



**HAL**  
open science

# Tree diversity effects on root production, decomposition and nutrient cycling under global change.

Andreas Altinalmazis Kondylis

## ► To cite this version:

Andreas Altinalmazis Kondylis. Tree diversity effects on root production, decomposition and nutrient cycling under global change.. Ecosystems. Université de Bordeaux, 2021. English. NNT : 2021BORD0067 . tel-03214567

**HAL Id: tel-03214567**

**<https://theses.hal.science/tel-03214567>**

Submitted on 2 May 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

THÈSE PRÉSENTÉE  
POUR OBTENIR LE GRADE DE

**DOCTEUR DE  
L'UNIVERSITÉ DE BORDEAUX**

ÉCOLE DOCTORALE SCIENCES ET ENVIRONNEMENTS  
Spécialité : Biogéochimie et écosystèmes

Par Andreas Altinmazis – Kondylis

**La biodiversité dans les écosystèmes forestiers est-elle susceptible de modifier les processus de production de racines et de décomposition des litières dans un contexte de changement climatique ?**

***Tree diversity effects on root production, decomposition and nutrient cycling under global change***

Sous la direction de : Mark R. Bakker  
Co-directeur de thèse: Stephan Hättenschwiler

Soutenue le 11 Mars 2021

**Membres du jury :**

Mme. Ivika OSTONEN	Professeur, University of Tartu (Estonia)	Rapporteur
Mme. Catherine ROUMET	Chargée de recherche (HDR), CNRS Montpellier	Rapporteur
M. Richard MICHALET	Professeur, Université de Bordeaux	Président
M. Antonino DI IORIO	Associate Professor, University of Insubria (Italy)	Examineur
M. Ivano BRUNNER	Chargé de recherche, WSL (Switzerland)	Examineur
M. Stephan HATTENSCHWILER	Directeur de recherche, CNRS Montpellier	Co-Directeur
M. Mark R. BAKKER	Ingénieur de Recherche (HDR), BSA-INRAE	Invité



# **Tree diversity effects on root production, decomposition and nutrient cycling under global change**

Andreas Altinmazis – Kondylis

2021

Université de Bordeaux



## Résumé

L'hypothèse de l'assurance prévoit que les forêts composées de mélanges d'espèces d'arbres pourraient mieux résister aux conditions environnementales stressantes que les forêts composées d'une seule espèce d'arbre. La majorité des travaux antérieurs ont testé cette hypothèse en se focalisant sur la productivité et les variables de réponse associées sans prendre en compte les processus souterrains. L'objectif principal de ma thèse était d'étudier l'effet de la diversité des espèces d'arbres sur les processus souterrains impliqués dans la décomposition des racines à travers des gradients climatiques. J'ai émis l'hypothèse que le mélange d'espèces ayant des systèmes racinaires contrastés entraînerait une faible compétition souterraine, et se traduirait par la production de plus biomasse de racines fines. En outre, j'ai émis l'hypothèse que les racines ayant des caractéristiques chimiques et morphologiques contrastées dans les peuplements mixtes se décomposent plus rapidement. Dans des conditions de stress hydrique, j'ai émis l'hypothèse d'une décomposition plus lente mais d'une atténuation des mélanges d'arbres sur la décomposition en raison de l'amélioration des conditions micro-environnementales. Pour tester ces hypothèses, j'ai examiné la variation des caractéristiques fonctionnelles des racines et leurs conséquences sur les flux de C, N et P à l'échelle de l'écosystème à travers l'étude de : 1) la ségrégation verticale des racines et la biomasse des racines fines, 2) la dynamique des racines fines et les flux de nutriments associés et 3) la décomposition des racines fines et des feuilles mortes. Dans ce cadre, deux expériences de terrain ont été réalisées, l'une avec une expérience de plantation d'arbres de 10 ans avec du bouleau et du pin près de Bordeaux (expérience ORPHEE), la seconde le long d'un gradient latitudinal de forêts de hêtres matures dans les Alpes françaises (expérience BIOPROFOR).

Les résultats obtenus montrent que les racines de bouleaux et de pins présentaient une distribution verticale similaire et une biomasse souterraine similaire de racines dans les mélanges d'arbres par rapport aux monocultures, contrairement à ma première hypothèse. Cependant, l'attribution plus importante du pin mais pas du bouleau à la croissance des racines dans les horizons du sol supérieur dans des conditions moins limitatives en eau suggère des conditions localement favorables qui peuvent conduire à une compétition asymétrique à la profondeur du sol. De plus, la production et la décomposition des racines fines étaient similaires dans les mélanges et dans les monocultures, en contradiction avec ma deuxième hypothèse. Il est intéressant de noter que les racines de bouleau, mais pas les racines de pin, ont libéré du P pendant leur décomposition, ce qui suggère un rôle important du bouleau dans le cycle du P et pour la nutrition en P des arbres sur ces sols sableux limités en P. Conformément à ma troisième hypothèse, j'ai observé une décomposition plus lente de la litière de feuilles et des racines fines en réponse à une sécheresse estivale prolongée, tout au long du gradient latitudinal dans les Alpes. Cependant, cette décomposition plus lente sous la sécheresse n'a pas été atténuée dans les peuplements forestiers à essences mixtes par rapport aux peuplements à essences uniques. Il est intéressant de noter qu'il y a une libération nette d'azote dans les racines fines en décomposition mais pas dans la litière de feuilles en décomposition, ce qui suggère un rôle distinct des racines fines dans le cycle de l'azote. En conclusion, j'ai constaté que le mélange des espèces d'arbres n'atténue pas les effets négatifs du changement climatique. Cette thèse démontre que la promotion de mélanges peut toujours être bénéfique pour au moins une des espèces d'arbres mélangées, par l'ajout d'espèces, car une espèce d'arbre peut en faciliter la nutrition minérale d'une autre par des flux souterrains de N et de P.

**Mots clés :** biodiversité, écosystèmes forestiers, changement climatique, qualité de la litière, production de racines fines, cycle des nutriments



## Abstract

The insurance hypothesis predicts that forests with tree species mixtures may resist better to stressful environmental conditions than forests composed of only one tree species. Most of the currently available literature tested this hypothesis for aboveground productivity and its related response variables, but less is known about belowground processes. In my PhD thesis, I studied the drivers of belowground productivity and decomposition across climatic gradients and how they are affected by tree mixtures. I hypothesized that mixing of tree species with contrasting rooting patterns and fine root morphologies, would result in a release of competitive pressure belowground, and translate into higher fine root standing biomass and increased fine root productivity. Moreover, I hypothesized that roots with contrasting chemical and morphological characteristics in mixed stands would decompose faster, which may be particularly important under nutrient-limited conditions. Under water-limiting conditions, such as during extreme summer drought, I hypothesized overall slower decomposition but an attenuating effect of tree mixtures on decomposition due to improved micro-environmental conditions, in particular for leaves, since roots decompose in a more buffered soil environment. To test these hypotheses I examined the variation in tree root functional traits (across- and within-species), and its consequences for fluxes of C, N and P at the ecosystem scale. I addressed three main objectives and associated research questions to quantify the interactive effect of tree mixtures and climate on: 1) vertical root segregation and fine root standing biomass, 2) fine root dynamics and their associated nutrient fluxes and 3) fine root- and leaf litter decomposition. I could benefit from two different field experiments for my work, one with a 10-year-old tree-plantation experiment with birch and pine close to Bordeaux (ORPHEE experiment), the second along a latitudinal gradient of mature beech forests in the French Alps (BIOPROFOR experiment).

I observed that roots from the birch and pine tree-plantation showed similar vertical distribution and similar belowground root standing biomass in tree mixtures compared to monocultures, contrary to my first hypothesis. However, the greater allocation of pine but not of birch to root growth within the top soil horizons under less water-limiting conditions suggests locally favourable conditions that may lead to soil depth-specific asymmetric competition. In the same experiment, fine root production and decomposition were similar in mixtures and in monocultures, in contradiction with my second hypothesis. Moreover, I did not observe any interactive effects of tree mixtures with stand density or water availability. Interestingly though, birch roots, but not pine roots released P during root decomposition, which suggests an important role of birch in the P-cycle and for P nutrition of trees on these P-limited sandy soils. In line with my third hypothesis, I observed a slower decomposition of leaf litter and fine roots in response to reinforced and prolonged summer drought, irrespective of the position along the latitudinal gradient in the Alps. However, this slower decomposition under drought was not attenuated in forest stands with mixed tree species compared to single species stands. Compared to leaf litter, fine roots decomposed slower and released less C. Interestingly, I found a net N release in decomposing fine roots but not in decomposing leaf litter, which suggests a distinct role of fine roots in the N cycle. In conclusion, I found that mixing tree species did not attenuate negative effects of climate change. However, this thesis demonstrates that promoting mixtures can still be beneficial for at least one of the admixed tree species, through species addition (i.e., complementing one tree species with another tree species), as one tree species may facilitate another via belowground fluxes of N and P.

**Keywords:** biodiversity, forest ecosystems, climate change, litter quality, fine root production, nutrient cycling





## Remerciements / Acknowledgments

This thesis is the product of many people, and it would never have come to fruition without them. I truly believe that relationships we build are sacred and should be cherished, and I want to dedicate this long acknowledgments section to all of you that were in my PhD life the last years.

It was not too long ago that I was in my MSc thesis, counting mycorrhizae through a microscope. A tedious process, which took me a couple of months to master. Also a brutal repeated sequence of four actions: counting, scanning, drying, weighing.....counting, scanning, drying, weighing. My friends would ask me “Andreas why are you still in the lab looking at these roots, and what the hell are you doing with your life?”. Actually, I knew exactly why I was looking at roots all day, but it is true that I did not really know what I was doing with my life, or what the future had in store for me. It was during that time that I first reflected on doing a PhD. Little did I know that I would spend that much and even more time, working with roots, thinking about roots, writing about roots. A few months later, I contacted Mark, and Stephan, we did a skype, and soon after, it all started!

First of all I give a big thank you to my wife Adriana who has been by my side through our adventures in The Netherlands, and at the moment in France, spending her Sundays cleaning roots with me in the living room, and even made few of the litterbags (large mesh !). You nourish my soul and give me balance, you hold me when I need to be held and I love you. Thank you to my family: my father Lefteris, my mother Gina, my sister Elena, who believed in me, and who supported me in my studies abroad. They taught me by example, to work hard and to never ever quit. Thank you to my extended Mexican family: Rafael, Patricia, Galilea, Rafa, Perlita, Chofies, Buki, and Felix. You have treated me like a son, and accepted me in your life. Thank you to my Greek extended family: Alexandros, his father Nikos, and his mother Maria. Although I have been away, it is so great to see our families unite and grow together.

Thank you to my international friends from the Netherlands: Lalo, Kodji, Mai, Nyandula, Shaibu, Daniel. Thank you for all the skype chats, and for being a real community of people that I can always rely on. Thank you to Gerlinde de Deyn and Janna Barel, for taking me into their project during my Msc thesis, and for (unintentionally) inspiring me to pursue a PhD. It is with your team that I discovered my fascination for science. Thank you to my French colocs, Arturo, Bastien, Joan, you made confinement so easy! I will always remember our BBQs à la plancha, the miniature garden, the movie nights, the raclettes, the tartiflettes, greek food, Spanish food, Peruvian food (ceviche !!!). Thank you to the franco-latino couples of Bordeaux!! Simon and Katie, Simon and Raquel, Arnaud and Krystal, Angel and Vicky. Que gente hermosa !!

I am grateful to our small but very productive team of the DiPTiCC project. Thank you Nicolas for motivating me to keep pushing, and for inspiring me. Laurent you have been a quiet force of stability all this time, although you have four kids at home, it feels like your father instinct crosses over at work as well. Thank you Hervé, Bastien,

Damien, Céline, Xavier, Maude, Soline for being such great collaborators. Even though I was lost in translation many times (you speak so fast French people!), I always felt at ease, and I learned a lot from you. Thank you Cathy for always being so eager to help, for being so patient with me, and for letting me use your lab to chop my roots! I give a very warm thank you to Sylvie Milin, for being a guardian angel in the lab. You are not only an excellent professional but a wonderful person.

Thank you to all the BIONUTs, previous and new ones! Arthur, David, Manon, Mohamed, Fred, Marko, Lucie, Pablo, Nicolas. My Mexican-marriage best men, Pietro and Ulysse, for creating the best office atmosphere, for teaching me R, for introducing me to climbing, for teaching me French. If I suck at all three, it is your fault!!! Very special thanks to my French-marriage best lady Tania (Yes, I married the same person twice in two different countries, one marriage was not a statistically robust design). Tania, I will terribly miss our conversations, our laughs and your never-ending optimism and ambition. Also, thank you to Lukas for keeping Tania sane, so that she could keep me sane...hahah. Finally yet importantly, I want to thank Kathina, Constance, Alison, and Meriam. You are the undoubtful carriers of this Thesis! You are all very talented and I hope to see you again in future root (or not) projects.

A very important thank you is addressed to my two supervisors. Stephan, although we could not spend more time together, you always did your best to help me. Even if that meant having a skype meeting on your son's birthday or travelling to Bordeaux from Montpellier for a one-day meeting! Thank you for being kind and patient with me, and for being a tutor when I needed you the most. Your kind emails always brightened my mood and gave a smile to my face. Mark, we have certainly been through a lot together. Thank you for the bike, the beers, the silly jokes, thank you for always being present, thank you for all the lab work we did together, and so much more. In reality, I cannot thank you enough and I cannot owe you enough beers (I guess the number is over three digits by now). I will remember our working together very dearly. You are both excellent men and scientists, I will always be grateful for giving me the opportunity to work on such an ambitious project, and to have rubbed shoulders with such charismatic people.



## Contents

<b>Résumé .....</b>	<b>v</b>
<b>Abstract .....</b>	<b>vii</b>
<b>Remerciements / Acknowledgments .....</b>	<b>ix</b>
<b>List of Tables .....</b>	<b>xvi</b>
<b>List of Figures .....</b>	<b>xxi</b>
<b>Chapter 1   Introduction .....</b>	<b>1</b>
1.1 <i>Changes in climate and biodiversity</i> .....	1
1.2 <i>Importance of roots for nutrient cycling</i> .....	2
1.3 <i>General aspects of biodiversity belowground</i> .....	4
1.4 <i>Fine root dynamics</i> .....	5
1.4.1 <i>Environmental effects on fine root dynamics</i> .....	6
1.4.2 <i>Biodiversity effects and resource availability</i> .....	6
1.5 <i>Decomposition</i> .....	7
1.5.1 <i>Climate and trait control on the decomposition of leaves and roots</i> .....	8
1.5.2 <i>Direct and indirect effects of tree diversity on decomposition</i> .....	9
1.6 <i>Rationale and research questions</i> .....	10
<i>References</i> .....	13
<b>Chapter 2   Materials and Methods.....</b>	<b>26</b>
2.1 <i>Study design</i> .....	26
2.2 <i>Site description</i> .....	26
2.3 <i>Methods</i> .....	29
2.3.1 <i>Tree species studied and tree inventory</i> .....	29
2.3.2 <i>Belowground productivity and turnover</i> .....	29
2.3.3 <i>Vertical root profiles</i> .....	30
2.3.4 <i>Decomposition</i> .....	30
2.3.5 <i>Soil analyses</i> .....	33
2.4 <i>Data treatment</i> .....	34
2.5 <i>Organization of the Result chapters</i> .....	35
<i>References</i> .....	36
<b>Chapter 3   Effect of tree mixtures and water availability on belowground complementarity of fine roots of birch and pine planted on sandy podzol.....</b>	<b>38</b>
3.1 <i>Introduction</i> .....	39
3.2 <i>Materials and Methods</i> .....	41
3.2.1 <i>Study site</i> .....	41
3.2.2 <i>Field sampling</i> .....	42
3.2.3 <i>Root sorting</i> .....	44

3.2.4 Data collection and computation of root morphology variables.....	44
3.2.5 Calculation of vertical root distribution index .....	45
3.2.6 Calculation of diversity metrics.....	45
3.2.7 Data analysis .....	46
3.3 Results .....	46
3.3.1 Overall treatment effects on fine root biomass and morphology .....	46
.....	47
3.3.2 Vertical root patterns.....	48
3.3.3 Relative fine root attributes .....	51
3.4 Discussion.....	53
3.4.1 General considerations .....	53
3.4.2 Effect of mixing tree species on vertical root segregation and belowground overyielding .....	55
3.4.3 Pronounced vertical root segregation and belowground overyielding under ambient water supply.....	56
3.4.4 Depth-specific effects on relative yield .....	56
3.5 Conclusion.....	59
Acknowledgements .....	59
References.....	60
<b>Chapter 4   Fine root dynamics in response to tree species mixing, stand density, and water availability.....</b>	<b>68</b>
<i>Abstract</i> .....	68
4.1 Introduction.....	69
4.2 Materials and Methods .....	72
4.2.1 Study site and experimental design .....	72
4.2.2 Fine root production and turnover .....	74
4.2.3 Fine root decomposition.....	76
4.2.4 Chemical analysis of roots .....	77
4.2.5 Root litter input and C, N, P fluxes in the soil .....	78
4.2.6 Data analysis .....	79
4.3 Results .....	79
4.3.1 Differences in fine root morphology and chemistry between species, and between ingrowth cores and root standing biomass cores .....	79
4.3.2 Fine root production and turnover .....	82
4.3.3 Fine root decomposition.....	84
4.4 Discussion.....	86
4.4.1 General considerations .....	86
4.4.2 Effect of tree species mixing .....	87
4.4.3 Effect of stand density.....	88
4.4.4 Effect of irrigation.....	89
4.4.5 Implications for carbon and nutrient cycling .....	90
4.5 Conclusion.....	91
Acknowledgements .....	91
References.....	92

<b>Chapter 5   Prolonged summer drought decreases C release but does not change N release during fine root and leaf litter decomposition in beech forests.....</b>	<b>101</b>
<i>Abstract</i> .....	101
5.1 <i>Introduction</i> .....	102
5.2 <i>Materials and Methods</i> .....	104
5.2.1 Study sites .....	104
5.2.2 Rain exclusion treatment .....	106
5.2.3 Litterbag construction.....	106
5.2.4 Chemical analysis of plant material.....	107
5.2.5 Carbon and nitrogen loss.....	107
5.2.6 Experimental design and statistical analyses .....	108
5.3 <i>Results</i> .....	109
5.3.1 Reinforced and prolonged summer drought .....	109
5.3.2 Initial fine root and leaf litter chemistry .....	109
5.3.3 Fine root- and leaf litter decomposition in response to reinforced and prolonged drought .....	111
5.3.4 Fauna contribution to leaf litter decomposition .....	114
5.4 <i>Discussion</i> .....	115
5.4.1 Drought effects on decomposition.....	115
5.4.2 Severe summer drought and tree species mixtures .....	117
5.4.3 Comparison between root and leaf decomposition.....	118
5.4.4 Fauna contribution to leaf litter decomposition .....	119
5.5 <i>Conclusion</i> .....	119
<i>Acknowledgements</i> .....	119
<i>References</i> .....	120
<b>Chapter 6   Discussion.....</b>	<b>125</b>
6.1 <i>Root adaptations to water availability and the role of interspecific interactions</i> .....	125
6.1.1 Vertical root distribution .....	126
6.1.2 Fine root growth in mixed stands .....	127
6.1.3 Fine root morphology .....	128
6.1.4 Fine root mortality and mycorrhizal associations as adaptations to changing conditions .....	129
6.2 <i>Belowground carbon and nutrient cycling</i> .....	130
6.2.1 Tree mixture effects on belowground nutrient cycling.....	132
6.3 <i>Root versus leaf litter decomposition</i> .....	134
6.3.1 Comparison between roots and leaves .....	134
6.3.2 The contribution of macrofauna to decomposition.....	135
6.4 <i>Concluding remarks &amp; recommendations</i> .....	136
<i>References</i> .....	138
<b>Appendix A   Materials and Methods .....</b>	<b>146</b>
<b>Appendix B   Effect of tree mixtures and water availability on belowground complementarity of fine roots of birch and pine planted on sandy podzol.....</b>	<b>152</b>

<b>Appendix C   Fine root dynamics in response to tree species mixing, stand density, and water availability .....</b>	<b>164</b>
<b>Appendix D   Prolonged summer drought decreases C release but does not change N release during fine root and leaf litter decomposition in beech forests.....</b>	<b>170</b>
<b>Appendix E   Preliminary results.....</b>	<b>174</b>



## List of Tables

<b>Table 2.1</b> English name, species, families and plant clades used in the study, per experimental site, and their abbreviations. ....	29
<b>Table 3.1</b> Overview table of stand characteristics at 10 years-old. Values are means ( $\pm$ standard deviations) of 36 centre trees per plot (alive and measurable trees at breast height 130 cm) repeated across 8 blocks (4 blocks are irrigated and 4 blocks are not). 43	
<b>Table 3. 2</b> Overview of the summed values of fine root biomass (FRB; $\text{g m}^{-2}$ ), adjusted fine root biomass (FRBadj.; $\text{g m}^{-2} \text{m}^{-2} \text{BA}$ ), understory fine root biomass ( $\text{g m}^{-2}$ ), fine root length (FRL; $\text{m m}^{-2}$ ), fine root surface area (FRA; $\text{m}^2 \text{m}^2$ ) and root tip abundance (FRT; $10^3$ tips $\text{m}^{-2}$ ) for the whole soil profile (0–90 cm) for <i>Betula pendula</i> , <i>Pinus pinaster</i> in pure and mixed plots, in control and irrigated blocks. The tables also includes fine root morphology for the upper 15 cm of soil: specific root length (SRL <sub>15</sub> ; $\text{m g}^{-1}$ ), and specific root area (SRA <sub>15</sub> ; $\text{cm}^2 \text{g}^{-1}$ ). Different lowercase letters indicate significant differences between the eight combinations of stand composition and water treatment. ....	47
<b>Table 3.3</b> Means and medians of the relative mixed stand values ( $\pm$ standard deviations) of fine root biomass for the whole soil profile. Mean values of RY > 0.5 (per species), or when RYT > 1 (for the whole community) indicate positive mixture effects. Bold values indicate significant deviation ( $p < 0.05$ ) from 0.5 (per species) or 1 (for the whole community). Asterisks * indicate significant effect of irrigation at species or community level ( $p < 0.05$ ). ....	51
<b>Table 4.1</b> Overview of fine root morphology between fine roots of birch and pine, originating from root standing biomass (RSB) and 2 <sup>nd</sup> year ingrowth cores harvests (IC2). The morphological traits are: specific root length (SRL; $\text{m g}^{-1}$ ), specific root area (SRA; $\text{cm}^2 \text{g}^{-1}$ ), root tissue density (RTD; $\text{g cm}^{-3}$ ), and root diameter (D; mm). We also present an estimation of initial root litter morphology based on the fine root standing biomass collected in March 2018 from birch ( $n = 64$ replicates) and pine trees ( $n = 61$ replicates) that were left unirrigated. Values are means with standard deviation in parenthesis. Different upper case letters indicate differences for a root cohort between both species for a given root metric, while different lower case letters indicate differences within a species between root cohorts for a given root metric. ....	80
<b>Table 4.2</b> Overview of fine root quality between fine roots of birch and pine, originating from root standing biomass (RSB), 2 <sup>nd</sup> year ingrowth cores harvests (IC2), from initial litter of fine roots collected from birch and pine trees at three km from the study site, and for root litterbags. All available replicates of each species were used for RSB, IC2 and root litterbag analysis of C, N, P. For initial litter quality three replicates ( $n = 3$ ) were used for the elemental analysis, and one replicate ( $n = 1$ ) was used for lignin measurements. Values are means with standard deviation in parenthesis. Different upper case letters indicate differences for a root cohort between both species for a given chemical variable, while different lower case letters indicate differences within a species between root cohorts for a given chemical variable. ....	81
<b>Table 5.1</b> Overview of site characteristics, and soil characteristics. Average precipitation and temperature is averaged between years (2018 – 2019) and collected from the closest available meteorological stations. Soil data are averages of two tree species mixture plots (i.e. two plots at each site) and were collected, using automated sensors (RT-1 and EC-5 or GS-1 sensors for temperature and moisture (TDR sensors), respectively, Decagon	

Devices Inc., Pullman, WA, USA) storing average values every three hours for the whole duration of the experiment. ....	105
<b>Table 5.2</b> Results of the PERMANOVA test, using Euclidean distances, to test for the dissimilarity between groups of litter type (leaves vs roots), between sites (Vercors, Mont Ventoux, Lubéron, Ste Baume), and their interaction. Significant results are in bold font ( $p < 0.05$ ). ....	109
<b>Table 5.3</b> Overview of initial litter quality of a) fine root-, and b) leaf litter. Means and standard deviation (in parentheses) of three replicates of: Carbon, Nitrogen, Phosphorus, Calcium, Potassium, Magnesium, Manganese, across the four sites (Vercors, Mont Ventoux, Lubéron Ste Baume). Significant differences are annotated with small letters ( $p < 0.05$ ). ....	110
<b>Table 5.4</b> Results of the linear mixed effects models to test for the effects of site, mixture, rainfall exclusion and their interactions on remaining carbon (C), and remaining nitrogen (N), after two years of decomposition, for: a) root litterbags, b) leaf litterbags with a fine mesh. Significant results are in bold font ( $p < 0.05$ ). ....	113
<b>Table 5.5</b> Results of the linear mixed effects models to test for the effects of site, rainfall exclusion, litterbag type and their interactions on remaining carbon (C), and remaining nitrogen (N), after two years of decomposition. The litterbag comparison is between fine root litterbags and leaf litterbags with a fine mesh. Significant results are in bold font ( $p < 0.05$ ). ....	113
<b>Table 5.6</b> Results of the linear mixed effects models to test for the effects of site, rainfall exclusion, litterbag type and their interactions on remaining carbon (C), and remaining nitrogen (N), after two years of decomposition. The litterbag comparison is between leaf litterbags with a fine mesh, and leaf litterbags with a coarse mesh. Significant results are in bold font ( $p < 0.05$ ). ....	115
<b>Table A2.1</b> Overview of the ingrowth core and hyphal bag installation per plot. Each plot code is replicated for eight blocks, for a total of 56 plots. A total of eight ingrowth cores and their hyphal bags are installed per plot, and divided among 4 subplots. ....	146
<b>Table A2.2</b> Overview of the of the ingrowth core and hyphal bag installation installation in the Alps, per site. Each site comprises of two altitudes (low and high elevation). Each altitude has a triplet of plots, and each plot comprises of 4 subplots (two with rain exclusion and two without). ....	147
<b>Table A2.3</b> Overview of the vertical root profiles per plot. Each plot code is replicated for eight blocks, for a total of 32 plots. A total of four soil columns were harvested per plot, and further divided to six layers. ....	147
<b>Table A2.4</b> Overview of the litterbag installation in the experimental site of ORPHEE, and divided for two litterbag types: a) root litterbags (mesh of 48 $\mu$ m Ø), b) leaf litterbags (mesh of 48 $\mu$ m Ø). Each plot code represents a different tree species composition, which can be monospecific or a mixture, and is replicated for 8 blocks, for a total of 56 plots. Standardized litter, which corresponds to the tree species composition of each plot, was placed in pairs of three litterbags. ....	148
<b>Table A2.5</b> Overview of the litterbag installation in the Alpes, per site, and divided for three litterbag types: a) root litterbags (mesh of 48 $\mu$ m Ø), b) leaf litterbags (mesh of 48 $\mu$ m Ø), c) leaf litterbags (mesh of 2mm Ø). Each site comprises of two altitudes (low and high elevation). Each altitude has a triplet of plots, and each plot comprises of 4 subplots (two with rain exclusion and two without). Site-specific litter, which corresponds to the tree species composition of each plot, is placed in pairs of three litterbags. ....	149

<b>Table B3.1</b> Overview table of understory species with means of soil cover (%) and std. deviations (in parentheses), under monocultures of <i>Betula pendula</i> , <i>Pinus pinaster</i> , and their mixture. Presence is the percentage of aboveground occurrence for each understory species. ....	158
<b>Table B3.2</b> Soil moisture (%) along the soil profile in the three different species composition treatments (pure <i>Betula pendula</i> , pure <i>Pinus pinaster</i> , mixed <i>Betula pendula</i> + <i>Pinus pinaster</i> ), each in both control and irrigated blocks. Soil was collected in March 2018 along with the roots. Data are means $\pm$ standard deviation. ....	159
<b>Table B3.3</b> Results of the linear mixed effects models to test for the effects of species, mixture, irrigation, depth and their interactions on Fine root mass density (FRMD), Specific root length (SRL), Specific root area (SRA) and Specific root tip density (SRTD). Significant results are in bold font. ....	160
<b>Table B3.4</b> Results of the linear mixed effects models to test for the effects of mixture, irrigation, depth and their interactions on Fine root mass density (FRMD), Specific root length (SRL), Specific root area (SRA) and Specific root tip density (SRTD). The upper part shows results for <i>Betula pendula</i> , and the lower part for <i>Pinus pinaster</i> . Significant results are in bold font. ....	161
<b>Table B3.5</b> Means of root distribution values, with std. deviations (in parentheses) calculated for g m <sup>-2</sup> soil surface. The parameters d30 and d60 are fine root cumulative proportions to depths of 30 and 60 cm respectively. The d50 parameter is the depth at which we find 50% of roots, and was estimated with the beta values. ....	162
<b>Table B3.6</b> Overview of fine root biomass (FRB; g m <sup>-2</sup> ) per soil layer, and summed for the whole profile (0-90 cm) that is found in pure and mixed plots of birch and pine, under control and irrigated conditions. Different lowercase letters indicate significant differences between the six combinations of stand composition and water treatment, across each soil layer. ....	163
<b>Table C4.1</b> Overview table of stand characteristics at 10 years-old. Measurements were performed in the summer of 2018; therefore in the middle of the two year observation period for roots. Values are means ( $\pm$ standard deviations) of 36 centre trees per plot (alive and measurable trees at breast height 130 cm) repeated across 8 blocks (4 blocks are irrigated and 4 blocks are not). ....	165
<b>Table C4.2</b> Relative canopy ratios when trees are growing in mixture, and the % of missing trees per measurement (dead trees and trees with a trunk diameter of less than 1.3 cm were excluded). The canopy extension (in m <sup>2</sup> ) of each tree was computed by using the four largest branches and assuming a vertical projection on the forest floor. The canopy ratio was then defined as the canopy extension divided by 4 m <sup>2</sup> (the theoretical space of each tree in the design). Values higher than 1 mean that the tree occupies more than this space (canopy closure). ....	166
<b>Table C4.3</b> Overview table of understory species with means of soil cover (%) and std. deviations (in parentheses), under monocultures of birch, pine, and their mixture (birch + pine). Presence is the percentage of aboveground occurrence for each understory species. ....	167
<b>Table C4.4</b> Results of the linear mixed effects models to test for the effects of tree species composition, stand density, irrigation, and their interactions on Fine root production (FRP), Fine root necromass (FRN), Fine root turnover (FRT) and Fine root decomposition (FRD). Significant results are in bold font ( $p < 0.05$ ). ....	168
<b>Table C4.5</b> Results of the linear mixed effects models to test for the effects of tree species composition, stand density, irrigation, and their interactions on cumulative release (after	

a 2-year decomposition period) of carbon (C), nitrogen (N), and phosphorus (P). Significant results are in bold font ( $p < 0.05$ ).....168

**Table C4.6** Annual fine root litter input to soil (fine root necromass) and cumulative N and Carbon release (after 2 years of fine root decomposition) between different tree species composition of birch and pine, planted at high and low stand density. Half of the stands are left unirrigated (Ctrl) and half are irrigated during the summer (Irr). Values are means with stand deviation in parentheses. ....169

**Table D5.1** Overview of stand basal area of the tree species present in the pure and mixed tree stands, across the four sites (Vercors, Mont Ventoux, Lubéron, Ste Baume).....173



## List of Figures

- Fig. 1.1** Projected change in meteorological drought frequency between the present (1981-2010) and the mid-century 21st century (2041-2070) in Europe, under two emissions scenarios; RCP 4.5 (moderate), RCP 8.5 (severe). Data source: Projections of future meteorological droughts in Europe provided by Joint Research Centre (JRC) ..... 2
- Fig. 1.2** Simplified scheme of pools and fluxes..... 6
- Fig. 1.3** Schematic overview of research questions addressed in the indicated chapters of my thesis.....11
- 
- Fig. 2.1** Schematic representation of the ORPHEE experimental design. Image is taken from the official ORPHEE online site 'https://sites.google.com/view/orpheeexperiment/experimental-design'. .....27
- Fig. 2.2** (a) Study area and location of the six sites in southeastern France. Northern points (sites S1, S2, S3) represent sites with European beech (*Fagus sylvatica*) and silver fir (*Abies alba*) forests. Southern points (sites S4, S5, S6) represent southern sites with European beech and pubescent oak (*Quercus pubescens*) forests. The sites are located in the following mountain ranges of the French pre-Alps: Bauges (S1), Vercors (S2), Mont Ventoux (S3), Lubéron Lagarde (S4), Grand Lubéron (S5), Sainte-Baume (S6) (b) Schematic representation of a site with two triplets per site. Each triplet is made up of two pure plots and one mixed plot. (c) Representation of an individual plot. Plots are circular with a central plot area (10 m radius) and a buffer zone (7.5 m radius).....28
- Fig. 2.3** Schematic representation of: (a) a plot in ORPHEE, (b) a plot in the Alps, (c) the ingrowth cores (represented by a red triangle) and hyphal bags, and (d) root litterbags installed on the side (brown font) and leaf litterbags (green font) installed in the middle, and under metal grids. ....32
- 
- Fig. 3.1** Depth distributions for root traits of *Betula pendula* and *Pinus pinaster* for all four Irrigation × Mixture combinations (Pure – C; Mixed – C; Pure – IRR; Mixed – IRR). Presented data are mean values with st. errors for: (a,e) fine root mass density (FRMD;  $\text{g m}^{-3}$ ), (b, f) specific root length (SRL;  $\text{m g}^{-1}$ ), (c, g) specific root area (SRA;  $\text{cm}^2 \text{g}^{-1}$ ), and (d, h) branching intensity (SRTD;  $1000 \times \text{tips g}^{-1}$ ). Effects of irrigation (IRR), mixture (MIX) and their interaction are indicated at the lower right side of each graph. Significant differences per soil depth, between combinations of Irrigation and Mixture are indicated with lower case letters. Absence of letters means that there are no significant differences. ....49
- Fig. 3.2** Cumulative fine root fraction with soil depth and the coefficients of the rooting distribution ( $\beta$ ) for: a) *Betula pendula* in pure stands b) *Betula pendula* in mixed stands, c) *Pinus pinaster* in pure stands, d) *Pinus pinaster* in mixed stands, under irrigation and control treatment. The  $\beta$  values are based on fine root biomass ( $\text{g m}^{-2}$ ); the higher the  $\beta$  value (the closer to 1), the deeper is the vertical distribution of fine roots. Absence of letters means that there are no significant differences. ....50
- Fig. 3.3** Boxplots with medians (-), and means ( $\diamond$ ) of the relative mixed stand values of fine root biomass ( $\text{g m}^{-2}$ ).  $\text{RY} > 0.5$  indicates positive mixture effects for a given species: a) *Betula pendula*, b) *Pinus pinaster*, c) and  $\text{RYT} > 1$  indicates positive mixture effects for the whole community. Significant relative yield effects (overyielding or underyielding) are noted using # ( $p < 0.05$ ), ## ( $p < 0.01$ ) and ### ( $p < 0.001$ ). Asterisks \* indicate significant differences ( $p < 0.05$ ) between irrigated and non-irrigated plots, per depth.....52
- Fig. 3.4** Depictions of the simplified shallow vs deep paradigm for species 1 (in red colour) and species 2 (in green colour) growing in mixtures, and the proposed depth-specific

paradigm for young mixed forests. On the left is the response in fine root investment of Species 1 and Species 2 growing together in mixture, in the middle is their calculated vertical fine root distributions ( $\beta$  values), on the right is the yield effect for the whole soil column (pure versus mix). The shallow vs deep paradigm is based on the assumption that in case of vertical root segregation, there will be a higher root occupation of the soil profile, which results in belowground overyielding in tree mixtures. In scenario A, vertical root segregation could occur if more roots of one species grow in either the top soil layers or the deeper soil layers (respectively curves on the left, curves on the right) relative to its distribution in a pure stand, leading to an overyielding for the whole soil column. However, vertical root segregation can also be obtained if one species would grow less roots in top or deeper soil layers relative to the pure stand, though in that case this implies an underyielding at the whole soil profile level (scenario B). Alternatively, other factors such as soil conditions and the depth of the water table, can constrain root growth at deeper soil layers, giving results that are not consistent with the shallow vs deep paradigm. For example, a lack of vertical root segregation may still result in overyielding for the whole soil profile, as both species actually invest in higher biomass when growing in mixture (scenario C). Under our conditions, this pattern, considering root segregation per soil layer, and not throughout the whole soil profile, better matches our observations of root distribution in tree mixtures. ....58

- Fig. 4.1** Conceptual diagram representing our experimental setup. We take advantage of the slower growth and higher mortality rate of oak (small black dots) to test tree diversity effect at high and low stand densities of pine (dark green dots) and birch (light green dots) in the ORPHEE experiment. In each plot, we chose four zones for the study of root growth ('*Ingrowth*' subplots) and two zones for the study of the decomposition of roots ('*Decomp*' subplots). The four '*Ingrowth*' subplots were regularly distributed over each plot and were used for tree and understory inventory, sampling of root standing biomass and the study of root dynamics with ingrowth cores. The two '*Decomp*' subplots were assigned in half of the root zones. Diversity effects were tested between pure plots of pine, birch (solid squares) and pine x birch mixture (dashed line). The effect of stand density was tested using high-density plots (thick squares) and low-density plots (thin squares); these were plots where pine and birch grew together with oak. ....73
- Fig. 4.2** Annual fine root productivity ( $\text{g m}^{-2} \text{y}^{-1}$ ) based on both biomass and necromass collected from the ingrowth-cores. The graph effects of stand density 'D'(on the left), and irrigation 'I' (on the right) on the three tree species compositions 'C': birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences between the three tree species compositions. Asterisks \*\* indicate a significant effect of stand density ( $p < 0.01$ ) for a given tree species composition. Values are means with st. errors. ....82
- Fig. 4.3** Boxplots with medians (-), and means ( $\diamond$ ) of fine root turnover ( $\text{y}^{-1}$ ). The effects of stand density (on the left), and irrigation (on the right) on the three tree species compositions: birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences between the three tree species compositions. The dashed lines indicate an overall significant difference between high (thick dashed line) and low (fine dashed line) stand density ( $p < 0.05$ ). ....83
- Fig. 4.4** Boxplots with medians (-), and means ( $\diamond$ ) of fine root decomposition (root remaining mass %). The effects of stand density (on the left), and irrigation (on the right) on the three tree species compositions: birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences after 1 and 2 years of decomposition. ....84

- Fig. 4.5** Boxplots with medians (-), and means ( $\diamond$ ) of cumulative release ( $\text{kg ha}^{-1}$ ) of Carbon, Nitrogen, and Phosphorus. The effects of stand density (on the left), and irrigation (on the right) on the three tree species compositions: birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences between the three tree species compositions. The dashed lines indicate an overall significant difference between high (thick dashed line) and low (fine dashed line) stand density ( $p < 0.05$ ). .....85
- Fig. 5.1** Boxplots with medians (-), and means ( $\diamond$ ) of remaining C%, and remaining N% of: a) root litterbags, b) leaf litterbags/fine mesh, as affected by rainfall exclusion, and depicted across sites. Different lowercase letters indicate significant differences between the sites. Asterisks indicate a significant difference between rainfall exclusion treatments (\*\*\*) ( $p < 0.001$ ). .....112
- Fig. 5.2** Boxplots with medians (-), and means ( $\diamond$ ) of: a) remaining C%, and b) remaining N%, on leaf litter as affected by contribution of fauna, and depicted across sites. Absence of letters and Asterisks means there were no significant effects. Different uppercase letters indicate significant differences between sites, for leaf litterbags with fine mesh, and different lowercase letters indicate significant differences between sites, for leaf litterbags with a coarse mesh. Asterisks indicate a significant difference between litterbag type per site (\*\*;  $p < 0.01$ ), (\*\*\*) ( $p < 0.001$ ). .....114
- Fig. B3.1** Curves of ground water level from the ORPHEE experimental site. The data presented are monthly values (January – December) with std. deviations (in parentheses) from 2015 (initiation year of the irrigation treatment) until 2018 (year of study). Water table depth (m). .....152
- Fig. B3.2** Ombrothermic diagram from the Cestas weather station, positioned 1.43 km from the ORPHEE experimental site. The data presented are monthly values (January – December) from 2015 (initiation year of the irrigation treatment) until 2018 (year of study). Temperature = monthly average temperature ( $^{\circ}\text{C}$ ), Precipitation = monthly total rainfall (mm). Precipitation scale =  $2 \times$  Temperature scale. Potential evapotranspiration per month (mm). By plotting in this manner, we identify the potential for water stress, when mean precipitation is lower than mean temperature. Source: CLIMATICK – INRAE. ...153
- Fig. B3.3** Stacked barchart showing the distribution of fine root understory biomass for each soil layer, under pure (birch or pine) and mixed plots (birch + pine), growing under control and irrigated conditions. ....154
- Fig. B3.4** Depth distributions for root traits of *Betula pendula* and *Pinus pinaster* for all four Irrigation  $\times$  Mixture combinations (Pure – C; Mixed – C; Pure – IRR; Mixed – IRR). Presented data are mean values with st. errors for: (a, c) fine root mass density (FRMD;  $\text{g m}^{-3}$ ), (b, d) fine root length density (FRLD;  $\text{cm cm}^{-3}$ ). Effects of irrigation (IRR), mixture (MIX) and their interaction are indicated at the lower right side of each graph. Significant differences per soil depth, between combinations of Irrigation and Mixture are indicated with lower case letters. Absence of letters means that there are no significant differences. ....155
- Fig. B3.5** Depth distributions of fine root length divided into diameter classes of *Betula pendula* and *Pinus pinaster* for all four Irrigation  $\times$  Mixture combinations (Pure – C; Mixed – C; Pure – IRR; Mixed – IRR). Presented data are mean fine root fraction values with st. errors for the following diameter classes: (a,e) [0.000 – 0.500 mm], (b, f) [0.500 – 1.000 mm], (c, g) [1.000 – 1.500 mm], and (d, h) [1.500 – 2.000 mm]. Effects of irrigation (IRR), mixture (MIX) and their interaction are indicated at the lower right side of each graph.. .....156



- Fig. B3.6** Cumulative fine root fraction with soil depth and the coefficients of the rooting distribution ( $\beta$ ) of pure and mixed stands of *Betula pendula* and *Pinus pinaster* under: a) Irrigation, b) non-irrigation. The  $\beta$  values are calculated for fine root biomass ( $\text{g m}^{-2}$ ); the higher the  $\beta$  value (the closer to 1), the deeper is the vertical root distribution of fine roots. .... 157
- Fig. C4.1** Annual fine root productivity ( $\text{g m}^{-2} \text{y}^{-1}$ ) per tree species composition, with the contribution of biomass and necromass measured in the ingrowth-cores, 1 and 2 years after ingrowth-core installation. Understory roots (on the left), tree roots (on the right). Values are means with st. errors..... 164
- Fig. D5.1** (a) Study area and block of the four sites in southeAstern France. Northern point Points (sites S1, S2) represent sites with European beech (*Fagus sylvatica*) and silver fir (*Abies alba*) forests. Southern points (sites S3, S4) represent southern sites with European beech and pubescent oak (*Quercus pubescens*) forests. The sites are located in the following mountain ranges of the French pre-Alps: Vercors (S1), Ventoux (S2), Grand Lubéron (S3), Sainte-Baume (S4) (b) Schematic representation of a site with two blocks per site. Each block comprises of one pure beech plot and one mixed plot. (c) Representation of an individual plot. Plots are circular with a central plot area (10 m radius) and a buffer zone (7.5 m radius). The red rectangles indicate rain-exclusion in summer. .... 170
- Fig. D5.2** The soil moisture (%) during the summers of 2018 and 2019 when rainout shelters were installed for each site (Vercors, Mont Ventoux, Lubéron and Ste Baume) and block (block 1, and block 2). The percentage values on top of each bar represent the reduction in soil moisture under the rainout shelters, relative to the non-exclusion zone, for each site and block..... 171
- Fig. D5.3** Principal Component Analysis (PCA) of initial leaf and root litter quality of beech trees, collected from two blocks per site (Vercors, Mont Ventoux, Lubéron, Ste Baume). Litter quality includes the following elements: N, C, P, Ca, K, Mg, Mn. A PERMANOVA test showed that initial quality was different between the two litter types ( $p = 0.001$ ). . 172
- Fig. E6.1** Boxplot with medians (-), and means ( $\diamond$ ) of annual hyphal productivity ( $\text{kg ha}^{-1} \text{y}^{-1}$ ), across all plots of the ORPHEE experimental design, and comparison between control (in red) and irrigated conditions (in blue)..... 174
- Fig. E6.2** Multifaceted barchart of root tip colonization level (%) between birch and pine trees, grown in pure and mixed stands. Colonization levels under control conditions are depicted at the top panels, and under irrigated conditions at the bottom panels..... 174
- Fig. E6.3** Stacked barcharts depicting exploration types of birch and pine, grown in pure and mixed stands. Results under control conditions is depicted at the top panels, and under irrigated conditions at the bottom panels..... 175
- Fig. E6.4** Effect of irrigation on soil moisture for the months receiving the irrigation treatment (July – August – September – October) for the year of 2019. Barchart presents means with standard errors ..... 175



# Chapter 1 | Introduction

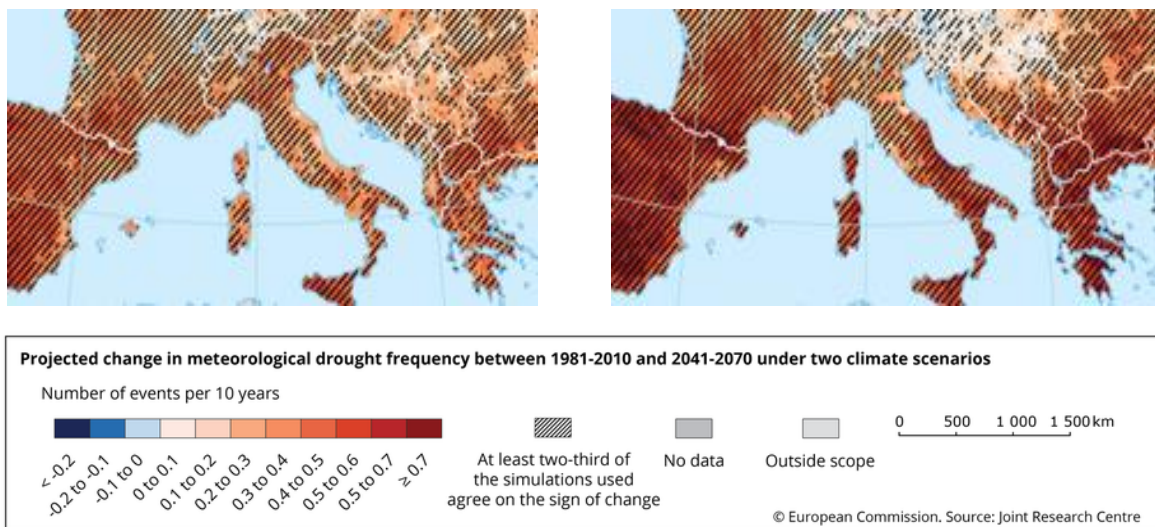
## 1.1 Changes in climate and biodiversity

Biodiversity enhances many of nature's benefits to people, including the regulation of climate and the production of wood in forests, and livestock forage in grasslands (Daily et al. 1997; Isbell et al. 2017). Biodiversity is declining at a fast pace mostly due to human activities leading to habitat conversion, degradation, fragmentation, overexploitation, pollution, and also to changes in atmospheric composition resulting from excessive use of fossil fuel. The combination of these human impacts accelerates climate change and biodiversity loss, with current species extinction rates likely exceeding those of the past (Butchart et al. 2010; Millennium Ecosystem Assessment 2005; Parmesan and Yohe 2003). Climate change affects ecosystems through a gradual climate warming that results in changing species distribution patterns and major extinctions (Thomas et al. 2004). In addition, climate change may also threaten the functioning of ecosystems through more frequent and more extreme climate events, such as prolonged drought (Hartmann 2011). Prolonged droughts reduce water availability in the soil, thereby limiting plant growth and affecting plant interactions and vegetation structure (Debouk et al. 2015). For example, trees that are long-lived species, may not be able to adapt to rapid changes in environmental conditions (changes in precipitation and/or temperature) (Aitken et al. 2008; McCormack et al. 2013). The adaptive ability of forest tree species to a changing climate could depend on whether trees can adapt to the new conditions by adapting aboveground organs, or its belowground uptake organs (through larger belowground allocation, or morphological, anatomical, physiological adaptations). Adaptations of the root system to a changing environment could be particularly relevant, because roots support plant growth with both, nutrient (higher potential demand under higher carbon supply), and water supply (higher potential demand is expected due to water stress, less precipitation and more transpiration). In fact, summer droughts have been shown to reduce forest productivity throughout Europe (Ciais et al. 2005; Dale et al. 2001; Jactel et al. 2012) and summer drought is predicted to increase in Central and Southern Europe over the next decades, with up to 40% less precipitation in summer for the years 2071-2100 (Dankers and Hiederer 2008; IPCC 2014) (Fig. 1.1). According to future climate scenarios, tree plantations and natural forests are expected to have to cope with drought events that may entail reduced forest productivity (woody biomass) due to reduced tree growth or higher tree mortality (Klein et al. 2019). There is a need for adaptive forestry strategies to meet the social demand for timber production (Gardiner and Moore 2014) and climate change mitigation through increased carbon sequestration (Bonan 2008; Seidl et al. 2014). Increasing tree species mixing in forests and tree plantations may be a solution for both of these requirements. The insurance hypothesis predicts that forests with tree species mixtures may resist better to stressful environmental conditions than forests composed of monocultures. Moreover, mixed tree species stands may increase forest productivity as well as contribute to higher C storage in soils (Dawud et al. 2017). For example, tree species richness can have an impact on soil carbon stocks through altered litter

decomposition rates, nitrogen fixation and rooting patterns, as well as on the water balance, soil microclimate and nutrient availability (Böttcher and Lindner 2010), with mixed plant communities potentially having higher carbon stocks than monocultures (Gamfeldt et al. 2013). Moreover, mixed species forests may create higher habitat diversity for other groups of species and increasing their abundance and/or diversity (Ampoorter et al. 2020), including soil biota such as macroarthropods, earthworms, enchytraeids, collembola, nematodes, isopods, acari, bacteria, protozoa, archaea, and fungi (Briones 2014). A higher diversity of soil biota, in particular, may mediate the supply of ecosystem services, for example by suppressing diseases, degrading pollutants, stimulating soil formation and water infiltration and through their effect on soil carbon dynamics contributing to climate regulation (Nielsen et al. 2015).

Scenario RCP 4.5 (moderate)

Scenario RCP 8.5 (severe)



**Fig. 1.1** Projected change in meteorological drought frequency between the present (1981-2010) and the mid-century 21st century (2041-2070) in Europe, under two emissions scenarios; RCP 4.5 (moderate), RCP 8.5 (severe). Data source: Projections of future meteorological droughts in Europe provided by Joint Research Centre (JRC)

## 1.2 Importance of roots for nutrient cycling

The primary function of roots are anchorage and uptake of nutrients and water from soil. Roots achieve this, by exploring and exploiting the soil space, vertically, horizontally, and by adapting morphological root traits (such as root length density, root diameter, architecture), and physiological root traits, such as root respiration, nutrient uptake kinetics and root tissue nutrient contents (Bolte and Villanueva 2006; Göransson et al. 2007; Liese et al. 2017). Additionally, roots release C in the form of exudates, respire C in the form of  $\text{CO}_2$ , and form associations with mycorrhizal fungi (Chen et al. 2000; Soudzilovskaia et al. 2015). The different ways with which roots can take up water and nutrients, and how they participate to soil C dynamics, positions roots as a primary essential link between living plants and the environment with respect to nutrient uptake.

Nutrients can be transported by mass flow or diffusive processes to the soil-root interface or associated symbiotic structures, where they are taken up by the roots. The part of the nutrients that are not used for root growth and function are then transported via xylem conduits to aboveground parts, where they are used for maintenance of existing living tissues and to produce stems, branches, twigs and foliage. During tissue senescence, plants recover a variable amount of nutrients through resorption, before dead organs such as leaves are deposited onto the soil, where they decompose and release nutrients during the mineralization of organic molecules that become potentially available to plants. The decomposition of leaf litter is recognized as a critical pathway linking above- and belowground processes (Polyakova and Billor 2007; Xuluc-Tolosa et al. 2003). Although leaf litter is an important source of nutrients for plants in most unmanaged terrestrial ecosystems, other plant organs, above all roots also contribute to organic matter derived nutrients. Their relative importance strongly depends on soil fertility and increases in particular on old and weathered soils, for example in some tropical ecosystems (Vitousek and Farrington 1997). Fine roots for example, represent 13–60 % of net primary production in different forest ecosystems (Brunner and Godbold 2007; Jackson et al. 1997; Ostonen et al. 2005), and the deposited fine root litter constitutes up to 30–46 % of total litter production (total of leaf and root litter) (Godbold et al. 2003; Joslin and Henderson 1987). These root deposits (also called ‘root litter’) contribute 18–58 % of total nitrogen (N) returned to forest soils from decomposing plant litter, and can thus be higher than that contributed by aboveground litterfall in some ecosystems (Vogt et al. 1986). Via associated mycorrhizal fungi that scavenge nutrients from soils and transfer a portion of these nutrients to their host plant in return for labile plant C (Smith & Read 1997), the plant-mycorrhizal association may promote belowground storage of C, in the form of root remains, their associated mycelium, and microbially transformed C. As such, roots and their associated mycorrhizae may determine soil C dynamics to a much larger extent than leaf litter decomposing at the soil surface as it was suggested for a boreal forest ecosystem (Clemmensen et al. 2013).

The predicted increasing drought occurrence with climate change, may force tree root systems to adapt through biomass allocation, changes in anatomy, physiology and even in the composition of the mycorrhizal community associated to tree roots (Hertel et al. 2013; Rewald et al. 2011; Weemstra et al. 2017), and these adaptations have implications for ecosystem functioning (Brunner et al. 2015). For example, more severe summer droughts in a future warmer climate may substantially alter C allocation between above- and below-ground, as trees may respond with a large increase in the size and productivity of the fine root system (Hertel et al. 2013), or by investing in deeper roots (Germon et al. 2020; Iversen 2010). If trees would invest in deeper roots, this may result in soil C sequestration within deeper soil layers. At the same time, under extreme drought events there may be increased fine root mortality in the top soil layers which increases the total flux of root carbon and nutrients that enter the top soil via necromass (Majdi and Öhrvik 2004). Drought can also influence root anatomy (anatomical conduits might narrow down, creating denser and less decomposable roots) (Kozłowski and Pallardy 2002) and root biochemistry (for example lignification or suberisation of roots can slow down decomposition) (Steudle 2000). While it is largely recognized that climate change can lead to substantially altered aboveground

litter production and decomposition, there is a lack of knowledge on how trees will respond belowground, and how this may affect belowground nutrient and carbon dynamics.

### **1.3 General aspects of biodiversity belowground**

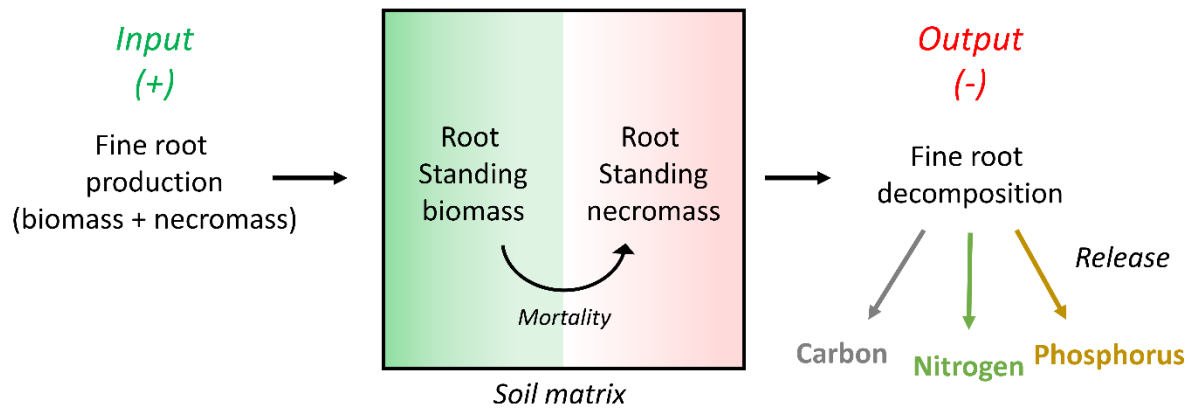
The generally positive relationship between plant diversity and productivity is well established with an increasing number of studies reporting increasing productivity with increasing plant diversity. This increased productivity is often referred to as 'overyielding' (i.e. when a species mixture results in higher aboveground productivity than the average productivity of their component monocultures) in the biodiversity – ecosystem functioning literature (Jactel et al. 2018). Higher aboveground productivity has been hypothesized to occur due to complementary use of resources with higher plant diversity, due to higher niche space filling and thus better resource partitioning by more species (Tilman et al. 2001). A key assumption of this hypothesis is that species differ in their traits and/or growth strategies permitting co-existence while allowing at the same time a more efficient uptake of resources such as light, nutrients, or water at the community level (Bauhus et al. 2017). Hence, a tree community that is composed of different species is likely functionally more diverse with a higher efficiency of resource capture than a community composed of a single species. Despite evidence that diverse forests are able to support higher levels of ecosystem functioning than forests with low species numbers (Gamfeldt et al. 2013), this positive relationship between biodiversity and ecosystem functioning may vary considerably among forest types, developmental stages of forests, geographic regions, and in relation to climatic conditions (Ammer 2019; Ratcliffe et al. 2017). The functional diversity of plant communities is largely described and quantified by aboveground plant traits that are then used for a better mechanistic understanding of biodiversity effects, but root traits remain critically understudied (Bardgett et al. 2014).

Positive diversity effects on root biomass (belowground overyielding) have been shown to be a consequence of an improved space filling of the soil volume, or of reduced competition due to a higher variation in root traits in species-rich stands, and thus lower interspecific competition compared to intraspecific competition of species-poor or single-species stands (Casper et al. 2000; Goldberg et al. 1999; Hodge 2004; Rajaniemi 2007). Previous studies on grasslands showed that herbaceous communities including both shallow- and deep-rooted species filled the available soil volume better (Dornbush and Wilsey 2010). If diverse plant communities use the available soil volume complementarily, the community may have higher total resource uptake, standing root biomass, belowground and aboveground productivity compared with less diverse communities (Husse et al. 2016; Mueller et al. 2013; Oram et al. 2018; Prechsl et al. 2015; von Felten et al. 2012). This phenomenon, named 'spatial resource partitioning', is commonly referred to as a potential driver of positive biodiversity–ecosystem functioning relationships in grasslands. However, the few existing studies on tree communities reported ambiguous results. For example, increasing tree species diversity was reported to increase (Brassard et al. 2011, 2013; Meinen et al. 2009a; Schmid and Kazda 2002), have no effects (Frutieux et al. 2018; Meinen et al. 2009b) or even to decrease root productivity (Bolte and Villanueva 2006).

Similarly, vertical root segregation has been found in some (Bolte and Villanueva 2006; Schmid and Kazda 2002), but not in all of the studies investigating root distribution along the soil profile (Fruleux et al. 2018; Meinen et al. 2009b), which appears to be an important aspect to consider among other root traits (Barry et al. 2020; Zeng et al. 2020). A recent meta-analysis on herbaceous plants provided little evidence for a relationship between 'spatial resource partitioning' and belowground overyielding (only 3 out of 21 tested datasets showed a positive relationship, Barry et al. (2020)). Similarly for trees, the study by Zeng et al. (2020), found that roots growing into deeper, still unexplored soil layers were not sufficient contributors to the positive diversity-function relationship. However, the existing tree studies that have investigated how tree species compete along the vertical soil profile, investigated stand density and tree stand age (Schmid and Kazda 2002; Curt and Prévosto 2003; Bolte and Villanueva 2006; Fruleux et al. 2018) but not water availability. For instance, if the top soil layers are regularly dry during the summer months, plants will likely have part of their fine roots distributed in deeper layers as an important adaptation for plant survival (Padilla and Pugnaire 2007), resulting in more evenly distributed resource use across the whole profile (Mueller et al. 2013). This could well be important, since benefits of species mixing may be more pronounced on nutrient-poor and dry sites than on nutrient-rich and wet sites (Pretzsch 2013). This leads to the question, as to whether asymmetrical vertical root distributions (this implies roots from neighbouring tree species occupying different soil layers) could explain part of the belowground overyielding in mixed forests, and if water limitation may reinforce this effect.

## 1.4 Fine root dynamics

Fine root dynamics comprise the following three processes: fine root productivity, turnover and decomposition (Fig. 1.2). Fine root productivity is the amount of fine root biomass produced per unit of time, typically on an annual basis. Fine root turnover refers to the rate at which fine roots are replaced and is usually calculated by dividing fine root production after variable periods of observation by living root standing biomass (Gill and Jackson 2000; Joslin et al. 2000). Fine root decomposition is defined as the rate at which dead roots decompose within the soil. A high and frequent input of fine root biomass, paired with slow decomposition rates may account for higher soil carbon buildup due to direct plant litter contribution to the soil organic matter, or due to the contribution from products of microbial transformation of plant litter (Cotrufo et al. 2015). On the other hand, faster decomposition rates imply that nutrients from plant litter will be rapidly available for subsequent uptake by the trees. The rate at which fine root biomass turns over and fuels nutrient fluxes via decomposition in forests, may influence soil fertility and the potential for aboveground productivity (Hobbie 2015); this is especially important in the context of climate change, as the expected longer and more frequent summer droughts may interrupt fine root growth and decelerate decomposition (IPCC 2014).



**Fig. 1. 2** Simplified scheme of pools and fluxes.

### 1.4.1 Environmental effects on fine root dynamics

The processes that comprise root nutrient cycling are generally influenced by climate, and soil fertility (Bakker et al. 2009; Brunner et al. 2015; McCormack and Guo 2014; Silver and Miya 2001). Previous studies on trees, that investigated simultaneously fine root production and turnover, reported that lower water availability may either: (i) simultaneously increase fine root production and turnover (Santantonio and Hermann 1985); (ii) decrease fine root production and turnover (Majdi and Andersson 2005); (iii) decrease production, but increase turnover (Meier and Leuschner 2008); or (iv) may have no influence on either (Joslin et al. 2000; Rytter 2013). Contrasting results have also been found relative to decomposition as fine root decomposition has been found to decrease (Gaul et al. 2008), increase (Zhang and Wang 2015a), or remain unchanged (King et al. 1997) under decreased water availability. These variable effects of water availability may be related to the general environmental context of the studies, including the intensity of water stress and to plant species-specific differences, including root traits such as root diameter and root tissue density. Moreover, there are not enough studies that measured fluxes of nutrients by roots, by combining all three processes in a holistic manner (Guo et al. 2006; Morozov et al. 2018; Palviainen et al. 2004). More studies manipulating water supply are needed to improve our understanding of the potential effect of lower water availability on fine root dynamics, and how nutrients move between the different pools.

### 1.4.2 Biodiversity effects and resource availability

Mixed species forests have been shown to better withstand drought episodes and fire-outbreaks with higher rates of tree survival than monocultures (Klein et al. 2019). It was further shown that nutrient cycling rates increased in species-rich compared to species-poor forests with increasingly unfavourable environmental conditions in relative terms, suggesting that species rich forests may adapt better to changing environmental conditions (Ratcliffe et al. 2017; Richards et al. 2010). Ratcliffe et al. (2017) found that water availability was the most important environmental factor in changing the relationship between tree species richness and forest functioning and



that the positive effects of species richness should increase with decreased water availability. However, the latter study did not include root decomposition among the processes that represented nutrient cycling (tree productivity and decomposition essentially). Whether such positive diversity effects under reduced water availability may also be observed for fine roots and to what extent, is unknown.

