PLANT MIXTURE EFFECTS ON FINE-ROOT BIOMASS AND ITS FUNCTIONAL TRAITS

By

Sai Peng

A Dissertation Submitted in
Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Forest Sciences

Faculty of Natural Resources Management
Lakehead University
January 20223

DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

In presenting this thesis in partial fulfillment of the requirements for the PhD degree at Lakehead University in Thunder Bay, I agree that the University will make it freely available for inspection.

This thesis is made available by my authority solely for the purpose of private study and research and may not be copied or reproduced in whole or in part (except as permitted by the Copyright Laws) without my written authority.

Name: Sai Peng

Signature:

Date: Jan. 20th, 2023

ABSTRACT

Fine roots play a critical role in the uptake of soil water and nutrients and make a crucial contribution to the carbon pool through their fast turnover rate and subsequent decay. Plant trait-based approach enables us to understand the plant growth strategy via the ratio of benefit to cost of carbon investment, especially under global change. Increasing biodiversity loss threatens the ecosystem productivity, which could further influence the fine-root functions. However, previous studies have reported inconsistent responses of fine-root biomass and root functional traits to the effects of plant species richness and functional trait dissimilarity (such as contrasting shade and drought tolerance). The purpose of this dissertation is to reveal the possible mechanisms of different responses of fine-root biomass (FRB) and root functional traits to plant species richness and functional trait dissimilarity, and further to test whether these mixture effects would change with water availability.

In my first study, I examined the effects of plant diversity on FRB and root functional traits (root/shoot ratio, community-weighted mean rooting depth (WRD), root length density (RLD), specific root length (SRL), root mean diameter (RMD) and root nitrogen content). By analyzing a global meta-analysis of 852 paired observations from 103 published studies, I found that the effects of species mixtures were highly dependent on species richness in mixtures, stand age, and soil depth. The positive effects of species mixtures on FRB increased with species richness, soil depth, and mean annual temperature. Species mixture effects on RLD shifted from negative to positive with increasing stand age and soil depth and with decreased temperatures. The effects of species mixtures on SRL shifted from positive to negative with increasing species richness and soil depth, and from negative to positive with increasing stand age. My meta-analysis highlights that the community-level consequences of changes in plant diversity on fine

root traits are not consistent, and that predicting these consequences requires taking into account the extent of changes in plant species richness, stand age, soil depth investigated, and background climates.

Apart from the number of plant species, the effects of species mixtures on root attributes could also be influenced by the plant functional trait dissimilarity, i.e., the variation magnitudes of shade and drought tolerance, growth rate, and leaf habit (coniferous, broadleaf, evergreen, deciduous) within the coexisting species. Therefore, in my second study, I compiled a meta-analysis of 652 paired observations to test the effects of different functional trait dissimilarity (presence and absence of contrasting shade tolerance (CST), contrasting drought tolerance (CDT), contrasting plant growth rate (CGR) and leaf habit dissimilarity (LHD)) on FRB and root functional traits (WRD, RLD and SRL) in plant mixtures. My results showed that, on average, FRB and SRL both differed with absence and presence of contrasting functional traits, whereas those on WRD and RLD were not. The effect of presence of CST on FRB was higher than the absence of CST, and the mixture effect decreased with stand age with CST. The mixture effects on FRB and SRL were both higher when CDT was present than absent. I also found increased mixture effects with soil depth on FRB when CDT was absent or on RLD when CDT was present, respectively. Plant mixtures with presence of contrasting plant growth rate had a higher FRB than absence, while the mixture effects on FRB and SRL were both lower in mixed-wood forests with high LHD than medium and low levels. My second study emphasizes the determinant role of plant functional trait dissimilarity in FRB and SRL.

Finally, my third study aimed to answer whether the effects of tree species mixtures on FRB and root functional traits were altered with changing water availability. This was accomplished by sampling soils to collect fine roots from natural boreal forests that were

dominated by *Populus tremuloides* and *Pinus banksiana*, respectively, and their relatively even mixtures under water addition (+25%), ambient water, and water reduction (-25%). I found a positive effect of tree mixtures on FRB in August, under ambient precipitation (41%), with more pronounced increases under water reduction (89%) and under water addition (71%), but species mixtures had no effects on FRB in October. Root surface area, fine-root volume, and RLD responded to species mixtures similarly to those of FRB. Whereas root tissue density (RTD), root mean diameter (RMD), length ratio (LR, ratio of root length with Ø < 0.5 mm to the total root length), SRL and specific root area had weak responses to plant mixtures and water treatment. My results suggest that plant mixtures increase both fine root biomass and the soil filling of root surface area and volume to improve resource uptake, in support of the enhanced primary production in mixtures with both increased and decreased water availability.

My findings suggest plants could invest in more fine roots and shift root traits for higher water and nutrient uptake in a more diverse plant community. When interpreting these positive species mixture effects on fine roots, we should incorporate the number of species in mixtures, stand age, soil depth, background environment, and especially, plant functional trait dissimilarity. My empirical results provide guidance for forest managements focusing on belowground carbon sequestration, regarding to precipitation change.

Keywords: meta-analysis, fine-root biomass, root functional traits, plant diversity, contrasting shade tolerance, contrasting drought tolerance, contrasting plant growth rate, leaf habit dissimilarity, water availability, resource uptake efficiency, resource uptake capacity

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my supervisor, Dr. Han Chen and Dr. Jian Wang, for their guidance, support, assistance and mentorship throughout the development of this dissertation. I would also like to extend my sincere thanks to my committee members, Dr. Brian McLaren, Dr. Seung-Il Lee and Dr. Don Henne, and external examiner Dr. Christian Messier for their instruction and insightful comments. I am also thankful to Dr. Mathew Leitch for his help for the field work and my comprehensive exam.

I would like to thank my lab colleagues that have had major contributions to my work and have supported me throughout: Xinli Chen, Chen Chen, Eric Searle, Yili Guo, Auldrey Lopo, Connor Jones, Neil Hills, Bilei Gao, Aaron Brecka, Masumi Hisano, Wenhuan Xu, Yakun Zhang. Also, I would like to thank Eva Scollie and Jennifer Manion for the help smooth my TA work and program research. Specially thanks for Lakehead University and City of Thunder Bay.

Lastly but foremost, I could not have undertaken this journey without my lovely wife, also my lab mate, Yakun Zhang, for her great encouragement for my study and life, and the on coming baby. Words cannot express my gratitude to my parents, Jiaren Peng and Taozhu Li, who both are humble farmer in the small village, for their love and support throughout the writing of this dissertation and my graduate studies. Thanks should also go to my elder brother and sister, who take care of my parents and stand behind me.

Financial support for this study was provided by Natural Sciences and Engineering Research Council of Canada (RGPIN-2014-04181, STPGP428641, RTI-2017-00358, and STPGP506284).

NOTE

This is a manuscript-based thesis. The chapters were written to suit the submission requirements of the targeted journals. Formatting and reference styles may differ. Since individual chapters reflect the joint contributions of myself and my academic supervisor, I use “we” instead of “I” for individual manuscripts.

Chapters:

2. **Peng, Sai**, and Han YH Chen. "Global responses of fine root biomass and traits to plant species mixtures in terrestrial ecosystems." *Global Ecology and Biogeography* 30.1 (2021): 289-304.

3. **Peng Sai**, Han Y.H. Chen. Plant functional trait dissimilarity as drivers of plant mixture effects of fine-root biomass and trait variations. (Preparing to submit to *Functional Ecology*)

4. **Peng Sai,** Xinli Chen, Yakun Zhang, Chen Chen, Yili Guo, Han Y.H. Chen. Altered precipitation regulates tree mixture effects on fine-root biomass and traits in young boreal forests. (Preparing to submit to *New Phytologist*)

Chapter 1. GENERAL INTRODUCTION

Fine roots, defined as diameter less than 2 mm, and are a major natural conduit of water and nutrient cycling, and play a critical role in regulating biogeochemical cycles via water and nutrient absorption, resource storage, high turnover rate and the subsequent fast decomposition (McCormack *et al.*, 2012; Clemmensen, 2013). Although fine-root biomass is a small part of the total plant biomass, fine-root production represents around 22% of the global terrestrial net primary production because of its high turnover rate (McCormack *et al.*, 2015; Erktan *et al.*, 2018). Plant trait-based methods are used to better understand how plant community changes and global changes impact ecosystem process (McCormack *et al.*, 2017; Choat *et al.*, 2018; Ma *et al.*, 2018), which can be projected to understand trade-offs underlying the ecological strategies of plant growth and survival (Wright *et al.*, 2004; Ebeling *et al.*, 2014; Reich, 2014). Fine-root functional traits could serve as drivers of plant and ecosystem functioning (Bardgett *et al.*, 2014; Freschet *et al.*, 2021), and root functional traits have been viewed as the indices for resource uptake efficiency and capacity, such as specific root length and root length density. Specific root length (SRL, root length divided by root dry weight, m g^{-1}) represent the root length contacting with water and nutrient per carbon invested, higher SRL increases the efficiency of soil exploration at the cost of lower root lifespan (Eissenstat *et al.*, 2000; McCormack *et al.*, 2012). Root length density (Root length per soil volume, m m^{-3}) determines the spatial configuration of root system as a whole, and relates to the capacity of water and nutrient uptake (Newman, 1966; Guderle *et al.*, 2018; Archambault *et al.*, 2019). It could enable us to know how plant uptake soil resources via studying root functional traits.

