Laubwälder (Italien). Die Bestände waren entweder Mischungen mit drei Hauptbaumarten oder Reinbestände. Pro Bestand wurden im Frühjahr 2017 Bodenproben an fünf Punkten entnommen, mit jeweils gleichem Abstand zu drei Bäumen der herrschenden Arten. Die 30 cm langen Bohrkerne wurden in drei Tiefenstufen (0-10, 10-20, 20-30 cm) unterteilt. Insgesamt wurden 928 Bodenproben im Labor bearbeitet. Die Feinwurzeln wurden nach Arten sortiert und anhand der Verzweigungsordnungen in absorbierende und transportierende Feinwurzeln unterteilt. Neben der Biomasse wurde die Morphologie, die chemische Zusammensetzung sowie die Mykorrhizierungsintensität der Feinwurzeln untersucht. Zudem wurden für die Zersetzungsstudie an allen Standorten im Frühjahr 2018 insgesamt 1.330 Streubeutel für ein Jahr im Oberboden vergraben. Die Zersetzungsraten wurden nach der einjährigen Inkubationszeit über den Massenverlust bestimmt.

## **Ergebnisse und Diskussion**

Über alle Standorte hinweg hatte die Baumartenmischung einen signifikanten Einfluss auf die Eigenschaften der Feinwurzeln und deren Zersetzungsraten. Mischbestände wiesen eine geringere Feinwurzelbiomasse auf als Reinbestände. In Mischungen deutet eine erhöhte Wurzellängendichte im Oberboden auf eine erhöhte Aufnahmefähigkeit von Ressourcen im Vergleich zu Reinbeständen hin. Die vertikale Verteilung der Wurzeln im Boden lieferte hingegen keine Hinweise auf eine Stratifizierung der Wurzelsysteme in Mischungen. Die gesamte Wurzellängendichte (d. h. über das gesamte beprobte Bodenprofil) unterschied sich zwischen Mischungen und Reinbeständen nicht signifikant, so dass die Bodenraumbesetzung durch die Feinwurzeln und damit die Erschließungskapazität von Bodennährstoffen nicht von der Mischung abhing.

Weitere Analysen zeigten, dass die Abnahme der Feinwurzelbiomasse in Mischungen im Vergleich zu Reinbeständen mit Veränderungen anderer Feinwurzeleigenschaften zusammenhing. So waren Feinwurzeln in Mischungen durch höhere spezifische Wurzellängen, geringere

Durchmesser, geringere Gewebedichten und höhere Stickstoffkonzentrationen gekennzeichnet. Insgesamt deutete dies darauf hin, dass Bäume hier andere Strategien zur Ressourcenaufnahme verfolgen. Die höhere Besiedlungsintensität der absorbierenden Feinwurzeln und die höhere Diversität und Abundanz der Ektomykorrhiza in Bodenproben deutet auf eine positive biotische Rückkopplung der Bäume durch Mykorrhiza hin, die die Erschließung der Bodenressourcen durch Bäume in Mischungen signifikant verbessern könnte. Ein wichtiges Ergebnis war die unterschiedliche Reaktion von Nadelbaumarten, die eher dickere Feinwurzeln ausbilden, zu Laubbaumarten, die durch eher dünnere Feinwurzeln gekennzeichnet sind auf die Mischung der Baumarten. So zeigten Laubbaumarten stärkere Reaktionen als Nadelbaumarten, insbesondere in Bezug auf die Wurzelmorphologie und die Mykorrhizierungsrate.

Die Zersetzungsstudie zeigte, dass Feinwurzelstreu in Mischbeständen einer anderen Dynamik als in Reinständen unterliegen kann. So zersetzte sich an den vier Untersuchungsstandorten die Streu, die mehrere Arten enthielt, etwas schneller als die mit nur einer Art in Reinbeständen. Für diese nicht-additiven Effekte scheinen Unterschiede in den Mikro-Umweltbedingungen zwischen Misch- und Reinbeständen verantwortlich zu sein und weniger Interaktionseffekte zwischen den Streuarten. Jedoch hatte die chemische Zusammensetzung des Ausgangsmaterials einen größeren Einfluss auf die Zersetzungsraten als die Baumartenvielfalt. Die Analyse von Standardmaterial zeigte zudem, dass makroklimatische und regionale Unterschiede sowie die Streuart wichtigere Prädiktoren für die Feinwurzelzersetzung waren als Baumartenvielfalt.

### **Schlussfolgerungen**

Diese Arbeit leistet einen wichtigen Beitrag zu unserem Verständnis über die Funktionsweise von Mischwäldern. Die vorliegenden Ergebnisse liefern Belege dafür, dass unterirdische Interaktionen zwischen verschiedenen Baumarten in Mischungen die Aufnahme von Bodenressourcen der Bäume verbessern können. Daher können diese Ergebnisse unter anderem zu einem besseren grundlegenden Verständnis der oft beobachteten höheren Produktivität von Mischbeständen im Vergleich zu Reinbeständen beitragen. Da die hier gemessenen Effekte der Baumartenmischungen auf die Feinwurzelfunktionen und -prozesse über einen großen Umweltgradienten und vier wichtigen europäischen Waldtypen hinweg relativ beständig waren, sind die Ergebnisse dieser Arbeit repräsentativ für die Wälder Europas.





## 1. General introduction

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## **1.1. Mixed-species forests and ecosystem functioning**

### **1.1.1. Biodiversity and ecosystem functioning**

Biodiversity is inevitably linked to ecosystem functioning and thereby with the provision of goods and services essential for human beings (Isbell *et al.*, 2017). The human-induced loss of biodiversity around the globe (Sala *et al.*, 2000; Ceballos *et al.*, 2015) may hence result in a significant reduction of ecosystem functioning (Cardinale *et al.*, 2012; Hooper *et al.*, 2012; Isbell *et al.*, 2017). To better predict the effects of biodiversity loss on ecosystem functioning, an enhanced understanding of underlying mechanisms of species' interactions and their ecological consequences under different abiotic and biotic conditions is a prerequisite (Isbell *et al.*, 2017). Yet, the research field of *Biodiversity and Ecosystem Functioning* (BEF) is relatively young, and our knowledge of BEF relationships is far from complete (Barry *et al.*, 2019). This is particularly the case for complex ecosystems such as forests, which are at the same time among the world's most threatened ecosystems (FAO, 2019).

### **1.1.2. Mixed-species forests as objects of study**

Forests cover about 30% of the earth's land surface, and 93% (3.75 billion ha) of the forest area worldwide are naturally regenerating forest, of which most are dominated by more than one species and hence can be classified as mixed-species forests (Food and Agriculture Organization of the United Nations, 2020). Similarly, about 70% of Europe's forests are mixed-species forests, and this proportion continues to grow (Ministerial Conference on the Protection of Forests in Europe, 2015). This increase is the result of forest management strategies aiming to promote mixed-species forests that fulfil multiple ecosystem functions and provide ecosystem goods (e.g., timber) and services (e.g., climate regulation) to society (Bauhus *et al.*, 2017a).

As early as in the 19<sup>th</sup> century, it had already been recognised that tree species mixtures might fulfil ecosystem functions to a higher degree than monocultures (Cotta, 1828). Scientific evidence gathered during the last decades has shown that mixed-species forests generally enhance ecosystem functioning compared to their mono-specific counterparts. As such, tree species mixtures can be more productive (Piotto, 2008; Zhang *et al.*, 2012; Gamfeldt *et al.*, 2013; Jucker *et al.*, 2014; Kambach *et al.*, 2019), more resistant to biotic and abiotic disturbances (Jactel & Brockerhoff, 2007; Knoke *et al.*, 2008; Castagneyrol *et al.*, 2014; Jactel *et al.*, 2017), promote nutrient cycling (Richards *et al.*, 2010; Handa *et al.*, 2014), increase soil C stocks (Gamfeldt *et al.*, 2013) and enhance the biodiversity of forest-dwelling species (Ampoorter *et al.*, 2020).

Especially given the uncertainties related to global change, silvicultural strategies promoting and maintaining diverse, productive forests with a high degree of adaptive capacity and resilience are required (Puettmann, 2011; Brang *et al.*, 2014). Such approaches are strongly advocated across Europe (Bauhus *et al.*, 2017a), yet our mechanistic understanding of mixed-species forests is greatly limited (Forrester & Bauhus, 2016; Ratcliffe *et al.*, 2017; Barry *et al.*, 2019). As it represents a fundamental necessity for forest management, we urgently need to improve our scientific knowledge of the underlying mechanisms of BEF relationships in forests.

Much evidence of BEF relationships is still based on experimental studies, including grassland studies and young tree diversity experiments (Scherer-Lorenzen *et al.*, 2007; Bruelheide *et al.*, 2014; Tobner *et al.*, 2014; Grossman *et al.*, 2018). These have substantially improved our knowledge, which emerged from theoretical considerations (e.g., Tilman *et al.*, 1997) and laboratory (e.g., mesocosms) experiments. Nevertheless, functioning of artificially assembled communities differs substantially from that of more complex naturally formed communities, and hence this knowledge cannot simply be extrapolated to forest landscapes (Duffy *et al.*, 2017). Therefore, broad implications for managed forest ecosystems need to be supported by real-world studies, including the analysis of forest inventory data and comparative observational studies, which are still relatively rare (van der Plas, 2019). In particular, comparative observational studies (so-called explanatories) carried out in mature forests are a feasible tool for enhancing our understanding of tree species mixing effects in managed forests, as they represent an intermediate approach between tree diversity experiments and forest inventories (Bauhus *et al.*, 2017b). Their *representativeness* of existing forests and thereby their relevance for forest managers is much higher than that of experimental studies (Baeten *et al.*, 2013; Bauhus *et al.*, 2017b; Kambach *et al.*, 2019). In contrast to forest inventories, comparative observational studies are characterised by higher *orthogonality* (i.e., a reduction of potentially confounding factors such as environmental influences) and a higher *comprehensiveness* (i.e., a larger number of ecosystem functions and services can be quantified) (Nadrowski *et al.*, 2010; Baeten *et al.*, 2013; Bauhus *et al.*, 2017b; Kambach *et al.*, 2019).

### **1.1.3. General underlying mechanisms of BEF**

In principle, several non-exclusive mechanisms may cause positive diversity effects in relation to ecosystem functioning. First, positive species interactions can cause competitive reduction in mixtures, i.e., when intraspecific competition is greater than interspecific competition (Forrester & Bauhus, 2016). Competitive reduction may result from spatial, temporal, or chemical resource partitioning, i.e., species use resources differently in time or space, or use other

forms of resources, leading to more complete use of resources (Barry *et al.*, 2019). Second, the mechanism of facilitation - when one or more species positively affect another species' performance (Vandermeer, 1989) - can contribute to positive mixing effects. Facilitation may further be separated into abiotic facilitation and positive biotic feedbacks by other trophic levels (Barry *et al.*, 2019). Competitive reduction and facilitation are together often referred to as complementarity (Forrester & Bauhus, 2016). In addition to complementarity, selection effects, i.e., the dominance of highly productive species, may also cause positive diversity effects (Loreau, 1998). These mechanisms are associated with species' functional traits (Forrester & Bauhus, 2016). Functional traits can be defined as “morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance” (Violle *et al.*, 2007). They thereby determine species' fundamental niches (Bauhus *et al.*, 2017b), and a higher diversity in functional traits can conceptually result in a greater niche differentiation (De Bello *et al.*, 2010). Hence, trait-based approaches are increasingly used to investigate diversity effects (e.g., by analysing the species' dissimilarity in functional traits).

The occurrence and the magnitude of these underlying mechanisms may depend on abiotic and biotic conditions as well as time (e.g., Cardinale *et al.*, 2007; Forrester & Bauhus, 2016; Barry *et al.*, 2019). Our understanding and the predictability of species' interactions along environmental gradients are still limited (Ratcliffe *et al.*, 2017; Barry *et al.*, 2019). By accounting for these factors, the mechanisms driving mixing effects can be better understood (Forrester & Bauhus, 2016). Several hypotheses have been proposed to describe how BEF relationships may change along environmental gradients. For instance, the *stress-gradient hypothesis* states that facilitative processes increase under more stressful and harsher conditions (Bertness & Callaway, 1994). Nevertheless, this hypothesis originally related to grassland studies, which differ significantly from studies in forests (e.g., stand density), and since facilitative processes and competitive reduction are hard to separate (Forrester & Bauhus, 2016), it may be challenging to use this theory as an explanation for patterns observed in forests. Paquette & Messier (2011) reported that complementarity effects were less important than selection effects in more productive (temperate) forests, whereas, in less productive (boreal) forests, complementarity effects were predominant. In addition, selection effects may become weaker over time (Reich *et al.*, 2012) and therefore especially dominate in young forests (Tobner *et al.*, 2016).

#### 1.1.4. The role of the *hidden half* in BEF

The *hidden half*, i.e., the below-ground component of ecosystems, is of fundamental importance for BEF relationships (Bardgett & Van Der Putten, 2014). Not only are above- and below-ground ecosystem components strongly interlinked (Wardle *et al.*, 2004; Bardgett *et al.*, 2014), but positive tree diversity effects on overall ecosystem functioning can be driven by below- and above-ground mechanisms (Eisenhauer, 2012; Forrester & Bauhus, 2016). For instance, positive tree diversity-productivity relationships may result from enhanced above- and below-ground resource availability, uptake, and resource-uptake efficiency (Richards *et al.*, 2010; Forrester, 2017).

Owing to vast methodological challenges related to measuring below-ground ecosystem and plant properties and processes, the below-ground compartment in mixed-species forests has long been neglected. This is even though roots, particularly fine roots, fulfil essential plant functions. As such, they acquire soil nutrients and water and thereby directly affect above-ground properties and processes, including plant growth and vitality (Bardgett *et al.*, 2014). Root competition for these resources may influence plant performance more than shoot competition (Wilson, 1988). Owing to their high plasticity to varying abiotic conditions (Hodge, 2004; Ostonen *et al.*, 2007), fine roots may also play a key role in driving the context-dependency of tree diversity effects.

The relatively limited number of studies examining fine roots in mixed-species forest ecosystems barely allow for broader conclusions about the role of below-ground mechanisms. This is because such studies were mainly conducted in the context of young tree diversity experiments (e.g., Archambault *et al.*, 2019) or focused on only one forest ecosystem with specific environmental conditions and a few species only (e.g., Meinen *et al.*, 2009a; Brassard *et al.*, 2013). Inconsistencies in root classifications and inaccurate sampling methods (Freschet & Roumet, 2017) further greatly limit comparability among different studies. Moreover, only a few below-ground plant traits, such as fine-root biomass, have been looked at, while other traits that may likely better capture ecosystem functions driven by fine roots have largely been neglected (Freschet *et al.*, 2021). As such, mycorrhizal symbionts – which play a crucial role for fine-root resource acquisition, in particular in forests (Brundrett, 2002) – have rarely been considered (Laliberté, 2017; Erktan *et al.*, 2018).

## **1.2. The role of fine roots for ecosystem functioning**

### **1.2.1. General functions of fine roots**

Roots as distinct plant organs have existed for a relatively long time – they evolved during the Devonian period, 416 to 360 million years ago (Raven & Edwards, 2001). Their main functions for plants include the uptake of soil resources, the transport and storage of these resources as well as plant anchorage. The acquisition of nutrients and water from soil is performed by the finest part of the root system - the fine roots. They are usually assisted by symbiotic microorganisms such as mycorrhizal fungi (Brundrett, 2002). Both fine roots and their symbionts, therefore, play a crucial role in the growth and vitality of plants.

Fine roots can further influence multiple ecosystem functions (reviewed by Bardgett *et al.*, 2014), including nutrient (Bardgett *et al.*, 2014) and carbon (C) cycling (Neumann *et al.*, 2021). For instance, by distributing organic material within the soil profile and enmeshing particles together, roots and fungal hyphae (Tisdall & Oades, 1982; Lynch, 1995) can directly influence the rate and stability of soil aggregates, in which organic C may be physically protected from decomposing organisms (occluded particulate organic matter fraction) (Jastrow, 1996). Consequently, fine roots can affect the residence time of organic C in soils and thereby influence the potential for soil C sequestration (Rasse *et al.*, 2005; De Deyn *et al.*, 2008; Cotrufo *et al.*, 2013). Fine roots can also affect nutrient availability in soils through the release of root exudates (Bardgett *et al.*, 2014) and owing to their relatively high nutrient concentrations (Gordon & Jackson, 2000; McCormack *et al.*, 2015), even after death (Brant & Chen, 2015). Besides their impacts on soil chemistry, fine roots, together with mycorrhizal fungi, can also alter soil physical properties, including soil stability, density, and porosity, which in turn determine soil water/nutrient availability and aeration (Bardgett *et al.*, 2014).

Despite the roots' crucial role for overall ecosystem functioning, root research has traditionally lagged behind above-ground plant research. This has recently triggered a surge in root ecological studies (Freschet *et al.*, 2020). For instance, in 2011, the TRY database – which was set up to compile global plant trait data - contained only little information on root traits (Kattge *et al.*, 2011). As such, data on species' rooting depths were only available for approximately 0.05% of vascular plants, while the availability of other root trait data was even lower than that (Kattge *et al.*, 2011). In the meantime, a database on root traits only – the *Fine-Root Ecology Database* (FRED) - has been launched (Iversen *et al.*, 2017). However, species-specific root data are still scarce, and much uncertainty remains about the relationships between measured root traits and root functions (Freschet *et al.*, 2021).

### 1.2.2. Fine-root soil exploration and exploitation

Soil represents a complex matrix in which plant-essential resources are not homogeneously available in space (distribution), time, and quality (the type of resources) (Jackson & Caldwell, 1993; Huber-Sannwald & Jackson, 2001). To acquire these resources, roots and microbial symbionts explore the soil space in search of nutrient-rich patches (i.e., soil exploration), which are then exploited (i.e., the nutrients are taken up). Accordingly, soil exploitation can be defined as “how thoroughly resources are acquired within a given domain, i.e., with no further soil exploration” (York *et al.*, 2013). Root foraging can be defined as “the process whereby an organism searches, or ramifies within its habitat, which enhance its acquisition of essential resources” (Hutchings & de Kroon, 1994). In other words, plant root foraging comprises morphological and physiological changes (plasticity) to the roots that enhance the acquisition of resources (Giehl & von Wirén, 2014).

Several root traits are related to soil exploration and exploitation functions of fine roots (Freschet *et al.*, 2021). In general, the soil exploration function is mainly associated with traits of the entire root system, whereas the exploitation function depends for the most part on traits of absorptive roots (Freschet & Roumet, 2017; Freschet *et al.*, 2021). For instance, the specific root length (SRL) of the entire root system is indicative of the roots’ soil volume explored and exploited per unit of biomass invested, while the SRL of the absorptive roots only indicates the roots’ exploitation capacity (Freschet & Roumet, 2017). A typical explorative trait is the spatial (horizontal and vertical) distribution of roots in soil (Gale & Grigal, 1987; Freschet *et al.*, 2021), whereas the root length density (RLD) – the root length deployed for a given soil volume - is one of the most important traits associated with soil exploitation by fine roots, as root uptake properties and the surface area of absorptive roots (i.e., root hairs and mycorrhizae) are key determinants of nutrient uptake by plants (Lambers & Oliveira, 2019).

During evolution, species have developed different soil resource-uptake strategies, translating into different degrees of intraspecific trait plasticity, allowing plants to maintain continuous soil resource uptake in a heterogeneous environment (Ma *et al.*, 2018). Hence, plants can adapt root morphology, demography, and physiology in response to changes in abiotic conditions (e.g., Fitter, 1994; Hodge, 2004). Intraspecific plasticity of tree fine-root traits to abiotic changes has widely been documented in response to fertilisation treatments (e.g., Noguchi *et al.*, 2013) and along environmental gradients (e.g., Ostonen *et al.*, 2006, 2007, 2011; Zadworny *et al.*, 2016). The *optimal-partitioning theory* further suggests that depending on the most limiting resource



(e.g., light above- vs. nutrients below-ground), trees can change their whole-tree biomass allocation pattern to favour the capture of either above- or below-ground resources (Bloom & Mooney, 1985; Poorter *et al.*, 2012). Intraspecific trait plasticity may also occur as a direct response to allospecific neighbours (Schenk, 2006; de Kroon *et al.*, 2012). As such, it has been shown that roots are capable of identifying the roots of neighbouring plants (Schenk, 2006). Although the underlying mechanisms of such neighbour recognition are not yet fully understood (de Kroon *et al.*, 2012), root exudates (Bais *et al.*, 2006; Wang *et al.*, 2020), which may be regulated by rhizosphere microbiomes (Korenblum *et al.*, 2020), are thought to play an essential role here.

### 1.2.3. Decomposition of fine-root litter

Decomposition of organic matter (OM) is a crucial ecosystem process due to its role in biogeochemical cycling (Attiwill & Adams, 1993; Berg & Mcclaugherty, 2014). Decomposition can be defined as “the process through which dead organic material is broken down into particles of progressively smaller size until the structure can no longer be recognised, and organic molecules are mineralised to their prime constituents: H<sub>2</sub>O, CO<sub>2</sub> and mineral components” (Cotrufo *et al.*, 2009).

Studies on decomposition dynamics have primarily focused on litter from above-ground plant organs, although root litter can constitute up to 70% of total plant biomass (Poorter *et al.*, 2012). Moreover, in boreal forests, 50-70% of soil C were found to originate from roots and related microorganisms (Clemmensen *et al.*, 2013). In addition to their contribution to soil organic matter (SOM) and soil organic carbon (SOC) quantity, roots can also influence SOM/SOC quality and thereby the residence time of C in soils (Rasse *et al.*, 2005). Observations from a long-term field experiment have shown that root-derived C contributes even more to stable SOC pools than does C originating from above-ground plant residues (Kätterer *et al.*, 2011). Hence, below-ground litter inputs may be more significant for soil C sequestration than above-ground inputs. This could particularly apply to the most distal root orders, i.e., root tips, second-, and third-order roots, which have relatively short lifespans (McCormack *et al.*, 2012) and were found to decompose slower than higher-order roots (Xiong *et al.*, 2013; Beidler & Pritchard, 2017), potentially leading to a disproportionate contribution to the root-derived OM pool. In particular, for C dynamics simulation models (Parton *et al.*, 1988), accurate data on the most distal root pool’s decomposition dynamics are therefore indispensable. Owing to its relatively high nutrient concentrations (Gordon & Jackson, 2000), decomposing fine-root litter additionally plays an essential role in nutrient cycling.

Decomposition rates are regulated by several factors, with initial litter quality, climate, and decomposer communities as the most crucial influences (Swift *et al.*, 1979). Root and leaf litter differ significantly in their chemical composition. As such, compared to leaf litter, root litter generally has a higher content of lignin and condensed tannins (Xia *et al.*, 2015) and is surrounded by a complex heterogeneous soil matrix. In contrast to above-ground litter, which is initially deposited on the forest floor surface and which may not get in direct contact with the mineral soil unless through physical processes such as bioturbation, root litter is usually surrounded by soil for the entire course of decomposition. Consequently, the decomposer community dynamics likely differ considerably between above- and below-ground plant litter (Fisk *et al.*, 2011). In addition, tips of ectomycorrhizal (EcM) tree fine roots are often surrounded by fungal sheath, which can significantly affect overall fine-root decomposition rates due to its unique chemical composition (Langley *et al.*, 2006; Koide *et al.*, 2011).

The few existing studies have indeed shown that leaf and root litter can decompose at different rates (Vivanco & Austin, 2006; Hobbie *et al.*, 2010) and that decomposition rates of leaf and root litter may each be determined by different factors (Hobbie *et al.*, 2010; Sun *et al.*, 2018; See *et al.*, 2019). The lack of transferability of decomposition rates of leaf to root litter in combination with the significant role of fine-root litter in biogeochemical cycling, therefore clearly represents a major research gap that needs to be addressed.

### **1.3. Diversity effects on below-ground functions driven by fine roots**

#### **1.3.1. Diversity effects on fine-root soil exploitation**

Similar to above-ground competition for light, plants also compete below-ground for soil resources. Interspecific fine-root trait plasticity enables plants to engage in below-ground competitive interactions with neighbours and consequently determines their coexistence (Callaway *et al.*, 2003). Yet, root-root interactions still represent one of the frontiers in root ecology (Erktan *et al.*, 2018). The *production ecology equation* (Monteith, 1977) can be used as a framework to understand how species' interactions drive mixing effects on productivity (Forrester, 2017):

Gross primary production = resource supply x fraction of resource acquired x resource use efficiency
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Following this equation, total soil resource uptake by trees may increase in mixtures compared to pure stands as a result of greater resource supply, a greater proportion of resources captured, or increased resource-use efficiency (Monteith, 1977; Binkley *et al.*, 2004; Richards *et al.*,

2010; Forrester, 2017). While it is difficult to quantify these three processes directly, the study of functional fine-root traits related to soil exploration/exploitation could provide indicators of enhanced resource acquisition in mixtures compared to pure stands.

In principle, three main mechanisms may result in enhanced or more efficient below-ground resource uptake by fine roots in mixtures, i.e., resource partitioning, abiotic facilitation, and positive biotic feedbacks from other trophic levels (Barry *et al.*, 2019). One of the most prominent examples of positive below-ground species interactions that could enhance soil resource uptake is the complementary use of soil space. For instance, complementary rooting patterns (i.e., deep vs. shallow-rooted species) may allow resource uptake from different soil depths (Berendse, 1979; Schmid & Kazda, 2002). Overall greater resource capture by fine roots in mixtures may also be achieved through chemical resource partitioning, i.e., complementary preferences in nutrient types (McKane *et al.*, 2002; Turner, 2008; von Felten *et al.*, 2009) or temporal resource partitioning, i.e., complementary timing in resource uptake rates (Rothe & Binkley, 2001). Abiotic facilitation can lead to increased soil resource availability and hence higher resource uptake by fine roots in mixtures (Barry *et al.*, 2019). The plant-available resource pool may be enhanced through the presence of N-fixing species (Forrester *et al.*, 2006), the calcium pump effect (Berger *et al.*, 2006), hydraulic redistribution (Burgess, 2011; Prieto *et al.*, 2012), root exudation (Callaway, 2007; Hinsinger *et al.*, 2011) or faster nutrient cycling, for instance, owing to positive litter mixing effects (Hättenschwiler, 2005). Positive biotic feedbacks may, in particular, play a role for tree fine roots in mixtures, as most trees form symbiosis with mycorrhizal fungi (Brundrett, 2009). These feedbacks may include nutrient transfer through a shared mycorrhizal network (Munroe & Isaac, 2014). The presence of mycorrhizae can also reduce pathogen pressure on root growth (Lambers *et al.*, 2017), thereby enhancing overall resource acquisition by fine roots in mixtures (de Kroon *et al.*, 2012). Above-ground interactions can also affect below-ground resource capture by fine roots. For instance, in response to shifts in C allocation patterns at the whole-tree level (Poorter *et al.*, 2012), fine-root resource acquisition in mixtures may become more efficient (Archambault *et al.*, 2019).

Total fine-root biomass (standing biomass or productivity) has been one of the most commonly examined below-ground tree traits in tree diversity studies, as a positive tree diversity effect on fine-root biomass (i.e., overyielding) is supposed to reflect the consequence of positive below-ground species' interactions, similar to above-ground mixing effects. Several grassland studies have shown positive diversity effects on fine-root biomass as a result of complementarity among species (Mommer *et al.*, 2010; Bakker *et al.*, 2018, 2019b; Oram *et al.*, 2018; Mahaut *et al.*, 2020). However, studies on trees ranging from pot experiments to young tree diversity

experiments to mature, natural forests show ambiguous results on tree species mixing on fine-root biomass. As such, higher (Fredericksen & Zedaker, 1995; Schmid, 2002; Schmid & Kazda, 2002; Wang *et al.*, 2002; Brassard *et al.*, 2011, 2013; Laclau *et al.*, 2013; Sun *et al.*, 2017; Liu *et al.*, 2020b; Zeng *et al.*, 2021), lower (Bolte & Villanueva, 2006; Archambault *et al.*, 2019), and no changes in fine-root biomass (Bauhus *et al.*, 2000; Meinen *et al.*, 2009a; Lei *et al.*, 2012a; Beyer *et al.*, 2013b; Brassard *et al.*, 2013; Jacob *et al.*, 2013; Domisch *et al.*, 2015; Ma & Chen, 2016; Finér *et al.*, 2017; Fruleux *et al.*, 2018) were reported in response to increasing tree species diversity. These inconsistencies among studies do not allow general conclusions to be drawn about below-ground tree responses to species mixing. Moreover, fine-root biomass does not directly indicate fine-root resource uptake, which instead depends on the roots' total absorptive surface area, including root hairs (Itoh & Barber, 1983) and microbial symbionts (Brundrett, 2002). Alternatively, RLD is considered a good indicator of species' below-ground competitive ability (Casper & Jackson, 1997; Hodge *et al.*, 1999; Mommer *et al.*, 2011), as it is directly linked to the fine roots' uptake capacity (Hodge *et al.*, 1999; Robinson *et al.*, 1999a). The few studies having investigated the effect of species diversity on RLD have reported both neutral effects for forests (Bauhus *et al.*, 2000; Bolte & Villanueva, 2006; Lei *et al.*, 2012b) and positive effects for grasslands (Mommer *et al.*, 2010; de Kroon *et al.*, 2012).

In addition to an increase in trees' below-ground resource uptake capacity, resource exploitation and uptake efficiency of fine roots could change in mixtures compared to mono-specific stands. So far, only morphological root trait adaptations, including increased SRL in mixtures, have been observed (e.g., Bolte & Villanueva, 2006; Bu *et al.*, 2017; Salahuddin *et al.*, 2018). Other important fine-root traits and the role of mycorrhizal fungi remain to be investigated.

To conclude, our knowledge of tree diversity effects on fine-root properties and processes is greatly limited, although fine roots have immense importance for below-ground resource acquisition by trees.