Some studies argued that the positive diversity effects on nutrient cycling may be modulated by other stand characteristics than tree species diversity, such as stand density, that can result in variable interspecific interactions depending on the stand density (Finér et al. 2011; Forrester and Bauhus 2016). The effect of stand density is likely to depend on the resources that are influenced by the species interactions (water or nutrients), as trees can preferentially acquire water and nutrients from different soil depths and in different forms (in the case of nutrients) (Göransson et al. 2007; Grossiord et al. 2014; Kulmatiski et al. 2017). Usually, resource requirement increases with increasing stand density, which may affect resource availability depending on the general environmental context, most importantly on soil fertility. This leads to the question: What is the relative contribution of stand characteristics (such as stand density) and water availability in how tree mixture affect fine root dynamics?

## 1.5 Decomposition

Decomposition of plant litter is the onset for C and N cycling in the soil. The major factors that regulate decomposition rates of dead plant tissues are: i) climate (above all temperature and moisture) (Aerts 1997; Berg and Meentemeyer 2001), ii) initial litter quality (e.g. nitrogen content (N) (Tierney et al. 2003), carbon:nitrogen ratio (C:N) (Berg and Ekbohm 1991), lignin content and lignin:N ratio (Aerts 1997), and iii) the decomposer organisms (Bradford et al. 2016; Hättenschwiler et al. 2005). The 'leaf economics spectrum' provides a simple framework to characterize plant growth strategies along a gradient ranging from the acquisitive strategy with fast growth rates and rapidly turning over leaves with low construction costs to the conservative strategy with slow growth rates and slowly turning over leaves with high construction costs (Wright et al. 2004). A similar framework, the 'Root Economic Space' (RES), was recently proposed for roots (Bergmann et al. 2020), where root traits (such as root N concentration, and root tissue density) vary along a gradient. Hence, root functional traits can be grouped in trait syndromes associated with fast resource acquisition (roots with low root tissue density (RTD), high N concentration, and shorter lifespan typically associated with rapid decomposition), or enhanced resource conservation (high RTD roots with lower N concentration, and longer lifespan typically associated with slow decomposition) (Bergmann et al. 2020; Freschet et al. 2013; Hobbie et al. 2010; Roumet et al. 2016). Initial litter traits are a key determinant for decomposition and the rate of mineralization of nutrients and carbon. The net release rate of nutrients typically is retarded in litter types with high C to nutrient ratios, because initially, microorganisms that colonize the litter immobilize nutrients with a net increase of the amount of nutrients during an initial stage of decomposition. The dynamics of immobilization and net mineralization depend on the C:N, and C:P stoichiometry of litter material and their microbial decomposers and is strongly modulated by soil fertility (Hobbie 2015; See et al. 2019). For example, litter with higher initial values of N, may lead to higher N release

in the initial stages of decomposition, since it matches the lower C:N ratio of microbes. But microbial immobilization of N (which is exogenous and presumably controlled by N availability in the environment) might occur right after (Guo et al. 2006; Palviainen et al. 2004; Parton et al. 2007). The timing of net nutrient release and immobilization, thus depends on the C:nutrient ratios of the litter substrate and to a minor part of that of the microbial decomposers, as well as on soil nutrient availability. Microbial decomposers inhabit the soil, and range from small microorganisms such as bacteria and fungi to microfauna (e.g., protozoans, nematodes), mesofauna (e.g., collembolans, mites), and large macrofauna (e.g., earthworms, millipedes, spiders). Bacteria, fungi and protozoa are the key drivers of energy and nutrient transformations, whereas the larger decomposer organisms such as earthworms, millipedes, and isopods are the dominant habitat transformers (Hattenschwiler et al. 2005). Microbes decompose litter through the production of exo-enzymes that brings particulate organic matter into solution (which contains dissolved organic C and N), which serves as the source for microbial uptake of nutrients. At the same time, larger soil fauna also affect the soil N cycle, as grazing microbial population by fauna releases N and the contribution of the fauna to net N mineralization may be relatively high (Osler and Sommerkorn 2007). For example in a study from Schröter et al. (2003), the authors estimated that fauna was more important for N than for C mineralization, as well as Berg et al. (2001) who showed an immobilizing effect of bacteria, but important contributions to N mineralization from amoebae, predaceous mites, and spiders in a Scots pine forest. In the latter study, soil fauna's contribution to N mineralization was even greater than the contribution of fungi. These studies show that in forests, where N may be more limiting, microbial contributions to net N mineralization decline and the contribution of soil fauna to N mineralization through microbial grazing increases. The inclusion of larger soil organisms is thus important as it might assist in N cycling, even more than C cycling.

### **1.5.1 Climate and trait control on the decomposition of leaves and roots**

Climatic factors may not be of equal importance for leaf and root decay. Silver and Miya (2001) found that environmental variables (mean annual temperature, mean annual precipitation, latitude, elevation, soil texture) play a secondary role for root litter decomposition, with litter quality (nutrient concentration, concentration of secondary compounds, and C:N ratios) being considered as primary, whereas the opposite may be observed for leaves (Zhang et al. 2008; but see Cornwell et al. 2008). The Silver and Miya (2001) study was a meta-analysis with a global data set, and included fine root data for conifer and broadleaf tree species, across multiple latitudinal and altitudinal gradients. Fine root decomposition decreased with increasing latitude and altitude, with mean annual temperature and mean annual precipitation having a positive effect on decomposition. In addition, fine roots decomposed significantly faster in clay loam soils than in other soil texture types (stony, sandy, sandy loam, clay etc), meaning that soil texture that encompasses complex edaphic factors such as moisture (Castanha et al. 2018), and aeration (O<sub>2</sub> concentrations ; Neira et al. (2015)) is an important factor for decomposition. In addition, climate indirectly influences initial leaf litter quality (Zhang and Wang 2015b), but the same may not be expected for roots. That is because roots are constrained by soil physical forces or nutrient limitations, and

can develop different uptake strategies (Weemstra et al. 2016), irrelevant to the climatic conditions. Therefore, even though the same quality parameters are measured for roots and leaves (mineral nutrient concentrations, the concentrations of secondary compounds, as well as C: nutrient and nutrient: nutrient ratios), roots perform fundamentally different physiological functions than leaves, which could lead to different chemical compositions (e.g. roots being more recalcitrant than leaves). For example, in Hobbie et al. (2010), high hemicellulose concentrations and thinner roots were associated with more rapid decomposition belowground, while low lignin and high Ca concentrations were associated with rapid aboveground leaf decomposition. In Sun et al. (2018), non-lignin carbon compounds controlled root tip decomposition, while in contrast the lignin:nitrogen ratio controlled leaf litter decomposition.

### **1.5.2 Direct and indirect effects of tree diversity on decomposition**

In natural ecosystems, litter from a particular plant species typically occurs in mixtures together with litter from other species. According to Grime's mass-ratio hypothesis, the relative contribution of the different plant species to the litter pool determines the community weighted mean (CWM) traits of the decomposing litter mixtures (Grime 1998). Decomposition rates of litter mixtures should then be perfectly predictable from single litter species decomposition, with purely additive effects. An additive effect means that the decomposition rate of a mixture is the average rate of the individual litters corrected by their relative abundance in the mixture. However, mixtures of litter from different species often decompose at different rates than expected from their component species (Gartner and Cardon 2004; Hättenschwiler et al. 2005). With a literature review, Gartner and Cardon (2004) reported non-additive effects of mixtures on litter mass loss in the majority of cases (67%), most of them synergistic (i.e. faster decomposition than expected), with up to 20% higher litter mass loss than expected, but also some antagonistic effects (i.e. slower decomposition than expected). Potential mechanisms for non-additive litter mixture effects on decomposition include fungi-driven nutrient transfer among litter species, inhibition or stimulation of microorganisms by specific litter compounds, and positive feedback of soil fauna due to greater habitat and food diversity (Hättenschwiler 2005). A number of studies showed that litter mixture effects are not determined by the number of litter species present in mixtures (species richness), but by the diversity of functional traits of the present species (functional diversity) (Gessner et al. 2010; Handa et al. 2014; Kou et al. 2020). In another study, Joly et al. (2017) suggested that this functional diversity might on one hand directly influence decomposition within the litter mixture, but on the other hand, indirectly affect decomposition via changes in understory environmental condition, like the specific microclimate in which litter decomposes and soil biota community composition that are in part determined by the functional diversity of living plants. Species-specific canopy characteristics can affect microclimate, i.e. ground temperature, evapotranspiration and moisture conditions, via shading, interception of precipitation and wind break (Prescott 2002). Moreover, the canopy composition dictates the physical litter layer structure of the forest floor, through deposition of leaf litter with distinct shapes and morphologies. In turn, litter layer structure can modify decomposer activity, for example through changes in litter water-holding capacity

(WHC) (Wardle et al. 2003). Also, the long-term input of diverse litter may lead to local adaptation of the soil decomposer community and consequently to a higher efficiency of litter decomposition. For example, decomposer communities may be more diverse with a more diverse litter input because it may promote greater resource partitioning among the soil organisms (Hooper et al. 2000). Some studies have shown that diverse litter can modify the composition of microbial communities, with non-additively lower bacterial abundance, and lower faunal abundance in the mixed litter (Ball et al. 2014), or in contrast, higher microbial diversity with increasing plant litter diversity (Chapman and Newman 2010). Other studies found a positive effect of litter diversity on soil invertebrate diversity (Armbrecht et al. 2004; Hansen 1999; Kaneko and Salamanca 1999). The indirect canopy composition effects on microclimate and the decomposition environment may be particularly important in forest ecosystems, as forests can have a very complex canopy architecture depending on the topography and the overstory structure (Gracia et al. 2007). For instance, the relative abundance of tree species of distinct functional types, such as evergreen versus deciduous tree species, may have an important effect on the decomposition environment (Augusto et al. 2015).

Microclimatic effects, as observed for leaf litter decomposition, may be less likely for roots that decompose belowground. Leaf litter decomposes within a litter layer created on top of the forest floor; hence, it is exposed to the local microclimate and to the forest floor's microbial community, which is 'adapted' to rapidly changing microclimatic conditions that occur aboveground. Contrarily, roots decompose in a climatically more buffered environment within the soil with soil microbial communities that may be less adapted to fluctuating environmental conditions (Gallardo and Schlesinger 1994; Grayston and Prescott 2005).

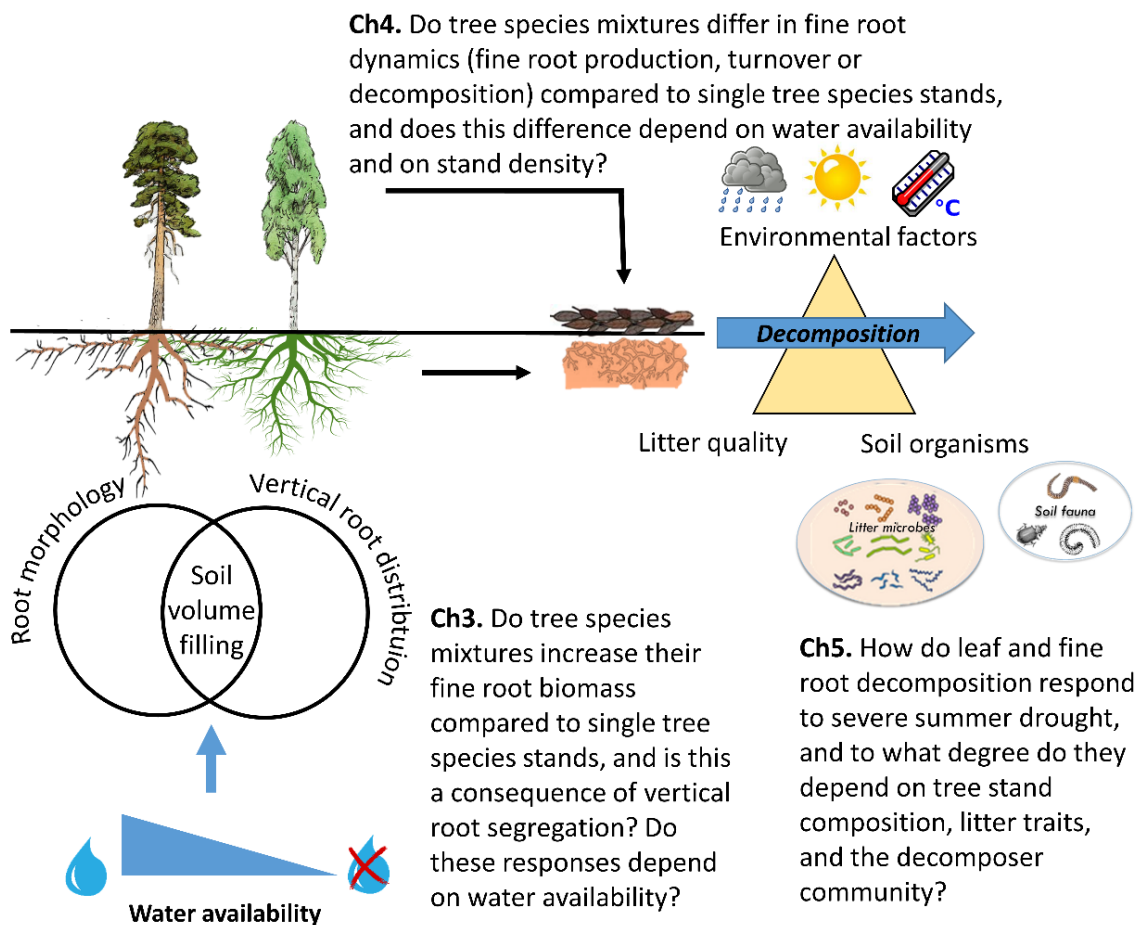
Recognizing the importance of tree stand composition on the decomposer environment for litter decomposition and nutrient cycling, we identify the following as the two main factors that control decomposition for leaves and roots: (i) litter quality and its physicochemical environment (forest floor layer for leaves; soil matrix for roots), (ii) the composition of its decomposer community, which may be tree species-dependent. How such interactions between plant community composition and changing precipitation play out at larger regional scales including different site conditions and plant communities is not well known (Jourdan and Hättenschwiler 2020), particularly for roots.

## **1.6 Rationale and research questions**

According to the insurance hypothesis, biodiversity ensures ecosystems against declines in their functioning because many species provide greater guarantees that some will maintain functioning even if others fail (Naeem and Li 1997). Following this hypothesis, mixed-species plantations are considered one of the main options for adapting to and reducing risks of climate change, as mixed stands tend to show higher resistance, and resilience to drought events than monospecific stands, and at the same time provide with multiple ecosystem services compared to monocultures (Gamfeldt et al. 2013; Hooper and Vitousek 1998; Pardos et al. 2021). It has been suggested that the coexistence of tree species with complementary root traits that improve water availability, water uptake or water use efficiency, is important to cope with drought.

Mixing with tree species that complement each other belowground, may also lead to higher belowground productivity due to reduced interspecific competition. Moreover, species rich tree communities may lead to faster litter decomposition (leading to increased release of nutrients back into the soil) compared to tree monocultures (Chapman et al. 2013; Handa et al. 2014). Also, species rich communities may exhibit an increased uptake rate of nutrients due to a more efficient soil exploration via roots and associated mycorrhizae (Bauhus and Messier 1999). However, such complementarity effects are not always present, and the size of the complementarity effects varies along spatial and temporal gradients in resource availability and climate (Forrester and Bauhus 2016). Furthermore, most studies focus on the productivity and decomposition of aboveground parts, neglecting the importance of roots and their associated mycorrhiza on soil C dynamics (Clemmensen et al. 2013).

Diversity relationships with fine root productivity and decomposition seem to strongly interact with local environmental conditions in a complex manner. Moreover, positive tree diversity effects are more likely when water availability is limiting but most of the evidence rests on aboveground processes (Ratcliffe et al. 2017). In my PhD, I aimed to better understand the processes that drive belowground productivity and decomposition across climatic gradients and how these interact with tree diversity (Fig.1.3). My overarching research question is the following: **What is the interactive effect of climate and tree diversity on roots, and what are the consequences for nutrient cycling?**



**Fig. 1. 3** Schematic overview of research questions addressed in the indicated chapters of my thesis.

My general expectation was that tree species mixtures would accelerate nutrient cycling, and attenuate the expected negative effects of drier conditions. I hypothesized that mixing of tree species with contrasting rooting patterns, and fine root morphologies, would result in a release of competitive pressure belowground, which would translate into higher fine root standing biomass, and increased fine root productivity. Moreover, I hypothesized that roots with contrasting chemical and morphological characteristics would decompose faster in mixed stands. Relative to the drought impact, I hypothesized that tree species mixture effects on decomposition would differ between root- and leaf litter under drought, because of different environmental conditions in the litter layer compared to the top soil.

To test these hypotheses, I carried out a series of experiments within two different field settings: one was a planted tree diversity experiment near Bordeaux (ORPHEE) and one was a latitudinal gradient of natural forests in the Western French Alps (BIOPROFOR). In the tree-plantation experiment, my primary focus was on fine roots, their vertical distribution and standing root biomass and how these are affected by tree species mixtures and reduced water availability. Within the same long-term experiment, I also studied the influence of stand density, another aspect of tree stand characteristics in addition to tree species mixtures, in interaction with water availability on fine root productivity and decomposition over a period of 2 years. Along the gradient of mature forests in the French Alps, I compared leaf and fine root decomposition and how they are affected by mixed species stands and experimentally reinforced and prolonged summer drought using complete rain exclusion during an extended period in summer. The results of these experiments are presented in chapters 3 to 5 that address the following three main questions:

**Chapter 3:** *Do tree species mixtures increase their fine root biomass compared to single tree species stands, and is this a consequence of vertical root segregation? Do these responses depend on water availability?*

**Chapter 4:** *Do tree species mixtures differ in fine root dynamics (fine root production, turnover or decomposition) compared to single tree species stands, and does this difference depend on water availability and on stand density?*

**Chapter 5:** *How do leaf and fine root decomposition respond to severe summer drought, and to what degree do they depend on tree stand composition, litter traits, and the decomposer community?*

## References

- Aerts R (1997) Climate, Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial Ecosystems: A Triangular Relationship. *Oikos* 79:439. <https://doi.org/10.2307/3546886>
- Aitken SN, Yeaman S, Holliday JA, et al (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol Appl* 1:95–111. <https://doi.org/10.1111/j.1752-4571.2007.00013.x>
- Ammer C (2019) Diversity and forest productivity in a changing climate. *New Phytol.* 221:50–66
- Ampoorter E, Barbaro L, Jactel H, et al (2020) Tree diversity is key for promoting the diversity and abundance of forest-associated taxa in Europe. *Oikos* 129:133–146. <https://doi.org/10.1111/oik.06290>
- Armbrecht I, Perfecto I, Varndermeer J (2004) Enigmatic Biodiversity Correlations: Ant Diversity Responds to Diverse Resources. *Science* (80- ) 304:284–286. <https://doi.org/10.1126/science.1094981>
- Augusto L, De Schrijver A, Vesterdal L, et al (2015) Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. *Biol Rev* 90:444–466. <https://doi.org/10.1111/brv.12119>
- Bakker MR, Jolicoeur E, Trichet P, et al (2009) Adaptation of fine roots to annual fertilization and irrigation in a 13-year-old *Pinus pinaster* stand. *Tree Physiol* 29:229–238. <https://doi.org/10.1093/treephys/tpn020>
- Ball BA, Carrillo Y, Molina M (2014) The influence of litter composition across the litter-soil interface on mass loss, nitrogen dynamics and the decomposer community. *Soil Biol Biochem* 69:71–82. <https://doi.org/10.1016/j.soilbio.2013.10.048>
- Bardgett RD, Mommer L, De Vries FT (2014) Going underground: Root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* 29:692–699
- Barry KE, van Ruijven J, Mommer L, et al (2020) Limited evidence for spatial resource partitioning across temperate grassland biodiversity experiments. *Ecology* 101:1–13. <https://doi.org/10.1002/ecy.2905>
- Bauhus J, Forrester DI, Gardiner B, et al (2017) Ecological stability of mixed-species forests. In: *Mixed-Species Forests: Ecology and Management*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 337–382

- Bauhus J, Messier C (1999) Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. *Can J For Res* 29:260–273. <https://doi.org/10.1139/x98-206>
- Berg B, Ekbohm G (1991) Litter mass-loss rates and decomposition patterns in some needle and leaf litter types. Long-term decomposition in a Scots pine forest. VII. *Can J Bot* 69:1449–1456. <https://doi.org/10.1139/b91-187>
- Berg B, Meentemeyer V (2001) Litter fall in some European coniferous forests as dependent on climate: A synthesis. *Can J For Res* 31:292–301. <https://doi.org/10.1139/x00-172>
- Berg M, De Ruiter P, Didden W, et al (2001) Community food web, decomposition and nitrogen mineralisation in a stratified scots pine forest soil. In: *Oikos*. pp 130–142
- Bergmann J, Weigelt A, van der Plas F, et al (2020) The fungal collaboration gradient dominates the root economics space in plants. *Sci Adv* 6:eaba3756. <https://doi.org/10.1101/2020.01.17.908905>
- Bolte A, Villanueva I (2006) Interspecific competition impacts on the morphology and distribution of fine roots in European beech (*fagus sylvatica* L.) and Norway spruce (*picea abies* (L.) karst.). *Eur J For Res* 125:15–26. <https://doi.org/10.1007/s10342-005-0075-5>
- Bonan GB (2008) Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. *Science* (80-. ). 320:1444–1449
- Böttcher H, Lindner M (2010) Managing forest plantations for carbon sequestration today and in the future
- Bradford MA, Berg B, Maynard DS, et al (2016) Understanding the dominant controls on litter decomposition. *J Ecol* 104:229–238. <https://doi.org/10.1111/1365-2745.12507>
- Brassard BW, Chen HYHH, Bergeron Y, Paré D (2011) Differences in fine root productivity between mixed- and single-species stands. *Funct Ecol* 25:238–246. <https://doi.org/10.1111/j.1365-2435.2010.01769.x>
- Brassard BW, Chen HYHH, Cavard X, et al (2013) Tree species diversity increases fine root productivity through increased soil volume filling. *J Ecol* 101:210–219. <https://doi.org/10.1111/1365-2745.12023>
- Briones MJJ (2014) Soil fauna and soil functions: A jigsaw puzzle. *Front. Environ. Sci.* 2:7



- Brunner I, Godbold DL (2007) Tree roots in a changing world. *J For Res* 12:78–82. <https://doi.org/10.1007/s10310-006-0261-4>
- Brunner I, Herzog C, Dawes MA, et al (2015) How tree roots respond to drought. *Front Plant Sci* 6:1–16. <https://doi.org/10.3389/fpls.2015.00547>
- Butchart SHM, Walpole M, Collen B, et al (2010) Global biodiversity: Indicators of recent declines. *Science* (80- ) 328:1164–1168. <https://doi.org/10.1126/science.1187512>
- Casper B, Cahill J, Jackson RB (2000) Casper BB, Cahill JF, Jackson RB. 2000. Plant competition in spatial heterogeneous environments. In: Hutchings MJ, John EA, Stewart AJA, eds. *Ecological consequences of habitat heterogeneity*. Oxford, UK: Blackwell, 111-130. Blackwell
- Castanha C, Zhu B, Hicks Pries CE, et al (2018) The effects of heating, rhizosphere, and depth on root litter decomposition are mediated by soil moisture. *Biogeochemistry* 137:267–279. <https://doi.org/10.1007/s10533-017-0418-6>
- Chapman SK, Newman GS (2010) Biodiversity at the plant-soil interface: Microbial abundance and community structure respond to litter mixing. *Oecologia* 162:763–769. <https://doi.org/10.1007/s00442-009-1498-3>
- Chapman SK, Newman GS, Hart SC, et al (2013) Leaf Litter Mixtures Alter Microbial Community Development: Mechanisms for Non-Additive Effects in Litter Decomposition. *PLoS One* 8:e62671. <https://doi.org/10.1371/journal.pone.0062671>
- Chen H, Harmon ME, Griffiths RP, Hicks W (2000) Effects of temperature and moisture on carbon respired from decomposing woody roots. *For Ecol Manage* 138:51–64. [https://doi.org/10.1016/S0378-1127\(00\)00411-4](https://doi.org/10.1016/S0378-1127(00)00411-4)
- Ciais P, Reichstein M, Viovy N, et al (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437:529–533. <https://doi.org/10.1038/nature03972>
- Clemmensen KE, Bahr A, Ovaskainen O, et al (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* (80- ) 340:1615–1618. <https://doi.org/10.1126/science.1231923>
- Cornwell WK, Cornelissen JHC, Amatangelo K, et al (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol Lett* 11:1065–1071. <https://doi.org/10.1111/j.1461-0248.2008.01219.x>

- Cotrufo MF, Soong JL, Horton AJ, et al (2015) Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat Geosci* 8:776–779. <https://doi.org/10.1038/ngeo2520>
- Curt T, Prévosto B (2003) Rooting strategy of naturally regenerated beech in Silver birch and Scots pine woodlands. *Plant Soil* 255:265–279. <https://doi.org/10.1023/A:1026132021506>
- Daily G, Postel S, Bawa KS, Kaufman L (1997) *Nature's Services: Societal Dependence on Natural Ecosystems* (Chapter 6)
- Dale VH, Joyce LA, McNulty S, et al (2001) Climate Change and Forest Disturbances Climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms, or landslides. *Bioscience* 51:723–734. [https://doi.org/10.1641/0006-3568\(2001\)051\[0723:CCAFD\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0723:CCAFD]2.0.CO;2)
- Dankers R, Hiederer R (2008) Extreme Temperatures and Precipitation in Europe: Analysis of a High-Resolution Climate Change Scenario. *JRC Sci Tech Reports* 82
- Dawud SM, Raulund-Rasmussen K, Ratcliffe S, et al (2017) Tree species functional group is a more important driver of soil properties than tree species diversity across major European forest types. *Funct Ecol* 31:1153–1162. <https://doi.org/10.1111/1365-2435.12821>
- Debouk H, de Bello F, Sebastià M-T (2015) Functional Trait Changes, Productivity Shifts and Vegetation Stability in Mountain Grasslands during a Short-Term Warming. *PLoS One* 10:e0141899. <https://doi.org/10.1371/journal.pone.0141899>
- Dornbush ME, Wilsey BJ (2010) Experimental manipulation of soil depth alters species richness and co-occurrence in restored tallgrass prairie. *J Ecol* 98:117–125. <https://doi.org/10.1111/j.1365-2745.2009.01605.x>
- Finér L, Ohashi M, Noguchi K, Hirano Y (2011) Fine root production and turnover in forest ecosystems in relation to stand and environmental characteristics. *For Ecol Manage* 262:2008–2023. <https://doi.org/10.1016/j.foreco.2011.08.042>
- Forrester DI, Bauhus J (2016) A Review of Processes Behind Diversity—Productivity Relationships in Forests. *Curr. For. Reports* 2:45–61
- Freschet GT, Cornwell WK, Wardle DA, et al (2013) Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. *J Ecol* 101:943–952. <https://doi.org/10.1111/1365-2745.12092>

- Fruleux A, Bogeat-Triboulot MB, Collet C, et al (2018) Aboveground overyielding in a mixed temperate forest is not explained by belowground processes. *Oecologia* 188:1183–1193. <https://doi.org/10.1007/s00442-018-4278-0>
- Gallardo A, Schlesinger WH (1994) Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biol Biochem* 26:1409–1415. [https://doi.org/10.1016/0038-0717\(94\)90225-9](https://doi.org/10.1016/0038-0717(94)90225-9)
- Gamfeldt L, Snäll T, Bagchi R, et al (2013) Higher levels of multiple ecosystem services are found in forests with more tree species. *Nat Commun* 4:. <https://doi.org/10.1038/ncomms2328>
- Gardiner B, Moore J (2014) *Creating the Wood Supply of the Future*. Springer, Dordrecht, pp 677–704
- Gartner TB, Cardon ZG (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos* 104:230–246
- Gaul D, Hertel D, Borken W, et al (2008) Effects of experimental drought on the fine root system of mature Norway spruce. *For Ecol Manage* 256:1151–1159. <https://doi.org/10.1016/j.foreco.2008.06.016>
- Germon A, Laclau JP, Robin A, Jourdan C (2020) Tamm Review: Deep fine roots in forest ecosystems: Why dig deeper? *For. Ecol. Manage.* 466:118135
- Gessner MO, Swan CM, Dang CK, et al (2010) Diversity meets decomposition. *Trends Ecol. Evol.* 25:372–380
- Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *Res New Phytol* 147:13–31. <https://doi.org/https://doi.org/10.1046/j.1469-8137.2000.00681.x>
- Godbold DL, Fritz H-WHW, Jentschke G, et al (2003) Root turnover and root necromass accumulation of Norway spruce (*Picea abies*) are affected by soil acidity. *Tree Physiol* 23:915–921. <https://doi.org/10.1093/treephys/23.13.915>
- Goldberg DE, Rajaniemi T, Gurevitch J, Stewart-Oaten A (1999) Empirical approaches to quantifying interaction intensity: Competition and facilitation along productivity gradients. *Ecology* 80:1118–1131
- Göransson H, Fransson AM, Jönsson-Belyazid U (2007) Do oaks have different strategies for uptake of N, K and P depending on soil depth? *Plant Soil* 297:119–125. <https://doi.org/10.1007/s11104-007-9325-2>
- Gracia M, Montané F, Piqué J, Retana J (2007) Overstorey structure and topographic gradients determining diversity and abundance of understory shrub species in

- temperate forests in central Pyrenees (NE Spain). *For Ecol Manage* 242:391–397.  
<https://doi.org/10.1016/j.foreco.2007.01.056>
- Grayston SJ, Prescott CE (2005) Microbial communities in forest floors under four tree species in coastal British Columbia. *Soil Biol Biochem* 37:1157–1167.  
<https://doi.org/10.1016/j.soilbio.2004.11.014>
- Grime JP (1998) Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. *J. Ecol.* 86:902–910
- Grossiord C, Granier A, Ratcliffe S, et al (2014) Tree diversity does not always improve resistance of forest ecosystems to drought. *Proc Natl Acad Sci U S A* 111:14812–14815. <https://doi.org/10.1073/pnas.1411970111>
- Guo LB, Halliday MJ, Gifford RM (2006) Fine root decomposition under grass and pine seedlings in controlled environmental conditions. *Appl Soil Ecol* 33:22–29.  
<https://doi.org/10.1016/j.apsoil.2005.09.004>
- Handa IT, Aerts R, Berendse F, et al (2014) Consequences of biodiversity loss for litter decomposition across biomes. *Nature* 509:218–221.  
<https://doi.org/10.1038/nature13247>
- Hansen RA (1999) Red oak litter promotes a microarthropod functional group that accelerates its decomposition. *Plant Soil* 209:37–45.  
<https://doi.org/10.1023/A:1004506414711>
- Hartmann H (2011) Will a 385 million year-struggle for light become a struggle for water and for carbon? - How trees may cope with more frequent climate change-type drought events. *Glob Chang Biol* 17:642–655. <https://doi.org/10.1111/j.1365-2486.2010.02248.x>
- Hättenschwiler S (2005) Effects of Tree Species Diversity on Litter Quality and Decomposition. In: *Forest Diversity and Function*. pp 149–164
- Hättenschwiler S, Tiunov A V., Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annu Rev Ecol Evol Syst* 36:191–218.  
<https://doi.org/10.1146/annurev.ecolsys.36.112904.151932>
- Hector A, Schmid B, Beierkuhnlein C, et al (1999) Plant diversity and productivity experiments in European grasslands. *Science* (80- ) 286:1123–1127.  
<https://doi.org/10.1126/science.286.5442.1123>
- Hertel D, Strecker T, Müller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient - Is optimal resource

- partitioning theory applicable to water-limited mature trees? *J Ecol* 101:1183–1200. <https://doi.org/10.1111/1365-2745.12124>
- Hobbie SE (2015) Plant species effects on nutrient cycling: revisiting litter feedbacks. <https://doi.org/10.1016/j.tree.2015.03.015>
- Hobbie SE, Oleksyn J, Eissenstat DM, Reich PB (2010) Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. *Oecologia* 162:505–513. <https://doi.org/10.1007/s00442-009-1479-6>
- Hodge A (2004) The plastic plant: Root responses to heterogeneous supplies of nutrients. *New Phytol.* 162:9–24
- Hooper DU, Bignell DE, Brown VK, et al (2000) Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *Bioscience* 50:1049–1061
- Hooper DU, Vitousek PM (1998) Effects of plant composition and diversity on nutrient cycling. *Ecol Monogr* 68:121–149. [https://doi.org/10.1890/0012-9615\(1998\)068\[0121:EOPCAD\]2.0.CO;2](https://doi.org/10.1890/0012-9615(1998)068[0121:EOPCAD]2.0.CO;2)
- Husse S, Huguenin-Elie O, Buchmann N, Lüscher A (2016) Larger yields of mixtures than monocultures of cultivated grassland species match with asynchrony in shoot growth among species but not with increased light interception. *F Crop Res* 194:1–11. <https://doi.org/10.1016/j.fcr.2016.04.021>
- IPCC (2014) Synthesis Report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.
- Isbell F, Gonzalez A, Loreau M, et al (2017) Linking the influence and dependence of people on biodiversity across scales. *Nature* 546:65–72
- Iversen CM (2010) Digging deeper: fine-root responses to rising atmospheric CO<sub>2</sub> concentration in forested ecosystems. *New Phytol* 186:346–357. [https://doi.org/10.1111/J.1469-8137.2009.03122.X@10.1002/\(ISSN\)1469-8137\(CAT\)VIRTUALISSUES\(VI\)SCALINGROOTPROCESSESGLOBALIMPACTS](https://doi.org/10.1111/J.1469-8137.2009.03122.X@10.1002/(ISSN)1469-8137(CAT)VIRTUALISSUES(VI)SCALINGROOTPROCESSESGLOBALIMPACTS)
- Jackson RB, Mooney HA, Schulze E-DD (1997) A global budget for fine root biomass, surface area, and nutrient contents. *Proc Natl Acad Sci U S A* 94:7362–7366. <https://doi.org/10.1073/pnas.94.14.7362>
- Jactel H, Petit J, Desprez-Loustau ML, et al (2012) Drought effects on damage by forest insects and pathogens: A meta-analysis. *Glob Chang Biol* 18:267–276. <https://doi.org/10.1111/j.1365-2486.2011.02512.x>

- Joly FX, Milcu A, Scherer-Lorenzen M, et al (2017) Tree species diversity affects decomposition through modified micro-environmental conditions across European forests. *New Phytol* 214:1281–1293. <https://doi.org/10.1111/nph.14452>
- Joslin JD, Henderson GS (1987) Organic matter and nutrients associated with fine root turnover in a white oak stand. *For Sci* 33:330–346. <https://doi.org/10.1093/forestscience/33.2.330>
- Joslin JD, Wolfe MH, Hanson PJ (2000) Effects of altered water regimes on forest root systems. *New Phytol* 147:117–129. <https://doi.org/10.1046/j.1469-8137.2000.00692.x>
- Jourdan M, Hättenschwiler S (2020) Decomposition in mixed beech forests in the south-western Alps under severe summer drought 2. *bioRxiv* 2020.05.23.111815. <https://doi.org/10.1101/2020.05.23.111815>
- Kaneko N, Salamanca EF (1999) Mixed leaf litter effects on decomposition rates and soil microarthropod communities in an oak-pine stand in Japan. *Ecol Res* 14:131–138. <https://doi.org/10.1046/j.1440-1703.1999.00292.x>
- King JS, Allen HL, Dougherty P, Strain BR (1997) Decomposition of roots in loblolly pine: Effects of nutrient and water availability and root size class on mass loss and nutrient dynamics. *Plant Soil* 195:171–184. <https://doi.org/10.1023/A:1004248232450>
- Klein T, Cahanovitch R, Sprintsin M, et al (2019) A nation-wide analysis of tree mortality under climate change: Forest loss and its causes in Israel 1948–2017. *For Ecol Manage* 432:840–849. <https://doi.org/10.1016/j.foreco.2018.10.020>
- Kou L, Jiang L, Hättenschwiler S, et al (2020) Diversity-decomposition relationships in forests worldwide. *Elife* 9:1–51. <https://doi.org/10.7554/eLife.55813>
- Kozłowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. *Bot Rev* 68:270–334. [https://doi.org/10.1663/0006-8101\(2002\)068\[0270:AAAROW\]2.0.CO;2](https://doi.org/10.1663/0006-8101(2002)068[0270:AAAROW]2.0.CO;2)
- Kulmatiski A, Adler PB, Stark JM, Tredennick AT (2017) Water and nitrogen uptake are better associated with resource availability than root biomass. *Ecosphere* 8:e01738. <https://doi.org/10.1002/ecs2.1738>
- Liese R, Alings K, Meier IC (2017) Root branching is a leading root trait of the plant economics spectrum in temperate trees. *Front Plant Sci* 8:1–12. <https://doi.org/10.3389/fpls.2017.00315>

- Majdi H, Andersson P (2005) Fine root production and turnover in a Norway spruce stand in northern Sweden: Effects of nitrogen and water manipulation. *Ecosystems* 8:191–199. <https://doi.org/10.1007/s10021-004-0246-0>
- Majdi H, Öhrvik J (2004) Interactive effects of soil warming and fertilization root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. *Glob Chang Biol* 10:182–188. <https://doi.org/10.1111/j.1365-2486.2004.00733.x>
- McCormack ML, Eissenstat DM, Prasad AM, Smithwick EAH (2013) Regional scale patterns of fine root lifespan and turnover under current and future climate. *Glob Chang Biol* 19:1697–1708. <https://doi.org/10.1111/gcb.12163>
- McCormack ML, Guo D (2014) Impacts of environmental factors on fine root lifespan. *Front. Plant Sci.* 5:205
- Meier IC, Leuschner C (2008) Genotypic variation and phenotypic plasticity in the drought response of fine roots of European beech. <https://doi.org/https://doi.org/10.1093/treephys/28.2.297>
- Meinen C, Hertel D, Leuschner C (2009a) Root growth and recovery in temperate broad-leaved forest stands differing in tree species diversity. *Ecosystems* 12:1103–1116. <https://doi.org/10.1007/s10021-009-9271-3>
- Meinen C, Hertel D, Leuschner C (2009b) Biomass and morphology of fine roots in temperate broad-leaved forests differing in tree species diversity: Is there evidence of below-ground overyielding? *Oecologia* 161:99–111. <https://doi.org/10.1007/s00442-009-1352-7>
- Millennium Ecosystem Assessment (2005) Millennium Ecosystem Assessment (2005). *Ecosystems and human well-being: Biodiversity synthesis.* Washington, DC.
- Morozov G, Aosaar J, Varik M, et al (2018) Long-term dynamics of leaf and root decomposition and nitrogen release in a grey alder (*Alnus incana* (L.) Moench) and silver birch (*Betula pendula* Roth.) stands. *Scand J For Res* 34:12–25. <https://doi.org/10.1080/02827581.2018.1521468>
- Mueller KE, Tilman D, Fornara DA, Hobbie SE (2013) Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. *Ecology* 94:787–793. <https://doi.org/10.1890/12-1399.1>
- Naeem S, Li S (1997) Biodiversity enhances ecosystem reliability *Shahid.* 390:507–509

- Neira J, Ortiz M, Morales L, Acevedo E (2015) Oxygen diffusion in soils: Understanding the factors and processes needed for modeling. *Chil J Agric Res* 75:35–44. <https://doi.org/10.4067/S0718-58392015000300005>
- Nielsen UN, Wall DH, Six J (2015) Soil Biodiversity and the Environment. *Annu Rev Environ Resour* 40:63–90. <https://doi.org/10.1146/annurev-environ-102014-021257>
- Oram NJ, Ravenek JM, Barry KE, et al (2018) Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. *J Ecol* 106:265–277. <https://doi.org/10.1111/1365-2745.12877>
- Osler GHR, Sommerkorn M (2007) TOWARD A COMPLETE SOIL C AND N CYCLE: INCORPORATING THE SOIL FAUNA GRAHAM. 88:1611–1621
- Ostonen I, Lõhmus K, Pajuste K (2005) Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: Comparison of soil core and ingrowth core methods. *For Ecol Manage* 212:264–277. <https://doi.org/10.1016/j.foreco.2005.03.064>
- Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct Ecol* 21:489–495. <https://doi.org/10.1111/j.1365-2435.2007.01267.x>
- Palviainen M, Finér L, Kurka AM, et al (2004) Release of potassium, calcium, iron and aluminium from Norway spruce, Scots pine and silver birch logging residues. *Plant Soil* 259:123–136. <https://doi.org/10.1023/B:PLSO.0000020938.78215.bd>
- Pardos M, del Río M, Pretzsch H, et al (2021) The greater resilience of mixed forests to drought mainly depends on their composition: Analysis along a climate gradient across Europe. *For Ecol Manage* 481:118687. <https://doi.org/10.1016/j.foreco.2020.118687>
- Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37–42. <https://doi.org/10.1038/nature01286>
- Parton W, Silver WL, Burke IC, et al (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* (80- ) 315:361–364. <https://doi.org/10.1126/science.1134853>
- Polyakova O, Billor N (2007) Impact of deciduous tree species on litterfall quality, decomposition rates and nutrient circulation in pine stands. *For Ecol Manage* 253:11–18. <https://doi.org/10.1016/j.foreco.2007.06.049>



- Prechsl UE, Burri S, Gilgen AK, et al (2015) No shift to a deeper water uptake depth in response to summer drought of two lowland and sub-alpine C3-grasslands in Switzerland. *Oecologia* 177:97–111. <https://doi.org/10.1007/s00442-014-3092-6>
- Prescott CE (2002) The influence of the forest canopy on nutrient cycling. *Tree Physiol* 22:1193–1200
- Pretzsch H (2013) Facilitation and competition in mixed-species forests analyzed along an ecological gradient. *Nov Acta Leopold* 114:159–174
- Rajaniemi TK (2007) Root foraging traits and competitive ability in heterogeneous soils. *Oecologia* 153:145–152. <https://doi.org/10.1007/s00442-007-0706-2>
- Ratcliffe S, Wirth C, Jucker T, et al (2017) Biodiversity and ecosystem functioning relations in European forests depend on environmental context. *Ecol. Lett.* 20:1414–1426
- Rewald B, Leuschner C, Wiesman Z, Ephrath JE (2011) Influence of salinity on root hydraulic properties of three olive varieties. *Plant Biosyst* 145:12–22. <https://doi.org/10.1080/11263504.2010.514130>
- Richards AE, Forrester DI, Bauhus J, Scherer-Lorenzen M (2010) The influence of mixed tree plantations on the nutrition of individual species: A review. *Tree Physiol.* 30:1192–1208
- Roumet C, Birouste M, Picon-Cochard C, et al (2016) Root structure-function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol* 210:815–826. <https://doi.org/10.1111/nph.13828>
- Rytter RM (2013) The effect of limited availability of N or water on C allocation to fine roots and annual fine root turnover in *Alnus incana* and *Salix viminalis*. *Tree Physiol* 33:924–939. <https://doi.org/10.1093/treephys/tpt060>
- Santantonio D, Hermann RK (1985) Standing crop, production, and turnover of fine roots on dry, moderate, and wet sites of mature Douglas-fir in western Oregon
- Schmid I, Kazda M (2002) Root distribution of Norway spruce in monospecific and mixed stands on different soils. *For Ecol Manage* 159:37–47. [https://doi.org/10.1016/S0378-1127\(01\)00708-3](https://doi.org/10.1016/S0378-1127(01)00708-3)
- Schröter D, Wolters V, De Ruiter PC (2003) C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos* 102:294–308. <https://doi.org/10.1034/j.1600-0579.2003.12064.x>

- See CR, Luke McCormack M, Hobbie SE, et al (2019) Global patterns in fine root decomposition: climate, chemistry, mycorrhizal association and woodiness. *Ecol. Lett.* 22:946–953
- Seidl R, Schelhaas MJ, Rammer W, Verkerk PJ (2014) Increasing forest disturbances in Europe and their impact on carbon storage. *Nat Clim Chang* 4:806–810. <https://doi.org/10.1038/nclimate2318>
- Silver WL, Miya RK (2001) Global patterns in root decomposition: Comparisons of climate and litter quality effects. *Oecologia* 129:407–419. <https://doi.org/10.1007/s004420100740>
- Soudzilovskaia NA, Douma JC, Akhmetzhanova AA, et al (2015) Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. *Glob Ecol Biogeogr* 24:371–382. <https://doi.org/10.1111/geb.12272>
- Steudle E (2000) Water uptake by roots: effects of water deficit
- Sun T, Hobbie SE, Berg B, et al (2018) Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proc Natl Acad Sci U S A* 115:10392–10397. <https://doi.org/10.1073/pnas.1716595115>
- Thomas CD, Cameron A, Green RE, et al (2004) Extinction risk from climate change. *Nature* 427:145–148. <https://doi.org/10.1038/nature02121>
- Tierney GL, Fahey TJ, Groffman PM, et al (2003) Environmental control of fine root dynamics in a northern hardwood forest. *Glob Chang Biol* 9:670–679. <https://doi.org/10.1046/j.1365-2486.2003.00622.x>
- Tilman D, Reich PB, Knops J, et al (2001) Diversity and productivity in a long-term grassland experiment. *Science* (80- ) 294:843–845. <https://doi.org/10.1126/science.1060391>
- Vitousek PM, Farrington H (1997) Nutrient limitation and soil development: Experimental test of a biogeochemical theory. *Biogeochemistry* 37:63–75. <https://doi.org/10.1023/A:1005757218475>
- Vogt KA, Grier CC, Vogt DJ (1986) Production, Turnover, and Nutrient Dynamics of Above- and Belowground Detritus of World Forests. *Adv Ecol Res* 15:303–377. [https://doi.org/10.1016/S0065-2504\(08\)60122-1](https://doi.org/10.1016/S0065-2504(08)60122-1)
- von Felten S, Niklaus PA, Scherer-Lorenzen M, et al (2012) Do grassland plant communities profit from N partitioning by soil depth? *Ecology* 93:2386–2396. <https://doi.org/10.1890/11-1439.1>

- Wardle DA, Nilsson MC, Zackrisson O, Gallet C (2003) Determinants of litter mixing effects in a Swedish boreal forest. *Soil Biol Biochem* 35:827–835. [https://doi.org/10.1016/S0038-0717\(03\)00118-4](https://doi.org/10.1016/S0038-0717(03)00118-4)
- Weemstra M, Mommer L, Visser EJW, et al (2016) Towards a multidimensional root trait framework: a tree root review. *New Phytol.* 211:1159–1169
- Weemstra M, Sterck FJ, Visser EJW, et al (2017) Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. *Plant Soil* 415:175–188. <https://doi.org/10.1007/s11104-016-3148-y>
- Wright IJ, Reich PB, Westoby M, et al (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827. <https://doi.org/10.1038/nature02403>
- Xuluc-Tolosa FJ, Vester HFM, Ramírez-Marcial N, et al (2003) Leaf litter decomposition of tree species in three successional phases of tropical dry secondary forest in Campeche, Mexico. *For Ecol Manage* 174:401–412. [https://doi.org/10.1016/S0378-1127\(02\)00059-2](https://doi.org/10.1016/S0378-1127(02)00059-2)
- Zeng W, Xiang W, Zhou B, et al (2020) Positive tree diversity effect on fine root biomass: via density dependence rather than spatial root partitioning. *Oikos* oik.07777. <https://doi.org/10.1111/oik.07777>
- Zhang D, Hui D, Luo Y, Zhou G (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *J Plant Ecol* 1:85–93. <https://doi.org/10.1093/jpe/rtn002>
- Zhang X, Wang W (2015a) The decomposition of fine and coarse roots: Their global patterns and controlling factors. *Sci Rep* 5:. <https://doi.org/10.1038/srep09940>
- Zhang X, Wang W (2015b) Control of climate and litter quality on leaf litter decomposition in different climatic zones. *J Plant Res* 128:791–802. <https://doi.org/10.1007/s10265-015-0743-6>

## Chapter 2 | Materials and Methods

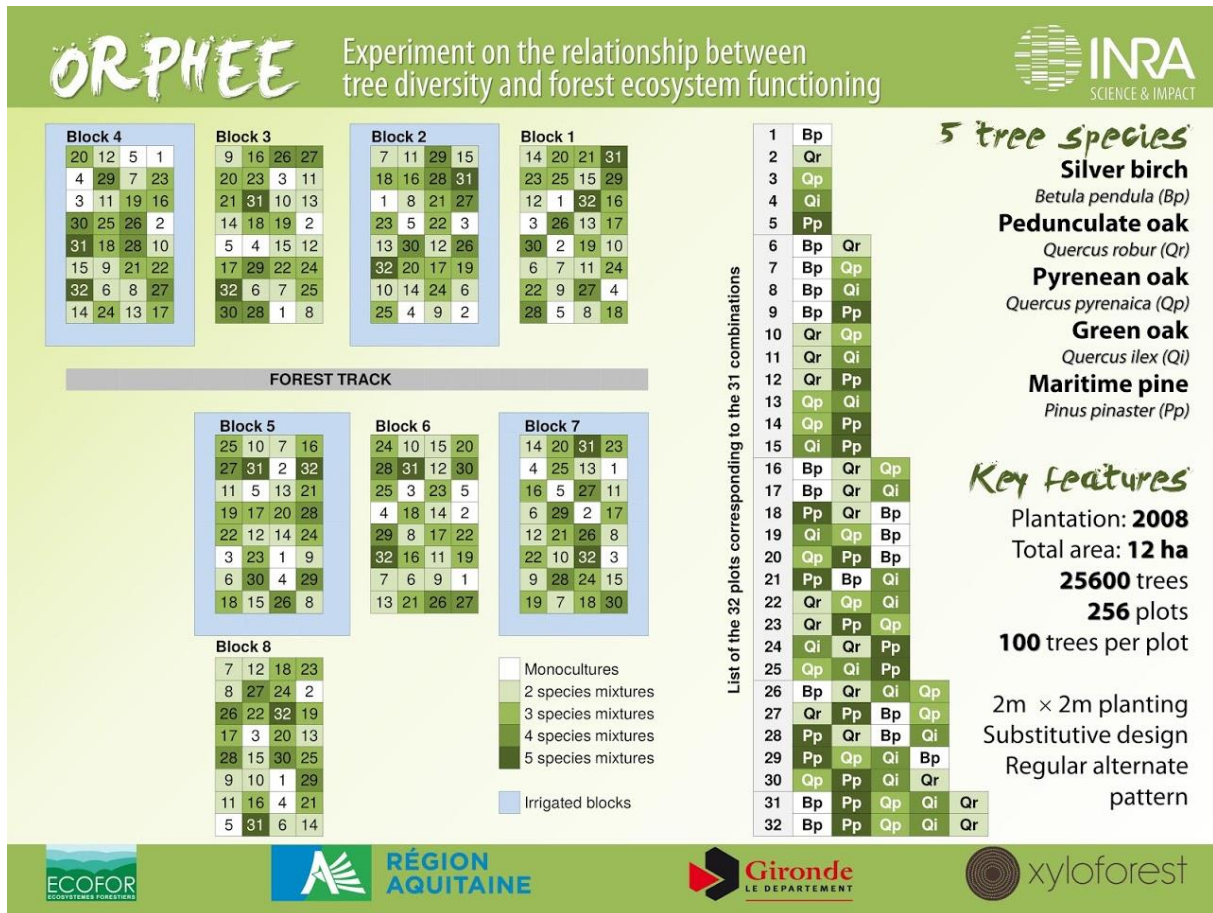
### 2.1 Study design

This study uses both a tree plantation experiment of young even aged trees and a gradient of natural forests to answer the main experimental questions. The tree plantation in a location close to Bordeaux (tree age 10–12 years during the PhD study period) was used to test how mixtures of birch x pine, and irrigation relate to: i) vertical root segregation and belowground overyielding, and ii) to fine root dynamics, measured as fine root production, turnover, decomposition, and their associated nutrient fluxes. The altitudinal/latitudinal gradient of natural forests of beech growing together with fir and oak in the Alps was used to test whether tree mixture (mixture of beech x fir, and mixture of beech x oak), and extended summer drought (rain exclusion with tents) affect the decomposition of beech leaves and beech fine roots in a similar manner. Further data on secondary species (oak on Bordeaux site, fir and oak in the Alpine gradient) or on other measured parameters (hyphal growth, ectomycorrhizal counts amongst others) were excluded from this general design.

### 2.2 Site description

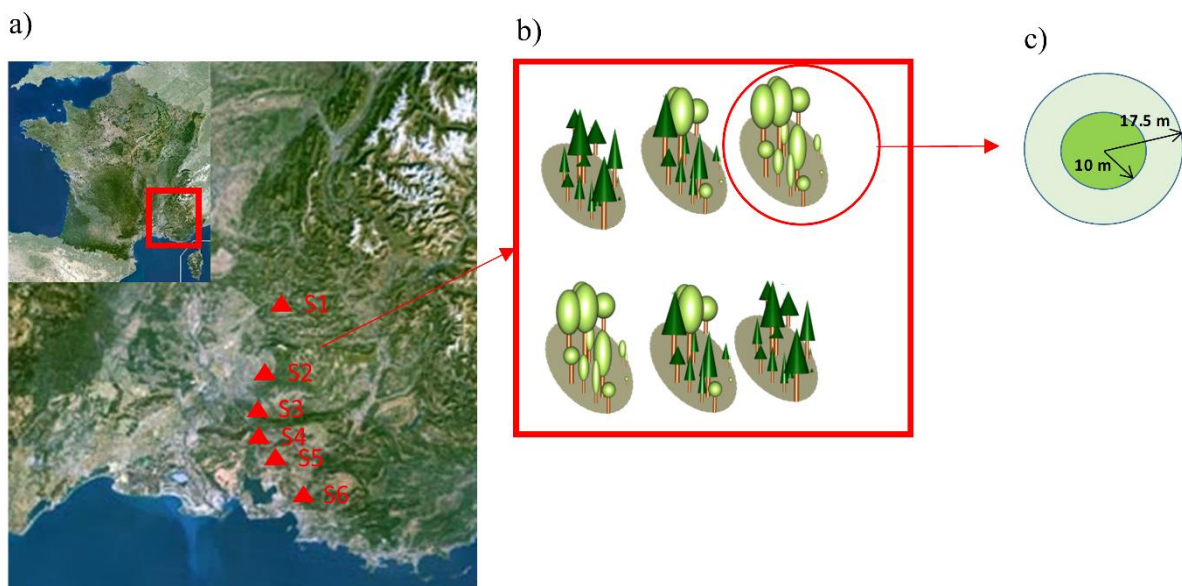
The research was conducted using two experimental platforms in the south of France:

1. ORPHEE experiment: This is a tree species diversity experiment, which was established in southwestern France in 2008 (close to Bordeaux; 44°44' N, 00°47' W) to investigate biodiversity and ecosystem functioning under the influence of summer drought. The experiment consists of a full factorial randomized block design, where five tree species were planted in monocultures or mixtures of 1 to 5 species; i.e. 32 treatments (plots) in each of the 8 blocks (256 plots in total); the tree species and schematic representation of ORPHEE is depicted in [Fig 2.1](#). Each plot contains 10 rows of 10 trees planted 2 m apart, resulting in 100 trees per plot, with a plot area of 400 m<sup>2</sup>. Tree species mixtures were established according to a substitutive design, keeping tree density equal across plots. Within plots, individual trees from different species were planted in a regular alternate pattern, such that a tree from a given species had at least one neighbour from each of the other species within a 2 m radius. Plots are separated by a distance of three metres and were randomly distributed within blocks. Blocks cover an area of 100 × 175 m. A climate manipulation is added by irrigating 4 of the blocks since May 2015, while the other 4 blocks experience ambient water conditions during the summer season. Irrigation consists in sprinkling ca 42 m<sup>3</sup> per night and per block from early May to late September, corresponding to ca 3 mm/day per plot. This volume was calculated based on regional climatic data (evapotranspiration) and is assumed to avoid any soil water deficit in the irrigated blocks during the entire growing season.



**Fig. 2. 1** Schematic representation of the ORPHEE experimental design. Image is taken from the official ORPHEE online site 'https://sites.google.com/view/orpheeexperiment/experimental-design'.

2. BIOPROFOR experiment: This experimental platform comprises of altitudinal transects of mature natural forests across a north - south latitudinal gradient in the French Alps. In its totality, the experimental platform comprises of six sites; from north to south: Bauges, Vercors, Mont Ventoux, Lubéron Lagarde, Grand Lubéron, Sainte Baume. At each location, monocultures of *Fagus sylvatica* L. (Beech, deciduous) can be compared with two-species mixtures of *Quercus pubescens* Willd. (pubescent oak, deciduous, the three southern locations) or *Abies alba* Mill. (silver fir, evergreen conifer, the northern three locations). At each location a series of three stands (two monocultures, one mixture) are present at two locations per site (i.e. one triplet per location) (Fig. 2.2). Two rain exclusion zones (with tents) are added per plot during the summer period. At each triplet, soil probes measuring temperature and moisture are present within the mixed forest plot.



**Fig. 2. 2** (a) Study area and location of the six sites in southeastern France. Northern points (sites S1, S2, S3) represent sites with European beech (*Fagus sylvatica*) and silver fir (*Abies alba*) forests. Southern points (sites S4, S5, S6) represent southern sites with European beech and pubescent oak (*Quercus pubescens*) forests. The sites are located in the following mountain ranges of the French pre-Alps: Bauges (S1), Vercors (S2), Mont Ventoux (S3), Lubéron Lagarde (S4), Grand Lubéron (S5), Sainte-Baume (S6) (b) Schematic representation of a site with two triplets per site. Each triplet is made up of two pure plots and one mixed plot. (c) Representation of an individual plot. Plots are circular with a central plot area (10 m radius) and a buffer zone (7.5 m radius).

## 2.3 Methods

### 2.3.1 Tree species studied and tree inventory

There are six tree species used in this study, comprising three plant families, with two species per plant family (Table 2.1). We regularly measured tree stem diameters for all trees in both experimental sites. The measurements in ORPHEE were done once per year, and in BIOPROFOR they were done once in the middle of the study period.

**Table 2.1** English name, species, families and plant clades used in the study, per experimental site, and their abbreviations.

Species	English name	Family	Clade		Experimental site
<i>Betula pendula</i> Roth	Silver birch	Betulaceae	Angiosperm	Deciduous	ORPHEE
<i>Quercus robur</i> L.	Common oak	Fagaceae	Angiosperm	Deciduous	ORPHEE
<i>Pinus pinaster</i> Ait.	Maritime pine	Pinaceae	Gymnosperm	Evergreen	ORPHEE
<i>Fagus sylvatica</i> L.	Common beech	Fagaceae	Angiosperm	Deciduous	BIOPROFOR
<i>Quercus pubescens</i> Willd.	Pubescent oak	Fagaceae	Angiosperm	Deciduous	BIOPROFOR
<i>Abies alba</i> Mill.	Silver fir	Pinaceae	Gymnosperm	Evergreen	BIOPROFOR

### 2.3.2 Belowground productivity and turnover

#### 2.3.2.1 Fine root growth

To measure belowground productivity, we used the ingrowth core method. The ingrowth cores had a 6 mm Ø mesh, to allow the entry of fine and small roots, and were installed down to a soil depth of 15 cm (Fig 2.3). In order to calculate fine root turnover we collected root standing biomass adjacent to the ingrowth cores, also to a soil depth of 15 cm. Fine root turnover was calculated as the ratio of newly produced roots in the ingrowth cores (biomass + necromass) / living root standing crop, according to Gill and Jackson (2000). Ingrowth cores were harvested at two time intervals (after 1, 2 years), and the root standing biomass was harvested in between. In total 448 ingrowth cores were installed in ORPHEE, and 288 ingrowth cores in the Alps (for number of samples see Tables A2.1 and A2.2 in Appendix A).

#### 2.3.2.2 Hyphal growth

Around each ingrowth core, we buried three hyphal bags at 2-3 cm of depth, with the purpose of quantifying hyphal production belowground (Fig 2.3). The hyphal bags were extracted together with their corresponding ingrowth cores; therefore, they were

harvested at the same time intervals (after 1, 2 years). In addition, and in order to measure hyphal turnover, we buried a third series of hyphal bags in each plot; the installation was next to the initial installation points. To quantify hyphal production and turnover, we adapted the ergosterol extraction protocol methods from Wallander et al. (2010). In total 1568 hyphal bags were installed in ORPHEE, and 864 hyphal bags were installed in the Alpes (for number of samples see [Tables A2.1](#) and [A2.2](#) in Appendix A)

#### *2.3.2.3 Ectomycorrhizal counts*

Subsamples from the collected roots were stored in solution of 10% ethanol. On these root samples, we: i) measured the ectomycorrhizal colonization percentage (number of root tips colonized relative to the total number of root tips), ii) assessed the intersects with mycorrhizal structures (using the intersect method from Tennant (1975) we identified percentage of root length that is colonized by ectomycorrhizae), iii) and we identified the different mycorrhizal exploration types (contact, short distance, medium distance, long distance) according to Agerer (2001).

#### *2.3.2.4 Morphological and chemical traits*

All fine root morphological traits were assessed with the WinRhizo Software (version 2005a, Regents Instruments Inc. Canada). Root samples belonging to root standing biomass, ingrowth cores, and root litterbags and leaf samples belong to leaf litterbags were analyzed for C and N with a Flash EA1112 analyzer, and for P using colorimetric determination with a SEAL AutoAnalyzer 3 HR (SEAL Analytical, Ltd.). Additional chemical analyses on these vegetation samples were carried out on a selected number of samples and concerned the analysis of total Ca, K, Mg and Mn levels in leaves or root tissue. We measured values of C, N, and P, but also Ca, K, Mg, Mn concentrations with the spectrophotometer SpectrAA20 Varian (3 repetitions were performed for each species). Content of cellulose and lignin was determined applying methods according to TAPPI T 222 om-88 for Klason lignin.

### **2.3.3 Vertical root profiles**

In order to investigate the vertical root profile, we combined a standard soil corer device ( $\varnothing$  8 cm, length 15 cm, see 2.3.3.1 standing root biomass) and a mechanical drill attached to a gouge ( $\varnothing$  4 cm, length 1 m) to collect root and soil samples down to 90 cm of soil depth. Forest floor samples were collected within a metal frame. We collected four soil columns from each of the pure plots of birch and pine, mixed plots of birch x pine, and mixed plots of birch x pine x oak. Each soil column was further divided into six layers giving a total of 768 samples (forest floor, and five soil layers per column) (for all soil samples see [Table A2.3](#) in Appendix A).

### **2.3.4 Decomposition**

#### *2.3.4.1 Litter collection*

For the collection of litter material, we followed separate approaches for each experimental site. The litter material for the ORPHEE experiment was collected from

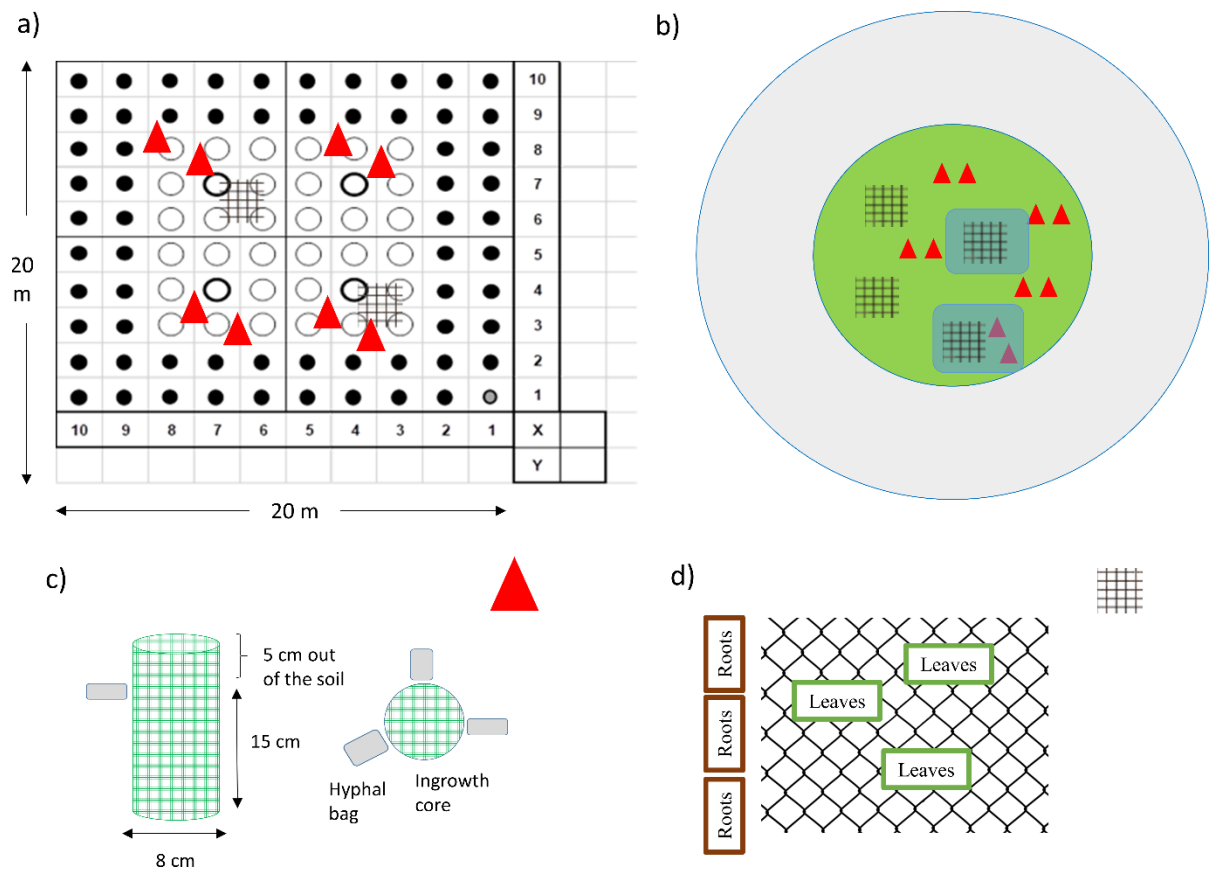


an identical site at Pierroton (3km from ORPHEE) from below Pine, Birch and Oak trees (approximately ~15 – 40 yrs old), and litter material was gently mixed (without damaging it) in order to standardize it. On the other hand, in the Alpine site, site-specific litter was collected from pure plots (of beech, fir, and oak) of each triplet, with the purpose to install it into its site of origin. Roots were collected from June-December 2017 using an 8cm Ø core, or by extracting soil blocks (approx. 20 cm<sup>3</sup>). Leaves were collected from October-December 2017 using litter traps (nets suspended between trees) in the same plots of root litter collection. We selected complete fine roots ( $\leq 2$  mm Ø), and discarded dead roots (criteria to distinguish between live and dead roots were root turgor, and root elasticity). Species identification was conducted by using a morphological key based on periderm structure and color, root ramification, root tip morphology and the type of mycorrhiza developed. Leaves that were not whole, or had at least 75% of leaf area infected from disease or herbivory were discarded.