The ongoing and unprecedent biodiversity loss due to land use influences the functioning of ecosystems such as resource capture and biomass production (Cardinale *et al.*, 2012). Despite the critical function of fine roots in numerous ecological processes, it is still uncertain that how fine-root biomass (FRB) and root functional traits respond to the plant diversity. Previous studies have reported inconsistent responses of FRB and functional traits with regard to plant mixture (Meinen *et al.*, 2009; Smith *et al.*, 2013; Bennett *et al.*, 2016; Finér *et al.*, 2017; Germon *et al.*, 2017; Guderle *et al.*, 2018; Oram *et al.*, 2018; Archambault *et al.*, 2019; Zwetsloot *et al.*, 2019; Altinalmazis-Kondylis *et al.*, 2020; Zwetsloot & Bauerle, 2021). To elucidate the effects of diversity effects on FRB and root functional traits, I conducted a meta-analysis to tested whether these divergent outcomes result from different species richness in plant mixtures, stand age, soil depth and background environment including ecosystem type and mean annual temperature and precipitation. Different plant functional trait dissimilarity in plant species mixtures (variation magnitudes of shade and drought tolerance, heterogeneous plant growth rate, leaf habit dissimilarity) could influence the niche occupancy, resource partitioning and enrichment (Silvertown, 2004; Searle & Chen, 2019). Therefore, I extended my first study to answer how FRB and root functional traits respond to plant functional trait dissimilarity in mixtures, and tested it across various stand age, soil depth and background environment.

Due to anthropogenic activity, there has been an increase in the frequency and intensity of extreme climatic events such as drought and heavy precipitation (O'Neill *et al.*, 2017). These events could affect soil water availability and plant composition, with profound consequences for processes and functioning of ecosystem (Fauset *et al.*, 2012; Choat *et al.*, 2018; Engelhardt *et al.*, 2018). Increasing precipitation generally drives more fine-root investment to uptake soil resources derived from elevated water availability (Wang *et al.*, 2020), whereas decreasing precipitation could decrease fine-root growth (Zhou *et al.*, 2018). Plant mixtures are proposed to mitigate the negative effects of climate change (e.g., reduced water availability) and enhance the positive effects of climate change (e.g., increased water availability) on forest biomass dynamics (Hisano *et al.*, 2018). To test this hypothesis on fine-root biomass and root functional traits, I conducted an experiment with manipulated precipitation. I collected soil sample in boreal forests dominated by *Populus tremuloides* and *Pinus banksiana*, respectively, and their relatively even mixtures under water addition (+25%), ambient water, and water reduction (-25%).

Chapter 2. GLOBAL RESPONSES OF FINE ROOT BIOMASS AND TRAITS TO PLANT SPECIES MIXTURES IN TERRESTRIAL ECOSYSTEM

2.1 Abstract

Aim Fine root traits underpin terrestrial ecosystem functioning. Despite ongoing plant diversity loss due to anthropogenic activities, our understanding of the effects of plant diversity on fine root traits remains elusive. We addressed: (1) Do fine roots modify their traits in response to species mixtures? (2) Do these responses change with species richness in mixtures, stand age, and soil depth? (3) Do plant-mixture induced responses of root traits differ across terrestrial ecosystems?

Location Global.

Time period publication years: 1985-2019.

Major taxa studied Plants.

Methods We conducted a global meta-analysis of 852 paired observations from 103 published studies to assess the effects of species mixtures on fine root biomass and traits (including root/shoot ratio, community-weighted mean rooting depth, root length density, specific root length, mean root diameter and root nitrogen content).

Results We found that the effects of species mixtures were highly dependent on species richness in mixtures, stand age, and soil depth. The positive effects of species mixtures on root biomass increased with species richness, soil depth, and mean annual temperature. Species mixture effects on root length density shifted from negative to positive with increasing stand age and soil depth and with decreased temperatures. The effects of species mixtures on specific root length shifted from positive to negative with increasing species richness and soil depth, and from negative to positive with increasing stand age.

Main conclusions Our meta-analysis highlights that the community-level consequences of changes in plant diversity on fine root traits are not consistent, and that predicting these consequences requires taking into account the extent of changes in plant species richness, stand age, soil depth investigated, background climates, and importantly, particular fine root traits.

Keywords: capacity, efficiency, fine root traits, meta-analysis, resource uptake, species diversity.

2.2 Introduction

The ongoing and increasing exploitation of terrestrial and marine resources, direct exploitation of organisms, as well as climate change, are reducing plant diversity (IPBES, 2019), which negatively impacts a diverse array of ecological functions that are critical for sustaining humanity (Cardinale et al., 2012). In terrestrial ecosystems, increased aboveground productivity with plant species diversity is accompanied by greater fine root biomass and productivity (Barry et al., 2020; Ma & Chen, 2016; Zhang, Chen & Reich, 2012). This suggests that plant species mixtures require additional soil resources (water and nutrients) and/or increased resource use efficiency to support higher above- and belowground productivity, in contrast to corresponding monocultures. Plant fine root biomass (or root mass per unit soil volume) and its architectural, morphological, and chemical traits influence their soil resource uptake capacity and efficiency (Bardgett, Mommer & De Vries, 2014; Kiba & Krapp, 2016; Kulmatiski, Adler, Stark & Tredennick, 2017; Reich, 2014). However, although plant diversity continues to decline (IPBES, 2019), the global effects of plant diversity on fine root biomass and traits, which underpin terrestrial productivity, remain uncertain.

Plant species mixtures can increase fine root biomass and simultaneously alter multiple fine root traits to increase soil resource uptake capacity and efficiency. However, empirical studies have reported contrasting responses of root traits to plant species mixtures (Table 2-1). For example, previous studies have reported positive (Ma, Chen, Kumar & Gao, 2019), neutral (Domisch, Finer, Dawud, Vesterdal & Raulund-Rasmussen, 2015), and negative (Bessler et al., 2009; Martin-Guay, Paquette, Reich & Messier, 2019) effects of plant species mixtures on roots to shoots, or aboveground to belowground biomass ratios. Species mixtures allocate more fine roots into deeper soil layers where far fewer roots compete for nutrients (Ma & Chen, 2017; Oram et al., 2018), and thus have a greater community-weighted mean rooting depth; however, others have reported no or opposite responses (Brassard et al., 2013; Siebenkäs & Roscher, 2016). At the individual root level, specific root length may increase in species mixtures (Shu et al., 2018), as higher specific root length increases resource uptake efficiency for a given unit of biomass investment (Ostonen et al., 2007). However, other researchers have reported insignificant (Bauhus, Khanna & Menden, 2000; Gould, Quinton, Weigelt, De Deyn & Bardgett, 2016; Siebenkäs & Roscher, 2016), or even negative (Baxendale, Orwin, Poly, Pommier & Bardgett, 2014; Salahuddin et al., 2018) effects of plant diversity on specific root length. Similarly, contrasting results have been reported for the effects of plant species mixtures on root length density, mean root diameter, and root nitrogen content (Table 2-1). These divergent findings may have resulted from differences in the species richness in mixtures (hereafter species richness), stand age, soil layers, as well as background environments or ecosystem types, as previously reported for the responses of soil carbon (Chen et al., 2019), soil respiration (Chen & Chen, 2019), and soil microbial communities (Chen, Chen, Chen & Huang, 2019).

Table 2-1 Fine-root traits and resource uptake strategies in response to plant species mixtures. When high soil resources are required, fine roots can adjust their traits for high capacity and/or efficiency for soil resource uptake. A higher root: shoot ratio, deeper community-weighted mean rooting depth, and higher root length density could increase water and nutrient uptake capacity, a higher specific root length and root nitrogen content, and thinner diameter could increase water and nutrient uptake efficiency.

Trait	Definition and comments	Resource uptake strategy	Mixture effects		
			positive	negative	neutral
Root system					
Root/shoot (R/S)	Root to shoot biomass ratio, representing the strategy to adjust investments between above- and belowground with changes in the environment and life stage (Bessler et al., 2009; Ma et al., 2019).	Capacity	(Ma et al., 2019)	(Bessler et al., 2009; Martin-Guay et al., 2019)	(Domisch et al., 2015)
Architecture					
Community-weighted mean rooting depth (WRD, cm)	The centroid of weighted root depth, describing the vertical distribution of roots (Archambault et al., 2019).	Capacity	(Ma & Chen, 2017; Oram et al., 2018)	(Brassard, Chen, Bergeron & Pare, 2011; Brassard et al., 2013)	(Siebenkäs & Roscher, 2016)
Root length density (RLD, m m^{-3})	Total fine root length per unit soil volume, reflecting the capacity of root systems for resource uptake (Guderle et al., 2018).	Capacity	(Shu et al., 2018)	(Salahuddin et al., 2018)	(Bauhus et al., 2000; Gould et al., 2016; Siebenkäs & Roscher, 2016)

Morphology					
Specific root length (SRL, m g^{-1})	Root length per root dry mass, absorptive efficiency relative to carbon investment (Ostonen et al., 2007).	Efficiency	(Shu et al., 2018)	(Baxendale et al., 2014; Salahuddin et al., 2018)	(Gould et al., 2016)
Mean root diameter (MRD, mm)	Mean diameter of fine root (< 2 mm) components. Thicker diameters facilitate root penetration of denser soil; thinner diameters reduce dependence on mycorrhizal symbiosis (Ma et al., 2018).	Efficiency	(Salahuddin et al., 2018)	(Gould et al., 2016)	(Beyer et al., 2013)
Chemistry					
Root nitrogen content (RN, %))	RN supports metabolic activity, including nutrient and water transport, enzyme functioning and mycorrhizal symbiosis (Bloom et al., 1985; Weemstra et al., 2016). In the shared soil, RN decreases with competition intensity due to nitrogen depletion (Hajek, Hertel & Leuschner, 2014).	Efficiency	(Bauhus et al., 2000)	(Hajek et al., 2014; Salahuddin et al., 2018)	(Callaway, Sullivan & Zedler, 2003)

Previous studies have reported that aboveground and belowground productivity in species mixtures are, on average, 23.7 % and 28.4 % higher than in monocultures, respectively (Ma & Chen, 2016; Zhang, Chen & Reich, 2012). This overyielding (i.e., productivity in mixtures over the average productivity of the corresponding monocultures) increases with species richness (Ma & Chen, 2016; Zhang, Chen & Reich, 2012). Enhanced plant productivity in mixtures is expected to increase the demand for water and nutrients, which leads to alterations in fine root traits toward higher capacity and efficiency. For instance, as an indicator of ecosystem carbon and nutrient cycling (Bardgett et al., 2014), root length density is higher in more diverse plant communities (Gould et al., 2016) (Table 2-1). At the same time, the higher resource demands of species-rich communities might be met by changes in other root traits toward higher resource uptake efficiency (Table 2-1). Thin roots are more active in exploring soil through the more efficient utilization of photosynthetic carbon (Ma et al., 2018), while root nitrogen is employed as a proxy for the nutrient acquisition rate (Bloom, Chapin & Mooney, 1985; Reich et al., 2008). Therefore, we hypothesized that the root/shoot ratio, community-weighted mean rooting depth, root length density, specific root length, and root nitrogen content would be higher, while the mean root diameter would be smaller in species mixtures than their averages in the corresponding monocultures. Moreover, we anticipated greater trait alterations in more diverse species mixtures due to the increased demand for soil water and nutrients associated with higher productivity.