### **1.3.2. Diversity effects on fine-root decomposition**

In theory, tree species mixing can alter decomposition rates of fine-root litter, thereby affecting ecosystem functioning via several interrelated direct and indirect mechanisms (Hector *et al.*, 2000; Gessner *et al.*, 2010). Direct mechanisms include interactions between different litter types (interaction effects), altering the decomposition rates of litter mixtures compared to single-species litter. In contrast, indirect mechanisms relate to the mixture environment and may include changes in microclimate, soil conditions, and the abundance and diversity of soil organisms.

Direct diversity effects on litter decomposition may be the result of positive (synergistic) or negative (antagonistic) interactions between litter species or of selection effects, i.e., the strong influence of a particular litter species characterised by high or low decomposition rates (Loreau & Hector, 2001). These interactions may result in non-additive effects, i.e., decomposition rates of the litter mixtures cannot be predicted based on observations of the decomposition of single-species litter (Handa *et al.*, 2014). When synergistic and antagonistic effects occur simultaneously, they can cancel each other out and result in overall additive net effects (Hättenschwiler, 2005; Hui & Jackson, 2009). Synergistic effects may derive from complementary resource use among detritivores (Hättenschwiler *et al.*, 2005; Vos *et al.*, 2013) or fungal nutrient transfer from nutrient-rich to nutrient-poor litter (Schimel & Hättenschwiler, 2007; Tiunov, 2009; Handa *et al.*, 2014). In addition, the dissimilarity in physical litter properties, such as in water holding capacities, may result in enhanced micro-climatic conditions and thereby synergistic effects (Makkonen *et al.*, 2013). Antagonistic effects may occur owing to the presence of inhibitory secondary compounds such as condensed tannins (Hättenschwiler, 2005), the inhibitory effect of N-rich litter on lignin-rich litter (Dijkstra *et al.*, 2009; Berg, 2014), or unfavourable micro-climatic conditions (Makkonen *et al.*, 2013). As initial litter quality has been found to affect decomposition rates of roots and leaves in different ways (Sun *et al.*, 2018), the types of interactions occurring in mixed-species root and leaf litter may also differ (Jiang *et al.*, 2019). In addition to direct effects, tree species mixing may affect the decomposing environment indirectly via changes in microclimate (Joly *et al.*, 2017), edaphic properties (Prescott & Grayston, 2013; Vesterdal *et al.*, 2013; Scheibe *et al.*, 2015; Dawud *et al.*, 2016) and consequently soil fauna (Korboulewsky *et al.*, 2016) and microbial communities (Scheibe *et al.*, 2015) or priming effects via root exudation (Zwetsloot *et al.*, 2020).

Studies examining mixing effects on leaf litter decomposition have yielded inconclusive results and mainly reported synergistic or additive effects (Gartner & Cardon, 2004; Hättenschwiler, 2005; Lecerf *et al.*, 2011; Kou *et al.*, 2020; Liu *et al.*, 2020a; Porre *et al.*, 2020). Moreover, two recent meta-analyses on leaf litter mixing reached opposite conclusions: A meta-analysis, which considered 69 individual studies, showed that leaf litter mixing in forests overall resulted in positive effects (Liu *et al.*, 2020a), whereas another meta-analysis reported generally additive effects for two-species mixtures (Porre *et al.*, 2020).

In contrast to studies on leaf litter mixing, there are few studies that have examined the effects of plant richness on root decomposition, particularly for tree roots. Negative (Chen *et al.*, 2017a,b; Prieto *et al.*, 2017) and positive (De Graaff *et al.*, 2011; Prieto *et al.*, 2017) effects of species mixing on fine-root litter mass loss have been reported for grasslands. Another study,

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including plant species from polar regions, reported both positive and negative litter mixing interactions (Robinson *et al.*, 1999b). Li *et al.* (2018) analysed tree and shrub fine-root decomposition of mixed-root and single-root litter under the same canopy for three years in a subtropical forest. They reported no effect of litter mixing on root litter mass loss in the absence of detritivores, whereas in the presence of detritivores, litter mass loss was significantly reduced in mixed-litter bags compared to single-species litter bags (Li *et al.*, 2018). Moreover, in a tropical tree diversity experiment (Sardinilla), neither mass loss of mixed-species root litter buried in tree species mixtures differed from single-species root litter in mono-specific stands nor did the within-bag litter richness affect root mass loss under the same canopy (Guerrero-Ramírez *et al.*, 2016). Most recently, the mixing of fine-root litter (the first two most distal root orders) of two tree species in subtropical plantations, both under control conditions and with nutrient addition, resulted in synergistic effects (Jiang *et al.*, 2019).

This extremely small number of studies dealing with diversity effects on fine-root litter decomposition in forest ecosystems highlights an immense research gap, despite the crucial role of fine-root litter for SOM dynamics. To improve our mechanistic understanding of litter mixing effects, the influences of abiotic and biotic conditions and initial litter quality on the decomposition dynamics of fine roots need to be additionally investigated.

## **1.4. Research objectives**

While ample evidence has been presented that mixed-species forests can provide multiple ecosystem services and goods to a higher degree than single-species forests (e.g., Gamfeldt *et al.*, 2013; Forrester & Bauhus, 2016; van der Plas *et al.*, 2016; Jactel *et al.*, 2017; Ammer, 2019), vast knowledge gaps on the mechanistic understanding of these positive tree diversity effects exist. In particular, the role of the below-ground ecosystem compartment controlled by fine roots is still largely unexplored. Therefore, the overall research aim of this work was to investigate the effects of tree diversity on below-ground properties and processes driven by tree fine roots in four wide-spread European forest types.

The following objectives were formulated:

### **Chapter two**

- To assess tree diversity effects on fine-root soil space occupation (i.e., standing fine-root biomass and root length density) and vertical root stratification
- To determine whether complementary or selection effects drive below-ground responses to tree species mixing

### **Chapter three**

- To analyse the effect of tree diversity on soil exploitation strategies of tree fine roots under consideration of their mycorrhizal symbionts
- To assess the roles of phylogenetic tree identity for tree species mixing effects
- To investigate the role of functional fine-root trait diversity for below-ground soil exploitation by tree fine roots

### **Chapter four**

- To determine early-stage fine-root litter decomposition rates in response to tree species mixing
- To investigate whether direct or indirect effects drive non-additive decomposition rates of mixed-species root litter
- To examine the relative influence of initial litter quality, macro- (regional scale) and micro-environmental conditions on fine-root litter decomposition



## **1.5. Study design and research framework**

In this subsection, I address overarching points that are relevant to the applied methodological approaches of all three result chapters. Detailed descriptions of the study design are provided in the following result chapters.

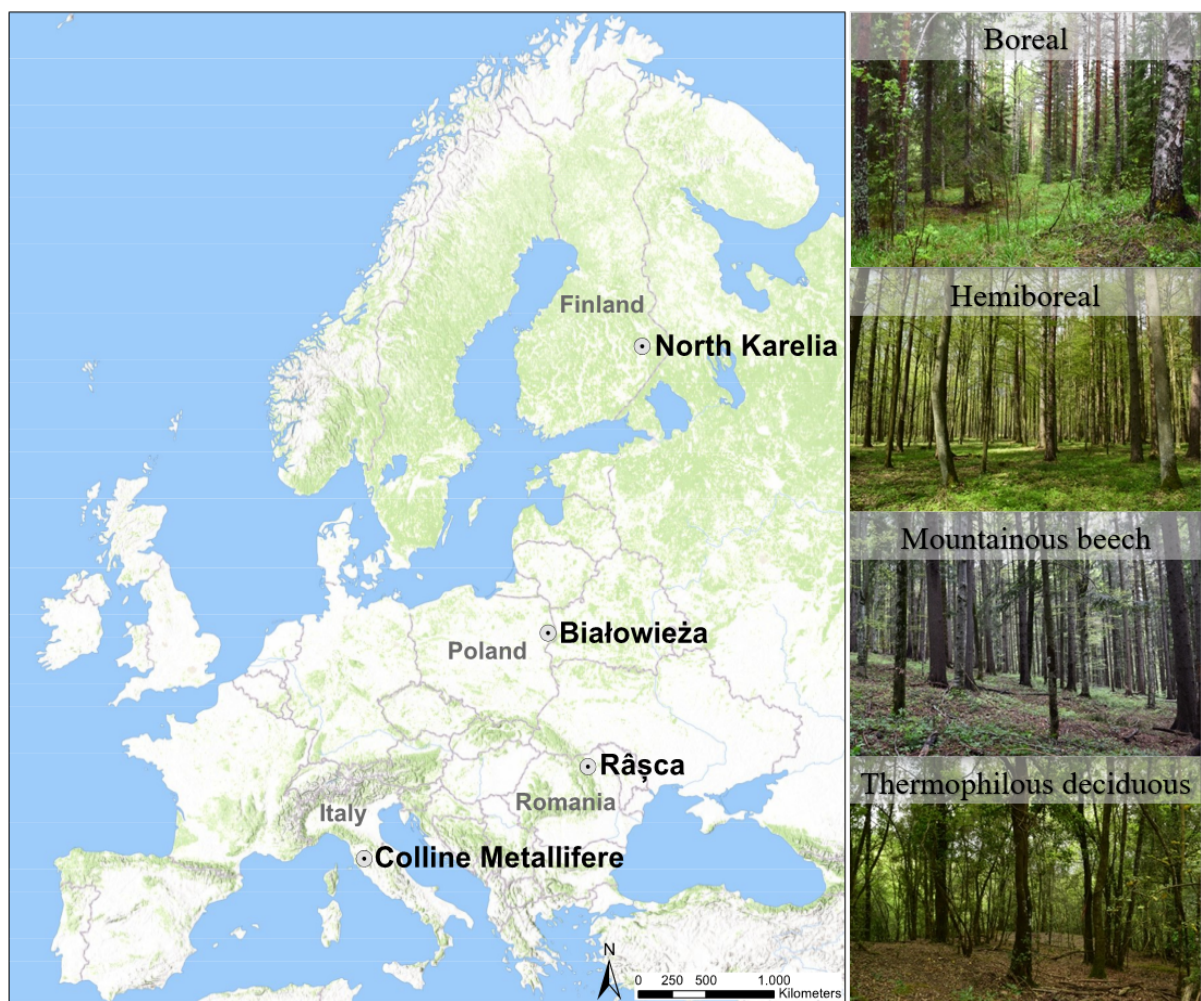
### **1.5.1. SoilForEUROPE Project**

This doctoral research was carried out in the framework of the interdisciplinary SoilForEUROPE project, which aimed at investigating the role of forest soil biodiversity for ecosystem goods and services in European forests. Briefly, the three main objectives of SoilForEUROPE were (1) to assess the effect of tree species richness on soil biodiversity across European forests, (2) to investigate ecosystem resistance and resilience to drought effects in connection with soil biodiversity, and (3) to determine the socio-economic value of soil biodiversity. Whereas this thesis focused on how tree species mixing affects below-ground properties and functions driven by fine roots, the data collected on fine roots were a crucial foundation for the work of other project partners (Gillespie *et al.*, 2020; Prada-Salcedo *et al.*, 2021; Prada-Salcedo *et al.*, 2021). This is because tree roots may be an important driver for below-ground biodiversity (Bardgett & Van Der Putten, 2014), which can promote essential ecosystem services in forests (Bakker *et al.*, 2019a). SoilForEUROPE was a follow-up project of FunDivEUROPE, which aimed at assessing forest biodiversity effects on ecosystem functioning, with a focus on above-ground ecosystem components (e.g., Baeten *et al.*, 2013; Jucker *et al.*, 2014; van der Plas *et al.*, 2016; Joly *et al.*, 2017; Ratcliffe *et al.*, 2017).

### **1.5.2. Study sites**

The study sites of the SoilForEUROPE project were a subset of the FunDivEUROPE exploratory forest plot network, which represents a unique comparative study platform along a large environmental gradient (Baeten *et al.*, 2013). This plot network covers tree species diversity gradients in mostly mature, uneven-aged forest stands in six European countries (Baeten *et al.*, 2013). Three essential criteria were considered for plot selection: Evenness (approximately equal abundances of the target species in mixtures), a maximum of 5-10% basal area of non-target species, and a minimal influence of confounding factors (Baeten *et al.*, 2013). The dimension of the plots was 30 x 30 m and plots were surrounded by 10 m buffer zones characterised by similar conditions as the plots themselves. Owing to the vast sampling effort associated with below-ground ecosystem properties, only mono-specific and mixed-species plots, consisting of three different tree species (hereafter referred to only as mixtures), were considered in this study. The SoilForEUROPE plots were distributed across four sites in four countries (Fig.

1.1) and were representative of four wide-spread European forest types, i.e., boreal (Finland), hemiboreal (Poland), mountainous beech (Romania), and thermophilous deciduous forest (Italy). Of the total 63 plots, 30 plots represented mono-specific and 33 mixed stands, consisting of a pool of 3-5 indigenous target tree species per site that were representative of the local tree community (Table 1.1). In total, 13 tree species were studied across the four sites. Each species had two replicate mono-specific plots (except for *Quercus robur* and *Picea abies* in Poland, with one replicate each). Mixtures consisted of different species combinations, and not all possible mixture combinations were represented. Replication number of identical species compositions varied from one to four (Table 1.1).



**Figure 1.1** Locations of the four study sites in North Karelia (Finland), Białowieża (Poland), Râșca (Romania), and Colline Metallifere (Italy). Photos on the right-hand side are representative of mixed-species stands of the four forest types. Source map: ESRI, 2020.

**Table 1.1** Stand and environmental characteristics of the four study sites. Abbreviations: MAT (mean annual temperature), MAP (mean annual precipitation).

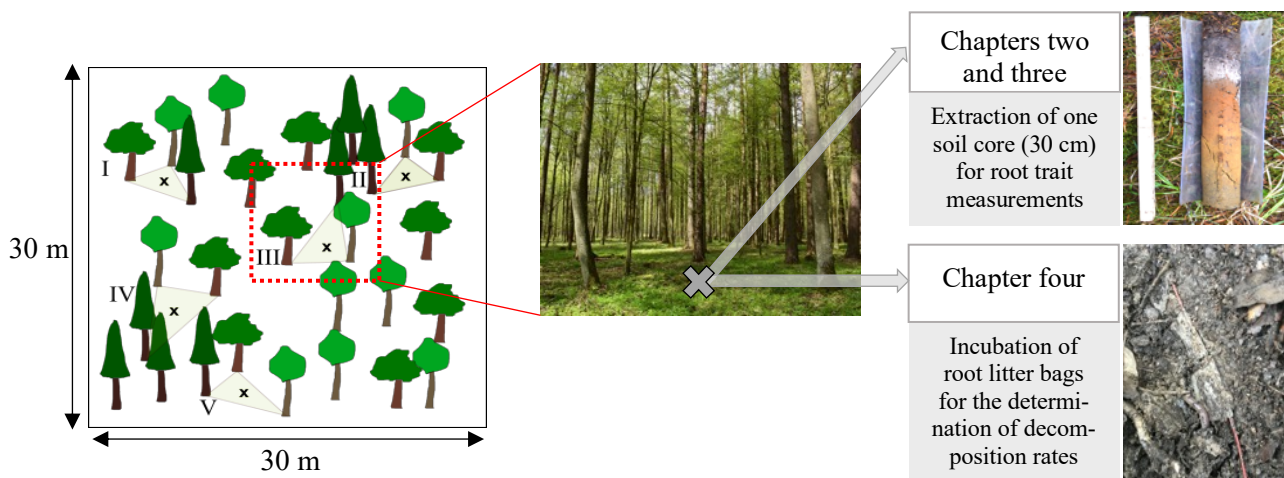
Forest type	Boreal forest	Hemiboreal forest	Mountainous beech forest	Thermophilous deciduous forest
Site	North Karelia	Białowieża	Râșca	Colline Metallifere
Country	Finland	Poland	Romania	Italy
Latitude, Longitude (°)	62.6, 29.9	52.7, 23.9	47.3, 26.0	43.2, 11.2
Number of mono-specific/mixed plots	6/3	6/13	8/8	10/9
Target tree species	<i>Betula pendula</i> , <i>pubescens</i> , <i>Picea abies</i> , <i>Pinus sylvestris</i>	<i>Betula pendula</i> , <i>Carpinus betulus</i> , <i>Picea abies</i> , <i>Pinus sylvestris</i> , <i>Quercus robur</i>	<i>Abies alba</i> , <i>Acer pseudoplatanus</i> , <i>Fagus sylvatica</i> , <i>Picea abies</i>	<i>Castanea sativa</i> , <i>Ostrya carpinifolia</i> , <i>Quercus cerris</i> , <i>Quercus ilex</i> , <i>Quercus petraea</i>
Species compositions (number of plot replicates/ number of triplet replicates)	<i>B. pendula</i> (2/10) <i>P. abies</i> (2/10) <i>P. sylvestris</i> (2/10)	<i>C. betulus</i> (2/10) <i>P. abies</i> (1/5) <i>P. sylvestris</i> (2/10) <i>Q. robur</i> (1/5)	<i>A. alba</i> (2/10) <i>A. pseudoplatanus</i> (2/10) <i>F. sylvatica</i> (2/10) <i>P. abies</i> (2/10)	<i>C. sativa</i> (2/10) <i>O. carpinifolia</i> (2/10) <i>Q. cerris</i> (2/10) <i>Q. ilex</i> (2/10) <i>Q. petraea</i> (2/10)
	<i>B. pendula</i> , <i>P. abies</i> , <i>P. sylvestris</i> (3/15)	<i>B. pendula</i> , <i>C. betulus</i> , <i>P. abies</i> (1/5) <i>B. pendula</i> , <i>C. betulus</i> , <i>P. sylvestris</i> (2/10) <i>B. pendula</i> , <i>C. betulus</i> , <i>Q. robur</i> (2/10) <i>B. pendula</i> , <i>P. abies</i> , <i>P. sylvestris</i> (1/5) <i>B. pendula</i> , <i>P. abies</i> , <i>P. sylvestris</i> (1/5) <i>B. pendula</i> , <i>P. abies</i> , <i>Q. robur</i> (1/5)	<i>A. alba</i> , <i>A. pseudoplatanus</i> , <i>F. sylvatica</i> (4/20) <i>A. alba</i> , <i>F. sylvatica</i> , <i>P. abies</i> (3/15) <i>A. alba</i> , <i>A. pseudoplatanus</i> , <i>P. abies</i> (1/5)	<i>C. sativa</i> , <i>O. carpinifolia</i> , <i>Q. ilex</i> (1/5) <i>C. sativa</i> , <i>Q. cerris</i> , <i>Q. ilex</i> (1/5) <i>C. sativa</i> , <i>Q. cerris</i> , <i>Q. petraea</i> (2/10) <i>C. sativa</i> , <i>Q. ilex</i> , <i>Q. petraea</i> (1/5) <i>O. carpinifolia</i> , <i>Q. cerris</i> , <i>Q. ilex</i> (1/5) <i>O. carpinifolia</i> , <i>Q. petraea</i> (1/5) <i>Q. cerris</i> , <i>Q. ilex</i> , <i>Q. petraea</i> (1/5)

		<i>C. betulus</i> , <i>P. abies</i> , <i>P. sylvestris</i> (1/5)		
		<i>C. betulus</i> , <i>P. abies</i> , <i>Q. robur</i> (1/5)		
		<i>C. betulus</i> , <i>P. sylvestris</i> , <i>Q. robur</i> (2/10)		
		<i>P. abies</i> , <i>P. sylvestris</i> , <i>Q. robur</i> (2/10)		
Approximate average stand age (y)	53	106	90	69
Stand development stage <sup>1</sup>	Mid/late stem exclusion, understory reinitiation	Mid/late stem exclusion, understory reinitiation	Mid/late stem exclusion, understory reinitiation	Mid/late stem exclusion
Elevation range (m)	80-200	135-185	600-1000	260-525
MAT (°C)	2.1	6.9	6.8	13
MAP (mm)	700	627	800	850
Soil type	Podzol	Cambisol/ Luvisol	Eutric Cambisol	Cambisol
Forest floor types	Mor and Mull	Mull and Moder	Mull	Amphi, Mull, Moder
Bedrock types	Mica schists, quartzites, granites and granodiorites	Fluvioglacial (sands, clays, loams)	Sandstone	Quartzite-anagenite (verrucano), sandstone conglomerate, diabase, limestone
Mean max. soil depth (cm)	80	80	75	67
Topsoil texture class	Sandy loam	Sandy loam	Silty clay loam	Silt loam
Topsoil clay %	5	6	27	18
Topsoil pH	3.9	3.8	4.6	4.6
Topsoil carbon (mg g <sup>-1</sup> )	37.8	28.4	49.2	50.4
Topsoil nitrogen (mg g <sup>-1</sup> )	1.7	1.7	3.5	2.6

<sup>1</sup> according to Oliver & Larson (1996)

### 1.5.3. Triplet approach

Within each plot, five subplots, so-called tree *triplets*, were selected following the approach of Vivanco & Austin, 2008 (Fig. 1.2). Each of these tree *triplets* consisted of three neighbouring similar-sized tree individuals, arranged in a triangle. In mixtures, these *triplets* were composed of the three target species and in mono-specific stands of the same species. This neighbourhoods approach was chosen instead of a random sampling design to maximise the influence of the target tree species and minimise other impacts on our sampling spots (Vivanco & Austin, 2008). It further ensured a higher probability of capturing interactions of the target species' root systems. Selection criteria for the *triplet* trees were (target) tree species, size (diameter at breast height, height, and crown size), social status according to Kraft's classes (Kraft, 1884), vitality, health status (i.e., visible signs of diseases), distances to each other and representativeness of microtopography. Ideally, the tree individuals forming one *triplet* were of equal size, dominant or co-dominant and healthy. The five tree neighbourhoods were typically evenly distributed across the plot and a few meters apart from each other. Owing to the clustering of species in some mixture plots, in some cases the 10 m buffer zones of the plots had to be used to find *triplets*.



**Figure 1.2** Illustration of the sampling design using a mixed-species plot as an example (in mono-specific stands, triplets consisted of three tree individuals of the same species).

### 1.5.4. Data collection

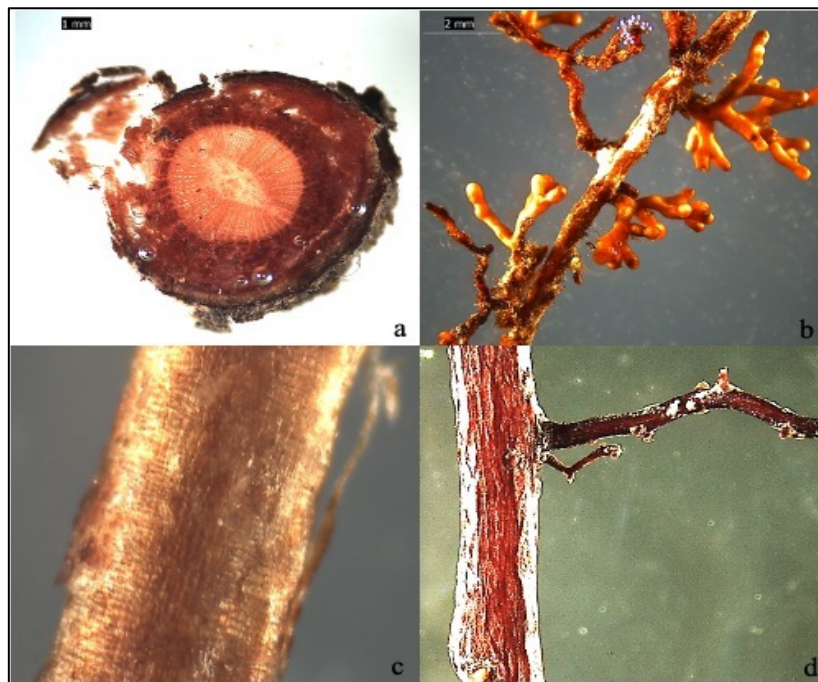
In total, four separate sampling campaigns were conducted as part of this work. The first sampling campaign in spring 2017 was a collective effort of the entire SoilForEUROPE-consortium to ensure a consistent sampling approach among the project partners. All other sampling campaigns (in autumn 2017, spring 2018, and 2019) were conducted by myself.



### 1.5.5. Laboratory work

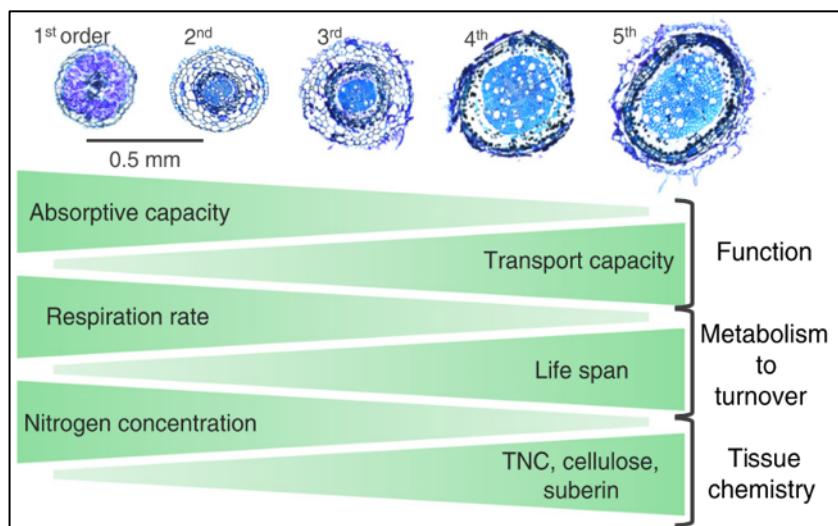
An essential part of this study was the identification and differentiation of different fine-root species. This was done manually by visual inspection in the laboratory using a microscope. Manual sorting of fine roots is particularly time-consuming when dealing with a large number of samples (in total, 928 for this thesis). Other methods, such as DNA-based techniques, are relatively expensive and require specific equipment (Rewald *et al.*, 2012a) and were therefore not an option for this work. The use of near-infrared spectroscopy could have been an alternative (Lei & Bauhus, 2010), yet, there are several draw-backs related to this method, including, for instance, vast calibration efforts required due to the high number of different species' compositions from four study sites (Finér *et al.*, 2017).

Criteria for the manual differentiation of fine-root species included periderm structure, cross-section, architecture, colour, and mycorrhizal associations (for examples, see Fig. 1.3 and for details, Appendix Table S2.1). Moreover, reference samples were collected for each species from each of the four sites during the first sampling campaign. For these samples, roots were traced back to their mother trees. Intact reference samples for each species were stored in 40% ethanol for preservation.



**Figure 1.3** Examples of four different root species with distinct identification criteria. (a) *Abies alba* has a distinct red-coloured cortex; (b) *Pinus sylvestris* is often associated with an EcM fungi ensheathing root tips with a dichotomous shape; (c) *Quercus petraea*, as other *Quercus* species, is characterised by a shiny, transparent layer of old periderm and fine lateral furrows; (d) *Fagus sylvatica* has a reddish, relatively rough surface with coarser, irregular longitudinal furrows.

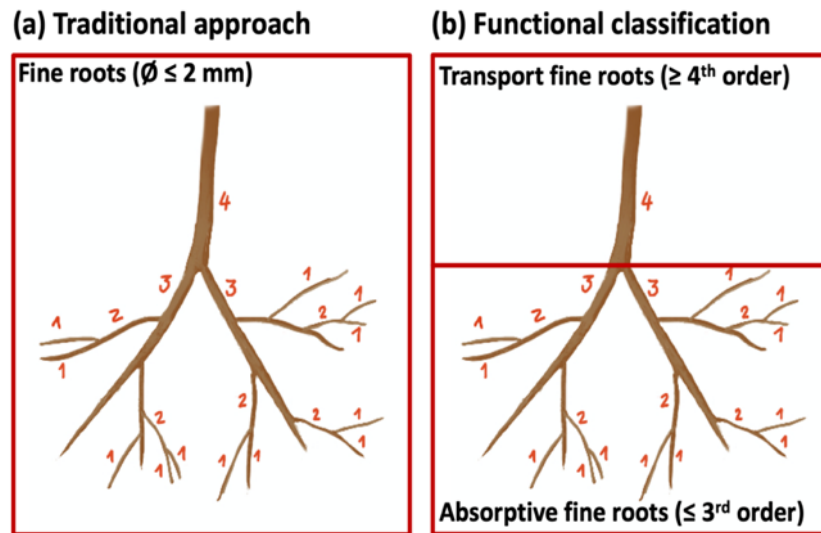
Inconsistent definitions and classifications of fine roots represent major challenges in root ecological research, severely limiting comparability among studies. Fine roots have traditionally been classified according to diameter classes, i.e., mainly as roots with a diameter  $\leq 2$  mm, while roots with a diameter  $> 2$  mm have been defined as coarse roots. It has recently been recognised that a definition based on such a diameter cut-off is inadequate for investigating the role of fine roots for ecosystem functioning (McCormack *et al.*, 2015; Laliberté, 2017). This is because roots within this 2-mm-diameter root pool differ significantly in anatomy, absorptive/transport capacity, and lifespan (McCormack *et al.*, 2015) and such a heterogeneous pool of roots therefore does not sufficiently represent the fine roots' functions (Guo *et al.*, 2008; Makita *et al.*, 2009). Instead, root functions can better be linked to individual root orders (Fig. 1.4, McCormack *et al.*, 2015). Therefore, an order-based instead of a diameter-based approach has recently been suggested for the classification of fine roots (McCormack *et al.*, 2015; Fahey *et al.*, 2017; Freschet & Roumet, 2017; Laliberté, 2017).



**Figure 1.4** Functions of roots by root order. Adopted from McCormack *et al.*, 2015, with permission from Wiley. First-three-order roots are characterised by an intact root cortex, whereas in fourth- and higher-order roots the cortex is absent.

The application of such an order-based approach is extremely laborious and thereby often unfeasible given the tight project schedules and limited budgets. An intermediate approach – the functional classification approach – has thus been proposed as an alternative and compromise in terms of time and budget (Fig. 1.5, McCormack *et al.*, 2015). This approach is based on the finding that across species, the first-three-order roots can mainly be linked to absorptive capacity, whereas higher-order roots are mainly characterised by the transport function (Fig. 1.4,

McCormack *et al.*, 2015). As the main objective of this doctoral thesis was to capture tree species mixing effects on the functions associated with the most-distal root orders, i.e., resource uptake capacity, the application of the functional classification approach was an inevitable foundation of this study.