#### 2.3.4.2 Construction and installation of litterbags

Litterbags with nylon mesh size of 48µm (DIATEX) were chosen to be able to compare leaf litter and root decomposition (and as such to avoid ingrowth of new roots). This mesh size also excludes meso- and macro-fauna from the decomposition process. Each root litterbag (dimensions: 5cm x 9cm) contains 0.5 gr of dry roots, and each leaf litterbag (dimensions: 10cm x 18cm) contains 9gr of dry leaves. All litterbags were installed mid-December 2017. Root litterbags were buried at a depth of 3 – 5cm, and leaf litterbags were placed on top and left to decompose within the natural litter layer of the forest floor. A metal grid was used to protect from wild animals (covered all litterbags). In monospecific stands, we installed litterbags with monospecific material of the same species. For stands with multiple species, we installed both litter with mixed species material and litterbags with monospecific material. In ORPHEE, we installed 192 root litterbags for each species (birch, pine, oak), 48 for mixtures of two species, and 48 for mixtures of three species. In the Alps, and similarly to the ORPHEE site, we took care that our litterbags represented the species combinations for each plot. Therefore, for monospecific stands, we installed litterbags with monospecific material of the same species. For stands with multiple species, we installed both litter with mixed species material and litterbags with monospecific material. So we installed in total 192 root litterbags for beech, 92 root litterbags for oak, 92 root litterbags for fir, and 48 for each mix of two species. In complement to the comparison of root and leaf litter decomposition, we added a total of 320 leaf litterbags with a larger mesh size (2 mm) on the same plots, so as to compare the effect of meso- and macro-fauna on the decomposition of the leaf litter.

In total, we installed 768 root litterbags (48µm Ø) and 768 leaf litterbags (48µm Ø) in ORPHEE, and 480 root litterbags (48µm Ø), 480 leaf litterbags (48µm Ø), and 320 leaf litterbags (2mm Ø) in the Alps (for number of samples see [Table A2.4](#) and [A2.5](#) in Appendix A). For each mixture combination, we performed a harvest of two replicates at different time intervals (after 1, 2 and 3 years). Two subplots were constructed within each plot; the litterbags were placed equally for each subplot ([Fig 2.3](#)).



**Fig. 2.3** Schematic representation of: (a) a plot in ORPHEE, (b) a plot in the Alps, (c) the ingrowth cores (represented by a red triangle) and hyphal bags, and (d) root litterbags installed on the side (brown font) and leaf litterbags (green font) installed in the middle, and under metal grids.

### 2.3.5 Soil analyses

We profited from the abundance of root standing biomass cores collected from ORPHEE and from BIOPROFOR to collect soil subsequently used for soil analyses.

#### 2.3.5.1 Soil analyses of ORPHEE

The harvested vertical root soil profile allowed us to extract 768 soil samples. The 128 of these samples, represented soil collected from the forest floor litter. These forest floor samples were dried at 40 °C until weight stabilization and then ground them up (3 species compositions x 2 water levels x 3 blocks = 18 samples). Soil pH-H<sub>2</sub>O was determined in a water–soil suspension with a mass-to-volume ratio of 1 g : 2.5 mL (NF ISO 10390). The C:N ratios were determined after measuring total C and N by dry combustion (NF ISO 10694 and 13878). Soil C levels were analysed with a CN autoanalyser after dry combustion. Total soil N and P were assessed by extracting their ionique phases (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>2-</sup>) in water, and measuring their concentrations by colorimetry (San++, Automated Wet Chemistry Analyzer, Breda, Netherlands). The total available mineral N was expressed as the sum of mineral N from NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> (µg N g<sup>-1</sup> dry soil) and available mineral P as PO<sub>4</sub><sup>2-</sup> (µg P g<sup>-1</sup> dry soil). After separating roots from soil, we assessed soil moisture for each sample by comparing the fresh soil weight to the dry soil weighed after 72 h at 105°C. Composites of the four cores were made for each soil layer per plot, and these samples were then air-dried for chemical analysis (4 species treat. x 2 water treat. x 4 blocks x 5 depths = 160 samples).

#### 2.3.5.2 Soil analyses of BIOPROFOR

The soil samples were extracted from the root standing biomass cores, and then composites of the four cores were made per plot. The soil samples were sent to an external laboratory of Arras, where the French standard methods (Association Française de NORmalisation; AFNOR, 1999) were used for most of the physico-chemical soil analyses. For soil texture, the five size fractions for clay (< 2 µm diameter), fine loam (2–20 µm), coarse loam (20–50 µm), fine sand (50–200 µm) and coarse sand (200–2000 µm) were assessed after decarbonation (NF X 31–107). Soil pH-H<sub>2</sub>O was determined in a water–soil suspension with a mass-to-volume ratio of 1 g : 2.5 mL (NF ISO 10390). Total organic C and N contents were determined by dry combustion with oxygen (NF ISO 10694 and NF ISO 13878, respectively). Total calcium carbonate contents were assessed using a volumetric method (NF X 31–105).

## 2.4 Data treatment

Due to time constraints and analytical delays, I could not utilize the whole dataset. Therefore, out of the vast possibilities that the two experimental sites had to offer (ORPHEE and Alps), and from the vast datasets collected during this PhD, I decided to work specifically on three data subsets. Two datasets are from the experimental tree plantation of ORPHEE (fine root production and decomposition of birch and pine after 1, 2 years, and vertical root profiles of birch and pine), while the third dataset is from the Alps (2 years of decomposing beech root and leaf litter). The statistical analysis was performed totally in R software (version 3.5.1), and we used mainly a linear mixed effects model (lme package) accompanied post-hoc Tukey's tests (multcomp package). The statistical model approach aimed to investigate for a possible interaction between water limitation and tree species mixtures on root dynamics (biomass, necromass), decomposition (roots and leaves), and root adaptations (vertical investment, fine root morphology).

The following metrics were calculated to assess root nutrient cycling, and the competitive interactions of root systems of young plantations and mature forests under water manipulation (summer irrigation, and/or rainfall exclusion):

- fine root turnover metrics of birch and pine; biomass and necromas/ living root standing biomass) (Gill and Jackson 2000)
- fine root and leaf decomposition (% mass remaining)
- $\beta$  value (Gale and Grigal 1987); indicator of vertical root distribution
- relative yield total (Vandermeer and Goldberg 2013); indicator of belowground overyielding in tree mixtures
- tree basal area (BA), BA adjusted fine root biomass
- specific root length, specific root area, specific root tip, root diameter

## 2.5 Organization of the Result chapters

After the introduction and overview of research sites and methods, the document will now continue with the three result chapters. These three result chapters are followed by a general discussion and conclusion. Tables with information on sampling scheme and number of samples, as well as additional information that could not be incorporated in the three result chapters (see chapter 2.1) are summarized in Appendix A.

**Chapter 3** focuses on the effect of tree species mixture and tree stand density, on fine root productivity and decomposition assessed after 2 years of root growth and decomposition. We test the hypotheses that: (i) annual fine root production, turnover, and decomposition rate will increase in mixed tree species stands compared to their respective monocultures, (ii) summer drought and higher tree density will accentuate the above effects.

**Chapter 4** focuses on the evidence of belowground complementarity. We test the hypotheses that: (i) mixtures of pine and birch trees, will result in higher total relative yield (belowground overyielding), and vertical root segregation, (ii) belowground overyielding, and vertical root segregation will be more pronounced under summer drought, (iii) positive mixture effects will be depth-specific.

**Chapter 5** focuses on the interspecific effect of *Abies alba* and *Quercus pubescens* on *Fagus sylvatica* fine root vs. leaf decomposition, across a latitudinal gradient in the French Alps. We test the hypotheses that: (i) leaf and fine root decomposition will increase when in mixture, (ii) summer rain-exclusion will have a negative effect on both leaf and fine root decomposition, (iii) decomposition will be higher for the northern (wetter) sites, compared to southern (drier) sites.

Finally, I close this thesis by reflecting on my research objectives in **chapter 6**. The General Discussion points out lessons learned by examining the interactive effect of mixture and stressful environmental conditions on belowground processes, discusses the conclusions of the previous chapters and their implications for nutrient cycling.

## References

- Agerer R (2001) Exploration types of ectomycorrhizae: A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107–114. <https://doi.org/10.1007/s005720100108>
- Gale MR, Grigal DF (1987) Vertical root distributions of northern tree species in relation to successional status. *Can J For Res* 17:829–834. <https://doi.org/10.1139/x87-131>
- Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *Res New Phytol* 147:13–31. <https://doi.org/https://doi.org/10.1046/j.1469-8137.2000.00681.x>
- Tennant D (1975) A Test of a Modified Line Intersect Method of Estimating Root Length. *J Ecol* 63:995–1001
- Vandermeer JH, Goldberg DE (2013) *Population ecology: first principles*
- Wallander H, Johansson U, Sterkenburg E, et al (2010) Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. *New Phytol* 187:1124–1134. <https://doi.org/10.1111/j.1469-8137.2010.03324.x>



## Chapter 3 | Effect of tree mixtures and water availability on belowground complementarity of fine roots of birch and pine planted on sandy podzol

Altinalmazis-Kondylis Andreas, Kathina Muessig, Céline Meredieu, Hervé Jactel, Laurent Augusto, Nicolas Fanin, and Mark R. Bakker. 2020. "Effect of Tree Mixtures and Water Availability on Belowground Complementarity of Fine Roots of Birch and Pine Planted on Sandy Podzol." *Plant and Soil* 457 (1–2): 437–55. <https://doi.org/10.1007/s11104-020-04741-8>.

### Abstract

*Aims* We investigated whether tree species growing in mixtures and under different water supply would segregate their fine roots vertically, produce more fine roots overall, or only in specific soil layers.

*Methods* We examined the biomass, morphology, and distribution of fine roots down to 90 cm (forest floor, 0–5, 5–15, 15–30, 30–60, 60–90 cm) in pure and mixed stands of 10-year-old birch and pine trees, planted on a sandy podzol with discontinuous hardpan and seasonal high water table, following a randomized block design with four blocks receiving irrigation and four blocks left unirrigated during summer.

*Results* Our results did not show any vertical root segregation between birch and pine in mixed plots. None of the species overyielded belowground throughout, but pine developed more roots in the top soil layer under irrigation. Both species had shallower fine root distributions in wet conditions, especially birch that was more plastic than pine in response to irrigation.

*Conclusions* Both species followed similar ecological strategies, occupying and competing for the same layers of the soil profile, under both control and irrigated conditions. However, the greater allocation of pine roots at the top soil horizons under irrigated conditions suggests locally favourable niches can lead to depth-specific asymmetric competition. This sheds new light on vertical niche partitioning of young tree mixtures under varying environmental conditions.

**Keywords:** Vertical fine root distribution, fine root biomass, fine root morphology, belowground overyielding, mixed-species plantations, summer irrigation



### 3.1 Introduction

Mixed-species plantations are considered one of the main options for adapting to and reducing risks of climate change (Bauhus et al. 2017; Messier et al. 2015). This is mainly because tree species mixtures have been found to be more resistant to adverse climatic conditions (e.g. drought) (Forrester 2017; Lebourgeois et al. 2013), and to biotic hazards (e.g. herbivory) (Jactel et al. 2017), than monospecific stands. In addition to the higher capacity of mixed forests to stabilize key ecosystem functions such as primary productivity (Jucker et al. 2014; Kardol et al. 2018), an increasing number of studies found that tree diversity increased plant biomass through overyielding (Jactel et al. 2018), i.e. when tree species mixture results in higher aboveground productivity than the average productivity of their component monocultures. Overyielding is often the result of species complementarity in light, water or nutrient resource acquisition (Hooper et al. 2005). Yet, even though scientists and foresters are interested in aboveground overyielding, as higher timber production is desirable, little is known about the belowground adaptations involved in overyielding and if they vary along the soil profile (Jose et al. 2006). Belowground overyielding might indicate the potential for aboveground overyielding, especially since trees with a large aboveground biomass may have a roughly proportional biomass belowground and vice versa (Rewald and Leuschner 2009).

Belowground overyielding in tree mixtures (e.g. increased Relative Yield Total), is a consequence of complementarity (Barry et al. 2019). This is mainly because different tree species may present contrasting root traits (i.e. through differences in fine root morphology), rooting depth strategies (i.e. through vertical root segregation), and/or maximum extensions of their fine root system (Richards et al. 2010; Sudmeyer et al. 2004). Further causes of complementarity may include different specific uptake capacities for water and nutrients, and utilization of different nutrient sources (e.g. different N forms). All of the above enable tree mixtures to explore a larger part of the soil volume, and increase total nutrient and water uptake. Yet, although the number of studies conducted to assess belowground overyielding in forests is increasing in the literature, the results are often contradictory. For example, the outcome of tree diversity on belowground overyielding was reported as positive (Brassard et al. 2011, 2013; Meinen et al. 2009a; Schmid and Kazda 2002), neutral (Fruleux et al. 2018; Meinen et al. 2009b) or even negative (Bolte and Villanueva 2006). Similarly, vertical root segregation has been found in some (Bolte and Villanueva 2006; Schmid and Kazda 2002), but not in all the studies investigating root traits along the soil profile (Fruleux et al. 2018; Meinen et al. 2009b). Such discrepancies in the outcome of tree diversity may be due to the variability in the environmental context such as precipitation, soil fertility, or soil structure, which can all play an important role in explaining the root distribution across contrasting ecosystems (Leuschner et al. 2004; Zanetti et al. 2014), especially since benefits of species mixing may be more pronounced on nutrient-poor and dry sites than on rich and wet sites (Pretzsch 2013).

Vertical root distribution can be shallower or deeper depending on the tree species successional status, soil types or even the biomes in which they are growing (Gale and Grigal 1987; Jackson et al. 1997). For instance, species like birch and spruce invest in

extensive, superficial root systems (Helmisaari et al. 2007; Puhe 2003), while others, like pine, beech, and oak are reported to grow rather deeply (Achat et al. 2008; Bakker et al. 2006; Curt and Prévosto 2003; Rosengren et al. 2005). Overall, tree species adapted to dry climatic regimes feature higher root-to-shoot ratios, greater maximum root depth and distribute their roots to deeper soil layers than trees adapted to humid or mesic-climate conditions (Hartmann 2011; Schmidt-Vogt 1977). This is because when water is limiting, trees could distribute their roots preferentially to those layers having more available soil water; for example, they may intensify root growth deeper into the soil layers to access water necessary for sustaining plant growth (Bakker et al. 2006; Persson et al. 1995; Puhe 2003), with further consequences on the soil volume occupation. Since the top soil layers become dry during the summer months due to evapotranspiration, fine root distribution to deeper layers is an important adaptation for plant survival (Padilla and Pugnaire 2007). Mixing tree species that possess complementary root distribution patterns (e.g. shallow vs. deep-rooted) could thus be essential for optimal water uptake. However, the response of a tree species to irrigation or drought conditions may not relate to its expected root distribution. For example, in a comparison between humid and dry soils, Bakker et al. (2006) showed that drought-resistant pine responds to dry growth conditions (deep water table) by redistributing fine roots at deeper soil layers. In one study on Norway spruce, the same pattern of deeper growing roots was observed in response to rain-exclusion (Persson et al. 1995), while in a rewetting experiment of Norway spruce there was no effect on root distribution (Blanck et al. 1995). Yet, although many studies present data on belowground overyielding and vertical root segregation, only a few have investigated presence of vertical root segregation, and fine root overyielding per soil layer under tree mixtures (Jose et al. 2006), and even less in a context of climate change with longer and more frequently occurring summer droughts.

Our main objective was to evaluate the interactive effect of species mixture and water supply on fine root distribution and morphology, and assess whether they overyield when growing in mixtures. In practice, we examined young dense plantations of previously reported deep-rooted pine (Bakker et al. 2006; Gale and Grigal 1987) and shallow-rooted birch (Curt and Prévosto 2003; Helmisaari et al. 2007; Mauer and Palátová 2018) in pure and mixed stands, irrigated or under ambient precipitation. Specifically, we tested three main hypotheses. First, we hypothesized that mixtures of pine and birch should result in vertical root segregation and higher total relative yield (i.e. “belowground overyielding”). This is because these two tree species present different vertical root distributions and fine root morphologies, thereby leading to a release of the belowground competition and higher fine root biomass for the whole soil column. Secondly, we tested the hypothesis that vertical root segregation and belowground overyielding should be larger in plots, which are not irrigated and thus potentially experience summer drought. This is because when tree species grow in mixed stands and under favourable growth conditions (e.g. increased water availability), interspecific competition would be likely smaller, while under harsher conditions (e.g. decreased water availability), interspecific competition would be stronger (Rewald and Leuschner 2009). In the case that the competition is stronger, the less competitive species may respond with a shift towards less occupied niches (Schenk 2006). Thirdly, we hypothesized that shifts in soil space occupation would

follow a shallow-rooted vs deep-rooted pattern. We expected that birch would grow better in top soil layers while pine would grow better in deeper soil layers. This is because the fine roots of birch are much finer in diameter and should be able to explore and exploit favourable growth conditions more rapidly, hence leading to more growth of birch roots in top soil layers under favourable conditions. Alternatively, because pine roots are thicker and have a more conservative growth strategy (George et al. 1997) they should be able to grow better into less favourable soil layers such as dense soil layers, therefore leading to relatively more root growth in deeper soil layers.

## 3.2 Materials and Methods

### 3.2.1 Study site

The study was carried out 40 km south-west of Bordeaux (44°44' N, 00°47' W) in the ORPHEE experiment (Castagneyrol et al. 2013; Verheyen et al. 2016). This experimental site was established on mesic moorlands, after a clear cut of the preceding maritime pine stands. The soil is a typical podzol with a coarse texture (95% sand), acidic conditions, and is very poor in phosphorus (Augusto et al. 2010). This soil is characterized by a discontinuous cemented spodic horizon (hardpan) at 50 cm depth (Table 3.1). The water table is relatively shallow in rainy wintertime, generally ranging from 40 to 80 cm below the surface, but drops between 150 and 180 cm during the summer months (see Fig. B3.1 in Appendix B).

Silver birch (*Betula pendula* Roth) and maritime pine (*Pinus pinaster* Ait.) possess complementary root systems as they have *a priori* contrasting vertical root distributions and different fine root morphological traits (Gale and Grigal 1987). Our experimental study encompasses eight blocks, with three plots in each block (3 plots × 8 blocks = 24 plots). In 2008, seedlings of birch and pine were planted in each 3 plots as monocultures or in mixtures. Each plot contains 10 rows of 10 planting positions 2 m apart, resulting in 100 trees per plot, with a plot area of 400 m<sup>2</sup>. A buffer zone of 2 m wide (2 lines of trees from each side) was not included in our study, leaving us to work within a surface of 144 m<sup>2</sup> per plot (36 trees per central plot). Tree species mixtures were established according to a substitutive design, keeping tree density equal across plots. In mixed plots, individual trees from the two species were planted in a regular alternate pattern. Thus, in a 16m<sup>2</sup> square, 4 trees of the same species can be found in monospecific plots, or 2 trees per species can be found in mixed plots. Since 2015, half of the blocks receive irrigation every night (3 mm day<sup>-1</sup>) from May to October, the other half experience the dry summer of south-western France (see Fig. B3.2 in Appendix B). Night irrigation was applied to avoid evaporation. The irrigation water was applied with a sprinkler system and is pumped from the water table with a pump system that is nearby to the site. The volume of irrigation (3 mm day<sup>-1</sup>) was calculated based on regional climatic data (evapotranspiration). It is assumed to avoid any soil water deficit in the irrigated blocks during the entire growing season (Castagneyrol et al. 2017; Rahman et al. 2018). The understory vegetation was dominated by bracken fern (*Pteridium aquilinum* L. Kuhn; average soil cover across plots of 18–67%), purple moor-grass (*Molinia caerulea* L. Moench); average soil cover of 3–22%), common gorse (*Ulex europaeus* L.) and dwarf gorse (*Ulex minor* L.; together 3–9% average soil cover) and three Ericaceous species,

common heather (*Calluna vulgaris* L. Hull), bell heather (*Erica cinerea* L.) and besom heath (*Erica scoparia* L.); together 3–5% soil cover. Alder buckthorn (*Frangula alnus* P. Mill.), common blackberry (*Rubus fruticosus* L.) and European honeysuckle (*Lonicera periclymenum* L.) occurred less frequently (see Appendix B, [Table B3.1](#) for a detailed description of understory presence across treatments). The understory was mown once per year in the two first years and not afterwards.

### 3.2.2 Field sampling

The sampling took place in mid-March 2018 (at the very beginning of spring season) when roots are growing slowly or not at all, and that allowed us to minimize soil core losses (soil not too dry neither too wet). We harvested litter and soil cores (90 cm deep) from four sampling points within each plot, with each sampling point located at the centre of square with four alive trees (see [Table 3.1](#) for number of live trees per plot). Firstly, we collected the forest floor litter within a rectangular frame of 10 × 20 cm. Then, the top 0–15 cm of soil was collected manually with a soil corer (8 cm Ø). The bottom 15–90 cm of soil was collected with a mechanical drill, attached onto a gouge (4 cm Ø). We aimed at drilling and collecting soil cores down to 120 cm, but we could not always sample the 90–120 cm layer. The soil from the lower part of the soil column fell out at the lower side of the gouge in many cases, rendering our sampling incomplete. The few samples from 90–120 cm layer that we managed to collect, did not have any roots and roots were rarely found in the lower part of the 60–90 cm layer. Hence, we chose to analyse only samples down to 90 cm, assuming this depth permits to sample all fine roots under our site conditions. The hardpan was discontinuous and not encountered at all the sampling points (51 out of 96 sampling points), but when occurring it was detected at an average depth of about 50 cm below the surface, and it varied from friable to very dense with an average thickness of 17 cm ([Table 3.1](#)). After collection, the soil cores were carefully separated into five layers (0–5; 5–15; 15–30; 30–60; 60–90 cm), and together with the forest floor samples stored at 4°C before further analysis.

A full inventory of stem diameters for all alive trees per plot was performed at the end of 2017 (10 years-old trees since plantation). In June-July 2018, we recorded soil cover of understory vegetation around each sample point (four for each plot) and made inside plot measurements of stem diameters and canopy dimensions of the four bordering trees around each sampling point. For measurements on understory, we used standardized patterns of cover and recordings were done by the same experienced operators. For measurement on trees, their diameters and the longest canopy branches in four directions (two perpendicular to the tree line, two in the direction of the tree) were measured. The canopy extension (in m<sup>2</sup>) of each tree was computed by using the four largest branches and assuming a vertical projection on the forest floor. The canopy ratio was then defined as the canopy extension divided by 4 m<sup>2</sup> (the theoretical space of each tree in the design). Values higher than 1 mean that the tree occupies more than this space (see [Table 3.1](#) for summary descriptors of the stand density, diameter, basal area, and canopy dimension), which is the case here, as pine extended its canopy (average canopy ratio: 1.46 under control conditions, and 1.46 under irrigation) into birch (average canopy ratio: 0.88 under control conditions, and 0.83 under irrigation) and directly competes for light (canopy closure) ([Table 3.1](#)).

**Table 3. 1** Overview table of stand characteristics at 10 years-old. Values are means ( $\pm$  standard deviations) of 36 centre trees per plot (alive and measurable trees at breast height 130 cm) repeated across 8 blocks (4 blocks are irrigated and 4 blocks are not).

	<i>Betula pendula</i>				<i>Pinus pinaster</i>			
	Pure stand		Mixed stand		Pure stand		Mixed stand	
	Control	Irrigation <sup>a</sup>	Control	Irrigation <sup>a</sup>	Control	Irrigation <sup>a</sup>	Control	Irrigation <sup>a</sup>
Number of live trees <sup>b</sup>	31.8 (2.6)	34.3 (1.3)	17.8 (0.5)	17.5 (1.0)	35.8 (0.5)	35.3 (0.9)	17.0 (0.0)	17.8 (0.5)
DBH at 130 cm <sup>b,c</sup> (cm)	5.39 (0.55)	6.62 (0.65)	5.26 (0.69)	5.37 (1.11)	12.7 (0.83)	12.9 (0.79)	14.9 (1.74)	15.3 (0.51)
Stand basal area <sup>d</sup> (m <sup>2</sup> ha <sup>-1</sup> )	5.56 (1.69)	8.74 (1.75)	2.86 (0.685)	3.08 (1.30)	32.6 (3.96)	33.7 (2.89)	21.2 (3.88)	23.4 (1.15)
Canopy ratio <sup>b,e</sup>	0.92 (0.46)	0.97 (0.41)	0.88 (0.44)	0.83 (0.30)	1.02 (0.36)	0.98 (0.55)	1.46 (0.76)	1.46 (0.88)
Hardpan depth <sup>f</sup> (cm)	51.0 (8.62)	51.7 (8.77)	49.1 (6.30)	53.1 (10.1)	51.1 (10.9)	54.8 (12.3)	49.1 (6.30)	54.6 (12.1)
Hardpan thickness <sup>g</sup> (cm)	17.6 (11.1)	17.6 (7.86)	16.3 (9.51)	17.5 (11.7)	17.5 (3.41)	17.0 (10.9)	16.3 (9.51)	18.3 (11.0)

<sup>a</sup> Daily irrigation from May to October equivalent to about 3 mm day<sup>-1</sup>

<sup>b</sup> Mean values based on plot averages for four blocks (max = 36 in pure stand, 18 in mixed stands)

<sup>c</sup> Using quadratic means for mean DBH at 130 cm

<sup>d</sup> Mean values based on the sum of alive and measurable trees at the plot level

<sup>e</sup> Measurements performed on the 4 bordering trees for each sampling point

<sup>f</sup> Depth of hardpan encountered at the sampling date

<sup>g</sup> Hardpan thickness at the sampling date

### 3.2.3 Root sorting

The soil cores were passed briefly on a 2 mm mesh size sieve, in order to separate roots from bulk soil by hand, which is adequate for our sandy soils. The roots retrieved this way were then soaked in water, and fine roots ( $\leq 2$  mm  $\varnothing$ ) were separated into the different target species (birch, pine) and understory species (Bracken, Purple moor-grass, Common Gorse, Bell heather, Common heather, Alder buckthorn, European honeysuckle). Fine roots ( $\leq 2$  mm  $\varnothing$ ) are essential for water and nutrient uptake (Jackson et al. 1997) and are the ones most affected by change in environmental conditions (Ostonen et al. 2007b). Root fractions greater than 2 mm in diameter were not common in our samples and were not considered here. This is also because small cores are not appropriate to investigate medium root and coarse root distributions. We removed dead roots, which we identified by the presence of dark discoloration of the central cylinder and decreased flexibility of root segments (Bauhus and Messier 1999). Birch and pine roots were identified visually according to root colour, epidermis texture, root tip ectomycorrhizal colonization, and root tip ramifications. Both birch and pine are associated with ectomycorrhizal fungi; birch is very ramified, with reddish colour, and smooth epidermis, while pine is much less ramified, with characteristic dichotomous root tips, and rougher epidermis. To recognize between the understory species, we uprooted whole plants of each species and kept them as reference material. In addition, distinctive descriptions were already available from Bakker et al. (2006).

Additionally, after separating roots from soil, we assessed soil moisture for each sample by comparing the fresh soil weight to the dry soil weighed after 72 h at 105°C. Composites of the four cores were made for each soil layer per plot, and these samples were then air-dried for chemical analysis (3 species treat.  $\times$  2 water treat.  $\times$  4 blocks  $\times$  5 depths = 120 samples) (see [Table B3.2](#) in Appendix B).

### 3.2.4 Data collection and computation of root morphology variables

Birch and pine fine roots were scanned using the WinRhizo Software (version 2005a, Regents Instruments Inc. Canada). To measure the fine root morphology, we selected two or three largely intact fine roots ( $< 2$  mm  $\varnothing$ ) as a subsample for scans. The roots were placed in a transparent water filled tray (20  $\times$  30 cm), and roots were spread as much as possible, while trying to keep complete roots intact. The root density was kept at approximately 0.5 mm root per mm<sup>2</sup> surface (Bouma et al. 2000), and the image resolution was 800 dpi. The roots were not stained. After scanning, the scanned roots were oven-dried for four days at 40°C and weighed. This analysis provided data on fine root length, fine root surface area, root tip abundance, which were used to calculate: Specific Root Length (SRL) (fine root length/ dry root weight), Specific Root Area (SRA) (fine root area/ dry root weight), and Specific Root Tip Density (SRTD) (number of root tips / dry root weight). The root morphology indicators were calculated based on existing indicators (Comas and Eissenstat 2009; Godbold et al. 2003; Jagodziński and Kałucka 2011). We further used the scans to extract data on the distribution of fine root length per diameter class (0.0 – 0.5 mm; 0.5 – 1.0 mm; 1.0 – 1.5 mm; 1.5 – 2.0 mm) (see [Fig. B3.5](#) in Appendix B), with a particular interest in the very fine root fraction (diameter class of 0.0 – 0.5 mm  $\varnothing$ ). The very fine root fraction is

usually occupied by 1<sup>st</sup> and 2<sup>nd</sup> root orders (most distal parts of the root system), which have been observed to be more sensitive to environmental factors and to soil depth than higher root orders (Makita et al. 2011; Ostonen et al. 2007b). Hence, we calculated fine root fraction (fine root length per diameter class), as a percentage of the total fine root length; all diameter classes ( $\leq 2$  mm  $\varnothing$  summed). The remaining root fragments (not subsampled for the scans), were also dried and weighed to compute total root parameters for each soil layer. With the dried fine root biomass, we calculated Fine root mass density (FRMD) (dry root weight / soil volume) which we used to investigate how fine root mass density changed with soil depth.

### 3.2.5 Calculation of vertical root distribution index

Following (Gale and Grigal 1987), the vertical distribution of fine roots at each soil core sampling point was described with the ' $\beta$ ' parameter which was determined by non-linear regression using the 'nls' function of the R software (R Core Team 2017) from the formula:

$$Y = 1 - \beta^d$$

where Y= the cumulative root fraction of fine root biomass ( $\text{g m}^{-2}$ ) from the soil surface to depth d (cm), and  $\beta$  a parameter that ranges between 0.90-0.98 (0.90 indicating shallower and 0.98 deeper root distributions). To describe the vertical root distribution further, we also computed the % of fine root biomass (FRB), that were encountered in the top 30 cm and 60 cm of the profile, and  $d50$  which is the depth at which we find 50% of fine roots.

### 3.2.6 Calculation of diversity metrics

In order to assess the direction of diversity effects, we chose to work with the *Relative Yield* metric (RY; de Wit 1960), which is calculated for each component species, and is the division of observed yield of species A in mixture, divided by the mean yield of species A in monoculture. This relative yield can further be calculated for the whole community, resulting in a *Relative Yield Total* (RYT; Vandermeer and Goldberg 2013).

Relative Yield (RY) by species and Relative Yield Total (RYT) for our two-species mixture (50:50) of species A and B, are calculated as follows:

$$RYT = RY_A + RY_B = \frac{FRB_A (mixture)}{FRB_A (monoculture)} + \frac{FRB_B (mixture)}{FRB_B (monoculture)}$$

Yield is expressed as fine root biomass per soil surface area ( $\text{g dry roots m}^{-2}$ ).

RY = 0.5 indicates no diversity effect (i.e. the performance of the species in the mixture is equal to their performance in the monoculture). RY < 0.5 indicates a negative and RY > 0.5 a positive mixture effect. Negative mixture effects mean the species has a lower production in the mixture, while positive mixture effects mean higher production, which could be sign of positive outcome of competition, of facilitation, or of reduced pathogen pressure (Barry et al 2019). Similarly, the mixture effect for the whole

community can be calculated based on (RYT) which is then compared to a reference value of 1 (RYT > 1 indicate community overyielding). Significant differences from reference values were assessed using one-Sample Wilcoxon Signed Rank Test.

### 3.2.7 Data analysis

Statistical data analyses were carried out with R software (version 3.5.1). We used mixed models to assess the effect of tree species (*Betula pendula* vs *Pinus pinaster*), the type of stands (pure vs mixed plots), water treatment (control vs irrigation), and their interaction. Block was included as a random factor to account for the spatial structure of our experimental design. Plots were then nested within the block random factor to enable a comparison of treatments within each block separately. Finally, the sampling replicate number was nested within the plot to account for the non-independency of soil horizons within a soil core of a given plot. Fine root attributes (biomass, length, and surface area) were transformed with the SQRT function; fine root morphology attributes (SRL, SRA, SRTD) were log-transformed. The  $\beta$  parameters were transformed with the logit function. The multcomp package was used to perform post-hoc Tukey's tests between all possible combinations of species  $\times$  mixture  $\times$  irrigation. Relative yield and FRBadj values were too variable; therefore we used the Mann-Whitney U non-parametric test for significant effects of mixture and irrigation. The one-Sample Wilcoxon Signed Rank Test was used to assess overyielding.

## 3.3 Results

### 3.3.1 Overall treatment effects on fine root biomass and morphology

Total fine root biomass (FRB) values of trees for the whole soil profile (0–90 cm and litter) were similar for birch and pine in monocultures, and averaged between 143–297 g m<sup>-2</sup> (Table 3.2). Pure birch stands had the highest value of 297 g m<sup>-2</sup> under irrigation which was nearly doubled compared to the non-irrigated birch monocultures (172 g m<sup>-2</sup>). Under control conditions, pine and birch in mixture averaged similar amounts of total fine root biomass (95 and 99 g m<sup>-2</sup> respectively). Under irrigation, pine represented a larger part of total fine root biomass compared to birch (143 vs 83 g m<sup>-2</sup>). Adjusting for tree basal area, showed that fine root biomass was scaled to aboveground tree size (Table 3.2). Understory species accounted for almost half the fraction of the total fine root biomass (trees and understory species taken together) with some variation (44–65%), but their proportion was roughly similar among the different stands. The distribution of understory species was rather even along the soil profile, and was representative of aboveground presence (see Fig. B3.3 in Appendix B). For the top 15 cm of the soil profile, birch averaged a specific root length (SRL) of 23 m g<sup>-1</sup> and a specific root area (SRA) of 248 cm<sup>2</sup> g<sup>-1</sup>. Alternatively, pine had an average SRL of 8.5 m g<sup>-1</sup> and an SRA of 173 cm<sup>2</sup> g<sup>-1</sup> ( $p < 0.001$ ) (Table 3.2). Values for total fine root length (FRL), total fine root area (FRA), and total root tip abundance (FRT) followed the same pattern as described for fine root biomass, with the highest mean values observed for the irrigated pure birch stands, with an average of 6050 m m<sup>-2</sup>, 6.73 m<sup>2</sup> m<sup>-2</sup> and 1660 thousand tips m<sup>-2</sup> respectively.



**Table 3. 2** Overview of the summed values of fine root biomass (FRB; g m<sup>-2</sup>), adjusted fine root biomass (FRBadj.; g m<sup>-2</sup> BA), understory fine root biomass (g m<sup>-2</sup>), fine root length (FRL; m m<sup>-2</sup>), fine root surface area (FRA; m<sup>2</sup> m<sup>-2</sup>) and root tip abundance (FRT; 10<sup>3</sup> tips m<sup>-2</sup>) for the whole soil profile (0–90 cm) for *Betula pendula*, *Pinus pinaster* in pure and mixed plots, in control and irrigated blocks. The tables also includes fine root morphology for the upper 15 cm of soil: specific root length (SRL<sub>15</sub>; m g<sup>-1</sup>), and specific root area (SRA<sub>15</sub>; cm<sup>2</sup> g<sup>-1</sup>). Different lowercase letters indicate significant differences between the eight combinations of stand composition and water treatment.

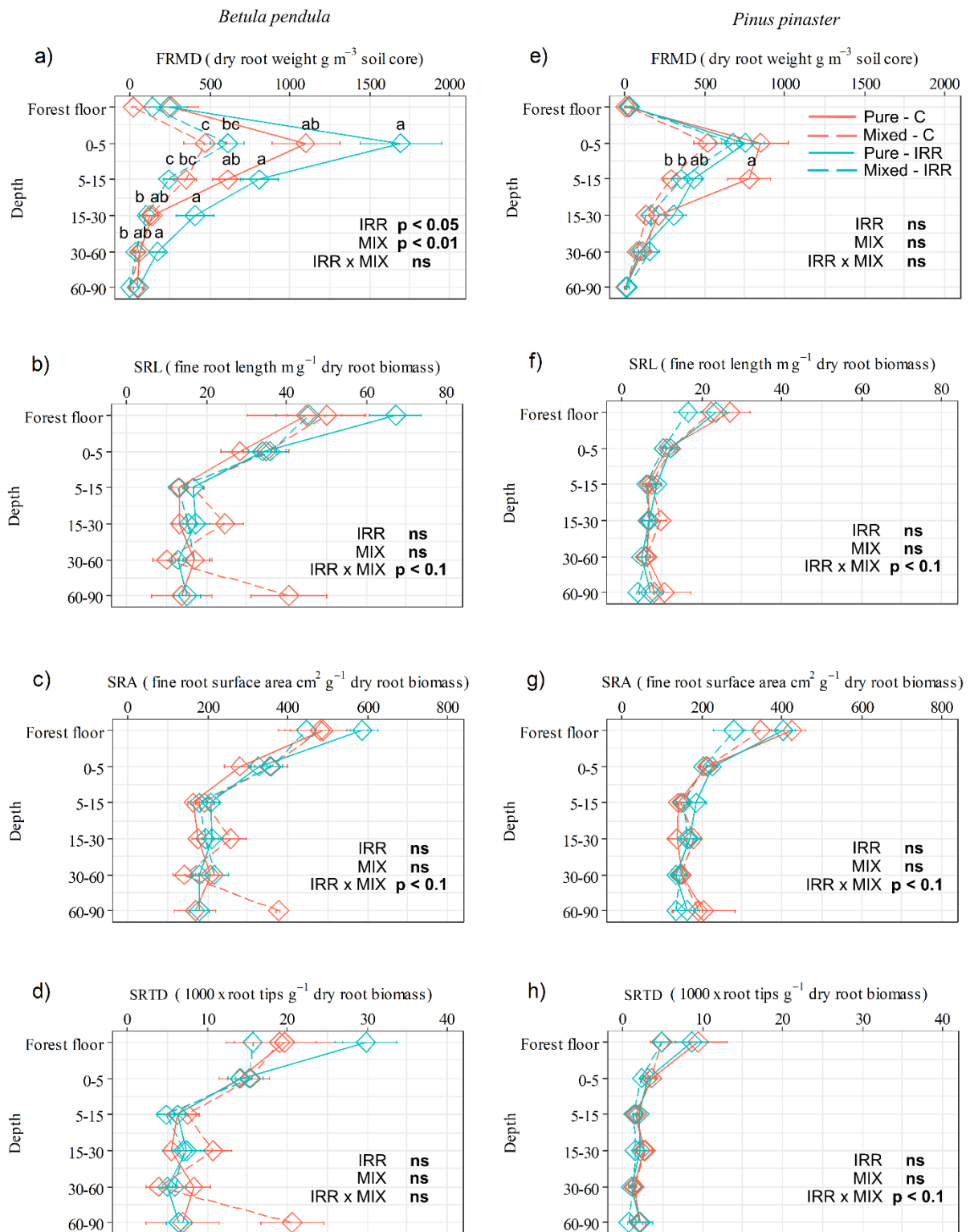
Species	<i>Betula pendula</i>				<i>Pinus pinaster</i>			
	Pure stand		Mixed stand		Pure stand		Mixed stand	
	Control	Irrigation	Control	Irrigation	Control	Irrigation	Control	Irrigation
FRB (g m <sup>-2</sup> )	n = 16	n = 16	n = 16	n = 16	n = 16	n = 16	n = 16	n = 16
FRBadj (g m <sup>-2</sup> BA) <sup>a</sup>	172 (82) ab	297 (129) a	99 (76) b	83 (41) b	186 (118) ab	143 (88) b	95 (49) b	143 (129) b
Understory (g m <sup>-2</sup> )	1.5 (0.7) a	1.8 (0.9) a	2.0 (1.3) a	1.3 (0.6) a	0.3 (0.2) b	0.3 (0.2) b	0.3 (0.2) b	0.3 (0.3) b
FRL (m m <sup>-2</sup> )	295 (185) a	230 (116) a	284 (137) a	287 (185) a	283 (118) a	263 (147) a	284 (137) a	287 (185) a
FRA (m <sup>2</sup> m <sup>-2</sup> )	2640 (1760) b	6050 (3590) a	1520 (964) b	1690 (924) b	1290 (847) b	1040 (640) b	725 (438) b	833 (631) b
FRT (10 <sup>3</sup> tips m <sup>-2</sup> )	3.2 (1.8) ab	6.73 (3.6) a	1.8 (1.0) b	1.9 (1.1) b	2.7 (1.7) ab	2.27 (1.37) b	1.5 (0.8) b	2.1 (1.7) b
SRL <sub>15</sub> (m g <sup>-1</sup> )	942 (569) b	1660 (1080) a	571 (365) bc	490 (285) bcd	261 (181) ce	171 (130) e	159 (76) de	124 (58) e
SRA <sub>15</sub> (cm <sup>2</sup> g <sup>-1</sup> )	18.8 (11.8) ab	25.9 (11.3) a	23.4 (11.4) a	24.2 (10.5) a	8.1 (2.4) c	9.8 (4.4) bc	8.4 (2.5) c	7.9 (2.2) c
	205 (94) ac	269 (87) a	253 (96) ab	265 (84) a	163 (36) c	196 (67) ac	163 (28) c	172 (33) bc

<sup>a</sup> FRBadj is the average fine root biomass (FRB) of four sampling points, adjusted to the tree basal area (BA) value for each sampling point. FRB is the fine root biomass (g m<sup>-2</sup>), and BA (m<sup>2</sup>) is the total basal area of the four bordering trees for each sampling point (Schmid 2002; Schmid and Kazda 2002).

### 3.3.2 Vertical root patterns

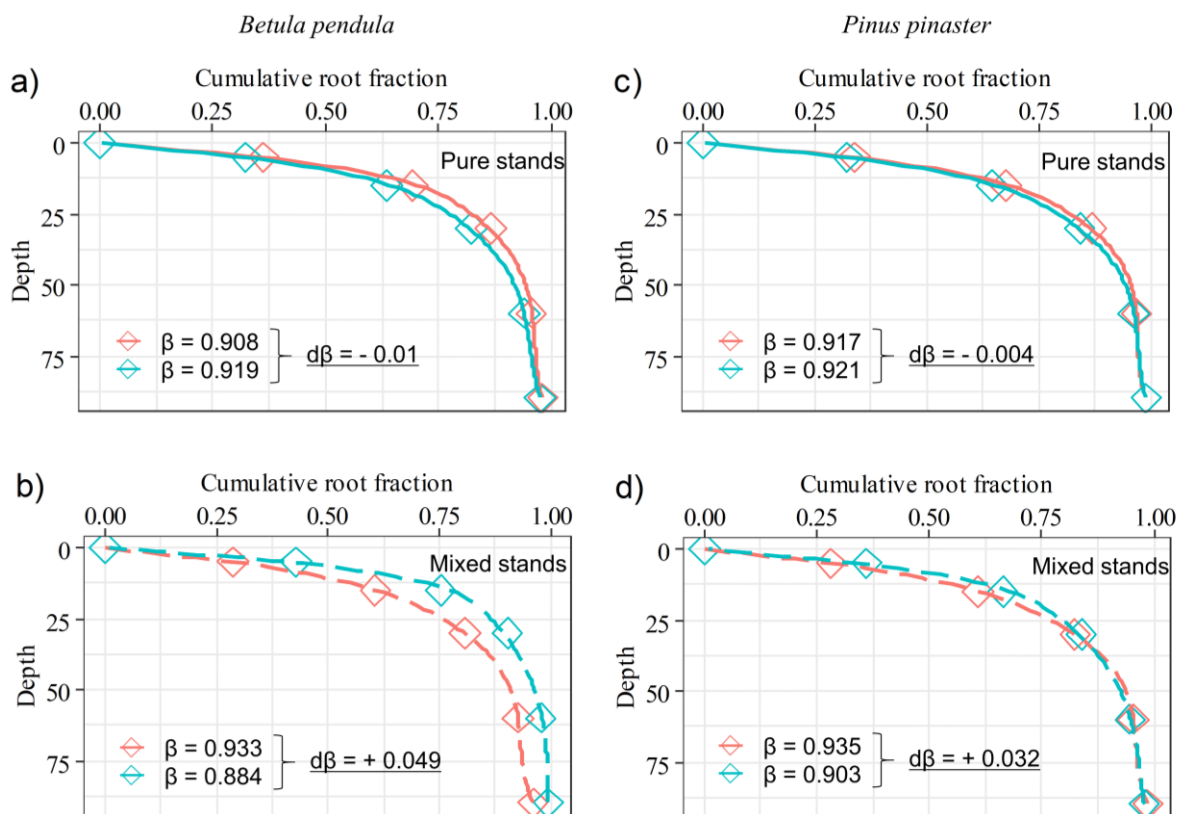
Fine root mass density (FRMD) of tree fine roots showed no significant effect of species, but significantly depended on mixture, depth and interactions between species × mixture and species × depth (see [Table B3.3](#) in Appendix B). FRMD decreased with increasing soil depth ([Fig. 3.1](#)). We observed that the response of pine and birch to irrigation and mixture was not analogous (see [Table B3.4](#) in Appendix B). Birch responded with a significantly higher FRMD under irrigation, and a lower FRMD under mixture. On the contrary, pine did not respond significantly to irrigation or mixture, although for the 5–15 cm soil layer, the pure control stands had a significantly higher FRMD than both mixed stands and the pure irrigated stands had an intermediate value ([Fig. 3.1](#)). Results were the same for FRLD (see [Fig. B3.4](#) in Appendix B). The vertical patterns of specific root length (SRL), specific root area (SRA) and specific root tip density (SRTD) are further given in [Fig. 3.1](#); all three metrics had the highest values at the forest floor, and significantly decreased with soil depth ( $p < 0.0001$ ). Roots in the forest floor and bottom soil layer were present either in variable or very low numbers, leading to a large standard deviation for these layers. SRL, SRA and SRTD, all had higher values for birch than for pine ( $p < 0.0001$ ) (see [Table B3.3](#) in Appendix B). In fact, the majority of birch fine root length was between 0.0 – 0.5 mm in diameter, and this proportion ranged between 60 – 90% along the soil profile. On the other hand, for pine the very fine root fraction ranged between 12.5 – 50 %, and the species developed the majority of its root length in the two finest diameter classes (0.0 – 0.5 mm; 0.5 – 1.0 mm Ø) (see [Fig. B3.5](#) in Appendix B). For both species, SRL, SRA and SRTD were only marginally affected by irrigation and mixture, and there were no clear morphological adaptations based on the entire fine root class (< 2 mm Ø) (see [Table B3.4](#) in Appendix B).

When looking at the different diameter classes, we find soil depth to be significant ( $p < 0.0001$ ), with values of fine root length of the very fine roots (0.0 – 0.5 mm Ø) decreasing with soil depth. For the other diameter classes, a subtle increase per soil depth was observed, which tended to give higher values for the deeper layers. As compared to the entire < 2 mm diameter root class, we found that fine root length values within certain diameter classes were plastic to our treatment effects. In particular for birch, the very fine root or the second finest root fraction (0.0 – 0.5 mm Ø; 0.5 – 1.0 mm Ø) (see [Fig. B3.5a-d](#) in Appendix B), and for pine, the very fine root or sometimes even the 1.0 – 1.5 mm Ø fraction (see [Fig. B3.5e-h](#) in Appendix B).



**Fig. 3.1** Depth distributions for root traits of *Betula pendula* and *Pinus pinaster* for all four Irrigation  $\times$  Mixture combinations (Pure – C; Mixed – C; Pure – IRR; Mixed – IRR). Presented data are mean values with st. errors for: (a,e) fine root mass density (FRMD;  $\text{g m}^{-3}$ ), (b, f) specific root length (SRL;  $\text{m g}^{-1}$ ), (c, g) specific root area (SRA;  $\text{cm}^2 \text{g}^{-1}$ ), and (d, h) branching intensity (SRTD;  $1000 \times \text{tips g}^{-1}$ ). Effects of irrigation (IRR), mixture (MIX) and their interaction are indicated at the lower right side of each graph. Significant differences per soil depth, between combinations of Irrigation and Mixture are indicated with lower case letters. Absence of letters means that there are no significant differences.

There was no significant difference in the vertical root distribution of root biomass between birch and pine ( $p = 0.65$ ) (see Fig. B3.6 in Appendix B). Furthermore, the effects of irrigation and of mixture were not significant either, although the interactive effect of irrigation and mixture was marginally significant ( $p = 0.054$ ). In particular, we found that under control conditions, both mixed tree species distributed roots deeper ( $d\beta = + 0.049$  for birch;  $d\beta = + 0.032$  for pine) than their respective irrigated stands (Fig. 3.2). In the pure stands, the opposite occurred and the trees distributed their fine roots to shallower soil layers ( $d\beta = - 0.01$  for birch,  $d\beta = - 0.004$  for pine) under control conditions compared to irrigation (Fig. 3.2). It is important to notice that the mixtures of birch and pine had almost identical vertical root distributions in the control treatment ( $\beta = 0.933 \approx \beta = 0.935$ ), while under irrigation, birch produced shallower roots than pine ( $\beta = 0.884 < \beta = 0.903$ ). In the top 30 cm of the soil profile (including the forest floor), around 80–87 % of all fine root biomass was found, and this was 95–100% for the upper 60 cm of the soil profile (see Table B3.5 in Appendix B).



**Fig. 3.2** Cumulative fine root fraction with soil depth and the coefficients of the rooting distribution ( $\beta$ ) for: a) *Betula pendula* in pure stands b) *Betula pendula* in mixed stands, c) *Pinus pinaster* in pure stands, d) *Pinus pinaster* in mixed stands, under irrigation and control treatment. The  $\beta$  values are based on fine root biomass ( $\text{g m}^{-2}$ ); the higher the  $\beta$  value (the closer to 1), the deeper is the vertical distribution of fine roots. Absence of letters means that there are no significant differences.

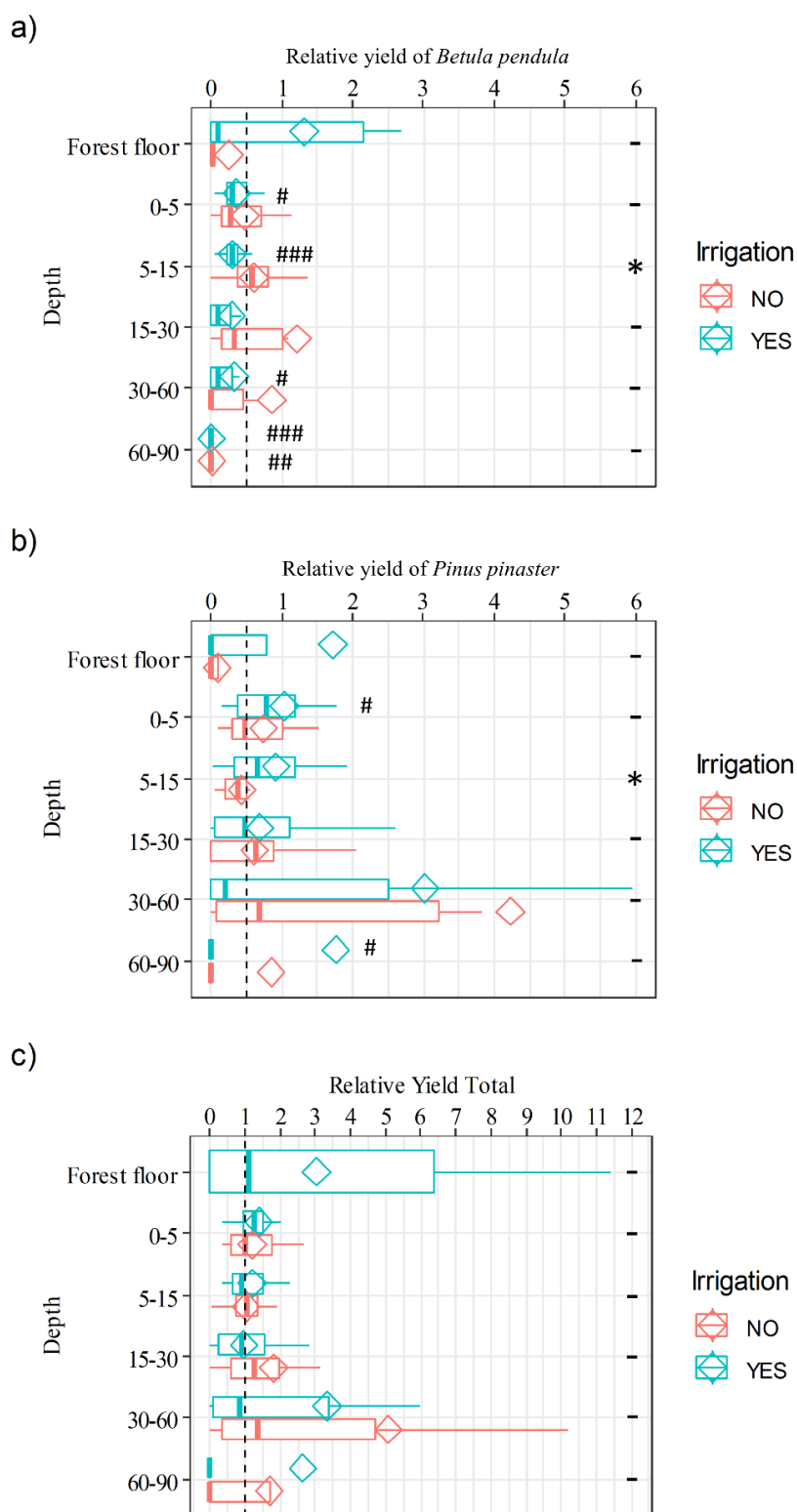
### 3.3.3 Relative fine root attributes

The Relative Yield Total at the community level did not significantly change under different water supply (RYT control: 1.02  $\approx$  RYT irrigation: 1.05), and there was no significant overyielding under neither control nor irrigated conditions (Table 3.3, see Table B3.6 in Appendix B). At the individual tree level, under ambient water supply birch tended to produce on average the same amount of total fine root biomass in mixed stands relative to its respective pure stands, while it produced significantly less in mixed stands under conditions of irrigation relative to pure stands ( $p < 0.0001$ ) (Table 3.3). The latter value was accompanied by significant negative effect of irrigation on relative yield (RY control: 0.50  $>$  RY irrigation: 0.27;  $p = 0.023$ ). Pine relative stand values were not significantly affected by ambient water supply, but irrigation resulted in significantly higher relative yield (RY control: 0.48  $<$  RY irrigation: 0.77;  $p = 0.033$ ) (Table 3.3).

**Table 3.3** Means and medians of the relative mixed stand values ( $\pm$  standard deviations) of fine root biomass for the whole soil profile. Mean values of RY  $>$  0.5 (per species), or when RYT  $>$  1 (for the whole community) indicate positive mixture effects. Bold values indicate significant deviation ( $p < 0.05$ ) from 0.5 (per species) or 1 (for the whole community). Asterisks \* indicate significant effect of irrigation at species or community level ( $p < 0.05$ ).

		<i>Relative Yield</i>				<i>Relative Yield Total</i>	
		<i>Betula pendula</i>		<i>Pinus pinaster</i>		<i>Betula pendula</i>	<i>Pinus pinaster</i>
		Control	Irrigation	Control	Irrigation	Control	Irrigation
Whole soil profile (0-90 cm)	<b>mean (sd)</b>	0.55 (0.48)	0.29 (0.13)	0.54 (0.24)	0.85 (0.59)	1.09 (0.47)	1.14 (0.53)
	<b>median</b>	0.50	<b>0.27*</b>	0.48	<b>0.77</b>	1.02	1.05

Along the soil profile, at the community level (RYT), there were no significant effects of mixture under ambient water supply or irrigation, and there was no significant effect of irrigation on the relative mixed stand value (Fig. 3.3a, see Table B3.6 in Appendix B). RYT values tended to be lower than 1, with the exception of the irrigated 0–5 cm soil layer, and the not irrigated 15–30 cm soil layer. At the individual tree level, birch significantly underyielded under irrigation for most of the soil layers (RY  $<$  0.5; 0–5, 5–15, 30–60, 60–90 cm) (Fig. 3.3a). Under ambient water supply, there were no significant mixture effects, even though the median values were slightly higher for the soil layer of 5–15 cm. The latter was accompanied by a significant negative effect of irrigation on the relative mixed stand value ( $p = 0.013$ ). For pine, there was significant overyielding under irrigation for the soil layer 0–5 cm (RY  $>$  0.5;  $p = 0.038$ ), and a significant underyielding under irrigation for the bottom soil layer (Fig. 3.3a). Finally, there was a significant positive effect of irrigation on the relative mixed stand values for the soil layer of 5–15 cm ( $p = 0.042$ ), but we did not observe significant overyielding.



**Fig. 3.3** Boxplots with medians (-), and means ( $\diamond$ ) of the relative mixed stand values of fine root biomass ( $\text{g m}^{-2}$ ). RY > 0.5 indicates positive mixture effects for a given species: a) *Betula pendula*, b) *Pinus pinaster*, c) and RYT > 1 indicates positive mixture effects for the whole community. Significant relative yield effects (overyielding or underyielding) are noted using # ( $p < 0.05$ ), ## ( $p < 0.01$ ) and ### ( $p < 0.001$ ). Asterisks \* indicate significant differences ( $p < 0.05$ ) between irrigated and non-irrigated plots, per depth.

## 3.4 Discussion

### 3.4.1 General considerations

Birch and pine developed very similar vertical root distributions when they grow together in mixture, and showed no belowground overyielding for the whole community (Relative yield total), while vertical root segregation and belowground overyielding did not significantly increase under ambient water supply. Furthermore, there was no overall shift towards shallow layers for birch and deeper layers for pine under ambient water supply. Instead, such responses were only observed under irrigation with birch producing very shallow fine root distributions compared to pine, but even then, we did not observe belowground overyielding for the whole community. At the same time birch responded by significantly underyielding under irrigation, for the 0–5, 5–15, 30–60, and 60–90 cm soil layers, while pine significantly overyielded for the 0–5 cm soil layers. This underlines that although vertical root segregation does not necessarily lead to belowground overyielding for the whole soil profile, other depth-related factors such as the occurrence of a hardpan, depth and fluctuation of the water table or other vertical patterns such as organic matter and nutrient distribution could be relevant. Such depth-specific variation of site conditions may be more important to consider than shallow vs deep fine root distributions, for predicting the effects of tree diversity on belowground complementarity in a context of climate change.

Differences in sampling depth, tree stand density, average stand basal area, average basal area per tree, stand age, soil resources and environmental conditions can result in variable fine root biomass values (FRB) (Curt and Prévosto 2003; Finér et al. 2007; Jackson et al. 1997; Zhou et al. 2018). In our study, birch and pine trees were planted (same tree age) at a fixed density, and on common soil type (sandy soil), which permits to compare the effects of tree mixture and differing water supply (summer irrigation vs. ambient precipitation) under a standardized setting. The trees could grow for 10 years under these conditions and received summer irrigation (in half of the blocks) for the last three summers prior to our sampling. At sampling, aboveground competition was apparent (as witnessed from canopy closure and branches intertwining into neighbouring grids); this was mostly observed for pine that extended its canopy twice as much as birch, when growing together with birch. We consequently expected clear belowground responses of both species with regard to how fine roots were deployed, and in particular for pine to be the superior competitor. Having said that, it is important to consider that our experimental field has a reoccurring hardpan at a depth of around 50 cm (with an average thickness of around 17 cm), and an underground water table that ranges high from 40 to 80 cm below the surface, which drops between 150 and 180 cm during the summer months. As a result, occurrence of fine roots below 60 cm of soil depth in our soil cores was low. In addition, we did not measure the horizontal spread of fine roots from trees of neighbouring plots (we sampled at least 10 m from a tree of an adjacent plot), so we have to remain cautious as roots may grow into adjacent plots, which could affect the interpretation of fine root data at the local scale.

Our FRB values were comparable to those reported in the literature. Since we could not find literature on mixtures of pine and birch, we present comparisons for pure stands only. Pure pine stands had a mean FRB of 186 g m<sup>-2</sup> on control plots and 143 g m<sup>-2</sup> on irrigated plots. These amounts of fine roots were in a similar range as those found for other *Pinus* stands: (Bakker et al. 2009) (111–296 g m<sup>-2</sup> for a 13 year-old *Pinus pinaster* stand, 120 cm deep), (Jagodziński and Kałucka 2011) (~100–250 g m<sup>-2</sup> for 6–18 year-old *Pinus sylvestris* stands, 90 cm deep), (Finér et al. 2007) (~200 g m<sup>-2</sup> for 20 year-old *Pinus sylvestris* stands, 100 cm deep). This is, however, much less than the range of 270–720 g m<sup>-2</sup> (humid to dry stands) as given by Achat et al. (2008) for 56 year-old *Pinus pinaster* stands (0–120 cm) and the 230–493 g m<sup>-2</sup> for *Pinus sylvestris* stands (0–30 cm) over 60 years-old as reported by Helmisaari et al. (2007) for Finnish podzols. Mean FRB values of our pure birch stands (control: 172 g m<sup>-2</sup>, irrigation: 297 g m<sup>-2</sup>) were of similar range to *Betula* studies: (Vogt et al. 1995) (318 g m<sup>-2</sup>, for 0–20 cm, 35 year-old stand), (Curt and Prévosto 2003) (306 g m<sup>-2</sup>, for 0–75 cm, 50-year old forest), (Hansson et al. 2013) (196 g m<sup>-2</sup>, for 0–30 cm, 58 year-old stand), but much less than Ding et al. (2019) (402 g m<sup>-2</sup>, for 0–20 cm, 82 year-old stand).

Regarding morphological plasticity for fine roots of pine for the summed layers of the profile, we see that the values for fine root length (FRL: 735–1290 m m<sup>-2</sup>), fine root area (FRA: 1.5–2.7 m<sup>2</sup> m<sup>-2</sup>) and fine root tip abundance (FRT: 159–261 10<sup>3</sup> tips m<sup>-2</sup>) were smaller but not too far from the values obtained in a 13-yr old stand subjected to annual fertilization and irrigation (Bakker et al. 2009) (FRL: 2471–2973 m m<sup>-2</sup>; FRA: 2.4–4.8 m<sup>2</sup> m<sup>-2</sup>; FRT: 62–320 10<sup>3</sup> tips m<sup>-2</sup>). The values for pine were in general lower than those for birch. Though we could not find a study with root morphological data of birch for the summed soil profile, we consider that higher FRL, FRA, and FRT to be normal, as birch has usually very ramified roots compared to conifers (Bauhus and Messier 1999). The specific root length (SRL) values for birch and pine for the top 15 cm averaged between 18.8–25.9 m g<sup>-1</sup> and 7.9–8.4 m g<sup>-1</sup> respectively, which are similar to values from Ostonen et al. (2007) (approx. for *Betula*: 13–14 m g<sup>-1</sup>, and *Pinus*: 8–9 m g<sup>-1</sup>). The specific root area (SRA) values for the top 15 cm averaged between 205–265 cm<sup>2</sup> g<sup>-1</sup> and 163–196 cm<sup>2</sup> g<sup>-1</sup> respectively for birch and pine, and were similar to Wang et al. (2015) (*Betula pendula* ranged between 125–266 cm<sup>2</sup> g<sup>-1</sup>) and Bakker et al. (2009) (*Pinus pinaster* ranged between 144–245 cm<sup>2</sup> g<sup>-1</sup> for the soil and 286–445 cm<sup>2</sup> g<sup>-1</sup> for the forest floor).