The effects of species mixtures on fine root traits may change with stand age. Under-utilized soil space and other resources in young stands often lead to an insignificant diversity effect on fine root biomass and productivity (Ma & Chen, 2017). In mature stands, increasing interspecific complementarity and decreasing functional redundancy increases the positive impacts of species mixtures on standing biomass and productivity (Reich et al., 2012), thus, increasing water and nutrient demands. Therefore, we expected greater trait alterations in species mixtures over time due to higher demands for soil water and nutrients associated with higher mixture effects on productivity. Alternatively, the higher fine-root production in mixtures might enhance carbon inputs into the soil through the high turnover rates of fine roots over time (Steinbeiss et al., 2008), which might promote mineralization and enhance the availability of nutrients (Fornara, Tilman & Hobbie, 2009). Consequently, the high availability of soil nutrients might counteract their high demand in older stands, resulting in no changes in the effects of species mixtures on fine-root traits with stand development.

The effects of species mixtures on fine-root morphological and architectural traits may differ between soil layers. For instance, the mixture effects on root length density can increase with soil depth (Wang et al., 2014). However, the positive effects of tree species mixtures on specific root length and mean root diameter are consistent across soil layers, down to 17 m in tropical plantations (Germon et al., 2017). Moreover, soil depth-dependent responses to species mixtures may increase with species richness, as the positive effects of species mixtures on fine root biomass increase in more diverse plant communities (Lei, Scherer-Lorenzen & Bauhus, 2012; Steinbeiss et al., 2008). The uncertainty of fine-root traits associated with soil depth in mixtures hampers the appreciation of fine root resource uptake strategy.

The effects of species mixtures may be altered through the background environment. Climatic parameters such as temperature and precipitation are crucial factors on fine root traits (Freschet et al., 2017); however, it remains uncertain how the effects of species mixtures on root traits change with different climates. Plant-plant interactions can become more positive in water- or temperature-limiting environments because the strength of abiotic facilitation is expected to increase under environmental stress, i.e., stress gradient hypothesis (Maestre, Callaway, Valladares & Lortie, 2009; Wright, Wardle, Callaway & Gaxiola, 2017). However, the positive relationship between tree diversity and forest productivity is stronger in resource-rich than resource-poor environments (Hisano & Chen, 2020). Moreover, the effects of plant diversity and their temporal trends between forests and grasslands are hypothesized to be different primarily due to variable species or individual recruitment rates (Forrester & Bauhus, 2016). Nevertheless, it remains unclear whether the effects of species mixtures on fine-root traits diverge between ecosystem types or climates.

Here, we compiled data from 103 studies, including deliberate experiments and data collected in naturally assembled environments, to examine the effects of species mixtures on fine-root traits associated with their resource uptake capacity and efficiency. Specifically, we endeavoured to address the following: (1) How do fine roots modify their traits in response to species mixtures? (2) Are these responses altered with species richness, stand age, and soil depth? (3) Do plant-mixture induced responses of root traits change with ecosystem types and/or background climates? We hypothesized that: (1) The root: shoot ratios, community-weighted mean rooting depths, root length density, specific root length and root nitrogen content would be higher, and that the mean root diameter would be smaller in species mixtures than their averages in the corresponding monocultures; (2) The root-trait alterations in response to plant species mixtures would increase with species richness and over time. We tested the above hypotheses across a wide range of soil depths and a variety of ecosystem types and environmental conditions.

2.3 Materials and Methods

2.3.1 Data collection

Using Google Scholar and Web of Science, we identified peer-reviewed papers through the combination of several search keywords, including (plant diversity OR species diversity OR species mixture OR species mixture OR mix plant OR polyculture OR intercrop), and (fine root OR root biomass OR root density OR root length density OR root/shoot OR biomass allocation OR specific root length OR SRL OR root diameter OR root nitrogen), up to 1st July 2019. The following criteria were applied for the selection of publications: (1) Studies were purposely implemented to separate the effects of plant species diversity from other factors, such as water treatment and nutrition addition (2) Values of fine-root traits could be extracted directly from the text, tables, and figures (3) Genotype mixtures with species were not included (4) Each plant species mixture was compared to corresponding monocultures.

For each study, we extracted the fine-root biomass at different soil depths to calculate the vertical distribution. Fine-root traits were also collected, including root to shoot biomass ratio, weighted rooting depth, root length density, specific root length, mean root diameter, and root nitrogen content. For studies that reported root traits by root order or diameter class, we calculated the community-level means of these values. Species richness in plant mixtures, stand age for forests or experimental age for grasslands and croplands, and species proportions in mixtures, were recorded from the original publications. We treated the different locations, mixture ratios, or abiotic treatments with independent controls involved in given publications as distinct comparisons (studies) in those publications. In total, 103 studies with 852 paired observations from 64 publications were selected for this meta-analysis (Fig. S2-1; Table S2-1).

The proportions of each species in mixtures were based on basal areas or stem densities in forests, seeds sown in grasslands and croplands, and the number of individuals in containers. Forest stand ages were recorded from the descriptions of sites in the publications, whereas the experimental ages in containers, grasslands, or croplands were determined by the period between the initiation of the experiments and sampling of the fine roots. Soil sampling depth intervals were converted to the middle values of corresponding depth intervals to facilitate analyses across studies, which involved a wide range of depth intervals (Chen & Brassard, 2013).

Ecosystem types were categorized as either planted containers (including woody plants (tree and shrub seedings) and herbaceous plants), cropland, planted grasslands, natural forests, or planted forests. We obtained geographical locations (altitude, latitude, and longitude) from the original papers that described experiments being conducted in croplands, grasslands, planted forests, and natural forests. We recorded the mean annual temperature and precipitation (when available) conveyed in the original publications, or derived these data from the geographical locations of each site using the WorldClim version 2 Dataset (Fick & Hijmans, 2017).

2.3.2 **Data analysis**

We calculated the community-weighted mean rooting depth (WRD) as:

$$WRD\ (cm) = \sum_{i=1}^{n}\left(\frac{B_i}{B_T} \times D_i\right) \tag{1}$$

where B_i is the fine-root biomass in the ith soil layer, B_T is the total biomass in all soil layers, and D_i is soil sampling depth (as the middle value of each sampling depth interval) of the ith layer. A larger WRD value represents a deeper centroid of community weighted mean rooting depth, which means that fine roots explore the resources in deeper soil layers.

Natural log-transformed response ratio (lnRR) (Hedges, Gurevitch & Curtis, 1999) was employed as the effect size for fine-root biomass and traits (root traits hereafter). We calculated the lnRR as:

$$lnRR = ln\left(\frac{X_t}{X_c}\right) \tag{2}$$

where X_t is the observed value in the mixture, and X_c is the expected value. Following Loreau and Hector (2001), we calculated the expected value X_c as the weighted mean of the corresponding species in monocultures according to the species proportion in mixtures for all root traits. For root biomass and root length density, X_t is the sum of each constituent species in mixtures. Since the root to shoot biomass ratio, weighted rooting depth, specific root length, mean root diameter, and root nitrogen content are not judged by soil area or volume, X_t was the weighted mean of each constituent species based on the species proportion in mixtures for these traits. For three of the 64 publications in which species proportions were unavailable, we assumed that the species in mixtures were equally distributed. Analyses without the data from these three publications yielded quantitatively similar results. For simplicity and inclusivity, we reported the data from all 64 publications.

Sampling variances (or standard deviations or standard errors) were not reported in 13 of the 64 publications. Importantly, weightings based on sampling variances might assign extreme importance to a few individual observations. Subsequently, the average lnRR would be primarily determined by a small number of studies. Therefore, similar to previous meta-analyses (Chen et al., 2020; Pittelkow et al., 2015), we employed the number of replications for weighting.

$$W_r = \frac{(N_c \times N_t)}{(N_c + N_t)} \tag{3}$$

where W_r is the weight for each observation, and N_t, and N_c are the numbers of replications in the mixtures and monocultures, respectively.

To ensure the assumption of linearity between each trait and species richness (R), stand or experimental age (A), and soil depth (D), we compared the linear, log-linear, and quadratic functions for R, A, and D for each root trait, using equation (4):

$$lnRR = \beta_0 + \beta_1 \times X + \pi_{study} + \varepsilon \tag{4}$$

where β is the estimated coefficient, π_{study} is the random effect factor of study, ε is the sampling error, and X is the linear, log-linear, or quadratic form of R, A, and D.

To test the simultaneous effects of R, A, and D on the lnRR of each root trait, we employed the following model:

$$lnRR = \beta_0 + \beta_1 \cdot R + \beta_2 \cdot A + \beta_3 \cdot D + \beta_4 \cdot R \times A + \beta_5 \cdot R \times D + \beta_6 \cdot A \times D$$
$$+\pi_{study} + \varepsilon \tag{5}$$

where βs are the coefficients to be estimated, π_{study} is the random effect factor of study, and ε is the sampling error. The function forms (linear, log-linear, and quadratic) of the three predictors in equation 5 were selected based on the lowest AIC values derived from equation 4 for each root trait (Table S2). The term D in equation (5) was excluded for root to shoot biomass ratio and weighted rooting depth since they are traits for the entire ecosystem and soil profile, respectively. To prevent overfitting, we derived the most parsimonious model based on the lowest AIC between all alternatives, rather than forward or backward stepwise selection, which has been heavily criticized (Mundry & Nunn, 2009; Whittingham, Stephens, Bradbury & Freckleton, 2006). We implemented model selection using the 'dredge' function of the *MuMln* package (Bartoń, 2019). As for the root to shoot biomass ratio, weighted rooting depth, mean root diameter, and root nitrogen content, we selected the null models since they had the lowest AIC values (Table S2-3). We conducted all analyses using restricted maximum likelihood estimation with the *lme4* package with W_r as the weight for each corresponding observation (Bates, Machler, Bolker & Walker, 2015).