**Figure 1.5** Comparison of fine-root classification approaches: (a) Traditional approach ( $\leq 2$  mm in diameter), and (b) the functional classification approach, which further divides all roots  $\leq 2$  mm in diameter into absorptive (i.e., first three root orders) and transport roots (i.e., higher-order roots). Red numbers indicate root orders. Adapted from McCormack *et al.* 2015.

### 1.5.6. Datasets and data use

Data collected during the initial field campaign (spring 2017) and subsequent laboratory work were finally fed into a common dataset. For the data analyses related to the third chapter of this thesis, I used data on the diversity and abundance of mycorrhizal fungi obtained from samples taken adjacent to the root sampling spots during the first field campaign. Likewise, for the analyses of the fourth chapter, I used data on forest floor properties derived from this common dataset. In addition, I used data not collected in the context of the SoilForEUROPE project, but accessible on the FunDivEUROPE platform (<https://data.botanik.uni-halle.de/fundiveurope>). These include soil property data, originating from measurements in 2012 and microclimate data taken at the plot level during root litter incubation (spring 2018 until spring 2019).



## 2. Tree species mixing reduces biomass but increases length of absorptive fine roots in European forests

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Wambsganss, J., Beyer, F., Freschet, G. T., Scherer-Lorenzen, M., Bausch, J. (2021): *Tree species mixing reduces biomass but increases length of absorptive fine roots in European forests*. Journal of Ecology. DOI: <https://doi.org/10.1111/1365-2745.13675>

Link to publication: <https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2745.13675>



## 2.1. Abstract

1. Mixed-species forests often enhance the provision of ecosystem functions, both above and below-ground. Several of these effects are mediated by the amount and spatial distribution of tree tissues. However, previous studies on tree diversity effects on fine-root biomass (FRB) have returned inconsistent results and did not distinguish between absorptive and transport fine roots. Furthermore, owing to the lack of species-specific data, it is not well understood whether complementarity or selection effects contribute more to these mixing effects.
2. Here, we analysed tree species mixing effects on fine-root traits while considering the respective tree species contributions and root functional types. Specifically, we tested whether tree species mixing increases FRB and root length density (RLD) and results in vertical root stratification. We quantified FRB and RLD in 30 cm deep soil profiles for 13 tree species in mixed and pure stands across four wide-spread European forest types. The differentiation of different fine-root species in mixtures allowed us to disentangle complementarity and selection effects.
3. Across all sites, mixtures supported on average less FRB than pure stands, which was reflected in negative complementarity and selection effects. RLD of absorptive fine roots did not change across the soil profile and even increased in the topsoil, which was associated with positive complementarity effects. There was no evidence for vertical root stratification. Conifer proportion, which was mainly associated with selection effects, dampened net diversity effects. Root functional type further influenced tree species mixing effects.
4. **Synthesis:** Despite the underyielding of FRB in mixtures, overall soil occupation by absorptive fine roots (RLD) did not decrease in mixtures, pointing to morphological root trait adaptations associated with higher resource-use efficiency. Increased RLD in the most nutrient-rich layer in mixtures further indicates complementary interactions among species and a greater resource uptake capacity. This work illustrates that considering only one aspect of trait-functioning relationships, e.g. root biomass, may not capture the full effect of plant diversity on ecosystem functioning. The integration of a larger range of relevant traits is required. Moreover, traditional classification of fine roots based on the 2-mm diameter cut-off may obscure responses of roots to environmental changes.

**Key words:** additive partitioning, biodiversity and ecosystem functioning, root length density, SoilForEUROPE, species identity effects, tree species richness, vertical root distribution.

### 3. Tree species mixing causes a shift in fine-root soil exploitation strategies across European forests

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Wambsganss, J., Freschet, G. T., Beyer, F., Goldmann, K., Prada-Salcedo, L. D., Scherer-Lorenzen, M., Bauhus, J. (2021): *Tree species mixing causes a shift in fine-root soil exploitation strategies across European forests*. Functional Ecology. DOI: <https://doi.org/10.1111/1365-2435.13856>

Link to publication: <https://besjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1365-2435.13856>



### 3.1. Abstract

1. Mixed-species forests have often been shown to enhance above-ground ecosystem properties and processes. Despite the significance of fine roots for tree and ecosystem functioning, the role of tree species diversity for below-ground processes driven by fine roots remains largely unknown. Previously, an underyielding of fine-root biomass (FRB) in tree mixtures across four major European forest types has been reported. To explain this phenomenon, we tested here the effect of tree species mixing on fine-root traits related to soil exploitation efficiency, including biotic feedbacks from ectomycorrhizal fungi (EcM), and assessed the role of root trait dissimilarity.
2. We analysed morphological and chemical traits as well as ectomycorrhizal colonisation intensity of absorptive fine roots (i.e. first three most distal orders) in soil samples from 315 mixed and mono-specific tree neighbourhoods in mainly mature, semi-natural forest stands across Europe. Additionally, we quantified mycorrhizal abundance and diversity in soil samples from the same stands.
3. At the community level, fine roots in tree mixtures were characterised by higher specific root lengths and root nitrogen concentrations, lower diameters, and root tissue densities indicating a *faster* resource acquisition strategy compared to mono-specific stands. The higher root EcM colonisation intensity and soil EcM diversity in mixtures compared to mono-specific stands may further provide evidence for positive biotic feedbacks. Moreover, the diversity of fine-root traits influenced FRB, as mixtures characterised by a higher trait dissimilarity were linked to a lower reduction in FRB. At the level of phylogenetic groups, thin-rooted angiosperm species showed stronger responses to mixing than thick-rooted gymnosperms, especially in terms of root morphology and EcM colonisation, indicating different strategies of response to tree mixing.
4. Our results indicate that a lower FRB can reflect a shift in soil resource acquisition strategies, rather than a lower performance of trees in mixtures. They show that several non-exclusive mechanisms can simultaneously explain negative net effects of mixing on FRB. This study sheds new light on the importance of using integrative approaches including both above- and below-ground biomass and traits to study diversity effects on plant productivity.

**Key words:** absorptive fine roots, biodiversity-ecosystem functioning, fungal diversity, functional dispersion, SoilForEUROPE, species identity effects, trait dissimilarity, tree species richness.

#### **4. Tree diversity, initial litter quality, and macroclimate drive early-stage fine-root decomposition in European forests**

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Wambsganss, J., Freschet, G. T., Beyer, F., Bauhus, J., Scherer-Lorenzen, M.: *Tree diversity, initial litter quality, and macroclimate drive early-stage fine-root decomposition in European forests*. Under revision, Ecosystems.





#### 4.1. Abstract

Decomposition of dead fine roots contributes significantly to nutrient cycling and soil organic matter stabilization. Most knowledge on tree fine-root decomposition stems from studies in mono-specific stands or single-species litter, although most forests are mixed. Therefore, we assessed how tree species mixing affects fine-root litter mass loss and which role initial litter quality and environmental factors play. For this purpose, we determined fine-root decomposition of 13 common tree species in four European forest types ranging from boreal to Mediterranean climates. Litter incubations in 315 tree neighborhoods allowed for separating the effects of litter species from environmental influences, and litter mixing (direct) from tree diversity (indirect). On average, mass loss of mixed-species litter was higher than those of single-species litter in mono-specific neighborhoods. This was mainly attributable to indirect rather than direct diversity effects. Overall diversity effects were relatively weak, and initial litter quality and macroclimate were more important predictors of fine-root litter mass loss than tree diversity. We showed that tree species mixing can alter fine-root litter mass loss across large environmental gradients, but these effects are context-dependent and of moderate importance. Interactions between species identity and macroclimate need to be considered to explain diversity effects on fine-root decomposition.

**Key words:** absorptive fine roots, environmental gradient, functional trait diversity, site-specific litter, SoilForEUROPE, species identity, standard litter, tree species mixing.

## 4.2. Introduction

The decomposition of plant litter is one of the key processes influencing carbon and nutrient cycling and thereby crucial for overall ecosystem functioning (Swift *et al.*, 1979). Drivers of decomposition rates have mainly been identified for above-ground litter, despite the substantial contribution of root litter to soil organic matter (SOM) (Jackson *et al.*, 1997; Clemmensen *et al.*, 2013). For example, in boreal forests, soil organic C (SOC) originated up to 50-70 % from root litter and root-associated microbes (Clemmensen *et al.*, 2013). Necromass of both fine roots and fungi, which are often associated with roots, can contribute to the stabilization of SOM, thereby possibly increasing C residence time in soils (Cotrufo *et al.*, 2013; Adamczyk *et al.*, 2019a). In particular, the most distal root orders have relatively low lifespans (McCormack *et al.*, 2012) and yet may decompose slower than higher-order roots (e.g., Xiong and others 2013). Hence, fine roots may play a significant role in soil C sequestration. In addition, fine-root turnover is very important for nutrient cycling (Jackson *et al.*, 1997; Gordon & Jackson, 2000). Owing to lower nutrient resorption, fine-root nutrient concentrations at senescence are likely higher than in freshly shed leaves (Brant & Chen, 2015). In addition, trees may allocate a greater share of biomass to roots than to foliage (Reich *et al.*, 2014). This further highlights the crucial role of tree root litter for C and nutrient cycling in forests.

Several studies have shown that leaf and root litter can decompose at different rates (Vivanco & Austin, 2006; Hobbie *et al.*, 2010; Freschet *et al.*, 2013). Their decomposition rates are controlled by distinct factors, and particularly lignin appears to play different roles (Hobbie *et al.*, 2010; Sun *et al.*, 2018). Contrasting drivers consequently prevent a simple transfer of results from leaf litter decomposition studies to those of fine-root litter. Moreover, inconsistent results regarding the major controls of fine-root decomposition have been reported. A recent meta-analysis suggested that climatic factors, i.e., mean annual temperature (MAT) and precipitation (MAP), were more significant predictors of fine-root decomposition rates than initial litter chemistry (See *et al.*, 2019). However, previous (meta-)analyses showed that initial litter quality (particularly root nitrogen (N), calcium (Ca) and lignin concentrations) determined fine-root decomposition rates more than climatic factors (Silver & Miya, 2001; Zhang *et al.*, 2008; Zhang & Wang, 2015).

Most studies of root litter decomposition dynamics have concentrated on single species, even though the majority of the world's natural forests are mixed (Bauhus *et al.*, 2017a) and so is

their litter. Moreover, current management strategies increasingly focus on promoting tree species mixtures (Bauhus *et al.*, 2017a), as they can enhance the provision of ecosystem services compared to their mono-specific counterparts (reviewed by Scherer-Lorenzen, 2014).

Tree diversity may alter litter decomposition rates via several direct and indirect effects (Hector *et al.*, 2000; Gessner *et al.*, 2010). Direct interaction effects occur when litter properties of at least one species in mixture alter decomposition rates in litter of other species. These non-additive effects, which have been observed in 15% of litter mixtures globally (Porre *et al.*, 2020), can be either synergistic (accelerate) (e.g., Scherer-Lorenzen, 2008) or antagonistic (decelerate) (e.g., Grossman and others 2020) decomposition process. Synergistic effects may stem from complementary resource use among detritivores (e.g., Vos and others 2013), nutrient transfer from rich to poor litter (e.g., Schimel and Hättenschwiler 2007) through fungal transport (Tiunov, 2009) or leaching (McTiernan *et al.*, 1997), or more favorable micro-climatic conditions (Makkonen *et al.*, 2013; Liu *et al.*, 2020a) in mixed vs. single-species litter. Antagonistic effects may stem from the presence of inhibitory secondary compounds such as condensed tannins (Hättenschwiler & Vitousek, 2000; Hättenschwiler, 2005), via the inhibitory effect of increased N availability on the synthesis of lignolytic enzymes by microorganisms or the recombination of N with partly decomposed compounds (Berg, 2014), and also altered micro-climatic conditions (Makkonen *et al.*, 2013). These interactions are under the influence of initial species' chemical and physical litter traits (Liu *et al.*, 2020a). As such, a higher dissimilarity in litter traits among species may lead to stronger synergistic or antagonistic mixing effects, as shown for leaf litter (Makkonen *et al.*, 2013) or cellulose paper (Joly *et al.*, 2017). Yet, litter trait dissimilarity has not always been found to affect mixed leaf litter decomposition (Barantal *et al.*, 2011; Lin & Zeng, 2018; Porre *et al.*, 2020), and functional trait identity was further suggested to play an important role (Grossman *et al.*, 2020), in some cases even more than trait dissimilarity (Schindler & Gessner, 2009; Frainer *et al.*, 2015). Whether chemical or physical trait dissimilarity in fine roots affects their decomposition in mixtures has not been tested so far.

In addition, tree species diversity at the location of litter incubation may indirectly affect root-litter decomposition. For instance, alterations in canopy structure can affect understory species composition (Leuschner & Ellenberg, 2017), forest floor temperature and moisture (Joly *et al.*, 2017) and edaphic properties, such as soil pH or texture (Prescott & Grayston, 2013; Scheibe *et al.*, 2015; Dawud *et al.*, 2016). In turn these influence soil fauna (Korboulewsky *et al.*, 2016), microbial communities (Scheibe *et al.*, 2015) or priming effects via root exudation (Zwetsloot *et al.*, 2020).



Globally, the occurrence, strength, and direction of diversity effects on leaf litter decomposition are still highly debated, as illustrated by several reviews and meta-analyses (Gartner & Cardon, 2004; Hättenschwiler, 2005; Gessner *et al.*, 2010; Kou *et al.*, 2020; Liu *et al.*, 2020a; Porre *et al.*, 2020), potentially due to a predominance of studies suggesting rather than testing the mechanisms behind these effects. Nonetheless, as described above, a range of studies have demonstrated the existence of causal links between leaf litter traits and non-additive effects (e.g., Schimel and Hättenschwiler 2007; Joly and others 2017). Such a mechanistic understanding remains to be extended to the below-ground decomposition of fine-root mixtures.

The majority of studies assessing diversity effects on fine-root litter decomposition comes from grasslands and report both positive and negative effects of species mixing on mass loss (Chen *et al.*, 2017a; Prieto *et al.*, 2017). The three studies on fine-root litter mixing in forests observed no effects on 4<sup>th</sup> and 5<sup>th</sup> order roots in a tropical tree diversity experiment (Guerrero-Ramírez *et al.*, 2016), negative effects in a subtropical forest (Li *et al.*, 2018) and positive effects in a subtropical plantation (Jiang *et al.*, 2019). Based on these studies, no mechanistic process can be identified as driving the non-additive effects of fine-root litter decomposition in mixtures.

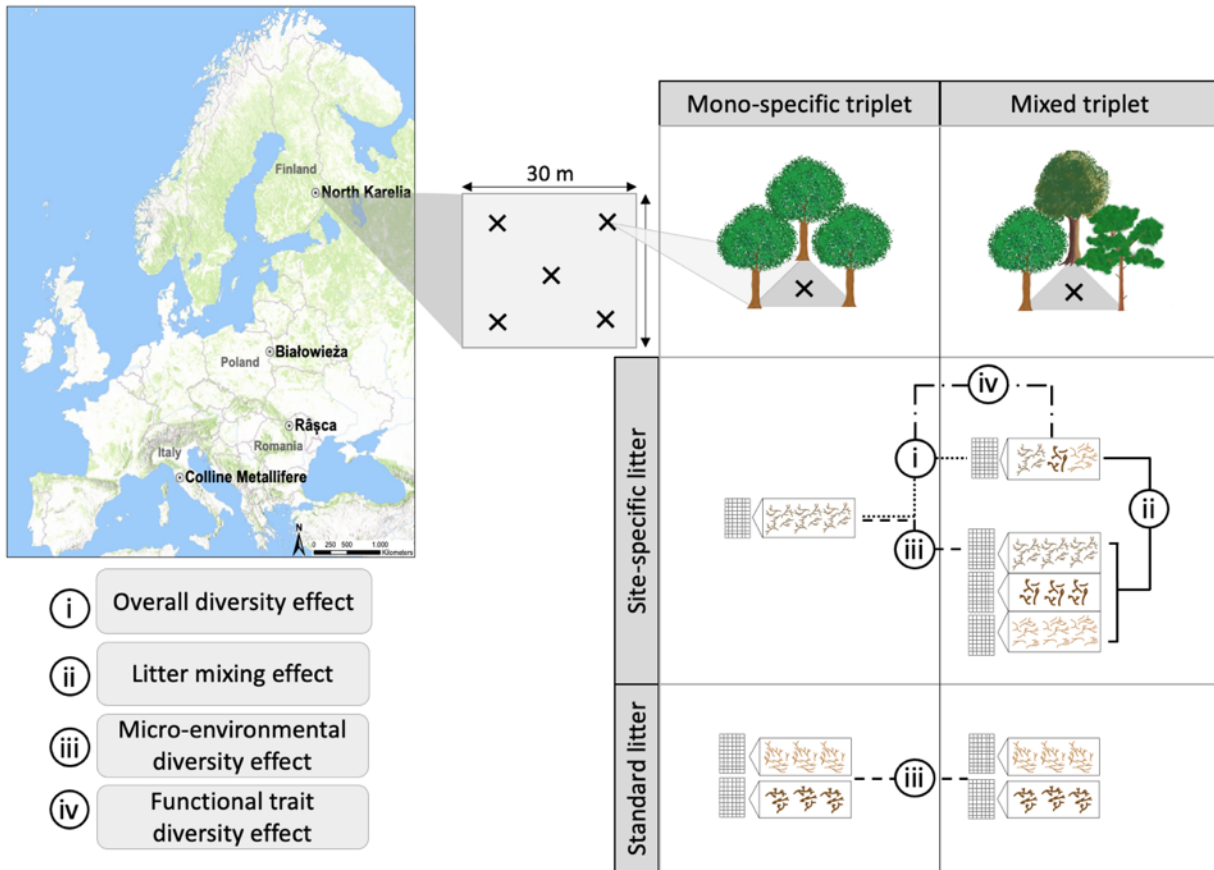
Inconsistent methodologies (e.g., variations in mesh size, incubation time, or root functional type) can limit the comparability of studies (Liu *et al.*, 2020a). A major constraint of root decomposition studies may be the lack of differentiating between fine-root orders, which are known to vary in chemical and physical properties (e.g., Pregitzer 2002; Beyer and others 2013) and thus likely also in decomposition patterns (McCormack *et al.*, 2012) and thereby have different impacts on C and nutrient cycling.

To address these knowledge gaps, we quantified fine-root litter mass loss of 13 common tree species in relation to tree species diversity, initial fine-root traits, and micro- and macro-environmental conditions in four European forest types ranging from boreal to Mediterranean climate. Since the very fine roots contribute disproportionately to soil litter input (McCormack *et al.*, 2015), we focus on the first three root orders. We hypothesized that (i) fine-root mass loss increases with tree species diversity, with (ii) direct diversity effects through litter mixtures being stronger determinants than (iii) indirect effects through alterations of the decomposition environment. In addition, we hypothesized that (iv) a higher functional dispersion in initial litter traits enhances diversity effects on root litter mass loss. To assess the relative importance of these diversity effects, we further investigated the influence of initial litter traits, regional-scale environmental differences, and micro-environmental variations on fine-root decomposition.

### 4.3. Materials and methods

We used a subset of study sites and plots of the FunDivEurope project, which studies the effect of tree diversity on ecosystem functioning in mature European forests (Baeten *et al.*, 2013). Here, we selected mono-specific and 3-species mixed plots in four study sites comprising boreal (Finland), hemiboreal (Poland), mountainous beech (Romania), and Mediterranean thermophilous forest (Italy). Each site had between nine and nineteen 30x30 m plots with a pool of three to five site-specific target species (Table S2.1). Three of the 13 target species occurred in more than one study site (*Betula pendula* in Finland and Poland, *Picea abies* Finland, Poland and Romania, *Pinus sylvestris* in Finland and Poland). Across all sites, 21 different tree species compositions were included. Plot selection was made following predefined criteria including evenness of species proportions, stand age and density, and site factors including soil characteristics, topography, and former management type. Besides establishing a gradient in tree species diversity, abiotic and biotic conditions were kept as constant as possible to minimize the influence of confounding factors (Baeten *et al.*, 2013). Within each plot, we selected five subplots at the level of tree neighborhoods following the *triplet* design of Vivanco and Austin (2008). These triplets consisted of a triangle shaped by three trees, either of the same species in mono-specific stands or different tree species in mixed-species plots. For these triplets, we aimed at selecting healthy, dominant, or co-dominant trees of similar dimensions, and the five triplets were ideally evenly distributed across the plot. For more details on the selection of the triplets, see Wambsganss and others (2021).

At each triplet, a set of litter bags filled with different litter species was deployed in the center of the triangle. See Fig. 4.1 for a description of the design and composition of litter bags to test the four hypotheses. We incubated site-specific root litter, i.e., of species occurring in the triplet and two *standard* litter species, i.e., *Pinus sylvestris* and *Carpinus betulus*, to clearly separate environmental influences from initial litter quality effects. The standard litter species were deployed at all sites and selected owing to their dissimilarity in root traits (thick vs. thin-rooted, different chemical composition and mycorrhization intensity).



**Figure 4.1.** Illustration of the study design for testing diversity effects on root litter mass loss after one year of incubation. For each of the overall 62 plots across the four sites, five tree triplet microsites were selected. In mono-specific stands, these triplets consisted of three tree individuals of the same species, whereas in the mixed plots, three different tree species shaped a triplet. To test *overall diversity effects* (Hypothesis i), site-specific fine-root litter matching the canopy was incubated in the center of each of the five triplets, and mass loss was compared between both diversity levels. To test *litter mixing effects* (Hypothesis ii), single-species root litter of the component species was also incubated in mixed neighborhoods and compared with mixed litters in the same stand (homogeneous environmental conditions). The site-specific single-species litter in mixed and mono-specific stands was also used to test the *micro-environmental diversity effect* (Hypothesis iii). In addition, two standard litter species, i.e., *Carpinus betulus* and *Pinus sylvestris*, were deployed in single-species litter bags in both mono-specific and mixed stands to test Hypothesis iii. The role of functional trait diversity in fine-root litter decomposition (Hypothesis iv) was assessed for overall effects.

*Root collection and preparation of litter bags*

Root material of the 13 species was collected in fall 2017 from the surface soil (top 15 cm) of pure stands in Southwest Germany (i.e., the midpoint of our pan-European latitudinal gradient) and Italy (for the species not present in Germany, i.e., *Ostrya carpinifolia*, *Quercus ilex*, and *Q. cerris*). We sampled additional roots for each species by tracing roots back to tree stems and used them as reference material for further sorting of roots. To extract roots, soil was washed carefully over a sieve cascade in the laboratory. In contrast to leaf litter decomposition studies, where freshly senesced or shed leaves are typically used, fine-root decomposition experiments inevitably include fresh fine roots because of difficulties associated with sampling recently senesced, but yet undecomposed roots (e.g., Hobbie and others 2010).

After removing roots from soil, we separated fresh fine roots of the target species according to their function into the first-three order (absorptive) roots and higher-order (transport) roots  $\leq 2$  mm according to McCormack and others (2015). Dead roots (hollow, dark stele, breakable, not elastic) were discarded. Representative subsamples of live absorptive and transport roots were taken and kept frozen ( $-20^{\circ}\text{C}$ ) until trait analyses could be performed. Roots of each target species were air-dried to constant mass and stored at room temperature. Before filling the litter bags with dried roots, the root samples of each species were well mixed, and three subsamples of 50 mg, respectively, were taken to determine the initial moisture content (oven-dried at  $105^{\circ}\text{C}$  for 48h) and subsequent ash content (in a muffle furnace at  $850^{\circ}\text{C}$  for 2h). The samples' air-dry moisture content was on average  $10.2 \pm 0.15\%$ , and ash content on average  $9.04 \pm 0.09\%$ . In total, 1,330 litter bags were filled with 50 mg each. For the 3-species mixture bags, each species represented one-third of that mass. Mesh bags consisted of precision woven synthetics monofilament fabrics (Sefar Nitex) with a mesh size of  $100\text{ }\mu\text{m}$ , which only allows small organisms, including fungal hyphae, to enter the bags but excludes roots and larger soil fauna. Hence, this study focused on microbial decomposition, which is presumably more important in soils than for above-ground leaf litter decomposition (Silver & Miya, 2001). All bags were labelled, and those placed at the same microsite were tied together with a wire while ensuring a distance of several centimeters between bags.

*Root incubation and harvest*

Litter bags were incubated at the beginning of spring 2018 at different dates along the North-South gradient (Table S2.1). Bags were moistened before inserting them vertically in the upper 10 cm of the topsoil (below the organic horizon) by creating an incision in the soil using a shovel and gently pressing the soil around the bag. The closed incision was covered again with

on-site leaf litter. Litter bags were retrieved after ~365 days and subsequently dried at 40°C. The adhering soil was carefully brushed off, and hyphae growing inside the bags were removed. To account for mineral particle contamination, the ash content was determined for each root litter sample incubated at the Italian and Romanian site, which had high soil clay contents (Table S1). The air-dried mass of each sample was corrected for initial water and mineral content. For sites in Finland and Poland (sandy soils) we only corrected for initial water content, as mineral particle contamination could be excluded (which was confirmed by quantifying the ash content of a subset of representative samples). We quantified decomposition as mass loss in % of the initial mass after one year of incubation.

#### *Non-additive effect in litter mass loss*

To assess diversity effects on root decomposition, we calculated the proportional deviation from the expected mass loss, according to Loreau (1998). We did this for (i) overall diversity effects, (ii) direct litter mixing effects, and (iii) indirect diversity effects on the microenvironment.

The following equation was used to quantify these diversity effects (Loreau, 1998; Palmborg *et al.*, 2005):

$$D_{mix} = \frac{O - E}{E} \quad (2)$$

where  $D_{mix}$  is calculated respectively as:

#### **(i) Overall diversity effects (=combined direct and indirect effects)**

$O$  is the observed mass loss of mixed-species litter located in tree species mixtures, and  $E$  equals the expected mass loss of mixed-species litter, derived from the mean of mass loss of the component single-species root litter in mono-specific plots.

#### **(ii) Litter mixing effects (= direct effects)**

where  $O$  is the observed mass loss of mixed-species litter located in tree species mixtures, and  $E$  equals the expected mass loss of mixed-species litter, derived from the mean of mass loss of the component single-species root litter in mixed plots.

#### **(iii) Micro-environmental diversity effects (=indirect effects)**

where  $O$  is the observed mass loss of single-species litter located in tree species mixtures, and  $E$  equals the expected mass loss based on the component single-species root litter mass loss in mono-specific plots.

In cases in which  $D_{\text{mix}}$  significantly differs from zero, effects are non-additive; when  $D_{\text{mix}}$  does not differ from zero, effects are additive.

### *Root trait measurements*

Initial functional traits related to root morphology, architecture, chemistry, and ectomycorrhizal (EcM) associations were quantified for each root species using five representative subsamples of the live or air-dried fine-root material (Table S2.2). Healthy tips and tips colonized by EcM were visually identified and counted under a binocular to determine root tip density (total number of tips per root length,  $\text{n m}^{-1}$ ) and EcM colonization rate (proportion of infected vs. total number of tips in %). Infected tips were identified for 12 of the 13 tree species based on the presence of a fungal sheath (*Acer pseudoplatanus* associates with arbuscular mycorrhizae). The first three-order fine-root samples were scanned in water with a flat-bed scanner (resolution 800 dpi). Scans were analyzed with the software WinRhizo (Regents Instruments, Quebec, Canada, 2009) to obtain root length, area, volume, and diameter. Root volume and (average) diameter values were recalculated based on individual diameter classes to avoid bias resulting from the global values calculated by WinRhizo assuming constant root diameter (Freschet *et al.*, 2020). Samples were dried ( $40^{\circ}\text{C}$ ,  $>72$  h) and weighed. For chemical analysis, the dried root samples were ground to fine powder with a Retsch MM400 mixer mill (Retsch GmbH, Germany). Total organic C and N in this homogenized material were determined by dry combustion with a Vario El Cube Elementar analyzer (Elementar Analysensysteme GmbH, Germany). To measure initial macronutrient (N, P, K, Ca, Mg) and micronutrient (Mn, Cu, Zn) concentrations, 75 mg of root material was mixed with 1.5 ml distilled  $\text{H}_2\text{O}$ , 1.5 ml  $\text{H}_2\text{O}_2$  (30%), and 3 ml  $\text{HNO}_3$  ( $>65\%$ ). Microwave digestion was applied for 12 minutes (CEM Discover SP-D, CEM Corporation, USA), and subsequently, solutions were filtered and diluted with distilled  $\text{H}_2\text{O}$  to 25 ml. An optical emission spectrometer with inductively coupled plasma (ICP-OES, SPECTROBLUE, SPECTRO Analytical Instruments GmbH, Germany) was used to determine element concentrations.

### *Environmental data*

Macroclimate, soil, and forest floor data were obtained from the FunDivEurope database (<https://data.botanik.uni-halle.de/fundiveurope>). Soil parameters were measured at the plot-level in 2012 (Dawud *et al.*, 2017), while forest floor properties were derived from samples

taken in 2017 at the same subplots used in this study (Gillespie and others, under revision). Microclimate variables were measured throughout the incubation period, with recordings every 15 minutes using TMS-4 data loggers (TOMST, Prague, Czech Republic). These loggers were installed in each plot center, measuring soil temperature, soil moisture (at 10 cm depth), and air temperature (1 m above ground). Mean values for the year and the growing season (daily mean temperatures  $>5^{\circ}\text{C}$ ) were calculated for each plot. For soil moisture, daily minimum values were used instead of means, as water accumulated around the sensors after rain events, possibly distorting mean values.