As expected, birch possessed longer and more ramified roots than pine, with all three variables (SRL, SRA, SRTD) showing higher values than pine. Fine roots for the entire diameter class (< 2mm Ø) did not show any significant morphological effects of mixture or irrigation, not per soil depth and not for any summed parameters. Similarly to our observations, in a study with mixtures (up to five tree species) with contrasting fine root morphologies, there were no adaptations in morphology with increasing diversity (Fruleux et al. 2016; Meinen et al. 2009b). Also similar to previous studies, irrigation had no effect on fine root morphology of the entire < 2 mm Ø fine root class (Brunner et al. 2019; Leuschner et al. 2004).

Regarding the diameter classes, both tree species showed a decrease of the very fine root fraction with soil depth. Pine in particular, showed an increase of the fraction of thicker fine roots with soil depth. The adaptation for pine of larger diameter roots at deeper soil layers is indicative of higher transport capacity for water from deeper soil



(Makita et al. 2011), while birch seems to lack this adaptive ability. Pine tended to invest in fine root fractions of thicker diameter classes when irrigated, and so did birch, but only when growing with pine. It is likely that the presence of pine roots decreases the soil available water and nutrients, resulting in birch root plasticity only when growing with pine. The irrigation effects are probably due to trees assigning less fine root length to the very fine root diameter class (usually responsible to nutrient-water acquisition), while the fraction of thicker fine roots that would usually be responsible for transportation is increased. It has been shown in previous studies that root order traits (e.g. morphology, nutrient content) can be influenced by interspecific competition or water availability (Salahuddin et al. 2018), especially absorptive roots (distal root orders), and this effect is species-specific (Ostonen et al. 2013), and can be affected by soil depth (Makita et al. 2011). Our results suggest that the very fine root fraction is influenced by environmental or interspecific effects and that this depends on the tree species.

### **3.4.2 Effect of mixing tree species on vertical root segregation and belowground overyielding**

The tree species in our study were previously reported to have contrasting fine root distributions, and fine root morphologies, with the potential to stratify further under mixture. Contrary to our first hypothesis, the vertical root distributions of pine and birch were relatively similar along the soil profile, with both species having very shallow root systems in both pure and mixed stands, and our results did not reveal any belowground overyielding.

The values of %FRB in the upper 30 cm of 80-87% for pine and birch were far higher than the global averages of 46% and 63% reported for temperate coniferous and deciduous forests, respectively (Jackson et al. 1997). This suggests that the root distributions of our site were very shallow compared to other distributions of the same geographical context, and that they are probably dependent on local soil conditions rather than genotypic characteristics (Achat et al. 2008; Bakker et al. 2006). The similar and shallow root systems may be due to a cemented layer observed around 50 cm deep (existing in 51 out of 96 sampling points) (Table 3.1), that may have forced both tree species to reallocate biomass from the taproot and stump to other compartments, and resort to using what is remaining of the soil profile (Danjon et al. 2005). Shallow fine root distributions have been observed before for *Pinus pinaster* growing on a humid moorland site of the SW of France, and were attributed to a hardpan and shallow water table (Achat et al. 2008). Moreover, for short periods the water table could reach -30 or -40 cm in wet years, while in summer, it does not necessarily descend below 120 cm (see Fig. B3.1 in Appendix B). Such fluctuations can inhibit fine root proliferation into zones that are regularly attained by the water table (anoxic conditions), while stimulating at the same time the proliferation of fine roots in the layers above the water table and near the soil surface (Imada et al. 2008). It is noteworthy that the lack of vertical root segregation could also be due to the young age of these stands, as vertical compartmentalization may increase with stand maturity (Curt and Prévosto 2003). The latter applies also for fine root morphology, which has been positively and negatively correlated with tree age (Jagodzinski et al. 2016; Rosenvald et al. 2013).

### 3.4.3 Pronounced vertical root segregation and belowground overyielding under ambient water supply

Contrary to our second hypothesis, vertical root segregation and belowground overyielding were not larger under ambient water supply (no irrigation). The vertical root distributions were almost identical for pine and birch under mixture ( $\beta = 0.933 \approx \beta = 0.935$ ), and there was no change in belowground overyielding (RYT control: 1.02  $\approx$  RYT irrigation: 1.05). It is interesting that under ambient water supply, both species tended to invest in deeper fine root distributions compared to their respective irrigated stands (Fig. 3.2), and that under irrigation, birch had a clear adaptation towards shallower distribution, compared to irrigated pine ( $\beta = 0.884 < \beta = 0.903$ ). However, this apparent fine root segregation between irrigated birch and pine was not accompanied by belowground overyielding for the whole community. The latter was due to the simultaneous overyielding of irrigated birch and underyielding of irrigated pine, concerning the root standing biomass of the whole soil profile (Table 3.3).

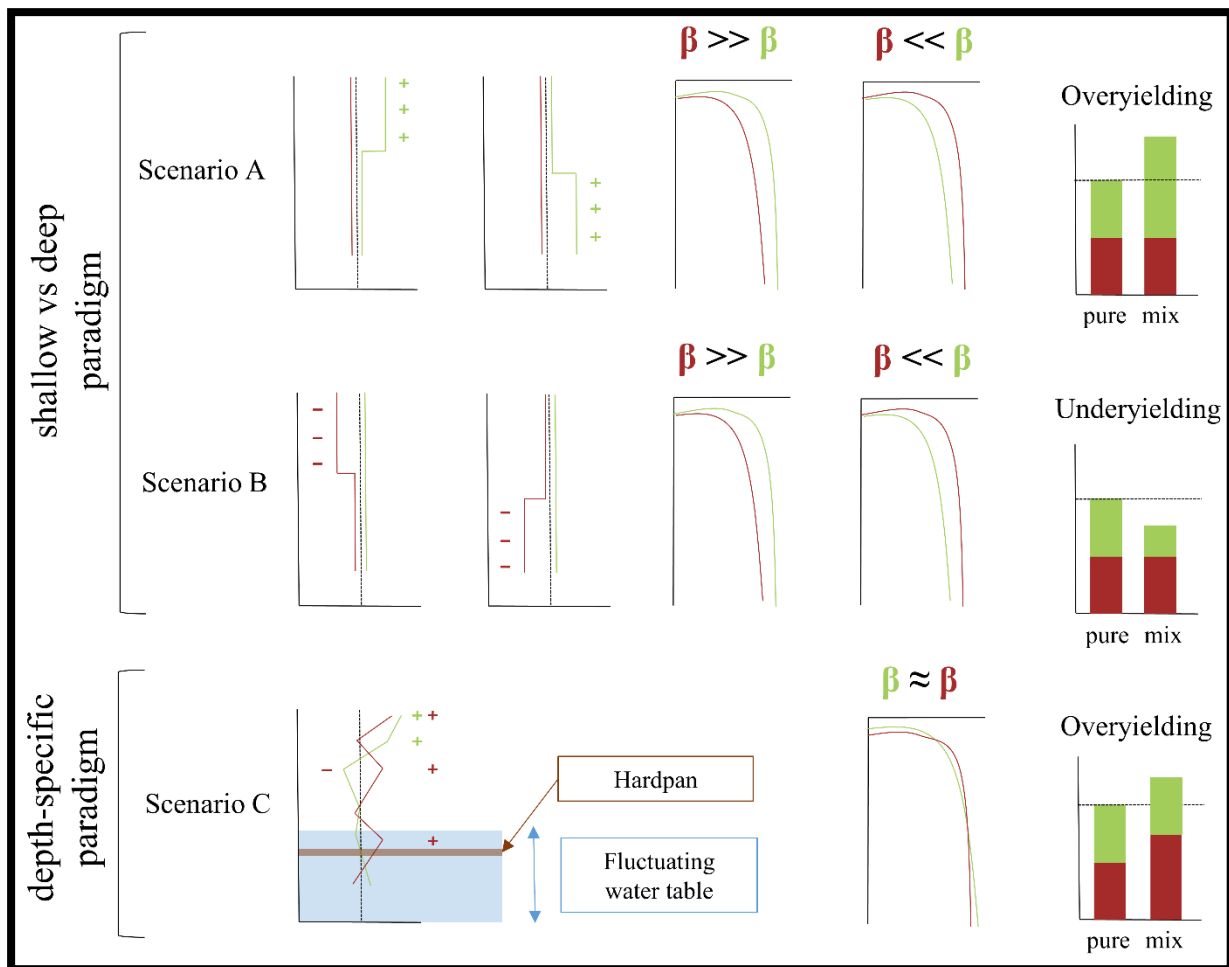
Interestingly, when not irrigated, the vertical root distribution was opposite between mixed (adaptations towards deeper distributions) and pure plots (adaptations towards shallower distributions), indicating that vertical root adaptations to water availability change with interspecific competition (Bolte and Villanueva 2006; Curt and Prévosto 2003). Monocultures may adapt less than mixtures due to negative feedback from species-specific pests and pathogens (biotic feedback) or because mixing species mediates environmental stress (abiotic facilitation; i.e. hydraulic lift) (Barry et al. 2020; Jactel and Brockerhoff 2007). We know from previous studies the importance that interspecific competition has on vertical root distributions (Bolte and Villanueva 2006; Curt and Prévosto 2003), as the inferior competitor might be forced to shift their roots to unoccupied soil space causing vertical root segregation (Schenk 2006). In this case, we reported higher flexibility of the birch root system to water availability, particularly when irrigated during the growing season and when enduring interspecific competition. However, we should be cautious in our interpretation. Although root location is an honest indicator of water and nutrient uptake, it has been shown that the spatial distribution of the roots does not always correlate to the spatial distribution of uptake (Göransson et al. 2007; Grossiord et al. 2014; Kulmatiski et al. 2017). The general tendency of roots adapting towards deeper distributions does not automatically imply that birch and pine compete hard for water and nutrients.

### 3.4.4 Depth-specific effects on relative yield

In contrast to our third hypothesis, shifts in soil space occupation did not follow a shallow-rooted vs deep-rooted pattern. However, we did observe depth-specific effects of mixtures on relative yield for birch and pine; in particular, this varied between ambient water supply and the irrigation treatment. When investigating per soil layer, we found that under ambient water supply, the relative yield for birch and pine was not significantly affected. These results suggest there is a rather symmetrical interspecific competition of birch and pine plantations along the soil profile when water availability is lower, i.e. no irrigation during the summer. On the contrary, under irrigation, we found that the interspecific competition was asymmetric along the soil profile and favourable

for pine; birch roots were significantly under-represented in the 0–5, 5–15, 30–60 cm soil layers, while pine roots tended to be over-represented in the soil layer 0–5 cm. Hence, under irrigation (i.e. summer irrigation over the last years), both species demonstrated depth-specific changes in fine root growth when growing in mixture, with birch being the most plastic. The general idea that interspecific competition is alleviated through fine root adaptations towards shallower or deeper layers has been described in previous studies (Bolte and Villanueva 2006; Schmid 2002; Schmid and Kazda 2002), but this is not supported by our study. Our results highlight the importance of looking into different soil depths to better understand the net outcome of competition in mixtures along different environmental conditions (Fig. 3.4).

The general decline in birch fine root biomass in mixture may be due to the competitive superiority of pine aboveground. Pine had a larger basal area than birch, which implies that it has larger needs for water. The latter is confirmed as fine root biomass per species was proportional to its basal area (Table 3.2). It is logical to expect that since pine grew faster than birch (Morin et al. 2020), it would also take up water faster, and would outcompete the relatively slower growing birch trees, leaving less available water in the soil. In addition, birch has finer roots than pine, and it might be more sensitive to unfavourable moisture conditions, which might occur more frequently in the very permeable sandy soils that “hold” less water. Thus, a more carbon efficient strategy for nutrient acquisition would be to shed fine roots or to form ectomycorrhizal associations (Withington et al. 2006). Other ectomycorrhizal tree species have demonstrated changes in fine root mass and mycorrhizal fungal biomass for the purpose of efficient nutrient acquisition, under different soil resource conditions (Weemstra et al. 2017), and there is still possibility that different ectomycorrhizal exploration systems for both species could also contribute to a better soil exploration (i.e. long distance rhizomorphs) (Agerer 2001). Moreover, pine extended its canopy towards birch, directly competing for light. Consequently, birch may have responded by investing less in belowground biomass, and instead allocated more carbon to aboveground parts to balance the competition aboveground (Epron et al. 2012). The competitive pressure might be lower for pine, which could then respond by investing more roots into less occupied soil layers (i.e. 0–5 cm soil layer). It is important to note that irrigated birch reduced fine root biomass overall, but at the same time produced shallower root distributions than pine, which was evidently due to high fine root growth of birch at the forest floor; though the presence of roots for that layer was rather sporadic. Furthermore, niche shifts might occur mostly for the upper soil layers under our conditions, especially since the hardpan poses a limit for deeper soil exploration (von Felten and Schmid 2008). Therefore, vertical root segregation might take place in the top soil layers, instead of a complete transition towards the deeper soil layers. However, we must remain cautious in our interpretations, as we do not know whether competitive ability is static or changes dynamically over seasons and years (Brassard et al. 2013). Interspecific competition could eventually lead to a vertical niche partitioning when the tree stand reaches maturity (Curt and Prévosto 2003), but currently does not follow under our experimental conditions the commonly shown shallow vs deep paradigm. Also, we do not know whether facilitation or positive/negative feedback from species specific pests and pathogens are behind our depth-specific mixture effects (Barry et al. 2019).



**Fig. 3.4** Depictions of the simplified shallow vs deep paradigm for species 1 (in red colour) and species 2 (in green colour) growing in mixtures, and the proposed depth-specific paradigm for young mixed forests. On the left is the response in fine root investment of Species 1 and Species 2 growing together in mixture, in the middle is their calculated vertical fine root distributions ( $\beta$  values), on the right is the yield effect for the whole soil column (pure versus mix). The shallow vs deep paradigm is based on the assumption that in case of vertical root segregation, there will be a higher root occupation of the soil profile, which results in belowground overyielding in tree mixtures. In scenario A, vertical root segregation could occur if more roots of one species grow in either the top soil layers or the deeper soil layers (respectively curves on the left, curves on the right) relative to its distribution in a pure stand, leading to an overyielding for the whole soil column. However, vertical root segregation can also be obtained if one species would grow less roots in top or deeper soil layers relative to the pure stand, though in that case this implies an underyielding at the whole soil profile level (scenario B). Alternatively, other factors such as soil conditions and the depth of the water table, can constrain root growth at deeper soil layers, giving results that are not consistent with the shallow vs deep paradigm. For example, a lack of vertical root segregation may still result in overyielding for the whole soil profile, as both species actually invest in higher biomass when growing in mixture (scenario C). Under our conditions, this pattern, considering root segregation per soil layer, and not throughout the whole soil profile, better matches our observations of root distribution in tree mixtures.

### 3.5 Conclusion

Our study revealed that 10 years after planting and after 3 years of summer irrigation, mixing silver birch and maritime pine did not affect vertical root distribution and did not result in significant belowground overyielding considering the sum of the soil profile, under ambient water supply (i.e. summer drought). This is probably due to the relatively high groundwater table, and the presence of a hardpan at 50 cm, which did not allow roots to explore deeper. Birch and pine were only moderately responsive in their morphological plasticity and in their vertical root distribution to mixture and ambient water supply.

Under irrigation, mixed birch had significantly the lowest relative yields for most of the soil layers, while relative yields of pine were significantly higher only for the top soil. This suggests that birch is more sensitive to environmental conditions, in particular when growing in mixtures. Under irrigation, birch and pine featured depth-specific differences for the 0–5, 5–15, 15–30 and 30–60 cm layers regarding relative yields of fine root biomass, suggesting an asymmetric competition that depends on the environmental context. The commonly used shallow vs deep paradigm was not present in these aggrading young forests, and evaluating the root profile in terms of shallower or deeper fine root distributions gave indecisive/unclear results that could not explain overyielding at the soil profile level. Instead, effects were specific to soil layers, which could indicate that effects of interspecific competition may need more time to be visible along the whole soil profile. This means that for these young dynamic mixed species forests, we need to fine-tune our evaluation of interspecific competition and not neglect depth- and species-specific interactions.

### Acknowledgements

This study was supported by the ANR project DiPTiCC (16-CE32-0003). We thank the Forest experimental Facility (UEFP-<https://doi.org/10.15454/1.5483264699193726E12>) and especially Bernard Issenhuth for maintenance of the ORPHEE experiment. We thank Coralie Chesseron and Nathalie Gallegos, for their assistance in the lab, Catherine Lambrot and Sylvie Millin for their priceless participation on the field, Pietro Barbieri, Ulysse Gaudaré and Tania Maxwell for their guidance in handling statistics in R, and David Vidal for creating the ombrothermic diagram.

## References

- Achat DL, Bakker MR, Trichet P (2008) Rooting patterns and fine root biomass of *Pinus pinaster* assessed by trench wall and core methods. *J For Res* 13:165–175. <https://doi.org/10.1007/s10310-008-0071-y>
- Agerer R (2001) Exploration types of ectomycorrhizae: A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107–114. <https://doi.org/10.1007/s005720100108>
- Augusto L, Bakker MR, Morel C, et al (2010) Is “grey literature” a reliable source of data to characterize soils at the scale of a region? A case study in a maritime pine forest in southwestern France. *Eur J Soil Sci* 61:807–822. <https://doi.org/10.1111/j.1365-2389.2010.01286.x>
- Bakker MR, Augusto L, Achat DL (2006) Fine root distribution of trees and understory in mature stands of maritime pine (*Pinus pinaster*) on dry and humid sites. *Plant Soil* 286:37–51. <https://doi.org/10.1007/s11104-006-9024-4>
- Bakker MR, Jolicoeur E, Trichet P, et al (2009) Adaptation of fine roots to annual fertilization and irrigation in a 13-year-old *Pinus pinaster* stand. *Tree Physiol* 29:229–238. <https://doi.org/10.1093/treephys/tpn020>
- Barry KE, Mommer L, van Ruijven J, et al (2019) The Future of Complementarity: Disentangling Causes from Consequences. *Trends Ecol. Evol.* 34:167–180
- Barry KE, van Ruijven J, Mommer L, et al (2020) Limited evidence for spatial resource partitioning across temperate grassland biodiversity experiments. *Ecology* 101:1–13. <https://doi.org/10.1002/ecy.2905>
- Bauhus J, Forrester DI, Gardiner B, et al (2017) Ecological stability of mixed-species forests. In: *Mixed-Species Forests: Ecology and Management*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 337–382
- Bauhus J, Messier C (1999) Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. *Can J For Res* 29:260–273. <https://doi.org/10.1139/x98-206>
- Blanck K, Lamersdorf N, Dohrenbusch A, Murach D (1995) Response of a Norway spruce forest ecosystem to drought/rewetting experiments at Solling, Germany. *Water, Air, Soil Pollut* 85:1251–1256. <https://doi.org/10.1007/BF00477153>
- Bolte A, Villanueva I (2006) Interspecific competition impacts on the morphology and distribution of fine roots in European beech (*fagus sylvatica* L.) and Norway spruce (*picea abies* (L.) karst.). *Eur J For Res* 125:15–26. <https://doi.org/10.1007/s10342-005-0075-5>
- Bouma TJ, Nielsen KL, Koutstaal B (2000) Sample preparation and scanning protocol for computerised analysis of root length and diameter. *Plant Soil* 218:185–196. <https://doi.org/10.1023/A:1014905104017>

- Brassard BW, Chen HYHH, Bergeron Y, Paré D (2011) Differences in fine root productivity between mixed- and single-species stands. *Funct Ecol* 25:238–246. <https://doi.org/10.1111/j.1365-2435.2010.01769.x>
- Brassard BW, Chen HYHH, Cavard X, et al (2013) Tree species diversity increases fine root productivity through increased soil volume filling. *J Ecol* 101:210–219. <https://doi.org/10.1111/1365-2745.12023>
- Brunner I, Herzog C, Galiano L, Gessler A (2019) Plasticity of Fine-Root Traits Under Long-Term Irrigation of a Water-Limited Scots Pine Forest. *Front Plant Sci* 10:701. <https://doi.org/10.3389/fpls.2019.00701>
- Castagneyrol B, Bonal D, Damien M, et al (2017) Bottom-up and top-down effects of tree species diversity on leaf insect herbivory. *Ecol Evol* 7:3520–3531. <https://doi.org/10.1002/ece3.2950>
- Castagneyrol B, Giffard B, Péré C, Jactel H (2013) Plant apparency, an overlooked driver of associational resistance to insect herbivory. *J Ecol* 101:418–429. <https://doi.org/10.1111/1365-2745.12055>
- Comas LH, Eissenstat DM (2009) Patterns in root trait variation among 25 co-existing North American forest species. *New Phytol* 182:919–928. <https://doi.org/10.1111/j.1469-8137.2009.02799.x>
- Curt T, Prévosto B (2003) Rooting strategy of naturally regenerated beech in Silver birch and Scots pine woodlands. *Plant Soil* 255:265–279. <https://doi.org/10.1023/A:1026132021506>
- Danjon F, Fourcaud T, Bert D (2005) Root architecture and wind-firmness of mature *Pinus pinaster*. *New Phytol* 168:387–400. <https://doi.org/10.1111/j.1469-8137.2005.01497.x>
- Ding Y, Leppälampi-Kujansuu J, Helmisaari HS (2019) Fine root longevity and below- and aboveground litter production in a boreal *Betula pendula* forest. *For Ecol Manage* 431:17–25. <https://doi.org/10.1016/j.foreco.2018.02.039>
- Epron D, Nouvellon Y, Ryan MG (2012) Introduction to the invited issue on carbon allocation of trees and forests. *Tree Physiol.* 32:639–643
- Finér L, Helmisaari HS, Löhmus K, et al (2007) Variation in fine root biomass of three European tree species: Beech (*Fagus sylvatica* L.), Norway spruce (*Picea abies* L. Karst.), and Scots pine (*Pinus sylvestris* L.). *Plant Biosyst.* 141:394–405
- Forrester DI (2017) Ecological and physiological processes in mixed versus monospecific stands. In: *Mixed-Species Forests: Ecology and Management*. pp 73–115
- Fruleux A, Bogeat-Triboulot MB, Collet C, et al (2018) Aboveground overyielding in a mixed temperate forest is not explained by belowground processes. *Oecologia* 188:1183–1193. <https://doi.org/10.1007/s00442-018-4278-0>
- Fruleux A, Bonal D, Bogeat-Triboulot MB (2016) Interactive effects of competition and water availability on above- and below-ground growth and functional traits of

- European beech at juvenile level. *For Ecol Manage* 382:21–30.  
<https://doi.org/10.1016/j.foreco.2016.09.038>
- Gale MR, Grigal DF (1987) Vertical root distributions of northern tree species in relation to successional status. *Can J For Res* 17:829–834.  
<https://doi.org/10.1139/x87-131>
- George E, Seith B, Schaeffer C, Marschner H (1997) Responses of *Picea*, *Pinus* and *Pseudotsuga* roots to heterogeneous nutrient distribution in soil. *Tree Physiol* 17:39–45. <https://doi.org/10.1093/TREEPHYS/17.1.39>
- Godbold DL, Fritz H-WHW, Jentschke G, et al (2003) Root turnover and root necromass accumulation of Norway spruce (*Picea abies*) are affected by soil acidity. *Tree Physiol* 23:915–921. <https://doi.org/10.1093/treephys/23.13.915>
- Göransson H, Fransson AM, Jönsson-Belyazid U (2007) Do oaks have different strategies for uptake of N, K and P depending on soil depth? *Plant Soil* 297:119–125. <https://doi.org/10.1007/s11104-007-9325-2>
- Grossiord C, Granier A, Ratcliffe S, et al (2014) Tree diversity does not always improve resistance of forest ecosystems to drought. *Proc Natl Acad Sci U S A* 111:14812–14815. <https://doi.org/10.1073/pnas.1411970111>
- Hansson K, Helmisaari HS, Sah SP, Lange H (2013) Fine root production and turnover of tree and understorey vegetation in Scots pine, silver birch and Norway spruce stands in SW Sweden. *For Ecol Manage* 309:58–65.  
<https://doi.org/10.1016/j.foreco.2013.01.022>
- Hartmann H (2011) Will a 385 million year-struggle for light become a struggle for water and for carbon? - How trees may cope with more frequent climate change-type drought events. *Glob Chang Biol* 17:642–655.  
<https://doi.org/10.1111/j.1365-2486.2010.02248.x>
- Helmisaari HS, Derome J, Nöjd P, Kukkola M (2007) Fine root biomass in relation to site and stand characteristics in Norway spruce and Scots pine stands. *Tree Physiol* 27:1493–1504. <https://doi.org/10.1093/treephys/27.10.1493>
- Hooper DU, Chapin FS, Ewel JJ, et al (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecol Monogr* 75:3–35.  
<https://doi.org/10.1890/04-0922>
- Imada S, Yamanaka N, Tamai S (2008) Water table depth affects *Populus alba* fine root growth and whole plant biomass. *Funct Ecol* 22:1018–1026.  
<https://doi.org/10.1111/j.1365-2435.2008.01454.x>
- Jackson RB, Mooney HA, Schulze E-D (1997) A global budget for fine root biomass, surface area, and nutrient contents. *Ecology* 94:7362–7366
- Jactel H, Bauhus J, Boberg J, et al (2017) Tree Diversity Drives Forest Stand Resistance to Natural Disturbances. *Curr. For. Reports* 3:223–243
- Jactel H, Brockerhoff EG (2007) Tree diversity reduces herbivory by forest insects. *Ecol Lett* 10:835–848. <https://doi.org/10.1111/j.1461-0248.2007.01073.x>



- Jactel H, Gritti ES, Drössler L, et al (2018) Positive biodiversity–productivity relationships in forests: Climate matters. *Biol Lett* 14:20170747. <https://doi.org/10.1098/rsbl.2017.0747>
- Jagodziński AM, Kałucka I (2011) Fine root biomass and morphology in an age-sequence of post-agricultural *Pinus sylvestris* L. stands. *Dendrobiology* 66:71–84. <https://doi.org/https://www.scopus.com/inward/record.uri?eid=2-s2.0-82855181011&partnerID=40&md5=fdda0635af5ba0fa063cfd229af69aa9>
- Jagodzinski AM, Ziolkowski J, Warnkowska A, Prais H (2016) Tree age effects on fine root biomass and morphology over chronosequences of *fagus sylvatica*, *quercus robur* and *alnus glutinosa* stands. *PLoS One* 11:e0148668. <https://doi.org/10.1371/journal.pone.0148668>
- Jose S, Williams R, Zamora D (2006) Belowground ecological interactions in mixed-species forest plantations. *For Ecol Manage* 233:231–239. <https://doi.org/10.1016/j.foreco.2006.05.014>
- Jucker T, Bouriaud O, Avacaritei D, Coomes DA (2014) Stabilizing effects of diversity on aboveground wood production in forest ecosystems: Linking patterns and processes. *Ecol Lett* 17:1560–1569. <https://doi.org/10.1111/ele.12382>
- Kardol P, Fanin N, Wardle DA (2018) Long-term effects of species loss on community properties across contrasting ecosystems. *Nature* 557:710–713. <https://doi.org/10.1038/s41586-018-0138-7>
- Kulmatiski A, Adler PB, Stark JM, Tredennick AT (2017) Water and nitrogen uptake are better associated with resource availability than root biomass. *Ecosphere* 8:e01738. <https://doi.org/10.1002/ecs2.1738>
- Lebourgeois F, Gomez N, Pinto P, Mérian P (2013) Mixed stands reduce *Abies alba* tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. *For Ecol Manage* 303:61–71. <https://doi.org/10.1016/j.foreco.2013.04.003>
- Leuschner C, Hertel D, Schmid I, et al (2004) Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. *Plant Soil* 258:43–56. <https://doi.org/10.1023/B:PLSO.0000016508.20173.80>
- Makita N, Hirano Y, Mizoguchi T, et al (2011) Very fine roots respond to soil depth: Biomass allocation, morphology, and physiology in a broad-leaved temperate forest. *Ecol Res* 26:95–104. <https://doi.org/10.1007/s11284-010-0764-5>
- Mauer O, Palátová E (2018) The role of root system in silver birch (*Betula pendula* Roth) dieback in the air-polluted area of Krušné hory Mts. *J For Sci* 49:191–199. <https://doi.org/10.17221/4693-jfs>
- Meinen C, Hertel D, Leuschner C (2009a) Root growth and recovery in temperate broad-leaved forest stands differing in tree species diversity. *Ecosystems* 12:1103–1116. <https://doi.org/10.1007/s10021-009-9271-3>

- Meinen C, Hertel D, Leuschner C (2009b) Biomass and morphology of fine roots in temperate broad-leaved forests differing in tree species diversity: Is there evidence of below-ground overyielding? *Oecologia* 161:99–111. <https://doi.org/10.1007/s00442-009-1352-7>
- Messier C, Puettmann K, Chazdon R, et al (2015) From Management to Stewardship: Viewing Forests As Complex Adaptive Systems in an Uncertain World. *Conserv Lett* 8:368–377. <https://doi.org/10.1111/conl.12156>
- Morin X, Damestoy T, Toigo M, et al (2020) Using forest gap models and experimental data to explore long-term effects of tree diversity on the productivity of mixed planted forests. *Ann For Sci* 77:1–19. <https://doi.org/10.1007/s13595-020-00954-0>
- Ostonen I, Püttsepp Ü, Biel C, et al (2007) Specific root length as an indicator of environmental change. *Plant Biosyst.* 141:426–442
- Ostonen I, Rosenvald K, Helmisaari HS, et al (2013) Morphological plasticity of ectomycorrhizal short roots in *Betula* sp and *Picea abies* forests across climate and forest succession gradients: Its role in changing environments. *Front Plant Sci* 4:335. <https://doi.org/10.3389/fpls.2013.00335>
- Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct Ecol* 21:489–495. <https://doi.org/10.1111/j.1365-2435.2007.01267.x>
- Persson H, Von Fircks Y, Majdi H, Nilsson LO (1995) Root distribution in a Norway spruce (*Picea abies* (L.) Karst.) stand subjected to drought and ammonium-sulphate application. *Plant Soil* 168–169:161–165. <https://doi.org/10.1007/BF00029324>
- Pretzsch H (2013) Facilitation and competition in mixed-species forests analyzed along an ecological gradient. *Nov Acta Leopold* 114:159–174
- Puhe J (2003) Growth and development of the root system of Norway spruce (*Picea abies*) in forest stands - A review. *For Ecol Manage* 175:253–273. [https://doi.org/10.1016/S0378-1127\(02\)00134-2](https://doi.org/10.1016/S0378-1127(02)00134-2)
- Rahman MM, Castagneyrol B, Verheyen K, et al (2018) Can tree species richness attenuate the effect of drought on organic matter decomposition and stabilization in young plantation forests? *Acta Oecologica* 93:30–40. <https://doi.org/10.1016/j.actao.2018.10.008>
- Rewald B, Leuschner C (2009) Belowground competition in a broad-leaved temperate mixed forest: Pattern analysis and experiments in a four-species stand. *Eur J For Res* 128:387–398. <https://doi.org/10.1007/s10342-009-0276-4>
- Richards AE, Forrester DI, Bauhus J, Scherer-Lorenzen M (2010) The influence of mixed tree plantations on the nutrition of individual species: A review. *Tree Physiol.* 30:1192–1208

- Rosengren U, Göransson H, Jönsson U, et al (2005) Functional biodiversity aspects on the nutrient sustainability in forests - Importance of root distribution. *J Sustain For* 21:77–100. [https://doi.org/10.1300/J091v21n02\\_06](https://doi.org/10.1300/J091v21n02_06)
- Rosenvald K, Ostonen I, Uri V, et al (2013) Tree age effect on fine-root and leaf morphology in a silver birch forest chronosequence. *Eur J For Res* 132:219–230. <https://doi.org/10.1007/s10342-012-0669-7>
- Salahuddin S, Rewald B, Razaq M, et al (2018) Root order-based traits of Manchurian walnut & larch and their plasticity under interspecific competition. *Sci Rep* 8:1–14. <https://doi.org/10.1038/s41598-018-27832-0>
- Schenk HJ (2006) Root competition: Beyond resource depletion. *J. Ecol.* 94:725–739
- Schmid I (2002) The influence of soil type and interspecific competition on the fine root system of Norway spruce and European beech. *Basic Appl Ecol* 3:339–346. <https://doi.org/10.1078/1439-1791-00116>
- Schmid I, Kazda M (2002) Root distribution of Norway spruce in monospecific and mixed stands on different soils. *For Ecol Manage* 159:37–47. [https://doi.org/10.1016/S0378-1127\(01\)00708-3](https://doi.org/10.1016/S0378-1127(01)00708-3)
- Schmidt-Vogt H (1977) *The Spruce [Picea]. A manual in two volumes. Vol. 1. Taxonomy, geographical distribution, morphology, ecology, and forest communities.* Paul Parey.
- Sudmeyer RA, Speijers J, Nicholas BD (2004) Root distribution of *Pinus pinaster*, *P. radiata*, *Eucalyptus globulus* and *E. kochii* and associated soil chemistry in agricultural land adjacent to tree lines. In: *Tree Physiology*. pp 1333–1346
- Vandermeer JH, Goldberg DE (2013) *Population ecology: first principles*
- Verheyen K, Vanhellemont M, Auge H, et al (2016) Contributions of a global network of tree diversity experiments to sustainable forest plantations. *Ambio* 45:29–41. <https://doi.org/10.1007/s13280-015-0685-1>
- Vogt KA, Vogt DJ, Palmiotto PA, et al (1995) Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant Soil An Int J Plant-Soil Relationships* 187:159–219. <https://doi.org/10.1007/BF00017088>
- von Felten S, Schmid B (2008) Complementarity among species in horizontal versus vertical rooting space. *J Plant Ecol* 1:33–41. <https://doi.org/10.1093/jpe/rtm006>
- Wang AF, Roitto M, Sutinen S, et al (2015) Waterlogging in late dormancy and the early growth phase affected root and leaf morphology in *Betula pendula* and *Betula pubescens* seedlings. *Tree Physiol* 36:86–98. <https://doi.org/10.1093/treephys/tpv089>
- Weemstra M, Sterck FJ, Visser EJW, et al (2017) Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. *Plant Soil* 415:175–188. <https://doi.org/10.1007/s11104-016-3148-y>

Withington JJM, Reich PB, Oleksyn J, Eissenstat DDM (2006) Comparisons of structure and life span in roots and leaves among temperate trees. *Ecol Monogr* 76:381–397. [https://doi.org/10.1890/0012-9615\(2006\)076\[0381:COSALS\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2006)076[0381:COSALS]2.0.CO;2)

Zanetti C, Vennetier M, Mériaux P, Provansal M (2014) Plasticity of tree root system structure in contrasting soil materials and environmental conditions. *Plant Soil* 387:21–35. <https://doi.org/10.1007/s11104-014-2253-z>

Zhou G, Meng S, Yu J, et al (2018) Quantitative relationships between fine roots and stand characteristics. *Eur J For Res* 137:385–399. <https://doi.org/10.1007/s10342-018-1112-5>



## Chapter 4 | Fine root dynamics in response to tree species mixing, stand density, and water availability

*In collaboration with: Stephan Hättenschwiler, Laurent Augusto, Bastien Castagneyrol, Nicolas Fanin, Hervé Jactel, Céline Meredieu, and Mark R. Bakker*

### Abstract

Tree diversity could have a positive effect on forest functioning or its resilience to disturbances, but such relationships are under-investigated for belowground processes, and can further depend on stand density and abiotic factors such as water availability. Here, we investigated whether different stand density and water supply modulate tree mixture effects on fine root production, turnover and decomposition, and how this affects carbon (C), nitrogen (N), and phosphorus (P) cycling. We used a 10-year-old tree diversity experiment where species composition, stand density and water supply were manipulated, to examine production, turnover, decomposition and fluxes of C, N and P of fine roots in the top 15 cm of the soil. In a complete block design, pure stands of maritime pine (*Pinus pinaster*) or silver birch (*Betula pendula*) were compared with stands where both species are mixed. This was done at two levels of stand density and two levels of water supply (no irrigation vs summer irrigation). Fine root dynamics were assessed for a 2-yr period (from age 10 to 12 of the trees). We hypothesized higher fine root dynamics in mixtures, and that these mixture effects would be stronger under harsher conditions (in denser stands and/or non-irrigated stands). Fine root production, turnover and decomposition did not differ between mixtures and pure stands of pine and birch, regardless of stand density or water supply, but fine root production was greater and turnover was lower in denser stands. Release of N did not differ between mixtures and pure stands, but N release was higher in denser stands. Remarkably, in pine roots there was no net phosphorus (P) release during decomposition in contrast to birch roots, but pine roots released twice as much carbon (C) than birch roots. Our results suggest that decomposing birch roots more quickly release P than decomposing pine roots, which is potentially beneficial for pine nutrition, when growing together with birch. Although there were no mixture effects on fine root dynamics, there is still a benefit in mixing birch with pine, since fine root dynamics of birch and pine mixtures were unaffected when experiencing stronger interspecific competition for water and nutrients,.

**Keywords:** fine root production, turnover, decomposition, mixed-species plantations, stand density, summer drought

## 4.1 Introduction

Estimations from temperate forests of Central Europe revealed that C storage in trees accounts for about 110 t C ha<sup>-1</sup> of which 1.2 t C ha<sup>-1</sup> is in fine roots (Brunner and Godbold 2007). Although the global carbon allocation to fine roots seems small (1%), these fine roots are estimated to represent 33% of the global annual Net Primary Production (Gill and Jackson 2000), and together with their associated mycorrhiza, fine roots may be at the origin of 50–70% of the soil carbon (C) pool (Clemmensen et al. 2013; Sokol et al. 2019). In addition to their contribution to carbon dynamics, fine roots are a considerable pool of nitrogen (N) and phosphorus (P) in forest ecosystems; these nutrient fluxes being essential for tree growth and forest productivity (Freschet et al. 2013; Hobbie 2015). Both, the production and turnover of fine roots are sensitive to environmental conditions (McCormack et al. 2014), which make observations of fine root dynamics particularly important with projected climate change.

Plant species have recently been proposed to be positioned along a gradient of a root economic space (RES), which hypothesizes that root functional traits are distributed between two opposing trait syndromes. These are associated with either fast resource acquisition (low root tissue density (RTD), high N concentration, and shorter lifespan typically associated with rapid decomposition) or enhanced resource conservation (high RTD roots with lower N concentration, and longer lifespan typically associated with slow decomposition) (Freschet et al. 2013; Hobbie et al. 2010; Roumet et al. 2016). These traits can be influenced by environmental conditions, with further consequences on root decomposition and fine root lifespan. For example, roots are able to proliferate when encountering nutrient rich patches (increase in SRL for capture of N, or prolific root branching for capture of P; see Comas et al. (2012)). They were also shown to decrease SRL and specific root area (SRA), and to increase root C:N, when experiencing dry conditions (Meier and Leuschner 2008), resulting in roots that are more rapidly or more slowly decomposing, respectively, and that have shorter or longer lifespan. However, these commonly measured root traits may be indirectly influenced by other traits that are less frequently measured. For instance stele and cortical tissues can be sensitive to drought, and might strongly affect root tissue density or root diameter, to the extent that these traits no longer coincide with the RES (Kong et al. 2019), suggesting that an extended vision beyond the RES framework is needed when evaluating the influence of environmental conditions. Furthermore, individual root traits may be important for predicting soil microbial taxa and functional guilds that are linked to decomposition of soil organic matter (Spitzer et al. 2020). Therefore, although fine root morphology and chemistry have strong links with carbon economy (i.e. root decomposition; root lifespan) (McCormack et al. 2012; Roumet et al. 2016), a considerable improvement in the evaluation of fine root dynamics would be to simultaneously measure rates of fine root production, turnover (as inverse of lifespan), and decomposition, and how these are related to root trait variation under environmental constraints.

The rate at which nutrient cycling takes place in forests can influence aboveground productivity, and this is important in the context of climate change with an expected increase of drought and temperatures (IPCC 2014). Reduced water availability may lead to lower growth and higher mortality of fine roots, in particular if this occurs for

species or at sites normally well supplied in water. However, in adaptation to such conditions, or as a response to drought events, fine root growth may be stimulated to compensate for lower water and nutrient capture under water limitation (Leuschner et al. 2001). Previous experimental studies that used irrigation treatments in forests, reported inconsistent results on fine root production, turnover, and decomposition (Gaul et al. 2008; King et al. 1997; Majdi and Andersson 2005; Meier and Leuschner 2008). The inconsistent effects of water supply may be related to the general environmental context of the studies, including the intensity of water stress, and to plant species-specific differences, including root traits such as root diameter and root tissue density. Importantly, litter nutrient dynamics are rarely measured together with root decomposition rates (but see Hobbie and Vitousek 2000), making the evaluation of nutrient release difficult, especially because nutrients may be immobilized (nutrient retention) rather than released (release) to the soil (Guo et al. 2006; Palviainen et al. 2004). The decomposition rate depends on the quality of fine root litter, with roots of higher N and/or P, and lower C:N and/or C:P concentrations typically decomposing faster and releasing nutrients more rapidly than roots of opposing quality (Freschet et al. 2012; See et al. 2019; Silver and Miya 2001). In addition to initial quality, the release or retention of carbon and nutrients may depend on site fertility. For instance, root P influence on root decomposition has recently been found to be weak at the global scale, but to have consistent positive effects at the local scale, while root N effects were strong at both global and local scales (See et al. 2019). This result is likely due to site-specific differences in the N:P stoichiometry of microbial nutrient demand and availability, or because certain sites are more P limited (Cleveland and Liptzin 2007). By evaluating both C and nutrient dynamics simultaneously, we may improve our understanding of the effect of water supply on fine root dynamics.

The diversity of plant communities can affect both forest growth (Jactel et al. 2018; Kardol et al. 2018; Liang et al. 2016) and forest litter decomposition (Hättenschwiler 2005; Gessner et al. 2010; Kou et al. 2020). While plant diversity effects are reasonably well understood for aboveground processes there is still a lot of uncertainty for belowground processes (Laliberté 2017). The majority of biodiversity experiments that investigated belowground processes such as fine root productivity, turnover and decomposition were in grasslands (Mommer et al. 2010; Prieto et al. 2017; Ravenek et al. 2014). Forest ecosystems are critically underrepresented (Gamfeldt et al. 2013; Isbell et al. 2011), with some showing increased fine root productivity or turnover in tree mixtures (Meinen et al. 2009; Lei et al. 2012a; Brassard et al. 2013; Jacob et al. 2014), while other studies found no effects of tree mixtures (Domisch et al. 2015; Lei et al. 2012b). Contrasting results from forests may arise because: i) root traits among the studied tree species varied more or less with more or less potential for niche differentiation, ii) the intensity of above- or below-ground interspecific competition differed as a function of variable space occupancy (young stands) and/or iii) different neighbourhood patterns among trees with more or less root interactions (Domisch et al. 2015; Forrester et al. 2013; Forrester and Bauhus 2016). For example, in a recent study, Zeng et al. (2020) showed that positive tree diversity effects on fine root biomass were dependent on stand density, with fine root biomass increasing with species richness at lower stand density, but not at higher stand density. We are aware of only two studies that investigated root decomposition in mixed forests, and both studies



showed no mixture effect (Guerrero-Ramírez et al. 2016; Li et al. 2018). Possibly the composition and diversity of chemical litter traits, soil environment (soil temperature, soil humidity), soil texture (Weemstra et al. 2017), and presence of living roots may be more important than species diversity per se on the decomposition of fine roots. For instance, increased soil humidity can increase fine root decomposition, but only in combination with increased soil temperature (Liu et al. 2017). Also, denser stands may lead to more living fine root biomass in the soil, hence to more exudation of carbon compounds that stimulate the growth of soil microbes and thereby increase dead root decomposition and mineralization (Moore et al. 2020; Van Der Krift et al. 2002).

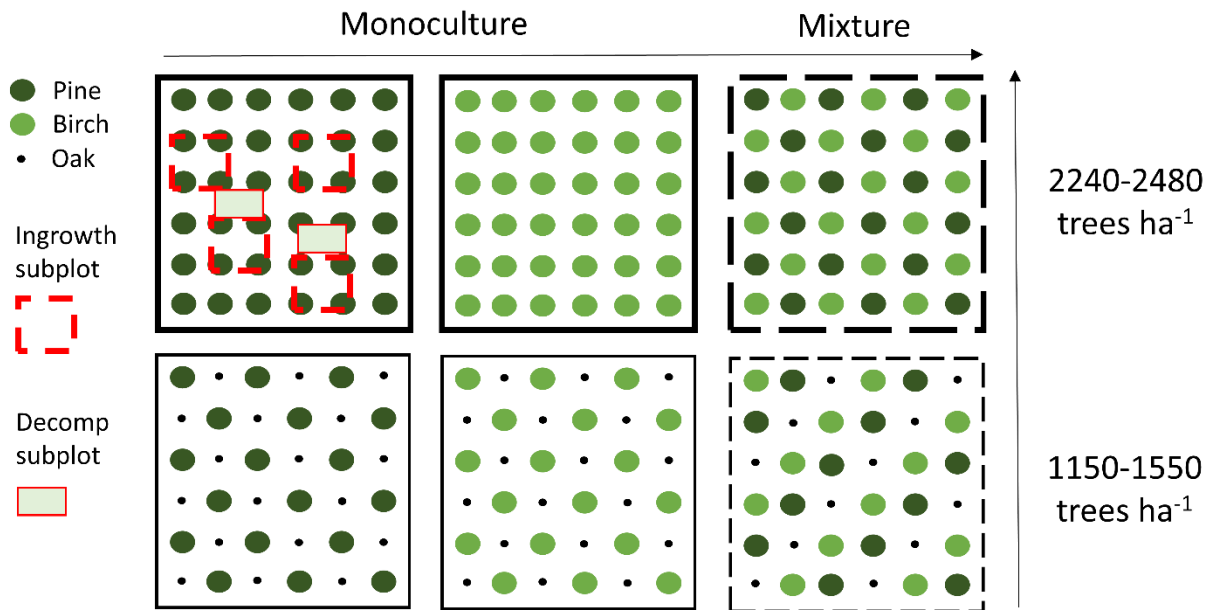
Here, we used two species in a fully factorial randomized block design, with tree composition (pure or mixed stands of birch and pine), stand density (two levels) and water supply (two levels) replicated in four blocks. Our objective was to evaluate how tree species mixture, stand density, and water supply interactively affect (1) fine root production, turnover and decomposition, and (2) how this may affect the fluxes of C, N, and P in the soil. With respect to fine root production (FRP), fine root turnover (FRT), and fine root decomposition (FRD), we hypothesized that there would be simultaneously a higher FRP, higher FRT and faster FRD in tree mixtures compared to single species stands. We also expected that in denser stands or under summer irrigation FRP, FRT and FRD would increase. We further hypothesized that mixture effects would be regulated by interspecific competition or water availability; for example, mixture effects on FRP, FRT and FRD might increase in denser stands where interspecific competition is stronger. Similarly, mixture effects would be weaker when irrigation leads to higher water availability. In a third hypothesis, we expected that since the soils of our study site are P-limited, there would be a higher release of P during root decomposition, especially for birch stands that were previously shown to have high activities of P-related enzymes (Maxwell et al. 2020).

## 4.2 Materials and Methods

### 4.2.1 Study site and experimental design

The study site is located 40 km south-west of Bordeaux (44°44' N, 00°47' W) at an altitude of 60 m above sea level. The climate is oceanic, usually with a dry season in summer and a wet season in winter and/or in spring with a mean annual precipitation of 941 mm and a mean day temperature of 13 °C (average 2009–2019 measured at the nearby weather station in Cestas, at 1.5 km from the site). The driest and warmest months alternate between July and August (average precipitation for these months is 45–49 mm, and temperature 20.3–20.6 °C). The wettest and coldest month is November (average precipitation is 151 mm, and temperature 9.9 °C). The soil is a typical nutrient poor podzol with coarse texture (95% sand) (Augusto et al. 2010).

We took advantage of an ongoing tree diversity experiment ORPHEE, where all 31 possible combinations of one to five tree species were planted in eight blocks in 2008 (Castagneyrol et al. 2013). The five species are: silver birch, *Betula pendula* Roth.; pedunculate oak, *Quercus robur* L.; Pyrenean oak, *Quercus pyrenaica* Willd., holm oak, *Quercus ilex* L.; and maritime pine, *Pinus pinaster* Ait. Tree species mixtures were established according to a substitutive design, keeping stand density equal across plots (2500 trees ha<sup>-1</sup>). Within mixture plots, individual trees from different species were planted in a regular alternate pattern, such that a tree from a given species had at least one neighbour from each of the other species within a 2 m radius. Four out of the eight blocks receive irrigation every year from May to October, since 2015, to increase water availability in this area where summer drought is regular and often severe, particularly on these sandy soils with low water holding capacity. For this study, we included 48 plots with combinations of the three species: silver birch, maritime pine and pedunculate oak. Oak trees experienced the highest mortality rate in the first 10 years of the experiment (mortality 45 %), and are growing slowly in comparison to birch and pine. At age 10, oaks were on average 164 ± 90 cm tall, and were much smaller than birches (727 ± 167cm) and pines (886 ± 109 cm). Given the contrasts in tree presence and height, oaks were thus confounded with the understory vegetation (around 100 cm) and therefore pure plots of oak were not included. However, we considered that plots containing either birch or pines (or both) together with oaks could be assimilated to low-density pine or birch monocultures, or low-density pine-birch mixtures (Fig. 4.1). Finally, our experimental design included three tree species compositions: pure silver birch, pure maritime pine, and the mixed birch-pine plots, at a comparatively high (2240–2480 trees ha<sup>-1</sup>, for a planting density of 2500 trees ha<sup>-1</sup>) and low (1150–1550 trees ha<sup>-1</sup>, for a planting density of 1666 trees ha<sup>-1</sup> without counting oak as the third species) stand densities, with four out of the eight replicates receiving irrigation during the summer months. This yielded 48 plots: three tree species compositions × two stand densities × two irrigation treatments × four replicates.



**Fig. 4.1** Conceptual diagram representing our experimental setup. We take advantage of the slower growth and higher mortality rate of oak (small black dots) to test tree diversity effect at high and low stand densities of pine (dark green dots) and birch (light green dots) in the ORPHEE experiment. In each plot, we chose four zones for the study of root growth ('*Ingrowth*' subplots) and two zones for the study of the decomposition of roots ('*Decomp*' subplots). The four '*Ingrowth*' subplots were regularly distributed over each plot and were used for tree and understory inventory, sampling of root standing biomass and the study of root dynamics with ingrowth cores. The two '*Decomp*' subplots were assigned in half of the root zones. Diversity effects were tested between pure plots of pine, birch (solid squares) and pine x birch mixture (dashed line). The effect of stand density was tested using high-density plots (thick squares) and low-density plots (thin squares); these were plots where pine and birch grew together with oak.

In each plot, in April 2017, we chose four 2 m × 2 m areas bounded by four trees for the study of root growth ('*Ingrowth*' subplots) and two other areas of the same dimensions for the study of the decomposition of roots ('*Decomp*' subplots, Fig. 4.1). The four '*Ingrowth*' subplots were regularly distributed within each plot and were used for tree and understory inventory, sampling of root standing biomass and the study of root dynamics with ingrowth cores. Next to two of the "*Ingrowth*" subplots, we established in total two '*Decomp*' subplots (Fig. 4.1), so that there were four "*Ingrowth*" and two "*Decomp*" subplots in each plot. In June-July 2018, we measured stem diameters (see Table C4.1 in Appendix C) and canopy dimensions (see Table C4.2 in Appendix C) of the four bordering trees of each subplot. We also recorded the understory vegetation cover for each subplot (enlarging each subplot by extending two meters in each direction; i.e. 6 m × 6 m areas, to have a larger observation area). Although the plantations were young, both high- and low stand density plots had already closed canopies. The understory vegetation was dominated by bracken fern (*Pteridium aquilinum* L. Kuhn) with an average ground cover ranging from 18% to 67%, and purple moor-grass (*Molinia caerulea* L. Moench) with an average cover ranging from 3% to 33%, (see Table C4.3 in Appendix C for a detailed description of understory presence across treatments). The understory was mown once per year in the two first years (2008–2009) and again at the beginning of 2018, every second row spacing.

#### 4.2.2 Fine root production and turnover

Between April 3<sup>rd</sup> and 7<sup>th</sup> 2017, holes for ingrowth core installation were created using a stainless steel soil corer (8 cm Ø, 15 cm deep). In each hole, an empty ingrowth core (mesh size 6 mm, closed at the bottom end) was deployed with the help of an 8 cm diameter plastic tube (15 cm deep into the hole), around which the ingrowth core was wrapped while inserting both into the soil. The soil retrieved previously from these holes, if necessary supplemented with soil from an extra hole made in the same plot to account for losses, was passed over a 2 mm sieve to remove organic debris and roots (Andreasson et al. 2016). The sieved soil was then inserted into each hole to reach a level slightly higher than the soil level, and was then gently 'pushed' by hand to compact each ingrowth core at a similar density as bulk soil. Subsequently, the plastic tube was removed, leaving only the ingrowth core filled with sieved soil. We established two ingrowth cores in each '*Ingrowth*' subplot (so each ingrowth core was surrounded by four trees), resulting in a total of eight root ingrowth cores (four pairs) in each plot (two ingrowth cores × four subplots × three tree species compositions × two stand densities × two irrigation treatments × four block replicates = 384 ingrowth cores).

In mid-March 2018, for the purpose of estimating the living fine root standing biomass, four soil cores were collected per plot, i.e. one core was collected from each of the four '*Ingrowth*' subplots. The collection of cores for fine root standing biomass was done with the same corer used for creating the ingrowth core holes (8 cm Ø, 15 cm length), permitting sampling of the top 0–15 cm of soil, i.e. the same soil depth as for the ingrowth cores (in total four soil cores × three tree species compositions × two stand densities × two irrigation treatments × four block replicates = 192 soil cores). We chose to perform the fine root standing biomass assessment in the middle of the two-

year period of ingrowth (2017–2019) to be as representative as possible of the total period for this rather young experimental plantation. One ingrowth core from each pair of ingrowth cores was harvested after 12 months (April 9<sup>th</sup> and April 10<sup>th</sup> 2018), while the second of the pair was harvested after 24 months (April 12<sup>th</sup> 2019). The ingrowth cores were removed by carefully cutting the soil surrounding each core with a knife and then tearing out the ingrowth core by hand. Any roots that passed beyond the mesh of the core were cut in the field in order to keep exclusively all the roots that were grown inside the core. All root sample cores (i.e. 384 ingrowth cores and 192 standing biomass cores) were transported in individual plastic bags to the laboratory and then stored at 4°C until further processing.

In the lab, we first separated roots from soil over a 2 mm mesh and then gradually passed the root material through a cleansing basin with water. This basin permitted gently manipulating the roots while soaking in the water and removing any adhering soil particles within a rather short period, efficient for our sandy soils. Any roots larger than 2 mm in diameter were discarded as we focussed here on fine roots ( $\leq 2$  mm  $\emptyset$ ) exclusively. Fine roots are responsible for plant water and nutrient uptake (Jackson et al. 1997) and are the ones most affected by change in environmental conditions (Ostonen et al. 2007c). Once all roots were recovered and cleaned from adhering soil particles we sorted them according to visual morphological criteria (Altinmazis-Kondylis et al. 2020; Bakker et al. 2006) into target species (silver birch, maritime pine), and understory species. Roots belonging to birch and pine were sorted to live and dead fractions. For the first ingrowth core harvest after 12 months, there were no apparent dead roots. For the second ingrowth core harvest after 24 months, there were some dead roots (see [Fig. C4.1](#) in Appendix C) that we kept separate for the target species, but we did not keep dead roots of understory as many of those were too fine and too degraded to retrieve. For the living fine root standing biomass cores, we only focussed on live roots and discarded dead root fragments from our samples. The cleaned live fine roots were stored in 10% ethanol at 4°C before being scanned with the WinRhizo Software (version 2005a, Regents Instruments Inc. Canada). After scanning, the scanned roots were oven-dried for two days at 60°C and weighed. The scans provided data on fine root: length, area, diameter and volume, which were used to calculate: Specific Root Length (SRL) (fine root length/ dry root weight), Specific Root Area (SRA) (fine root area/ dry root weight), and Root Tissue Density (RTD) (fine root volume / dry root weight). The dead root fraction was also dried at 60 °C for 2 days, and weighed to obtain values of fine root necromass.

Because of the disruptive nature of the ingrowth core method, it has been suggested that it is better to leave the ingrowth cores two to three years in the soil before harvests, and to use the last harvest for the overarching computations of production and turnover (Andreasson et al. 2016; Majdi et al. 2005). Yet, it can be worthwhile to investigate how fast fine roots reach similar densities as those encountered in undisrupted soil cores (such as in standing biomass cores) and thus to include intermediate harvests. The first year of ingrowth could be dominated by root-iteration of damaged roots upon installation while only few of the new roots turn over to dead roots (Andreasson et al. 2016). Consequently, we chose to work with the roots from the 24-month ingrowth cores in our computations of fine root production and turnover and used the 12-month ingrowth data only to investigate the growth pattern between installation and the 24-month ingrowth period (e.g. see Fig. C4.1 in Appendix C). All root production data were expressed per unit ground area and per year (i.e. as an approximation we divided the fine root production after 2 years by two). Annual fine root turnover ( $\text{yr}^{-1}$ ) was calculated by dividing the fine root production after 2 years by the living fine root standing biomass, according to Gill and Jackson (2000):

$$FRT = \frac{FRP/2}{\text{Standing FRB}}$$

With FRP = fine root production (cumulated value of biomass and necromass after 2 years), and Standing FRB = live root standing crop

### 4.2.3 Fine root decomposition

For the decomposition experiment we collected root material of *Pinus pinaster* and *Betula pendula* at a common location 3 km far from the ORPHEE experiment under similar climatic and edaphic conditions from at least five individual trees per species in early fall 2017. Fine roots including the first five root orders were harvested, washed and dried at 40 °C for 7 days. We constructed fine root litterbags (tightly stitched pockets of nylon 5 cm × 9 cm) with nylon mesh (mesh width of 48µm) and filled each litterbag with 0.5 g of dried fine root material of either tree species (no mixtures). We chose the particular mesh size to avoid roots from growing into the litterbag without hindering the passage of fungal hyphae. In mid-December 2017, four fine root litterbags of each species were buried at 0–5 cm depth distributed in two designated “Decomp” subplots within their species-specific monocultures (total of 4 root litterbags for each species; 2 fine root litterbags for each of two “Decomp” subplots). Therefore, for the monocultures we had: 2 replicates × 2 years of decomposition × 2 tree species (birch and pine growing in their monocultures) × 2 stand densities × 2 irrigation treatments × 4 block replicates = 128 fine root litterbags.

To evaluate the contribution of species mixture effects on fine root decomposition, we buried two litterbags of single species root litter of each of the two tree species present in the mixed plots (total of 4 root litterbags for each species; 2 root litterbags for each of two “Decomp” subplots and per species). This gave another: 2 replicates × 2 years of decomposition × 2 tree species (birch and pine growing in mixed stands) × 2 stand densities × 2 irrigation treatments × 4 block replicates = 128 fine root litterbags.

Half of the bags (128 fine root litterbags) were harvested after 12 months and the other half (128 remaining fine root litterbags) after 24 months. The remaining roots were carefully removed from the bags, dried at 40 °C for 4 days and weighed to determine mass loss (% of initial root mass).

$$Root\_remaining\_mass\% = 100 \frac{M_t}{M_o}$$

With  $M_o$  = initial litter mass before burial (g), and  $M_t$  = litter mass after one or two years of decomposition (g)

#### 4.2.4 Chemical analysis of roots

Roots from root litterbags were analysed at the individual sample level ( $n = 128$  samples). Roots from standing biomass and ingrowth cores were arranged as composites by pooling the two closest individual samples (from the two closest “*Ingrowth*” subplots) around each “*Decomp*” subplot together as one analytical composite for nutrient analysis. As a result, the nutrient values on these composites were based on two values per plot ( $n = 128$  samples). Dry root samples were ground in a planetary mill with spheres from Retsch series MM400 (two spheres per bowl with a frequency of 30 vibrations per second, and for a duration of 3 minutes). Root samples belonging to root standing biomass, ingrowth cores, and litterbags were analyzed for C and N with a Flash EA1112 analyzer, and for P, after mineralisation with sulphuric acid, concentrations were determined following a colorimetric determination with ammonium molybdate using a SEAL AutoAnalyzer 3 HR (SEAL Analytical, Ltd). Before root litterbag construction, we measured values of initial C, N, and P, but also the initial root Ca, K, Mg, Mn concentrations with the spectrophotometer SpectrAA20 Varian (3 repetitions were performed for each species). Content of lignin was determined applying methods according to TAPPI T 222 om-88 for Klason lignin.

#### 4.2.5 Root litter input and C, N, P fluxes in the soil

Taking into account the fine root necromass (FRN) found after two years of fine root production, and the C, N, P concentration of live roots in the ingrowth cores, we calculated the annual C, N, P inputs via FRN deposition, and the total C, N, P release to soil from roots after two years of decomposition. To calculate the input flux of C, N, P that entered the soil after two years, we multiplied the amount of FRN ( $\text{kg ha}^{-1}$ ) with the mineral concentration found in live roots. Due to lack of FRN material we could not check whether dead and alive roots had the same chemistry, but we assume that there was little, if any, nutrient resorption after death nor sufficient decomposition from the time of death till harvest (Gordon and Jackson 2000):

$$\text{root\_CNP\_input}(\text{kg ha}^{-1}) = \text{FRN\_input}(\text{kg ha}^{-1}) \times \text{CNP\_concentration} \in \text{live roots}$$

The release of elements after 2 years was calculated as loss relative to their initial mass or content; therefore the initial mineral pool minus the final mineral pool within each root litterbag (kg) was then divided by the initial root litter mass (kg). In order to measure the cumulative release of C, N, P at the stand level ( $\text{kg ha}^{-1}$ ) we multiplied the latter with FRN input after 2 years ( $\text{kg ha}^{-1}$ ):

$$\text{CumulativeCNPrelease}(\text{kg ha}^{-1})$$

$$= \frac{(\text{initial\_root\_mass} \times \text{CNPconc.})(\text{kg}) - (\text{remaining\_root\_mass} \times \text{CNPconc.})(\text{kg})}{\text{initial\_root\_mass}(\text{kg})} \times \text{FRNinput}(\text{kg ha}^{-1})$$



## 4.2.6 Data analysis

All statistical analyses were done using the R software (version 3.6.2). For the comparison between tree species compositions (pure birch, pure pine, mixed birch-pine plot), stand density (high vs low), and water supply (non-irrigation vs irrigation), we used a three-way interaction mixed effects model (lme package), with block set as random factor, and subplots nested within plot, and block. The residuals were checked for normality and homogeneity of variances and outliers were removed following the outlier labelling rule with a tuning parameter of  $g = 2.2$  (Hoaglin and Iglewicz 1987). The multcomp package was used to perform post-hoc Tukey's tests between all possible combinations of tree species composition  $\times$  stand density  $\times$  water supply.

## 4.3 Results

### 4.3.1 Differences in fine root morphology and chemistry between species, and between ingrowth cores and root standing biomass cores

Specific root length (SRL), specific root area (SRA) and root tissue density (RTD) were generally higher for birch compared to pine ( $p < 0.0001$ ), while the average root diameter (D) was larger for pine than for birch ( $p < 0.0001$ ) (Table 4.1). In addition, birch roots had significantly higher concentrations than pine for all measured elements, except for K and Mn, for which they had similar concentrations, and for C, which was significantly higher for pine. Both trees had similar percentages of root lignin concentrations (Table 4.2).