To examine whether the species mixture effects changed with ecosystem type, we conducted two types of analysis. First, we tested whether the lnRRs differed with ecosystem type using:

$$lnRR = \beta_0 + \beta_1 \cdot E + \pi_{study} + \varepsilon \tag{6}$$

where E is the ecosystem type, and the others are the same as in the equation (5). Second, similar to previous meta-analyses (Chen et al., 2019; Chen et al., 2020), we expanded equation (5) by adding the ecosystem type (E) and its interactions with the terms of equation 5.

$$lnRR = \beta_0 + \beta_1 \cdot R + \beta_2 \cdot A + \beta_3 \cdot D + \beta_4 \cdot E + \beta_5 \cdot R \times A + \beta_6 \cdot R \times D + \beta_7 \cdot A \times D + \beta_8 \cdot R \times E + \beta_9 \cdot A \times E + \beta_{10} \cdot D \times E + \beta_{11} \cdot R \times A \times E + \beta_{12} \cdot R \times D \times E + \beta_{13} \cdot A \times D \times E + \pi_{study} + \varepsilon \tag{7}$$

where all of the terms (except E) are the same as in equation (5).

Collinearity between explanatory variables leads to biased models (James, Witten, Hastie & Tibshirani, 2013). We derived variance inflation factor (VIF) values using the '*car*' package (Fox, Weisberg & Price, 2020). All full models had predictors with their VIFs > 5, i.e., strong collinearity between predictors. As recommended (James et al., 2013), we deleted the predictors from the highest order of interaction terms one by one according to their VIF values until all of the VIF values of the remaining terms were < 5. Following the model selection procedure described above, we derived the most parsimonious models, which were the same as those from Eq. 5, and none of which contained the ecosystem type.

For those studies conducted in natural environments, we replaced the ecosystem term in equation 7 by the mean annual temperature and precipitation. The responses of the effects of species mixtures on root traits to the mean annual temperature and precipitation were assessed by three function forms (linear, log-linear, and quadratic), where the function forms with the lowest AIC values were selected (Table S2-4). Similar to the analysis for ecosystem type, we initially examined collinearity between predictors, and then derived the most parsimonious models among alternatives for the effects of mean annual temperature and precipitation on the responses of root traits to species mixtures. Neither the mean annual temperature nor precipitation were included in the most parsimonious models for any root traits, except for the root biomass and root length density (Table S2-5).

We also examined the potential influences of publication bias on our results by using a funnel test of asymmetry on mixed effect models (Pellegrini et al., 2018). Due to the fact that standard errors were not reported in 13 of the 64 publications in our data set, we employed the treatment response ratios against their sample sizes rather than standard errors as the predictor (Peters, 2006). We found no significant publication bias in any test (Table S2-6), which suggested no publication biases that might have prejudiced our results toward significant effects.

All continuous predictors were scaled (observed values minus the mean and divided by one standard deviation) (Cohen, West & Aiken, 2014). In this way, β_0 is the overall mean lnRR at the means of all predictors. To facilitate interpretation, the lnRR and its corresponding 95% confidence interval was transformed to a percentage change using the equation:

$$(e^{lnRR} - 1) \times 100\% \tag{8}$$

If the CIs did not cover zero, the mixture effect was significant at $\alpha = 0.05$. Histograms of model residuals and the Shapiro-Wilk test were employed to check the normality of all models, and bootstrapped estimates were derived when the normality was violated by using the *boot* package

(Canty & Ripley, 2012; Davison & Hinkley, 1997). All analyses were performed in R 3.6.1 (R Core Team, 2019).

2.4 Results

On average, root biomass was significantly greater in mixtures than the average of corresponding monocultures; however, the root to shoot biomass ratio, community-weighted mean rooting depth, root length density, specific root length, mean root diameter, and root nitrogen contents did not vary significantly across study pairs (Fig. 2-1). The effects of species mixtures on root biomass increased significantly with species richness (95% confidence interval [CI], 8.6% - 16.1%, $P < 0.001$) and soil depth (CI = 1.9% - 7.4%, $P = 0.001$), but not with stand age (CI = – 0.4% - 10.7%, $P = 0.175$) (Fig. 2-2). The effects of species mixtures on root length density were not altered, while those on specific root length decreased significantly with species richness (Fig. 2-2). Both the effects of species mixtures on root length density and specific root length increased with stand age and increased with soil depth for root length density, but decreased for specific root length (Fig. 2-2). The effects of species mixtures on root to shoot biomass ratios, community-weighted mean rooting depth, mean root diameter, and root nitrogen content were not significantly altered in response to species richness, stand age, or soil depth, as the null models (with intercepts only) were the most parsimonious (Table S2-3).

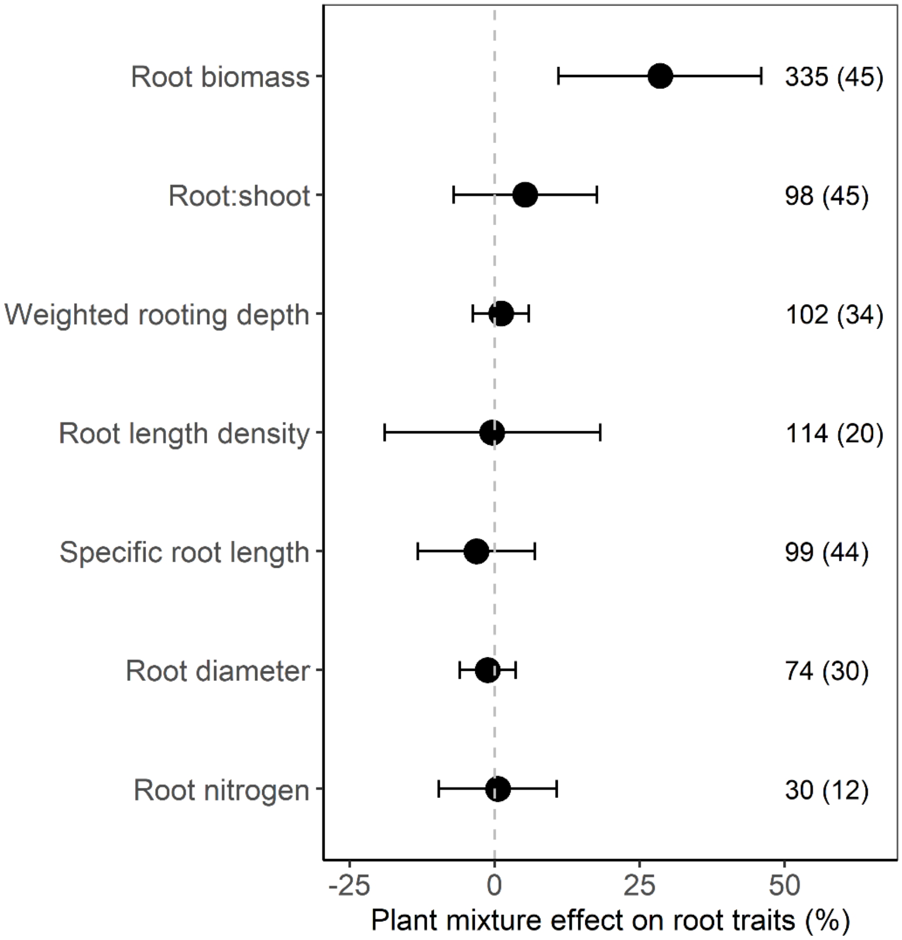

Figure 2-1 Comparison of fine-root traits in species mixtures and monocultures. Values (estimated β0 in Eq. 5) are mean ± 95% confidence intervals of the percentage effects between species mixtures and monocultures. The number of observations is shown beside each trait without parentheses, with the number of studies in parentheses.