### *Data analysis*

All statistical analyses were performed using R version 3.5.1. (R Core Team, 2018) and significance levels were set at  $P=0.05$ . To test whether mean mass loss differed among sites, Kruskal-Wallis and post-hoc tests (Dunn) were used. To test whether net diversity effects were significantly different from zero, we used Wilcoxon-signed rank tests or t-tests. Non-parametric tests were used in case data distribution was not normal, even after transformation.

### *Modelling approach*

We used linear mixed-effects models (LMMs) to account for the nested study design (R packages *LME4*, Bates and others (2015)). In case of violations of the LMMs' assumptions, response variables were transformed (R package *bestNormalize*, Peterson, 2017). Marginal and conditional  $R^2$  values for all models were retrieved using the R package *r2glmm* (Jaeger, 2017). Fixed effects were scaled, and collinearity among variables was tested using variance inflation factors (vif), considering  $\text{vif} > 5$  as threshold for collinearity (R package *car*, Fox and Weisberg, 2011).

#### Influence of initial root traits

The role of initial root traits on fine-root litter mass loss was assessed using site-specific root litter data. Based on a literature search, we initially considered the following traits as most crucial for the decomposition of the most-distal fine-root orders: Root C/N ratio as a proxy for litter quality (Silver & Miya, 2001; Zhang & Wang, 2015), EcM colonization intensity (Langley *et al.*, 2006), root N, Ca (Silver & Miya, 2001; Zhang & Wang, 2015), K (Chen *et al.*, 2017b), Mg (Berg, 1984), Mn (Keiluweit *et al.*, 2015), and P concentrations (See *et al.*, 2019), root diameter (Hobbie *et al.*, 2010) and root tissue density (RTD, Jiang and others (2020)). We tested these variables as predictors of the fine-root mass loss for single-species and mixed-species litter in mono-specific and mixed tree neighborhoods, respectively, after checking variance inflation. Subsequently, RTD, root N, and root diameter were excluded from the model owing to

their higher VIF factors. Model selection using *dredge* and subsequently averaging using *mod.avg* were employed to obtain the most parsimonious model (R package *MuMIn*, Bartoń, 2019). These functions allow for a ranking of all possible models based on the lowest Akaike Information Criteria (AIC) and subsequently select a subset of models with a  $\Delta AIC < 4$  based on a 95 % confidence set, which are then averaged (Burnham & Anderson, 2002). Subsequently, a synthesis model including all significant predictors (Table S2.3) and tree diversity was fitted to determine the relative importance of predictor variables.

The final model for the site-specific litter was as follows:

$$\text{orderNorm}(\text{mass loss}) \sim \text{overall tree diversity} + \text{initial root C/N} + \text{initial root P conc.} + \text{initial root Mg conc.} + (1|\text{Site/Plot})$$

#### Functional trait dissimilarity as a driver of diversity effects

To quantify dissimilarity in initial fine-root traits for mixed-species litter, we used the functional dispersion (FDis) index by Laliberté and Legendre (2010) based on species' mean initial trait values (R package, *FD*, Laliberté and others (2014)). First, we calculated multidimensional trait divergence of a combination of eight morphological, chemical and microbial initial root traits including root tissue density (RTD), specific root length (SRL), EcM colonization intensity, Ca, K, N, Mg, and Mn. These traits were selected after testing correlation coefficients among the measured initial root traits (Fig. S2.1) (we also calculated an alternative FDis index replacing initial RTD by P and N by C/N). We additionally quantified FDis indices for single traits. To test whether diversity effects were driven by FDis indices (Hypothesis iv), we fitted FDis of single traits and FDis of all eight traits combined in response to overall diversity effects. Model selection and averaging were used to obtain the most parsimonious model.

#### Influence of environmental conditions

The role of macro-and micro-environmental conditions on fine-root litter mass loss was tested using the standard litter data, as this dataset allowed us to disentangle the effects of substrate quality from environmental conditions on litter mass loss.

For the topsoil (pH, bulk density, C/N, clay %) and forest floor (P, lignin, mass, N) variables, we performed a principal component analysis (PCA) to summarize the variables and prevent model overfitting (Fig. S2.2). The respective axes of the two PCAs were extracted and tested as explanatory variables in the respective models (see below). Soil PCA axis 1 indicates a physicochemical gradient from sandy, acidic soils to denser, clay-rich, and less acidic soils. Soil PCA axis 2 represents a gradient ranging from low to high soil fertility, represented by high



and low soil C/N, respectively. Forest floor PCA axis 1 describes a gradient of properties linked to herbivory defense strategies, ranging from the dominance of chemical defense indicated by high phenol and lignin concentrations to more structural/physical strategies indicated by high lignin concentrations. Forest floor PCA axis 2 represents a gradient from more favorable, i.e., higher forest floor P and N, to more adverse conditions for decomposition, i.e., low forest floor P and N. For the microclimate variables, we considered mean annual soil temperature and moisture and for macroclimate variables mean annual temperature (MAT) and precipitation (MAP).

To avoid model overfitting, a hierarchical modelling approach was used following Joly and others (2017). In a first step, we separately tested the effects of the selected soil and forest floor PCs and macro-climatic and micro-climatic variables on litter mass loss. Hence, we fitted three separate models, i.e., (i) soil and forest floor model, (ii) microclimate model, and (iii) macro-climate model. We again applied model selection and averaging and kept the individual models' significant predictor variables (Tables S2.4-S2.6).

Consequently, the final model for the standard litter was as follows:

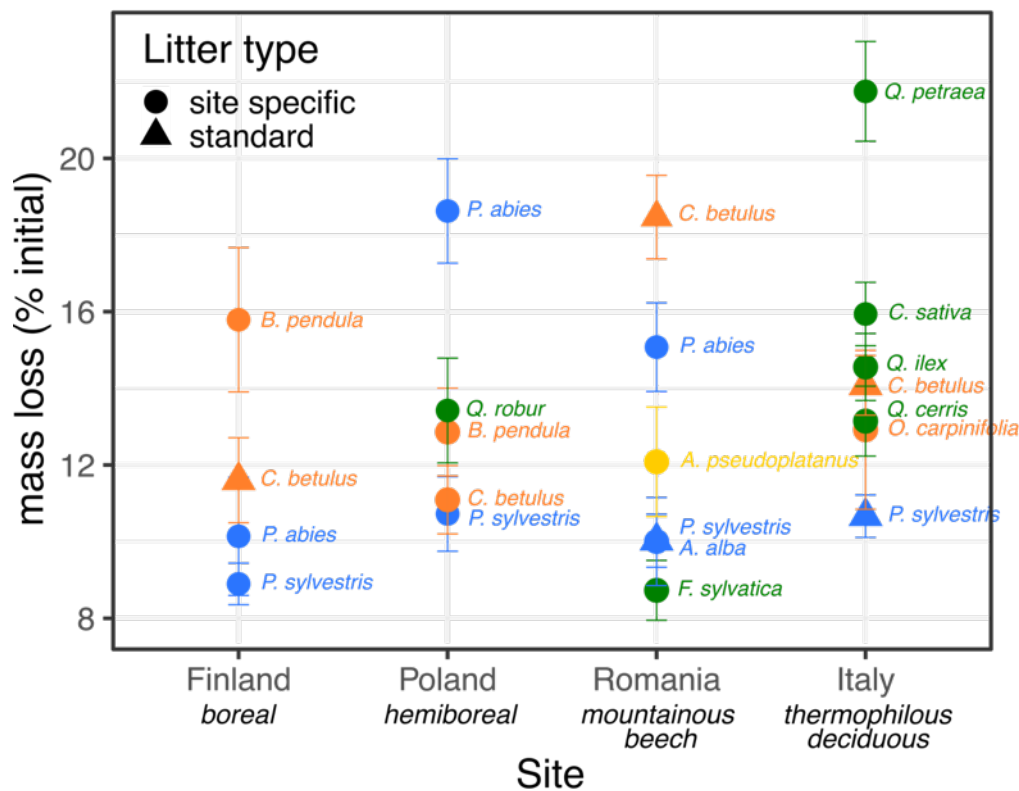
$$\text{Sqrt}(\text{mass loss}) \sim \text{tree diversity} + \text{species} * \text{soil PC1} + \text{species} * \text{soil PC2} + \text{species} * \text{forest floor PC2} + \text{species} * \text{MAT} + \text{species} * \text{MAP} + (1 | \text{Site/Plot/Triplet})$$

Model selection and averaging were applied again to find the most parsimonious model. To estimate the relative importance of each fixed effect, coefficients of determination ( $R^2$ ) were derived (*r2glmm* package in R using the Nakagawa and Schielzeth approach, Nakagawa and Schielzeth, 2013).

#### 4.4. Results

##### *Mass loss by forest type and tree species*

Overall, mass loss of fine roots varied among forest types (Fig. S2.3). Across sites, mean mass loss of site-specific litter ranged from  $10 \pm 0.8\%$  to  $16 \pm 0.7\%$  % of the initial mass and decreased in the following order: Mediterranean thermophilous forest (Italy) > hemiboreal forest (Poland) > boreal forest (Finland) > mountainous mixed beech forest (Romania).



**Fig. 4.2** Mean mass loss ( $\pm$ SE) of the site-specific (filled circles) and standard litter species (triangles) by site. Colors indicate common phylogenetic background (family): *Fagaceae* (green), *Betulaceae* (orange), *Pinaceae* (blue), *Sapindaceae* (yellow).

Fine-root litter mass loss further varied among species (Fig. 4.2). Fine roots of *Fagus sylvatica* in mountainous mixed beech forests showed the slowest mass loss during the one-year incubation period, losing on average  $8.73 \pm 0.78\%$  of the initial litter mass, while roots of *Quercus petraea* in Mediterranean thermophilous forests decomposed the fastest, with  $21.75 \pm 1.30\%$  of the initial mass lost. Fine-root mass loss of the two standard species also differed significantly. Across all sites, roots of *Carpinus betulus* showed significantly faster mass loss ( $14 \pm 0.6\%$  of initial mass) than those of *Pinus sylvestris* ( $10.3 \pm 0.4\%$  of initial mass) (Figs. 4.2, S2.4). Yet, in hemiboreal forests (Poland), the difference between the two species was not significant. In

addition, root mass loss of both standard litter species varied among sites, and differences between sites were considerably higher in *C. betulus*, (Figs. 4.2, S2.4).

#### *Diversity effects*

Across all four sites, *overall diversity effects* on fine-root litter mass loss were significantly positive ( $P=0.01$ ), i.e., single-species litter in mono-specific stands decomposed slower than mixed-species litter in mixed-species stands (Fig. 4.3). Within mixed stands, mass loss of single-species litter did not significantly differ from mixed-species litter, indicating no *litter mixing effects* (Fig. 4.3). Moreover, tree diversity tended to accelerate mass loss through a change in the decomposition environment, as shown by higher decomposition of site-specific single-species fine-root litter in mixed stands compared to single-species fine-root litter in mono-specific stands (*micro-environmental diversity effect*,  $P=0.03$ ), though mass loss of standard species litter did not significantly differ between mixed and mono-specific stands.

Diversity effects differed among sites (Fig. 4.3). *Overall effects* tended to positive in mountainous beech forest ( $P=0.09$ ), while they were significantly positive in hemiboreal forests ( $P=0.03$ , Fig. 4.3) and additive in thermophilous deciduous and boreal forests. In contrast, *litter mixing effects* did not occur at any of the four sites (Fig. 4.3). Moreover, positive *micro-environmental diversity effects* on site-specific root litter were significant in mountainous beech forests ( $P<0.01$ ) as well as in hemiboreal forests ( $P=0.03$ ). We also observed a tendency for a positive *micro-environmental diversity effect* on the two standard litter species ( $P=0.08$ ), despite no such effect across all sites.

#### *Functional trait dissimilarity*

Neither the multidimensional trait divergence based on eight initial root traits, nor the divergence in single root traits, except for initial root N, predicted overall diversity effects of root litter mass loss across the four sites (Fig. S2.5, Tables S2.7&S2.8). The dissimilarity in initial root N concentrations had a negative but weak ( $P=0.05$ ,  $mR^2 = 0.14$ ) effect on overall diversity effects (Fig. S2.5b).

#### *Relative importance of diversity, initial litter quality, macro-, and micro-environmental conditions*

##### Initial litter quality (site-specific litter)

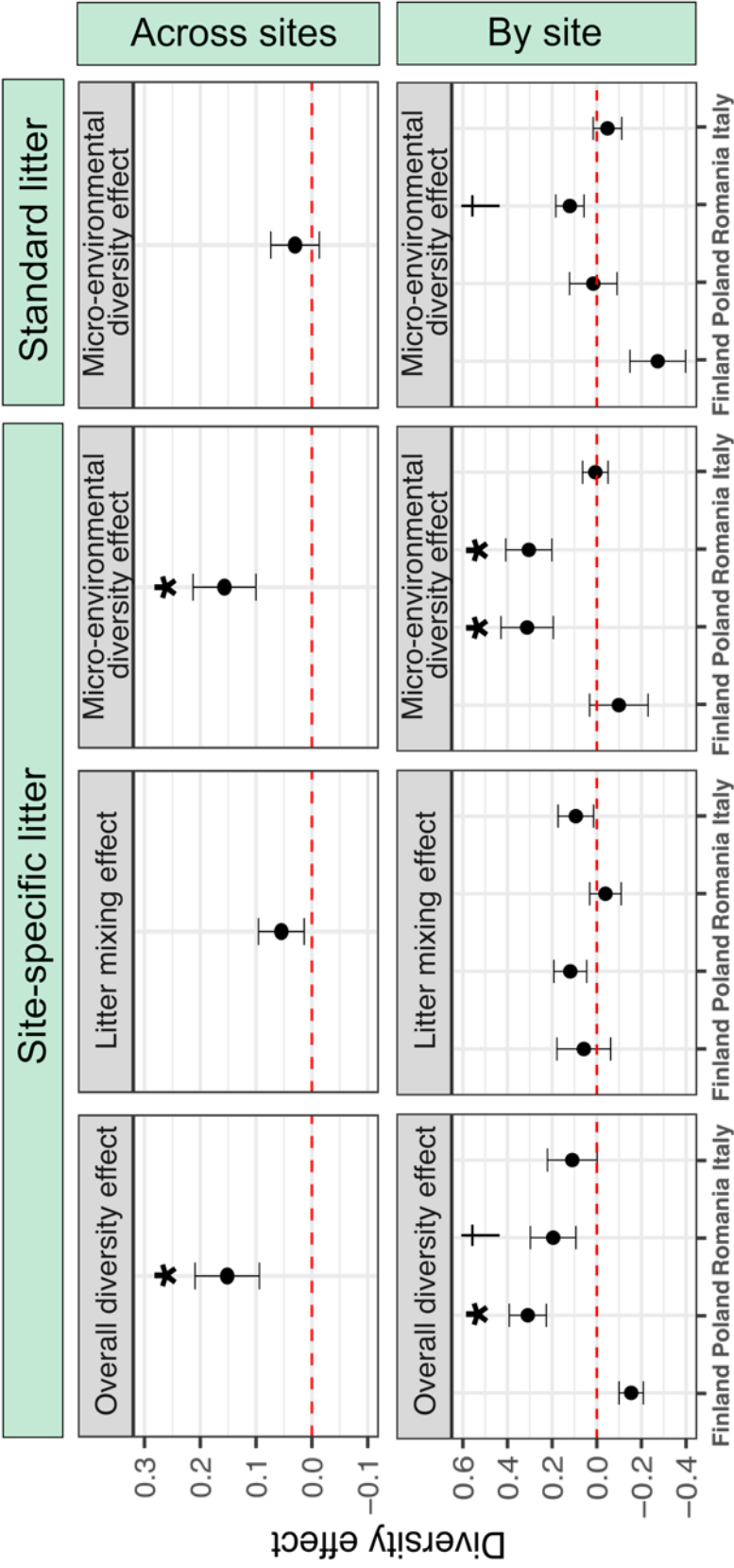
The overall positive diversity effect across sites accounted only for 2% of the data variation ( $P=0.047$ ). LMM analysis showed that, besides an effect of overall tree diversity, mass loss of site-specific fine-root litter was mainly determined by initial root litter chemistry, i.e., initial

root C/N, Mg, and P concentrations (Table 4.1). Initial root Mg concentrations had the strongest (negative) effect on site-specific litter mass loss ( $P<0.001$ ,  $mR^2=0.14$ ), followed by initial root P concentrations, which had a positive influence on mass loss across sites ( $P<0.001$ ,  $mR^2=0.11$ ). Moreover, root C/N had a significant positive but weak effect on mass loss ( $P<0.01$ ,  $mR^2=0.06$ ).

### Environmental influences (standard litter)

The synthesis model showed that MAP and the species identity of root litter were the most important predictors of mass loss across the four sites ( $P=0.01$ ,  $mR^2=0.08$  and  $P<0.001$ ,  $mR^2=0.07$ , respectively) (Table 4.1). Topsoil properties (soil PC1) significantly affected mass loss of standard species' litter ( $P<0.01$ ), yet only explained 4% of the variation, whereas forest floor properties (forest floor PC2) had only a marginal effect ( $P=0.04$ ,  $mR^2=0.01$ ). Significant interactions between species and MAP and MAT, respectively, further indicate that mass loss of the two species were differently affected by macro-environmental conditions. In the most parsimonious model, triplet species diversity was dropped as a predictor variable for standard species' mass loss. Similarly, microclimate variables did not affect standard litter mass loss across sites and were not included in the synthesis model (Table S2.5).

**Fig. 4.3** Mean diversity effects ( $\pm$ SE) across sites (upper panels) and by site (lower panels), respectively, for overall (single-species litter in mono-specific stands vs. mixed-species litter in mixed stands), litter mixing (single-species litter in mixed stands vs. mixed-species litter in mixed stands), micro-environmental diversity effects (single-species litter in mono-specific stands vs. single-species litter in mixed stands) for site-specific (left) and standard litter species (right). Asterisks indicate significant difference from zero ( $\dagger P < 0.1$ ,  $* P < 0.05$ ).



**Table 4.1.** Results of syntheses models including site-specific and standard litter species.

<i>mass loss (% initial) of site-specific litter</i>						
<b>Fixed effects</b>	<i>Esti- mate</i>	<i>t-value</i>	<i>P</i>	<i>R</i> <sup>2</sup>	<i>Upper Cl</i>	<i>Lower Cl</i>
Overall tree diversity	0.28	2.04	<b>&lt;0.05</b>	0.02	0.06	0.00
Root C/N	0.54	3.47	<b>&lt;0.01</b>	0.06	0.13	0.02
Root Mg	-0.56	-3.88	<b>&lt;0.001</b>	0.14	0.22	0.07
Root P	0.57	4.26	<b>&lt;0.001</b>	0.11	0.17	0.03
<b>mR</b> <sup>2</sup>		0.18		0.18	0.28	0.11
<b>cR</b> <sup>2</sup>		0.55				
<i>mass loss (% initial) of standard litter</i>						
<b>Fixed effects</b>	<i>Esti- mate</i>	<i>t-value</i>	<i>P</i>	<i>R</i> <sup>2</sup>	<i>Upper Cl</i>	<i>Lower Cl</i>
Tree diversity						
Species	-0.48	-6.73	<b>&lt;0.001</b>	0.07	0.11	0.03
Soil PC1	-0.27	-3.11	<b>&lt;0.01</b>	0.04	0.08	0.01
Soil PC2						
Forest floor PC2	-0.11	-2.13	<b>0.04</b>	0.01	0.04	0.00
MAT	-0.19	-1.20	0.29	0.01	0.40	0.0
MAP	0.65	3.64	<b>0.01</b>	0.08	0.13	0.04
Species * Forest floor PC2						
Species * Soil PC1						
Species * Soil PC2						
Species * MAT	0.37	3.93	<b>&lt;0.001</b>	0.02	0.06	0.01
Species * MAP	-0.56	-6.03	<b>&lt;0.001</b>	0.06	0.10	0.02
<b>mR</b> <sup>2</sup>		0.16		0.16	0.22	0.11
<b>cR</b> <sup>2</sup>		0.31				

## 4.5. Discussion

In this study, we tested the decomposition of absorptive fine roots of site-specific and standard tree species in response to species mixing across a broad environmental gradient. Across all sites, we observed positive but rather weak overall effects of tree species diversity on fine-root litter mass loss. Indirect effects through tree diversity at litter incubation sites contributed more to this acceleration of litter mass loss than direct litter species mixing effects. The dissimilarity in initial root traits contributed little to explain diversity effects. The differences among forest types and between litters (site-specific versus standard litter) indicate interactions between regional-scale conditions and litter species influencing diversity effects. Overall, initial root substrate quality and macroclimate played a stronger role in root litter mass loss than tree diversity.

### *Diversity effects on fine-root litter mass loss across sites*

In accordance with our first hypothesis, tree diversity effects on fine-root litter mass loss were overall non-additive and positive across the four forest types and 21 litter mixture compositions (Fig. 4.3). These results corroborate findings of two recent meta-analyses reporting generally positive diversity effects for leaf litter decomposition across biomes (Kou *et al.*, 2020; Liu *et al.*, 2020a).

Our study design allowed us to separate overall diversity effects into those attributable to litter species mixing (direct species interactions, Hättenschwiler, 2005) and those related to micro-environmental changes in mixed-species compared to mono-specific plots (indirect effects, Hector and others (2000)). A positive micro-environmental diversity effect and the lack of a litter mixing effect is not in line with our second and third hypothesis and indicate that the overall diversity effects were mainly attributable to indirect drivers (Fig. 4.3).

### *Indirect effects*

Several indirect effects may explain the accelerated mass loss of root litter in mixed compared to mono-specific neighborhoods. Changes in micro-environmental conditions, such as modifications of the tree canopy in mixtures compared to mono-specific stands, may be drivers of these non-additive effects. This was suggested by another study across the FunDivEUROPE plot network reporting a tendency for accelerated cellulose paper mass-loss rates in response to tree species richness (Joly *et al.*, 2017). Such indirect effects may also entail changes in edaphic conditions in mixed compared to mono-specific stands (Vesterdal *et al.*, 2013; Dawud *et al.*, 2016), which could be associated with changes in soil fauna (Hättenschwiler & Gasser, 2005) and microbial community composition (Scheibe *et al.*, 2015). Higher P concentrations and lower C/N in forest floor in mixed than in mono-specific plots across our sites (Gillespie and

others, under revision) could indicate more favorable conditions for decomposers (Hobbie & Vitousek, 2000).

During the incubation year and its vegetation period, soil temperature tended to be lower in mixtures than in mono-specific stands (Table S9). During the severe summer drought of 2018, the lower soil temperatures may have created more favorable conditions for decomposer organisms. Linked to this could be lower fluctuations in temperature and soil moisture under a denser canopy in mixed stands, as speculated by Joly and others (2017) who reported positive effects of leaf area index on cellulose decomposition across European forests. Higher canopy density was indeed observed with increasing species richness across the same plot network (Jucker *et al.*, 2015). Changes in soil microbial community structure, for example, higher abundance or diversity of decomposing microbes in response to higher tree diversity, could have also accelerated decomposition rates (Chapman *et al.*, 2013). The higher mycorrhizal fungi diversity reported for mixed compared to mono-specific stands across our sites (Wambsganss and others, under revision) may indeed substantiate this speculation.

Interestingly, overall, micro-environmental diversity effects were only significant for site-specific but not standard root litter species (Fig. 4.3), implying that these effects depend on context- and litter type.

### ***Direct effects***

The lack of a litter mixing effect suggests that under the same canopy, mass loss of mixed-species litter can be predicted from the component single-species mass loss. Such additive effects under homogeneous conditions (i.e., canopy) have also been reported for root litter in studies in subtropical (Li *et al.*, 2018) and tropical forests (Guerrero-Ramírez *et al.*, 2016). However, these bulk additive effects cannot be interpreted as the absence of interaction among the different species. Since we did not determine mass loss of the individual species in the mixed-litter bags, we cannot exclude the possibility that both synergistic and antagonistic interactions among litter species occurred simultaneously, resulting in an overall neutral effect (Hättenschwiler *et al.*, 2005). To account for such divergent patterns, future studies should attempt to separate component species from mixed litter bags, possibly using indirect methods of species identification in the fragmented and partly decomposed material (Gruselle & Bauhus, 2010).

### ***Role of root trait dissimilarity***

The dissimilarity in initial root traits (FDis) did not predict overall diversity effects on fine-root litter mass loss across sites (Fig. S2.5a), contradicting our fourth hypothesis but corroborating



a range of other studies on leaf litter mixing (Chapman *et al.*, 2013; Tardif & Shipley, 2015; Lin & Zeng, 2018). Here, these findings may be linked to the weak diversity effects and could point to the omission of key chemical traits, including lignin or condensed tannins (Sun *et al.*, 2018). Another explanation may entail the absence of soil macrofauna due to the litter bag approach, which can determine the magnitude of positive litter trait dissimilarity effects (Barantal *et al.*, 2014), likely owing to complementary resource use by detritivores (Vos *et al.*, 2013). A possibly better soil nutrient availability in mixtures could also make nutrient transfer among litter species dispensable (Frainer *et al.*, 2015).

Interestingly, litter mixtures that varied most in initial N concentrations showed the lowest mass loss compared to observations from single-species litter (Fig. S2.5b). Such negative effects of litter functional diversity, including several chemical traits, were also observed for leaf litter decomposition (Grossman *et al.*, 2020), including N (Kou *et al.*, 2020). Though this negative effect detected here was rather weak, it points to a complex interplay of litter interaction effects with environmental conditions (Lummer *et al.*, 2012).

### ***Mean mass loss***

In general, mass loss of both site-specific and standard litter species varied among sites and was extremely low (Figs. 4.2, S2.3) in face of the decomposition rates reported by related global meta-analyses for roots  $\leq 2$  mm in diameter ( $0.69 \pm 0.17 \text{ yr}^{-1}$ , Silver and Miya, 2001;  $0.75 \pm 0.04 \text{ yr}^{-1}$ , Zhang and Wang, 2015). This is especially true given that decomposition rates in early-stage decomposition phases may even be higher than those during later stages (Sun *et al.*, 2018). Nevertheless, findings by others focusing on lower-order roots ( $k$  of  $0.11 \pm 0.01 \text{ yr}^{-1}$  in temperate climates, Sun and others (2018) or  $0.002\text{--}0.085 \text{ yr}^{-1}$ , in temperate and subtropical tree roots, Xiong and others (2013)) also reported comparably low decomposition rates.

Yet, in view of the relatively short lifespans (much less than one year) and high turnover rates of the most distal root orders (McCormack *et al.*, 2012), this extremely low mass loss is presumably highly underestimated. It suggests unrealistically high fine-root necromass values in soils exceeding fine-root biomass values by several orders of magnitudes. This is unrealistic, as the average fine-root necromass across European forests was estimated to be only slightly higher than the fine-root biomass ( $379$  vs.  $332 \text{ g m}^{-2} \text{ year}^{-1}$ , for the bulk of roots  $\leq 2$  mm in diameter) (Neumann *et al.*, 2021).

The underestimation of mass loss may partially be attributable to the litter-bag technique and the artificial decomposition environment it creates (Dornbush *et al.*, 2002; Beidler & Pritchard, 2017). The small mesh size used here excludes meso- and macrofauna (Song *et al.*, 2020),

essentially affecting decomposition of SOM (Basile-Doelsch *et al.*, 2020). In addition, the severe summer drought in 2018 across Europe could have further hampered microbial decomposition at our sites. Consequently, the generally very low mass loss measured here could be partially responsible for the lack of strong diversity effects and overall relatively low variation explained by the predictor variables.

### ***Differences among forest types***

Nevertheless, the relatively weak overall diversity effects across sites and thereby among forest types (Fig. 4.3) further corroborate findings of context-dependency of tree diversity effects for above-ground litter decomposition (Kou *et al.*, 2020; Liu *et al.*, 2020a; Zhou *et al.*, 2020). Yet, this stands in contrast to reports of no changes in the magnitude of diversity effects on root decomposition under variable environmental conditions (e.g., soil fertility) (Guerrero-Ramírez *et al.*, 2016)

In hemiboreal forests (Poland), where the overall diversity effects were strongest, a previous study reported increasing forest floor pH values with increasing tree species diversity (Dawud *et al.*, 2016), thereby likely improving conditions for microbial decomposition. The weak (and not significant) overall negative diversity effect on fine-root decomposition in boreal forests (Finland) support findings from a meta-analysis on leaf litter (Liu *et al.*, 2020a) and may be explained by the generally lower decomposer activity in these climates. A slightly lower soil temperature in mixtures compared to mono-specific stands during the vegetation period 2018 (Table S2.9) could have had a particularly large (negative) effect on the activity of decomposing microorganisms (Pietikäinen *et al.*, 2005; Conant *et al.*, 2011) in boreal forests, as soil and air temperatures in these forests are generally much lower during the year than in the other forest ecosystems studied here. By contrast, in thermophilous deciduous forests (Italy), a slightly lower annual soil and air temperature in mixed than mono-specific stands could have had a positive effect on decomposers, as high temperatures coupled with low soil moisture during summer months could hamper decomposition at these sites.

### ***Relative importance of influences on mass loss***

The analyses of site-specific and standard root litter showed that both initial litter quality and macro-climatic conditions determined fine-root litter mass loss more strongly than tree diversity. The variability of diversity effects across sites is largely responsible for this overall low influence of tree diversity on decomposition, yet, these findings are consistent with other studies (Schindler & Gessner, 2009; Frainer *et al.*, 2015; Zhou *et al.*, 2020). The positive effect of initial root P on mass loss has been pointed out in a global meta-analysis (See *et al.*, 2019),

whereas the positive relationship of root C/N with mass loss does not seem straight-forward. While initial C/N may be a good predictor of leaf litter decomposition rates, it may not take the same role for the decomposition of first-order roots (Sun *et al.*, 2018) as, for example, incubation of freshly killed rather than senesced roots elude the C and nutrient resorption processes (Freschet *et al.*, 2020). A high EcM colonization, which was the case for many of the incubated roots here (Table S2.2), can slow down litter mass loss, despite a relatively low C/N ratio (Langley *et al.*, 2006), owing to the presence of N-rich chitin from the fungus (Langley *et al.*, 2006). The negative effect of initial root Mg concentration on fine-root litter mass loss contrasts most other studies (Beidler & Pritchard, 2017; Chen *et al.*, 2017b; Sun *et al.*, 2018), yet has been observed before (Goebel *et al.*, 2011; Sun *et al.*, 2013; Jiang *et al.*, 2020). The chemical composition of EcM could also play a role here, as speculated by Jiang and others (2020).