We found that roots from the ingrowth cores did not resemble roots from root standing biomass cores. Roots proliferated more abundantly in the ingrowth cores compared to root standing biomass cores, and produced finer roots with higher SRL, higher SRA, lower RTD, and lower D compared to root standing biomass ( $p < 0.001$ ). Moreover, birch roots from ingrowth cores had lower C concentrations than root standing biomass and tended to have higher concentrations of N and P, while pine ingrowth core roots had significantly lower concentrations of C and P, and slightly lower concentrations of N than root standing biomass roots. Nonetheless both trees exhibited significantly lower C:N ratios in ingrowth cores compared to root standing biomass, and C:P ratios were significantly lower for birch ingrowth roots, compared to root standing biomass, while for pine the C:P did not change between ingrowth cores and root standing biomass. The C:N and C:P ratios from the extraneous root material used for the root litterbags (harvested from birch and pine plantations 3 km from our experimental site), tended to resemble more to the root standing biomass, than to the ingrowth core roots, with the exception of the very low C:N ratios for birch initial root litter.

**Table 4.1** Overview of fine root morphology between fine roots of birch and pine, originating from root standing biomass (RSB) and 2<sup>nd</sup> year ingrowth cores harvests (IC2). The morphological traits are: specific root length (SRL; m g<sup>-1</sup>), specific root area (SRA; cm<sup>2</sup> g<sup>-1</sup>), root tissue density (RTD; g cm<sup>-3</sup>), and root diameter (D; mm). We also present an estimation of initial root litter morphology based on the fine root standing biomass collected in March 2018 from birch (n = 64 replicates) and pine trees (n = 61 replicates) that were left unirrigated. Values are means with standard deviation in parenthesis. Different upper case letters indicate differences for a root cohort between both species for a given root metric, while different lower case letters indicate differences within a species between root cohorts for a given root metric.

<b>Origin</b>	<b>n</b>	<b>Species</b>	<b>SRL m g<sup>-1</sup></b>	<b>SRA cm<sup>2</sup> g<sup>-1</sup></b>	<b>RTD g cm<sup>-3</sup></b>	<b>D mm</b>
RSB	128	<i>Birch</i>	22.9 Ab (10.5)	247 Ab (88)	0.568 Aa (0.220)	0.386 Ba (0.072)
IC2	116	<i>Birch</i>	55.7 Aa (18.8)	504 Aa (125)	0.281 Ab (0.056)	0.300 Bb (0.0486)
Initial root litter	64	<i>Birch</i> <sup>a</sup>	21.0 Ab (10.1)	231 Ab (86)	0.565 Aa (0.178)	0.388 Ba (0.069)
RSB	125	<i>Pine</i>	8.8 Bb (3.2)	178 Bb (43)	0.446 Ba (0.240)	0.683 Aa (0.106)
IC2	112	<i>Pine</i>	14.8 Ba (3.0)	272 Ba (49)	0.259 Bb (0.046)	0.590 Ab (0.063)
Initial root litter	61	<i>Pine</i> <sup>a</sup>	8.3 Bb (2.6)	168 Bb (36)	0.448 Ba (0.187)	0.663 Aa (0.101)

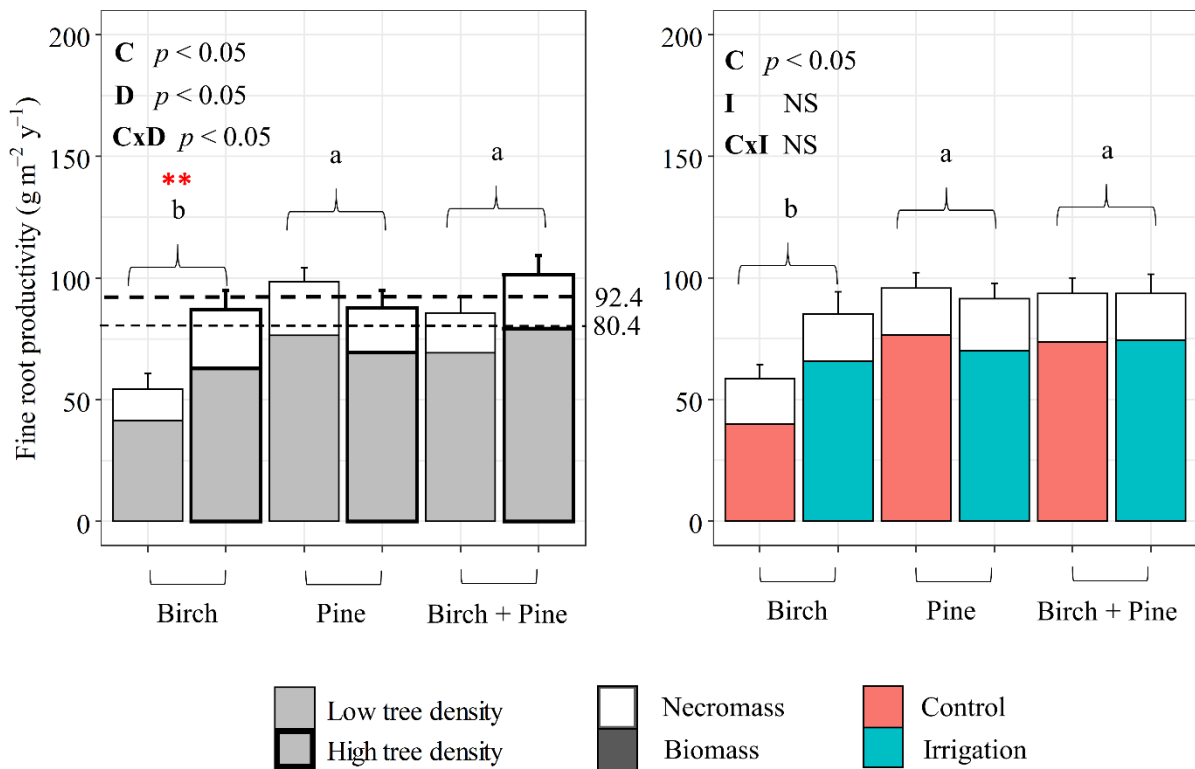
<sup>a</sup> Due to material constrains, replicates of birch and pine that were retrieved from unirrigated plots, were used as an estimator of root litter morphology prior to litterbag installation

**Table 4. 2** Overview of fine root quality between fine roots of birch and pine, originating from root standing biomass (RSB), 2<sup>nd</sup> year ingrowth cores harvests (IC2), from initial litter of fine roots collected from birch and pine trees at three km from the study site, and for root litterbags. All available replicates of each species were used for RSB, IC2 and root litterbag analysis of C, N, P. For initial litter quality three replicates ( $n = 3$ ) were used for the elemental analysis, and one replicate ( $n = 1$ ) was used for lignin measurements. Values are means with standard deviation in parenthesis. Different upper case letters indicate differences for a root cohort between both species for a given chemical variable, while different lower case letters indicate differences within a species between root cohorts for a given chemical variable.

Origin	Species	C %	N %	P %	C:N	C:P	Ca mg/g	K mg/g	Mg mg/g	Mn mg/g	Lignin %
RSB	<i>Birch</i>	49.1 Ab (2.9)	0.99 Ab (0.23)	0.095 Aa (0.043)	52.1 Aa (12.7)	596 Aa (157)	na	na	na	na	na
IC2	<i>Birch</i>	46.0 Ac (2.9)	1.11 Aab (0.14)	0.115 Aa (0.059)	42.1 Ab (5.81)	464 Ab (144)	na	na	na	na	na
Initial root litter	<i>Birch</i>	41.4 Bd (0.8)	1.16 Aa (0.04)	0.099 Aa (0.008)	35.6 Bb (0.829)	416 Aab (30.5)	4.81 A (0.13)	1.81 A (0.13)	1.05 A (0.03)	0.03 A (0.0006)	56.1 (-)
Root litterbag	<i>Birch</i>	51.2 Aa (1.4)	0.97 Ab (0.09)	0.112 Aa (0.021)	53.0 Aa (5.29)	474 Ab (90.1)	na	na	na	na	na
RSB	<i>Pine</i>	44.4 Bb (5.1)	0.83 Bb (0.16)	0.095 Ac (0.025)	55.7 Aa (14.0)	496 Ba (136)	na	na	na	na	na
IC2	<i>Pine</i>	38.9 Bc (4.4)	0.81 Bb (0.12)	0.082 Ba (0.018)	49.0 Ab (7.85)	489 Aa (89.0)	na	na	na	na	na
Initial root litter	<i>Pine</i>	47.6 Aab (0.4)	0.89 Bab (0.002)	0.081 Babc (0.008)	53.7 Aab (0.495)	603 Aa (125)	3.54 B (0.19)	1.64 A (0.16)	0.97 B (0.009)	0.03 A (0.003)	52.6 (-)
Root litterbag	<i>Pine</i>	48.6 Ba (1.6)	0.93 Ba (0.12)	0.120 Ab (0.035)	53.7 Aab (9.71)	421 Bb (100)	na	na	na	na	na

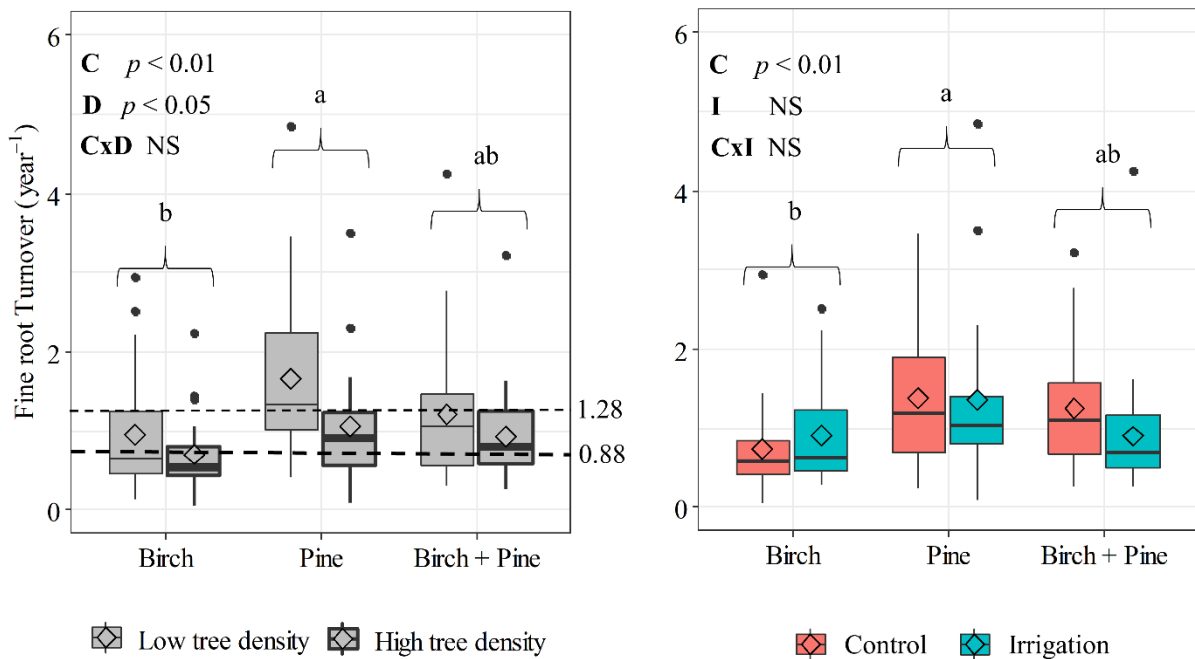
### 4.3.2 Fine root production and turnover

Fine root production (FRP) depended significantly on the tree species composition ( $p = 0.0103$ ), the stand density ( $p = 0.0449$ ) and the interaction between tree species composition  $\times$  stand density ( $p = 0.0293$ ) (Fig. 4.2, see Table C4.4 in Appendix C). The interaction was because pure birch stands had lower FRP values at low stand density compared to high stand density ( $p = 0.0025$ ) (Fig. 4.2). Irrigation did not significantly affect FRP, and there were no further significant interactions between tree species composition, stand density, and irrigation. Fine root necromass (FRN) represented a low percentage of FRP (around 25%), and was not significantly affected by any treatment (Fig. 4.2, see Table C4.4 in Appendix C).



**Fig. 4.2** Annual fine root productivity ( $\text{g m}^{-2} \text{y}^{-1}$ ) based on both biomass and necromass collected from the ingrowth-cores. The graph effects of stand density 'D' (on the left), and irrigation 'I' (on the right) on the three tree species compositions 'C': birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences between the three tree species compositions. Asterisks \*\* indicate a significant effect of stand density ( $p < 0.01$ ) for a given tree species composition. Values are means with st. errors.

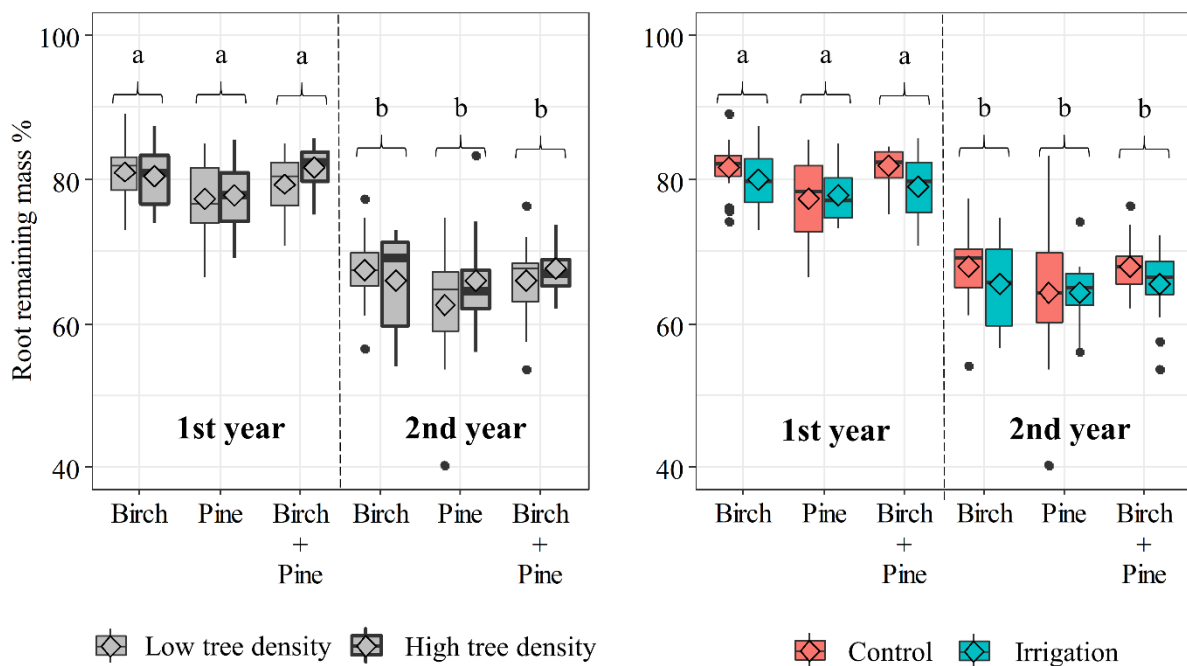
Fine root turnover (FRT) differed significantly among the three tree species compositions ( $p = 0.003$ ) (pure birch, pure pine, birch-pine mixture), with highest turnover values observed for pure pine, intermediate for the mixture, and lowest for pure birch (Fig. 4.3, see Table C4.4 in Appendix C). Moreover, FRT differed significantly between the two stand densities ( $p = 0.106$ ), as high stand density led to significantly lower FRT values overall (Fig. 4.3, see Table C4.4 in Appendix C). Irrigation did not significantly influence FRT, and there were no significant interactions whatsoever.



**Fig. 4.3** Boxplots with medians (-), and means (◇) of fine root turnover ( $y^{-1}$ ). The effects of stand density (on the left), and irrigation (on the right) on the three tree species compositions: birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences between the three tree species compositions. The dashed lines indicate an overall significant difference between high (thick dashed line) and low (fine dashed line) stand density ( $p < 0.05$ ).

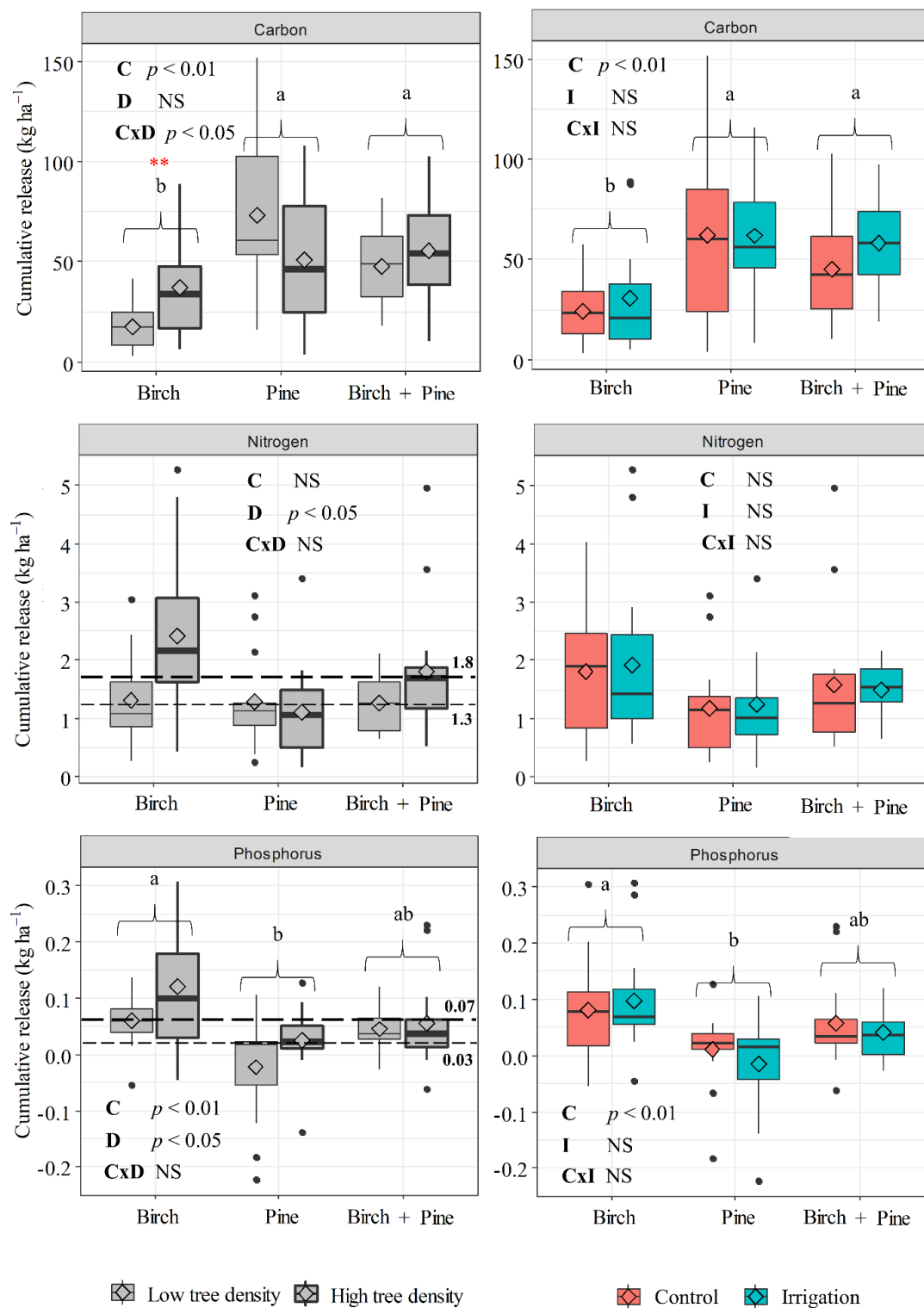
### 4.3.3 Fine root decomposition

Fine root decomposition (FRD) proceeded slowly overall and there were no differences among stands of different species composition or stand density (Fig. 4.4, see Table C4.4 in Appendix C). Likewise, irrigation did not influence fine root decomposition. It is noteworthy that the remaining root mass significantly decreased from 1<sup>st</sup> year (around 20% of root litter mass loss) to 2<sup>nd</sup> year of decomposition (around 35% of root litter mass loss).



**Fig. 4.4** Boxplots with medians (-), and means ( $\diamond$ ) of fine root decomposition (root remaining mass %). The effects of stand density (on the left), and irrigation (on the right) on the three tree species compositions: birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences after 1 and 2 years of decomposition.

Although fine root decomposition was not different between treatments, the cumulative release of C, N, P via decomposition was significantly affected, particularly by stand density and tree species composition, and interestingly the treatment effects were different for each element (Fig 4.5, see Table C4.5 in Appendix C). Cumulative release of C was significantly affected by an interaction between tree species composition  $\times$  stand density, as only for pure birch we recorded higher values at high stand density compared to lower values for low stand density ( $p = 0.0044$ ). At high stand density, there was an overall higher cumulative release of N, and P, compared to low stand density. Additionally, the effect of tree species composition was significant for P, with the highest values being for pure birch, intermediate for the mixed stand, and the lowest for pure pine (Fig 4.5, see Table C4.5 in Appendix C). Irrigation was not important for the cumulative release of any of the elements, and did not interact with any of the treatments.



**Fig. 4.5** Boxplots with medians (-), and means ( $\diamond$ ) of cumulative release ( $\text{kg ha}^{-1}$ ) of Carbon, Nitrogen, and Phosphorus. The effects of stand density (on the left), and irrigation (on the right) on the three tree species compositions: birch monoculture, pine monoculture, and birch-pine mixture. Different lowercase letters indicate significant differences between the three tree species compositions. The dashed lines indicate an overall significant difference between high (thick dashed line) and low (fine dashed line) stand density ( $p < 0.05$ ).

## 4.4 Discussion

Our hypothesis that fine root production (FRP), turnover (FRT) and decomposition (FRD) increase in mixed stands of birch and pine was not confirmed. Stand density effects on FRP of new roots were dependent on tree species composition. FRT showed overall opposite results than expected and contrary to FRP tree species composition had no effect. FRD was unchanged between treatments, but interestingly the cumulative release of C, N, P was significantly affected by stand density and tree species compositions. Summer irrigation did not affect whatsoever the production of fine roots, their turnover and their decomposition.

### 4.4.1 General considerations

Our study resulted in average FRP values of 50–85 g m<sup>-2</sup> y<sup>-1</sup> for birch, and of 85–100 g m<sup>-2</sup> y<sup>-1</sup> for pine, which are comparable to previous data of birch and pine fine root production (Andreasson et al. 2016; Hansson et al. 2013; Makkonen and Helmisaari 1999; Varik et al. 2015). Roots of birch were finer and longer than those of pine, which is a general difference between angiosperms and gymnosperm (Comas and Eissenstat 2004). Birch roots had a lower average turnover of 0.873 y<sup>-1</sup> (estimated lifespan = 1.14 years) than pine, which had a higher average turnover of 1.38 y<sup>-1</sup> (estimated lifespan = 0.72 years). This is in agreement with Varik et al. (2015) for a 13-year-old stand of *Betula pendula*, and with *Pinus sylvestris* estimates for 38–120 year old stands (Brunner et al. 2013). However, it is higher than the global average of 0.8 y<sup>-1</sup> for tree fine roots including a large panel of tree species (Gill and Jackson 2000). Root remaining mass% decreased to about 80% after 12 months and to 65–70 % after 24 months, and these values were similar to the values shown by Berg (1984) as well as those reported by Goebel *et al.* (2011) on fine root remaining mass after 8, 14, 19, and 36 months. Birch roots had higher nutrient concentrations than pine, except for K and Mn, for which the concentrations were similar, and for C with higher concentration in pine than birch. Both trees had similar lignin concentrations. Compared to previous studies with birch, we found similar concentrations for P, higher concentrations for N and K (Morozov et al. 2018), and lower concentrations for K (Palviainen et al. 2004). Pine had similar values for P, Ca, K, Mg compared to two previous studies, that however, were in the same study region (Augusto et al. 2015; Genet et al. 2005). Lignin concentrations were considerably higher in both species than usually reported in the literature.



#### 4.4.2 Effect of tree species mixing

In previous studies that reported higher fine root production in tree species mixtures, it was attributed to the dominant species of the mixture, producing more roots in mixtures than could be expected from monocultures (Lei et al. 2012a), due to improved soil volume filling that can be obtained by exploring and exploiting the soil environment more completely in space and time (Brassard et al. 2013), or due to vertical root segregation (Meinen et al. 2009). In spite of contrasting fine root morphologies and fine root turnovers between birch and pine, we did not find higher fine root production nor higher turnover rates at the community level. Our results agree with those reported by Domisch et al. (2015) and Lei et al. (2012b) who also found no effects of tree mixtures on fine root productivity. Several factors may explain the absence of any tree mixture effects; like for example root understory interference, weak interspecific interaction, because the available root space is not yet fully occupied, or mixture effects occurring earlier (during 1<sup>st</sup> year of root growth) or later (at stand maturity) (Domisch et al. 2015; Lei et al. 2012b, a). In our study, roots of birch and pine recolonized the “root-free” soil volume of the ingrowth cores at the same rate and intensity, and recolonization of the ingrowth cores from birch and pine was at least quicker than for understory roots (see Fig. C4.1 in Appendix C). In addition, no mixture effect was observed for the 1<sup>st</sup> year of root growth. Birch and pine mixtures had fully closed canopies, indicating that the soil volume was likely fully colonized as well, which should have resulted in sufficient interspecific interactions belowground. It is likely that since these tree stands are still young, other mechanisms that could explain higher fine root production in mixtures (such as vertical root segregation), may take longer to occur (Altinmazis-Kondylis et al. 2020; Curt and Prévosto 2003), or that competition effects were not properly measured after only 2 years of root growth. In fact, fine root necromass was low (~ 25%) compared to expected values (~ 50%) for similar pine forests in our study region (Andreasson et al. 2016) or for other tree species (Brunner et al. 2013). Also, the roots in ingrowth cores did not resemble the roots from root standing biomass in neither morphology nor chemistry (ingrowth cores contained finer roots, with lower C:N, and C:P ratios than root standing biomass). Finer morphology and lower C:nutrient ratios are indicative of the presence of young roots, some probably still not suberized. Nonetheless, even though fine root biomass production did not highlight any direct overyielding (i.e. no higher levels of fine root biomass production under tree mixtures as compared to monocultures), birch and pine do have very different fine root morphologies, which could result in different exploitation efficiencies. Further studies are needed to investigate whether differences in root morphology combined with physiology of such roots growing in tree mixtures, would permit to take up more nutrients than when growing in monocultures.

Although the litterbags contained roots that were apparently different in their morphology and chemistry, there were no fine root decomposition (FRD) effects in the mixed stands. The low soil fertility in our sandy soils may have slowed decomposition rates (Wardle et al. 2004), as characteristics such as soil pH (Rousk et al. 2010), soil C:N ratio (Fierer et al. 2009) and soil texture (Gijsman et al. 1997) can affect decomposer communities, such as the composition and ratios of fungal and bacterial communities, or the activity and mobility of decomposers. Furthermore, lignin

concentrations were very high in our study for both birch and pine roots, probably increasing recalcitrance of roots from both species (See et al. 2019). Also, birch and pine root litter had similar concentrations of Mn, which is arguably important for decomposition dynamics of leaf litter (Berg et al. 2010, 2015), and has been shown to explain a large part of variation in root decomposition of low-fertile sites in the tropics (Guerrero-Ramírez et al. 2016). Within woody plants, fine roots of ectomycorrhizal plants, such as pine and birch, decompose very slowly in general (See et al 2019). In conclusion, it may be that the properties of the soil matrix, and similarities in initial root litter quality between the two ectomycorrhizal species, in particular for Mn and C quality, were important factors determining decomposition, ultimately leading to similar decomposition rates between the two species, planted either in their respective monocultures or in mixed stands.

#### 4.4.3 Effect of stand density

We hypothesized that fine root production and turnover would increase due to stronger interspecific interactions at high stand density (Domisch et al. 2015). Effects of stand density on fine root production were significant but depended on the tree species composition, as we observed positive stand density effects only for the pure birch stand. Increased fine root productivity of birch at higher stand density may indicate that birch is exploiting the soil surface closer to the stems than pine, as pine had a larger basal area and may have explored further with its roots than the 2 m × 2 m area where trees meet (Day et al. 2010) (see the 4m<sup>2</sup> demonstrated as red squares in Fig. 4.1). This is depicted aboveground, as pine extended its canopy towards birch, while birch had a more restricted canopy development close to its gravitational center (average canopy ratios for birch: 0.83 – 0.95; for pine: 1.46 – 1.81). Interestingly, and opposite to our hypothesis, fine root turnover was lower at higher stand density (0.88 compared to 1.28 at lower stand density). It is important to consider that turnover is calculated as a ratio of fine root production / fine root standing biomass (Gill and Jackson 2000). When neither fine root production nor fine root standing biomass are affected, or when both metrics are affected in the same direction, this would obviously have no net effect on turnover. Here, fine root production at least increased in denser stands, but turnover overall decreased. Although, the influence of stand density on root standing biomass was generally positive (see Table C4.1 in Appendix C), at the same time it was still larger than its influence on fine root production, thus yielding smaller turnover values. It might be that effects of stand density on fine root production are weaker (current growth dynamics) than for root standing biomass (incorporating both actual and previous growth), especially since they comprise of mostly new roots whose growth accounts for a major part of aboveground annual tree growth (Helmisaari et al. 2002).

We further hypothesized that the presence of living roots (either through a direct rhizosphere priming effect *sensu* Kuzyakov (2010) or through the fluxes of root exudates fuelling microbial activity) is important for the decomposition of fine roots (FRD), and that increased root biomass of birch and pine at high stand density would positively influence fine root decomposition through higher root exudation. Contrary to our hypothesis, stand density did not influence fine root decomposition. Our results showed that stand density increased fine root biomass only for birch when it grew in

monocultures, and that even though fine production was higher for pure birch, there was no effect on fine root decomposition. In a study from Khlifa et al. (2020), understory vegetation cover (especially fern and herb) best explained FRD, due to modifications of the soil properties. Also, van der Van der Krift et al. (2001) found that the presence of growing grass species stimulated root decomposition significantly. We believe that the strong presence of understory roots in our study (especially bracken fern and molinia grass), which was similar across treatments (see [Table C4.3](#) in Appendix C), might have overridden potential effects caused by rooting of birch or pine.

#### **4.4.4 Effect of irrigation**

Contrary to our hypothesis, irrigation had no significant influence on productivity and turnover, which agrees with previous studies of Joslin et al. (2000), who found that exposure of trees to either wet or dry treatments did not lead to changes in net fine-root production and turnover. We also agree with Rytter (2013), who found that water limitation significantly decreased fine root production (but also aboveground biomass), and did not affect turnover. A reason might be that the relatively young roots found in the ingrowth cores (1-2 years old) were unaffected by the drier summer (Coleman and Aubrey 2018), given the overall small values and no differences in necromass between ambient precipitation and additional summer irrigation. In systems comprising larger amounts of fine root necromass (typically for root populations including older cohorts as well), root death as a result of drought could be equilibrated by equally large increases of new root growth to compensate for the drought-induced losses and then lead to higher values of annual fine root production (Gaul et al. 2008). Whether a tree maintains old roots or sheds old roots and produces new ones in response to drought is, according to Eissenstat et al. (2000), determined by the benefit to cost ratio in terms of water uptake and carbon investment. Root shedding and the construction of new roots mean the investment of a considerable amount of energy in the process of root turnover. Since young roots are able to take up water more efficiently than older ones, root shedding and regrowth may represent a less suitable acclimation of plants to reduced water supply if the energy costs are too important.

Also contrary to our hypothesis, irrigation did not increase fine root decomposition. Lack of irrigation effects on fine root decomposition agree with King et al. (1997), who found that root decomposition was rarely affected by an irrigation treatment, due to the buffering capacity of soil moisture. It is also in agreement with the study of Zhang and Wang (2015) who found that mean annual precipitation is not important for fine root decomposition. Although summer irrigation resulted in important differences in soil moisture for the top soil layer (0 – 5 cm) it was not sufficient to cause more rapid decomposition than in the plots that were left unirrigated (Maxwell et al. 2020). The low-fertility soils in combination with the poor litter quality of our root litter supports the idea that water availability is less important for root litter decomposition, but that other factors such as nutrient availability and/or biotic interactions with microbial communities drive decomposition rates of roots (Fanin et al. 2019).

#### 4.4.5 Implications for carbon and nutrient cycling

The cumulative release of C, N, P was significantly affected by stand density and tree species composition. Interestingly, these effects differed for each element. The different effect on each element meant that after 2 years of decomposition the remaining root mass differed considerably in stoichiometry in response to changes in stand density and tree species composition. Interestingly, the cumulative release of C was higher at high stand density, but this depended on the tree species composition (density effects were significant for pure birch), while N, and P, both showed overall higher values at high stand density (for N: 1.8 vs 1.3 kg ha<sup>-1</sup>, for P: 0.07 vs 0.03 kg ha<sup>-1</sup>). In accordance with our hypothesis, P showed the highest average release from decomposing roots in the pure birch stand (0.08 kg ha<sup>-1</sup>), while for pure pine, P release was the lowest (0.00009 kg ha<sup>-1</sup>). The highest recorded mean value for C release from root decomposition was for the pure pine stands (62 kg ha<sup>-1</sup>), while the lowest recorded values for C release were for the pure birch stands (27 kg ha<sup>-1</sup>). On average 28% of C necromass inputs, 40% of N necromass inputs and 12% of P necromass inputs were released during the 2-year decomposition period (see [Table C4.6](#) in Appendix C).

In summary, stand density modified N and P release rates and C release rate only in pure birch stands, the differences in C, N, P release from decomposing roots were driven by contrasting birch and pine in their respective mono-specific stands, and water availability during the typically dry summer does not affect release rates of any of the three elements. The tree density effect may be explained by different microclimatic conditions in denser stands that affect microbial respiration (Hanson et al. 2000). In a previous study, soil respiration in young stands increased with increasing stand density, and was correlated with biotic variables (aboveground, belowground and microbial biomass), but not with abiotic variables (litter and mineral soil C and N content, bulk density and soil texture) (Litton et al. 2003). We found a generally positive effect of fine root production at higher stand densities; there might exist a link between the generally higher root biomass at higher stand density and nutrient release via decomposition.

Carbon and nutrient dynamics during fine root decomposition differed clearly between the two tree species we studied. The higher C release rate from decomposing pine roots compared to birch roots is difficult to explain on the basis of the root traits we measured, that would all rather point to higher C and mass loss in birch roots. We may have missed some traits in our evaluation that were previously found to have an important role in root decomposition. For example, Sun et al. (2018) identified interspecific differences in non-structural carbohydrates and condensed tannins as the two most important root traits explaining interspecific differences in the decomposition of first-order roots across 35 different woody species from temperate forests. Both, non-structural carbohydrates and condensed tannins can show high concentrations in root tissues. Non-structural carbohydrate concentrations of *Pinus palustris* was about 13% for the first four root orders (Guo et al. 2004) compared to a range between 1.8 – 5.1% in *Betula pendula* trees (Peterson et al. 1993). These differences, although for another pine species than studied here, would be in line with higher pine than birch decomposition in our study, based on the strongly positive correlation between the concentration of non-structural carbohydrates and first-order root decomposition (Sun

et al. 2018). Also, a high initial C:N ratio and a low initial N concentration favour net N retention in the early phases of decomposition (Blair et al. 1992). In our study, pine root litter exhibited a significantly higher initial C:N ratio (53.7) than birch root litter (30.6), however, both species had relatively lower C:N values than observed in literature, and relatively high N values (Akburak et al. 2013; Rosenvald et al. 2011). This might explain the rather similar release of N for both species. The retention of P during pine root decomposition was a result of microbial immobilization which occurred strongly for pine, and might be related to: i) the P-limited soils of our study (Ostertag and Hobbie 1999), and ii) due to pine trees influencing differently the soil microbial community structure compared to birch trees. As a result of considerably higher initial C:P ratios in pine roots (603) compared to birch roots (416), the duration of the initial stage of decomposition (when P is immobilized) is probably longer for pine roots than for birch roots, which may explain why P release was higher from birch than from pine roots within the range of decomposition covered by our study. Moreover, Maxwell et al. (2020), measured higher activities of P-related enzymes in the soil underneath a pure birch canopy, possibly indicating that soil microorganisms are more limited by P compared to microbial communities in pine soils.

## **4.5 Conclusion**

Mixed stands of birch and pine showed no clear positive belowground diversity effects (such as higher fine root production or fine root decomposition). Instead, the absence of higher interspecific competition in denser stands or with lower water availability when the two species are growing together may indicate that mixtures perform better with increasing biotic or abiotic constraints compared to their respective monocultures. We showed that for P-limited soils, planting birch together with pine is potentially beneficial for pine nutrition, because decomposing birch roots tend to have higher initial concentrations of P that is also released more rapidly during the initial stages of decomposition. This is in line with a recent report of higher productivity in mixtures of birch and pine compared to pine monocultures (Morin et al 2020).

## **Acknowledgements**

This study was supported by the ANR project DiPTiCC (16-CE32-0003). We thank the Forest experimental Facility (UEFP-<https://doi.org/10.15454/1.5483264699193726E12>) and again Bernard Issenhuth for maintenance of the ORPHEE experiment. We thank Nathalie Gallegos, Coralie Chesseron, Catherine Lambrot, Dilane Lopes and Sylvie Milin for participating during the installation and harvesting of the samples. We would like to thank twice Catherine Lambrot and Sylvie Milin for performing a great deal of chemical analysis, and special thanks to Malika Aimaiti as well for participating among others in the grinding of the samples.

## References

- Akburak S, Oral HV, Ozdemir E, Makineci E (2013) Temporal variations of biomass, carbon and nitrogen of roots under different tree species. *Scand J For Res* 28:8–16. <https://doi.org/10.1080/02827581.2012.679680>
- Altinalmazis-Kondylis A, Muessig K, Meredieu C, et al (2020) Effect of tree mixtures and water availability on belowground complementarity of fine roots of birch and pine planted on sandy podzol. *Plant Soil* 457:437–455. <https://doi.org/10.1007/s11104-020-04741-8>
- Andreasson F, Gonzalez M, Augusto L, Bakker MR (2016) Comparison of ingrowth cores and ingrowth meshes in root studies: 3 years of data on *Pinus pinaster* and its understory. *Trees - Struct Funct* 30:555–570. <https://doi.org/10.1007/s00468-015-1256-6>
- Augusto L, Achat DL, Bakker MR, et al (2015) Biomass and nutrients in tree root systems-sustainable harvesting of an intensively managed *Pinus pinaster* (Ait.) planted forest. *GCB Bioenergy* 7:231–243. <https://doi.org/10.1111/gcbb.12127>
- Augusto L, Bakker MR, Morel C, et al (2010) Is “grey literature” a reliable source of data to characterize soils at the scale of a region? A case study in a maritime pine forest in southwestern France. *Eur J Soil Sci* 61:807–822. <https://doi.org/10.1111/j.1365-2389.2010.01286.x>
- Bakker MR, Augusto L, Achat DL (2006) Fine root distribution of trees and understory in mature stands of maritime pine (*Pinus pinaster*) on dry and humid sites. *Plant Soil* 286:37–51. <https://doi.org/10.1007/s11104-006-9024-4>
- Berg B (1984) Decomposition of root litter and some factors regulating the process: Long-term root litter decomposition in a scots pine forest. *Soil Biol Biochem* 16:609–617. [https://doi.org/10.1016/0038-0717\(84\)90081-6](https://doi.org/10.1016/0038-0717(84)90081-6)
- Berg B, Davey MP, de Marco A, et al (2010) Factors influencing limit values for pine needle litter decomposition: A synthesis for boreal and temperate pine forest systems. *Biogeochemistry* 100:57–73. <https://doi.org/10.1007/s10533-009-9404-y>
- Berg B, Erhagen B, Johansson MB, et al (2015) Manganese in the litter fall-forest floor continuum of boreal and temperate pine and spruce forest ecosystems - A review. *For. Ecol. Manage.* 358:248–260
- Blair JM, Crossley DA, Callaham LC (1992) Effects of litter quality and microarthropods on N dynamics and retention of exogenous <sup>15</sup>N in decomposing litter. *Biol Fertil Soils* 12:241–252. <https://doi.org/10.1007/BF00336039>
- Brassard BW, Chen HYHH, Cavard X, et al (2013) Tree species diversity increases fine root productivity through increased soil volume filling. *J Ecol* 101:210–219. <https://doi.org/10.1111/1365-2745.12023>

- Brunner I, Bakker MR, Björk RG, et al (2013) Fine-root turnover rates of European forests revisited: An analysis of data from sequential coring and ingrowth cores. *Plant Soil* 362:357–372
- Brunner I, Godbold DL (2007) Tree roots in a changing world. *J For Res* 12:78–82. <https://doi.org/10.1007/s10310-006-0261-4>
- Cardinale BJ, Wright JP, Cadotte MW, et al (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proc Natl Acad Sci U S A* 104:18123–18128. <https://doi.org/10.1073/pnas.0709069104>
- Castagneyrol B, Giffard B, Péré C, Jactel H (2013) Plant apparency, an overlooked driver of associational resistance to insect herbivory. *J Ecol* 101:418–429. <https://doi.org/10.1111/1365-2745.12055>
- Clemmensen KE, Bahr A, Ovaskainen O, et al (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* (80- ) 340:1615–1618. <https://doi.org/10.1126/science.1231923>
- Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85:235–252. <https://doi.org/10.1007/s10533-007-9132-0>
- Coleman MD, Aubrey DP (2018) Stand development and other intrinsic factors largely control fine-root dynamics with only subtle modifications from resource availability. *Tree Physiol* 38:1805–1819. <https://doi.org/10.1093/treephys/tpy033>
- Comas LH, Eissenstat DM (2004) Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. *Funct Ecol* 18:388–397. <https://doi.org/10.1111/j.0269-8463.2004.00835.x>
- Comas LH, Mueller KE, Taylor LL, et al (2012) Evolutionary patterns and biogeochemical significance of angiosperm root traits. *Int J Plant Sci* 173:584–595. <https://doi.org/10.1086/665823>
- Curt T, Prévosto B (2003) Rooting strategy of naturally regenerated beech in Silver birch and Scots pine woodlands. *Plant Soil* 255:265–279. <https://doi.org/10.1023/A:1026132021506>
- Day SD, Wiseman PE, Dickinson SB, Harris RJ (2010) Contemporary Concepts of Root System Architecture of Urban Trees Storm water Management with Structural Soils View project Consequences of Deep Planting of Trees View project
- Domisch T, Finér L, Dawud SM, et al (2015) Does species richness affect fine root biomass and production in young forest plantations? *Oecologia* 177:581–594. <https://doi.org/10.1007/s00442-014-3107-3>
- Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL (2000) Building roots in a changing environment: Implications for root longevity. *New Phytol.* 147:33–42

- Fanin N, Bezaud S, Sarneel JM, et al (2019) Relative Importance of Climate, Soil and Plant Functional Traits During the Early Decomposition Stage of Standardized Litter. *Ecosystems* 23:1004–1018. <https://doi.org/10.1007/s10021-019-00452-z>
- Fierer N, Strickland MS, Liptzin D, et al (2009) Global patterns in belowground communities. *Ecol. Lett.* 12:1238–1249
- Forrester DI, Bauhus J (2016) A Review of Processes Behind Diversity—Productivity Relationships in Forests. *Curr. For. Reports* 2:45–61
- Forrester DI, Kohnle U, Albrecht AT, Bauhus J (2013) Complementarity in mixed-species stands of *Abies alba* and *Picea abies* varies with climate, site quality and stand density. *For Ecol Manage* 304:233–242. <https://doi.org/10.1016/j.foreco.2013.04.038>
- Freschet GT, Aerts R, Cornelissen JHC (2012) A plant economics spectrum of litter decomposability. *Funct Ecol* 26:56–65. <https://doi.org/10.1111/j.1365-2435.2011.01913.x>
- Freschet GT, Cornwell WK, Wardle DA, et al (2013) Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. *J Ecol* 101:943–952. <https://doi.org/10.1111/1365-2745.12092>
- Gamfeldt L, Snäll T, Bagchi R, et al (2013) Higher levels of multiple ecosystem services are found in forests with more tree species. *Nat Commun* 4:. <https://doi.org/10.1038/ncomms2328>
- Gaul D, Hertel D, Borken W, et al (2008) Effects of experimental drought on the fine root system of mature Norway spruce. *For Ecol Manage* 256:1151–1159. <https://doi.org/10.1016/j.foreco.2008.06.016>
- Genet M, Stokes A, Salin F, et al (2005) The influence of cellulose content on tensile strength in tree roots. In: *Plant and Soil*. Springer Netherlands, pp 1–9
- Gessner MO, Swan CM, Dang CK, et al (2010) Diversity meets decomposition. *Trends Ecol. Evol.* 25:372–380
- Gijsman AJ, Alarcón HF, Thomas RJ (1997) Root decomposition in tropical grasses and legumes, as affected by soil texture and season. *Soil Biol Biochem* 29:1443–1450. [https://doi.org/10.1016/S0038-0717\(97\)00039-4](https://doi.org/10.1016/S0038-0717(97)00039-4)
- Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *Res New Phytol* 147:13–31. <https://doi.org/https://doi.org/10.1046/j.1469-8137.2000.00681.x>
- Gordon WS, Jackson RB (2000) Nutrient concentrations in fine roots. *Ecology* 81:275–280. [https://doi.org/10.1890/0012-9658\(2000\)081\[0275:NCIFR\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[0275:NCIFR]2.0.CO;2)
- Guerrero-Ramírez NR, Craven D, Messier C, et al (2016) Root quality and decomposition environment, but not tree species richness, drive root decomposition in tropical forests. *Plant Soil* 404:125–139. <https://doi.org/10.1007/s11104-016-2828-y>



- Guo LB, Halliday MJ, Gifford RM (2006) Fine root decomposition under grass and pine seedlings in controlled environmental conditions. *Appl Soil Ecol* 33:22–29. <https://doi.org/10.1016/j.apsoil.2005.09.004>
- Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry* 48:115–146. <https://doi.org/10.1023/A:1006244819642>
- Hansson K, Helmisaari HS, Sah SP, Lange H (2013) Fine root production and turnover of tree and understorey vegetation in Scots pine, silver birch and Norway spruce stands in SW Sweden. *For Ecol Manage* 309:58–65. <https://doi.org/10.1016/j.foreco.2013.01.022>
- Hättenschwiler S (2005) Effects of Tree Species Diversity on Litter Quality and Decomposition. In: *Forest Diversity and Function*. pp 149–164
- Helmisaari HS, Makkonen K, Kellomäki S, et al (2002) Below- and above-ground biomass, production and nitrogen use in Scots pine stands in eastern Finland. *For Ecol Manage* 165:317–326. [https://doi.org/10.1016/S0378-1127\(01\)00648-X](https://doi.org/10.1016/S0378-1127(01)00648-X)
- Hoaglin DC, Iglewicz B (1987) Fine-Tuning Some Resistant Rules for Outlier Labeling. *J Am Stat Assoc* 82:1147. <https://doi.org/10.2307/2289392>
- Hobbie SE (2015) Plant species effects on nutrient cycling: revisiting litter feedbacks. <https://doi.org/10.1016/j.tree.2015.03.015>
- Hobbie SE, Oleksyn J, Eissenstat DM, Reich PB (2010) Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. *Oecologia* 162:505–513. <https://doi.org/10.1007/s00442-009-1479-6>
- Hobbie SE, Vitousek PM (2000) Nutrient limitation of decomposition in Hawaiian forests. *Ecology* 81:1867–1877. [https://doi.org/10.1890/0012-9658\(2000\)081\[1867:NLODIH\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[1867:NLODIH]2.0.CO;2)
- IPCC (2014) Synthesis Report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.
- Isbell F, Calcagno V, Hector A, et al (2011) High plant diversity is needed to maintain ecosystem services. *Nature* 477:199–202. <https://doi.org/10.1038/nature10282>
- Jackson RB, Mooney HA, Schulze E-DD (1997) A global budget for fine root biomass, surface area, and nutrient contents. *Proc Natl Acad Sci U S A* 94:7362–7366. <https://doi.org/10.1073/pnas.94.14.7362>
- Jactel H, Gritti ES, Drössler L, et al (2018) Positive biodiversity–productivity relationships in forests: Climate matters. *Biol Lett* 14:20170747. <https://doi.org/10.1098/rsbl.2017.0747>
- Joslin JD, Wolfe MH, Hanson PJ (2000) Effects of altered water regimes on forest root systems. *New Phytol* 147:117–129. <https://doi.org/10.1046/j.1469-8137.2000.00692.x>

- Kardol P, Fanin N, Wardle DA (2018) Long-term effects of species loss on community properties across contrasting ecosystems. *Nature* 557:710–713. <https://doi.org/10.1038/s41586-018-0138-7>
- Khelifa R, Angers DA, Munson AD (2020) Understory species identity rather than species richness influences fine root decomposition in a temperate plantation. *Forests* 11:1–13. <https://doi.org/10.3390/f11101091>
- King JS, Allen HL, Dougherty P, Strain BR (1997) Decomposition of roots in loblolly pine: Effects of nutrient and water availability and root size class on mass loss and nutrient dynamics. *Plant Soil* 195:171–184. <https://doi.org/10.1023/A:1004248232450>
- Kong D, Wang J, Wu H, et al (2019) Nonlinearity of root trait relationships and the root economics spectrum. *Nat Commun* 10:1–9. <https://doi.org/10.1038/s41467-019-10245-6>
- Kou L, Jiang L, Hättenschwiler S, et al (2020) Diversity-decomposition relationships in forests worldwide. *Elife* 9:1–51. <https://doi.org/10.7554/eLife.55813>
- Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. *Soil Biol Biochem* 42:1363–1371. <https://doi.org/10.1016/j.soilbio.2010.04.003>
- Laliberté E (2017) Below-ground frontiers in trait-based plant ecology. *New Phytol.* 213:1597–1603
- Lei P, Scherer-Lorenzen M, Bauhus J (2012a) The effect of tree species diversity on fine-root production in a young temperate forest. *Oecologia* 169:1105–1115. <https://doi.org/10.1007/s00442-012-2259-2>
- Lei P, Scherer-Lorenzen M, Bauhus J (2012b) Belowground facilitation and competition in young tree species mixtures. *For Ecol Manage* 265:191–200. <https://doi.org/10.1016/j.foreco.2011.10.033>
- Leuschner C, Backes K, Hertel D, et al (2001) Drought responses at leaf, stem and fine root levels of competitive *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl. trees in dry and wet years. *For Ecol Manage* 149:33–46. [https://doi.org/10.1016/S0378-1127\(00\)00543-0](https://doi.org/10.1016/S0378-1127(00)00543-0)
- Li Y, Chen X, Veen GFC, et al (2018) Negative effects of litter richness on root decomposition in the presence of detritivores. *Funct Ecol* 32:1079–1090. <https://doi.org/10.1111/1365-2435.13057>
- Liang E, Leuschner C, Dulamsuren C, et al (2016) Global warming-related tree growth decline and mortality on the north-eastern Tibetan plateau. *Clim Change* 134:163–176. <https://doi.org/10.1007/s10584-015-1531-y>
- Litton CM, Ryan MG, Knight DH, Stahl PD (2003) Soil-surface carbon dioxide efflux and microbial biomass in relation to tree density 13 years after a stand replacing fire in a lodgepole pine ecosystem. *Glob Chang Biol* 9:680–696. <https://doi.org/10.1046/j.1365-2486.2003.00626.x>

- Liu Y, Liu S, Wan S, et al (2017) Effects of experimental throughfall reduction and soil warming on fine root biomass and its decomposition in a warm temperate oak forest. *Sci Total Environ* 574:1448–1455. <https://doi.org/10.1016/j.scitotenv.2016.08.116>
- Majdi H, Andersson P (2005) Fine root production and turnover in a Norway spruce stand in northern Sweden: Effects of nitrogen and water manipulation. *Ecosystems* 8:191–199. <https://doi.org/10.1007/s10021-004-0246-0>
- Majdi H, Pregitzer K, Morén AS, et al (2005) Measuring fine root turnover in forest ecosystems. In: *Plant and Soil*. pp 1–8
- Makkonen K, Helmisaari HS (1999) Assessing fine-root biomass and production in a Scots pine stand - Comparison of soil core and root ingrowth core methods. *Plant Soil* 210:43–50. <https://doi.org/10.1023/A:1004629212604>
- Maxwell TL, Augusto L, Bon L, et al (2020) Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest. *Soil Biol Biochem* 148:107864. <https://doi.org/10.1016/j.soilbio.2020.107864>
- McCormack M, Adams TS, Smithwick EAH, et al (2014) Variability in root production, phenology, and turnover rate among 12 temperate tree species. *Ecology* 95:2224–2235. <https://doi.org/10.1890/13-1942.1>
- McCormack M, Adams TS, Smithwick EAH, Eissenstat DM (2012) Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytol* 195:823–831. <https://doi.org/10.1111/j.1469-8137.2012.04198.x>
- Meier IC, Leuschner C (2008) Genotypic variation and phenotypic plasticity in the drought response of fine roots of European beech. <https://doi.org/https://doi.org/10.1093/treephys/28.2.297>
- Meinen C, Hertel D, Leuschner C (2009) Root growth and recovery in temperate broad-leaved forest stands differing in tree species diversity. *Ecosystems* 12:1103–1116. <https://doi.org/10.1007/s10021-009-9271-3>
- Mommer L, van Ruijven J, de Caluwe H, et al (2010) Unveiling below-ground species abundance in a biodiversity experiment: A test of vertical niche differentiation among grassland species. *J Ecol* 98:1117–1127. <https://doi.org/10.1111/j.1365-2745.2010.01702.x>
- Moore JAM, Sulman BN, Mayes MA, et al (2020) Plant roots stimulate the decomposition of complex, but not simple, soil carbon. *Funct Ecol* 34:899–910. <https://doi.org/10.1111/1365-2435.13510>
- Morozov G, Aosaar J, Varik M, et al (2018) Long-term dynamics of leaf and root decomposition and nitrogen release in a grey alder (*Alnus incana* (L.) Moench) and silver birch (*Betula pendula* Roth.) stands. *Scand J For Res* 34:12–25. <https://doi.org/10.1080/02827581.2018.1521468>

- Ostertag R, Hobbie SE (1999) Early stages of root and leaf decomposition in Hawaiian forests: Effects of nutrient availability. *Oecologia* 121:564–573. <https://doi.org/10.1007/s004420050963>
- Ostonen I, Püttsepp Ü, Biel C, et al (2007) Specific root length as an indicator of environmental change. *Plant Biosyst.* 141:426–442
- Palviainen M, Finér L, Kurka AM, et al (2004) Release of potassium, calcium, iron and aluminium from Norway spruce, Scots pine and silver birch logging residues. *Plant Soil* 259:123–136. <https://doi.org/10.1023/B:PLSO.0000020938.78215.bd>
- Petterson R, McDonald AJS, Stadenberg I (1993) Response of small birch plants (*Betula pendula* Roth.) to elevated CO<sub>2</sub> and nitrogen supply. *Plant Cell Environ* 16:1115–1121. <https://doi.org/10.1111/j.1365-3040.1996.tb02069.x>
- Prieto I, Birouste M, Zamora-Ledezma E, et al (2017) Decomposition rates of fine roots from three herbaceous perennial species: combined effect of root mixture composition and living plant community. *Plant Soil* 415:359–372. <https://doi.org/10.1007/s11104-016-3163-z>
- Ravenek JM, Bessler H, Engels C, et al (2014) Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. *Oikos* 123:1528–1536. <https://doi.org/10.1111/oik.01502>
- Rosenvald K, Kuznetsova T, Ostonen I, et al (2011) Rhizosphere effect and fine-root morphological adaptations in a chronosequence of silver birch stands on reclaimed oil shale post-mining areas. *Ecol Eng* 37:1027–1034. <https://doi.org/10.1016/j.ecoleng.2010.05.011>
- Roumet C, Birouste M, Picon-Cochard C, et al (2016) Root structure-function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol* 210:815–826. <https://doi.org/10.1111/nph.13828>
- Rousk J, Bååth E, Brookes PC, et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 4:1340–1351. <https://doi.org/10.1038/ismej.2010.58>
- Rytter RM (2013) The effect of limited availability of N or water on C allocation to fine roots and annual fine root turnover in *Alnus incana* and *Salix viminalis*. *Tree Physiol* 33:924–939. <https://doi.org/10.1093/treephys/tpt060>
- See CR, Luke McCormack M, Hobbie SE, et al (2019) Global patterns in fine root decomposition: climate, chemistry, mycorrhizal association and woodiness. *Ecol. Lett.* 22:946–953
- Silver WL, Miya RK (2001) Global patterns in root decomposition: Comparisons of climate and litter quality effects. *Oecologia* 129:407–419. <https://doi.org/10.1007/s004420100740>
- Sokol NW, Kuebbing SE, Karlsen-Ayala E, Bradford MA (2019) Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. *New Phytol* 221:233–246. <https://doi.org/10.1111/nph.15361>

- Spitzer CM, Lindahl B, Wardle DA, et al (2020) Root trait–microbial relationships across tundra plant species. *New Phytol* 169:16982. <https://doi.org/10.1111/nph.16982>
- Sun T, Hobbie SE, Berg B, et al (2018) Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proc Natl Acad Sci U S A* 115:10392–10397. <https://doi.org/10.1073/pnas.1716595115>
- Van der Krift TAJ, Giocchini P, Kuikman PJ, Berendse F (2001) Effects of high and low fertility plant species on dead root decomposition and nitrogen mineralisation. *Soil Biol Biochem* 33:2115–2124. [https://doi.org/10.1016/S0038-0717\(01\)00145-6](https://doi.org/10.1016/S0038-0717(01)00145-6)
- Van Der Krift TAJ, Kuikman PJ, Berendse F (2002) The effect of living plants on root decomposition of four grass species. *Oikos* 96:36–45. <https://doi.org/10.1034/j.1600-0706.2002.10978.x>
- Varik M, Kukumägi M, Aosaar J, et al (2015) Carbon budgets in fertile silver birch (*Betula pendula* Roth) chronosequence stands. *Ecol Eng* 77:284–296. <https://doi.org/10.1016/j.ecoleng.2015.01.041>
- Wardle DA, Bardgett RD, Klironomos JN, et al (2004) Ecological linkages between aboveground and belowground biota. *Science* (80-. ). 304:1629–1633
- Weemstra M, Sterck FJ, Visser EJW, et al (2017) Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. *Plant Soil* 415:175–188. <https://doi.org/10.1007/s11104-016-3148-y>
- Zeng W, Xiang W, Zhou B, et al (2020) Positive tree diversity effect on fine root biomass: via density dependence rather than spatial root partitioning. *Oikos* oik.07777. <https://doi.org/10.1111/oik.07777>
- Zhang X, Wang W (2015) The decomposition of fine and coarse roots: Their global patterns and controlling factors. *Sci Rep* 5:. <https://doi.org/10.1038/srep09940>



# Chapter 5 | Prolonged summer drought decreases C release but does not change N release during fine root and leaf litter decomposition in beech forests

*In collaboration with: Mark R. Bakker, and Stephan Hättenschwiler*

## Abstract

We tested the hypothesis that longer and more severe summer droughts slow carbon (C) and nitrogen (N) release during decomposition in different beech forests along a North-South gradient in the Western French Alps. We further hypothesized that drought affects leaf litter decomposition more than that of fine roots and that the contribution of macrofauna to the decomposition process counteracts drought effects. We used a multisite field experiment of beech-dominated forests in the Western French Alps ranging from northern sites where European beech co-occurs with silver fir to southern sites where beech co-occurs with pubescent oak. We applied rainout shelters during about four months from late June to early October to simulate reinforced and prolonged summer droughts over two years, and compared leaf litter and fine root decomposition (C and N release) with and without rainfall exclusion. With litterbags of different mesh sizes, we evaluated the contribution of macrofauna to C and N releases during decomposition. Experimentally reinforced and prolonged summer drought consistently slowed C loss, but did not alter N dynamics in decomposing beech fine roots and leaf litter in different beech forests across a wide north-south gradient in the Western French Alps. The drought effect was the same among forests differing in tree species composition, notably between pure and mixed beech forests, with or without the contribution of fauna to the decomposition process. Irrespective of reinforced and prolonged summer drought, C and N dynamics in decomposing fine roots and leaves differed considerably with less C loss but higher N loss from roots than from leaves. This difference was accentuated when fauna had access to decomposing leaf litter. Our results suggest that the predicted increasing frequency of extreme drought events slow C release during decomposition above- and belowground in beech forests independently of their tree species composition and specific environmental context. Because N dynamics remained unaffected by drought, it is likely that the relative availability of C substrates and N for decomposer communities are modified with increasing occurrence of extreme drought events, which may change stoichiometric constraints and biogeochemical cycling in these forests in the future. However, our study covered only early decomposition stages and it is critically important to address how drought modifies the whole decomposition process in future studies.

**Keywords:** fine roots, fauna-driven decomposition, leaf litter, tree mixtures, rainfall exclusion, extreme climate events

## 5.1 Introduction

According to climate change predictions an increased frequency and severity of extreme events, such as drought spells, are expected in the near future (IPCC (2014). Extreme events may have more serious implications for ecosystem functioning than the gradual increase of mean temperature (Jentsch et al. 2007). More frequent and severe drought events have already been recorded during the past decades, and this trend might potentially exacerbate in the future. Increasing frequencies of drought events are for example particularly likely in the Mediterranean area and the Western part of the Alps, with regional climate models predicting a decrease in the total amount of precipitation and an increase in the duration and frequency of summer drought (Dubrovský et al. 2014; Giorgi and Lionello 2008; Polade et al. 2017). Changes in the amount and distribution of precipitation is expected to have important implications for species distribution, and the structure, composition, and diversity of plant, animal, and microbial communities and ecosystem processes they drive (Weltzin et al. 2003).

Anthropogenic forces have resulted in climate change and biodiversity loss, which is expected to accelerate even more in the future. Basic ecological processes, such as primary production, resistance to perturbation, and decomposition, will all be potentially affected by climate change and biodiversity loss, with major implications for ecosystem functioning (Ratcliffe et al. 2017). Among all the processes involved in ecosystem functioning, the decomposition process is important, as it is the dominant driver of carbon and nutrient cycling in unmanaged terrestrial ecosystems such as forests (Hobbie 1996; Aerts 1997; Gessner et al. 2010). Decomposition is controlled by environmental conditions (e.g. humidity, temperature), litter quality (i.e. chemical and physical characteristics of litter) and the decomposer community (i.e. composition and activity) (Cornelissen 1996; Couteaux et al. 1995; Gholz et al. 2000; Hättenschwiler et al. 2005). Decomposition has been studied at the global scale, for example comparing between biomes, or along large-scale latitudinal gradients (Silver and Miya 2001; Zhang and Wang 2015b). However, because multiple other factors, including soil parameters, plant community composition, and the abundance and diversity of decomposer communities change alongside with precipitation across these gradients, it is often difficult to determine the driving mechanisms and to distinguish clearly among the different factors. Some studies decided to manipulate precipitation locally via the method of rain exclusion (Santonja et al. 2017, 2019). For example, Santonja et al. (2017) found that severe prolonged continuous droughts negatively impacted the decomposer community as a whole, but that detritivore abundance was more sensitive to change in rainfall regime than fungal biomass. In another manipulative study, Joly et al. (2019) showed that microbially-driven decomposition was important when water was applied in large amounts, while isopod-driven decomposition reached the highest values at smaller quantities of water supply. The latter two studies indicate that different organism groups may respond distinctively to drought with potentially different effects on decomposition. It is unknown whether responses to drought differ among groups of organisms in the decomposer food web (e.g., microbial decomposers that break down leaf litter through saprotrophic processes versus detritivores that directly ingest leaf litter).



A few studies have shown that plant canopy characteristics predict variation in decomposition better than the large differences in macroclimate (Gora et al. 2019; Joly et al. 2017; Seidelmann et al. 2016). The presence and relative abundance of particular species and/or functional types of plants can influence the decomposition environment in various ways. Species-specific canopy characteristics can directly influence microclimate such as soil temperature, evapotranspiration and moisture conditions, via shading, interception of precipitation and wind break (Prescott 2002). Moreover, the species composition and canopy structure determines the physical litter layer structure of the forest floor, through deposition of leaf litter that may differ in morphology, size and shape (Fujii et al. 2020). In turn, litter layer structure can modify decomposer activity, for example through changes in litter water-holding capacity (WHC) (Wardle et al. 2003), and the long-term input of diverse litter may lead to local adaptation of the soil decomposer community and consequently to a higher efficiency of litter degradation; as suggested in the home-field advantage hypothesis (Ayres et al. 2009; Gholz et al. 2000). For example, diverse litter can modify the composition of microbial communities (Ball et al. 2014; Chapman and Newman 2010) and alter the composition and abundance of soil fauna communities (Wardle 2006). However, the effects of local site conditions on leaf decomposition may be different for roots that decompose belowground (Silver and Miya 2001). Leaf litter decomposes within a litter layer created on top of the forest floor; hence, it is exposed to the forest floor's microbial community that differs in composition and abundance from microbial communities within the soil (Manzoni et al. 2012; Osono et al. 2006). Contrarily, roots are exposed to soil microbial communities that experience more stable conditions of moisture and temperature in the top soil (Gallardo and Schlesinger 1994; Grayston and Prescott 2005). All these tree species mixture effects on microclimate and the decomposition environment may be particularly important in forest ecosystems, as forests can have complex canopy architectures depending on the topography and the overstory structure (Gracia et al. 2007).