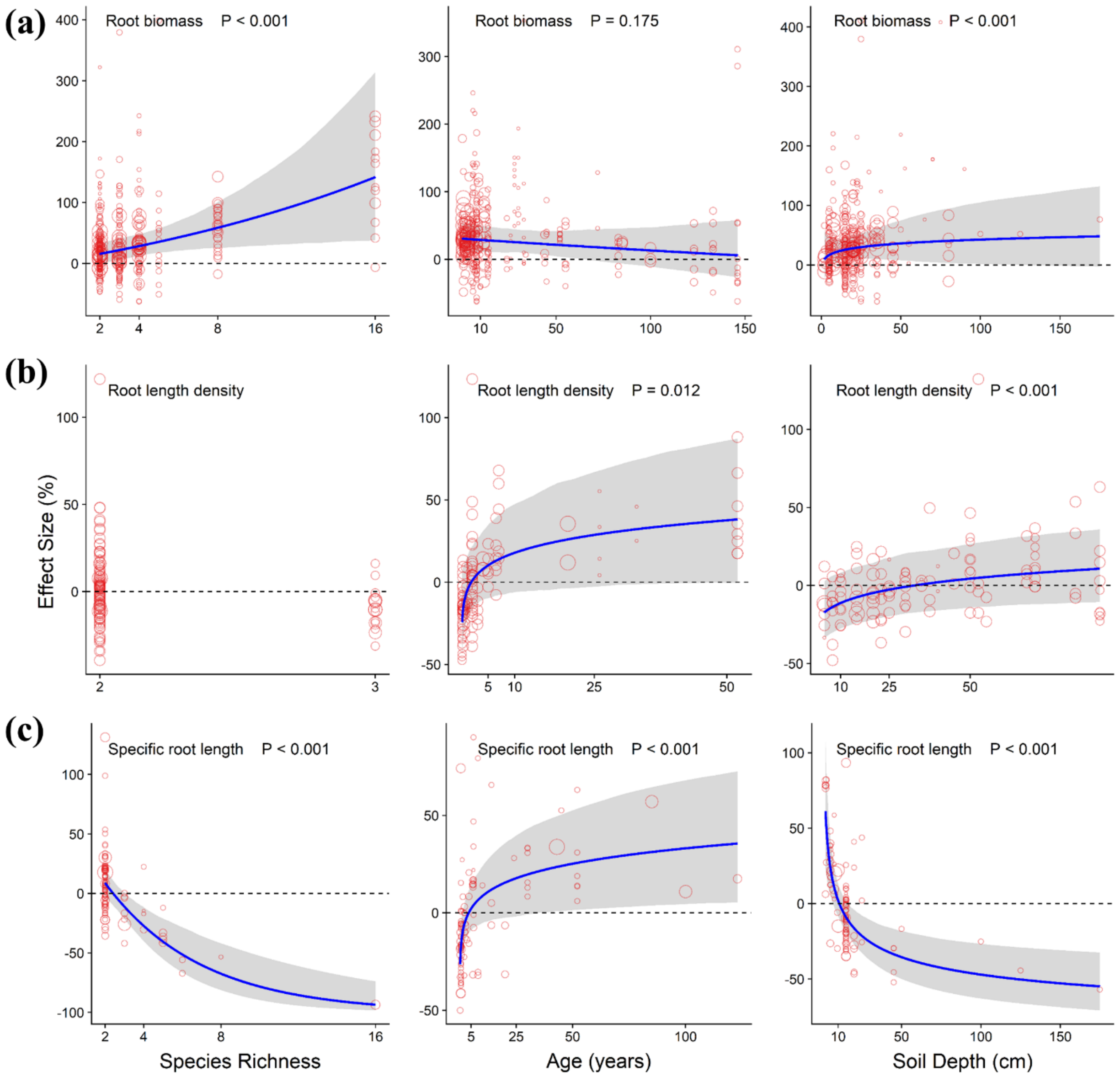

Figure 2-2 Species mixture effects on fine-root traits as relates to species richness in the mixtures, stand age, and soil depth. (a) fine-root biomass, (b) root length density and (c) specific root length. The blue lines for each trait were estimated β1, β2, and β3 in Equation 5, respectively, with bootstrapped 95% confidence intervals shaded. The sizes of red circles represent the relative weights of corresponding observations. The missing trendline of species mixture effects on root length density in relation to the species richness in mixtures was attributed to the fact that species richness was not included in the most parsimonious model derived from Eq. 5.

The effect sizes of root biomass with species richness were more progressive for older stand ages and at deeper soil depths (both *P* < 0.001, Figs. 2-3a,3b). The effects of species mixtures on specific root length strongly decreased with species richness in young stands but increased in stands older than 10 years (*P* < 0.001, Fig. 2-3c). The effects of species mixtures on specific root length decreased with species richness in deep soils but increased in topsoil (*P* < 0.001; Fig. 2-3d).

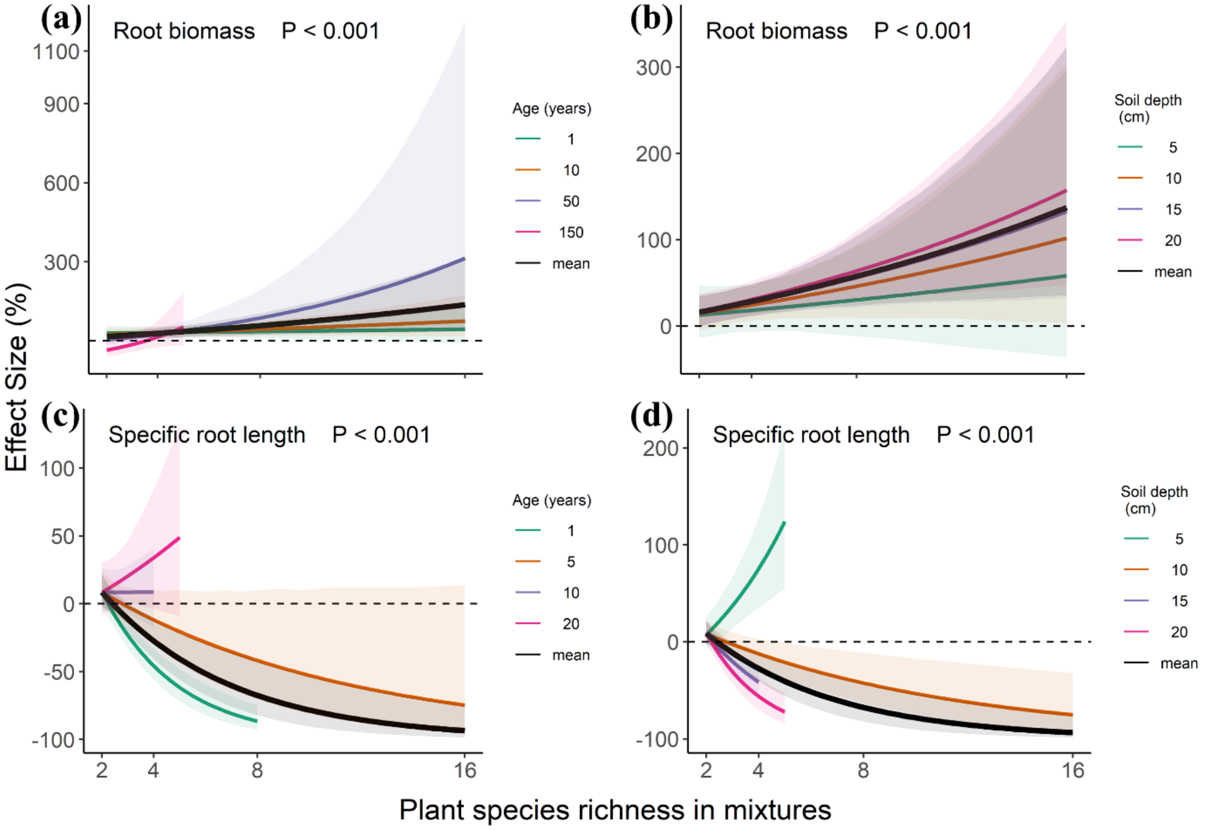

Figure 2-3 Effects of plant species mixtures on root biomass and specific root length in relation to species richness in mixtures, stand age, and soil depth. (a) interactive effects of species richness and stand age on root biomass, (b) interactive effects of species richness and soil depth on root biomass, (c) interactive effects of species richness and stand age on specific root length, and (d) interactive effects of species richness and soil depth on specific root length. Coloured and black lines refer to the responses of specific age or soil depth and their averages, respectively, with their bootstrapped 95% confidence intervals shaded. P values are the interactive terms tested. The figures were plotted based on the most parsimonious models derived from Eq. 5.

Despite significant differences in species richness and stand ages in mixtures between ecosystem types, the effects of species mixtures did not differ with ecosystem type for root traits, except for root biomass, with positive effects of species mixtures on root biomass in containers, planted grasslands, and planted forests, but not in natural forests (Fig.2- 4). However, when species richness, stand age, and soil depth were simultaneously included, none of the most parsimonious models for root biomass and traits included ecosystem type as a predictor.

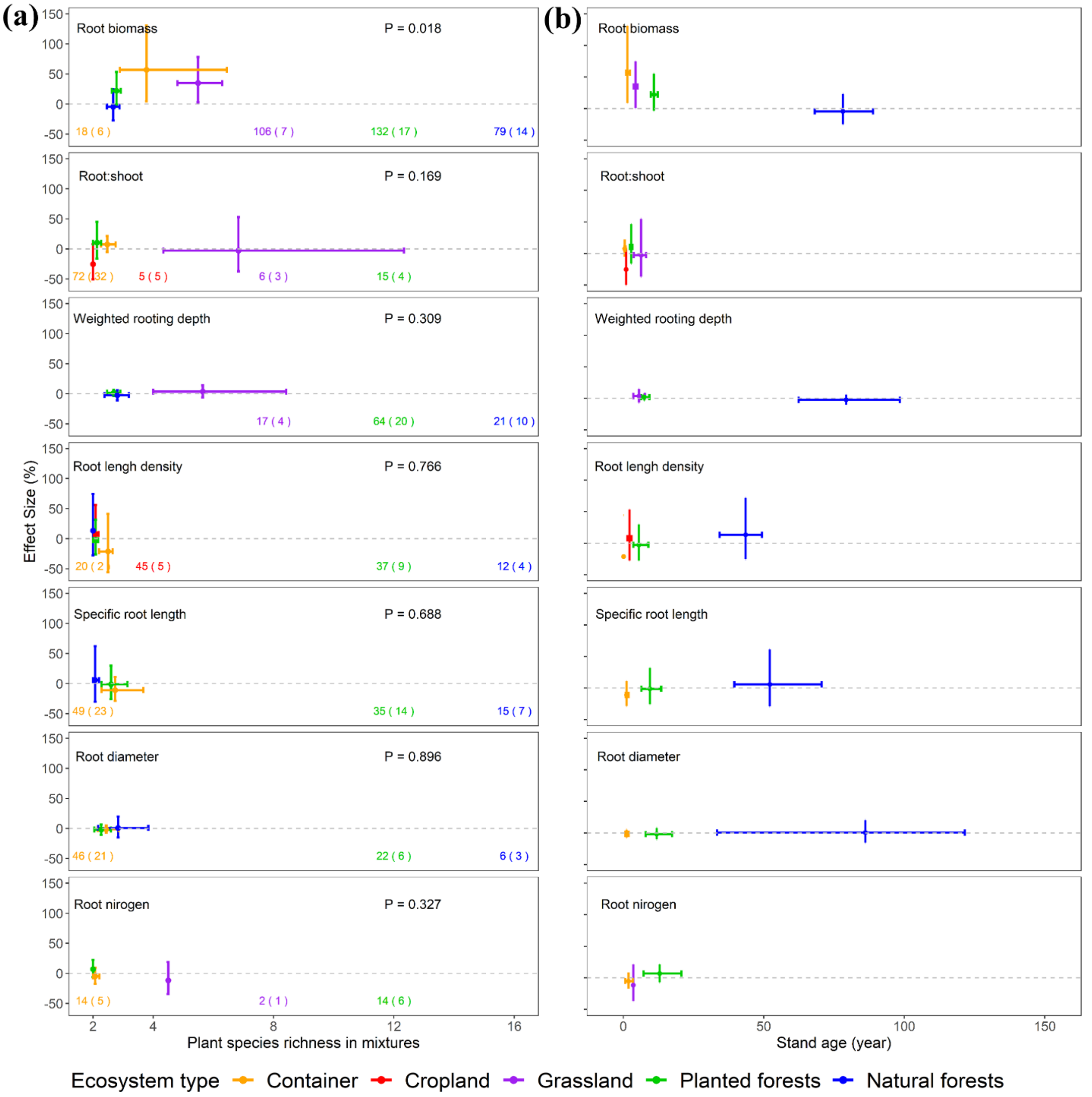
(a)
(b)
Root biomass
P = 0.018
18 (6)
106 (7)
132 (17)
79 (14)
Root:shoot
P = 0.169
72 (32)
5 (5)
6 (3)
15 (4)
Weighted rooting depth
P = 0.309
17 (4)
64 (20)
21 (10)
Root lengh density
P = 0.766
20 (2)
45 (5)
37 (9)
12 (4)
Specific root length
P = 0.688
49 (23)
35 (14)
15 (7)
Root diameter
P = 0.896
46 (21)
22 (6)
6 (3)
Root nirogen
P = 0.327
14 (5)
2 (1)
14 (6)
150
100
50
0
-50
Effect Size (%)
2
4
8
12
16
Plant species richness in mixtures
0
50
100
150
Stand age (year)
Ecosystem type
Container
Cropland
Grassland
Planted forests
Natural forests