Yet, the large number of root traits investigated here did not contribute much to predict mass loss. Altogether, the relatively low variance explained by these root traits may indicate the omission of key traits in our study influencing the decomposition process of fine roots. For the most-distal root orders, these may include lignins, condensed tannins, and non-structural carbohydrates (Sun *et al.*, 2018).

MAP and species' identity were the most important predictors of standard root litter mass loss across sites (Table 4.1). In particular for the finest root orders, initial litter quality may be the primary determinant of fine-root litter mass loss, and thereby more important than environmental conditions, including macroclimate (Silver & Miya, 2001; Zhang & Wang, 2015). In contrast, topsoil and forest floor properties only explained a marginal proportion of the variation in root litter mass loss (Table 4.1). This contradicts another study of standard root litter in forests (Solly *et al.*, 2014) but is in line with findings from grasslands, where soil abiotic conditions did also not affect root litter decomposition (Chen *et al.*, 2017b). The significant interactions of litter identity with MAP and MAP (Table 4.1), respectively, point to the complex interplay of macro-environmental conditions, affecting decomposer organisms, with initial litter quality. The study of such interactions is likely key to interpret the context-dependency of plant diversity effects on decomposition over large spatial scales.

#### **4.6. Conclusions**

Overall, our results extend the findings of other studies of mixture effects on leaf litter decomposition. Also in fine roots, the environmental context and initial litter chemistry can be more important predictors of litter decomposition than species mixing. Nonetheless, litter diversity effects differed strongly across forest types and in interaction with macroclimate variables, suggesting that incorporating diversity effects and their context-dependency in ecological models of carbon and nutrient cycling is likely to be crucial to avoid biased outcomes. Future studies should, therefore, investigate further the interplay of forest characteristics and the decomposer community in mixed-species forests. In addition, expanding the list of functional fine-root traits to include relevant properties such as lignins and condensed tannins could also improve our mechanistic understanding of diversity effects.

#### **4.7. Acknowledgements**

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#### **4.8. Data Availability**

The datasets used for this study will be archived on a data portal associated with the FunDiveEUROPE and SoilForEUROPE projects (<https://data.botanik.uni-halle.de/fundiveurope>) and freely accessible to the public.



## 5. General discussion

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The main objective of this doctoral thesis was to investigate the effects of tree diversity on properties and processes driven by tree fine roots across four wide-spread European forest types. More specifically, the first overall goal was to assess the effect of tree diversity on standing fine-root biomass, root length deployed by trees (i.e., fine-root soil space occupation) (chapter two). The thesis' second overall goal was to investigate the effect of tree diversity on fine-root soil exploitation strategies under consideration of their mycorrhizal symbionts (chapter three). The third objective was to assess tree diversity effects on tree fine-root litter decomposition and to quantify the relative influences of initial substrate quality, macro-and micro-environmental conditions for fine-root litter decomposition (chapter four).

### 5.1. Summary of results

The results of my thesis show that tree species mixing can significantly affect fine-root soil exploitation and decomposition across an environmental gradient, different forest types and species compositions. Despite consistent tree species mixing effects across sites, the magnitude of these mixing effects on fine roots was influenced by root functional type (absorptive vs. transport roots), forest type (regional-scale conditions), and phylogenetic identity (gymnosperms vs. angiosperms).

The results in **chapter two** highlight that, across four European forest types, tree species mixing led to an underyielding of standing fine-root biomass, which was mainly reflected in negative complementarity effects. Simultaneously, total length density of absorptive roots (across the soil profile) did not decrease in mixtures compared to mono-specific stands and even increased in the most nutrient-rich soil layer. This increase of root length density in the topsoil was associated with positive complementarity effects. There was no evidence for vertical root stratification among species in mixtures. Conifer proportion, which was mainly associated with selection effects, dampened net diversity effects. Furthermore, tree species mixing effects were affected by root functional type, i.e., for fine-root biomass, responses of absorptive fine roots (the first most distal root orders) were stronger than responses of transport fine roots (higher-order roots).

The analysis in **chapter three** further show that overall tree fine-root trait values changed in mixtures compared to mono-specific stands, indicating a *faster* fine-root resource foraging strategy in mixtures and simultaneously greater support in soil exploitation by EcM partners (positive biotic feedbacks). These changes in trait values were associated with the observed negative net diversity effects on standing fine-root biomass (chapter two). The diversity of root



functional traits further influenced net diversity effects on fine-root biomass. Mixtures characterised by a lower dissimilarity in fine-root traits were associated with a greater reduction in fine-root biomass and *faster* fine-root resource foraging. Moreover, the results revealed distinct responses by conifer and broadleaved species to tree species mixing, i.e., thin-rooted broadleaved species showed generally higher morphological plasticity compared to thick-rooted conifer species.

The results of **chapter four** show that tree species mixing can increase tree fine-root decomposition rates via indirect effects, i.e., likely as a result of micro-environmental changes caused by tree species mixing at the stand level, rather than interaction effects among different litter types. Yet, overall mixing effects were relatively weak and context dependent. Initial fine-root substrate quality and macro-environmental conditions were shown to have a stronger influence on fine-root decomposition rates than tree diversity.

## **5.2. Study strengths, limitations and recommendations for future research**

The key strengths and novel aspects of this study range from the application of state-of-the-art methodological approaches to the coverage of a wide variation in tree species and environmental conditions in different European forest types. Nevertheless, some limitations and possible uncertainties need to be addressed to provide directions for future studies. In this subsection, I will discuss the strengths, limitations, and recommendations for future research. To facilitate a better overview, this subchapter is further divided into several sections, focusing on different levels: First, the general design of this study is discussed (5.2.1.), followed by the (soil) sampling approach (5.2.2.). The subsequent section deals with linking the measured properties with functions (5.2.3.), whereas the last section discusses the underlying mechanisms of diversity effects (5.2.4.).

### **5.2.1. General study design**

#### **5.2.1.1. Real-world study with a broad scope**

Studies examining BEF relationships have primarily focused on the above-ground component of ecosystems, while the below-ground has received much less attention (Bardgett & Van Der Putten, 2014). Most insights into below-ground BEF relationships still stem from grassland studies (e.g., Mommer *et al.*, 2015; Bakker *et al.*, 2018; Oram *et al.*, 2018; Barry *et al.*, 2020) or young tree diversity experiments (e.g., Lei *et al.*, 2012b; Sun *et al.*, 2017). Grassland ecosystems are fundamentally different from forests (Forrester & Bauhus, 2016), and species interactions in artificially assembled tree communities of (young) tree diversity experiments may

significantly differ from naturally formed tree communities (van der Plas, 2019; Kou *et al.*, 2020). These previous findings are thus not directly transferrable to natural or semi-natural forest ecosystems, which represent the vast majority of the total forest area worldwide (Food and Agriculture Organization of the United Nations, 2020). By covering four major European forest types in semi-natural mature forests, including 13 wide-spread tree species, the design of this study was therefore suitable to comprehensively assess the impact of tree diversity on below-ground ecosystem properties. This thesis thereby provides valuable new insights for our understanding of mixed-species forests across a wide range of environmental conditions and species compositions. The high representativeness is a key strength of this work and enhances the results' generalisability and relevance. While this study's scope reaches beyond regional studies, future research efforts investigating whether the observed effects are applicable to even larger environmental gradients and other forest types, such as subtropical and tropical forests, are needed.

Positive biodiversity effects on ecosystem functioning are thought to increase with increasing species richness, as complementarity among species may increase with an increasing number of species (Cardinale *et al.*, 2007). This has been shown for tree diversity-productivity relationships (Zhang *et al.*, 2012). For below-ground effects, a recent meta-analysis found that positive diversity effects on root biomass increased with increasing species richness levels, whereas diversity effects on SRL turned negative with increasing richness (Peng & Chen, 2020). As the SoilForEUROPE design did not include a tree species richness gradient, it was impossible to analyse the effect of more or less diverse forest stands. Yet, as around 50% of Europe's forests are dominated by two to three species (EUROPE FOREST, 2015), the results of this study are still highly representative of Europe's forests. Nevertheless, it would be of value to assess the effect of tree species diversity on below-ground responses of trees for more (four or five species) and less diverse (two species) forest stands, as the magnitude of these effects may vary at different levels of richness.

In particular, in natural forests, diversity effects on ecosystem functioning can be confounded with environmental conditions (Forrester & Bauhus, 2016). The FunDivEUROPE/Soil-ForEUROPE exploratory plot network was established with the aim to minimise such confounding influences (Baeten *et al.*, 2013). This is one of the significant strengths of the comparative observational approach used here, as it not only increases the representativeness of the results compared to tree diversity experiments but also limits the possibility of confounding effects driving the observations (i.e., increased orthogonality), in contrast to forest inventories (Nadrowski *et al.*, 2010; Kambach *et al.*, 2019). Yet, stand structure and age slightly correlated

with tree diversity at our sites, which was regarded as “an integral part of the diversity effect” (Baeten *et al.*, 2013). Whether these stand characteristics played a role in below-ground interspecific root interactions could not be resolved here.

#### 5.2.1.2. Analysing the context-dependency of diversity effects

Studies have shown that tree diversity effects are largely context-dependent, i.e., their magnitude depends on space and time (Forrester & Bauhus, 2016; Ratcliffe *et al.*, 2017; Ammer, 2019). It has been found that species complementarity increases with the maturity of the community for grassland (Cardinale *et al.*, 2007) and young forest experiments (Huang *et al.*, 2018). A recent study reported stronger diversity effects on tree growth with increasing stand age across the FunDivEUROPE plot network (Jucker *et al.*, 2020), and canopy closure was suggested to be associated with the emergence of positive diversity effects on tree growth (Jucker *et al.*, 2020). A lack of diversity effects on below-ground properties related to fine roots in young tree diversity experiments before the occurrence of canopy closure (Lei *et al.*, 2012b; Domisch *et al.*, 2015; Archambault *et al.*, 2019) and the strong effects of tree species mixing on fine-root traits observed for mature stands here (chapters two and three) and by others (Bolte & Villanueva, 2006; Brassard *et al.*, 2011, 2013; Ma & Chen, 2017) indicate that these patterns may also apply to below-ground responses to tree species mixing. A recent meta-analysis further showed that diversity effects on RLD and SRL switched from negative in younger stands to positive in older stands (Peng & Chen, 2020). It has been suggested that greater competition for light and a lower degree of below-ground interspecific interactions between species could be the cause of the absence of diversity effects on fine-root traits at young stand development stages (Domisch *et al.*, 2015; Ma & Chen, 2016; Archambault *et al.*, 2019). However, fine-root biomass values resembling those observed in mature forests indicate a complete fine-root soil space occupation and the occurrence of below-ground species interactions in young stands (Lei *et al.*, 2012a,b). In addition, the observations of no changes in fine-root properties in response to tree species mixing in mature forest stands (Meinen *et al.*, 2009b; Jacob *et al.*, 2013; Finér *et al.*, 2017) suggest that other factors than stand age may also cause inconsistent results among studies. These may, for instance, include mean annual temperature of the site and the soil depth sampled (Peng & Chen, 2020).

In general, studies, including those conducted as part of the FunDivEUROPE project (Jucker *et al.*, 2016; Ratcliffe *et al.*, 2017), have shown how diversity effects on ecosystem properties can change with environmental conditions (Forrester, 2014; Toïgo *et al.*, 2015). The results of all three result chapters of this thesis indicate that regional-scale influences can determine the

magnitude of diversity effects, affirming the context-dependency of tree diversity effects for the below-ground ecosystem component. Although it would have been an asset to further investigate environmental drivers of the across-site variation in the observed below-ground mixing effects, the design of the SoilForEUROPE project was not ideal for this purpose. First, four sites represent a relatively small number for testing the influence of environmental context on tree diversity effects. Second, the four study sites did not constitute a true environmental gradient (i.e., soil and macroclimate) (Table 1.1). On the one side, the sites in Romania and Italy were characterised by clay-rich and less-acidic soils, whereas the sites in Finland and Poland were characterised by sandy, more acidic soils. On the other side, mean annual temperatures were almost identical for the Polish and Romanian forest stands. Third, the available environmental data were measured at different scales, representing a statistical issue for the identification of potential environmental drivers. Hence, for testing the influence of environmental conditions on below-ground responses to tree diversity, future studies need to include a broader range of conditions, preferably at a high-resolution scale.

### **5.2.2. Sampling approach**

#### **5.2.2.1. Representativeness of the sampling spot**

Mature, (semi-) natural mixed-species forests are composed of trees of different ages and dimensions, unevenly distributed across stands. Such forest stands may, therefore, be characterised by a relatively high small-scale heterogeneity in terms of micro-environmental conditions, e.g., light and soil conditions (Boyden *et al.*, 2012). Soil conditions substantially influence fine-root traits and distribution, even at a relatively small scale (Freschet *et al.*, 2020b). A recent study has further shown that micro-topography can significantly affect fine-root dynamics (Li *et al.*, 2020). Despite these findings, many related studies in semi-natural mature forests are based on random sampling approaches, i.e., soil sampling spots for root analyses are randomly distributed across stands (e.g., Brassard *et al.*, 2013; Finér *et al.*, 2017). Inconsistent results reported in terms of fine-root responses to tree species mixing may therefore, at least partially, be attributable to confounding factors as a result of random root sampling. To minimise the variation due to environmental factors such as micro-topography and small-scale soil heterogeneity (Vivanco & Austin, 2008), the soil cores used for the root trait analysis in this study were taken within tree neighbourhoods.

Several studies have shown that tree diversity effects on productivity can be confounded with stand density effects (e.g., Forrester & Bauhus, 2016; Ouyang *et al.*, 2019). Stand basal area, which is often used as an estimate of stand density, was found to influence values of total tree

fine-root biomass at the stand level both positively (Chen *et al.*, 2004; Finér *et al.*, 2011; Xu *et al.*, 2020) or negatively (Finér *et al.*, 2007). Likewise, the distribution of tree roots in soil samples may be influenced by the dimensions of and the distance to surrounding individual trees (Yanai *et al.*, 2006; Meinen *et al.*, 2009b; Lee, 2018). The sampling approach used here allowed for the consideration of potential effects of heterogeneous tree dimensions on the samples by ensuring equal influences of the surrounding (target) trees on the soil sampling spot.

Micro-environmental conditions of the sampling spots in the centre of the tree triplets likely differed from other spots within the plot, mainly due to the small-scale heterogeneity of soil conditions (Zinke, 1962). Hence, the sampling spots may not be representative of the entire plot, and measured total fine-root biomass values cannot be directly extrapolated to the entire forest stand. Moreover, this may also apply to the other traits measured, as fine roots are highly plastic in response to small-scale changes in nutrient availability, and measured root traits may even differ on the same root system (Hodge, 2004, 2009). Another disadvantage of the triplet approach may concern the ignorance of subordinate, non-target species present in the stand, as rare or subordinate species may have relatively large influences on ecosystem functioning (Comita *et al.*, 2010). Future studies could address this aspect.

The selection of sampling spots in the centre of three tree individuals certainly has the advantage of capturing the interaction among their fine-root systems with a high probability. Yet, this sampling approach does not allow for detecting tree species mixing effects on the horizontal distribution of root systems (i.e., horizontal stratification). As such, greater horizontal fine-root soil volume occupation could also lead to greater resource acquisition in mixtures compared to pure stands (Brassard *et al.*, 2011, 2013; Peng & Chen, 2020), which could particularly be the case in older stands (Ma & Chen, 2017). Likewise, taking samples at points of overlapping root systems does not allow for conclusions to be drawn as to whether changes in standing fine-root biomass values in mixtures compared to mono-specific stands (chapter two) are, for instance, a result of changes in above-ground productivity (Richards *et al.*, 2010). For this, capturing alterations in fine-root trait values at other locations than the overlapping zones, i.e., spots closer to individual trees, would additionally be required (Richards *et al.*, 2010).

#### **5.2.2.2. Sampling time**

Fine-root growth, mortality and turnover and thereby standing fine-root biomass and necromass values may fluctuate seasonally (Makkonen & Helmisaari, 1998; Brassard *et al.*, 2013; Ma & Chen, 2017) and can differ from leaf production peaks (Steinaker & Wilson, 2008; Abramoff

& Finzi, 2014; Zhao *et al.*, 2017). Likewise, seasonal changes in fine-root architecture, including SRL, diameter, and tip density, as well as mycorrhizal colonisation rates, have been observed (Coll *et al.*, 2012). Consequently, sampling time can have a crucial impact on the measured fine-root trait values and subsequently on the occurrence and magnitude of tree species mixing effects on fine roots. For instance, in mature boreal forest stands an overyielding of fine-root biomass was only detectable in summer months (Brassard *et al.*, 2013; Ma & Chen, 2017).

Fine-root samples from one sampling campaign, as in the case of this doctoral thesis, can thus only mirror the conditions at the time of sampling. In this sense, the fine-root measurements represent more of a snapshot. We took soil samples for root trait measurements in spring, approximately in sync with leaf emergence at each site. The results of chapters two and three affirm the occurrence of tree species mixing effects on standing fine-root biomass and other trait values at the start of the growing season. Yet, given species-specific and seasonal variations in fine-root trait values (Coll *et al.*, 2012; McCormack *et al.*, 2014), the diversity effects could differ throughout the year. For instance, lower standing fine-root biomass of deciduous than in evergreen trees in spring due to greater investment in the construction of leaves could significantly alter the contributions of selection and complementarity effects to the net diversity effects (Liu *et al.*, 2020b).

Indeed, multiple sampling campaigns would have been beneficial for investigating such potential seasonal variations in diversity effects. Yet, project time and budget were limited, and hence multiple sampling campaigns were unfeasible in this project. Consequently, this calls for studies addressing this root ecological frontier (Erktan *et al.*, 2018).

### **5.2.2.3. Sampling depth**

Despite the fact that the majority of fine roots in the forest types studied here are likely found in the upper 30 cm of the soil (Jackson *et al.*, 1997), a greater sampling depth has been recommended to better quantify the role of fine roots in ecosystem functioning (Freschet *et al.*, 2020). It has been shown that tree roots grow on average to a maximum soil depth of  $7 \pm 1.2$  m across biomes and the average maximum rooting depth of trees in boreal forests was estimated to be  $2.1 \pm 0.2$  m, while it was  $2.9 \pm 0.2$  m and  $2.6 \pm 0.2$  m in temperate coniferous and deciduous forests, respectively (Canadell *et al.*, 1996). A recent review has further highlighted the vast research gaps related to the role of deep fine roots in forest ecosystems (Germon *et al.*, 2020). Indeed, it has been shown that below-ground mixing effects even led to an increased fine-root soil exploration to a depth of 17 m (Germon *et al.*, 2018). A great sampling depth may particularly be

crucial when investigating plant-plant interactions related to water acquisition, including hydraulic redistribution (Freschet *et al.*, 2020). Yet, it may also be essential for the quantification of total below-ground biomass allocation of trees. As fine roots play a substantial role for soil C sequestration in forests (Clemmensen *et al.*, 2013), quantifying deep fine-root biomass could also contribute to more precise estimates of the global C budget (Balesdent *et al.*, 2018; Germon *et al.*, 2020).

Whether the sampling depth of 30 cm used in this study was adequate for capturing a representative snapshot of the below-ground interactions at the four sites examined here cannot be ascertained. In particular, for the investigation of vertical root stratification in mixtures (chapter two), the sampling depth may have been insufficient (as discussed in chapter two), as the results did not provide evidence for vertical stratification among root species. However, several other studies have also failed to observe vertical root stratification among different tree species (Bauhus *et al.*, 2000; Meinen *et al.*, 2009b; Valverde-Barrantes *et al.*, 2015; Archambault *et al.*, 2019), suggesting that it may not be a common phenomenon. It could also indicate that root distribution patterns are not the most important determinant of positive tree diversity effects. Instead, spatial resource partitioning may occur at much finer scales (Kulmatiski *et al.*, 2010) which may not be detectable using conventional methodological approaches. Moreover, the overlap of fine-root distribution with zones of high nutrient availability and nutrient uptake by mycorrhizal symbionts likely plays a more relevant role than fine-root distribution itself (Richards *et al.*, 2010).

Nevertheless, the relatively large number of soil samples (in total 928) already required an enormous amount of effort, and it would have been unfeasible to process more root samples as part of this doctoral thesis. In addition, at some of the sites (i.e., Italy and Romania), the sampling depth was already limited due to a high stone density in the subsoil, which would have made it impossible to take deeper samples. Future studies should nevertheless try to *dig deeper*, if feasible within the time frame and budget of the respective project.

#### **5.2.2.4. Quantifying fine-root decomposition**

Knowledge of fine-root litter decomposition dynamics is particularly relevant for our understanding of C and nutrient cycling and thus also in view of climate change (Clemmensen *et al.*, 2013). In contrast to leaf litter, the decomposition of root litter has been much less studied, particularly in mixed-species forests. Hence, this thesis makes an essential contribution towards

understanding the controls of fine-root litter decomposition in mixed stands. Nevertheless, studying the decomposition dynamics of plant organs is, in general, a challenging task, which is why it is important to reflect on possible methodological constraints.

As discussed in chapter four, the decomposition rates of fine-root litter quantified here appear relatively low, though the number of comparable studies is rather limited. Several factors most likely led to a general underestimation of root decomposition rates in this study. These include the disruption of the rhizosphere, the exclusion of macro-and mesofauna due to the small mesh size (100  $\mu\text{m}$ ) of the litter bags, and the type of root material used for incubation. In particular, the lack of adequate methods determining whether a root is still alive or not may be pointed out as a significant challenge (Sun *et al.*, 2018). Nevertheless, the applied method permitted to test the effect of tree species mixing on litter decomposition, as the conditions presumably remained identical for both mixed and mono-specific neighbourhoods. Exceptions could be, for instance, rhizosphere interaction effects (e.g., priming effects via root exudation Zwetsloot *et al.*, 2020) as well as soil fauna activity and thereby fragmentation and bioturbation (Basile-Doelsch *et al.*, 2020), which can significantly differ between mixtures and mono-specific stands (De Wandeler *et al.*, 2018).

As pointed out in chapters two and three, morphology (e.g., diameter) and chemical composition (e.g., root N concentrations) of fine roots can be affected by tree species mixing, resulting in a different initial quality of fine-root litter material in mixed and mono-specific plots. By incubating litter material of identical quality in mixed and mono-specific stands, such variations in litter quality were not taken into account. However, they could potentially alter litter interaction effects among litter species and overall decomposition rates. To examine this, future studies could use litter material originating from sites where the litter is incubated. To obtain more realistic decomposition rates in general, alternatives to the mesh bag approach, such as trenched plots and intact cores, could be used (Dornbush *et al.*, 2002; Freschet *et al.*, 2020).

The one-year incubation period represents another limitation of this study that does not allow general conclusions to be drawn on the entire decomposition process, as this short incubation time only captures the early-stage decomposition of roots. In addition, initial decomposition rates, especially of EcM species, may be faster than at later stages (Sun *et al.*, 2018). Likewise, diversity effects may also change during the course of decomposition (Wu *et al.*, 2013; Santonja *et al.*, 2018; Kou *et al.*, 2020). To obtain more realistic overall decomposition rates, future studies should therefore extend the incubation period (Freschet *et al.*, 2020).



### 5.2.2.5. *Home-field advantage for below-ground decomposition?*

To quantify environmental influences on root litter decomposition rates, two standard root litter species, i.e., *C. betulus* and *P. sylvestris*, were deployed at all four sites in addition to the site-specific root litter species (chapter four). However, while the root material did not directly stem from the incubation sites, the two species naturally occurred at some of the sites, potentially resulting in home-field advantage (HFA) effects.

The HFA hypothesis states that litter decomposes faster in its *home* environment, i.e., where it naturally occurs (Gholz *et al.*, 2000). It has also been suggested that low-quality litter displays more significant HFA effects than high-quality litter, as microbial communities would have to be specialised to degrade such litter (Milcu & Manning, 2011). In view of this, Lin *et al.* (2020) hypothesised that “root litters should display greater HFA effects than leaf litters because they are often more recalcitrant”. Evidence in favour of the HFA is limited for leaf litter (e.g., Ayres *et al.*, 2006; Vivanco & Austin, 2008b; Bachega *et al.*, 2016) and even scarcer for fine-root litter (Freschet *et al.*, 2012; Jacobs *et al.*, 2018; Minerovic *et al.*, 2018; Lin *et al.*, 2020). Recent studies, however, suggested that the HFA effect found for leaf litter may not apply to fine-root litter (Bachega *et al.*, 2016; Jacobs *et al.*, 2018; Minerovic *et al.*, 2018). As such, overall inconsistent HFA effects between fine-root and leaf litter have been observed, with fine roots showing nearly no effects (Lin *et al.*, 2020). These observations could be explained by the divergent conditions for microbes in the forest floor layer (where leaf litter decomposes) and the soil matrix (where root litter decomposes). Thus, nutrient availability for decomposing organisms is likely higher in the forest floor, whereas it is more limited in soil.

In the context of my decomposition study (chapter four), it is difficult to assess potential HFA effects for the two species, as such effects cannot easily be disentangled from other environmental influences on decomposition. Yet, while the results in chapter four do not necessarily indicate HFA effects (Fig. S4.4), HFA cannot be ruled out completely and should further be investigated in future studies.

### 5.2.3. **Linking measured properties with functions**

#### 5.2.3.1. *Capturing suitable root entities*

Previous studies have clearly shown that root orders differ in anatomy (e.g., Guo *et al.*, 2008), morphology (e.g., Pregitzer *et al.*, 2002), their association with mycorrhizal fungi (e.g., Ouimette *et al.*, 2013), chemical composition (e.g., Jia, Wang, Li, Zhang, & McLaughlin, 2011; Salahuddin *et al.*, 2018), uptake capacity (e.g., Rewald, Raveh, Gendler, Ephrath, &

Rachmilevitch, 2012), respiration rates (e.g., Jia et al., 2011; Salahuddin et al., 2018), longevity (e.g., Xia, Guo, & Pregitzer, 2010) and decomposition rates (e.g., Xiong et al., 2013). Consequently, when assessing specific plant and ecosystem functions, it is crucial to consider these differences among root orders (McCormack *et al.*, 2015; Laliberté, 2017; Freschet *et al.*, 2020).

The aim of this work was to investigate plant and ecosystem functions mainly driven by the most distal root orders, i.e., resource acquisition and nutrient cycling. Using the functional classification method was adequate to efficiently distinguish between the absorption and transport function within the 2-mm root pool (McCormack *et al.*, 2015). Indeed, the results presented in chapter two confirm that absorptive and transport roots can respond differently to tree species mixing. As such, the strong mixing effects on biomass of the absorptive root pool and the lack of effects on the transport root pool clearly indicate that intermediate effects on the traditionally defined root pool (all root orders within the 2-mm diameter class) are simply a result of the adding up of these two different entities. These results lead to the speculation that inconsistent and partially contradictory responses of fine roots to tree species mixing and, more generally, changes in environmental conditions reported previously may be attributable to the traditional classification of fine roots.

Although the application of the functional classification method was appropriate and necessary for this work, some limitations of this approach need to be addressed. First, despite the likely much faster laboratory procedures associated with this method compared to an entirely order-based approach, the time required to apply this method is still considerably greater compared to a diameter-based approach. This may particularly be the case when using soil cores with small diameters (e.g., 5 cm), as they lead to a large number of small root fragments of different sizes that need to be examined (often using a microscope). Given budget and time constraints, this may present a limitation for many projects. Second, in addition to the challenges of determining root orders of non-intact fine roots, the presence of EcM tips and pioneer roots may represent another difficulty for a consistent application of the approach (Zadworny & Eissenstat, 2011; Freschet *et al.*, 2020). For instance, the mycorrhizal mantle surrounding root tips can alter root tip morphology and branching, causing difficulties when counting root orders (Freschet *et al.*, 2020) (e.g., in dichotomous mycorrhizal tips of *Pinus sylvestris*). It is therefore preferable to reduce the number of persons involved in root sorting and assign only a few well-trained laboratory technicians to process the samples. Third, whether the absorption function is, in fact, dominant in the first three root orders may depend on the tree species (McCormack *et al.*, 2015) and environmental conditions (Zadworny *et al.*, 2016). While only direct measurements of uptake rates can shed light on the prevalence of absorption and transport functions

(see subsection 5.2.3.3.), anatomical measurements (e.g., presence/absence of intact cortical cells) could provide good indications in this regard (McCormack *et al.*, 2015; Zadworny *et al.*, 2016). Given the time limitations of this doctoral thesis, it was impossible to assess such root anatomical characteristics for each site or even plot and species.

Consequently, studies are needed that examine fine-root anatomy of different species in more detail in order to better account for species-specific variability and to more accurately separate the two root functional types.

#### **5.2.3.2. Significance of early-stage decomposition rates**

The decomposition of plant litter represents a complex process with fundamental importance for overall ecosystem functioning. However, inferences for C and nutrient cycling based only on decomposition rates are severely limited, as the rates at which litter decomposes generally do not represent the decomposition process (Prescott, 2005, 2010; Cotrufo *et al.*, 2009). The accumulation of SOC and thereby the potential for soil C sequestration generally depends on C inputs (e.g., litter, exudates), biotransformation and stabilisation mechanisms, and mineralisation (CO<sub>2</sub> release to the atmosphere) (reviewed by Basile-Doelsch *et al.*, 2020).