The consequences of changing patterns in precipitation on decomposition may therefore depend to a certain degree on plant canopy characteristics, litter traits, the decomposer community and other local conditions. However, it is currently not well understood how the consequences of reduced precipitation on decomposition may depend on plant community characteristics, and whether these affect root- and leaf decomposition similarly. Here, we aimed at testing whether carbon (C) and nitrogen (N) release during fine root- and leaf litter decomposition are affected by experimentally simulated reinforced and prolonged re summer droughts, and whether these effects are modified by tree species composition along a north-south gradient in the south-west part of the French Alps. The north-south gradient implies a natural gradient in climatic conditions with generally longer and more pronounced summer drought periods in the south, which is also accompanied by a change in the co-dominant tree species associated with European beech (*Fagus sylvatica* L.), with silver fir (*Abies alba* Mill.) in the northern part and pubescent oak (*Quercus pubescens* Willd.) in the southern part. We tested three hypotheses. Firstly, we hypothesized that release rates of C and N from decomposing litter will be lower with experimentally prolonged summer drought, in particular at northern sites with generally wetter summers than at southern sites with usually drier summers. We expected these differences between the northern

and the southern part of the gradient because the relative difference of experimental rainfall exclusion compared to the naturally occurring summer drought is larger in the North than in the South (26% and 11% of the total annual rainfall was excluded in the North and in the South, respectively, with rainout shelters between 2016 – 2017; Jourdan and Hattenschwiler, 2020). As a consequence of drier summers in the South, decomposer communities may also be better adapted to severe drought than those in the North. We further hypothesized that mixed species stands would attenuate the impact of drought on decomposition due to indirect canopy-composition effects creating more favourable micro-environmental conditions on decomposition. For fine root- compared to leaf litter decomposition, we hypothesized that micro-environmental factors are better buffered within the soil and that drought thus has smaller effects on fine root- than on leaf litter decomposition. In addition, we hypothesized that allowing access to macrofauna would lead to higher leaf decomposition, than without macrofauna, and that the prolonged rain exclusion would affect the litterbags with macrofauna access more strongly, as rainfall frequency is more important for detritivore-driven litter decomposition. In order to test these hypotheses, we used litterbags exposed in mono-specific beech forest stands and two-species tree mixtures (beech with fir or oak in the northern and the southern part of the gradient, respectively).

## 5.2 Materials and Methods

### 5.2.1 Study sites

We used four sites of beech forests in the Western French Alps, two in the northern part of the gradient where beech was associated with silver fir and two in the southern part of the gradient where beech was associated with pubescent oak ([Table D5.1](#)). All sites have a closed forest canopy and forests grow on limestone bedrock with a north to west exposition.

The sites vary to some extent in climate and soil characteristics ([Table 5.1](#)). The Northern sites have higher annual mean precipitation and lower annual mean temperatures (1206–1464 mm; 6.3–6.5 °C), than the Southern sites (793–940 mm; 10.1–10.2 °C). These annual means are calculated for the period of our experiment covering the the years 2017–2019 measured at the nearest meteorological station. All sites were rather clayey, with soil texture classified as clayey-loam to clayey. Lubéron and Mont Ventoux had a higher percentage of stones relative to its total soil volume (32 % and 11 %, respectively) than the other two sites. Lubéron and Mont Ventoux tended to have the highest percentage of organic matter (24.9–36.8 %) with lower values for the rest of the sites. Soil pH tended to be higher for Lubéron (7.67–7.70), while Mont Ventoux and Ste Baume had intermediate values (6.53–7.19), and Vercors had the lowest values with quite acidic soils (5.00–5.18). Consequently, Mont Ventoux also showed the highest concentrations in most of the measured soil nutrients (especially high values were found for Ca). Soil C/N ratios were the highest for Mont Ventoux and Ste Baume (~20), and the lowest for Lubéron and Vercors (~15) ([Table 5.1](#)).

**Table 5.1** Overview of site characteristics, and soil characteristics. Average precipitation and temperature is averaged between years (2018 – 2019) and collected from the closest available meteorological stations. Soil data are averages of two tree species mixture plots (i.e. two plots at each site) and were collected, using automated sensors (RT-1 and EC-5 or GS-1 sensors for temperature and moisture (TDR sensors), respectively, Decagon Devices Inc., Pullman, WA, USA) storing average values every three hours for the whole duration of the experiment.

	Vercors	Vercors	Mont Ventoux	Mont Ventoux	Lubéron	Lubéron	Ste Baume	Ste Baume
Blocks	1	2	1	2	1	2	1	2
<b>Site characteristics</b>								
Mean annual Precipitation (mm)	1464	1464	1216	1216	793	793	940	940
Mean annual Temperature (°C)	6.3	6.3	6.5	6.5	10.2	10.2	10.1	10.1
Av. soil moist. (%) <sup>a</sup> 2018–2019 [non – exclusion subplot]	76.1	69.7	59.6	60.1	60.8	57.7	72	54.3
Av. soil temp (°C) <sup>b</sup> 2018–2019 [non – exclusion subplot]	5.8	6.4	7	7.1	9.5	10.4	10.8	11.1
Slope (°)	28	28	18	18	30	30	10	10
Latitude	44.9	44.9	44.2	44.2	43.8	43.8	43.3	43.3
Altitude	1380	1190	1320	1270	972	852	748	719
<b>Soil characteristics of the 0–15 cm soil layer</b>								
Stoniness (vol %) <sup>c</sup>	0	0.8	11.5	10.7	30.8	34.1	0.5	2.9
Clay %	29.0	27.3	60.6	55.8	48.2	43.2	32.5	44.7
Sand %	31.1	26.5	7.95	11.0	9.95	15.0	28.0	24.1
Soil organic matter %	8.67	12	24.9	36.8	16.7	19.8	13.4	17.6
pH	5	5.18	6.88	6.6	7.7	7.67	6.53	7.19
Organic C %	5.01	6.91	14.4	21.3	9.69	11.4	7.75	10.1
Total N %	0.36	0.51	0.74	1.09	0.64	0.76	0.41	0.52
C/N	14	13.4	19.5	19.5	14.9	15.3	18.7	19.3
Available P (g/kg)	0.0205	0.02	0.0415	0.0475	0.0405	0.038	0.022	0.031
Ca (g/kg)	2.35	2.79	37.2	40.4	10.9	11.4	12.6	9.39
Mn (mg/kg)	0.799	0.417	1.35	1.96	1.93	2.32	0.899	1.28

<sup>a</sup> Soil moisture data are in % of the average from the ten highest measured individual values (3-hour intervals) during the entire two years from 1 Nov 2017 to 31 Oct 2019. These data were collected from subplots that were exposed to ambient climatic conditions

<sup>b</sup> Soil temperature data are averages during the entire two years from 1 Nov 2017 to 31 Oct 2019. These data were collected from subplots that were exposed to ambient climatic conditions

<sup>c</sup> Soil type is shown for both plots in the order of pure beech, and mixed beech – fir (Vercors, Mont Ventoux), or mixed beech – oak (Lubéron, Ste Baume)

## 5.2.2 Rain exclusion treatment

We used custom-made rainout shelters to exclude rainfall from relatively small areas (about 1.5 m<sup>2</sup>) of the forest floor within all forest plots during an extended period (roughly between end of June and early October) in the two summers 2018 and 2019 (see [Fig. D5.1](#) in Appendix D). In order to test for severe drought effects on litter decomposition, we applied a complete rainfall exclusion to designated subplots within each plot, during the summer (roughly between end of June and end of September, i.e. for about three months, [Fig. D5.1](#)) using a removable custom-made rainout shelter (Jourdan et al 2020). The removable rainout shelters were constructed with transparent plastic sheets covering an about twice as large area than that used for the placement of litterbags on the forest floor. Upslope of each subplot subjected to summer rainfall exclusion, we fixed the plastic sheets down to the forest floor where we additionally dug a small 10 cm deep ditch to direct runoff from the plastic cover and potentially from the forest floor away from the area with the litterbags. The other three sides were kept open with the plastic sheet 50 to 80 cm above the forest floor to allow unhindered air circulation in order to minimize microclimate effects other than rain exclusion. We continuously monitored soil moisture and temperature at 5 cm soil depth in one of each of the rainfall exclusion and the control subplots in each of the tree species mixture plots (i.e. two plots at each site) using automated sensors (RT-1 and EC-5 or GS-1 sensors for temperature and moisture (TDR sensors), respectively, Decagon Devices Inc., Pullman, WA, USA) storing average values every three hours for the whole duration of the experiment (see [Table D5.1](#) in Appendix D).

## 5.2.3 Litterbag construction

We collected fine roots and leaf litter of *Fagus sylvatica* trees from outside of the pure beech tree plots at the block level in each site (i.e. 2 x 4 = 8 fine root- and leaf litter pools). This permitted to produce litterbags filled with site- and block-specific fine root- and leaf litter material, which subsequently were transferred into the corresponding two plots (pure beech plot and mixture plot) of each block. All root samples were collected during fall of 2017 with the use of pick and spade from five random trees, outside of each plot. From the collected root batches, we selected intact fine roots (< 2 mm in diameter) that included roots up to the fifth root order. In the laboratory, the fine roots were gently cleaned with tap water and then dried at 40 °C for 7 days. Leaf litter was collected with litter traps (suspended nets underneath the beech canopy) during fall 2017. Leaves that were still green or that showed visible signs of herbivory or disease were excluded. The leaves were dried at 40 °C and stored dry until litterbags were constructed.

We used three types of litterbags filled with fine roots or leaf litter and using two different mesh sizes. These included fine mesh (48 µm) litterbags filled with either fine roots or leaf litter and coarse mesh (5 mm x 8 mm) litterbags filled with leaf litter. The litterbags containing fine roots were tightly stitched pockets (5 cm x 9 cm) of nylon mesh filled with 0.5 g of dried root material. We chose the particular mesh size to prohibit roots from growing into the litterbag but to allow fungal hyphae access (coarse mesh was not used for fine roots because new roots would grow into these making it

impossible for correct mass loss measurements). We used the same fine nylon mesh described above to construct leaf litterbags, which were 10 cm x 18 cm large and filled with 9 g of leaf litter. A second type of coarse mesh litterbags was used to evaluate the relative contribution of soil fauna to leaf litter decomposition. These litterbags were constructed with the coarse mesh (5 mm x 8 mm) on the top, but using a finer mesh (0.5 mm) on the soil-facing bottom to avoid gravitational loss of smaller litter particles. The coarse mesh litterbags were 13 cm x 13 cm large similar to the fine mesh litterbags used for leaf litter (see above) they were filled with 4.2 g of leaf litter.

#### **5.2.4 Chemical analysis of plant material**

Dry root samples were ground in a planetary mill with spheres (Retsch series MM400) using two spheres per bowl with a frequency of 30 vibrations per second and for a duration of 3 minutes. Dry leaf samples were ground with the same planetary mill, but with four spheres per bowl and a frequency of 300 vibrations per minute, and for a duration of 15 minutes. The ground powder was then used to determine initial fine root and leaf litter chemistry, including carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) concentrations. Analysis of C and N was done with a Flash EA1112 analyser using a dry combustion method, while K, Ca, Mg and Mn concentrations were measured with a flame atomic absorption spectrophotometer (SpectrAA20 Varian) after digestion in sulphuric acid and hydrogen peroxide. For P, after mineralisation with sulphuric acid, concentrations were determined following a colorimetric determination with ammonium molybdate using a SEAL AutoAnalyzer 3 HR (SEAL Analytical, Ltd). Because we used the same plot- and block-specific litter pool for the construction of litterbags, we analysed three samples from each pool for initial chemistry, representing variability among litterbags for the evaluation of differences among the different litter pools. For the quantification of C and N loss during decomposition, we analyzed C and N concentrations in the remaining litter material after the final harvest in the same way as described above for initial litter chemistry.

#### **5.2.5 Carbon and nitrogen loss**

We evaluated C and N dynamics by calculating the amount of C and N remaining after 24 months of decomposition. Remaining C and N were expressed as a percentage of initial C and N:

$$\text{Remaining C, N \%} = 100 \frac{M_t \times C_t}{M_o \times C_o}$$

With  $M_o$  = initial litter mass (g),  $M_t$  = litter mass after two years of decomposition (g),  $C_o$  = the concentration of C (or N) in initial litter material and  $C_t$  = the concentration of C (or N) in remaining litter material after two years of decomposition.

## 5.2.6 Experimental design and statistical analyses

At each of the four study sites there were two blocks with one plot of a two-species tree mixture (beech with fir in the northern part and beech with oak in the southern part of the gradient) and their corresponding single species stands (see Fig. D5.1 in Appendix D), yielding 2 plots x 2 blocks x 4 sites = 16 plots. Within each plot, we randomly selected four subplots (i.e. a total of 64 subplots) where we installed the litterbags for our decomposition study. Two of these subplots were randomly assigned to a reinforced and prolonged drought treatment and the two remaining subplots were used as controls. Within each of the subplots, we placed the three litterbag types (one root litterbag and two leaf litterbags) in early November 2017. For each type of litterbag, one replicate was harvested after 24 months from each of the four subplots, yielding a total of 4 subplots x 16 plots x 3 litterbag types = 192 litterbags. Upon harvest, litterbags were cleaned on the outside, transferred into paper bags, and transported to the laboratory. Back in the laboratory, we dried the litterbags at 40 °C for 4 days, removed remaining fine roots and leaf litter from the bags, and weighed them to determine mass loss (% of initial root or leaf litter mass).

All statistical analyses were done using the R software (version 3.6.3). To test for general dissimilarity between root- and leaf litter chemical characteristics, we used a PERMANOVA (with Euclidean distances), and tested for the interaction between litter type and site. We further used a post-hoc test (with block as random factor) to compare for differences between sites, and blocks. To test our hypotheses based on C, N loss during decomposition, we used two different statistical models.

To test for the effects of site, trees species mixture, and reinforced and prolonged summer drought on C, N fluxes, we used a mixed effects model (lme package) allowing for all interactions, with blocks set as random factor (2 blocks per site), and subplot nested within plot, and block. We ran this model for a subset of each litter type: fine root litterbags, and leaf litterbags with fine mesh.

Model 1:  $C, N \text{ remaining } \% \sim \text{Intercept} + \text{Site} + \text{Mixture} + \text{Rainfall exclusion} + (1|\text{Block/Plot/Subplot})$

To test our hypothesis on the differences between C and N dynamics in decomposing fine roots and leaf litter and between fine mesh width (microbial dominated decomposition) and coarse mesh width (fauna contribution to decomposition) litterbags used for leaf litter, we ran two separate models. Based on the previous global model, mixture was not significant overall for either roots, nor leaves, hence we removed 'Mixture' and added 'Litterbag type', as fixed factor with the same general structure of the model mentioned above:

Model 2 and 3:  $C, N \text{ remaining } \% \sim \text{Intercept} + \text{Site} + \text{Rainfall exclusion} + \text{Litterbag type} + (1|\text{Block/Plot/Subplot})$

Where model 2 was ran with fine mesh width litterbags of fine roots and leaf litter as the two levels within the factor "litterbag type" and model 3 was run with fine and coarse mesh width litterbags of leaf litter as the two levels within the factor "litterbag type".

## 5.3 Results

### 5.3.1 Reinforced and prolonged summer drought

The rainout shelters we installed during the two summers in 2018 and 2019 to simulate more severe and longer summer droughts, excluded rainfall efficiently according to the soil moisture measurements (see Fig. D5.2 in Appendix D). Nonetheless, the differences between control subplots and subplots where we excluded rainfall varied among sites and blocks. At the Lubéron site, the rainout shelters were extremely successful in excluding rain during the rain exclusion period for block 2 of the Lubéron site (relative soil moisture reduction of 79%) but for block 1, the soil moisture reduction was only 19%. At Vercors and Ste Baume, the rainout shelter reduced soil moisture by 32–46 % compared to the control subplots; while for Mont Ventoux the moisture reduction was slightly lower (16–28 %) (Fig. D5.2).

### 5.3.2 Initial fine root and leaf litter chemistry

The initial quality of fine roots was significantly different from that of leaf litter overall ( $p = 0.001$ ) (Table 5.2, see Fig. D5.3 in Appendix D), but the relative difference varied among sites ( $p = 0.03$ ). Furthermore, initial quality also varied between blocks (within sites), in particular for leaf litter (Table 5.3). For example, root N concentration varied slightly among the sites, but leaf N varied considerably, with highest values at Vercors, intermediate at Lubéron and Mont Ventoux, and lowest at Ste Baume. For root P the pattern was different, as root P concentration varied slightly among the sites, while leaf P concentration was the same at all sites. Root Ca, and leaf Ca were both unaffected by site. In general, leaf litter seemed to be richer in minerals than roots for the two northern sites (Vercors, Mont Ventoux), while rather the inverse was observed for southern sites, particularly at Ste Baume. For example, Ca and K (to a lesser extent P and N) are higher in roots at Ste Baume (somewhat at Lubéron as well) than in leaf litter. Leaf litter had clearly higher C/N ratios than fine roots, mostly due to a consistently higher C concentration than roots (leaves: 49% vs roots: 42%) (Table 5.3).

**Table 5.2** Results of the PERMANOVA test, using Euclidean distances, to test for the dissimilarity between groups of litter type (leaves vs roots), between sites (Vercors, Mont Ventoux, Lubéron, Ste Baume), and their interaction. Significant results are in bold font ( $p < 0.05$ ).

	$R^2$	$F$ -value	$p$ -value
Litter type	<b>0.35307</b>	<b>14.8268</b>	<b>0.001</b>
Site	0.05024	0.7033	0.656
Litter type x Site	<b>0.40619</b>	<b>5.686</b>	<b>0.003</b>

**Table 5. 3** Overview of initial litter quality of a) fine root-, and b) leaf litter. Means and standard deviation (in parentheses) of three replicates of: Carbon, Nitrogen, Phosphorus, Calcium, Potassium, Magnesium, Manganese, across the four sites (Vercors, Mont Ventoux, Lubéron, Ste Baume). Significant differences are annotated with small letters ( $p < 0.05$ ).

a)

Root litter	Vercors		Mont Ventoux		Lubéron		Ste Baume	
	1	2	1	2	1	2	1	2
	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)
C (%)	42.6 (0.470) bd	42.8 (0.746) ade	43.8 (0.301) ab	43.7 (0.124) ac	41.3 (0.318) e	42.3 (0.0889) cde	44.2 (0.601) a	41.8 (0.414) de
N (%)	0.960 (0.0096) ac	1.00 (0.0699) ab	0.873 (0.0373) bc	0.859 (0.0365) c	0.910 (0.0289) bc	1.02 (0.0292) ab	1.04 (0.0200) a	0.994 (0.0454) ab
P (%)	0.105 (0.0399) b	0.114 (0.0145) ab	0.130 (0.0092) ab	0.0797 (0.0083) ab	0.173 (0.0080) a	0.131 (0.0367) ab	0.106 (0.0043) b	0.0943 (0.0107) ab
Ca (%)	0.602 (0.216) ns	0.673 (0.0982) ns	1.15 (0.326) ns	1.44 (0.289) ns	1.31 (0.726) ns	1.40 (0.317) ns	1.00 (0.145) ns	0.963 (0.406) ns
K (%)	0.255 (0.0504) bd	0.224 (0.0138) cd	0.372 (0.0537) bc	0.274 (0.0725) cd	0.393 (0.0754) bc	0.367 (0.0483) bc	0.448 (0.0129) ac	0.471 (0.0955) ab
Mg (%)	0.0845 (0.0059) b	0.108 (0.0087) ab	0.129 (0.0226) ab	0.108 (0.0335) ab	0.116 (0.0248) ab	0.0907 (0.0067) b	0.172 (0.0150) a	0.109 (0.0273) ab
Mn (%)	0.0128 (0.0017) cd	0.0314 (0.0017) b	0.0110 (0.0018) d	0.0119 (0.0027) de	0.00391 (0.0012) e	0.00573 (0.0009) de	0.0874 (0.0039) a	0.0228 (0.0053) c

b)

**Leaf litter**

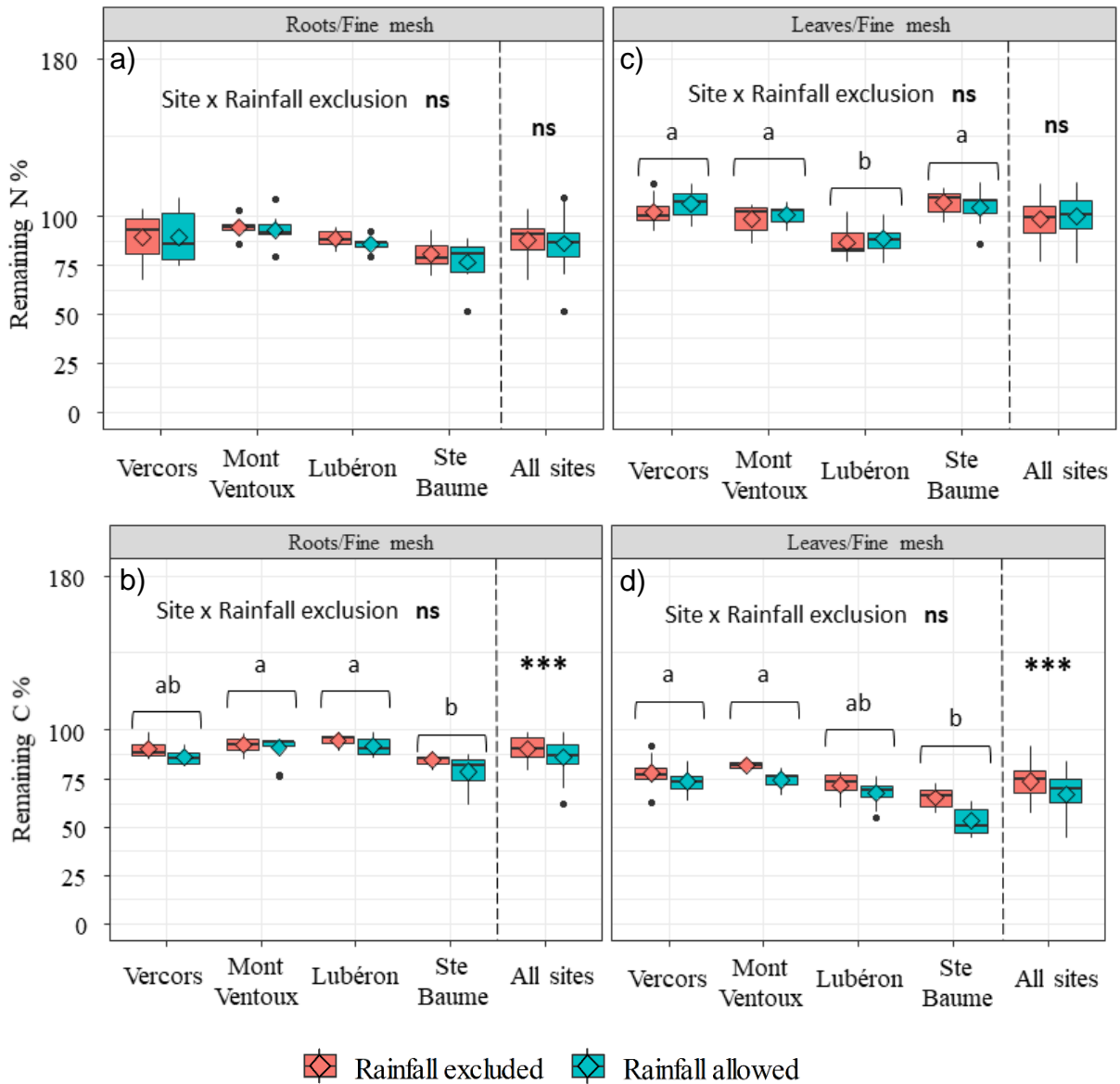
Leaf litter	Vercors		Mont Ventoux		Lubéron		Ste Baume	
	1	2	1	2	1	2	1	2
	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)
C (%)	49.0 (0.157) ac	48.8 (0.228) ac	48.3 (0.158) cd	48.4 (0.367) bc	49.6 (0.598) ab	49.1 (0.111) ac	48.8 (0.710) ac	49.7 (0.101) ad
N (%)	1.28 (0.0193) a	1.22 (0.0232) a	0.883 (0.0111) c	0.993 (0.0186) b	1.24 (0.0418) a	0.842 (0.0107) c	0.660 (0.0137) d	0.684 (0.00669) d
P (%)	0.107 (0.0059) ns	0.117 (0.0172) ns	0.104 (0.0208) ns	0.0972 (0.0385) ns	0.126 (0.0433) ns	0.0617 (0.0112) ns	0.0799 (0.00295) ns	0.123 (0.0281) ns
Ca (%)	1.29 (0.577) ns	1.32 (0.362) ns	1.32 (0.639) ns	1.90 (0.232) ns	1.50 (0.157) ns	1.50 (0.596) ns	1.61 (0.298) ns	1.38 (0.293) ns
K (%)	0.201 (0.0090) ef	0.186 (0.0172) f	0.382 (0.0254) ab	0.292 (0.0157) c	0.291 (0.0079) cd	0.256 (0.0111) de	0.352 (0.0124) b	0.426 (0.0126) a
Mg (%)	0.125 (0.0028) d	0.139 (0.0058) bd	0.160 (0.0092) abc	0.151 (0.0106) ab	0.169 (0.0011) a	0.151 (0.0072) ab	0.149 (0.0030) bc	0.136 (0.0025) cd
Mn (%)	0.0865 (0.0094) a	0.0576 (0.0058) b	0.0158 (0.0021) d	0.0170 (0.0010) d	0.0108 (0.0003) d	0.0177 (0.0016) d	0.0347 (0.0007) c	0.0334 (0.0021) c



### 5.3.3 Fine root- and leaf litter decomposition in response to reinforced and prolonged drought

Rainfall exclusion led to significantly lower C release from fine roots ( $p = 0.0026$ ) as well as for leaf litter ( $p < 0.0001$ ), but did not affect N (Fig. 5.1; Table 5.4). Carbon release differed among sites for leaf litter ( $p = 0.0287$ ), and marginally significantly for fine roots ( $p = 0.0564$ ). This site effect was mostly driven by Ste Baume, which had the highest C release from all the sites. No site effects were observed for the remaining N of root litter, however, N release from leaf litter differed significantly among sites ( $p = 0.0335$ ), mostly because of the very low values measured at Lubéron. There was no interaction between rainfall exclusion and site. Also, tree species mixture did not influence C and N release, and it did not interact with rainfall exclusion.

Carbon release from fine roots was significantly lower than that from leaf litter (Fig. 5.1; Table 5.5). It is noteworthy that contrary to the general C release for both roots and leaves, we observed that remaining N% was lower for roots, but was much higher for leaves, with the sole exception of Lubéron.



**Fig. 5.1** Boxplots with medians (-), and means ( $\diamond$ ) of remaining C%, and remaining N% of: a) root litterbags, b) leaf litterbags/fine mesh, as affected by rainfall exclusion, and depicted across sites. Different lowercase letters indicate significant differences between the sites. Asterisks indicate a significant difference between rainfall exclusion treatments (\*\*\*) ( $p < 0.001$ ).

**Table 5.4** Results of the linear mixed effects models to test for the effects of site, mixture, rainfall exclusion and their interactions on remaining carbon (C), and remaining nitrogen (N), after two years of decomposition, for: a) root litterbags, b) leaf litterbags with a fine mesh. Significant results are in bold font ( $p < 0.05$ ).

a) Root litterbags

	Carbon				Nitrogen			
	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
Site (S)	3	4	6.114	0.0564	3	4	2.57	0.192
Mixture (M)	1	4	1.78	0.253	1	4	0.1153	0.7512
Rainfall exclusion (R)	<b>1</b>	<b>35</b>	<b>10.485</b>	<b>0.0026</b>	1	35	0.4466	0.5084
(S x M)	3	4	3.587	0.1245	3	4	0.5843	0.6564
(S x R)	3	35	0.751	0.5292	3	35	0.1505	0.9287
(M x R)	1	35	2.687	0.1102	1	35	0.0005	0.9816
(S x M x R)	3	35	2.109	0.1167	3	35	0.5641	0.6423

b) Leaf litterbags/fine mesh

	Carbon				Nitrogen			
	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
Site (S)	<b>3</b>	<b>4</b>	<b>9.2167</b>	<b>0.0287</b>	<b>3</b>	<b>4</b>	<b>8.412</b>	<b>0.0335</b>
Mixture (M)	1	4	1.957	0.2344	1	4	3.224	0.147
Rainfall exclusion (R)	<b>1</b>	<b>37</b>	<b>35.4937</b>	<b>&lt;0001</b>	1	37	0.536	0.4687
(S x M)	3	4	0.8446	0.5367	3	4	0.817	0.5478
(S x R)	3	37	2.4431	0.0794	3	37	0.83	0.4861
(M x R)	1	37	0.8518	0.362	1	37	0.148	0.7023
(S x M x R)	3	37	1.454	0.2429	3	37	0.051	0.9843

**Table 5.5** Results of the linear mixed effects models to test for the effects of site, rainfall exclusion, litterbag type and their interactions on remaining carbon (C), and remaining nitrogen (N), after two years of decomposition. The litterbag comparison is between fine root litterbags and leaf litterbags with a fine mesh. Significant results are in bold font ( $p < 0.05$ ).

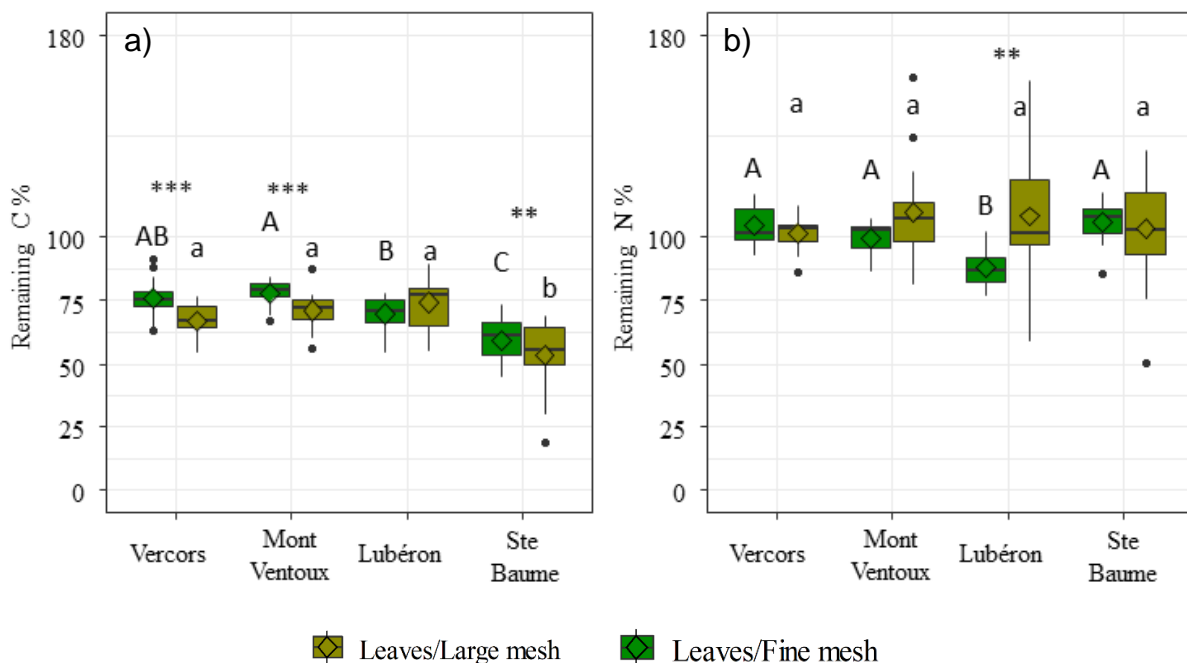
Root litterbags vs Leaf litterbags/fine mesh

	Carbon				Nitrogen			
	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
Site (S)	<b>3</b>	<b>4</b>	<b>15.23</b>	<b>0.0118</b>	3	4	4.315	0.0958
Rainfall exclusion (R)	<b>1</b>	<b>44</b>	<b>33.307</b>	<b>&lt;0001</b>	1	44	0.109	0.7426
Litterbag type (L)	<b>1</b>	<b>48</b>	<b>307.852</b>	<b>&lt;0001</b>	<b>1</b>	<b>48</b>	<b>66.07</b>	<b>&lt;0001</b>
(S x R)	3	44	1.211	0.3171	3	44	0.825	0.487
(S x L)	<b>3</b>	<b>48</b>	<b>8.224</b>	<b>0.0002</b>	<b>3</b>	<b>48</b>	<b>14.34</b>	<b>&lt;0001</b>
(R x L)	1	48	2.863	0.0971	1	48	1.003	0.3215
(S x R x L)	3	48	0.677	0.5702	3	48	0.09	0.9654

### 5.3.4 Fauna contribution to leaf litter decomposition

Macrofauna presence increased leaf litter decomposition, which was reflected by the higher values of C release compared to leaf litter that decomposed without macrofauna contribution ( $p = 0.0012$ ) (Fig. 5.2a; Table 5.6). The highest values of C release were observed in the most southern part of the gradient at Ste Baume ( $p = 0.007$ ). There was a significant interaction between site and macrofauna contribution to C release ( $p = 0.0012$ ), which was explained by the absence of any macrofauna effect at Lubéron in contrast to all other sites. Nitrogen dynamics changed when fauna had access ( $p = 0.0215$ ), but differently so among the four sites ( $p = 0.0065$ ) (Fig. 5.2b; Table 5.6). Overall, remaining N tended to be higher when fauna had access, a pattern that was mostly driven by the Lubéron site, and to a lesser extent by the Ventoux site, where remaining N was highly variable when macrofauna had access to decomposing litter, with values of remaining N ranging from 60% up to 170% (Fig. 5.2b; Table 5.6).

**Fig. 5.2** Boxplots with medians (-), and means ( $\diamond$ ) of: a) remaining C%, and b) remaining N%, on leaf litter as affected by contribution of fauna, and depicted across sites. Absence of letters and Asterisks means there were no significant effects. Different uppercase letters indicate significant differences between sites, for leaf litterbags with fine mesh, and different lowercase letters indicate significant differences between sites, for leaf litterbags with a coarse mesh. Asterisks indicate a significant difference between litterbag type per site (\*\*;  $p < 0.01$ ), (\*\*\*)  $p < 0.001$ ).



**Table 5.6** Results of the linear mixed effects models to test for the effects of site, rainfall exclusion, litterbag type and their interactions on remaining carbon (C), and remaining nitrogen (N), after two years of decomposition. The litterbag comparison is between leaf litterbags with a fine mesh, and leaf litterbags with a coarse mesh. Significant results are in bold font ( $p < 0.05$ ).

Leaf litterbags/fine mesh vs Leaf litterbags/large mesh

	Carbon				Nitrogen			
	<i>numDF</i>	<i>denDF</i>	<i>F-value</i>	<i>p-value</i>	<i>numDF</i>	<i>denDF</i>	<i>F-value</i>	<i>p-value</i>
Site (S)	<b>3</b>	<b>4</b>	<b>20.248</b>	<b>0.007</b>	3	4	0.3912	0.7667
Rainfall exclusion (R)	<b>1</b>	<b>44</b>	<b>25.398</b>	<b>&lt;.0001</b>	1	44	0.0848	0.7723
Litterbag type (L)	<b>1</b>	<b>49</b>	<b>11.875</b>	<b>0.0012</b>	<b>1</b>	<b>49</b>	<b>5.6412</b>	<b>0.0215</b>
(S x R)	3	44	2.202	0.1013	3	44	1.428	0.2474
(S x L)	<b>3</b>	<b>49</b>	<b>6.195</b>	<b>0.0012</b>	<b>3</b>	<b>49</b>	<b>4.5948</b>	<b>0.0065</b>
(R x L)	1	49	0.404	0.5281	1	49	0.0721	0.7894
(S x R x L)	3	49	0.647	0.5888	3	49	1.1292	0.3464

## 5.4 Discussion

### 5.4.1 Drought effects on decomposition

The rainout shelters that were placed in the summers of 2018 and 2019, were successful in blocking rainfall for the designated subplots, and this is reflected in our microclimatic data (see Fig. D5.2 in Appendix D). The control subplots had as expected higher soil moisture % in general, but the reduction in soil moisture under the rainout shelters relative to the control subplots was variable and ranged from 16% relative soil moisture reduction (in block 2 of Mont Ventoux), to 79% (in block 2 of Lubéron). The variability in the effectiveness of the rainout shelter, is likely due to the fact that the soils of some sites had considerable gravel (~10% at Mont Ventoux, ~30% at Lubéron), combined with rather steep slopes (18° at Mont Ventoux, 30° at Lubéron). At the same time the rainfall exclusion was more consistent in reducing soil moisture for their adjacent blocks; 28% soil moisture reduction for block 1 of Mont Ventoux, and 32% soil moisture reduction for block 2 of the Grand Lubéron. Though their adjacent blocks have similar topography, we cannot infer why the rainout shelters were less effective only there. A reason might be related to long drought events during the exclusion period, or heavy rainfall in large pulses, instead of frequent rainfall events that would have given a clearer difference between rainout shelter and control subplots. Our soil moisture data indicate the possibility for such conditions, but since we do not have currently the specific rainfall data, we cannot yet test for this hypothesis.

We hypothesized that rainfall exclusion would result in a lower C release (%) and lower N release (%) during decomposition, and that the rainfall exclusion effect would be stronger for the northern part of the gradient because rainfall events during summer have previously been reported to be more frequent and their complete exclusion should have a stronger impact on soil moisture and biological activity (Jourdan and Hättenschwiler 2020). In partial agreement with our hypothesis, we observed

significantly lower C loss from decomposing fine roots and leaf litter in response to reinforced and prolonged summer drought. However, this effect was similar across all sites with no indication that longer and more intense summer drought affects decomposition more in the wetter northern part of the gradient. The negative effect of rainfall exclusion on fine root decomposition is in accordance with previous results on root C release where 29% reduction of net precipitation was achieved using PVC gutters covering 33% of the area (García-Palacios et al. 2016); however our drought treatment gave a smaller reduction of C release (~5 %), while at the latter study the reduction was stronger (~30 %). The results on leaves are in accordance with previous results on pure beech leaf litter decomposing on the same plots (and using the identical rainout shelters) but for the years 2016–2017 (Jourdan and Hättenschwiler 2020); the rainout shelters resulted in a 12% of reduction in C remaining in the leaf litter in our study, and about 20% in the previous study. It is interesting to note, that the authors used common beech leaf litter across the gradient that was sourced from another site, though we used site-specific litter that varied greatly in quality across the sites. One would think that the variable litter qualities would accentuate variability in decomposition among sites and maybe change the drought effect, since these microbial communities are adapted to site-specific litter (Barantal et al. 2011). For example, N concentration of leaf litter was much higher in leaf litter from Vercors (1.28% of N; at the most Northern part of the gradient), compared to leaves from Ste Baume (0.6% of N; at the most Southern part of the gradient), which makes the Vercors litter potentially more decomposable, with potentially stronger consequences on drought. However, leaves still decomposed statistically similarly between the two sites, and drought effects were not different. It is likely, that since we assessed C loss in the early stage of decomposition, the decomposition study was not long enough to observe our hypothesized stronger effects of prolonged summer drought on the wetter, northern part of the gradient. Possibly, the summer drought effects are accumulating over time and differences between the North and the South may become more marked at the later stages of decomposition (García-Palacios et al. 2016). Our current results agree with other studies suggesting that applied rainfall exclusion in Mediterranean forests and shrublands reduces leaf decomposition (Santonja et al. 2017, 2019; Saura-Mas et al. 2012). The values of C release (%) after two years from roots and leaves were rather low (around 25% and 45%, respectively), but in a similar range as the C release values recorded from previous two-year decomposition studies of roots (of pine) and leaves (of oak) (Guo et al. 2006; Santonja et al. 2017).

The negative effects of rainfall exclusion on decomposition were reflected in the release of C, but not in N release. The latter is linked to the complex relationship between litter quality, soil fertility, and the stoichiometric demands of the soil microbial community, which makes N cycling very dynamic (Hobbie 2015; See et al. 2019). For example, litter with higher initial values of N, may lead to higher N release initially (Sanauallah et al. 2012), since it matches better with the usually lower C:N ratio of microbes, but microbial immobilization of N (usually is exogenous and is presumably controlled by N availability in the environment) might occur at a later stage of decomposition (Parton et al. 2007). Therefore, litter N can be immobilized by microbes when the decomposing litter has a stoichiometry that is too recalcitrant (very high C:N ratios for instance). Also, N immobilization may occur under prolonged summer

drought, as microbial decomposers do not have access to leached exogenous mineral N. For example, Zheng et al. (2017) reported that precipitation reduction can enhance the magnitude and duration of N immobilization in decomposing litter, which can last up to 2 years, especially for litter with high initial C:N ratios. Though we cannot know whether N has been previously released, or will be released at later stages of decomposition, we may assume that other reasons for the N immobilization could be related to a more stable, and well-adapted microbial community into the leaf litter layer. Lower decomposition rates with more severe summer drought may have stronger impacts on the C cycle than on N cycle from decomposing litter. Jourdan and Hättenschwiler (2020) suggested that C and N dynamics might be more unbalanced with increasing drought at least during the beginning of the decomposition process, which could have longer-term consequences for decomposer communities and biogeochemical cycling at the ecosystem scale.

#### **5.4.2 Severe summer drought and tree species mixtures**

Contrary to our second hypothesis, tree species mixtures had no impact on decomposition of either litter type. This result disagrees with other studies that found species richness to indirectly positively affect decomposition (Hector et al 2000; Scherer-Lorenzen 2008; Joly et al 2017). Also, in contrast to our second hypothesis, tree species mixture did not modify the observed drought effects on decomposition. Recent measurements of beech leaf decomposition on the same sites, showed that fir slowed beech litter decomposition and oak accelerated it compared to the respective pure beech plots (Jourdan and Hättenschwiler 2020). However, the aforementioned study used a common litter pool, while we used site-specific litter. Based on the home field advantage (HFA) effect, one might expect a stronger mixture effect with plot-specific litter, because decomposers are adapted to the specific litter quality produced at a particular site (Barantal et al. 2011), but in our study tree species mixture effects were absent. Our hypothesis was based on some previously reported indirect positive diversity effects of mixed plant canopies on the decomposition environment (Hector et al. 2000; Joly et al. 2017), and the direct mixture effects studied in detail by Jourdan and Hättenschwiler (2020). The lack of mixture effects on decomposition may likely be due to the plant community structure and composition in our plots, which possess plenty of understory species (small trees, shrubs, forbs etc). While beech, fir, and oak dominated the respective plots in terms of total basal area (see [Table D5.1](#) in Appendix D) and canopy size, a similar understory species composition that also contributes litter to the forest floor may homogenize the litter layer and the associated microenvironmental conditions.

### 5.4.3 Comparison between root and leaf decomposition

In accordance to our third hypothesis, root litter decomposed slower than leaf litter, and this was reflected in the lower C release (%) in roots compared to leaves. Differences between root and leaf decomposition were not the same among the different sites. The distinct decomposition between root and leaf litter is likely related to climatic conditions, soil characteristics, initial litter quality, and different abundance and structure of decomposer communities. For example, the highest decomposition that was observed at the Ste Baume site, is probably due to macroclimatic conditions stimulating decomposer activity, as for that site there was a combination of sufficient ambient precipitation (~ 940 mm), and relatively higher (~ 10 °C) temperatures compared to the other sites, that were either drier (Lubéron), or wetter and colder (Vercors, Mont Ventoux). The lowest root decomposition at Lubéron might be related to the very steep slope of the site, or the fact that for that site, the soil had a lot of big stones and gravel, which means that roots are decomposing in an environment that retains little water, and may not support the microbial community well. Finally, the low leaf decomposition at Mont Ventoux could be related to the low initial leaf litter quality, as the C:N ratio for that site was consistently high for both blocks (C:N ~ 60), making it harder for microbes to decompose.

Contrary to our hypothesis, N was released at a low rate from root litter, and was mostly immobilized in leaf litter, even when allowing for macrofauna. Though unexpected, the N immobilization is not completely surprising, as beech leaves have been shown before to accumulate N during decomposition, and for long periods of time (Albers et al. 2004). In the latter paper, the authors state that after an initial period of N mineralization (around 3 months) there was N immobilization, which led to high amounts of accumulated N that remained unchanged until the end of the experiment. We cannot know at what point N immobilization initiated for our samples, but N immobilization was observed for beech leaf litter, as early as six months for our plots, and was mildly sustained for the following three years (Jourdan and Hattenschwiler 2020). This does not exclude the possibility for N to have been mineralized earlier, or for N mineralization to occur at the same time as N immobilization (Myrold and Bottomley 2008), but our observation after two years shows that the immobilization rate is probably higher than the mineralization rate. The N immobilization in litter is usually linked to the stoichiometry of the decomposing litter. Litter with higher C:N ratio tend to be harder to decompose since concentrations in plant litter tend to be low compared to the decomposer requirements: hence nutrients may be initially immobilized from the environment (an exogenous source) by decomposers until the nutrient concentrations in the litter reach a critical value and net release occurs (Parton et al. 2007). However, roots experience a very different environment than leaves, as roots decompose belowground and leaves decompose in the litter layer. Microbial decomposers in the soil may have greater access to moisture, organic matter, and mineral N than microbes involved in leaf litter decomposition at the soil surface, which would facilitate net N release during root decomposition (Silver and Miya 2001). Similar patterns in net N release in roots have been described for native root litter decomposed in situ in grasslands (Seastedt et al. 1992), temperate broadleaf forests (Dornbush et al. 2002), and temperate conifer forests (Chen et al. 2002). This result suggests that



although beech leaves are decomposing more than beech roots, the latter represent a specific pool of N with its own dynamics for the studied forests. However, we must acknowledge that both, roots and leaves are at a very initial stage of decomposition, and that it is difficult to conclude about N dynamics with only this single point of measurement in time.

#### **5.4.4 Fauna contribution to leaf litter decomposition**

As expected, the inclusion of macrofauna significantly increased leaf decomposition by 10% or more, however increased the N variability between sites. It is well known that macrofauna contribute strongly to the decomposition process as macrofauna physically modify the litter layer via the breakdown of leaf litter into smaller particles and the production of faeces, which may affect microbial abundance and activity (David 2014). The variable leaf N after inclusion of macrofauna suggests the variable impact of fauna at small spatial scales (even within subplots probably), because their occurrence, abundance and activity varies much more in space and time than microbes. As a result, the presence of macrofauna homogenizes differences among sites that are more expressed when only microbes have access.

### **5.5 Conclusion**

We found that under a future scenario of climate change, and if summer drought is prolonged, there would be a reduction in decomposition rates, with negative implications for the nutrition of beech forests. However, we found weak evidence that a prolonged summer drought may have stronger impacts on the C cycle than on the N cycle from decomposing litter, and that this depends on the litter type but not on tree species mixture. Our results suggest that although beech fine root litter decomposes less than beech leaf litter, fine root litter represents a specific pool of N that may sustain N supply to the soil at a different rate, while for N from leaf litter N seems to be grossly immobilized.

### **Acknowledgements**

This study was supported by the ANR project DiPTiCC (16-CE32-0003). We thank Laurent Augusto, Nicolas Fanin, Catherine Lambrot, Sylvie Milin, Céline Gire for their participation in the field. Special thanks to Catherine Lambrot, and Dilane de Deus Lopes for their valuable assistance in the lab.

## References

- Albers D, Migge S, Schaefer M, Scheu S (2004) Decomposition of beech leaves (*Fagus sylvatica*) and spruce needles (*Picea abies*) in pure and mixed stands of beech and spruce. *Soil Biol Biochem* 36:155–164. <https://doi.org/10.1016/j.soilbio.2003.09.002>
- Ayres E, Steltzer H, Simmons BL, et al (2009) Home-field advantage accelerates leaf litter decomposition in forests. *Soil Biol Biochem* 41:606–610. <https://doi.org/10.1016/j.soilbio.2008.12.022>
- Ball BA, Carrillo Y, Molina M (2014) The influence of litter composition across the litter-soil interface on mass loss, nitrogen dynamics and the decomposer community. *Soil Biol Biochem* 69:71–82. <https://doi.org/10.1016/j.soilbio.2013.10.048>
- Barantal S, Roy J, Fromin N, et al (2011) Long-term presence of tree species but not chemical diversity affect litter mixture effects on decomposition in a neotropical rainforest. *Oecologia* 167:241–252. <https://doi.org/10.1007/s00442-011-1966-4>
- Chapman SK, Newman GS (2010) Biodiversity at the plant-soil interface: Microbial abundance and community structure respond to litter mixing. *Oecologia* 162:763–769. <https://doi.org/10.1007/s00442-009-1498-3>
- Chen H, Harmon ME, Sexton J, Fasth B (2002) Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A. *Can J For Res* 32:320–331. <https://doi.org/10.1139/x01-202>
- Cornelissen JHC (1996) An Experimental Comparison of Leaf Decomposition Rates in a Wide Range of Temperate Plant Species and Types. *J Ecol* 84:573. <https://doi.org/10.2307/2261479>
- Couteaux MM, Bottner P, Berg B (1995) Litter decomposition, climate and litter quality. *Trends Ecol. Evol.* 10:63–66
- David JF (2014) The role of litter-feeding macroarthropods in decomposition processes: A reappraisal of common views. *Soil Biol Biochem* 76:109–118. <https://doi.org/10.1016/j.soilbio.2014.05.009>
- Dornbush ME, Isenhardt TM, Raich JW (2002) Quantifying fine-root decomposition: An alternative to buried litterbags. *Ecology* 83:2985–2990. [https://doi.org/10.1890/0012-9658\(2002\)083\[2985:QFRDAA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[2985:QFRDAA]2.0.CO;2)
- Dubrovský M, Hayes M, Duce P, et al (2014) Multi-GCM projections of future drought and climate variability indicators for the Mediterranean region. *Reg Environ Chang* 14:1907–1919. <https://doi.org/10.1007/s10113-013-0562-z>
- Fujii S, Berg MP, Cornelissen JHC (2020) Living Litter: Dynamic Trait Spectra Predict Fauna Composition. *Trends Ecol. Evol.* 35:886–896

- Gallardo A, Schlesinger WH (1994) Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biol Biochem* 26:1409–1415. [https://doi.org/10.1016/0038-0717\(94\)90225-9](https://doi.org/10.1016/0038-0717(94)90225-9)
- García-Palacios P, Prieto I, Ourcival J, Hättenschwiler S (2016) García-Palacios, P., Prieto, I., Ourcival, JM. et al. Disentangling the Litter Quality and Soil Microbial Contribution to Leaf and Fine Root Litter Decomposition Responses to Reduced Rainfall. *Ecosystems* 19, 490–503 (2016). <https://doi.org/10.1007/s10021-016-00359-9>
- Gholz HL, Wedin DA, Smitherman SM, et al (2000) Long-term dynamics of pine and hardwood litter in contrasting environments: Toward a global model of decomposition. *Glob Chang Biol* 6:751–765. <https://doi.org/10.1046/j.1365-2486.2000.00349.x>
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Glob Planet Change* 63:90–104. <https://doi.org/10.1016/j.gloplacha.2007.09.005>
- Gora EM, Lucas JM, Yanoviak SP (2019) Microbial Composition and Wood Decomposition Rates Vary with Microclimate From the Ground to the Canopy in a Tropical Forest. *Ecosystems* 22:1206–1219. <https://doi.org/10.1007/s10021-019-00359-9>
- Gracia M, Montané F, Piqué J, Retana J (2007) Overstory structure and topographic gradients determining diversity and abundance of understory shrub species in temperate forests in central Pyrenees (NE Spain). *For Ecol Manage* 242:391–397. <https://doi.org/10.1016/j.foreco.2007.01.056>
- Grayston SJ, Prescott CE (2005) Microbial communities in forest floors under four tree species in coastal British Columbia. *Soil Biol Biochem* 37:1157–1167. <https://doi.org/10.1016/j.soilbio.2004.11.014>
- Guo LB, Halliday MJ, Gifford RM (2006) Fine root decomposition under grass and pine seedlings in controlled environmental conditions. *Appl Soil Ecol* 33:22–29. <https://doi.org/10.1016/j.apsoil.2005.09.004>
- Hättenschwiler S, Tiunov A V., Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annu Rev Ecol Evol Syst* 36:191–218. <https://doi.org/10.1146/annurev.ecolsys.36.112904.151932>
- Hector A, Beale AJ, Minns A, et al (2000) Consequences of the reduction of plant diversity for litter decomposition: Effects through litter quality and microenvironment. *Oikos* 90:357–371. <https://doi.org/10.1034/j.1600-0706.2000.900217.x>
- Hobbie SE (2015) Plant species effects on nutrient cycling: revisiting litter feedbacks. <https://doi.org/10.1016/j.tree.2015.03.015>
- IPCC (2014) Synthesis Report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.
- Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate-change experiments: Events, not trends. *Front. Ecol. Environ.* 5:365–374

- Joly FX, Milcu A, Scherer-Lorenzen M, et al (2017) Tree species diversity affects decomposition through modified micro-environmental conditions across European forests. *New Phytol* 214:1281–1293. <https://doi.org/10.1111/nph.14452>
- Joly FX, Weibel AK, Coulis M, Throop HL (2019) Rainfall frequency, not quantity, controls isopod effect on litter decomposition. *Soil Biol Biochem* 135:154–162. <https://doi.org/10.1016/j.soilbio.2019.05.003>
- Jourdan M, Hättenschwiler S (2020) Decomposition in mixed beech forests in the south-western Alps under severe summer drought 2. *bioRxiv* 2020.05.23.111815. <https://doi.org/10.1101/2020.05.23.111815>
- Manzoni S, Schimel JP, Porporato A (2012) Responses of soil microbial communities to water stress: Results from a meta-analysis. *Ecology* 93:930–938
- Myrold DD, Bottomley PJ (2008) Nitrogen Mineralization and Immobilization. John Wiley & Sons, Ltd, pp 157–172
- Osono T, Hirose D, Fujimaki R (2006) Fungal colonization as affected by litter depth and decomposition stage of needle litter. *Soil Biol Biochem* 38:2743–2752. <https://doi.org/10.1016/j.soilbio.2006.04.028>
- Parton W, Silver WL, Burke IC, et al (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* (80- ) 315:361–364. <https://doi.org/10.1126/science.1134853>
- Polade SD, Gershunov A, Cayan DR, et al (2017) Precipitation in a warming world: Assessing projected hydro-climate changes in California and other Mediterranean climate regions. *Sci Rep* 7:1–10. <https://doi.org/10.1038/s41598-017-11285-y>
- Prescott CE (2002) The influence of the forest canopy on nutrient cycling. *Tree Physiol* 22:1193–1200
- Ratcliffe S, Wirth C, Jucker T, et al (2017) Biodiversity and ecosystem functioning relations in European forests depend on environmental context. *Ecol. Lett.* 20:1414–1426
- Sanoullah M, Rumpel C, Charrier X, Chabbi A (2012) How does drought stress influence the decomposition of plant litter with contrasting quality in a grassland ecosystem? *Plant Soil* 352:277–288. <https://doi.org/10.1007/s11104-011-0995-4>
- Santonja M, Fernandez C, Proffit M, et al (2017) Plant litter mixture partly mitigates the negative effects of extended drought on soil biota and litter decomposition in a Mediterranean oak forest. *J Ecol* 105:801–815. <https://doi.org/10.1111/1365-2745.12711>
- Santonja M, Milcu A, Fromin N, et al (2019) Temporal Shifts in Plant Diversity Effects on Carbon and Nitrogen Dynamics During Litter Decomposition in a Mediterranean Shrubland Exposed to Reduced Precipitation. *Ecosystems* 22:939–954. <https://doi.org/10.1007/s10021-018-0315-4>

- Saura-Mas S, Estiarte M, Peñuelas J, Lloret F (2012) Effects of climate change on leaf litter decomposition across post-fire plant regenerative groups. *Environ Exp Bot* 77:274–282. <https://doi.org/10.1016/j.envexpbot.2011.11.014>
- Seastedt TR, Parton WJ, Ojima DS (1992) Mass loss and nitrogen dynamics of decaying litter of grasslands: the apparent low nitrogen immobilization potential of root detritus. *Can J Bot* 70:384–391. <https://doi.org/10.1139/b92-052>
- See CR, Luke McCormack M, Hobbie SE, et al (2019) Global patterns in fine root decomposition: climate, chemistry, mycorrhizal association and woodiness. *Ecol. Lett.* 22:946–953
- Seidelmann KN, Scherer-Lorenzen M, Niklaus PA (2016) Direct vs. Microclimate-Driven Effects of Tree Species Diversity on Litter Decomposition in Young Subtropical Forest Stands. *PLoS One* 11:e0160569. <https://doi.org/10.1371/journal.pone.0160569>
- Silver WL, Miya RK (2001) Global patterns in root decomposition: Comparisons of climate and litter quality effects. *Oecologia* 129:407–419. <https://doi.org/10.1007/s004420100740>
- Wardle DA (2006) The influence of biotic interactions on soil biodiversity. *Ecol. Lett.* 9:870–886
- Wardle DA, Nilsson MC, Zackrisson O, Gallet C (2003) Determinants of litter mixing effects in a Swedish boreal forest. *Soil Biol Biochem* 35:827–835. [https://doi.org/10.1016/S0038-0717\(03\)00118-4](https://doi.org/10.1016/S0038-0717(03)00118-4)
- Weltzin JF, Loik ME, Schwinning S, et al (2003) Assessing the Response of Terrestrial Ecosystems to Potential Changes in Precipitation. *Bioscience* 53:941–952
- Zhang X, Wang W (2015) Control of climate and litter quality on leaf litter decomposition in different climatic zones. *J Plant Res* 128:791–802. <https://doi.org/10.1007/s10265-015-0743-6>
- Zheng J, Guo R, Li D, et al (2017) Nitrogen addition, drought and mixture effects on litter decomposition and nitrogen immobilization in a temperate forest. *Plant Soil* 416:165–179. <https://doi.org/10.1007/s11104-017-3202-4>



## Chapter 6 | Discussion

Biodiversity plays a pivotal role in ecosystem functioning, with mixed-species forests providing higher levels of multiple ecosystem services, and being more resistant and resilient to drought events than monocultures (Gamfeldt et al. 2013; Pardos et al. 2021). However, the relation between plant diversity and aboveground production is unclear, and there is evidence for larger diversity effects under stronger environmental constraints, for example when growth is strongly nutrient limited in forests growing on infertile soils, or under recurrent water limitation (Harpole et al. 2016; Pretzsch et al. 2013; Ratcliffe et al. 2016). Ratcliffe et al. (2017) recently suggested that as water limitation increases under climate change, biodiversity appears to become more important to support high levels of ecosystem functioning in European forests. However, belowground processes that are essential to nutrient and carbon cycling such as root production and root decomposition were not included in their study; these belowground processes are less commonly considered in general. With the continuing species loss predicted under the scenario of a future drier climate, it is critical to reinforce the efforts for a better understanding of how plant diversity modifies biomass production, nutrient recycling, and carbon storage (Cardinale et al. 2011; Hooper et al. 2005). This thesis took a belowground perspective and examined across- and within-species variation in tree root functional traits in relation to belowground productivity and decomposition across climatic gradients and between tree monocultures and tree mixtures.

### 6.1 Root adaptations to water availability and the role of interspecific interactions

Adjustments in fine root biomass allocation, in fine root morphology or through fine root mortality, and/or altered investments in mycorrhizal symbioses, are among the important adaptations to cope with drought conditions (Brunner et al. 2015; Hertel et al. 2013; Rewald et al. 2011; Weemstra et al. 2017). Moreover, these root adaptations have a direct relevance for carbon and nutrient dynamics (Bardgett et al. 2014; Brunner and Godbold 2007). In my thesis, I focused on the morphological adaptations and vertical distributions of fine roots from monocultures or mixed plantations of birch and pine, which are irrigated or are left unirrigated during the summer months when they potentially experience summer drought (Chapter 3). Different tree species growing together may have contrasting fine root morphologies (e.g. thicker and shorter vs thinner and longer fine roots) and a distinct vertical root distribution (e.g. deep- vs shallow rooting patterns), which may lead to an increased soil volume filling, compared to when they grow in mono-specific stands. This may then allow trees to increase resource uptake per unit of ground area, potentially leading to higher aboveground productivity compared to the respective single tree species stands. Using the same experimental design, I also focused on whether these birch and pine tree mixtures increase their fine root turnover (by producing more new roots, or shedding more roots), and how the response on fine root turnover relates to water availability and stand density (Chapter 4). As an alternative to adaptations of fine root morphology, vertical root distribution, fine root growth and root shedding, trees might also utilize the mycorrhizal pathway in purpose of a more cost-efficient nutrient uptake strategy. For instance, the development of specific exploration types of ectomycorrhizae, with

differing amounts of rhizomorphs and hyphal biomass, enlarges the soil volume available to the plant (Agerer 2001; Weemstra et al. 2017). Due to time constraints, I did not dedicate a Chapter to mycorrhizae, but instead, I give a sneak-peak of some preliminary results in the discussion (6.1.4).

### **6.1.1 Vertical root distribution**

Birch and pine trees were previously reported to have contrasting fine root distributions, and fine root morphologies, with the potential to stratify further under mixture, thus assuming a vertical root segregation of birch and pine along the soil profile and higher fine root biomass for the whole soil column under mixture. Contrarily to my first hypothesis, pine and birch had similar rather shallow root systems compared to previously reported distributions (Curt and Prévosto 2003; Jackson et al. 1997), in both pure and mixed stands, and no vertical root segregation or increase in fine root biomass for the entire soil column could be observed when both species were growing together. In contradiction to my second hypothesis, under the harsher control conditions (no irrigation), there was no pronounced vertical root segregation and no higher fine root biomass for the whole soil column when both species grew together. This lack of a clear difference in vertical root segregation between birch and pine can have various explanations, notably the presence of a hardpan at 50 cm, and the seasonally high ground water table preventing roots from growing to deeper soil layers (Bakker et al. 2006). Arguably, the young age of the studied stands can be a further explanation, as this would match with a previous study by Curt and Prévosto (2003), where it is highlighted that differences in the vertical rooting patterns of competitors between birch- and pine-dominated stands tended to accentuate along stand maturation. A key finding of this part of the thesis is that under irrigation, both species tended to have shallower vertical root distributions when growing in mixture but deeper vertical root distributions when growing in monocultures. This indicates that vertical root distribution was marginally sensitive to increased water availability when the two species were growing together. This result was stronger for birch that grew its roots in shallower soil layers when growing together with pine and under irrigated conditions, indicating a higher plasticity of the birch root system compared to pine. Increased fine root growth and shallower vertical root distribution have been shown with increased water availability (Bakker et al. 2006, 2009; Meier and Leuschner 2008; Persson et al. 1995), but our study is the first to show that the response to water availability depends on tree species mixing. Although the effects were only marginally significant, we show that there is a tendency for vertical root segregation to appear under irrigation, and that this was likely due to birch showing the strongest response to irrigation under mixture, by producing shallower roots than pine.