Figure 2-4 Comparison of root biomass, root: shoot ratio, weighted rooting depth, root length density, specific root length, root diameter, and root nitrogen in plant species mixtures versus monocultures between ecosystem types. Means and vertical and horizontal error bars represent means and 95% confidence intervals for (a) species mixture effects and the species richness in mixtures, (b) species mixture effects and stand age in mixtures, respectively. For each system type, the number of observations is shown, with the number of studies in parentheses. P values, derived from the linear mixed model with the experimental system as the fixed effect and study as the random effect, represent the significance of the differences in the natural log response ratios (lnRRs) between ecosystem types. Despite significant differences in the species richness or stand age in mixtures among ecosystem types (as indicated by non-overlapping CIs), the lnRRs did not differ significantly for any of the root traits studied except for root biomass.

For studies conducted in natural environments, on average, mixture effects on root biomass increased with mean annual precipitation ($P = 0.007$), with more pronounced increases in species-rich mixtures ($P < 0.001$, Fig. 2-5a), while the mixture effect on root length density decreased significantly, changing from positive to negative with mean annual temperature ($P = 0.001$, Fig. 2-5b). The effects of species mixtures on other root traits did not respond significantly to either mean annual temperature or precipitation (Table S2-7).

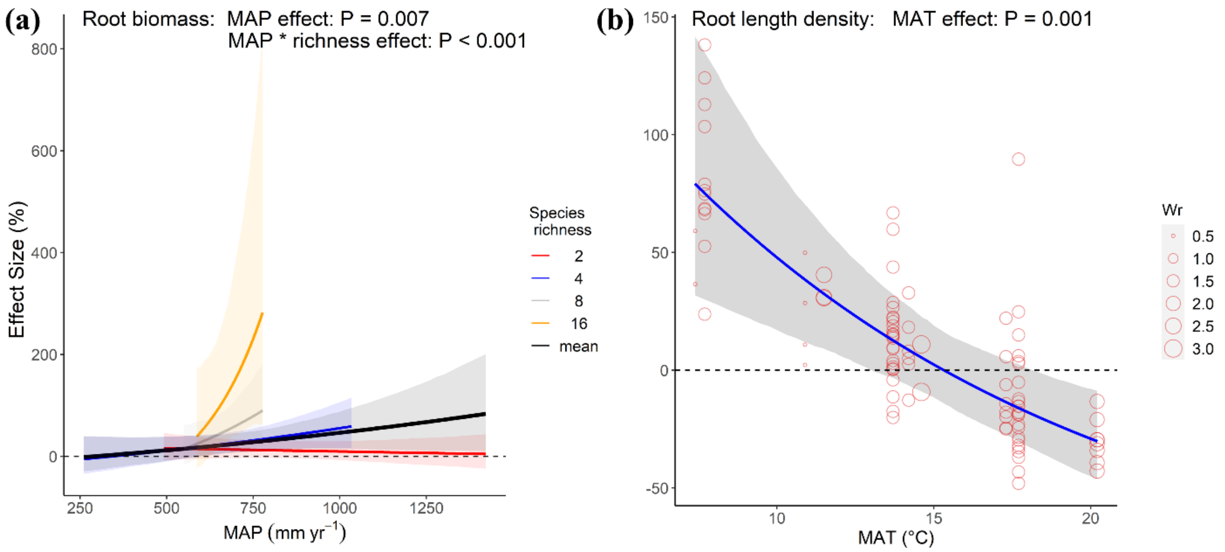

Figure 2-5 Species mixture effects on root biomass and root length density in relation to mean annual precipitation (MAP) and mean annual temperature (MAT), respectively. (a) Interactive effects of species richness and MAP on the effect size of species mixtures on root biomass. (b) Effect sizes of species mixtures on root length density (RLD) in relation to MAP. In Fig. 5a, coloured and black lines represent specific species richness and their average responses, respectively, with their bootstrapped 95% confidence intervals shaded in gray. In Fig. 5b, the blue line represents the estimated mean response, with bootstrapped 95% confidence intervals shaded. The figures were plotted based on the most parsimonious models derived from Eq. 7.

2.5 Discussion

Our meta-analysis revealed global evidence that fine-root functional traits could be altered in response to plant species diversity. We demonstrated that fine root biomass increased in species mixtures, with more pronounced increases observed in older stands and deeper soil layers. Although on average, other fine root traits were not altered by species mixtures, the effects of mixtures on specific root length shifted from negative to positive with stand age, positive in two-species mixtures, to negative in more species-rich mixtures, and positive to negative with soil depth. The effects of species mixtures on root length density shifted from negative to positive with stand age and soil depth.

We found significant average positive effects of species mixtures on fine root biomass but not root functional traits, including the root: shoot ratio, community weighted-mean rooting depth, root length density, specific root length, mean root diameter, and root nitrogen content. The sole positive mixture effects on root biomass suggest that plants increase carbon investments to roots for higher soil resource capacity rather than efficiency (or both) in species mixtures on a global scale (Table 2-1). The lack of a mixture effect on the root: shoot ratio indicated that fine root overyielding was of the same magnitude as its aboveground counterpart on a global scale, which was similar to the results reported in a grassland study (Barry et al., 2019a). Nevertheless, the effects of species mixtures on several functional traits were highly dependant on species richness, stand age, soil depth, or environmental stress.

We found that the effects of species mixtures on root biomass increased with species richness, whereas those on specific root length shifted from positive to negative from two to higher numbers of species in mixtures. The positive species-richness effects on root biomass were consistent with our understanding that plant productivity increases with species richness due to a more complete resource utilization by niche complementarity (Tilman et al., 2001). The decreased specific root length with species richness implied that fine roots reduced resource uptake efficiency in more diverse communities (Ostonen et al., 2007), which might have resulted from reduced soil resource competition due to the spatial and temporal complementarity between species (Barry et al., 2019b). This lower interspecific competition for resources might be also attributed to more nutrient-rich soil through more rapid litter decomposition in species-rich mixtures (Liu et al., 2020). The lack of species richness effects on root length density could have been primarily attributable to the limited range of species richness (2 and 3) in our meta-data

(Fig. 2-2b). Alternatively, higher root biomass alone could improve fine root resource uptake sufficiently to meet the high resource demands of more diverse communities (Table 2-1).

Although, on average, the effects of species mixtures on root biomass did not change over time, we found that the root biomass in species-rich mixtures increased over time, and the effects of species mixtures on root length density and specific root length shifted from negative to positive from younger to older stands. The progressive species-richness effects on root biomass with stand development were consistent with those previously reported for the relationships between plant diversity and above- and belowground productivity (Ma & Chen, 2016; Reich, 2012; Zhang et al., 2012). This also suggested that the loss of a few species in diverse communities could significantly decrease root production. The more pronounced effects of species mixtures on root length density and specific root length in older stands could be attributed to the fact that higher diversity effects on productivity in older stands (Barry et al., 2019a) should facilitate higher water and nutrient demands. The elevated resource demands in older stands could be achieved by the utilization of larger horizontal soil volume by fine roots (root length density) (Brassard et al., 2013; Ma & Chen, 2017), or high resource uptake efficiency (specific root length) due to the elevated magnitude of complementarity in species mixtures (Cardinale et al., 2007). The negative mixture effects in young stands could be attributable to a negative selection effect; that is, in young communities, pioneer species with thick roots are more dominant, which results in a lower specific root length and root length density. Collectively, the increasing effects of species mixtures on both root length density and specific root length with stand development, combined with more progressive positive diversity effects on root biomass in older stands, supported greater aboveground production with stand age in species mixtures (Zhang et al., 2012).

We also found that with increasing soil depth, the effect of species mixtures on root biomass increased, with more pronounced species-richness effects in deeper soil layers, and the effects on root length density shifted from negative to positive, whereas that on specific root length shifted from positive to negative. Increased mixture effects on fine root biomass and root length density with soil depth could be attributed to a stronger interspecific facilitation at the deeper soil levels, where nutrients are less available (Forrester & Bauhus, 2016; Jobbágy & Jackson, 2001; Ma & Chen, 2017; Makita et al., 2010). The positive mixture effects on specific root length in surface soil mean more acquisitive strategy for resource uptake in this root-rich soil layer, which might have resulted from the high resource availability in the topsoil in more diverse mixtures. In contrast, a conservative resource uptake strategy could have developed in the deep soils of species mixtures due to their lower resource availability from more complete resource utilization (Reich, 2014; Weemstra et al., 2016), which resulted in higher specific root length in deeper soils. Our findings suggested that both root biomass and root functional traits should be incorporated to explain the consequences of diversity loss on soil resource uptake.