The one-year incubation period likely reflects the initial part of the *early stage* of the entire decomposition process (Berg & Matzner, 1997). During this phase, the mass loss is dominated by the decomposition of non-structural soluble compounds (Berg & Mcclaugherty, 2014). Until recently, this phase had barely been associated with the formation of stable SOM/SOC, suggesting that early-stage decomposition rates are of little value for statements about the soil C sequestration potential. Instead, the *humus-near (limit-value) stage* was associated with the amount of litter that was primarily linked with the formation of stable SOM (Berg & Mcclaugherty, 2014). In that sense, the concept of limit values of decomposition could allow for estimations of persistent SOM/SOC (humus) built-up based on the amount of litter material left in bags decomposing at very low rates (Berg *et al.*, 2001). Accordingly, only long-term decomposition studies could provide further insights into the actual built-up of SOM/SOC.

However, this approach does not consider the interaction of OM with mineral particles, i.e., chemical and physical stabilisation of OM (Cotrufo *et al.*, 2009), which can hamper microbial decomposition and significantly increase the residence time of SOM/SOC (von Lützow *et al.*, 2006). These stabilisation mechanisms were thought to mainly occur during later stages of the decomposition process (von Lützow *et al.*, 2006), yet, it has recently been shown that even early-stage decomposition products can substantially contribute to soil C (Bird *et al.*, 2008; Rubino *et al.*, 2010) and the formation of stable SOM via dissolved organic matter (DOM)-

microbial paths (Cotrufo *et al.*, 2015). This emphasises that solely quantifying decomposition rates and in particular using the litter-bag approach, which excludes the role of chemical and physical stabilisation processes (Cotrufo *et al.*, 2009, 2019), greatly limits potential conclusions about effects on soil C sequestration.

Therefore, future efforts should not only focus on the quantification of decomposition rates in response to tree diversity but also quantify C inputs to soil (i.e., root turnover, exudation rates) and investigate the stabilisation processes of SOM/SOC. For instance, stable C isotopes could be used to assess C fluxes (Cotrufo *et al.*, 2009), while SOM density fractionation could provide more insights into the stabilisation of SOM/SOC (von Lützow *et al.*, 2007) in mixtures and mono-specific stands.

#### ***5.2.3.3. Measuring suitable traits for below-ground functioning***

Trait-based approaches are a useful tool to assess desired plant and ecosystem functions. Yet, the measured traits often represent only proxies for the desired functions, and the direct role of some of the measured fine-root traits for plant functioning is not entirely understood (Freschet *et al.*, 2021). Moreover, the most commonly measured fine-root traits do often not represent the most relevant functions (Freschet *et al.*, 2021). For instance, root N concentration is used as an indicator of root metabolic activity (Reich *et al.*, 2008) and nutrient uptake rates (Legay *et al.*, 2020). Yet, it may also relate to the storage of defence compounds, and hence its link to specific functions is hypothetical (Freschet *et al.*, 2021). As our current understanding of trait-functioning relationships is still limited, a call for “broadening the suite of traits” (Laliberté, 2017) has been made for below-ground root ecology research.

Within the scope of this doctoral thesis, it was possible to measure several key fine-root traits, that according to our current understanding, can be linked to resource uptake capacity and efficiency. Yet, these commonly measured traits bear certain limitations in terms of their consequences for plant and ecosystem functions.

- **Resource uptake capacity**

First of all, one of this thesis’ main objective was to assess tree species mixing effects on soil exploitation strategies by tree fine roots. Resource acquisition by fine roots is complex and associated with high spatial and temporal variability, making any measurements of it rather challenging (Freschet *et al.*, 2020). Several techniques exist for the quantification of soil resource uptake kinetics (i.e., specific uptake rates) by plants in the field including isotopic tracer applications and depletion methods (reviewed by Lucash *et al.*, 2007). Yet, particularly for

mature trees, the quantification of specific uptake rates entails significant methodological challenges (Lucash *et al.*, 2007). As such, destructive methods can result in severe bias, as roots are non-intact and detached from trees during measurements (Lucash *et al.*, 2007; Tiziani *et al.*, 2020), while measurements on intact roots (e.g., depletion methods) may be unfeasible for large-scale applications involving mature trees and create artificial soil conditions (Lucash *et al.*, 2007). *In-situ* isotopic tracer applications could be an alternative, yet isotope dilution may pose a problem (Lucash *et al.*, 2007), apart from the extensive efforts and financial resources required for its implementation. In addition, the uptake rates determined by each of these methods are greatly affected by the timing (season) and duration of the measurements (Lucash *et al.*, 2007; Case *et al.*, 2020).

For studies with relatively large environmental gradients and a high number of replicates, as in this work, quantification of nutrient uptake kinetics is literally unfeasible. Therefore, the use of proxies for fine-root resource uptake capacity, such as root length density, seems obvious.

As physiological measures were not performed in this study, it cannot be ascertained whether the higher RLD in surface soil measured in mixed compared to mono-specific stands can indeed be associated with a greater soil resource uptake capacity in mixtures (chapter two). Naturally, this does not only depend on the capacity of roots and their mycorrhizal partners themselves but also on the availability of resources in soil and the overlap of these resources with the root system. Here, it also remains to be tested whether tree species mixing enhances the uptake of limiting resources in particular, including immobile nutrients such as phosphorus, which would likely have the greatest effect on overall plant productivity.

In relation to uptake capacity, other traits could be measured more directly representing the effective radius of roots and thereby the function of nutrient acquisition. Root hairs (Itoh & Barber, 1983) and mycorrhizal hyphae (Brundrett, 2002) are of particular importance here. Root hairs can directly increase a root's effective radius (Itoh & Barber, 1983) and roots with greater root hair density and length can enhance plant nutrient uptake compared to roots without or shorter hairs (Bates & Lynch, 2000; Yan *et al.*, 2004). While EcM colonisation rates on fine roots were quantified and EcM abundance and diversity were measured in soil samples (chapter three), it is the mycorrhizal hyphae that are directly linked to resource acquisition, as they extend the absorbing surface area in soil beyond the roots' influence (Read, 1992) and can also take up nutrients in organic form (Smith & Read, 2008). In addition to root hairs and mycorrhizae, root exudates can significantly affect the nutrient uptake capacity of roots. For instance, the release of root-derived soluble C to soil has been shown to increase the availability of N as

a result of enhanced microbial decomposition (Phillips *et al.*, 2011). Consequently, the measurement of root exudation rates may provide further insights into resource acquisition by fine roots (Freschet *et al.*, 2020).

Root architectural and anatomical traits could also indicate soil exploitation capacity by fine roots and are more easily measurable than root hairs, mycorrhizal hyphae, and exudation rates. Architectural traits such as branching/tip density and growth angles have often been linked to the nutrient acquisition of fine roots (Hodge, 2004; Lynch, 2005; Eissenstat *et al.*, 2015; Liese *et al.*, 2017). In this study, such architectural traits were not measured since the sampling methodology used would have significantly reduced the accuracy of such measurements (Freschet *et al.*, 2020). As such, the destructive use of a small-diameter split-tube sampler to obtain soil and root samples yielded few intact root samples, while freezing of samples likely further impaired root architecture (Freschet *et al.*, 2020). An anatomical trait that could be considered is the root secondary (cambial) growth, which significantly affects resource acquisition capacity (Strock & Lynch, 2020). Here, the presence or absence of phellem and cortical parenchyma cells (cortex) can indicate the prevailing function of the fine root, i.e., transport or absorption (Freschet *et al.*, 2020).

In summary, future studies that cannot directly quantify water and nutrient uptake kinetics by roots could measure additional root traits to get a better estimate of fine-root resource uptake capacity. These may include root hair density and length, mycorrhizal hyphal length, root exudation rates, or architectural and anatomical traits.

- **Resource uptake efficiency**

In contrast to fine-root soil exploitation capacity, the efficiency of soil exploitation by fine roots depends on the C costs associated with the construction and maintenance of these roots (Fitter, 2002). Following the discussion in chapter three, whether the soil exploitation by fine roots in mixtures compared to mono-specific stands is more efficient in terms of C costs (as indicated by the higher SRL, the lower RTD, the higher EcM colonisation intensity, and the higher root N concentrations) can theoretically only be determined by quantifying all C-fluxes in relation to nutrient acquisition rates. As we neither quantified nutrient uptake rates nor the C costs involved, the question of efficiency remains highly speculative and needs to be addressed in future studies. Carbon costs to be considered here include those associated with root construction and respiration, turnover, exudation, and the C transfer to mycorrhizal symbionts (Eissenstat & Yanai, 1997; Lambers *et al.*, 2002).

How roots are built can significantly affect the C costs that need to be invested. For instance, dense tissue with advanced secondary root growth is more expensive than less dense tissue with suppressed secondary growth (Strock *et al.*, 2018). Anatomy and morphology of roots further affect root lifespan and turnover rates, which can fundamentally influence C costs for the plant (McCormack *et al.*, 2012; Weemstra *et al.*, 2016). On the one side, root exudation rates, which may particularly be high in thin roots with low mycorrhizal colonisation rates (Meier *et al.*, 2020), can account for one-third of a tree's assimilated photosynthates (Liese *et al.*, 2018). On the other side, C transfer rates from roots to mycorrhizae, which usually colonise roots of larger diameters (Kong *et al.*, 2014) owing to the greater cortex proportion (Guo *et al.*, 2008), can be substantial (Eissenstat, 1992) and may constitute 7-30% of the photosynthates (Tedersoo & Bahram, 2019).

Measuring C costs vs. the benefits in terms of nutrient acquisition represents an extremely challenging task also because many of the variables involved are strongly interlinked (as discussed in chapter three). For instance, low RTD may be associated with lower root construction costs on the one side (Strock *et al.*, 2018), yet it may also be linked to a reduction in root lifespan (Eissenstat *et al.*, 2000, 2015), resulting in high C costs for root replacement (McCormack *et al.*, 2012; Weemstra *et al.*, 2016). As discussed before, root hairs, mycorrhizae, and root exudates can positively influence the nutrient uptake capacity of roots, yet the C costs of the three traits may significantly differ (Lynch & Ho, 2005). In terms of phosphorus acquisition, for example, root hair formation may be relatively cheap, whereas mycorrhizae and root exudates can be more expensive for plants (Lynch & Ho, 2005). On the other side, it has also been demonstrated that mycorrhizal hyphae are more efficient in resource uptake than roots themselves (Chen *et al.*, 2018). The efficiency in resource uptake can also be influenced by fine-root architecture, which can determine the overlap of depletion zones (Lynch, 2005).

More generally, soil nutrient status can also affect nutrient uptake efficiency by roots, with higher nutrient availability enhancing efficiency (Bloom & Mooney, 1985; Binkley *et al.*, 2004). To add to the complexity, concerning the overall benefits to the tree (whole-tree level), not only the overall uptake rates of nutrients need to be considered, but also whether the uptake of plant limiting nutrients is improved for a given C cost.

#### **5.2.3.4. Considering the whole-tree level**

A recent study has demonstrated the importance of integrating both above- and below-ground variables when aiming to explain whole-tree performance (Weemstra *et al.*, 2020). Furthermore, studying above- and below-ground compartments in isolation may lead to contrasting

conclusions in view of the understanding of BEF relationships (Ma *et al.*, 2019; Martin-Guay *et al.*, 2020). This particularly applies to linkages between below-ground functioning and positive above-ground diversity-productivity relationships. According to the *optimal partitioning theory* (Bloom & Mooney, 1985) or theory of *functional equilibrium* (Poorter *et al.*, 2012), positive species interactions in mixtures either above- or below-ground increase the acquisition of resources of the respective component and may hence allow for a reduced C allocation to the component where resource acquisition was improved while enhancing C allocation to the other component. Indeed, such shifts in C allocation patterns from above- to below-ground in mixtures compared to single-species stands have been observed in plantations (Nouvellon *et al.*, 2012), natural boreal forests (Ma *et al.*, 2019) and a young tree diversity experiment (Archambault *et al.*, 2019). It was not possible to integrate above-ground data in the analyses of this thesis to further explore this possible explanation for the results of chapters two and three. Hence, future efforts should combine above-ground data such as tree ring data as a measure for above-ground wood production with root trait data indicating resource uptake capacity and efficiency to provide further evidence of whole-tree C partitioning.

#### **5.2.4. Disentangling underlying mechanisms of tree diversity effects**

##### **5.2.4.1. Species identity effects**

Species identity effects can play an essential role in above-ground diversity effects (Ratcliffe *et al.*, 2015; Tobner *et al.*, 2016), but also in below-ground effects (Meinen *et al.*, 2009a; Jacob *et al.*, 2013; Finér *et al.*, 2017). The significant effects of conifer tree proportion on net diversity effects (chapters two) highlight the importance of species identity effects (or phylogenetic identity effects), which have also been reported in several studies related to the FunDivEUROPE sites (Ratcliffe *et al.*, 2015; Dawud *et al.*, 2017; Finér *et al.*, 2017; Joly *et al.*, 2017; De Wandeler *et al.*, 2018). In contrast to the FunDivEUROPE studies, which included six sites across Europe, the analyses of this thesis are based on data from only four of these sites, which did not constitute a proper gradient in conifer tree proportions. As such, at the site in thermophilous deciduous forests in Italy, plots did not contain any conifer species but accounted for approximately 30% of the analysed plots, while the conifer proportion was highest at the boreal forest site in Finland, though this site only had a share of approximately 14% to the total number of analysed plots. Hence, the study design was not ideal for investigating the role of conifer proportion in tree diversity effects (as discussed in chapter two).



Nevertheless, one of the fundamental challenges for forest managers is the selection of species mixtures providing the highest degree of multifunctionality, rather than the promotion of mixtures *per se* (van der Plas *et al.*, 2018; Baeten *et al.*, 2019). Since the focus of this dissertation was on broad tree diversity effects across European forests, the study approach was not designed to consider the role of specific species compositions. Therefore, future efforts are required to investigate the role of different species compositions for below-ground functioning. This could be achieved by increasing the number of replicates for each tree species composition.

#### **5.2.4.2. Limitations of the additive partitioning approach**

Loreau & Hector (2001) proposed the additive partitioning approach to statistically disentangle the two primary processes presumed to explain positive diversity effects, i.e., the complementarity and selection effect. Consequently, the application of this method has the potential to significantly improve our mechanistic understanding of BEF relationships. However, species-specific data are a prerequisite for the use of this approach. As differentiating fine roots by species is challenging and extremely time-consuming (Rewald *et al.*, 2012a), studies that have actually applied the additive partitioning approach to below-ground data are scarce. Previously, this method had only been used in relation to grass roots (e.g., Bakker *et al.*, 2018; Oram *et al.*, 2018). To the best of my knowledge, my work is one of the first to consider the additive partitioning approach for tree fine-root data. I am aware of only one recent study in subtropical forests that used this approach to explain overyielding of fine-root production (Liu *et al.*, 2020b). Consequently, the disentangling of complementarity and selection effects in relation to below-ground diversity effects represents a major achievement of this thesis.

Since the additive partitioning method is a mathematical approach to calculate complementarity and selection effects (Loreau & Hector, 2001), it should be noted that with this approach, only the consequences of diversity effects can be quantified but not their actual biological causes (Petchey, 2003). Moreover, as pointed out by Mahaut *et al.* (2020), the interpretation of the additive partitioning method's outcomes needs to be done with caution, as the presence of a particular species can also largely determine the contribution of calculated complementarity effects to the overall net diversity effects. Consequently, the interpretation of the results of the additive partitioning approach, in particular, when averaged across different species composition (chapter two), is rather limited.

#### **5.2.4.3. *Identifying underlying mechanisms of tree diversity effects***

As discussed in the previous section, the additive partitioning approach is a mathematical tool (Loreau & Hector, 2001) and does not contribute to our understanding of the occurrence of a particular underlying ecological process driving tree species mixing effects (Barry *et al.*, 2019). These underlying mechanisms, i.e., resource partitioning, abiotic facilitation, and biotic feedbacks, are challenging to quantify and do likely not occur alone but rather simultaneously (Forrester & Bauhus, 2016; Barry *et al.*, 2019). Hence, when quantifying diversity effects on ecosystem properties, as done within the frame of this thesis, the net effects of the three underlying causes are measured (Barry *et al.*, 2019). The results of my thesis provide hints at the occurrence of all three of these main mechanisms of positive species' interactions, which could drive positive diversity-ecosystem functioning relationships (chapter three). Quantifying the contributions of these underlying mechanisms to enhanced ecosystem functioning is a difficult task, as they may coincide (Barry *et al.*, 2019). As Barry *et al.* (2019) suggested, this could be done by incorporating ecological gradients (of resources, stress, and enemies) in diversity studies that allow for a varying contribution of the different underlying causes.

#### **5.2.4.4. *Other measures of tree diversity***

Species richness, i.e., the number of species, is the most straightforward measure of tree diversity and the results of this thesis demonstrate that this measure of diversity was suitable for detecting diversity effects on below-ground functions. However, by only considering the sheer number of species, it is impossible to incorporate the differences between species, i.e., species functional characteristics, which essentially influence ecosystem properties (Hooper *et al.*, 2005). Functional trait diversity and identity have been shown to predict ecosystem functioning better than species richness in some cases (e.g., Mokany *et al.*, 2008; Roscher *et al.*, 2012; Tobner *et al.*, 2016). For the below-ground component, however, few studies have addressed this notion. Most of these studies were conducted in grassland ecosystems (Bakker *et al.*, 2018, 2019b; Oram *et al.*, 2018; Mahaut *et al.*, 2020) and only recently the role of functional diversity has been examined in forest ecosystems (Bu *et al.*, 2017; Sun *et al.*, 2017; Xu *et al.*, 2019; Zeng *et al.*, 2020). For the analysis in chapter three, I calculated functional trait diversity using the functional dispersion index, which is one of the most commonly used functional diversity indices as it is not correlated with species richness (Laliberté & Legendre, 2010). Consequently, this is one of the first studies in which the functional dispersion index was applied to below-ground data from mature forest stands across a broad environmental gradient. In addition to

calculating root trait diversity using multiple traits, a further option would have been to calculate the diversity of individual traits. This might have provided further insights into which traits are associated with below-ground resource partitioning. Given the distinct responses of gymnosperm and angiosperm tree species to mixing, phylogenetic diversity indices (e.g., Zeng *et al.*, 2020) could also be a useful tool to further investigate the distinct roles of gymnosperm and angiosperms in below-ground interactions.

#### **5.2.4.5. Considering understory roots**

Tree species diversity can substantially influence understory vegetation diversity and abundance (Barbier *et al.*, 2008; Leuschner & Ellenberg, 2017). Understory roots, particularly those of herbaceous plants, can compete with tree roots for soil resources. Indeed, in a previous study, a higher tree fine-root turnover rate in mixtures compared to mono-specific stands was attributed to increased competition from understory roots (Ma & Chen, 2018). Tree fine-root trait values were also found to be affected by changes in understory-species diversity levels (Yan *et al.*, 2019). In addition, differences in understory abundance or composition between mixed and mono-specific plots may also affect litter decomposition dynamics (Zhao *et al.*, 2013) for instance via alterations in soil biota composition and micro-environmental conditions (Wardle, 2006; Scherer-Lorenzen, 2008). At the four study sites investigated here, understory was indeed abundant and constituted on average approximately 30% of the total fine-root biomass (all roots of <2 mm Ø) in soil samples. As the understory root mass did not significantly differ between mixed and mono-specific stands at any sites (Appendix, Fig. S2.2b), competition between tree and understory roots may have played a negligible role in this study. Nevertheless, differences in understory diversity between mixed and mono-specific plots could have affected root dynamics. Consequently, it would be worthwhile to investigate the influence of understory roots in future tree diversity studies.



## 6. Synthesis

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## **6.1. The role of fine roots for ecosystem functioning in mixed-species forests**

Mixed-species forests have attracted much attention in recent decades, and our knowledge of how tree diversity affects ecosystem functioning has substantially improved (e.g., Gamfeldt *et al.*, 2013; Ratcliffe *et al.*, 2017; van der Plas, 2019). However, our understanding of the underlying ecological mechanisms, particularly related to below-ground mechanisms, is still incomplete. Such knowledge is crucial to better predict how ecosystems may respond to climate change (Isbell *et al.*, 2017; Barry *et al.*, 2019) but also to provide directions and recommendations for forest management towards diverse, productive, resilient forests with a high adaptive capacity (Puettmann, 2011; Brang *et al.*, 2014).

The results of this thesis present new insights into how fine roots may contribute to positive BEF relationships in forests. These are discussed in the following sections. The focus of this synthesis is the linkages between my findings and above-ground productivity as well as soil C sequestration. As a higher productivity of mixed-species forests may generally be related to the provision of multiple other ecosystem functions, i.e., multifunctionality (Baeten *et al.*, 2019), my results may likely also support observations related to other ecosystem functions provided to a higher degree by mixed-species compared to mono-specific forests. At the same time, it is necessary to acknowledge that my thesis' results are on the tree neighbourhood level, while most of the observations on above-ground productivity and soil C relate to the stand level. As neighbourhood-level interactions can drive tree species mixing effects at the community (plot)-level (Fichtner *et al.*, 2018), the discussion of my findings in relation to findings at different scales is still justified.

The last part of this section briefly addresses the context-dependency and the role of phylogenetic identity.

### **6.1.1. The role of tree fine roots for diversity-productivity relationships**

In addition to photosynthesis, nutrient, and water acquisition by fine roots are crucial plant physiological mechanisms influencing tree growth and vitality. The frequently observed positive tree diversity–productivity relationships (Jucker *et al.*, 2014; Forrester & Bauhus, 2016; Ammer, 2019; Kambach *et al.*, 2019) suggest an increased uptake of soil resources to sustain the higher biomass production in mixtures. Several studies have attempted to test whether the positive above-ground diversity-productivity relationship is mirrored below-ground (e.g., Meinen *et al.*, 2009b; Brassard *et al.*, 2011, 2013; Ma & Chen, 2016). For this purpose, fine-root biomass has been used as a suitable variable. However, inconsistent tree diversity effects on fine-root biomass among studies (e.g., Bolte & Villanueva, 2006; Meinen *et al.*, 2009b; Ma

& Chen, 2016), including a reduction in fine-root biomass despite above-ground overyielding (e.g., Archambault *et al.*, 2019), show that studying the below-ground *hidden half* is rather complex. These observations and this thesis' results (chapter two) suggest that fine-root biomass itself is not an adequate variable reflecting fine-root soil exploitation and tree community performance.

Across the FunDivEUROPE exploratory plot network, which includes the four study sites investigated here, above-ground productivity increased with increasing tree species richness (Jucker *et al.*, 2014). At the same time, increasing tree species richness also led to increased canopy packing (i.e., denser, more complex canopies) (Jucker *et al.*, 2015), possibly enhancing overall light interception (Binkley *et al.*, 2013) and hence serving as an explanation for the positive tree diversity effects on above-ground productivity. In addition to these positive species interactions above-ground, increased acquisition of water and nutrients by fine roots and their symbionts could have also contributed to these positive tree diversity effects on productivity. According to the *production ecology equation* (Monteith, 1977), greater soil resource acquisition in mixtures may result from increased resource availability in soil, resource uptake capacity by fine roots, or improved resource use efficiency by fine roots (Richards *et al.*, 2010; Forrester, 2017). Consequently, the results of chapters two to four provide evidence of positive below-ground species interactions, in support of the above-ground observations at the four sites. These are discussed in more detail in the following subsections.

- **Soil resource availability**

First, the accelerated fine-root litter decomposition in mixed compared to mono-specific stands (chapter four) may indicate a faster nutrient cycling and thereby a faster return of nutrients enhancing overall resource availability for trees in mixtures. Elevated P and N levels in the forest floor in mixtures, when compared to mono-specific stands (Gillespie *et al.*, *submitted*), may support this claim. In addition, the higher EcM root colonisation in mixtures (chapter three) suggests a greater abundance of fungal hyphae and thereby an additional supply of (poorly-available) resources from zones in soil likely inaccessible to their host plants' roots. The higher EcM diversity in soil samples further provides evidence of the presence of different EcM exploration types (Agerer, 2001). Consequently, soil resource acquisition by EcM could enhance overall resource availability for roots as a result of complementary resource acquisition (Baxter & Dighton, 2001; Köhler *et al.*, 2018). It may also point to the role of mycorrhizal networks facilitating nutrient transfer among different species in mixtures (Simard *et al.*, 2015). Hence,

these observations may provide evidence of below-ground abiotic facilitation and positive biotic feedback from mycorrhizae (Barry *et al.*, 2019).

- **Resource uptake capacity**

Second, the results in chapter two provide evidence of below-ground resource partitioning among species, increasing overall soil resource uptake in mixtures compared to mono-specific stands. The greater RLD of trees in mixtures in the most-nutrient rich soil layer could indicate a greater resource uptake capacity of roots in mixtures as a result of complementary use of space and resources (chapter two). Though resource uptake was not directly measured, the root hair density – which directly determines resource uptake – may have also increased with increasing RLD (York *et al.*, 2013), strengthening this argument. In addition, the higher EcM colonisation intensity of roots and greater soil EcM diversity in mixtures compared to pure stands (chapter three) could provide further evidence of enhanced and more efficient resource uptake not only by mycorrhizae (as discussed in the previous section) but subsequently also by their host roots. Finally, the shift in soil exploitation strategies in response to mixing (chapter three) towards faster and possibly more efficient foraging by fine roots could have improved resource uptake in mixtures compared to mono-specific stands. Higher SRL in mixtures could have also increased nutrient availability via elevated root exudation rates and microbial priming effects (Phillips *et al.*, 2011; Tückmantel *et al.*, 2017; Meier *et al.*, 2020).

- **Resource-use efficiency**

Third, greater resource availability and uptake of resources in mixtures than in mono-specific stands may result in greater resource-use efficiency (Binkley *et al.*, 2004; Richards *et al.*, 2010). The lower standing fine-root biomass in mixtures could indicate a shift in C allocation from below- to above-ground biomass, as a result of an increased below-ground resource-use efficiency (Poorter *et al.*, 2012). The changes in fine-root soil exploitation strategies in mixtures (chapter three) suggest increased resource uptake efficiency and thereby enhanced nutrient-use efficiency (Richards *et al.*, 2010) in mixed compared to mono-specific stands. In particular, the higher EcM diversity in mixtures compared to mono-specific stands may increase nutrient uptake efficiency (Agerer, 2001; Köhler *et al.*, 2018).

### **6.1.2. The role of tree fine roots for soil C sequestration in mixed forests**

Roots and their mycorrhizal partners play an essential role not only for tree growth and vitality but also for soil C sequestration (Clemmensen *et al.*, 2013; Bardgett *et al.*, 2014; Frey, 2019; Neumann *et al.*, 2021). Soils represent the largest pool of OC in forest ecosystems around the



globe (Pan *et al.*, 2011). It has been estimated that up to 70% of the stored C in boreal forest soils is derived from roots and their mycorrhizal associations (Clemmensen *et al.*, 2013). Below-ground root litter and rhizodeposition are more significant for SOM than above-ground inputs (Rasse *et al.*, 2005; Kätterer *et al.*, 2011; Clemmensen *et al.*, 2013) and may, in particular, be fundamental for the stability of OC (Schmidt *et al.*, 2011). Consequently, the role of root litter inputs for soil C stocks cannot be neglected (Vesterdal *et al.*, 2013).

At the same time, positive tree diversity effects on soil C stocks have been reported in forests (Forrester *et al.*, 2013; Gamfeldt *et al.*, 2013; Dawud *et al.*, 2017; Liu *et al.*, 2017; Li *et al.*, 2019; Mayer *et al.*, 2020). Likewise, increasing topsoil C stocks with increasing tree species richness levels were measured across the FunDivEUROPE plot network (Dawud *et al.*, 2017). My results are generally in agreement with these previous observations, yet they do not allow conclusions to be drawn about causal relationships. Nevertheless, on the basis of my results, the role of fine roots for soil C sequestration in mixtures is discussed in more detail in the following sections.

In general, soil C stocks may increase with increased input of dead roots (root litter) and rhizodeposition from live roots, an increased contribution to more stabilised SOC pools (i.e., physically or chemically protected OC), or reduced decomposition.

- **Root-derived C inputs to soil**

The root trait adaptations in mixed compared to mono-specific stands indicating a faster foraging strategy (chapter three) may have consequences for the total root-derived OC input to the soil. First, root exudation rates of live roots could increase in mixtures compared to mono-specific stands in relation to the higher SRL (Tückmantel *et al.*, 2017; Meier *et al.*, 2020) and higher root N content (Sun *et al.*, 2021) (chapter three). Root exudates represent an essential source of C input to soils (Shahzad *et al.*, 2015). Second, higher SRL, lower RTD, and higher root N indicate lower root lifespans (McCormack *et al.*, 2012; Eissenstat *et al.*, 2015) and thereby faster root turnover in mixtures compared to pure stands. Although fine-root turnover was not measured here, other studies have observed higher root mortality and faster fine-root turnover in mixtures than in mono-specific stands (Lei *et al.*, 2012a; Jacob *et al.*, 2014; Ma & Chen, 2018). Faster root turnover translates into greater input of root litter into soils, representing another essential soil C source (Shahzad *et al.*, 2015). Third, the increased EcM colonisation rates in mixtures compared to mono-specific stands could indicate increased mycorrhizal exudation and increased hyphal necromass, contributing to SOM formation (Frey, 2019).

- **Stabilisation of SOC**

The changes in root morphology and chemical composition, as well as their associations with mycorrhizal symbionts in mixed compared to mono-specific stands (chapter three), could alter not only total C input rates but also affect SOM stabilisation mechanisms and consequently the residence time of C in soils (Six *et al.*, 2004; Poirier *et al.*, 2018; Adamczyk *et al.*, 2019b).

A higher RLD (chapter two) may enhance macro- and microaggregation through several mechanisms such as the enmeshment of soil particles and the production of root exudates serving as binding agents for soil aggregates (Baumert *et al.*, 2018; Poirier *et al.*, 2018). Moreover, a higher RLD may enhance the stability of these soil aggregates (Gould *et al.*, 2016). Similarly, mycorrhizal fungi play an essential role in microaggregate formation by binding particles together via hyphae (Rillig *et al.*, 2015) and by excreting extracellular mucilage rich in polysaccharides which act as gluing agents for microaggregates (Bossuyt *et al.*, 2006). Consequently, the higher RLD and increased EcM colonisation rate in mixed compared to mono-specific stands (chapters two and three) could increase the proportion of physically protected SOM/SOC (von Lützow *et al.*, 2006).