### 6.1.2 Fine root growth in mixed stands

Birch and pine showed marginal effects on vertical root distribution in response to irrigation, but not to the benefit of increased belowground productivity, thus rejecting our hypothesis. The consequences of mixing tree species on belowground productivity has been reported as positive (Brassard et al. 2011, 2013; Meinen et al. 2009a; Schmid and Kazda 2002), neutral (Fruloux et al. 2018; Meinen et al. 2009b) or negative (Bolte and Villanueva 2006). Similarly, vertical root segregation has been found in some (Bolte and Villanueva 2006; Schmid and Kazda 2002), but not in all the studies investigating root distribution patterns along the soil profile (Fruloux et al. 2018; Meinen et al. 2009b). It might be that the environmental context is important in elucidating the possible link between vertical root segregation and belowground overyielding. In an in-situ root chamber study of oak and beech tree mixtures, where two terminal fine roots per species were placed in each chamber to investigate in-site root competition, Hertel and Leuschner (2006) found that interspecific root competition was in favour of beech, as beech root growth was enhanced when growing together with oak. In a following experiment, where the authors compared between ambient and reduced soil moisture during a period of 11 months via below-canopy throughfall reduction, interspecific root competition was in favour of oak, as oak root growth was enhanced when growing together with beech, and this occurred under ambient soil moisture (Rewald and Leuschner 2009). In our experiment, fine root growth of pine was positively affected by irrigation in the mixture compared to pine in the monoculture, while irrigation resulted in less fine roots for birch in mixture than birch in the monoculture. The increased fine root growth of pine under irrigation might mean that intraspecific competition in pine monocultures is strong, but that interspecific competition when admixed with birch is lower, thus allowing pine to grow more roots in mixtures than in monocultures. Birch was likely the inferior competitor under irrigation, which is apparent by the reduction in fine root growth of birch in mixtures relative to its monocultures. However, neither fine root growth of pine nor birch was significantly affected, when growing in mixtures under drier conditions, indicating that interspecific and intraspecific competition might have the same intensity in the more stressful environment. Similar to our experiment, Rewald and Leuschner (2009) found that drought seems to impact both species of their study in a similar manner with the consequence that species-specific differences in root growth in mixtures disappeared in the more stressful environment. Overall, the findings in my work agree with previous studies investigating interactions between tree mixing and changes in water availability, showing that differences in root growth among species are apparent only under comparatively high soil moisture conditions, but not under drier conditions. The general decline in fine root biomass of birch in mixture may be due to the competitive superiority of pine aboveground. Pine grew faster than birch (Morin et al. 2020) and had a larger basal area than birch, which implies larger water use and uptake for pine trees than for birch trees. As a result, pine would outcompete the relatively slower growing birch trees, leaving less available water in the soil. The latter finding is important to biodiversity research under the context of climate change, as it means that competition intensity belowground might increase under irrigated conditions but not under conditions of ambient water supply. In addition, the mixed stands never resulted in higher total fine root mass compared to their respective monocultures, which implies that water availability is not important for the total fine root

growth of the tree community. An alternative strategy for trees under reduced water availability might be to spatially segregate water and nutrient uptake instead of vertical root segregation or of an increase in fine root biomass for the whole soil column (Göransson et al. 2007; Grossiord et al. 2014; Kulmatiski et al. 2017).

### 6.1.3 Fine root morphology

Although the fine root morphology of entire root fragments ( $\leq 2$  mm  $\emptyset$ ) was not affected by species mixture, there was an interesting change in the diameter classes (see [Fig. B3.5](#) in Appendix B). It is well known that the very fine roots (0–0.5 mm  $\emptyset$ ) represented by the most distal root parts (1<sup>st</sup> and 2<sup>nd</sup> order roots) are most effective with regard to water uptake, and are more plastic in response to soil biotic and abiotic conditions (Ostonen et al. 2013, 2017). On the other hand, the basal root parts (when extending to 3<sup>rd</sup> – 5<sup>th</sup> or higher root orders or higher root orders with  $\leq 2$  mm  $\emptyset$ ) are mainly used to transport water. Pine growing in monoculture and mixture, and birch growing in mixture, tended to invest in higher fine root fractions of the very fine root diameter classes (0–0.5 mm  $\emptyset$ ) when left unirrigated. This is a key finding, since it shows that pine is adapting to drier conditions by assigning a larger fraction of its fine root system to function for uptake/exploitation of water and nutrients. An adaptation of the share between uptake and transport root parts may have occurred within the root system, and facilitated for water uptake from a drier soil and water transport within the pine roots. Consequently, pine roots are likely altering the soil moisture status, and consequently result in birch to adapt as well. Although we did not measure the morphology of root tips, I speculate that the underlying mechanisms for the observed changes in the relative abundance of different root orders may include: i) increased mortality/shedding of root tips, ii) regrowth of short roots (1<sup>st</sup> and 2<sup>nd</sup> order roots) with increased specific root length, for birch, and/or increased root tissue densities for pine (Ostonen et al. 2007b, a), iii) increased root tip diameter due to ectomycorrhizal mantle tissue). For example, the colonization with *Cenococcum geophilum*, which is very commonly observed on sandy dry soils (Pigott 1982), and also was commonly observed on both birch and pine roots of our study, could affect increase the average root tip diameter.

#### 6.1.4 Fine root mortality and mycorrhizal associations as adaptations to changing conditions

Beyond changes in vertical root distributions, and altered fine root morphology, differences in fine root dynamics (new growth and mortality) or modified mycorrhizal colonization are additional potential responses of tree root systems to changes in environmental conditions such as drought (Agerer 2001; Meinen et al. 2009a). Our results presented in Chapter 4, showed that fine root necromass was not significantly affected by irrigation or tree mixing (Fig 4.2). However, the fine root necromass collected in the ingrowth cores after two years of root growth was rather low accounting only 25% to total fine root mass. The amount of dead fine roots recovered in root ingrowth cores obviously depends on the time span between installation and harvest of ingrowth cores, and the proportion of live and dead roots may not have reached an equilibrium after two years (see Fig. C4.1 in Appendix C). For example, Andreasson et al. (2016) measured 10% dead roots two years after the installation of root ingrowth cores, but already 50–60% after three years. Therefore, we might have measured a more realistic percentage of fine root necromass after an additional year, which was unfortunately not possible with the tight schedule of a PhD thesis.

A preliminary analysis of the annual productivity of hyphal mass showed that mycorrhizal fungal biomass increased under irrigation, rather than fine root biomass and fine root morphology, that remained unchanged under irrigation, and this effect was apparently stronger for birch roots (see preliminary results in Appendix E, Fig. E6.1). This result partially agrees with an experiment combining irrigation with fertilization (Bakker et al. 2009), where irrigation combined with fertilization during the growing season resulted in both, higher fine root biomass in the top 30cm of soil, and higher hyphal mass, while fine root morphology was only marginally affected by irrigation. In a different experimental setup comparing drier sandy soils to wetter clay soils, Weemstra et al. (2017) also found that fine-root mass and mycorrhizal fungal biomass were correlated and responded similarly to the changes in environmental conditions (higher fine root biomass and fungal biomass in sandy soils compared to clay soils), but with only minor modifications in fine-root morphology. Assumingly, the mycorrhizal fungal biomass may respond more rapidly to environmental change (e.g. water supply) than fine root biomass and morphology. I note that these effects seem to be stronger for birch roots (look for instance at the pure birch plot in Fig. E6.1 in Appendix E), which already showed to be more plastic than pine when accounting for the whole soil column (adaptation of vertical root distribution, and fine root standing biomass; Chapter 3). It is likely that fine root biomass and mycorrhizal fungal biomass are correlated, but this remains untested for now. In addition to mycorrhizal fungal biomass, I also evaluated the effects of irrigation and tree species mixing on the ectomycorrhizal (ECM) colonization rate (See preliminary results in Appendix E, Fig. E6.2), and for the mycorrhizal exploration types on subsamples of the ingrowth cores (see preliminary results in Appendix E, Fig. E6.3). There were overall no clear effects on the rate of ECM root colonization with most root tips colonized at  $\geq 90\%$  with ECM. On the other hand, there were some interesting differences in exploration types. On birch tree roots, I found mostly the contact exploration type and some short- and long distance exploration types, while in pine tree roots the medium distance exploration

type dominated mostly at the expense of the long distance exploration type. This structural differentiation of the mycelia and their rhizomorphs could be of functional relevance (Agerer 2001). The surface area of the mantle and a few short hyphae (contact or short-distance exploration types) provide tight contact with the substrate, while on the contrary, longer hyphae (medium distance exploration type) or highly differentiated F-type rhizomorphs (long distance exploration type), producing vessel-like hyphae with partially or completely dissolved septa, are more relevant for long distance exploration. This preliminary evaluation indicates clear differences between the roots of birch and pine trees. On one hand, the dominance of contact- and short distance exploration types in birch (both exploration types together comprise 65–80 % of all exploration types) matches well its highly ramified and extensive root system allowing wide soil exploration and tight contact with soil and patches of decomposing organic matter. On the other hand, in pine trees the medium- and long distance exploration types are more abundant than in birch trees (both exploration types together comprise 50 % of all exploration types). The higher relative abundance of longer distance exploration types in pine than in birch roots may compensate for the less ramified pine roots, assuring sufficient soil exploration and mycorrhiza-mediated resource uptake.

## **6.2 Belowground carbon and nutrient cycling**

The combined assessment of the annual production rates of fine roots and their necromass and fine root decomposition rates, made it possible to estimate root-driven carbon and nutrient dynamics in response to tree species mixing, stand density, and soil water availability. The data I present in detail in Chapter 4, showed no tree species mixing and no irrigation effects on fine root productivity, necromass accumulation, and fine root decomposition, regardless of stand density or water availability. The lack of tree mixture effects on root decomposition was consistent in my thesis with similar results for root and leaf litter decomposition in natural beech forests studied in the Western French Alps (Chapter 5). A higher stand density on the other hand increased fine root production and decreased fine root turnover. It is important to consider that turnover is calculated as a ratio of fine root production / fine root standing biomass (Gill and Jackson 2000). In this case, stand density increased fine root production and root standing biomass (see [Table C4.1](#) in Appendix C), but the effect on root standing biomass was smaller than its influence on fine root production, thus yielding smaller turnover values. It might be that effects of stand density on fine root production are stronger (current growth dynamics) than for root standing biomass (incorporating both actual and previous growth), especially since they comprise of mostly new roots whose growth accounts for a major part of aboveground annual tree growth (Helmisaari et al. 2002). On the other hand, the fact that water availability did not have consistent effects on root decomposition in the two different experiments from the ORPHEE site (Chapter 4) and along the latitudinal gradient in the Alps (Chapter 5), may have different reasons, including methodological differences. Regular water supply from May to October in the ORPHEE tree-plantation experiment to avoid dry conditions during the summer (that occurred in the non-irrigated control plots), had no effect on root decomposition after two years (Chapter 4), while experimental rain exclusion to

simulate prolonged summer drought in the Alps from June to October, resulted in decreased root decomposition (Chapter 5). Both approaches accepted variation in natural rainfall in one of the treatments (the supposed drought treatment in ORPHEE and the control treatment in the Alps), which resulted in more or less marked differences in soil moisture between the treatments depending on the frequency and amount of rainfall. In case of abundant precipitation during the summer at ORPHEE, this would decrease the differences between the control and irrigated treatments, while rainfall events during the summer in the Alps would increase the differences in soil moisture between the two treatments. Under our experimental conditions, incident precipitation did not affect our treatments greatly. For instance, in ORPHEE our soil sensors showed 50–80% lower soil moisture percentage under ambient precipitation, compared to the irrigation treatment during the summer of 2019 (for the few available data of from soil sensors at ORPHEE please see [Fig. E6.4](#) in Appendix E). In the Alps, the rain-exclusion treatment reduced the soil moisture percentage by 16% to 79%, compared to subplots where rainfall was allowed during the summers of 2018 and 2019 (see [Fig. D5.2](#) in Appendix D). After removing the rain-exclusion tents in the Alps, we could see that the soil was clearly drier under the rain-exclusion tents, compared to the subplots where rain was allowed. The variable effect of the rain-exclusion treatment on the soil moisture reduction, might be due to the spatial replication within plots that was stronger in the Alps than at the ORPHEE site. For example, the designated subplots in the Alps, could at times be positioned at different small slopes within the same plot, or comprise of different levels of stones and gravel, that further influence the soil moisture measurement. On the opposite, in ORPHEE, the subplots had rather identical soil moisture conditions. Another important distinction is that in ORPHEE, water was applied via daily application of water ( $3\text{mm day}^{-1}$ ) with a sprinkler system, while in the Alps, the ambient precipitation that reaches the soil, was likely intercepted and reduced by the canopy composition. The root litterbags in ORPHEE were thus likely exposed to large and infrequent pulses of natural rainfall (starting as early as May), that may have accelerated root decomposition to the point that we no longer identify differences in root decomposition between the irrigation and the control treatment. For instance, Gaul et al. (2008) observed that rain exclusion of one month significantly decreased root decomposition, but that allowing roots to experience ambient water supply right after this drought period, led to similar root decomposition rates after four months. In the Alps, the root litterbags were kept at drier conditions, without soil rewetting, which probably explains the significant reduction of decomposition under the rain exclusion tent. The relation between water supply and fine root decomposition can thus not be clearly established from our experimental work, even though, there could be a relationship between water supply and root decomposition but for the lower range of water supply as demonstrated in the Alps.

## 6.2.1 Tree mixture effects on belowground nutrient cycling

Collectively, the results of fine root production, turnover and decomposition all indicated that nutrient cycling is not different in mixed tree species plantations compared to monocultures (Chapter 4), independently of differences in stand density or water availability. These results do not support the hypothesis of increasingly positive effects of tree species mixtures under increasing resource limitation, when complementary resource use among different species is thought to outweigh competitive interactions (Forrester and Bauhus 2016; Pretzsch et al. 2013). On the contrary, the birch–pine tree mixtures seem to be rather robust, as it pertains to belowground processes, and this robustness might be due to resource partitioning belowground. Although evidence for vertical root segregation was limited for these young stands, birch and pine trees possess different fine root morphologies (Fig. 3.1), which could indicate complementary resource uptake, which may reduce the competitive pressure belowground. Moreover, birch and pine trees differed considerably in the relative proportion of mycorrhizal exploration types (see preliminary results in Appendix E, Fig. E6.3), which may also increase complementarity.

Carbon and nutrient cycling are intrinsically connected, and thus, nutrient cycling is influenced by root carbon inputs via rhizodeposition and the decomposition of dead roots (root litter). Under our experimental conditions, nothing suggested that tree diversity had a significant effect on carbon and nutrient cycling through fine root tissues. Nevertheless, pine and birch differed substantially in fine root chemistry, which drove species-specific differences in carbon and nutrient cycling. In fact, I found that root P was released more rapidly for birch than for pine roots during decomposition. As the soils in ORPHEE are P-limited, I think that the P release is due to the root litter chemistry of birch roots as the initial C:P ratios in pine roots (603) were considerably higher compared to birch roots (416). As a result, the duration of the initial stage of decomposition (when P is immobilized) is probably longer for pine roots than for birch roots, which may explain why P release was higher from birch than from pine roots within the range of decomposition covered by my study. Also, in a previous study at ORPHEE, the authors observed that P-related enzymatic activity in the soil was higher for birch plots than for pine plots, possibly indicating that soil microorganisms under pine are more limited by P compared to microbial communities in pine soils (Maxwell et al. 2020). In turn, this higher P-related enzymatic activity may partially explain why P is released more rapidly during decomposition in birch plots but not in pine plots. Our result is in agreement with another study on P-limited soils in the tropics, that found that root quality (related to species identity) and the decomposition environment, but not tree species richness, drove root decomposition (Guerrero-Ramírez et al. 2016). This is a very important result, because it shows that birch trees may have a functional role if grown together with pine on P-limited soils, by increasing P availability to pine through decomposition of birch roots. Uptake of P originating from decomposing root tissues by roots of other plants is straightforward, especially when roots are intermingled and colonized by mycorrhizae (Johansen and Jensen 1996; Newman and Eason 1989). Also, taking into consideration that P is rather immobile compared to N for example (Achat et al. 2009), decomposing birch roots that release P in close vicinity of live pine roots, might be easier accessible compared to P from decomposing forest

floor litter. However, the average cumulative release of P from decomposing birch roots was rather small (only  $0.08 \text{ kg ha}^{-1}$  after two years of decomposition), compared to the potential P annual uptake rate of pine trees. For example, an 8-year-old pine stand took up  $1.98 \text{ kg P ha}^{-1} \text{ y}^{-1}$  (Gholz et al. 1985), and another 10–12 years-old-pine stand took up  $4 \text{ kg P ha}^{-1} \text{ y}^{-1}$  (Albaugh et al. 2008). This means that within two years, the decomposing birch roots may supply with approximately 1–2 % of the P uptake of pine. At the best case, the latter approximation would double (2–4 %), if we take into account the rather low necromass values observed in our study (25% of necromass, compared to values of around 50% occurring naturally outside of ingrowth cores). At the same time, my calculations based on the litterbags probably underestimate total P release from all decomposing roots. I measured only the decomposition of freshly dead birch and pine roots of one single annual cohort, which is not representative for all the cohorts in different decomposition stages decomposing simultaneously. Moreover, my estimation for the total fine root P release was based on the necromass measured for the 0–15 cm soil layer. This necromass value would be higher if I would also take into account the necromass of deeper soil layers (e.g. 0–90 cm). Alternatively, one could estimate the amount of P returning to the soil by dead roots by using the fine root turnover values (Chapter 4) and the root standing biomass for the whole soil column (0–90 cm of soil; Chapter 3). The estimation of the amounts of P deposited this way would range from  $1.13 \text{ kg P ha yr}^{-1}$  (birch and pine mixtures), to  $1.87$  (pure birch stands) to  $2.27$  (pure pine stands). The total root population (including all root cohorts from the different years) produces a total flux of released P that is likely even higher, but close to the amount of P that turns over ( $1.13$ – $1.87 \text{ kg P ha}^{-1} \text{ y}^{-1}$  depending on the plot), than the very small P released from the new dead roots of the 0–15 cm soil layer that decomposed for only two years ( $0.08 \text{ kg ha}^{-1}$ ). Also, the values of P deposited annually via the turnover of roots, are about 25–50 % lower than plot averages of annual deposition of P from leaf litterfall during the year of 2019 (pure birch plots:  $2.23 \text{ kg P ha}^{-1} \text{ y}^{-1}$ , pure pine plots:  $5.2 \text{ kg P ha}^{-1} \text{ y}^{-1}$ , birch and pine mixed plots:  $4.08 \text{ kg P ha}^{-1} \text{ y}^{-1}$ ) (data not shown). A rough calculation for the ORPHEE site, based on Augusto et al. (2010), indicates of a P pool of about  $2 \text{ kg ha}^{-1}$  in the forest floor, and close to  $800 \text{ kg ha}^{-1}$  for the total soil profile (0–90 cm). Collectively, this means that fine roots are an important contributor to the larger pool of P in the soil. From my research, I can infer only a very small portion of the P that is released from the larger soil pool, as the portion I calculated is limited to the early stages of decomposition and to only a small fraction of the total root necromass that exists in the soil.

Nonetheless, the fact that neither water supply nor the intensity of interspecific competition influenced mixture effects on fine root dynamics implies that fine root dynamics in young plantations of birch and pine are relatively insensitive to changes in water availability, at least within the range manipulated in my study, and to competitive pressure by neighbours. At the same time, the species-specific effects on P release imply that admixing birch trees to pine stands could improve P dynamics and P availability belowground through the decomposition of birch fine roots. Although the release of P from birch roots may be rather small compared to the total soil P pool, it may provide an additional source of P for pine, especially since the rest of the P is strongly bound to the soil phase. Simulations on the future yield of the ORPHEE tree stands predict that pines will overgrow birches in the future (Morin et al. 2020), and this

is partly visible presently, by the higher stand basal area of pines compared to birch trees (Table 3.1).

### **6.3 Root versus leaf litter decomposition**

As primary producers, plants feed terrestrial food webs, both above- and belowground. Litter quality, environmental conditions and decomposer organisms are the main control factors of litter decomposition (Bradford et al. 2016; Swift; et al. 1979). Decomposition rates and the fate of carbon and nutrients may differ between leaf- and root litter. In natural ecosystems, plants return leaves back to the forest floor, where they are left to decompose within their own layer of leaves or sometimes within the upper soil horizons due to physical transfer by earthworms for example. Roots, however, die and remain at the same spot within the soil, and experience a different decomposition environment than leaves. The difference in environment is both abiotic (humidity, temperature, oxygen), and biotic (difference in microbial community and detritivorous fauna). Microclimatic fluctuations are typically more pronounced in the litter layer than in the soil and ongoing climate change could reinforce these differences with potentially stronger impacts on leaf litter than root litter decomposition. Tree species diversity can also have important effects on the decomposition environment through differences in tree canopy structure that can determine microclimatic conditions (soil temperature, evapotranspiration and moisture conditions, via shading, interception of precipitation and wind break) in the litter layer and soil. Moreover, changing tree diversity has a direct impact on decomposition through the production of litter of distinct chemical and physical properties. While diversity effects on decomposition are reasonably well understood for leaf litter (Gessner et al. 2010; Hättenschwiler et al. 2005; Kou et al. 2020), there are only few studies addressing root decomposition, despite the fact that root litter can account for a substantial part of litter inputs in natural forests (Jackson et al. 1997). Estimations of carbon- and nutrient-cycling in mixed forests under climate change can be improved by insights on local decomposition effects driven by microclimate, and manipulated prolonged summer drought. An additional improvement would be to compare leaf and root decomposition and their related nutrient fluxes during decomposition.

#### **6.3.1 Comparison between roots and leaves**

The comparison of decomposition between roots and leaves (Chapter 5) showed that both tissue types decompose more slowly with reinforced and prolonged summer droughts. Regardless of any treatment effects, fine roots decomposed slower than leaf litter. However, despite slower mass loss of fine roots they showed a higher net N release compared to leaf litter that still was largely in the N immobilization stage during the two years of exposure in the field. Fine roots represent a large nutrient pool (Jackson et al. 1997), however, due to their higher recalcitrance than leaves, their contribution to nutrient cycling is considered of comparatively smaller importance (Berg and McLaugherty 2008). Although severe summer drought negatively affected the decomposition of both litter types, the distinction made for C, and N release in this Thesis, suggests another way of viewing roots, in particular that fine root litter may



represent a specific pool of N that may sustain N supply to the soil at an early stage of decomposition, while N from leaf litter seems to be grossly immobilized.

At the beginning of litter decomposition, nitrogen and phosphorus tend to be immobilized (i.e., both C:N and C:P critical ratios at which respective net N and P mineralisation commence, are lower than the initial litter ratios; see Ågren et al. (2013)), mostly because microbial colonization of the litter is followed by a net increase in nutrients due to their lower biomass C:nutrient stoichiometry compared to litter (Berg and McClaugherty 2008). Hence, the nutrient immobilization which is usually observed during the initial stage of decomposition is due to increasing microbial biomass colonizing the litter. In Chapter 4, I showed that birch and pine roots generally reached net N release after 2 years of decomposition, and the same was observed for beech roots in Chapter 5, while beech leaves still showed net N immobilization. Our results differ from a root/leaf litter comparison in the tropics, which showed net N immobilization after 12 months for both roots and leaves, but to be higher for roots, even though this was partly explained by the lower lignin:N ration in roots (Ostertag and Hobbie 1999). Generally, most studies showed rapid net N release for decomposing roots, or at least net N immobilization was less frequent in roots than in leaves where net N immobilization is rather common (Fornara et al. 2009; Parton et al. 2007; Seastedt et al. 1992). The reason for this discrepancy is usually attributed to chemical stoichiometry, since leaves may have higher C:N ratios than roots (Parton et al. 2007, Sun et al. 2018), which agrees with our higher leaf C:N ratios compared to those measured in roots. A complementary explanation can be that the decomposing roots experience different moisture conditions, and different microbial communities belowground, than leaves which decompose in the leaf litter layer (Gallardo and Schlesinger 1994; Grayston and Prescott 2005). Indeed, the leaf litter layer is characterized by a more fluctuating decomposition environment with larger amplitudes and more pronounced extreme values in temperature and moisture than the soil. Mineralized N might act as an exogenous N-addition, that accelerates litter decomposition, but at the same is likely immobilized by microbes and converted into microbial biomass or exo-enzymes (Frey et al. 2000). Patterns in net N release and immobilization are thus dependent upon the relative C:N ratios of the decomposer organisms and that of litter, as well as N availability in the decomposition environment. An important finding of this thesis is that beech root litter more rapidly enters the decomposition stage of net N release than beech leaf litter making N available from an earlier stage of decomposition. However, it will be important to follow N dynamics of decomposing beech leaves and roots over a longer time period covering also late decomposition stages to get a more complete picture of N dynamics during decomposition in these beech forests.

### **6.3.2 The contribution of macrofauna to decomposition**

The inclusion of detritivores increased the release of C from beech leaves, but resulted in higher N immobilization compared to leaf litter without access of detritivorous fauna (Chapter 5). It is noteworthy that the N dynamics in leaf litter varied much more when macrofauna had access, compared to when they were excluded. This result is opposite to other studies that show that including macrofauna lead to increased net N

mineralization (Berg et al. 2001; Carrillo et al. 2011; Schröter et al. 2003). It has been suggested that fauna can make litter and the products of its physical and chemical degradation more available to soil microbes, through the transformation into faeces and altering litter chemistry which accelerates decomposition, or by chopping it into smaller pieces that increase the litter surface area available for microbes to decompose (Hättenschwiler 2005; Joly et al. 2020). In our case, fauna inclusion did not consistently lead to N net mineralization, but overall increased variability with a mean net N immobilization. This increased variability exists probably because macrofauna occurrence, abundance and activity varies much more in space and time than that of microbes (Fujii et al. 2020; Smith and Bradford 2003). Another important factor might be related to the choice of the subplots within each plot. These subplots were chosen randomly and were sometimes placed to very different locations within each plot, with sometimes varying slopes, or different percentages of stones and gravel (for instance the Lubéron site). For instance in a study of beech forests, invertebrates were more abundant in micro-sites located at the base of slopes – where nutrients and water accumulate – than in micro-sites located on the slopes – which, due to surface runoff, are drier and accumulate less nutrients (Melguizo-Ruiz et al. 2012). As a result, the influence of macrofauna might vary even within the studied plots, and this variation may be higher than for microbes, since the more mobile macrofauna, such as millipedes or isopods, may be spatially structured at relatively small scales of 10–30 m (Ettema and Wardle 2002). Consequently, the presence of macrofauna tended to homogenize differences among sites that were more expressed when only microbes had access.

## **6.4 Concluding remarks & recommendations**

In this thesis, I studied the processes that drive belowground productivity and decomposition across climatic gradients and how these interact with tree diversity. To that end, this thesis specifically examined across- and within-species variation in tree root functional traits, and related these fine root traits to nutrient cycling. Functional traits are the manifestation of evolutionary and temporal (plasticity) adaptation to stresses and disturbances in the plant's environment. I studied whether differences in root growth strategies of trees growing in mixture, compared to monocultures, can attenuate negative effects of reduced water availability, and subsequent effects on decomposition and overall ecosystem functioning. Hereby, this thesis contributes to the scientific understanding of the belowground carbon and nutrient cycling of mixed forests under the context of climate change. The comparison between mixed and pure forests, and the inclusion of water availability as a controlling factor of positive diversity effects, bridges a gap in our general understanding of ecosystem functioning in resource-limited environments. Where many other studies focused on intra- and inter-specific adaptations, and decomposition of aboveground parts, I dedicated the largest part of this thesis on roots. My work did not only provide answers, the results from this thesis also raised new questions that require scientific attention in future work.

The tree mixtures investigated in this study did not lead to vertical root segregation under the naturally drier conditions of southwestern France and did not produce more

root biomass compared to their respective monocultures. However, both tree species responded marginally to increased water supply during the growing season, with birch being the most plastic. Tree diversity and water supply did not affect the inputs of fine root necromass, and its decomposition rates, but there was an important species-dependency on mineralization, as pine roots retained P, while birch roots released P during root decomposition. Prolonged summer drought decreased the C release from both leaf and root decomposition in beech forests. When comparing between leaves and roots, there was faster decomposition of leaf litter than root litter that did not match with N-mineralization rates. There was net immobilization of N during beech leaf decomposition, while there was net N mineralization during beech root decomposition. The inclusion of macrofauna increased the release of C from beech leaves, but resulted in higher N immobilization compared to leaf litter without access of macrofauna. It is noteworthy that the inclusion of macrofauna to leaf litter decomposition gave variable results of N dynamics. A species-specific approach that takes into account soil fertility and the microbial community, can be helpful in studying the role of roots as N- and P- suppliers, and it is worthwhile to extend this to other (mineral) nutrients. My study does not allow me to make generalizations on the role of roots in nutrient cycling as I studied only one time point and at an early stage of decomposition. In addition, these results should be taken with a grain of salt, as although the difference between N release rates and N immobilization rates resulted in net N immobilization during leaf decomposition, there is still N being released during the initial stages of decomposition. Even more importantly, I followed the decomposition of only one small cohort of roots and leaves, while in reality there is probably a lot of nutrients being released by older and at a later decomposition stage of roots and leaves. Finally, I cannot generalize for all tree species and for all site conditions, as for example I investigated only pine, birch, and beech root decomposition, but not pine, birch leaf decomposition, and not fir, and oak decomposition.

In conclusion, I found that mixing tree species did not attenuate negative effects of climate change. Nonetheless, a certain plasticity in response to neighbouring tree species occurs, and may also contribute to complementarity effects when tree stands reach maturity. In addition, the role of root tips and mycorrhizal associations, as a more dynamic part of the root system should be better explored, as it gave another level of complementarity in our study. Root- and leaf litter decomposition were similarly affected by severe drought conditions, with negative implications of climate change on the carbon and nutrient cycling of forests. Interestingly, the effects on litter decomposition did not match nutrient mineralization, and were shown to be dependent on litter type, tree species, and soil fertility, although I underline that no major conclusions can be made at the early stage of decomposition and without taking into account the larger pool of older roots and leaves that are at a later decomposition stage in the soil. This thesis demonstrates that promoting mixtures could still be beneficial for at least one of the admixed tree species, through species addition (i.e., complementing one tree species with another tree species), as one tree species may facilitate another via release of N and P during the early stage of root decomposition. My results imply that longer lasting studies should be designed as well, when comparing root and leaf nutrient release.

## References

- Achat DL, Bakker MR, Augusto L, et al (2009) Evaluation of the phosphorus status of P-deficient podzols in temperate pine stands: Combining isotopic dilution and extraction methods. *Biogeochemistry* 92:183–200.  
<https://doi.org/10.1007/s10533-008-9283-7>
- Agerer R (2001) Exploration types of ectomycorrhizae: A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107–114.  
<https://doi.org/10.1007/s005720100108>
- Ågren GI, Hyvönen R, Berglund SL, Hobbie SE (2013) Estimating the critical N: C from litter decomposition data and its relation to soil organic matter stoichiometry. *Soil Biol Biochem* 67:312–318. <https://doi.org/10.1016/j.soilbio.2013.09.010>
- Albaugh TJ, Allen HL, Fox TR (2008) Nutrient use and uptake in *Pinus taeda*. In: *Tree Physiology*. Heron Publishing, pp 1083–1098
- Andreasson F, Gonzalez M, Augusto L, Bakker MR (2016) Comparison of ingrowth cores and ingrowth meshes in root studies: 3 years of data on *Pinus pinaster* and its understory. *Trees - Struct Funct* 30:555–570. <https://doi.org/10.1007/s00468-015-1256-6>
- Augusto L, Bakker MR, Morel C, et al (2010) Is “grey literature” a reliable source of data to characterize soils at the scale of a region? A case study in a maritime pine forest in southwestern France. *Eur J Soil Sci* 61:807–822.  
<https://doi.org/10.1111/j.1365-2389.2010.01286.x>
- Bakker MR, Augusto L, Achat DL (2006) Fine root distribution of trees and understory in mature stands of maritime pine (*Pinus pinaster*) on dry and humid sites. *Plant Soil* 286:37–51. <https://doi.org/10.1007/s11104-006-9024-4>
- Bakker MR, Jolicoeur E, Trichet P, et al (2009) Adaptation of fine roots to annual fertilization and irrigation in a 13-year-old *Pinus pinaster* stand. *Tree Physiol* 29:229–238. <https://doi.org/10.1093/treephys/tpn020>
- Bardgett RD, Mommer L, De Vries FT (2014) Going underground: Root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* 29:692–699

- Berg B, McClaugherty C (2008) Berg, B. & McClaugherty, C. Plant Litter: Decomposition, Humus Formation, Carbon Sequestration (Springer, 2008). Springer-Verlag Berlin Heidelberg
- Berg M, De Ruiter P, Didden W, et al (2001) Community food web, decomposition and nitrogen mineralisation in a stratified scots pine forest soil. In: *Oikos*. pp 130–142
- Bolte A, Villanueva I (2006) Interspecific competition impacts on the morphology and distribution of fine roots in European beech (*fagus sylvatica* L.) and Norway spruce (*picea abies* (L.) karst.). *Eur J For Res* 125:15–26.  
<https://doi.org/10.1007/s10342-005-0075-5>
- Bradford MA, Berg B, Maynard DS, et al (2016) Understanding the dominant controls on litter decomposition. *J Ecol* 104:229–238. <https://doi.org/10.1111/1365-2745.12507>
- Brassard BW, Chen HYHH, Bergeron Y, Paré D (2011) Differences in fine root productivity between mixed- and single-species stands. *Funct Ecol* 25:238–246.  
<https://doi.org/10.1111/j.1365-2435.2010.01769.x>
- Brassard BW, Chen HYHH, Cavard X, et al (2013) Tree species diversity increases fine root productivity through increased soil volume filling. *J Ecol* 101:210–219.  
<https://doi.org/10.1111/1365-2745.12023>
- Brunner I, Godbold DL (2007) Tree roots in a changing world. *J For Res* 12:78–82.  
<https://doi.org/10.1007/s10310-006-0261-4>
- Brunner I, Herzog C, Dawes MA, et al (2015) How tree roots respond to drought. *Front Plant Sci* 6:1–16. <https://doi.org/10.3389/fpls.2015.00547>
- Cardinale BJ, Matulich KL, Hooper DU, et al (2011) The functional role of producer diversity in ecosystems. *Am J Bot* 98:572–592.  
<https://doi.org/10.3732/ajb.1000364>
- Carrillo Y, Ball BA, Bradford MA, et al (2011) Soil fauna alter the effects of litter composition on nitrogen cycling in a mineral soil. *Soil Biol Biochem* 43:1440–1449. <https://doi.org/10.1016/j.soilbio.2011.03.011>

- Curt T, Prévosto B (2003) Rooting strategy of naturally regenerated beech in Silver birch and Scots pine woodlands. *Plant Soil* 255:265–279.  
<https://doi.org/10.1023/A:1026132021506>
- Ettema CH, Wardle DA (2002) Spatial soil ecology. *Trends Ecol. Evol.* 17:177–183
- Fornara DA, Tilman D, Hobbie SE (2009) Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. *J Ecol* 97:48–56. <https://doi.org/10.1111/j.1365-2745.2008.01453.x>
- Forrester DI, Bauhus J (2016) A Review of Processes Behind Diversity—Productivity Relationships in Forests. *Curr. For. Reports* 2:45–61
- Frey SD, Elliott ET, Paustian K, Peterson GA (2000) Fungal translocation as a mechanism for soil nitrogen inputs to surface residue decomposition in a no-tillage agroecosystem. *Soil Biol Biochem* 32:689–698.  
[https://doi.org/10.1016/S0038-0717\(99\)00205-9](https://doi.org/10.1016/S0038-0717(99)00205-9)
- Fruleux A, Bogeat-Triboulot MB, Collet C, et al (2018) Aboveground overyielding in a mixed temperate forest is not explained by belowground processes. *Oecologia* 188:1183–1193. <https://doi.org/10.1007/s00442-018-4278-0>
- Fujii S, Berg MP, Cornelissen JHC (2020) Living Litter: Dynamic Trait Spectra Predict Fauna Composition. *Trends Ecol. Evol.* 35:886–896
- Gallardo A, Schlesinger WH (1994) Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biol Biochem* 26:1409–1415. [https://doi.org/10.1016/0038-0717\(94\)90225-9](https://doi.org/10.1016/0038-0717(94)90225-9)
- Gamfeldt L, Snäll T, Bagchi R, et al (2013) Higher levels of multiple ecosystem services are found in forests with more tree species. *Nat Commun* 4:.  
<https://doi.org/10.1038/ncomms2328>
- Gaul D, Hertel D, Borken W, et al (2008) Effects of experimental drought on the fine root system of mature Norway spruce. *For Ecol Manage* 256:1151–1159.  
<https://doi.org/10.1016/j.foreco.2008.06.016>
- Gessner MO, Swan CM, Dang CK, et al (2010) Diversity meets decomposition. *Trends Ecol. Evol.* 25:372–380

- Gholz HL, Fisher RF, Pritchett WL (1985) Nutrient dynamics in slash pine plantation ecosystems. *Ecology* 66:647–659. <https://doi.org/10.2307/1940526>
- Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *Res New Phytol* 147:13–31.  
<https://doi.org/https://doi.org/10.1046/j.1469-8137.2000.00681.x>
- Göransson H, Fransson AM, Jönsson-Belyazid U (2007) Do oaks have different strategies for uptake of N, K and P depending on soil depth? *Plant Soil* 297:119–125. <https://doi.org/10.1007/s11104-007-9325-2>
- Grayston SJ, Prescott CE (2005) Microbial communities in forest floors under four tree species in coastal British Columbia. *Soil Biol Biochem* 37:1157–1167.  
<https://doi.org/10.1016/j.soilbio.2004.11.014>
- Grossiord C, Gessler A, Granier A, et al (2014) Impact of interspecific interactions on the soil water uptake depth in a young temperate mixed species plantation. *J Hydrol* 519:3511–3519. <https://doi.org/10.1016/j.jhydrol.2014.11.011>
- Guerrero-Ramírez NR, Craven D, Messier C, et al (2016) Root quality and decomposition environment, but not tree species richness, drive root decomposition in tropical forests. *Plant Soil* 404:125–139.  
<https://doi.org/10.1007/s11104-016-2828-y>
- Harpole WS, Sullivan LL, Lind EM, et al (2016) Addition of multiple limiting resources reduces grassland diversity. *Nature* 537:93–96.  
<https://doi.org/10.1038/nature19324>
- Hättenschwiler S (2005) Effects of Tree Species Diversity on Litter Quality and Decomposition. In: *Forest Diversity and Function*. pp 149–164
- Hättenschwiler S, Tiunov A V., Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annu Rev Ecol Evol Syst* 36:191–218.  
<https://doi.org/10.1146/annurev.ecolsys.36.112904.151932>
- Helmisaari HS, Makkonen K, Kellomäki S, et al (2002) Below- and above-ground biomass, production and nitrogen use in Scots pine stands in eastern Finland. *For Ecol Manage* 165:317–326. [https://doi.org/10.1016/S0378-1127\(01\)00648-X](https://doi.org/10.1016/S0378-1127(01)00648-X)

- Hertel D, Leuschner C (2006) The in situ root chamber: A novel tool for the experimental analysis of root competition in forest soils. *Pedobiologia (Jena)* 50:217–224. <https://doi.org/10.1016/j.pedobi.2005.11.002>
- Hertel D, Strecker T, Müller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient - Is optimal resource partitioning theory applicable to water-limited mature trees? *J Ecol* 101:1183–1200. <https://doi.org/10.1111/1365-2745.12124>
- Hooper DU, Chapin FS, Ewel JJ, et al (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecol Monogr* 75:3–35. <https://doi.org/10.1890/04-0922>
- Jackson RB, Mooney HA, Schulze E-DD (1997) A global budget for fine root biomass, surface area, and nutrient contents. *Proc Natl Acad Sci U S A* 94:7362–7366. <https://doi.org/10.1073/pnas.94.14.7362>
- Johansen A, Jensen ES (1996) Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biol Biochem* 28:73–81. [https://doi.org/10.1016/0038-0717\(95\)00117-4](https://doi.org/10.1016/0038-0717(95)00117-4)
- Joly FX, Coq S, Coulis M, et al (2020) Detritivore conversion of litter into faeces accelerates organic matter turnover. *Commun Biol* 3:1–9. <https://doi.org/10.1038/s42003-020-01392-4>
- Kou L, Jiang L, Hättenschwiler S, et al (2020) Diversity-decomposition relationships in forests worldwide. *Elife* 9:1–51. <https://doi.org/10.7554/eLife.55813>
- Kulmatiski A, Adler PB, Stark JM, Tredennick AT (2017) Water and nitrogen uptake are better associated with resource availability than root biomass. *Ecosphere* 8:e01738. <https://doi.org/10.1002/ecs2.1738>
- Maxwell TL, Augusto L, Bon L, et al (2020) Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest. *Soil Biol Biochem* 148:107864. <https://doi.org/10.1016/j.soilbio.2020.107864>
- Meier IC, Leuschner C (2008) Belowground drought response of European beech: Fine root biomass and carbon partitioning in 14 mature stands across a



- precipitation gradient. *Glob Chang Biol* 14:2081–2095.  
<https://doi.org/10.1111/j.1365-2486.2008.01634.x>
- Meinen C, Hertel D, Leuschner C (2009a) Root growth and recovery in temperate broad-leaved forest stands differing in tree species diversity. *Ecosystems* 12:1103–1116. <https://doi.org/10.1007/s10021-009-9271-3>
- Meinen C, Hertel D, Leuschner C (2009b) Biomass and morphology of fine roots in temperate broad-leaved forests differing in tree species diversity: Is there evidence of below-ground overyielding? *Oecologia* 161:99–111.  
<https://doi.org/10.1007/s00442-009-1352-7>
- Melguizo-Ruiz N, Verdeny-Vilalta O, Arnedo MA, Moya-Laraño J (2012) Potential drivers of spatial structure of leaf-litter food webs in south-western European beech forests. *Pedobiologia (Jena)* 55:311–319.  
<https://doi.org/10.1016/j.pedobi.2012.06.003>
- Morin X, Damestoy T, Toigo M, et al (2020) Using forest gap models and experimental data to explore long-term effects of tree diversity on the productivity of mixed planted forests. *Ann For Sci* 77:1–19. <https://doi.org/10.1007/s13595-020-00954-0>
- Newman EI, Eason WR (1989) Cycling of nutrients from dying roots to living plants, including the role of mycorrhizas. *Plant Soil* 115:211–215.  
<https://doi.org/10.1007/BF02202589>
- Ostertag R, Hobbie SE (1999) Early stages of root and leaf decomposition in Hawaiian forests: Effects of nutrient availability. *Oecologia* 121:564–573.  
<https://doi.org/10.1007/s004420050963>
- Ostonen I, Lohmus K, Helmisaari H-S, et al (2007a) Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. *Tree Physiol* 27:1627–1634.  
<https://doi.org/10.1093/treephys/27.11.1627>
- Ostonen I, Püttsepp Ü, Biel C, et al (2007b) Specific root length as an indicator of environmental change. *Plant Biosyst.* 141:426–442

- Ostonen I, Rosenvald K, Helmisaari HS, et al (2013) Morphological plasticity of ectomycorrhizal short roots in *Betula* sp and *Picea abies* forests across climate and forest succession gradients: Its role in changing environments. *Front Plant Sci* 4:335. <https://doi.org/10.3389/fpls.2013.00335>
- Ostonen I, Truu M, Helmisaari HS, et al (2017) Adaptive root foraging strategies along a boreal–temperate forest gradient. *New Phytol* 215:977–991. <https://doi.org/10.1111/nph.14643>
- Pardos M, del Río M, Pretzsch H, et al (2021) The greater resilience of mixed forests to drought mainly depends on their composition: Analysis along a climate gradient across Europe. *For Ecol Manage* 481:118687. <https://doi.org/10.1016/j.foreco.2020.118687>
- Parton W, Silver WL, Burke IC, et al (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* (80- ) 315:361–364. <https://doi.org/10.1126/science.1134853>
- Persson H, Von Fircks Y, Majdi H, Nilsson LO (1995) Root distribution in a Norway spruce (*Picea abies* (L.) Karst.) stand subjected to drought and ammonium-sulphate application. *Plant Soil* 168–169:161–165. <https://doi.org/10.1007/BF00029324>
- Pigott CD (1982) Survival of mycorrhiza formed by *cenococcum geophilum* fr. in dry soils. *New Phytol* 92:513–517. <https://doi.org/10.1111/j.1469-8137.1982.tb03409.x>
- Pretzsch H, Schütze G, Uhl E (2013) Resistance of European tree species to drought stress in mixed versus pure forests: Evidence of stress release by inter-specific facilitation. *Plant Biol* 15:483–495. <https://doi.org/10.1111/j.1438-8677.2012.00670.x>
- Ratcliffe S, Liebergesell M, Ruiz-Benito P, et al (2016) Modes of functional biodiversity control on tree productivity across the European continent. *Glob Ecol Biogeogr* 25:251–262. <https://doi.org/10.1111/geb.12406>
- Ratcliffe S, Wirth C, Jucker T, et al (2017) Biodiversity and ecosystem functioning relations in European forests depend on environmental context. *Ecol. Lett.* 20:1414–1426

- Rewald B, Leuschner C (2009) Does root competition asymmetry increase with water availability? *Plant Ecol Divers* 2:255–264.  
<https://doi.org/10.1080/17550870903022865>
- Rewald B, Leuschner C, Wiesman Z, Ephrath JE (2011) Influence of salinity on root hydraulic properties of three olive varieties. *Plant Biosyst* 145:12–22.  
<https://doi.org/10.1080/11263504.2010.514130>
- Schmid I, Kazda M (2002) Root distribution of Norway spruce in monospecific and mixed stands on different soils. *For Ecol Manage* 159:37–47.  
[https://doi.org/10.1016/S0378-1127\(01\)00708-3](https://doi.org/10.1016/S0378-1127(01)00708-3)
- Schröter D, Wolters V, De Ruiter PC (2003) C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos* 102:294–308.  
<https://doi.org/10.1034/j.1600-0579.2003.12064.x>
- Seastedt TR, Parton WJ, Ojima DS (1992) Mass loss and nitrogen dynamics of decaying litter of grasslands: the apparent low nitrogen immobilization potential of root detritus. *Can J Bot* 70:384–391. <https://doi.org/10.1139/b92-052>
- Smith VC, Bradford MA (2003) Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. *Appl Soil Ecol* 24:197–203. [https://doi.org/10.1016/S0929-1393\(03\)00094-5](https://doi.org/10.1016/S0929-1393(03)00094-5)
- Swift MJ, Heal OW, Anderson JM (1979) *Decomposition in terrestrial ecosystems*, Volume 5. University of California Press
- Weemstra M, Sterck FJ, Visser EJW, et al (2017) Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. *Plant Soil* 415:175–188. <https://doi.org/10.1007/s11104-016-3148-y>

## Appendix A | Materials and Methods

**Table A2. 1** Overview of the ingrowth core and hyphal bag installation per plot. Each plot code is replicated for eight blocks, for a total of 56 plots. A total of eight ingrowth cores and their hyphal bags are installed per plot, and divided among 4 subplots.

Code	Treatment	Blocks	Subplot	Harvests (1st, 2nd yr)	<i>Replicate nr</i>		
					Ingrowth cores	Hyphal bags*	Hyphal turnover (1 yr)
1	Birch	8	4	2	64	192	32
2	Oak	8	4	2	64	192	32
5	Pine	8	4	2	64	192	32
6	Birch x Oak	8	4	2	64	192	32
9	Birch x Pine	8	4	2	64	192	32
12	Oak x Pine	8	4	2	64	192	32
18	Birch x Oak x Pine	8	4	2	64	192	32
<u>Total plots: 56</u>				<u>Total:</u>	<u>448</u>	<u>1344</u>	<u>224</u>

\* Three hyphal bags were installed next to each ingrowth core

**Table A2. 2** Overview of the of the ingrowth core and hyphal bag installation installation in the Alps, per site. Each site comprises of two altitudes (low and high elevation). Each altitude has a triplet of plots, and each plot comprises of 4 subplots (two with rain exclusion and two without).

Site	Altitude (m)		Subplots		Harvests (1st, 2nd yr)		Replicate nr			
							Ingrowth cores		Hyphal bags	
							Rain excluded	Rain allowed	Rain excluded	Rain allowed
	Low	High								
Bauges	Beech			4	2	0	8	0	24	
	Fir	1017	1236	4	2	0	8	0	24	
	Beech x			4	2	0	8	0	24	
	Fir									
Vercors	Beech			1	4	2	8	8	24	
	Fir	1131–	1350–	1	4	2	8	8	24	
	Beech x	1254	1416	1	4	2	8	8	24	
	Fir									
Mont Ventoux	Beech			1	4	2	8	8	24	
	Fir	1265	1297–	1	4	2	8	8	24	
	Beech x		1352	1	4	2	8	8	24	
	Fir									
Grand Lubéron	Beech			1	4	2	8	8	24	
	Oak	821–	972	1	4	2	8	8	24	
	Beech x	882		1	4	2	8	8	24	
	Oak									
Ste Baume	Beech			1	4	2	8	8	24	
	Oak	719	748	1	4	2	8	8	24	
	Beech x			1	4	2	8	8	24	
	Oak									
	<u>Total plots: 24</u>				<u>Total:</u>		<u>48</u>	<u>240</u>	<u>144</u>	<u>720</u>

\* three hyphal bags were installed next to each ingrowth core

**Table A2. 3** Overview of the vertical root profiles per plot. Each plot code is replicated for eight blocks, for a total of 32 plots. A total of four soil columns were harvested per plot, and further divided to six layers.

Code	Treatment	Blocks	Sampling points	Soil Layers
1	Birch	8	4	6
5	Pine	8	4	6
9	Birch x Pine	8	4	6
18	Birch x Oak x Pine	8	4	6
	<u>Total plots: 32</u>		<u>Total samples: 768</u>	

**Table A2. 4** Overview of the litterbag installation in the experimental site of ORPHEE, and divided for two litterbag types: a) root litterbags (mesh of 48µm Ø), b) leaf litterbags (mesh of 48µm Ø). Each plot code represents a different tree species composition, which can be monospecific or a mixture, and is replicated for 8 blocks, for a total of 56 plots. Standardized litter, which corresponds to the tree species composition of each plot, was placed in pairs of three litterbags.

a)

Code	Treatment	Blocks	Subplot	Harvests (1, 2, 3 yrs)	Monospecific litter								Mixed litter						
					Birch	Oak	Pine	Birch x Oak	Birch x Pine	Oak x Pine	Birch x Oak x Pine								
1	Birch	8	2	3	48													Birch x Oak x Pine	
2	Oak	8	2	3		48													
5	Pine	8	2	3			48												
6	Birch x Oak	8	2	3	48	48		48											
9	Birch x Pine	8	2	3	48		48			48									
12	Oak x Pine	8	2	3		48	48					48							
18	Birch x Oak x Pine	8	2	3	48	48	48											48	
<u>Total plots: 56</u>															<u>Total root litterbags: 768</u>				

b)

Code	Treatment	Blocks	Subplot	Harvests (1, 2, 3 yrs)	Monospecific litter			Mixed litter											
					Birch	Oak	Pine	Birch x Oak	Birch x Pine	Oak x Pine	Birch x Oak x Pine								
1	Birch	8	2	3	48													Birch x Oak x Pine	
2	Oak	8	2	3		48													
5	Pine	8	2	3			48												
6	Birch x Oak	8	2	3	48	48		48											
9	Birch x Pine	8	2	3	48		48			48									
12	Oak x Pine	8	2	3		48	48					48							
18	Birch x Oak x Pine	8	2	3	48	48	48											48	
<u>Total plots: 56</u>															<u>Total leaf litterbags: 768</u>				

Table A2. 5 Overview of the litterbag installation in the Alpes, per site, and divided for three litterbag types: a) root litterbags (mesh of 48µm Ø), b) leaf litterbags (mesh of 48µm Ø), c) leaf litterbags (mesh of 2mm Ø). Each site comprises of two altitudes (low and high elevation). Each altitude has a triplet of plots, and each plot comprises of 4 subplots (two with rain exclusion and two without). Site-specific litter, which corresponds to the tree species composition of each plot, is placed in pairs of three litterbags.

Site	Altitude (m)		Subplots		Harvests (1st, 2nd, 3rd yr)	Replicate nr				
	Low	High	Rain excluded	Rain allowed		Monospecific litter		Mixed litter		
Root litterbags (48µm Ø)										
Vercors	Beech		2	2	3			Beech	Beech	
	Fir	1131–	2	2	3	12				
	Beech x Fir	1350– 1416	2	2	3	12	12			
Mont Ventoux	Beech		2	2	3			Beech	Beech	
	Fir	1265	2	2	3	12				
	Beech x Fir	1297– 1352	2	2	3	12	12			
Grand Lubéron	Beech		2	2	3			Beech	Beech	
	Oak	821–882	2	2	3	12				
	Beech x Oak	972	2	2	3	12	12			
Ste Baume	Beech		2	2	3			Beech	Beech	
	Oak	719	2	2	3	12				
	Beech x Oak	748	2	2	3	12	12			
Total plots: 24						Total root litterbags: 480				

b)

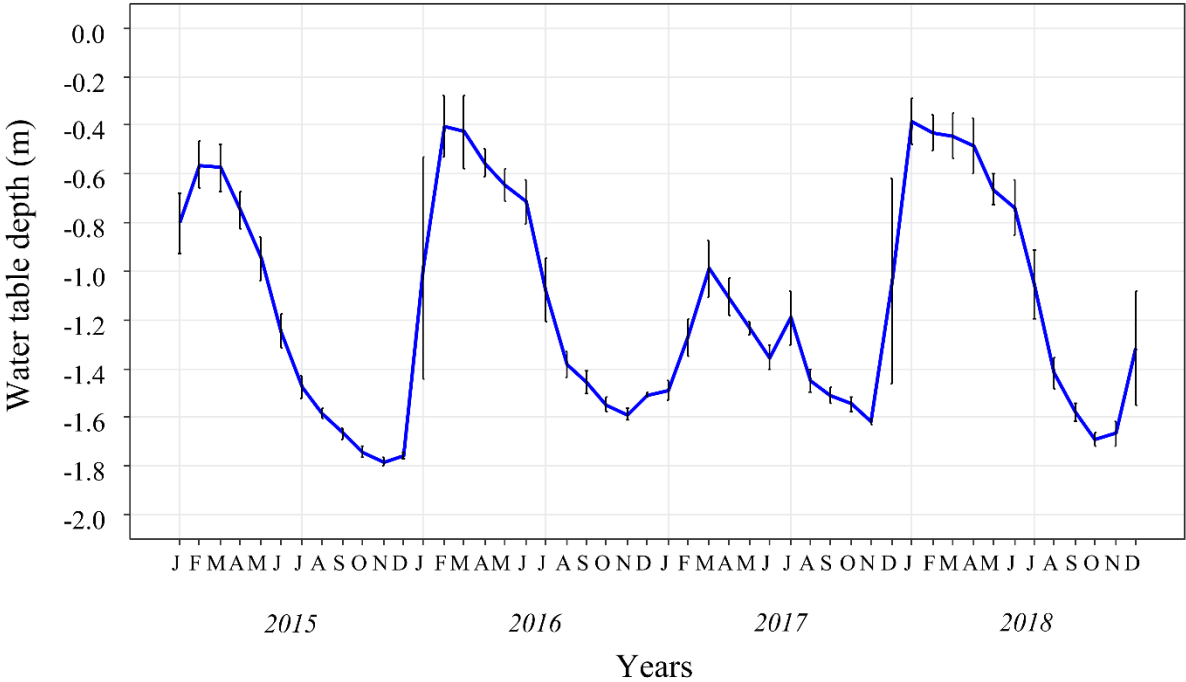
Leaf litterbags (48µm Ø)		Replicate nr												
Site	Altitude (m)	Subplots		Harvests (1st, 2nd, 3rd yr)	Monospecific litter			Mixed litter						
		Rain excluded	Rain allowed		Beech	Fir	Oak	Beech	x Fir	Oak				
Vercors	Beech		2	2	3			12			Beech			
	Fir	1131 -	2	2	3				12					
	Beech	1254	2	2	3			12	12					
	x Fir		2	2	3			12	12					12
Mont Ventoux	Beech		2	2	3			12			Beech			
	Fir	1265	2	2	3				12					
	Beech	1297 -	2	2	3					12				
	x Fir	1352	2	2	3			12	12					12
Grand Luberon	Beech		2	2	3			12						
	Oak	821 - 882	2	2	3					12				
	Beech	972	2	2	3			12	12					12
	x Oak		2	2	3			12	12					12
Ste Baume	Beech		2	2	3			12						
	Oak	719	2	2	3					12				
	Beech	748	2	2	3			12	12					12
	x Oak		2	2	3			12	12					12
<u>Total plots: 24</u>											<u>Total leaf litterbags: 480</u>			



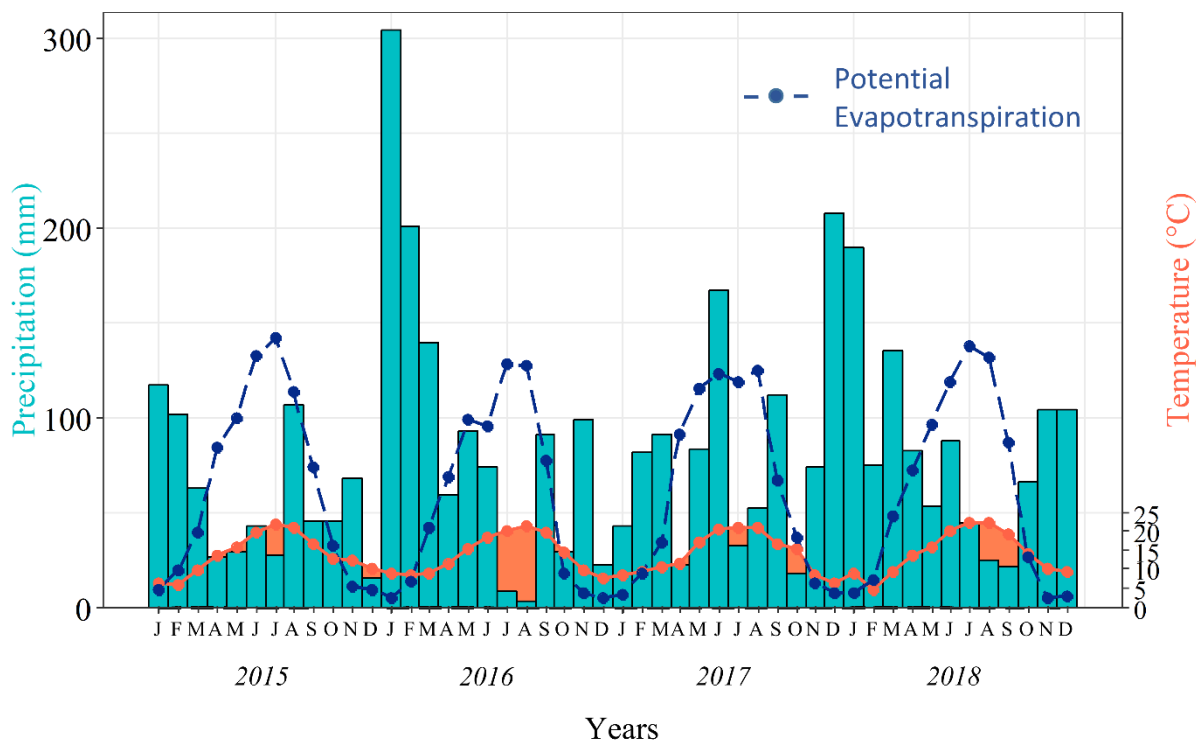
c)

Leaf litterbags (2mm Ø)		Replicate nr									
Site	Altitude (m)	Subplots		Harvests (1st, 2nd, 3rd yr)			Monospecific litter		Mixed litter		
	Low	High	Rain excluded	Rain allowed			Beech	Fir	Oak	Beech x Fir	Beech x Oak
Vercors	Beech		2	2	3		8				
	Fir	1131 -	2	2	3	8					
	Beech	1254	2	2	3	8	8				
	x Fir		2	2	3	8	8				
Mont Ventoux	Beech		2	2	3	8					
	Fir	1265	2	2	3	8					
	Beech	1297 -	2	2	3	8	8				
	x Fir	1352	2	2	3	8	8				
Grand Luberon	Beech		2	2	3	8					
	Oak	821 - 882	2	2	3	8			8		
	Beech	972	2	2	3	8	8				8
	x Oak		2	2	3	8	8				
Ste Baume	Beech		2	2	3	8					
	Oak	719	2	2	3	8			8		
	Beech	748	2	2	3	8	8				8
	x Oak		2	2	3	8	8				
Total plots: 24		Total leaf litterbags: 320									

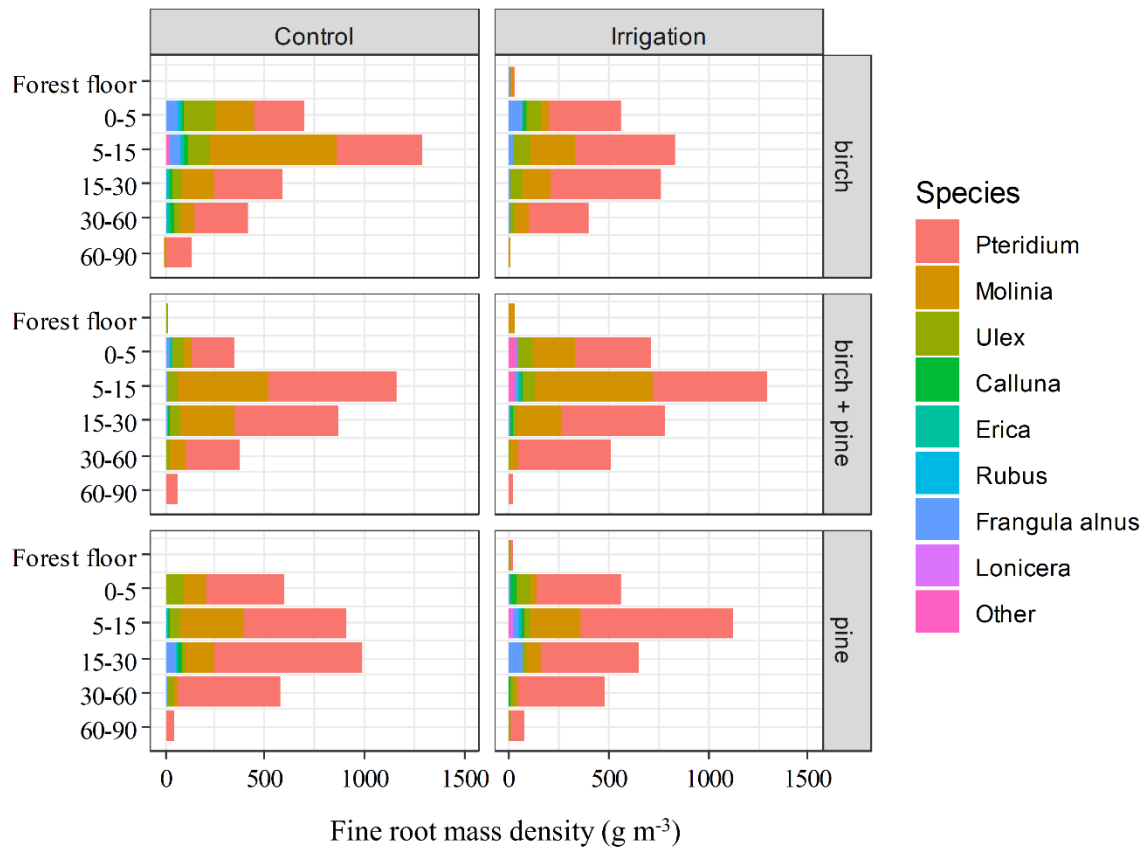
# Appendix B | Effect of tree mixtures and water availability on belowground complementarity of fine roots of birch and pine planted on sandy podzol



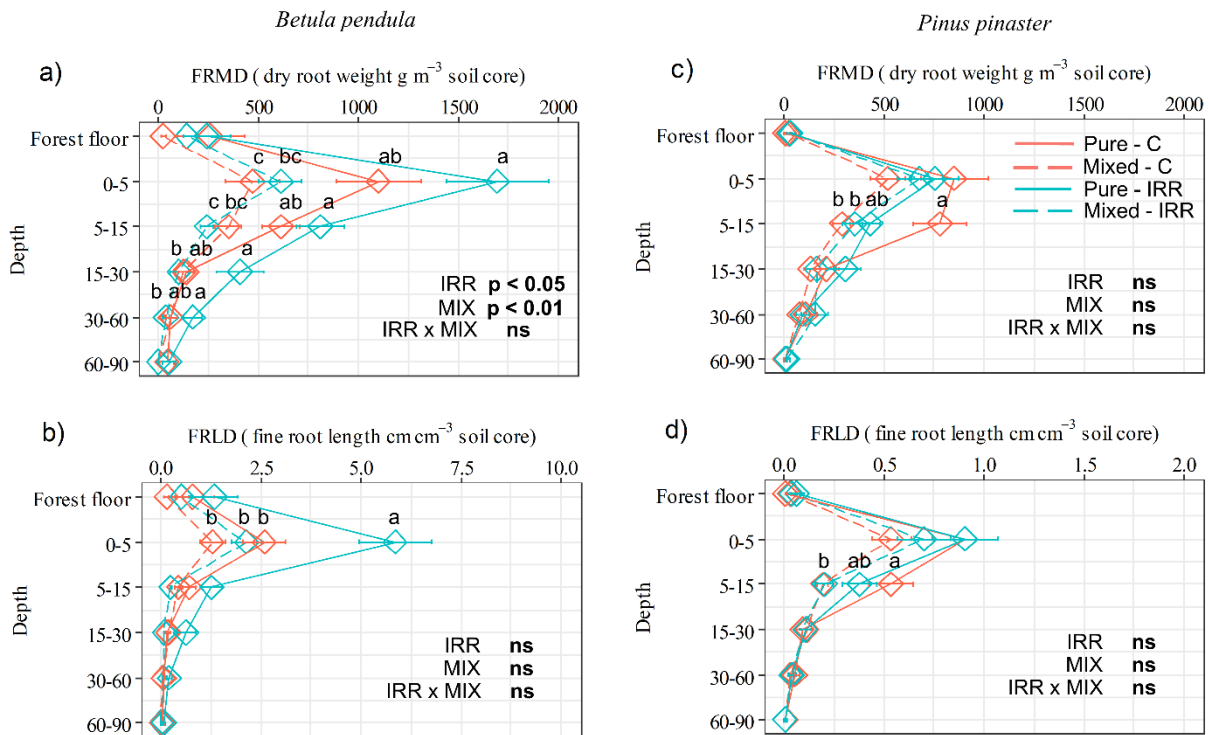
**Fig. B3. 1** Curves of ground water level from the ORPHEE experimental site. The data presented are monthly values (January – December) with std. deviations (in parentheses) from 2015 (initiation year of the irrigation treatment) until 2018 (year of study). Water table depth (m).