Our analysis revealed that ecosystem types had negligible effects on all root traits, except for root biomass, including containers, planted croplands, planted grasslands, planted forests, and natural forests. The effects of species mixtures on root biomass were neutral in natural forests, while they were positive in planted containers, planted grasslands, and planted forests. The insignificant mixture effects in natural forests might primarily result from the fact that most studies contain low species richness, in contrast to other artificial ecosystems.

For studies conducted in natural environments, we found that the effects of species mixtures on root biomass increased with mean annual precipitation, with more pronounced increases in species-rich mixtures. This finding was in contrast with the prediction of the stress gradient hypothesis, which posits that diversity effects increase with environmental harshness. Although interspecific facilitation may be enhanced under resource limitations (Forrester & Bauhus, 2016; Maestre et al., 2009), it is possible that the increased availability of water and nutrients with accelerated litter decay rate augmented niche differentiation under humid conditions (Liu et al., 2020). The enhanced effects of species mixtures on root biomass with higher water availability in more diverse communities might also be attributable to stronger resource partitioning in resource-rich environments(Barry et al., 2019b; Hisano & Chen, 2020). Therefore, species diversity loss could increase the uncertainty of root production under more variable climate changes. The effects of species mixtures on root length density shifted from positive to negative with mean annual temperature. In colder climates where fine roots face lower resource availability due to a slower fine root decay rate (See et al., 2019), increased interspecific facilitation via microclimate amelioration in mixtures (Forrester & Bauhus, 2016; Wright et al., 2017) might increase root length density for an improved resource capacity. In warm climates, the reduced root length density in species mixtures might be an outcome of dominant interspecific competition rather than facilitation (Brooker, 2006). Our results aligned with the findings of a grassland experiment, which revealed that interspecific interactions transitioned from facilitation to competition along a temperature gradient (Olsen, Topper, Skarpaas, Vandvik & Klanderud, 2016).

There were several uncertainties in our synthesis. First, root trait data was limited. For instance, there were only 30 observations with 12 studies for root nitrogen. Second, our meta data was dominated by two-species mixtures with short experimental durations, in which mixture effects were expected to be minimal due to limited interspecific interactions between individual plants (Beyer, Hertel, Jung, Fender & Leuschner, 2013; Lei et al., 2012; Siebenkäs & Roscher, 2016). Third, phylogenetic diversity could influence how root traits respond to mixtures more than species diversity (Kong et al., 2019; Valverde-Barrantes, Freschet, Roumet & Blackwood, 2017; Valverde-Barrantes, Smemo, Feinstein, Kershner & Blackwood, 2015). However, only few studies have clearly stated species combinations to allow for the determination of phylogenetic diversity, particularly in more diverse planted grassland ecosystems (Jesch et al., 2018; Mueller, Tilman, Fornara & Hobbie, 2013; Ravenek et al., 2014). Future studies shall overcome these limitations.

In conclusion, we found that the effects of species mixtures on root functional traits were highly dependant on species richness, stand age, soil depth, or environmental stress. To address the high water and nutrient demands toward supporting the faster growth rate in plant species mixtures (Hisano, Chen, Searle & Reich, 2019), fine roots increased in biomass and/or root length density, but decreased the specific root length, in relation to both species richness and soil depth. We also found that species mixture effects on root length density and specific root length increased with stand development. These findings suggested that elevated water and nutrient demands in species-rich and old mixtures, led to deeper soil exploration. Our results extended aboveground overyielding to belowground (Liang et al., 2016; Zhang et al., 2012), particularly, the pronounced diversity effects in older stands and deeper soils (Ma & Chen, 2016). Across global climatic variations, the effects of species mixtures on root biomass increased with mean annual precipitation, where increased trends were more pronounced in more diverse plant communities, while the effects of species mixtures on root length density decreased with the mean annual temperature. Our analysis highlights the need to incorporate the number of species in mixtures, stand age, and soil depth profiles, toward a better understanding of the mixture effects on root traits. Because of the important role of fine roots in soil water, carbon and nutrient cycling, our results suggest that increased fine root biomass, with shifts in fine root traits, could be one of the drivers for the positive diversity-productivity relationship in species mixtures.

2.6 Data Accessibility Statement

The data and R codes supporting the results are available from the Figshare Repository: https://figshare.com/articles/journal_contribution/_/12974639.

Chapter 3. PLANT FUNCTIONAL TRAIT DISSIMILARITY AS DRIVERS OF PLANT MIXTURE EFFECTS ON FINE-ROOT BIOMASS AND TRAIT VARIATIONS

3.1 Abstract

1. Water and soil nutrient uptake are primarily carried out by fine roots in terrestrial ecosystems. Although studies have shown predominantly positive effects of plant diversity on fine root biomass (FRB) and influence fine root functional traits, the lack of plant diversity effects has also been reported. It remains unclear how plant functional trait variations influence the effects of plant diversity on FRB and functional traits across and at given species diversity levels.
2. By combining 652 paired observations of plant mixtures and monocultures, we conducted a meta-analysis to examine how plant functional trait variations, including presence or absence of contrasting shade tolerance (CST), drought tolerance (CDT), plant growth rate (CGR), and leaf habit dissimilarity (LHD) in mixtures, on the extent of plant mixture on FRB, root length density (RLD), community-weighted mean rooting depth (WRD), and specific root length (SRL) in plant mixtures at a global scale.
3. We found that the presence of functional trait variations had significant influences on the mixture effects on FRB and SRL, but those not on WRD and RLD. Plant mixture effects on FRB were greater with the presence of CDT and CGR than their absence, but was contrary for shade tolerance. The presence of CDT also increased mixture effects on SRL higher than its absence. While the mixture effects on FRB and SRL were both lower in mixed-wood forests with high LHD than lower levels. We also found the mixture effects on FRB decreased with stand age when CST was present. The mixture effect on FRB

increased with soil depth when CDT was absent and on RLD when CDT was present, respectively. Moreover, the mixture effect on RLD increased with aridity index when CST was present, but that was decreased when CST was absent. While in the presence of CGR, the mixture effect on SRL decreased with aridity index. The mixture effects on WRD increased with both mean annual temperature and aridity index in mixed-wood forests of high leaf habit dissimilarity.

4. Our findings highlight that presence of plant functional trait dissimilarity is a strong determinant of plant mixture effects on FRB and root traits. Our results provide a guidance for plant diversity management focusing on belowground productivity and carbon sequestration.

Keywords: drought tolerance, leaf habit dissimilarity, plant growth rate, root biomass, root traits, shade tolerance

3.2 Introduction

The rising crisis of biodiversity loss due to global change during the past decades makes us realize the positive role of biodiversity on ecosystem services, and on our human welfare (Hooper *et al.*, 2012; Isbell *et al.*, 2015). The main cornerstone describing mechanisms to support the positive plant diversity-ecosystem functioning is the niche complementarity hypotheses (Loreau & Hector, 2001). A stronger complementarity represents a more complete resource utilization via larger differences of plant resource needs and acquisition strategies (i.e. niche differentiation), and a stronger beneficial relationship between coexisting species via nutrient enrichment or microclimate amelioration (i.e. facilitation) in more diverse plant mixtures (Wright *et al.*, 2017; Barry *et al.*, 2019). Due to the multi-dimensional diversity, this complementarity could be influenced by the number of plant species and the composition of plant functional trait dissimilarity in mixtures. Previous studies have revealed that plant species richness and the extent of life-history trait variation (e.g., presence of contrasting shade tolerance) positively affect aboveground plant productivity in plant mixtures (Zhang *et al.*, 2012; Toïgo *et al.*, 2018). However, although species richness plays a key role on fine-root productivity and traits (Ma & Chen, 2016; Peng & Chen, 2021), we still do not know how plant mixtures consisting of different plant functional traits (e.g., shade and drought tolerance, plant growth rate, leaf habit dissimilarity) influence fine-root biomass and traits.

Fine roots play a critical role in nutrient and water uptake and make a significant contribution to the terrestrial biomass pool (McCormack *et al.*, 2015). Fine-root architectural traits (community-weighted mean rooting depth (WRD), root length density (RLD)) and morphological trait (specific root length (SRL)) are crucial drivers for carbon and nutrient cycling, which influence soil resource acquisition (Bardgett *et al.*, 2014; Reich, 2014). WRD and RLD represent the spatial distribution of the whole root system, which impacts plant water and nutrient uptake (Guderle *et al.*, 2018; Archambault *et al.*, 2019). SRL, defined as root length per dry root mass, is used as a proxy for soil resource uptake efficiency (Ostonen *et al.*, 2007). The inconsistent variations of fine-root biomass and traits in response to the effects of plant mixtures (Peng & Chen, 2021) could be partly attributed to ignoring of plant functional trait dissimilarity. Given that the link between plant functional trait dissimilarity and niche occupancy, resource partitioning and enrichment (Silvertown, 2004), there could be a higher complementarity in mixtures with contrasting shade and drought tolerance, contrasting plant growth rate among constituent species, and in mixed-wood forests with high leaf habit dissimilarity (Searle & Chen, 2019; Ding *et al.*, 2021). With contrasting plant functional trait dissimilarity in mixtures, the coexisting species have a more complete utilization of light, water and nutrients because of differences in leaf phenology, root distribution, preferences of nutrient forms (Ashton *et al.*, 2010; Ishii & Asano, 2010; Brassard *et al.*, 2013). Therefore, we expect a higher FRB, RLD and SRL, a deeper WRD toward high activities of soil exploration and exploitation in the mixtures with contrasting shade and drought tolerance, contrasting plant growth rate, and high leaf habit dissimilarity.