Root and mycorrhizal exudates mainly represent labile C sources (Schmidt *et al.*, 2011), likely stimulating microbial activity (Kuzyakov, 2010; Phillips *et al.*, 2011). As microbes may be the dominant force of SOM formation and stabilisation (Cotrufo *et al.*, 2013), a higher exudation rate associated with increased SRL (Tückmantel *et al.*, 2017; Meier *et al.*, 2020) could also enhance the residence time of SOC in mixtures compared to pure stands via microbial paths.

- **Fine-root decomposition rates**

In addition to possibly higher overall root- and mycorrhizae-derived C inputs to soil and possibly enhanced SOC stabilisation in mixtures compared to mono-specific stands, the slightly higher decomposition rates of fine-root litter in mixtures (chapter four) could have further consequences for the formation of SOM/SOC and thereby soil C sequestration. However, the conclusions that can be drawn here based on my results are rather limited, particularly because the measurements captured only a relatively short, early phase of the overall decomposition process (as discussed in section 5.2.3.2.). Nevertheless, my findings could provide some hints as to how decomposition dynamics in mixtures could affect soil C sequestration.

A faster early-stage microbial decomposition in mixtures compared to mono-specific stands, as indicated by the higher decomposition rates (chapter four), could, on the one hand, indicate an accelerated loss of C in the form of CO<sub>2</sub> to the atmosphere as a result of increased microbial

respiration. On the other hand, it could also indicate faster leaching of dissolved organic C (DOC) into the soil, potentially stabilised via the DOC-microbial path (Cotrufo *et al.*, 2015). This process has been proposed as the primary SOM formation process during the early-stage decomposition phase (Cotrufo *et al.*, 2015) and, in particular, may enhance long-term SOM stabilisation (Poirier *et al.*, 2018).

The changes in root quality (chapter three) in mixtures compared to mono-specific stands could significantly alter fine-root decomposition rates in mixtures. Thinner roots in mixtures (chapter three) could decompose faster than thicker roots in mono-specific stands in the long run (Hobbie *et al.*, 2010; Zhuang *et al.*, 2018), and a lower RTD could also accelerate decomposition. Hence, in addition to the findings in chapter four, i.e., the positive effect of tree species mixing on fine-root litter decomposition rates, the results of chapters two and three suggest that the quality of dead root material differs between mixed and mono-specific stands and may further accelerate decomposition rates in mixed-species forests. Conversely, increased initial root N and a higher EcM colonisation intensity in mixtures compared to mono-specific stands (chapter three) could affect fine-root litter decomposition rates in both positive and negative directions. Two meta-analyses showed that initial root N can be positively related to fine-root decomposition rates (Zhang & Wang, 2015; See *et al.*, 2019), yet the opposite has also been observed (Yang *et al.*, 2020). Likewise, higher EcM colonisation rates could either slow (Langley *et al.*, 2006) or increase decomposition rates (Koide *et al.*, 2011). While my results only allow for speculations here, a recent review pointed out that a high root N concentration may accelerated root litter decomposition and eventually promote long-term SOM stabilisation (Poirier *et al.*, 2018).

### **6.1.3. Context-dependency and phylogenetic identity effects**

The magnitude of tree diversity effects on ecosystem functioning may depend – among other factors - on the environmental context (Paquette & Messier, 2011; Grossiord *et al.*, 2014b; Jucker *et al.*, 2016; Ratcliffe *et al.*, 2017; Peng & Chen, 2020), but also the species composition (Ratcliffe *et al.*, 2015; Dawud *et al.*, 2016, 2017; Tobner *et al.*, 2016; Joly *et al.*, 2017; De Wandeler *et al.*, 2018). While the main focus of this thesis was to assess overall diversity effects across different environmental conditions and species compositions, the results of this thesis show that phylogenetic identity and site conditions can influence the magnitude of the below-ground responses to mixing, similar to observations made above-ground. As discussed before, species identity effects and the role of site conditions cannot be entirely disentangled in this work. Site conditions are essential because resource limitation may be crucial in determining

whether species interactions result in positive tree diversity effects on ecosystem functioning (Forrester & Bauhus, 2016). Regarding soil resource availability, the occurrence of gymnosperms and angiosperms at our sites is likely confounded with the nutrient status of the soil because gymnosperms have probably been preferentially cultivated at more nutrient-poor sites. Nevertheless, the results in chapters two and three support ample evidence from other studies that thin-rooted angiosperms and thick-rooted gymnosperms can generally be associated with distinct soil resource acquisition strategies (Ma *et al.*, 2018) governing their responses to tree species mixing (Valverde-Barrantes *et al.*, 2015, 2017). The results in chapter three further indicate that these inherently different strategies were linked to the prevalence of different underlying mechanisms driving tree species mixing effects across the study sites.

Boreal gymnosperm tree species (followed by temperate gymnosperm species) are characterised by the *slowest* traits of the plant economic trait spectrum and thereby the least flexible nutrient acquisition strategies (Netherway *et al.*, 2021). They tend to follow an *outsourcing* strategy, which is reflected in a high dependence on EcM (Bergmann *et al.*, 2020). Hence, mixtures comprising these gymnosperms could particularly benefit from positive biotic feedback from EcM, which improve the overall nutrient availability of growth-limiting soil resources such as N in these systems (Gill & Finzi, 2016; Högberg *et al.*, 2017), as EcM are capable of accessing various sources of nutrients (Phillips *et al.*, 2013) including organic N forms (Courty *et al.*, 2010). Consequently, neighbouring tree species may benefit from improved N availability, possibly as a result of shared mycorrhizal networks (Molina & Horton, 2015). Yet, the low-quality litter of gymnosperm trees could counteract this benefit by generally slowing down nutrient cycling rates (Vesterdal *et al.*, 2008; Augusto *et al.*, 2015).

On the other side, deciduous and evergreen angiosperm trees are, in comparison to boreal gymnosperm trees, relatively flexible in their nutrient acquisition (Netherway *et al.*, 2021). They tend to follow a *do-it-yourself* strategy to obtain soil resources with a lower dependence on mycorrhizae (Bergmann *et al.*, 2020) compared to gymnosperms. Hence, morphological and physiological fine-root trait adaptations enhancing resource foraging and likely increasing soil exploitation efficiency could improve the acquisition of possibly more heterogeneously (in space and time) available resources in mixtures comprised of angiosperms.

Interestingly, these patterns could also explain the contrasting observations in relation to tree diversity effects on above-ground productivity (Jucker *et al.*, 2014) compared to below-ground fine-root properties (chapters two and three) across the FunDivEUROPE/SoilForEUROPE sites. As such, the most substantial effects of tree diversity on above-ground productivity were

reported for gymnosperm-dominated boreal forests (Finland) and angiosperm-dominated thermophilous deciduous forests (Italy) (Jucker *et al.*, 2014). In contrast, mixing effects on fine-root traits were weakest in boreal forests and strongest in thermophilous deciduous forests (chapters two and three). These observations imply that the underlying mechanisms of these below-ground responses may differ, including the prevalence of positive biotic feedback from mycorrhizae in boreal forests mainly composed of gymnosperms and a possibly more efficient soil resource exploitation by fine roots themselves in thermophilous deciduous forests composed of angiosperms.

This distinct below-ground functioning of angiosperms and gymnosperms in tree species mixtures could also influence tree diversity effects on other ecosystem functions. As such, these different modes of functioning could also play a role in the observed effects of mixture conifer proportion on soil C stocks, C/N ratios, and soil pH (Dawud *et al.*, 2017), above-ground litter decomposition (Joly *et al.*, 2017), and biomass and functional composition of earthworms communities (De Wandeler *et al.*, 2018) across the FunDivEUROPE exploratory plot network.

## **6.2. Some implications for forest management**

This thesis provides evidence that tree fine roots may play an essential role in positive tree diversity effects on ecosystem functioning, thereby serving as a crucial foundation for the many ecosystem functions provided by healthy forests. Hence, my results are in general agreement with the close-to-nature silvicultural strategies currently applied in Europe (Brang *et al.*, 2014).

Tree diversity effects on fine-root soil exploitation and decomposition were observed in tree neighbourhoods consisting of three tree individuals. Consequently, if these below-ground patterns can indeed be linked to an enhanced provision of ecosystem functions such as productivity and soil C sequestration (as discussed before), these findings would support the promotion of mixed-species patches at small spatial scales, i.e., the scale of tree neighbourhoods, where below-ground interactions among different tree species can occur.

Forest managers are often primarily interested in the utilisation of wood as (high-quality) timber. While below-ground interactions may positively affect above-ground (biomass) productivity, concerning forest management, the impact of mixing on wood quality in mixtures, therefore, needs to be further investigated (but see Pretzsch & Rais, 2016; Benneter *et al.*, 2018).

- **Mixing species under consideration of site conditions**

Depending on the overall forest management objectives, it is generally advisable to select tree species for mixtures with particular traits that maximise the potential for positive species interactions. Both above- and below-ground components are relevant here, which significantly increases the complexity accordingly. For instance, when aiming for greater above-ground productivity, mixing species that generally improve soil resource uptake, e.g., through below-ground resource partitioning or facilitation, can be beneficial. This example also shows the importance of considering site conditions because these below-ground interactions will only play a role if they enhance the uptake of the growth-limiting resources (Forrester, 2014; Forrester & Bauhus, 2016). For instance, on nutrient-poor sites, where water is not a limiting factor, mixtures improving water uptake through hydraulic redistribution are unlikely to enhance above-ground productivity unless they simultaneously enhance nutrient uptake. Here, the type of nutrients that are limiting need to be considered as well. As such, positive biotic feedbacks from mycorrhizae may be more advantageous when they can access these limiting nutrients.

- **A greater functional root trait diversity is not necessarily beneficial**

The results of this thesis suggest that higher diversity in functional fine-root traits does not necessarily result in a greater positive net effect and hence is not a good selection criterion by itself (Bauhus *et al.*, 2017c). The analysis in chapter three showed that gymnosperm-dominated mixtures were characterised by the highest functional fine-root trait diversity but at the same time displayed the weakest response in root trait adaptations, likely benefitting more from positive biotic feedbacks from EcM. In contrast, the lowest root trait dissimilarity was associated with pure angiosperm mixtures, which also showed the greatest response in root trait adaptations to tree species mixing (chapter three).

- **Uncertainties in light of climate change**

Predicted changes associated with global warming including increased biotic and abiotic stressors and disturbances such as windstorms, fire, pathogen outbreaks, and droughts (Bréda *et al.*, 2006; Blenkinsop & Fowler, 2007; Jactel *et al.*, 2012; Millar & Stephenson, 2015), could significantly affect above- and below-ground species interactions and thus the mechanisms of how mixtures promote ecosystem functioning. In the face of these changes, forest management efforts are increasingly focused on mitigation and adaptation strategies to maintain the provision of ecosystem goods and services (Puettmann, 2011). In this regard, one crucial aspect concerns

how trees will cope with these predicted changes and whether they can adapt and thereby maintain their functional capacity.

From a below-ground perspective, the generally lower flexibility in resource acquisition strategies of EcM compared to arbuscular mycorrhizal (AM) trees suggests that the consequences of climate change could generally have adverse effects on EcM-dominated forests (Netherway *et al.*, 2021). This may include an overall lower performance of EcM-associated tree species (Fernandez *et al.*, 2017; DeForest & Snell, 2020) and lower importance of EcM (Kilpeläinen *et al.*, 2017; Boeraeve *et al.*, 2019). Hence, positive biotic feedback from EcM may become less relevant in future. In view of the predicted increases in pest outbreaks and pathogen attacks (Jactel *et al.*, 2012), positive biotic feedbacks resulting from mixing species with different enemies (pests and pathogens) could be essential. Moreover, given the likely increase in the occurrence of drought events (Blenkinsop & Fowler, 2007), complementarity in water uptake (Grossiord *et al.*, 2014a), and hydraulic redistribution (Neumann & Cardon, 2012) could generally become pivotal mechanisms in mixtures.

- **Below-ground strategies of gymnosperm vs. angiosperm trees**

Considering the different below-ground strategies and flexibility in regard to resource acquisition strategies of the gymnosperm and angiosperm tree species studied here, one could speculate that climate change may generally affect these phylogenetic groups differently. This could have consequences for overall tree species mixing effects.

Owing to their generally greater adaptability to fluctuations in resource supply (Ma *et al.*, 2018) and their lower dependence on collaboration with mycorrhizae (*do-it-yourself strategy*, Bergmann *et al.*, 2020), the angiosperms species considered in this study may have a greater overall adaptive capacity to climate change impacts than the gymnosperms (Sáenz-Romero *et al.*, 2019). It could be expected that the least flexible boreal EcM tree species will be particularly disadvantaged. This seems likely in view of the possibly declining relevance of EcM fungi (Kilpeläinen *et al.*, 2017; Boeraeve *et al.*, 2019), which may be a consequence of the shift towards less C-demanding, short-contact exploration types (Fernandez *et al.*, 2017). Such a shift could negatively affect trees under drought stress as long-distance exploration of mycorrhizal types can improve plant water uptake under drought (Lehto & Zwiazek, 2011). A recent study further suggested that the gymnosperm *P. abies* may heavily rely on mycorrhizal associations under drought conditions, in contrast to the angiosperm *F. sylvatica* (Nikolova *et al.*, 2020). If this is also the case for other gymnosperm species, this observation could be another indicator of the disadvantage of *outsourcing* gymnosperms under drought conditions. Yet, a *fast* strategy

in response to drought, for instance, by enhancing specific root area and root shedding (Nikolova *et al.*, 2020), could be deadly (C starvation) for angiosperm tree species in case the increased C costs required for the regrowth of roots after drought events cannot be covered (Brunner *et al.*, 2015; Nikolova *et al.*, 2020). Moreover, the fast economic strategy of thin-rooted species could potentially be associated with greater susceptibility to insects and pathogens (Brodribb *et al.*, 2012). On the other side, as mycorrhizal fungi also play an essential role in protecting their hosts from pathogens (Lambers *et al.*, 2017), the declining importance of EcM could also increase the susceptibility of gymnosperms to pathogens.

These speculations show that there is still a great demand for studies that further improve our mechanistic understanding of diversity effects on the below-ground and overall functioning of forests, in particular given the substantial uncertainties surrounding the impacts of global change on forest ecosystems. The contrasting below-ground strategies of the studied gymnosperms and angiosperm species could generally be regarded as an overall risk-spreading strategy in view of these significant uncertainties.



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## Appendix

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## General supplementary material

**Table S1.1** Photos of the four sites including mono-specific and mixed stands.

	Mono-specific plot	Mixed plot
<b>Finland – boreal forest</b>		
<b>Poland – hemiboreal forest</b>		
<b>Romania – mountainous beech forest</b>		
<b>Italy – thermophilous deciduous forest</b>		



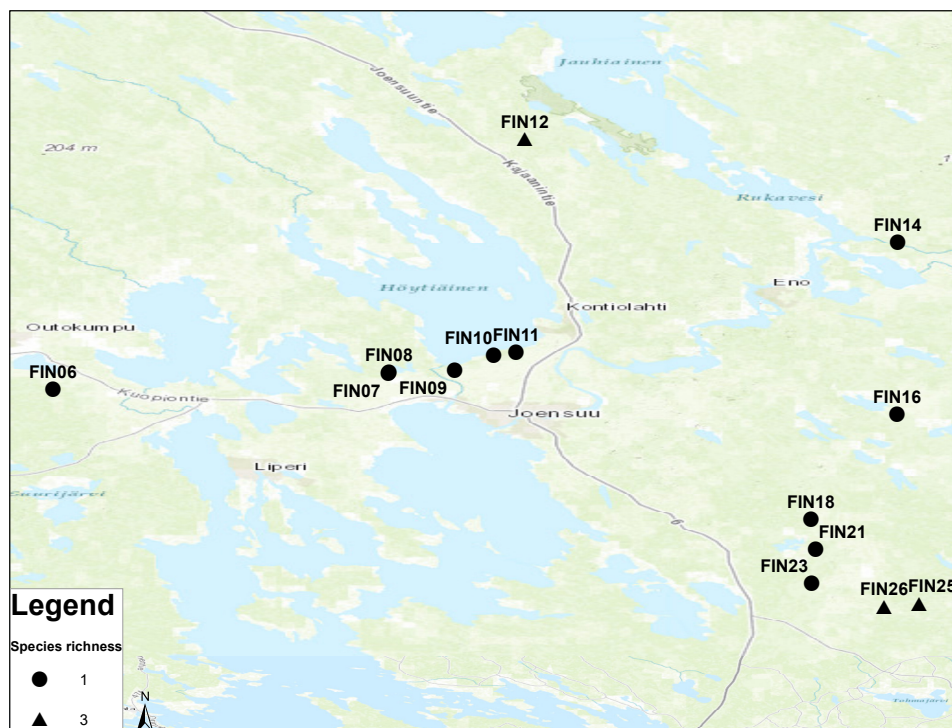


Figure S1.1 Study sites in North Karelia, Finland (boreal forests).

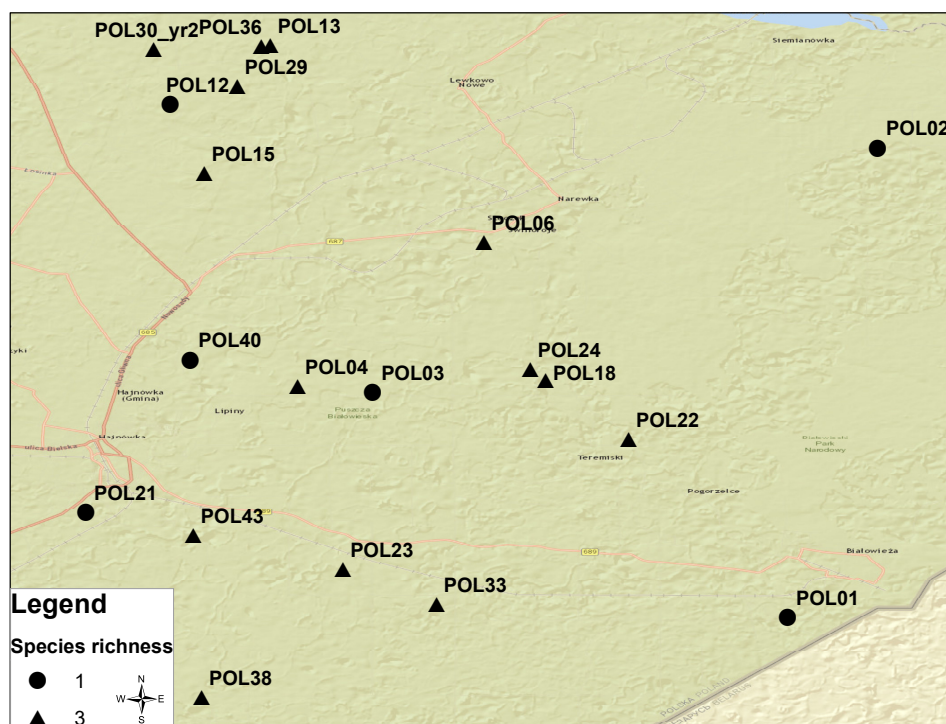
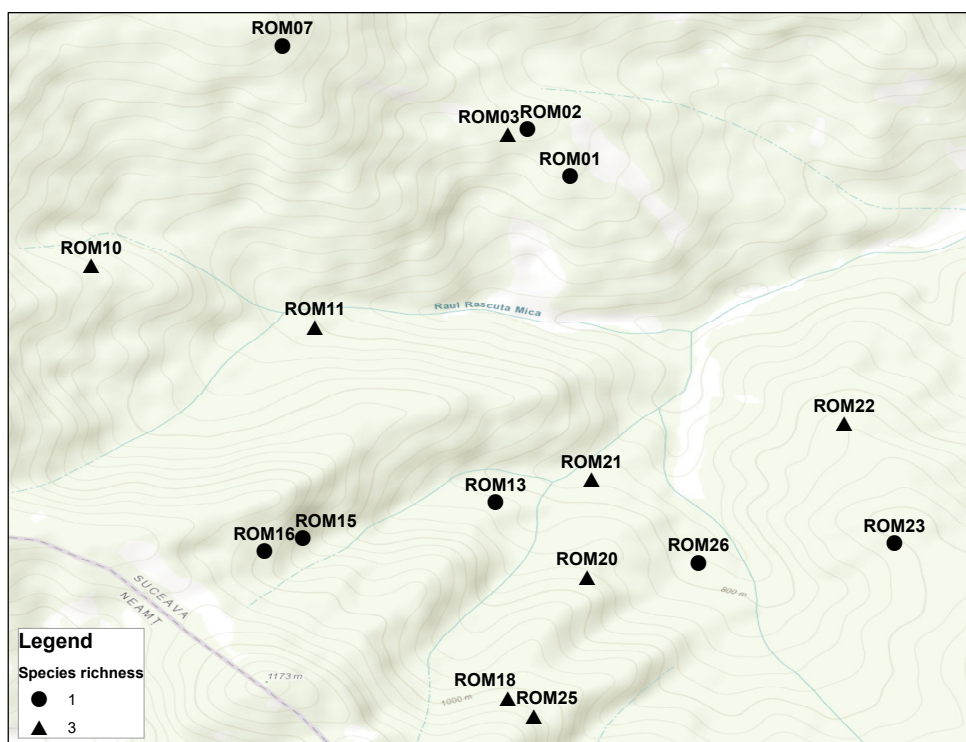
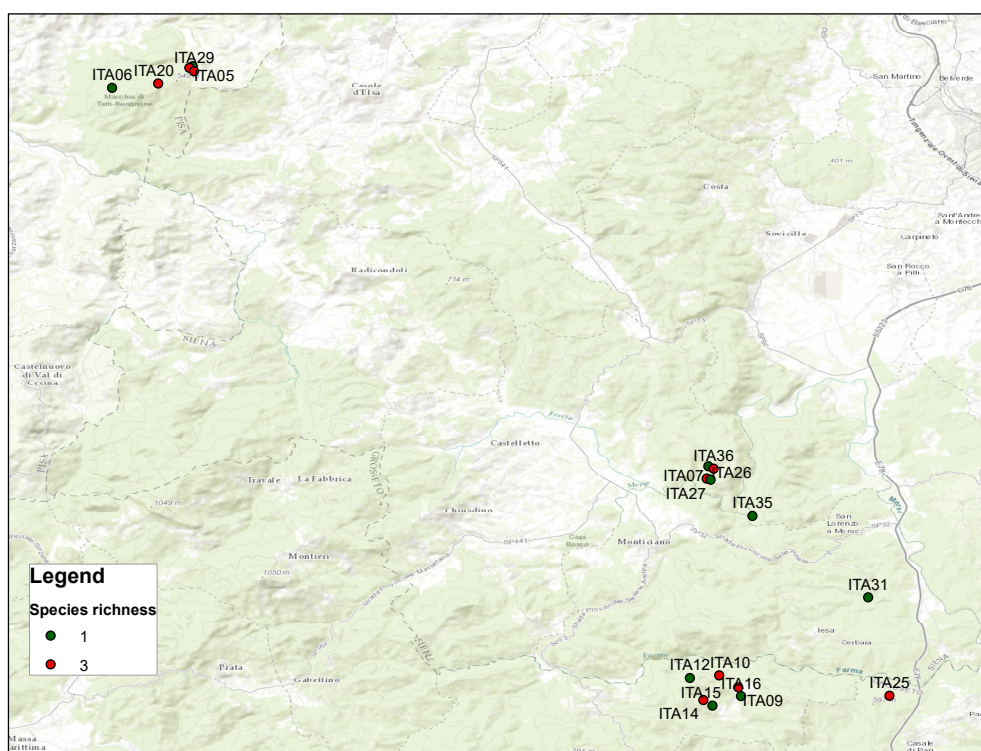


Figure S1.2 Study sites in Białowieża forest, Poland (hemiboreal forests).

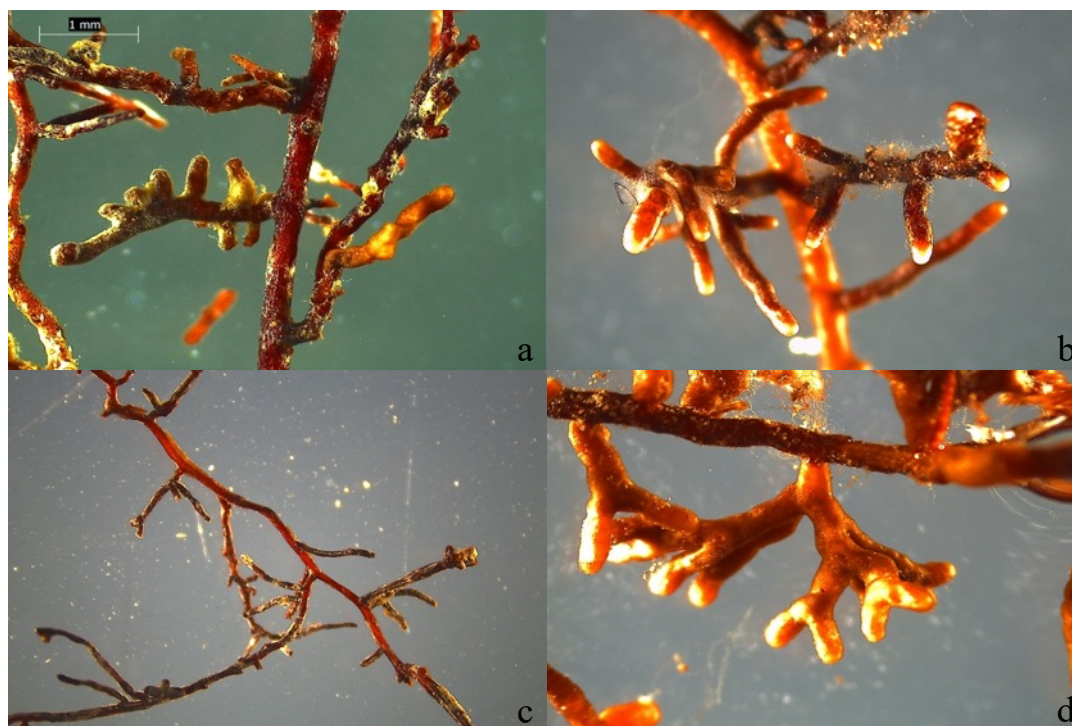


**Figure S1.3** Study sites in north-eastern Carpathian Mountains, Romania (mountainous beech forest).



**Figure S1.4** Study sites in the Tuscany, Italy (thermophilous deciduous forest).





**Figure S1.5** Examples of fine roots with root tips colonised by ectomycorrhizal fungi: (a) *Fagus sylvatica*, (b) *Picea abies*, (c) *Fagus sylvatica*, (d) *Pinus sylvestris*. Ectomycorrhizal species were not identified.