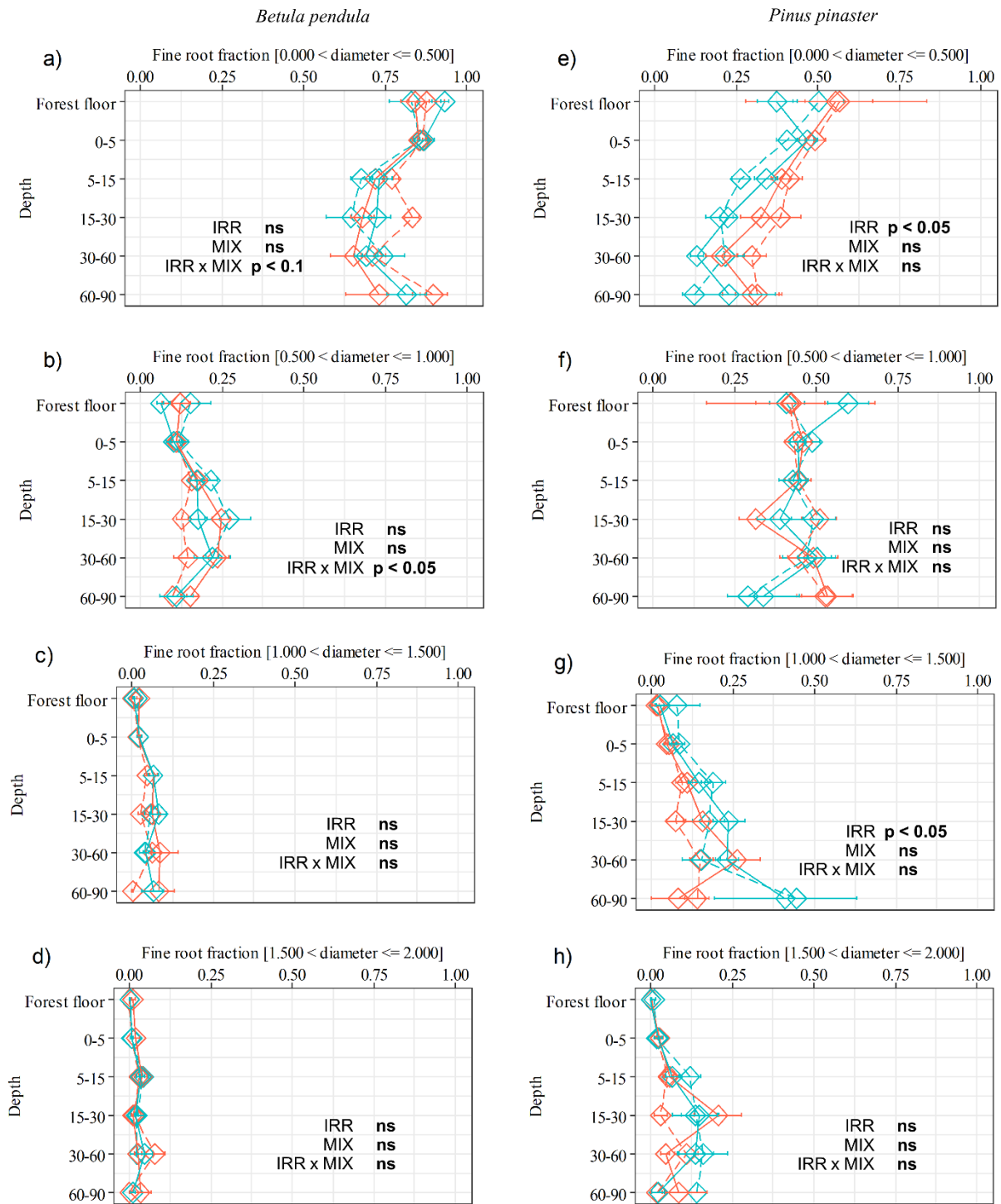
**Fig. B3. 2** Ombrothermic diagram from the Cestas weather station, positioned 1.43 km from the ORPHEE experimental site. The data presented are monthly values (January – December) from 2015 (initiation year of the irrigation treatment) until 2018 (year of study). Temperature = monthly average temperature (°C), Precipitation = monthly total rainfall (mm). Precipitation scale = 2 × Temperature scale. Potential evapotranspiration per month (mm). By plotting in this manner, we identify the potential for water stress, when mean precipitation is lower than mean temperature. Source: CLIMATICK – INRAE.



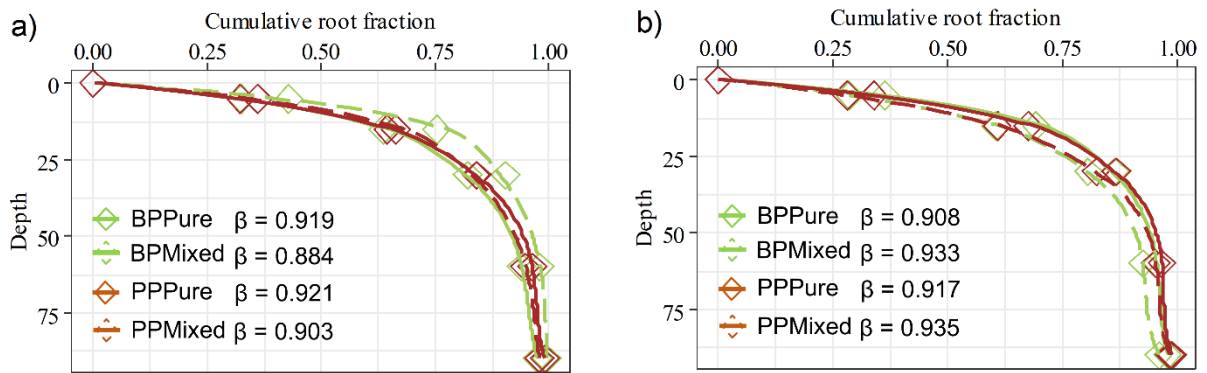
**Fig. B3. 3** Stacked barchart showing the distribution of fine root understory biomass for each soil layer, under pure (birch or pine) and mixed plots (birch + pine), growing under control and irrigated conditions.



**Fig. B3. 4** Depth distributions for root traits of *Betula pendula* and *Pinus pinaster* for all four Irrigation  $\times$  Mixture combinations (Pure – C; Mixed – C; Pure – IRR; Mixed – IRR). Presented data are mean values with st. errors for: (a, c) fine root mass density (FRMD;  $\text{g m}^{-3}$ ), (b, d) fine root length density (FRLD;  $\text{cm cm}^{-3}$ ). Effects of irrigation (IRR), mixture (MIX) and their interaction are indicated at the lower right side of each graph. Significant differences per soil depth, between combinations of Irrigation and Mixture are indicated with lower case letters. Absence of letters means that there are no significant differences.



**Fig. B3. 5** Depth distributions of fine root length divided into diameter classes of *Betula pendula* and *Pinus pinaster* for all four Irrigation × Mixture combinations (Pure – C; Mixed – C; Pure – IRR; Mixed – IRR). Presented data are mean fine root fraction values with st. errors for the following diameter classes: (a,e) [0.000 – 0.500 mm], (b, f) [0.500 – 1.000 mm], (c, g) [1.000 – 1.500 mm], and (d, h) [1.500 – 2.000 mm]. Effects of irrigation (IRR), mixture (MIX) and their interaction are indicated at the lower right side of each graph..



**Fig. B3. 6** Cumulative fine root fraction with soil depth and the coefficients of the rooting distribution ( $\beta$ ) of pure and mixed stands of *Betula pendula* and *Pinus pinaster* under: a) Irrigation, b) non-irrigation. The  $\beta$  values are calculated for fine root biomass ( $\text{g m}^{-2}$ ); the higher the  $\beta$  value (the closer to 1), the deeper is the vertical root distribution of fine roots.

**Table B3. 1** Overview table of understory species with means of soil cover (%) and std. deviations (in parentheses), under monocultures of *Betula pendula*, *Pinus pinaster*, and their mixture. Presence is the percentage of aboveground occurrence for each understory species.

	Control			Irrigation		
	Betula pendula	Pinus pinaster	Betula end. + Pinus pin.	Betula pendula	Pinus pinaster	Betula pend. + Pinus pin.
<i>Pteridium aquilinum</i> L. Kuhn						
Soil cover	56.5 (28.2)	17.8 (11.7)	25.9 (13.5)	66.7 (15.0)	22.5 (9.17)	20.4 (10.3)
Presence	82.1%	100%	87.5%	85%	85%	85%
<i>Molinia caerulea</i> L. (Moench)						
Soil cover	21.7 (24.5)	3.21 (2.69)	10.3 (10.0)	17.3 (24.6)	6.31 (7.02)	12.8 (15.3)
Presence	100%	87.5%	100%	100%	100%	100%
<i>Ulex Europeaus</i> L.						
Soil cover	7.33 (7.28)	3.73 (3.52)	4.08 (3.94)	7.17 (5.42)	1.60 (0.548)	2.43 (1.40)
Presence	56.2%	68.8%	85%	37.5%	31.2%	43.8%
<i>Ulex Minor</i> Roth.						
Soil cover	1.13 (0.354)	1.14 (0.378)	-	1.00 (0.00)	1.20 (0.447)	1.00 (1.00)
Presence	50.0%	43.8%	0%	18.8%	31.2%	18.8%
<i>Erica cinerea</i> L.						
Soil cover	1.21 (0.802)	1.50 (0.707)	1.33 (0.577)	1.20 (0.447)	1.00 (0.00)	0.800 (0.447)
Presence	87.5%	12.5%	18.8%	31.2%	37.5%	31.2%
<i>Calluna vulgaris</i> L. Hull						
Soil cover	2.13 (0.991)	1.67 (1.15)	1.33 (0.577)	2.20 (1.64)	1.00 (-)	1.00 (0.00)
Presence	50.0%	18.8%	18.8%	31.2%	6.2%	12.5%
<i>Frangula alnus</i> P. Mill.						
Soil cover	1.73 (1.79)	2.62 (1.94)	2.42 (1.88)	3.30 (2.45)	1.73 (0.786)	3.43 (1.79)
Presence	93.8%	81.2%	85%	62.5%	68.8%	87.5%
<i>Lonicera periclymenum</i> L.						
Soil cover	0.600 (0.548)	1.00 (0.00)	1.00 (0.00)	1.67 (0.577)	1.00 (0.00)	1.25 (0.500)
Presence	31.2%	12.5%	25%	18.8%	12.5%	25%
<i>Rubus fruticosus</i> L.						
Soil cover	0.429 (0.535)	1.33 (0.577)	1.00 (0.00)	1.00 (0.00)	1.25 (0.500)	1.50 (0.707)
Presence	43.8%	18.8%	18.8%	12.5%	25%	12.5%
<i>Erica scoparia</i> L.						
Soil cover	0.200 (0.447)	2.00 (0.00)	-	-	1.00 (0.00)	1.00 (-)
Presence	31.2%	12.5%	0%	0%	12.5%	6.2%



**Table B3. 2** Soil moisture (%) along the soil profile in the three different species composition treatments (pure *Betula pendula*, pure *Pinus pinaster*, mixed *Betula pendula* + *Pinus pinaster*), each in both control and irrigated blocks. Soil was collected in March 2018 along with the roots. Data are means  $\pm$  standard deviation.

Species composition	<i>Betula pendula</i>		<i>Pinus pinaster</i>		<i>Betula pendula</i> + <i>Pinus pinaster</i>	
	Control	Irrigation	Control	Irrigation	Control	Irrigation
Soil moisture (%)						
0–5	15.8 $\pm$ 4.0	15.7 $\pm$ 4.3	12.3 $\pm$ 4.3	17.9 $\pm$ 5.4	13.1 $\pm$ 4.2	14.8 $\pm$ 3.0
5–15	14.3 $\pm$ 4.3	11.4 $\pm$ 4.5	13.6 $\pm$ 8.2	13.4 $\pm$ 4.9	14.7 $\pm$ 6.2	14.1 $\pm$ 7.4
15–30	13.7 $\pm$ 3.8	12.4 $\pm$ 2.6	13.5 $\pm$ 2.2	12.4 $\pm$ 3.2	11.8 $\pm$ 2.0	11.0 $\pm$ 3.2
30–60	11.1 $\pm$ 0.6	12.0 $\pm$ 3.3	10.6 $\pm$ 3.4	14.2 $\pm$ 4.0	10.3 $\pm$ 2.9	13.6 $\pm$ 6.0
60–90	13.4 $\pm$ 2.5	13.5 $\pm$ 2.7	12.8 $\pm$ 2.5	13.3 $\pm$ 1.8	13.0 $\pm$ 1.4	12.5 $\pm$ 1.8

**Table B3. 3** Results of the linear mixed effects models to test for the effects of species, mixture, irrigation, depth and their interactions on Fine root mass density (FRMD), Specific root length (SRL), Specific root area (SRA) and Specific root tip density (SRTD). Significant results are in bold font.

	FRMD <sup>a</sup>		SRL <sup>b</sup>		SRA <sup>b</sup>		SRTD <sup>b</sup>	
	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>
<b>Species (S)</b>	1.9942	0.1584	<b>180.9309</b>	<b>&lt;0001</b>	<b>75.0386</b>	<b>&lt;0001</b>	<b>48.7995</b>	<b>&lt;0001</b>
<b>Mix (M)</b>	<b>22.6477</b>	<b>0.0005</b>	0.0615	0.8083	0.0390	0.8468	0.0636	0.8052
<b>Depth (D)</b>	<b>108.5585</b>	<b>&lt;0001</b>	<b>170.5558</b>	<b>&lt;0001</b>	<b>185.5276</b>	<b>&lt;0001</b>	<b>26.9431</b>	<b>&lt;0001</b>
<b>Irrigation (I)</b>	3.9833	0.0930	1.2871	0.2999	2.7810	0.1464	1.2673	0.3033
<b>(S x M)</b>	<b>6.993</b>	<b>0.0214</b>	0.0393	0.8461	0.3802	0.5490	0.3087	0.5887
<b>(S x D)</b>	<b>4.9984</b>	<b>0.0257</b>	<b>43.7795</b>	<b>&lt;0001</b>	<b>19.4934</b>	<b>&lt;0001</b>	<b>7.7223</b>	<b>0.0057</b>
<b>(M x D)</b>	3.1248	0.0776	2.3451	0.1265	2.1397	0.1443	0.1535	0.6954
<b>(S x I)</b>	1.9476	0.1633	1.3090	0.2533	0.8543	0.3559	0.2195	0.6397
<b>(M x I)</b>	0.5499	0.4726	<b>7.6191</b>	<b>0.0173</b>	<b>10.5152</b>	<b>0.0071</b>	1.1161	0.3116
<b>(D x I)</b>	1.0648	0.3025	<b>6.4402</b>	<b>0.0115</b>	<b>4.6597</b>	<b>0.0315</b>	<b>3.975</b>	<b>0.0469</b>
<b>(S x M x D)</b>	0.7939	0.3733	1.0731	0.3009	0.0072	0.9326	1.6685	0.1972
<b>(S x M x I)</b>	2.6194	0.1315	3.0213	0.1077	0.8522	0.3741	1.6461	0.2237
<b>(S x D x I)</b>	0.8626	0.3534	<b>5.2329</b>	<b>0.0227</b>	2.1478	0.1436	1.5497	0.2139
<b>(M x D x I)</b>	0.1687	0.6814	2.3553	0.1257	2.4172	0.1208	0.5291	0.4674
<b>(S x M x D x I)</b>	0.0036	0.9525	0.1336	0.7149	0.0046	0.9460	0.0301	0.8624

<sup>a</sup> The FRMD number of individual samples underlying the calculations was: n = 768

<sup>b</sup> The SRL, SRA, SRT number of individual samples underlying the calculations was: = 251

**Table B3. 4** Results of the linear mixed effects models to test for the effects of mixture, irrigation, depth and their interactions on Fine root mass density (FRMD), Specific root length (SRL), Specific root area (SRA) and Specific root tip density (SRTD). The upper part shows results for *Betula pendula*, and the lower part for *Pinus pinaster*. Significant results are in bold font.

<i>Betula pendula</i>									
	FRMD <sup>a</sup>		SRL <sup>b</sup>		SRA <sup>b</sup>		SRTD <sup>b</sup>		
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	
Mix (M)	<b>31.0494</b>	<b>0.0014</b>	0.2076	0.6647	0.5499	0.4864	0.0007	0.9801	
Depth (D)	<b>75.282</b>	<b>&lt;.0001</b>	<b>110.191</b>	<b>&lt;.0001</b>	<b>111.831</b>	<b>&lt;.0001</b>	<b>30.2245</b>	<b>&lt;.0001</b>	
Irrigation (I)	<b>6.7081</b>	<b>0.0412</b>	1.0995	0.3348	1.8919	0.2181	1.1592	0.3230	
(M x D)	3.3229	0.0693	1.9149	0.1681	0.8688	0.3525	1.4037	0.2376	
(M x I)	2.9998	0.1340	4.8662	0.0695	4.7281	0.0726	2.1352	0.1943	
(D x I)	1.8071	0.1798	<b>6.4923</b>	<b>0.0117</b>	<b>4.3613</b>	<b>0.0381</b>	<b>4.8720</b>	<b>0.0285</b>	
(M x D x I)	0.1040	0.7473	1.0083	0.3166	0.8522	0.3571	0.0665	0.7968	

<i>Pinus pinaster</i>									
	FRMD <sup>a</sup>		SRL <sup>b</sup>		SRA <sup>b</sup>		SRTD <sup>b</sup>		
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	
Mix (M)	3.13568	0.1270	0.3446	0.5786	0.5131	0.5007	0.1406	0.7206	
Depth (D)	<b>36.05474</b>	<b>&lt;.0001</b>	<b>99.3049</b>	<b>&lt;.0001</b>	<b>80.457</b>	<b>&lt;.0001</b>	3.2796	0.0719	
Irrigation (I)	0.08984	0.7745	0.7196	0.4288	0.4232	0.5394	0.9239	0.3736	
(M x D)	0.41381	0.5205	0.8667	0.3532	2.3436	0.1276	0.5117	0.4754	
(M x I)	0.33711	0.5826	5.8965	0.0513	4.068	0.0903	0.013	0.9128	
(D x I)	0.00573	0.9397	0.2203	0.6394	0.6811	0.4103	0.3427	0.559	
(M x D x I)	0.06638	0.7968	3.4245	0.0659	2.2701	0.1337	0.3623	0.548	

<sup>a</sup> The FRMD number of individual samples underlying the calculations for: *Betula pendula* (n = 384), and for *Pinus pinaster* (n = 384)

<sup>b</sup> The SRL, SRA, SRTD number of individual samples underlying the calculations for: *Betula pendula* (n = 251), and for *Pinus pinaster* (n=242)

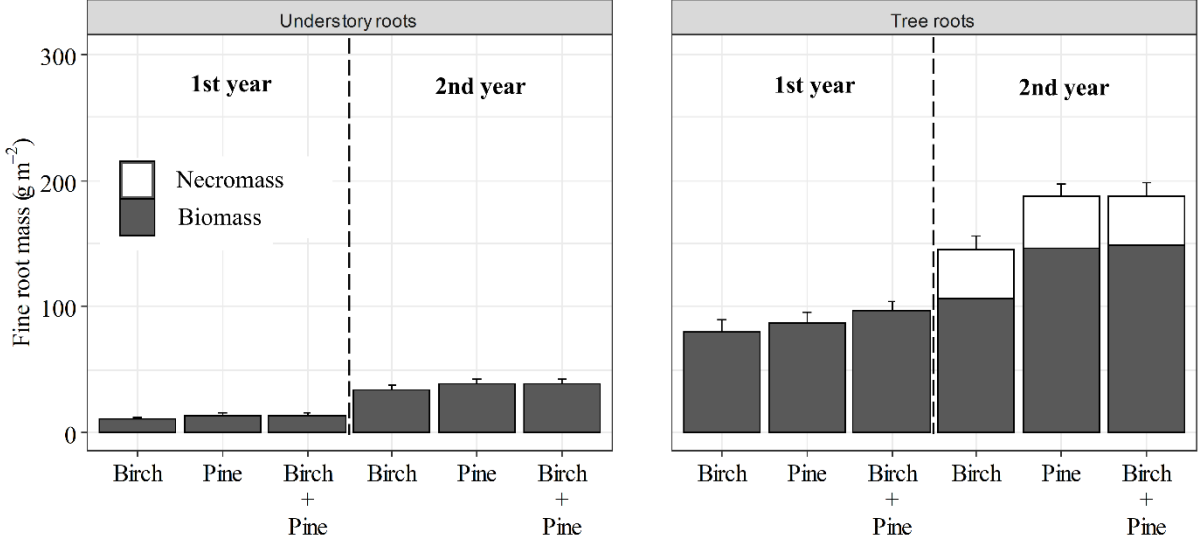
**Table B3. 5** Means of root distribution values, with std. deviations (in parentheses) calculated for g m<sup>-2</sup> soil surface. The parameters d30 and d60 are fine root cumulative proportions to depths of 30 and 60 cm respectively. The d50 parameter is the depth at which we find 50% of roots, and was estimated with the beta values.

Species	<i>Betula pendula</i>				<i>Pinus pinaster</i>			
	Pure stand		Mixed stand		Pure stand		Mixed stand	
	Control	Irrigation	Control	Irrigation	Control	Irrigation	Control	Irrigation
<i>Soil depth</i>	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
30 cm	83.1 (21.6)	79.5 (20.3)	81.9 (28.9)	87.8 (15.7)	88.3 (16.9)	87.4 (14.0)	79.5 (16.8)	81.8 (24.7)
60 cm	95.9 (13.6)	95.6 (11.6)	94.2 (22.4)	100 (0.00)	99.1 (2.53)	98.8 (2.58)	98.5 (4.14)	99.2 (3.19)
<i>FRB proportion</i>	[cm]	[cm]	[cm]	[cm]	[cm]	[cm]	[cm]	[cm]
50% (cm)	10.8 (9.05)	12.7 (8.87)	15.2 (15.9)	8.26 (5.40)	10.3 (5.77)	11.1 (5.29)	12.2 (5.45)	11.7 (8.61)

**Table B3. 6** Overview of fine root biomass (FRB; g m<sup>-2</sup>) per soil layer, and summed for the whole profile (0-90 cm) that is found in pure and mixed plots of birch and pine, under control and irrigated conditions. Different lowercase letters indicate significant differences between the six combinations of stand composition and water treatment, across each soil layer.

Species composition	<i>Betula pendula</i>		<i>Pinus pinaster</i>		<i>Betula pendula</i> + <i>Pinus pinaster</i>	
	Control	Irrigation	Control	Irrigation	Control	Irrigation
FRB (g m <sup>2</sup> )						
Forest floor	3 (7) a	2 (5) a	0.02 (0.06) a	0.3 (0.8) a	0.4 (0.7) a	1.7 (2.1) a
0-5	55 (42) ab	85 (51) a	43 (34) ab	38 (24) b	49 (33) ab	65 (26) ab
5-15	61 (38) a	81 (48) a	78 (54) a	43 (18) a	64 (27) a	60 (29) a
15-30	21 (23) a	61 (72) a	31 (37) a	46 (50) a	38 (35) a	40 (36) a
30-60	19 (29) a	52 (57) a	33 (68) a	28 (38) a	41 (53) a	57 (84) a
60-90	13 (44) a	16 (40) a	1.7 (5.4) a	2.5 (6.9) a	2 (4) a	4 (16) a
Total root biomass	172 (82) b	297 (129) a	186 (118) ab	143 (88) b	194 (103) ab	226 (116) ab

# Appendix C | Fine root dynamics in response to tree species mixing, stand density, and water availability



**Fig. C4. 1** Annual fine root productivity ( $\text{g m}^{-2} \text{y}^{-1}$ ) per tree species composition, with the contribution of biomass and necromass measured in the ingrowth-cores, 1 and 2 years after ingrowth-core installation. Understory roots (on the left), tree roots (on the right). Values are means with st. errors.

**Table C4. 1** Overview table of stand characteristics at 10 years-old. Measurements were performed in the summer of 2018; therefore in the middle of the two year observation period for roots. Values are means ( $\pm$  standard deviations) of 36 centre trees per plot (alive and measurable trees at breast height 130 cm) repeated across 8 blocks (4 blocks are irrigated and 4 blocks are not).

	Low stand density						High stand density					
	Birch		Pine		Birch + Pine		Birch		Pine		Birch + Pine	
	Ctl	Irr <sup>a</sup>	Ctl	Irr	Ctl	Irr	Ctl	Irr	Ctl	Irr	Ctl	Irr
Live trees <sup>b</sup>	17.3 (1.50)	16.5 (2.38)	18.0 (0.00)	17.8 (0.50)	22.3 (0.957)	21.0 (3.16)	32.3 (2.75)	34.8 (0.96)	35.8 (0.50)	34.8 (0.50)	35.0 (0.82)	35.8 (0.50)
Stand Density <sup>c</sup> (trees ha <sup>-1</sup> )	1200 (104)	1150 (165)	1250 (0)	1230 (35)	1550 (66)	1460 (220)	2240 (191)	2410 (67)	2480 (35)	2410 (35)	2430 (57)	2480 (35)
Stand basal area <sup>c</sup> (m <sup>2</sup> ha <sup>-1</sup> )	5.39 (2.05)	4.45 (2.19)	23.8 (3.49)	24.4 (5.97)	18.5 (0.59)	20.4 (1.81)	5.56 (1.69)	8.74 (1.75)	29.3 (6.54)	33.7 (2.89)	24.1 (4.26)	26.5 (0.63)
Root standing biomass (g m <sup>2</sup> )	80.8 (33.4)	68.6 (35.0)	68.8 (45.3)	87.6 (42.9)	73.5 (36.1)	113 (36.3)	126 (71.5)	175 (78.2)	120 (71.6)	82.2 (30.2)	115 (44.4)	131 (25.8)

<sup>a</sup> Daily irrigation from May to October equivalent to about 3 mm day<sup>-1</sup>

<sup>b</sup> Mean values based on plot averages for four blocks

<sup>c</sup> Mean values based on the sum of alive and measurable trees at the plot level

**Table C4. 2** Relative canopy ratios when trees are growing in mixture, and the % of missing trees per measurement (dead trees and trees with a trunk diameter of less than 1.3 cm were excluded). The canopy extension (in m<sup>2</sup>) of each tree was computed by using the four largest branches and assuming a vertical projection on the forest floor. The canopy ratio was then defined as the canopy extension divided by 4 m<sup>2</sup> (the theoretical space of each tree in the design). Values higher than 1 mean that the tree occupies more than this space (canopy closure).

		<i>Birch</i>		<i>Pine</i>	
		Crtl	Irr	Crtl	Irr
<b>High tree density</b>	Relative canopy ratio	0.88 (0.44)	0.83 (0.29)	1.46 (0.76)	1.46 (0.88)
	Missing trees	0%	0%	0%	0%
<b>Low tree density</b>	Relative canopy ratio	0.95 (0.39)	0.88 (0.39)	1.62 (0.55)	1.81 (0.85)
	Missing trees	12%	11%	6%	0%



**Table C4. 3** Overview table of understory species with means of soil cover (%) and std. deviations (in parentheses), under monocultures of birch, pine, and their mixture (birch + pine). Presence is the percentage of aboveground occurrence for each understory species.

	Control						Irrigation					
	Low tree stand density			High tree stand density			Low tree stand density			High tree stand density		
	Birch	Pine	+	Birch	Pine	+	Birch	Pine	+	Birch	Pine	+
<b>Pteridium aquilinum L.</b>												
<b>Kuhn</b>												
Soil cover	43.8	31.5	31.8	56.5	17.8	25.9	45.8	39.4	48.3	66.7	22.5	20.4
	(31.7)	(23.9)	(21.6)	(28.2)	(11.7)	(13.5)	(41.7)	(32.2)	(31.7)	(15.0)	(9.17)	(10.3)
Presence	100%	100%	100%	82%	100%	87%	100%	100%	75%	75%	75%	75%
<b>Molinia caerulea L.</b>												
<b>(Moench)</b>												
Soil cover	14.0	14.6	13.1	21.7	3.21	10.3	29.3	33.0	23.9	17.3	6.31	12.8
	(9.13)	(7.88)	(9.16)	(24.5)	(2.69)	(10.0)	(24.7)	(31.2)	(27.2)	(24.6)	(7.02)	(15.3)
Presence	100%	100%	100%	100%	87%	100%	100%	100%	100%	100%	100%	100%
<b>Ulex Europeanus L.</b>												
Soil cover	4.29	2.44	4.40	7.33	3.73	4.08	3.55	1.80	3.27	7.17	1.60	2.43
	(3.25)	(1.33)	(4.03)	(7.28)	(3.52)	(3.94)	(3.33)	(1.23)	(2.49)	(5.42)	(0.54)	(1.40)
Presence	43%	56%	62%	56%	68%	85%	68%	62%	68%	37.5%	31.2%	43%
<b>Ulex Minor</b>												
<b>Roth.</b>												
Soil cover	1.63	3.45	1.73	1.13	1.14	-	2.80	1.40	1.33	1.00	1.20	1.00
	(1.06)	(5.52)	(0.79)	(0.35)	(0.37)	(-)	(2.49)	(0.54)	(0.57)	(0.00)	(0.44)	(1.00)
Presence	50%	68%	68%	50%	43%	-	31%	31%	18%	18.8%	31.2%	18%
<b>Erica cinerea L.</b>												
Soil cover	1.00	1.20	1.86	1.21	1.50	1.33	4.33	1.00	2.17	1.20	1.00	0.800
	(0.00)	(0.45)	(1.07)	(0.80)	(0.71)	(0.57)	(3.67)	(0.00)	(1.60)	(0.44)	(0.00)	(0.45)
Presence	37%	31%	43%	87%	12%	18%	37%	18%	37%	31.2%	37.5%	31%
<b>Calluna vulgaris L.</b>												
<b>Hull</b>												
Soil cover	4.56	1.83	2.60	2.13	1.67	1.33	8.38	2.25	1.67	2.20	1.00	1.00
	(3.54)	(1.60)	(1.34)	(0.99)	(1.15)	(0.58)	(9.02)	(1.26)	(0.81)	(1.64)	(-)	(0.00)
Presence	56%	37%	31%	50%	18%	18%	50%	25%	37%	31%	6.20%	12%
<b>Frangula alnus P.</b>												
<b>Mill.</b>												
Soil cover	1.50	1.67	4.50	1.73	2.62	2.42	2.00	2.82	2.50	3.30	1.73	3.43
	(0.70)	(0.86)	(3.63)	(1.79)	(1.94)	(1.88)	(1.32)	(2.68)	(1.56)	(2.45)	(0.78)	(1.79)
Presence	62%	56%	75%	93%	81%	85%	56%	68%	87%	62%	68.8%	87%
<b>Lonicera periclymenum L.</b>												

Soil cover	1.40 (0.89)	1.00 (0.00)	1.33 (0.57)	0.600 (0.54)	1.00 (0.00)	1.00 (0.00)	2.00 (0.00)	1.00 (0.00)	1.50 (0.57)	1.67 (0.57)	1.00 (0.00)	1.25 (0.50)
Presence	31%	12%	18%	31%	12%	25%	18%	12%	25%	18%	12.5%	25%
<b>Rubus fruticosus L.</b>												
Soil cover	1.00 (NA)	1.50 (0.70)	1.00 (0.00)	0.429 (0.53)	1.33 (0.57)	1.00 (0.00)	2.00 (1.73)	1.17 (0.40)	1.33 (0.57)	1.00 (0.00)	1.25 (0.50)	1.50 (0.70)
Presence	6.20%	12%	31.2%	43.8%	18.8%	18.8%	31.2%	37.5%	18.8%	12.5%	25%	12.5%
<b>Erica scoparia L.</b>												
Soil cover	1.33 (0.57)	1.25 (0.50)	1.00 (0.00)	0.200 (0.44)	2.00 (0.00)	- (-)	1.88 (0.99)	1.60 (0.54)	1.00 (-)	- (-)	1.00 (0.00)	1.00 (-)
Presence	18%	25%	12%	31%	12.5%	0%	50%	31%	6.2%	0%	12.5%	6.2%

**Table C4. 4** Results of the linear mixed effects models to test for the effects of tree species composition, stand density, irrigation, and their interactions on Fine root production (FRP), Fine root necromass (FRN), Fine root turnover (FRT) and Fine root decomposition (FRD). Significant results are in bold font ( $p < 0.05$ ).

	FRP		FRN		FRT		FRD	
	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>
<b>Composition (C)</b>	5.3501	<b>0.0103</b>	0.132	0.8764	7.0781	<b>0.003</b>	1.854	0.1741
<b>Density (D)</b>	4.3812	<b>0.0449</b>	3.045	0.0912	7.4210	<b>0.0106</b>	1.03	0.3183
<b>Irrigation (I)</b>	1.5686	0.257	0.031	0.8651	0.2173	0.6575	1.679	0.2427
<b>(C × D)</b>	3.982	<b>0.0293</b>	2.728	0.0815	0.9419	0.4011	1.672	0.2048
<b>(C × I)</b>	2.4149	0.1066	0.059	0.9421	2.9435	0.068	0.023	0.9775
<b>(D × I)</b>	3.2956	0.0795	0.603	0.4434	2.0028	0.1673	0.48	0.4938
<b>(C × D × I)</b>	0.1807	0.8355	0.672	0.5181	1.8288	0.1781	2.049	0.1466

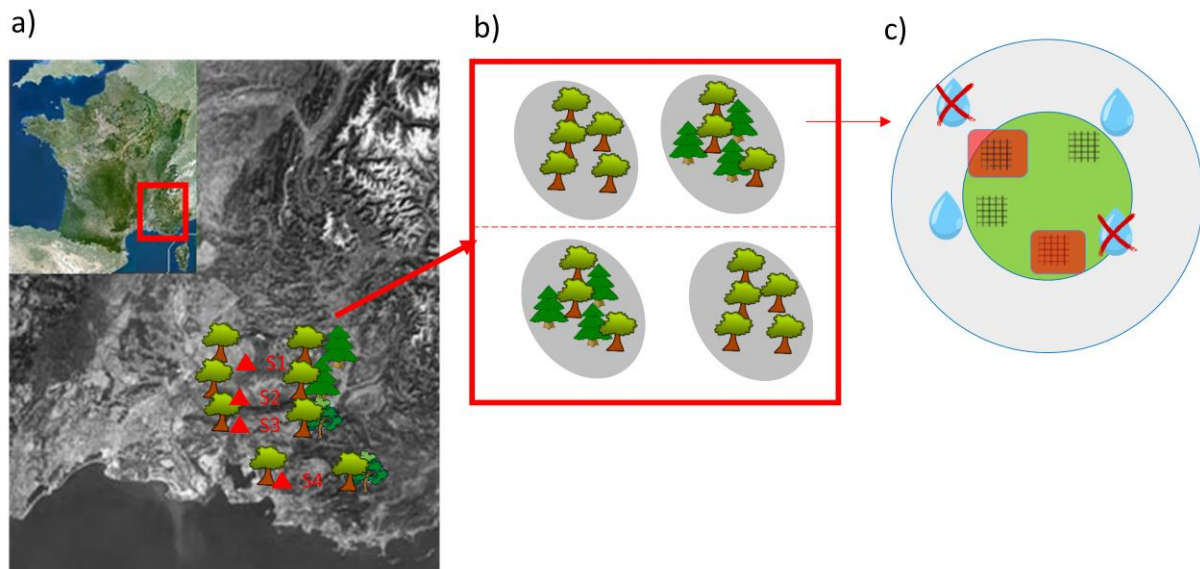
**Table C4. 5** Results of the linear mixed effects models to test for the effects of tree species composition, stand density, irrigation, and their interactions on cumulative release (after a 2-year decomposition period) of carbon (C), nitrogen (N), and phosphorus (P). Significant results are in bold font ( $p < 0.05$ ).

	Cumulative release					
	Carbon		Nitrogen		Phosphorus	
	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>
<b>Composition (C)</b>	10.52568	<b>0.0003</b>	2.6297	0.0887	7.785544	<b>0.0019</b>
<b>Density (D)</b>	0.2204	0.6421	5.35237	<b>0.0277</b>	5.189496	<b>0.03</b>
<b>Irrigation (I)</b>	0.58935	0.4718	0.00131	0.9723	0.125306	0.7355
<b>(C × D)</b>	3.93134	<b>0.0305</b>	2.66796	0.0858	0.597375	0.5567
<b>(C × I)</b>	0.09814	0.9068	0.1492	0.862	0.644467	0.5321
<b>(D × I)</b>	0.64929	0.4267	0.99655	0.3261	0.038969	0.8448
<b>(C × D × I)</b>	0.25541	0.7763	1.74337	0.1922	0.519633	0.6

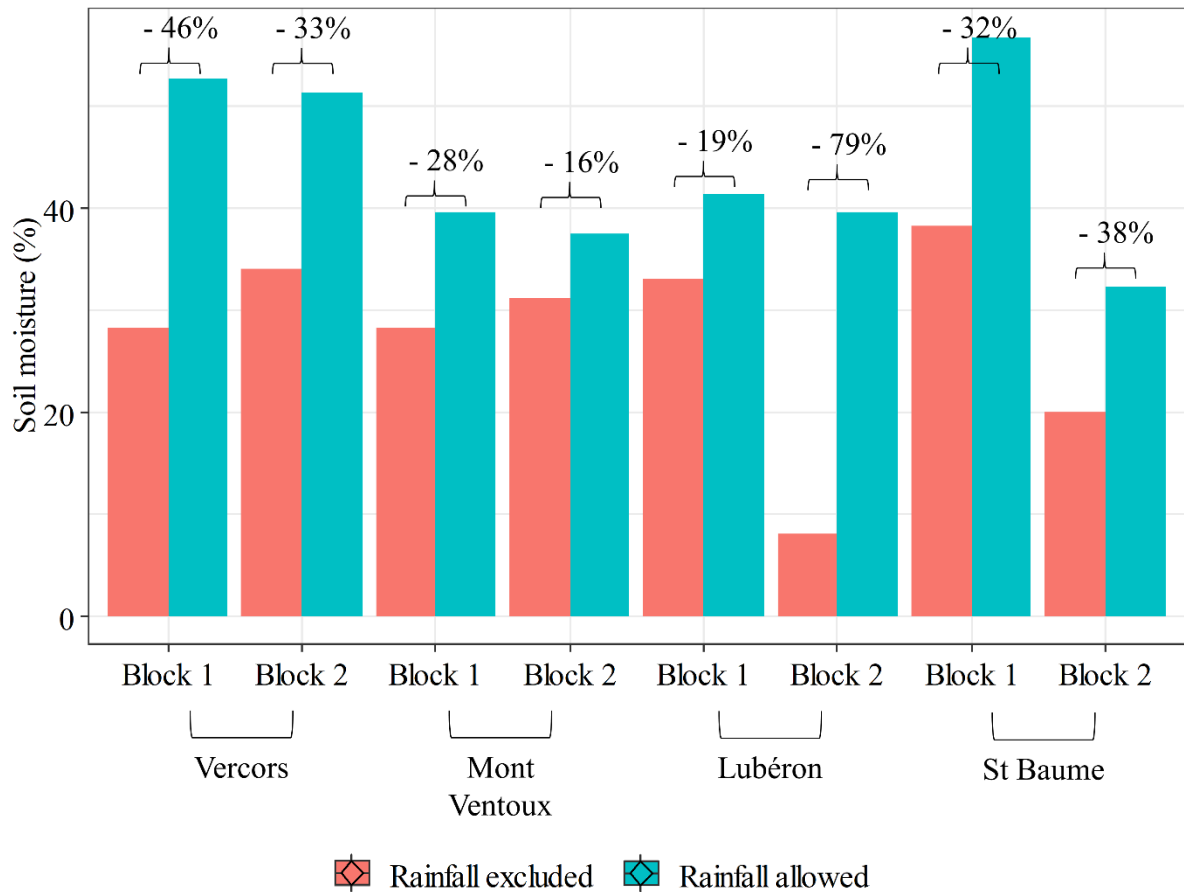
**Table C4. 6** Annual fine root litter input to soil (fine root necromass) and cumulative N and Carbon release (after 2 years of fine root decomposition) between different tree species composition of birch and pine, planted at high and low stand density. Half of the stands are left unirrigated (Ctrl) and half are irrigated during the summer (Irr). Values are means with stand deviation in parentheses.

	Annual soil inputs				Cumulative release after 2 years			
	Root litter input (kg ha <sup>-1</sup> y <sup>-1</sup> )	C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	N input (kg ha <sup>-1</sup> y <sup>-1</sup> )	P input (kg ha <sup>-1</sup> y <sup>-1</sup> )	C release (kg ha <sup>-1</sup> )	N release (kg ha <sup>-1</sup> )	P release (kg ha <sup>-1</sup> )	
<i>Birch</i>	Ctrl	207 (139)	93.6 (58.2)	2.14 (1.32)	0.181 (0.118)	30.4 (17.8)	1.76 (1.09)	0.084 (0.091)
	Irr	273 (174)	123 (68.5)	2.63 (1.38)	0.252 (0.181)	50.9 (30.4)	2.37 (1.04)	0.137 (0.131)
<i>Pine</i>	Ctrl	182 (97)	68.1 (38.1)	1.50 (0.92)	0.138 (0.063)	37.4 (32.5)	0.89 (0.59)	0.037 (0.043)
	Irr	178 (126)	69.6 (50.8)	1.45 (0.89)	0.187 (0.089)	44.2 (23.0)	1.16 (0.89)	0.036 (0.180)
<i>Birch</i> + <i>Pine</i>	Ctrl	239 (160)	103 (74.5)	2.38 (1.67)	0.253 (0.142)	45.9 (35.6)	1.93 (1.57)	0.071 (0.110)
	Irr	229 (56.3)	98.0 (25.7)	2.11 (0.63)	0.195 (0.049)	56.0 (21.6)	1.53 (0.43)	0.039 (0.037)
<i>Birch</i>	Ctrl	158 (96)	71.1 (45.4)	1.88 (1.30)	0.201 (0.104)	21.4 (15.1)	1.64 (1.08)	0.087 (0.083)
	Irr	103 (33)	48.4 (16.4)	1.05 (0.32)	0.112 (0.057)	16.4 (8.45)	0.87 (0.21)	0.063 (0.036)
<i>Pine</i>	Ctrl	210 (136)	73.7 (45.5)	1.59 (0.96)	0.154 (0.081)	55.3 (32.4)	1.15 (0.82)	-0.009 (0.066)
	Irr	231 (89)	87.3 (37.0)	1.90 (0.86)	0.185 (0.073)	54.6 (28.2)	1.04 (0.45)	-0.052 (0.134)
<i>Birch</i> + <i>Pine</i>	Ctrl	163 (45)	69.7 (12.4)	1.57 (0.38)	0.156 (0.039)	37.1 (11.7)	1.15 (0.36)	0.064 (0.064)
	Irr	158 (69)	69.9 (29.8)	1.44 (0.59)	0.151 (0.051)	50.6 (22.5)	1.31 (0.52)	0.059 (0.062)

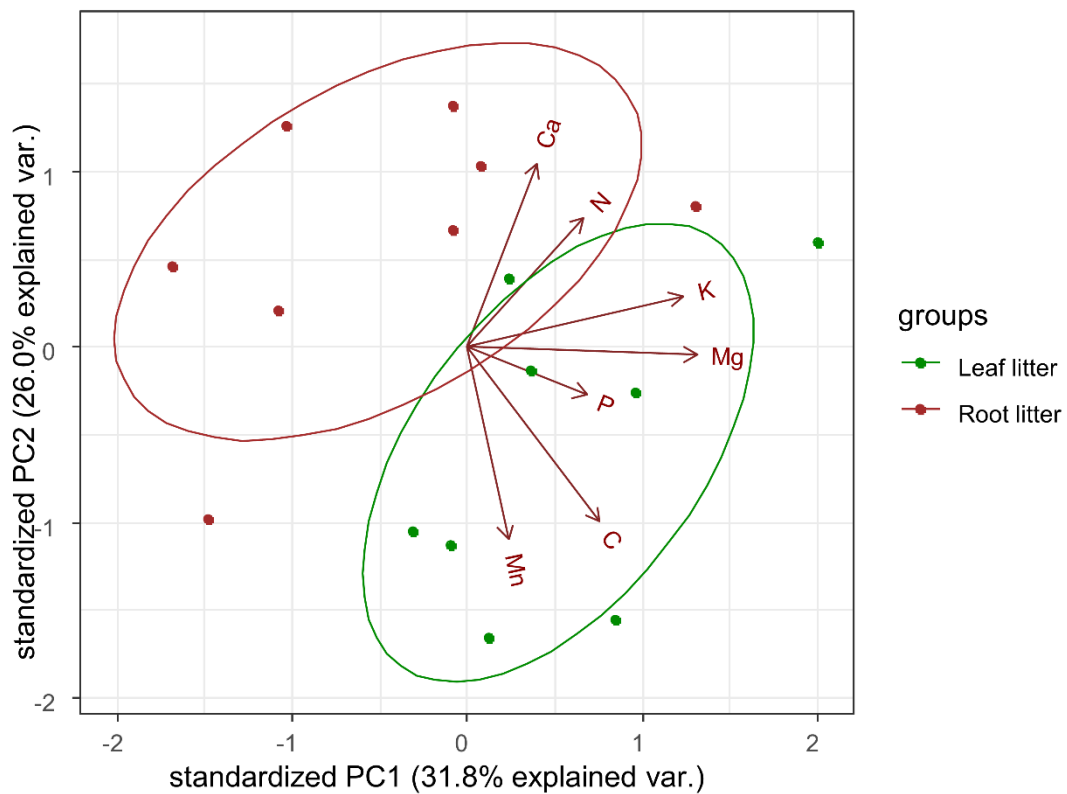
## Appendix D | Prolonged summer drought decreases C release but does not change N release during fine root and leaf litter decomposition in beech forests



**Fig. D5. 1** (a) Study area and block of the four sites in southeAstern France. Northern point Points (sites S1, S2) represent sites with European beech (*Fagus sylvatica*) and silver fir (*Abies alba*) forests. Southern points (sites S3, S4) represent southern sites with European beech and pubescent oak (*Quercus pubescens*) forests. The sites are located in the following mountain ranges of the French pre-Alps: Vercors (S1), Ventoux (S2), Grand Lubéron (S3), Sainte-Baume (S4) (b) Schematic representation of a site with two blocks per site. Each block comprises of one pure beech plot and one mixed plot. (c) Representation of an individual plot. Plots are circular with a central plot area (10 m radius) and a buffer zone (7.5 m radius). The red rectangles indicate rain-exclusion in summer.



**Fig. D5. 2** The soil moisture (%) during the summers of 2018 and 2019 when rainout shelters were installed for each site (Vercors, Mont Ventoux, Lubéron and Ste Baume) and block (block 1, and block 2). The percentage values on top of each bar represent the reduction in soil moisture under the rainout shelters, relative to the non-exclusion zone, for each site and block.



**Fig. D5. 3** Principal Component Analysis (PCA) of initial leaf and root litter quality of beech trees, collected from two blocks per site (Vercors, Mont Ventoux, Lubéron, Ste Baume). Litter quality includes the following elements: N, C, P, Ca, K, Mg, Mn. A PERMANOVA test showed that initial quality was different between the two litter types ( $p = 0.001$ ).

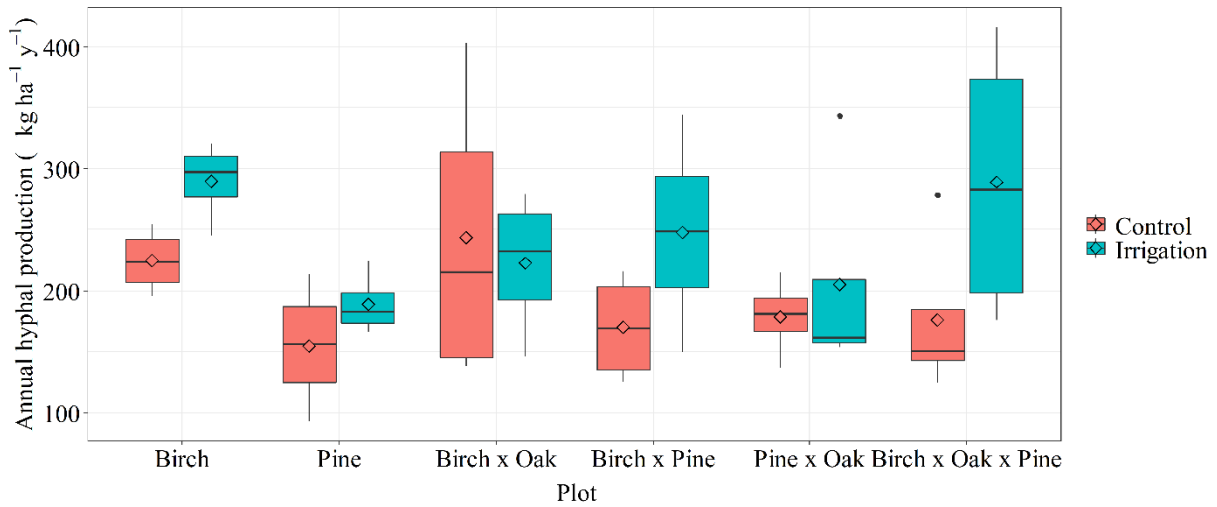
**Table D5. 1** Overview of stand basal area of the tree species present in the pure and mixed tree stands, across the four sites (Vercors, Mont Ventoux, Lubéron, Ste Baume).

Site	Block	Plot	G m <sup>2</sup> ha <sup>-1</sup>			
			Fagus	Abies	Quercus	Other *
Vercors	1	pure	39.6			
Vercors	1	mix	19.1	20.4		0.5
Vercors	2	pure	37.9	0.5		
Vercors	2	mix	15.9	21.9		
Ventoux	1	pure	45.8	3.5		
Ventoux	1	mix	13.3	35.5		0.5
Ventoux	2	pure	50.9	12.1		
Ventoux	2	mix	40.2	31.4		
Lubéron	1	pure	34.0		3.6	3.6
Lubéron	1	mix	21.1		8.5	6.2
Lubéron	2	pure	41.4			2.2
Lubéron	2	mix	11.4		11.5	4.8
Ste Baume	1	pure	42.6			13.6
Ste Baume	1	mix	22.1		17.7	16.3
Ste Baume	2	pure	19.6		0.7	8.7
Ste Baume	2	mix	22.1		13.7	8.1

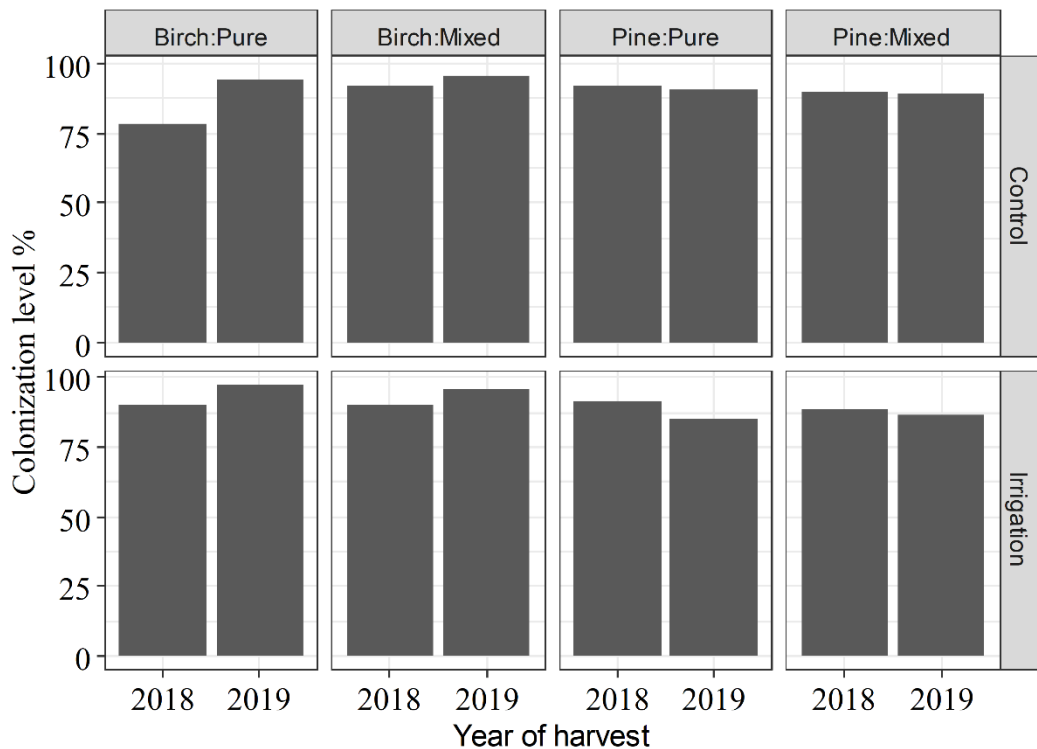
\* For the two northern sites: other species Acer, Sorbus aria, Taxus baccata, Fraxinus excelsior

for the two southern sites: other species Acer opalus, Sorbus torminalis, Sorbus aria, Taxus Baccata, Cornus mas

## Appendix E | Preliminary results



**Fig. E6. 1** Boxplot with medians (-), and means ( $\diamond$ ) of annual hyphal productivity ( $\text{kg ha}^{-1} \text{y}^{-1}$ ), across all plots of the ORPHEE experimental design, and comparison between control (in red) and irrigated conditions (in blue).

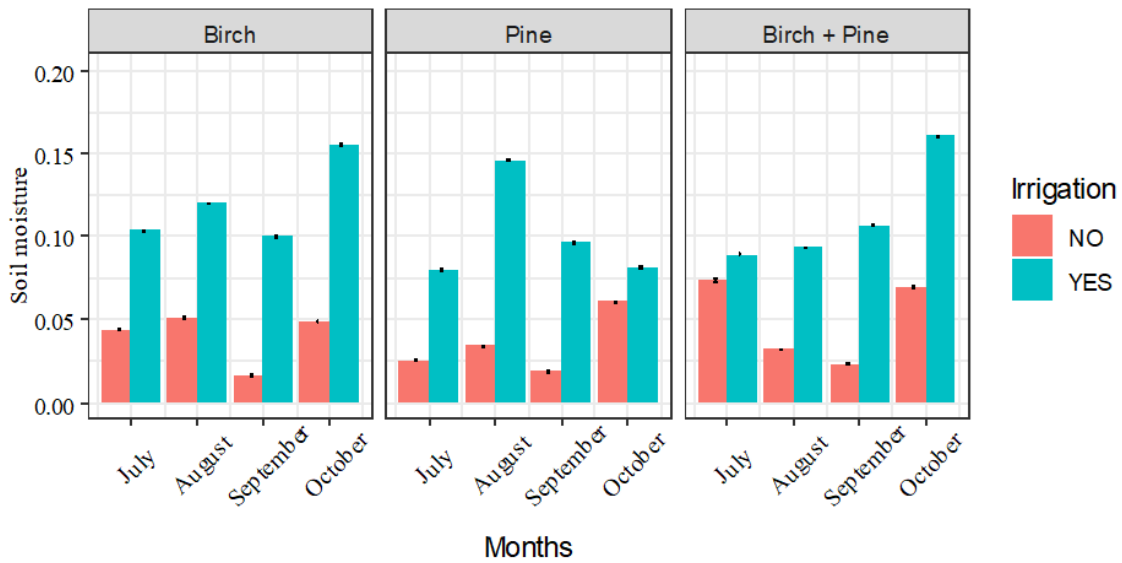


**Fig. E6. 2** Multifaceted barchart of root tip colonization level (%) between birch and pine trees, grown in pure and mixed stands. Colonization levels under control conditions are depicted at the top panels, and under irrigated conditions at the bottom panels.





**Fig. E6. 3** Stacked barcharts depicting exploration types of birch and pine, grown in pure and mixed stands. Results under control conditions is depicted at the top panels, and under irrigated conditions at the bottom panels.



**Fig. E6. 4** Effect of irrigation on soil moisture for the months receiving the irrigation treatment (July – August – September – October) for the year of 2019. Barchart presents means with standard errors

**Titre** : La biodiversité dans les écosystèmes forestiers est-elle susceptible de modifier les processus de production de racines et de décomposition des litières dans un contexte de changement climatique ?

**Résumé** : L'hypothèse de l'assurance prévoit que les forêts composées de mélanges d'espèces d'arbres pourraient mieux résister aux conditions environnementales stressantes que les forêts composées d'une seule espèce d'arbre. La majorité des travaux antérieurs ont testé cette hypothèse en se focalisant sur la productivité et les variables de réponse associées sans prendre en compte les processus souterrains. L'objectif principal de ma thèse était d'étudier l'effet de la diversité des espèces d'arbres sur les processus souterrains impliqués dans la décomposition des racines à travers des gradients climatiques. J'ai émis l'hypothèse que le mélange d'espèces ayant des systèmes racinaires contrastés entraînerait une faible compétition souterraine, et se traduirait par la production de plus biomasse de racines fines. En outre, j'ai émis l'hypothèse que les racines ayant des caractéristiques chimiques et morphologiques contrastées dans les peuplements mixtes se décomposent plus rapidement. Dans des conditions de stress hydrique, j'ai émis l'hypothèse d'une décomposition plus lente mais d'une atténuation des mélanges d'arbres sur la décomposition en raison de l'amélioration des conditions micro-environnementales. Pour tester ces hypothèses, j'ai examiné la variation des caractéristiques fonctionnelles des racines et leurs conséquences sur les flux de C, N et P à l'échelle de l'écosystème à travers l'étude de : 1) la ségrégation verticale des racines et la biomasse des racines fines, 2) la dynamique des racines fines et les flux de nutriments associés et 3) la décomposition des racines fines et des feuilles mortes. Dans ce cadre, deux expériences de terrain ont été réalisées, l'une avec une expérience de plantation d'arbres de 10 ans avec du bouleau et du pin près de Bordeaux (expérience ORPHEE), la seconde le long d'un gradient latitudinal de forêts de hêtres matures dans les Alpes françaises (expérience BIOPROFOR).

Les résultats obtenus montrent que les racines de bouleaux et de pins présentaient une distribution verticale similaire et une biomasse souterraine similaire de racines dans les mélanges d'arbres par rapport aux monocultures, contrairement à ma première hypothèse. Cependant, l'attribution plus importante du pin mais pas du bouleau à la croissance des racines dans les horizons du sol supérieur dans des conditions moins limitatives en eau suggère des conditions localement favorables qui peuvent conduire à une compétition asymétrique à la profondeur du sol. De plus, la production et la décomposition des racines fines étaient similaires dans les mélanges et dans les monocultures, en contradiction avec ma deuxième hypothèse. Il est intéressant de noter que les racines de bouleau, mais pas les racines de pin, ont libéré du P pendant leur décomposition, ce qui suggère un rôle important du bouleau dans le cycle du P et pour la nutrition en P des arbres sur ces sols sableux limités en P. Conformément à ma troisième hypothèse, j'ai observé une décomposition plus lente de la litière de feuilles et des racines fines en réponse à une sécheresse estivale prolongée, tout au long du gradient latitudinal dans les Alpes. Cependant, cette décomposition plus lente sous la sécheresse n'a pas été atténuée dans les peuplements forestiers à essences mixtes par rapport aux peuplements à essences uniques. Il est intéressant de noter qu'il y a une libération nette d'azote dans les racines fines en décomposition mais pas dans la litière de feuilles en décomposition, ce qui suggère un rôle distinct des racines fines dans le cycle de l'azote. En conclusion, j'ai constaté que le mélange des espèces d'arbres n'atténue pas les effets négatifs du changement climatique. Cette thèse démontre que la promotion de mélanges peut toujours être bénéfique pour au moins une des espèces d'arbres mélangées, par l'ajout d'espèces, car une espèce d'arbre peut en faciliter la nutrition minérale d'une autre par des flux souterrains de N et de P.

**Mots clés** : biodiversité, écosystèmes forestiers, changement climatique, qualité de la litière, production de racines fines, cycle des nutriments

**Title :** Tree diversity effects on root production, decomposition and nutrient cycling under global change

**Abstract :** The insurance hypothesis predicts that forests with tree species mixtures may resist better to stressful environmental conditions than forests composed of only one tree species. Most of the currently available literature tested this hypothesis for aboveground productivity and its related response variables, but less is known about belowground processes. In my PhD thesis, I studied the drivers of belowground productivity and decomposition across climatic gradients and how they are affected by tree mixtures. I hypothesized that mixing of tree species with contrasting rooting patterns and fine root morphologies, would result in a release of competitive pressure belowground, and translate into higher fine root standing biomass and increased fine root productivity. Moreover, I hypothesized that roots with contrasting chemical and morphological characteristics in mixed stands would decompose faster, which may be particularly important under nutrient-limited conditions. Under water-limiting conditions, such as during extreme summer drought, I hypothesized overall slower decomposition but an attenuating effect of tree mixtures on decomposition due to improved micro-environmental conditions, in particular for leaves, since roots decompose in a more buffered soil environment. To test these hypotheses I examined the variation in tree root functional traits (across- and within-species), and its consequences for fluxes of C, N and P at the ecosystem scale. I addressed three main objectives and associated research questions to quantify the interactive effect of tree mixtures and climate on: 1) vertical root segregation and fine root standing biomass, 2) fine root dynamics and their associated nutrient fluxes and 3) fine root- and leaf litter decomposition. I could benefit from two different field experiments for my work, one with a 10-year-old tree-plantation experiment with birch and pine close to Bordeaux (ORPHEE experiment), the second along a latitudinal gradient of mature beech forests in the French Alps (BIOPROFOR experiment).

I observed that roots from the birch and pine tree-plantation showed similar vertical distribution and similar belowground root standing biomass in tree mixtures compared to monocultures, contrary to my first hypothesis. However, the greater allocation of pine but not of birch to root growth within the top soil horizons under less water-limiting conditions suggests locally favourable conditions that may lead to soil depth-specific asymmetric competition. In the same experiment, fine root production and decomposition were similar in mixtures and in monocultures, in contradiction with my second hypothesis. Moreover, I did not observe any interactive effects of tree mixtures with stand density or water availability. Interestingly though, birch roots, but not pine roots released P during root decomposition, which suggests an important role of birch in the P-cycle and for P nutrition of trees on these P-limited sandy soils. In line with my third hypothesis, I observed a slower decomposition of leaf litter and fine roots in response to reinforced and prolonged summer drought, irrespective of the position along the latitudinal gradient in the Alps. However, this slower decomposition under drought was not attenuated in forest stands with mixed tree species compared to single species stands. Compared to leaf litter, fine roots decomposed slower and released less C. Interestingly, I found a net N release in decomposing fine roots but not in decomposing leaf litter, which suggests a distinct role of fine roots in the N cycle. In conclusion, I found that mixing tree species did not attenuate negative effects of climate change. However, this thesis demonstrates that promoting mixtures can still be beneficial for at least one of the admixed tree species, through species addition (i.e., complementing one tree species with another tree species), as one tree species may facilitate another via belowground fluxes of N and P.

**Keywords:** biodiversity, forest ecosystems, climate change, litter quality, fine root production, nutrient cycling

---

### Unité de recherche

Institut National de la Recherche Agronomique – Bordeaux Sciences Agro  
UMR 1391 Interactions Sol Plante Atmosphère  
71 Avenue Édouard Bourlaux, 33140 Villenave d'Ornon, France