## Supplementary material for Chapter four

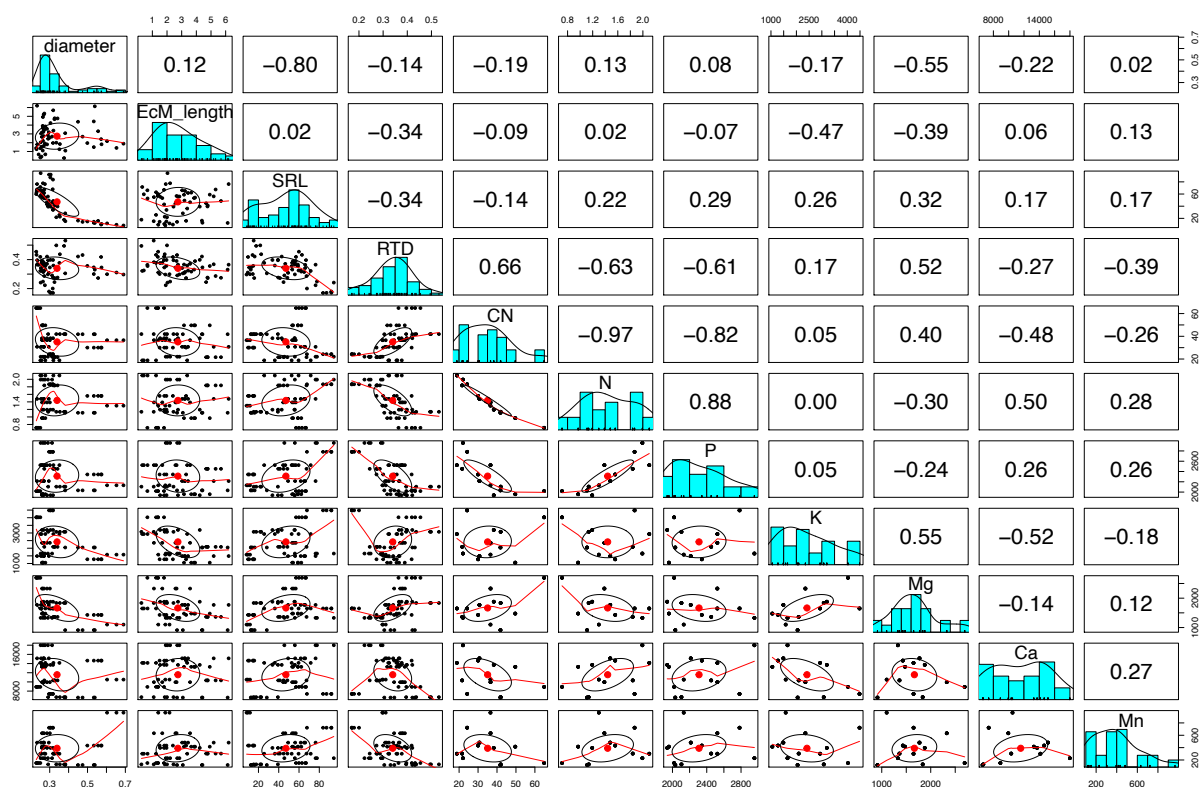
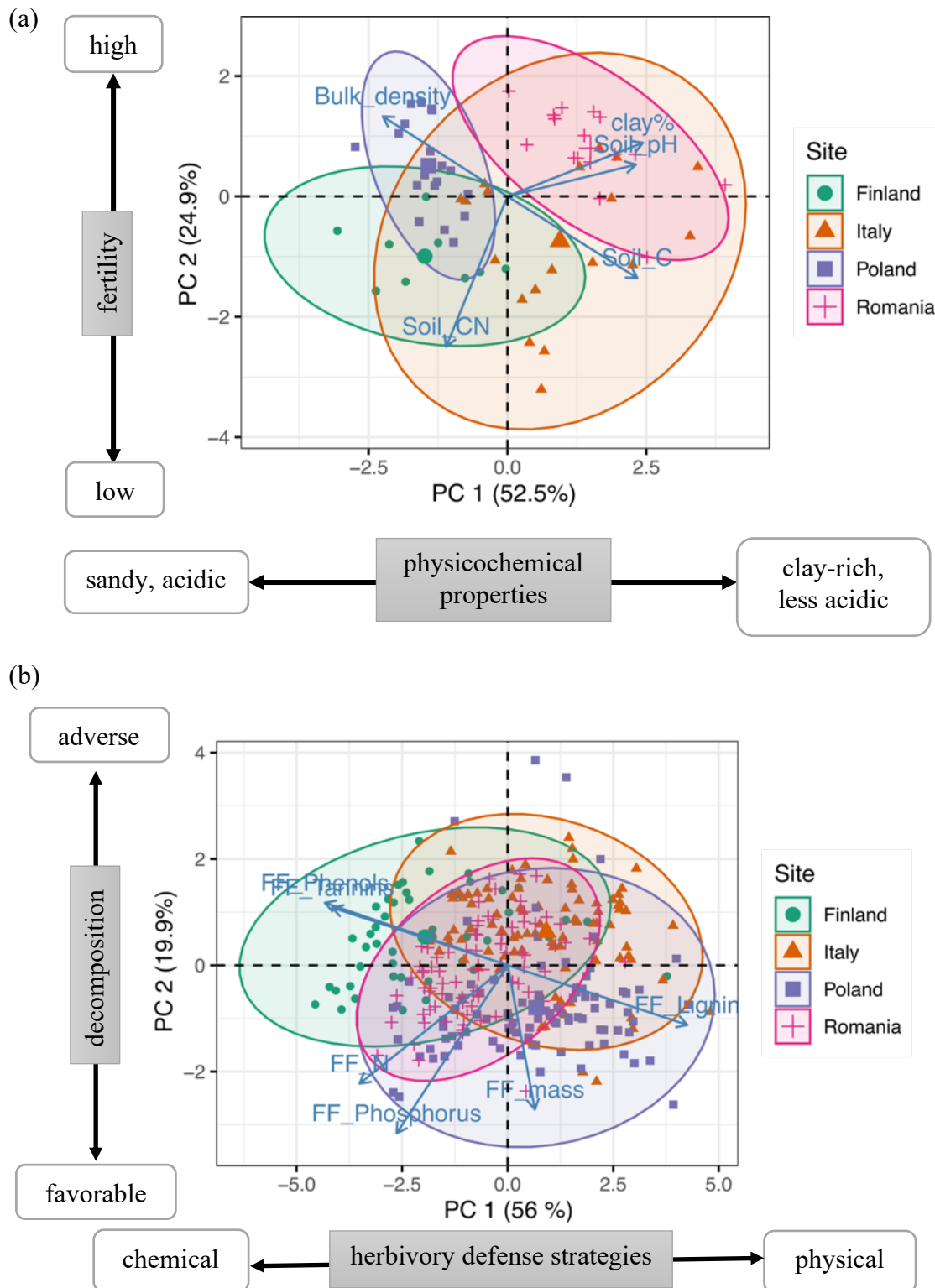
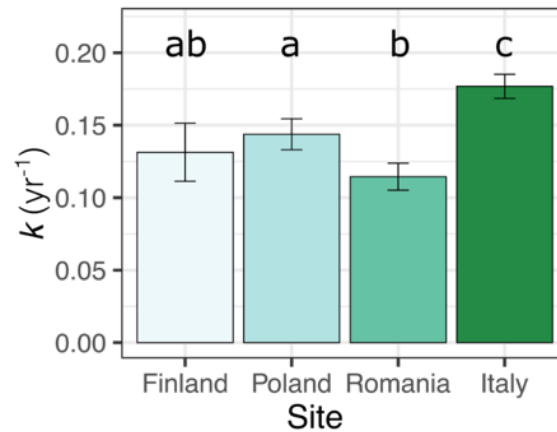


Figure S2.1 Correlation coefficient matrix of initial root traits using Spearman's rank correlation.

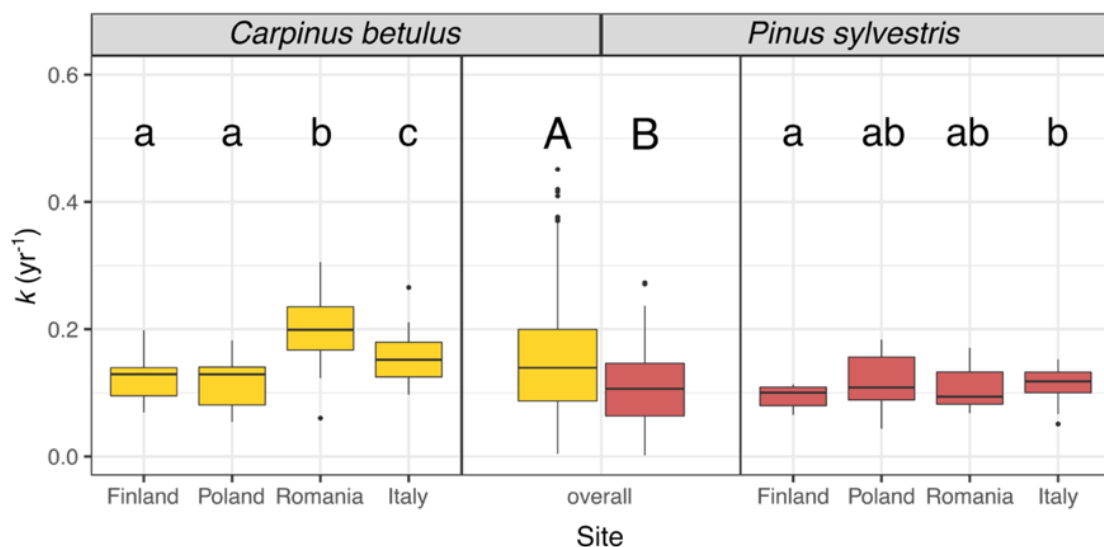


**Figure S2.2** (a) Biplot of the soil principal component analysis (PCA) including soil bulk density ( $\text{g cm}^{-3}$ ), soil pH, soil C/N, soil C concentration ( $\text{mg g}^{-1}$ ) and soil clay % with axis 1 representing a physicochemical gradient from sandy, acid soils (negative values, low clay %, low pH, low C concentrations, high BD) to clay-rich, denser and less acidic soils (positive values, high pH, high clay %, high soil C) and axis 2 representing a gradient of soil fertility from low (negative values, high C/N, low BD) to high (positive values, low C/N, high BD) soil fertility. (b) Biplot of the forest floor PCA including litter mass

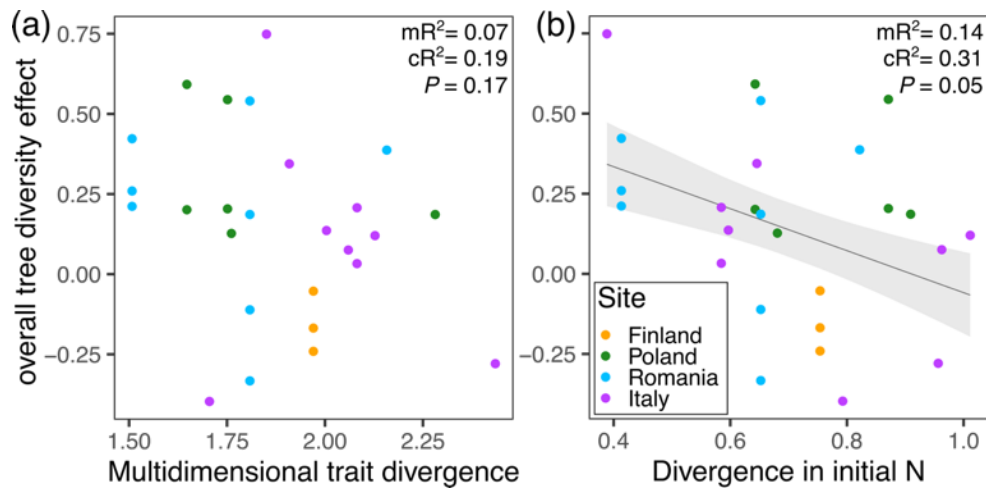
(kg m<sup>-2</sup>), Nitrogen (%), lignin concentration (g kg<sup>-1</sup> dry litter), C/N, phosphorus concentration, total phenolics content (mg g<sup>-1</sup> dry litter) and condensed tannin concentration (%), with axis 1 of the PCA representing a litter property gradient linked to leaf herbivory defence strategies, from the importance of more chemical (negative values) to more (structural) physical properties (positive values). Forest floor PC axis 2 represents a gradient from more favourable (negative values) to more adverse (positive values) conditions for the decomposition of organic matter.



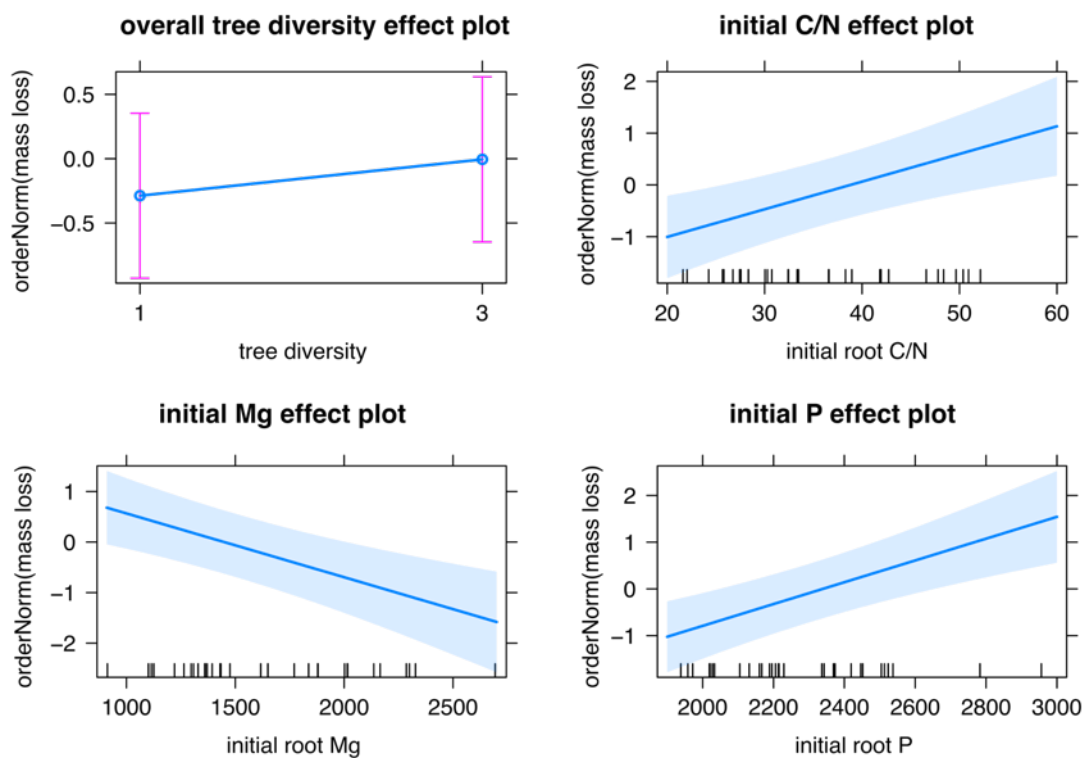
**Figure S2.3** Mean decomposition rates ( $\pm$ SE) of the site-specific litter at the four sites. Lower case letters indicate significant differences in decomposition rates among the sites.



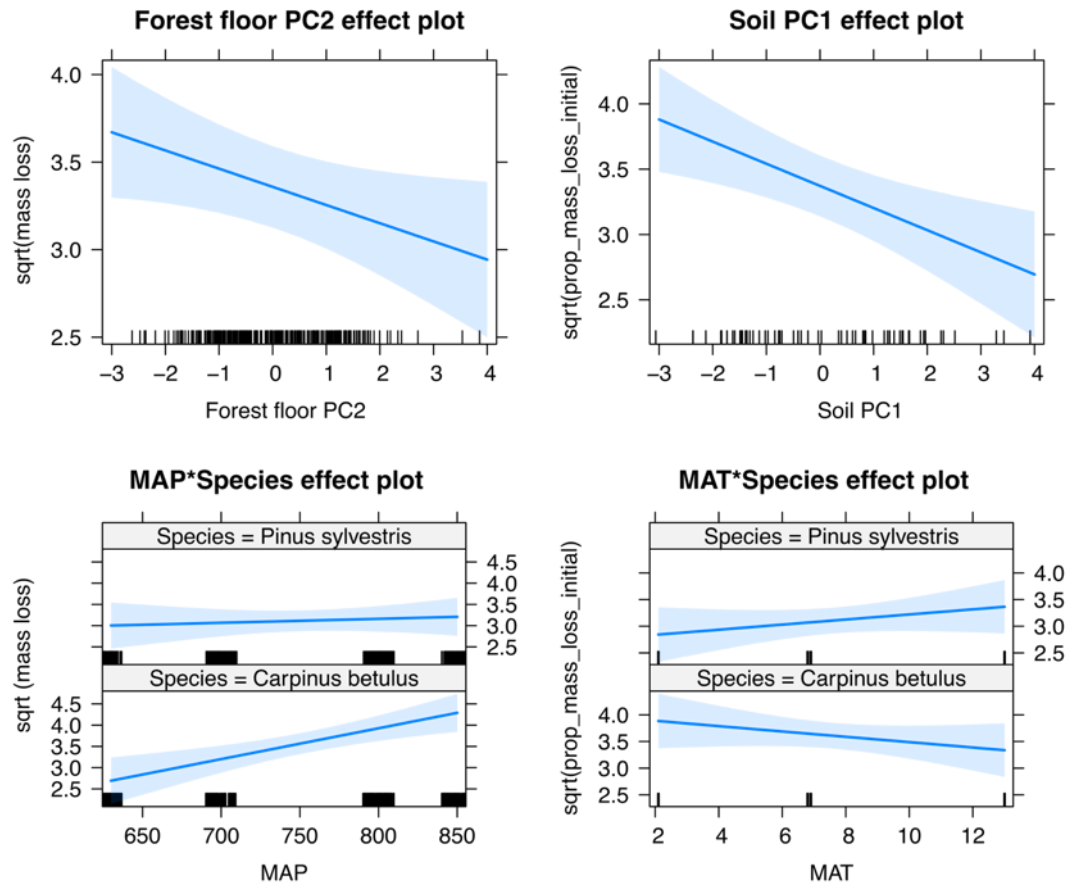
**Figure S2.4** Mean decomposition constant  $k$  ( $\pm$  SE) by site (left and right panel) and overall (central panel) for the two standard litter species, *Carpinus betulus*, and *Pinus sylvestris*. Lower case letters indicate significant differences among sites for each species separately. Capital letters indicate significant differences between the two species across all four sites.



**Fig. S2.5** Overall diversity effects in response to (a) multidimensional trait divergence including initial root tissue density (RTD), specific root length (SRL), EcM colonization intensity, Ca, K, N, Mg, Mn, and (b) divergence in initial N, across sites. Trait divergence was calculated as functional dispersion index (FDis). Marginal (m) and conditional (c)  $R^2$  were derived from linear mixed-effect models and describe the variation explained by fixed and combined fixed and random effects, respectively. The solid lines represent linear regression lines including 95% confidence intervals (grey shaded area).



**Figure S2.6** Effect plot showing the linear mixed-effect model results of the synthesis model for site-specific litter of Table 4.1.



**Figure S2.7** Effect plot showing the linear mixed-effect model results of the synthesis model for stand and litter of Table 4.1.



**Table S2.1.** Soil, climate, and forest stand characteristics of the four study sites.

Site	North Karelia	Białowieża	Râsca	Colline Metallifere
Country	Finland	Poland	Romania	Italy
Latitude/Longitude (°)	62.6/29.9	52.7/23.9	47.3/26.0	43.2/11.2
Elevation range (m)	80-200	135-185	600-1000	260-525
Mean annual temperature (°C)	2.1	6.9	6.8	13
Mean annual precipitation (mm)	700	627	800	850
Mean soil temperature (°C) during the incubation period	5.5±0.2	9.4±1.8	8.3±0.5	12.1±0.6
Mean air temperature (°C) during the incubation period	4.2±0.1	9.8±0.6	8.2±0.5	12.5±0.8
Mean soil moisture (%) during the incubation period	32.8±1.7	24.6±1.1	31.1±2.2	21.8±1.7
Mean soil temperature (°C) during the vegetation period of 2018	10.5±0.2	12.4±0.2	11.0±0.5	12.9±0.5
Mean air temperature (°C) during the vegetation period of 2018	13.2±0.1	14.6±0.2	13.0±0.4	13.7±0.7
Mean soil moisture (%) during the vegetation period of 2018	30.9±2.2	22.3±1.2	31.0±2.3	21.3±1.6
Forest type	Boreal	Hemiboreal	Mountainous mixed beech	Mediterranean thermophilous deciduous
Average stand age (y)	51	104	88	67
Target tree species	<i>Betula pendula</i> / <i>pubescens</i> , <i>Picea abies</i> , <i>Pinus sylvestris</i>	<i>Betula pendula</i> . <i>Carpinus betulus</i> , <i>Picea abies</i> , <i>Pinus sylvestris</i> ,	<i>Abies alba</i> , <i>Acer pseudoplatanus</i> , <i>Fagus sylvatica</i> , <i>Picea abies</i>	<i>Castanea sativa</i> , <i>Ostrya carpinifolia</i> , <i>Quercus cerris</i> , <i>Quercus ilex</i> ,

Soil type	<i>Quercus robur</i>			<i>Quercus petraea</i>
	Podzol	Cambisol/ Luvisol	Eutric Cambisol	Cambisol
Topsoil texture class	Sandy loam	Sandy loam	Silty clay loam	Silt loam
Topsoil clay %	5	6	27	18
Topsoil pH	3.9	3.8	4.6	4.6
Topsoil C concentration (mg g <sup>-1</sup> )	37.8	28.4	49.2	50.4
Topsoil N concentration (mg g <sup>-1</sup> )	1.7	1.7	3.5	2.6
Incubation start (yyyy/mm/dd)	2018/05/29	2018/04/15-16	2018/04/26-27	2018/02/10-11
Incubation end (yyyy/mm/dd)	2019/05/31	2019/04/08-09	2019/05/10-11	2019/02/10-11
Total incubation period (days)	367	358	379	365
Number of mono-specific stands/mixed stands	6/3	6/11	8/8	10/9

**Table 2.2.** Initial mean fine-root traits ( $\pm$ SE) of all tree species, i.e., site-specific and standard litter species (\*), used in this study.

Species	Root diameter cm	Specific root length (SRL) m g <sup>-1</sup>	Root tissue density (RTD) g cm <sup>-3</sup>	Tip density n cm <sup>-1</sup>	Ectomy-corrhizal colonization rate %	Ectomy-corrhizal colonization intensity n cm <sup>-1</sup>	Root N %	Root C/N %	Root C %	Root Ca %	Root K %	Root P %	Root Mg %	Root Mn %
<i>Abies alba</i>	0.55±0.01	12.7±1.16	0.35±0.03	2.2±0.31	100±0	2.2±0.31	1.56	30.0	46.8	14.6	1.28	2.34	1.43	0.43
<i>Acer pseudo-platanus</i>	0.31±0.00	76.2±4.01	0.2±0.01	3±0.13	NA	NA	1.87	24.3	45.3	7.39	4.48	2.78	1.65	0.72
<i>Betula pendula</i>	0.28±0.01	60.7±7.47	0.3±0.02	2.51±0.19	93±1	2.34±1.04	1.99	22.0	43.9	18.0	1.65	2.96	1.33	0.23
<i>Carpinus betulus</i> *	0.29±0.02	41.4±6.91	0.41±0.01	3.34±0.22	34±15	1.21±15.43	2.1	18.8	39.6	14.1	2.93	2.52	1.65	0.3
<i>Castanea sativa</i>	0.27±0.00	51.1±1.47	0.4±0.01	4.59±0.29	85±1	3.90±1.22	0.97	49.7	48.1	13.3	1.05	1.96	1.48	0.42
<i>Fagus sylvatica</i>	0.26±0.01	58.6±3.54	0.36±0.01	4.18±0.55	97±1	4.05±0.81	1.48	30.7	45.5	15.1	1.49	2.21	1.77	0.48
<i>Ostrya carpinifolia</i>	0.29±0.01	40.7±3.95	0.44±0.01	3.05±0.69	82±2	2.46±2.40	1.19	38.3	45.7	13.7	3.2	1.94	1.88	0.4
<i>Picea abies</i>	0.65±0.03	9.47±0.96	0.31±0.00	1.82±0.22	100±0	1.82±0.22	1.3	36.7	47.5	10.5	1.58	2.13	1.1	0.97
<i>Pinus sylvestris</i> *	0.48±0.03	16.2±2.84	0.38±0.03	4.18±0.6	99±1	4.14±0.60	1.11	41.8	46.5	6.59	2.05	2.03	0.91	0.12
<i>Quercus cerris</i>	0.35±0.02	22.2±3.97	0.5±0.03	2.14±0.00	85±0	1.53±0.39	1.14	42.0	47.7	6.68	3.08	2.1	1.84	0.13
<i>Quercus ilex</i>	0.24±0.00	58.7±1.75	0.44±0.02	5.9±0.53	22±3	1.30±3.45	0.71	64.9	46.1	8.91	4.03	2.02	2.69	0.15
<i>Quercus petraea</i>	0.28±0.01	49.6±2.35	0.39±0.01	2.45±0.4	93±3	2.32±3.37	1.39	36.6	50.7	9.95	2.35	2.52	2.33	0.63
<i>Quercus robur</i>	0.32±0.01	40.2±4.19	0.33±0.01	5.69±0.28	89±4	5.04±4.09	1.84	21.6	39.7	10.3	2.09	2.45	1.36	0.3

**Table S2.3.** Linear mixed-effect model results for the site-specific root litter testing the influence of initial root traits on mass loss. Model selection and averaging were applied. Shaded rows (grey) indicate dropped variables after model selection. Colors were included for estimate values (*Estimate*) only when the p-value (*P*) was significant (i.e.,  $< 0.05$ ); green indicates positive slopes. Marginal and conditional  $R^2$  indicate variation described by fixed as well as fixed and random effects, respectively. Plot and site were included as random effects in the model. Grey-shaded cells indicate variables that were dropped after model selection and averaging. The response variable was transformed (yeojohnson) to comply with model assumptions.

Response	mass loss (site-specific litter)		
Predictors	<i>Estimate</i>	<i>t-value</i>	<i>P</i>
Root C/N	0.63	3.75	<b>&lt;0.001</b>
Root Ca			
Root P	0.67	4.60	<b>&lt;0.001</b>
Root Mn			
Root K			
Root Mg	-0.61	-3.85	<b>&lt;0.001</b>
Ectomycorrhizal colonization intensity			
mR <sup>2</sup>		0.20	
cR <sup>2</sup>		0.56	

**Table S2.4.** Linear mixed-effect model results for the standard root litter testing the influence of soil and forest floor principal components on mass loss. Model selection and averaging were applied. Shaded rows (grey) indicate dropped variables after model selection. Colors were included for estimate values (*Estimate*) only when the p-value (*P*) was significant (i.e.  $< 0.05$ ); red indicates negative slopes, and green indicates positive slopes. Marginal and conditional  $R^2$  indicate variation described by fixed as well as fixed and random effects, respectively. Triplet, plot, and site were included as random effects in the model. Grey-shaded cells indicate variables that were dropped after model selection and averaging. The response variable was transformed (square root) to comply with model assumptions.

Response	mass loss (standard litter)		
Predictors	<i>Estimate</i>	<i>t-value</i>	<i>P</i>
Species	-0.48	-6.59	<b>&lt;0.001</b>
Soil PC1	-0.17	-1.86	<b>0.07</b>
Soil PC2	-0.06	-0.81	0.42
Forest floor PC1			
Forest floor PC2	0.07	0.95	0.35
Species * Soil PC1	-0.20	-2.66	<b>&lt;0.01</b>
Species * Soil PC2	0.19	2.33	<b>0.02</b>
Species * Forest floor PC2	-0.29	-3.57	<b>&lt;0.001</b>
mR <sup>2</sup>		0.15	
cR <sup>2</sup>		0.43	

**Table S2.5.** Linear mixed-effect model results for the standard root litter testing the influence of microclimate on mass loss. Colors were included for estimate values (*Estimate*) only when the p-value (*P*) was significant (i.e.  $< 0.05$ ); red indicates negative slopes. Marginal and conditional  $R^2$  indicate variation described by fixed as well as fixed and random effects, respectively. Triplet, plot and site were included as random effects in the model. The response variable was transformed (boxcox) to comply with model assumptions.

Response	mass loss (standard litter)		
Predictors	<i>Estimate</i>	<i>t-value</i>	<i>P</i>
Species	-0.51	-6.63	<0.001
Mean annual soil temperature (°C)	0.01	-0.73	0.94
Mean annual soil moisture content (%)	0.12	1.51	0.13
Species * Mean annual soil temperature	0.01	0.07	0.95
Species * Mean annual soil moisture	-0.13	-1.47	0.14
mR <sup>2</sup>		0.07	
cR <sup>2</sup>		0.21	

**Table S2.6.** Linear mixed-effect model results for the standard root litter testing the influence of macroclimate on mass loss. Colors were included for estimate values (*Estimate*) only when the p-value (*P*) was significant (i.e. <0.05); red indicates negative slopes. Marginal and conditional  $R^2$  indicate variation described by fixed as well as fixed and random effects, respectively. Triplet, plot and site were included as random effects in the model. Abbreviations: Mean annual temperature (MAT), mean annual precipitation (MAP). The response variable was transformed (square root) to comply with model assumptions.

Response	mass loss (standard litter)		
Predictors	<i>Estimate</i>	<i>t-value</i>	<i>P</i>
Species	3.27	4.82	<0.001
MAT	-0.05	-1.66	0.15
MAP	0.01	3.46	0.02
Species * MAT	0.10	3.91	<0.001
Species * MAP	-0.01	-5.74	<0.001
mR <sup>2</sup>		0.13	
cR <sup>2</sup>		0.29	

**Table S2.7.** Linear mixed-effect model (LMM) testing the influence of functional trait diversity (FDis) of initial root N on overall diversity effects. The response variable was transformed (arcsinh) to comply with model assumptions. Marginal and conditional  $R^2$  indicate variation described by fixed as well as fixed and random effects, respectively. Site was included as random effect. Grey-shaded cells indicate variables that were dropped after model selection and averaging.

Response	overall diversity effects		
Predictor	<i>Esti- mate</i>	<i>t- value</i>	<i>P</i>
FDis N	-0.12	-2.05	0.05
mR <sup>2</sup>		0.14	
cR <sup>2</sup>		0.31	

**Table S2.8.** Linear mixed-effect model results testing the influence of functional dispersion (FDis) of eight root traits including (a) root tissue density (RTD), specific root length (SRL), EcM colonization intensity, Ca, K, N, Mn, Mg, and (b) specific root length (SRL), EcM colonization intensity, Ca, K, C/N, Mn, Mg, P on overall diversity effects on site-specific litter. Marginal and conditional  $R^2$  indicate variation described by fixed as well as fixed and random effects, respectively. The response variables were transformed (arcsinh) to comply with model assumptions.

Response	overall diversity effects		
Predictor	<i>Esti- mate</i>	<i>t-value</i>	<i>P</i>
FDis (RTD, SRL, EcM, Ca, K, N, Mn, Mg)	-0.08	-1.43	0.17
mR <sup>2</sup>		0.07	
cR <sup>2</sup>		0.19	
FDis (SRL, EcM, C/N, Ca, K, Mn, Mg, P)	-0.10	-1.4	0.10
mR <sup>2</sup>		0.11	
cR <sup>2</sup>		0.18	



**Table S2.9.** Relationship of microclimate variables (air temperature, soil temperature, soil moisture) with tree species richness (mono-specific vs. mixed) across all sites, tested with linear mixed-effects models. Plot and site were included as random effects in the models. Colors were included for estimate values (*Estimate*) only when the p-value (*P*) was significant (i.e.  $< 0.05$ ); red shades indicate negative slopes. Marginal and conditional  $R^2$  indicate variation described by fixed as well as fixed and random effects, respectively.

Response	Transformation	Predictor	Estimate	t-value	<i>P</i>	mR <sup>2</sup>	cR <sup>2</sup>
Mean air temperature during the vegetation period of 2018	orderNorm	tree species richness	-0.54	-2.48	<b>0.02</b>	0.08	0.30
Mean soil temperature during the vegetation period of 2018	orderNorm	tree species richness	-0.37	-1.74	<b>0.09</b>	0.04	0.32
Mean moisture content during the vegetation period of 2018	sqrt	tree species richness	0.12	0.66	0.51	<0.01	0.27
Mean annual soil temperature of the incubation period	orderNorm	tree species richness	-0.26	-1.74	<b>0.09</b>	0.02	0.72
Mean annual air temperature of the incubation period	orderNorm	tree species richness	-0.26	-1.89	<b>0.07</b>	0.02	0.74
Mean annual soil moisture content of the incubation period	sqrt	tree species richness	0.14	0.82	0.42	<0.01	0.29