The effects of plant functional trait dissimilarity on fine-root attributes could also be influenced by stand development and soil depth. Plant canopy closure is not complete at the stand initiation stage, then the shade-tolerant species might not be able to benefit the shade-intolerant species in mixtures. While with crown overlapping in the older stands, the FRB and root traits could be altered to meet the elevated water and resource demands in the plant mixtures with contrasting shade tolerance (Zhang *et al.*, 2012). We expected that the FRB, WRD, RLD and SRL increase with stand development when contrasting shade tolerance among the coexisting species presented. Moreover, water availability is different among soil layers, and plants are found to uptake half their water in the surface soil (Kulmatiski, 2017). Through hydraulic lift of deeper soil water, drought-tolerant species may facilitate the neighbouring drought-intolerant species by reducing water competition in the surface soil (Pretzsch *et al.*, 2013). Hence, more FRB, greater RLD and SRL are expected to favour hydraulic lift in plant mixtures with contrasting drought tolerance.

The response of FRB and traits to plant functional trait dissimilarity may also differ contingent with the background environment. Since there are different vegetation recruitment rates and structure, root growth limitations among ecosystem types (container, cropland, grassland and forests), the effects of functional trait dissimilarity on FRB and traits could differ among ecosystem types induced by the magnitudes of diversity–productivity relationship variations (Forrester & Bauhus, 2016; Freschet *et al.*, 2017). Moreover, plants could adjust their FRB and traits for growth and survival under variable climate conditions (Freschet *et al.*, 2017; Zhou *et al.*, 2018). In cold and dry areas, the more stress-tolerant species could benefit the less stress-tolerant and stress-intolerant species by alleviating the microclimatic stress in plant mixtures (Wright *et al.*, 2017). Increased aboveground biomass in mixtures with contrasting shade tolerance increases shade and further reduces temperature, increases surface soil moisture (Zhang *et al.*, 2012; Wright *et al.*, 2015). However, it remains unclear that how effects of plant functional trait dissimilarity on FRB and traits responds to climates.

Understanding how effects of plant functional trait dissimilarity on fine roots enables us to make decisions on plant species selection focusing on belowground part, especially under the increasing crisis of climate change. In this paper we compiled data from 652 paired observations to describe how the effects of species mixtures on FRB, WRD, RLD and SRL change with heterogeneous plant functional traits. We expected that: (1) since the coexisting species with contrasting shade and drought tolerance, contrasting plant growth rate and high leaf habit dissimilarity could have a more complete utilization of light, water and nutrients induced by resource partitioning and abiotic facilitation (Wright *et al.*, 2017; Barry *et al.*, 2019), the mixture effects on FRB, WRD, RLD and SRL would be higher in the plant mixtures with heterogeneous plant functional traits than those without; (2) FRB, WRD, RLD and SRL increase with stand development to meet the elevated resource demands induced by higher aboveground productivity when contrasting shade tolerance is present (Chen & Popadiouk, 2002; Zhang *et al.*, 2012); (3) more FRB and higher RLD and SRL with soil depth are required to favour hydraulic lift in plant mixtures with contrasting drought tolerance (Pretzsch *et al.*, 2013). We tested the above hypotheses across different ecosystem types and environmental conditions.

3.3 Materials and methods

3.3.1 Data collection

We examined all peer-reviews journal articles focusing on the effects of plant mixtures on FRB and traits, by searching the Web of Science, Google Scholar and China National Knowledge Infrastructure (CNKI), up to April 2020. We used several keyword combinations for the research, such as '(plant diversity OR species diversity OR species mixture OR mix plants OR polyculture OR intercrop)' and '(fine root OR root biomass OR root distribution OR root length density OR specific root length OR SRL)'. The following criteria were applying for study filtering: (1) studies were purposely implemented to separate the effects of plant mixtures from other factors, such as nitrogen addition or water alterations; (2) values of FRB and traits could be extracted directly from the text, tables, and figures; (3) each plant species in the mixtures was presented in the study (4) each plant species mixture was compared to corresponding monocultures.

Ecosystem types were recorded as either planted container (woody plants (tree and shrub seedings) and herbaceous plants), planted cropland, planted grassland, natural forest or planted forest. We extracted the plant species identities from the original studies. The proportions of each species in mixtures were calculated according to the number of individuals in planted containers, the seeds sown in planted cropland and grassland, and stem densities or basal areas in planted and natural forests. FRB, WRD, RLD and SRL were collected for each study. Species richness in mixtures, stand or experimental age and species proportions in plant mixtures were also extracted from the original studies. We used the average values of root biomass or traits if one given study had different root order or diameter classifications. We recorded studies that carried out with different plant mix ratios, or in different locations as distinct studies in a given publication. In order to facilitate the analysis across studies over multiple soil depth intervals, we converted the soil sampling depth intervals to the middle values of corresponding depth intervals (Chen & Brassard, 2013). Plot Digitizer version 2.0 (Department of Physics at the University of South Alabama, Mobile, AL, USA) was used to extract data from the figures. There were 652 paired observations with 90 studies included in this meta-analysis (Table S3-1).

Based on the species identities, we recorded the plant functional traits (shade and drought tolerance, plant relative grow rate and leaf habit type) of each species in plant mixtures for each study. If there was no information in the original data, we extracted the corresponding description form the TRY Plant Trait Database (Kattge *et al.*, 2011), the PFAF Plant For A Future database (https://pfaf.org/user/Default.aspx) and other published literatures (Table S3-2). According to the plant shade and drought tolerance, plant growth rate for each species (Appendix S3-1), we classified the mixture as presence or absence of contrasting shade and drought tolerance, contrasting plant growth rate for each study. For leaf habit dissimilarity, we classified the plant mixtures into high, medium and low level (Table S3-3). Geographical locations (altitude, latitude, and longitude) from the original papers were obtained. Then the mean annual temperature and precipitation (when available) were recorded from the original publications or derived based on the geographical location for each site from the WorldClim version 2 Dataset (Fick & Hijmans, 2017).

3.3.2 Data analysis

Community-weighted mean rooting depth (WRD) was calculated as:

$$WRD\ (cm) = \sum_{i=1}^{n} \left(\frac{B_i}{B_T} \times D_i \right) \qquad (1)$$

where B_i and B_T is the FRB in the i^{th} soil layer and all soil layers, respectively, D_i is the middle value of each sampling depth interval of the i^{th} soil layer.

We estimated the effect size of FRB and WRD, RLD, SRL by the natural log-transformed response ratio (lnRR) as following (Hedges *et al.*, 1999):

$$lnRR = ln\left(\frac{X_t}{X_c}\right) \qquad (2)$$

where X_t and X_c were the observed values for selected variables in the mixture and expected values in the mixture for each study, respectively. We calculated Xc as the weighted mean of the corresponding species in monocultures based on the species proportion in mixtures for FRB, WRD, RLD and SRL (Loreau & Hector, 2001). The Xt values for FRB and RLD were calculated as the sum value of each constituent species in mixtures. Whereas calculation of WRD and SRL were not influenced by the soil area or volume, Xt for these two rot traits were calculated as the weighted mean of each constituent species based on the species proportion in mixtures.

In meta-analysis, how to weight the individual observations could impact the estimation of effect size (Ma & Chen, 2016). Weightings based on sampling variances might assign extreme importance to a few individual observations, so we employed the number of replications for weighting in this meta-analysis (Chen & Chen, 2018):

$$W_r = (N_t \times N_c)/(N_t + N_c) \qquad (3)$$

where W_r was the weight for each paired observation, N_t and N_c were the numbers of replication of mixture and the corresponding monoculture, respectively.

To validate the assumption of linearity between lnRR and the predictors (species richness: R, stand or experimental age: A, soil depth: D, mean annual temperature: MAP, Aridity index: AI), we compared the linear, loglinear and quadratic functions with the predictor of interest as the fixed effect and ‘study’ as the random effect in a linear mixed-effect model. Then we selected the function of each predictor for each root attribute according to the Akaike Information Criterion (AIC) values (Table S4 & 5).

To test whether the responses of FRB, WRD, RLD and SRL to plant mixtures were affected by plant functional trait dissimilarity (FT, shade tolerance, drought tolerance, plant growth rate and leaf habit dissimilarity), as well as R, A D, we employed the following model:

$$lnRR = \beta_0 + \beta_1 \cdot R + +\beta_2 \cdot A + \beta_3 \cdot D + \beta_4 \cdot FT + \beta_5 \cdot R \times FT + \beta_6 \cdot A \times FT + \beta_7 \cdot D \times FT + \pi_{study} + \varepsilon \quad (4)$$

where β_i, π_{study}, ε are the coefficient to be estimated, the random effect factor of ‘study’ and sampling error, respectively. The random effect explicitly accounted for autocorrelation among observations within each ’study’. Terms related to soil depth (D) in eq. 5 were excluded for WRD as it is for the entire soil profile. The analysis was conducted by the restricted maximum likelihood estimation with the *lme4* package, with Wr as the weight for each corresponding observation (Bates *et al.*, 2015). To prevent overfitting, we derived the most parsimonious model by using the ‘dredge’ function of *MuMln* package (Bartoń, 2019). To test the heterogeneous plant growth rate and leaf habit dissimilarity effects on FRB and traits, we selected the models with lowest AIC values by keeping FT terms. To examine how the heterogeneous shade tolerance effects on FRB and traits change over time and how heterogeneous drought tolerance effects on FRB and traits changing among soil layers, we kept the terms ‘FT×A’ and ‘F×TD’, respectively.

To test whether the effects of plant functional trait dissimilarity on FRB and traits differ among different ecosystem types, we used the linear mixed-effects model with ecosystem type and their interactions with the terms in the selected most parsimonious models from equation 4. We did not find that ecosystem type accounted for additional variance for any plant functional trait dissimilarity on FRB and traits, except the effect of leaf habit dissimilarity on FRB between natural forests and planted forests (Table S3-7). To further test the interactive effects of ecosystem type with functional trait dissimilarity, linear mixed-effect model with only ecosystem type and functional trait dissimilarity was used for each root attributes. The effects of functional trait dissimilarity on root attributes did not differ among ecosystem types. Finally, to test whether the effects of plant functional trait dissimilarity on FRB and traits vary with mean annual temperature (MAT) and aridity index (AI) in the natural ecosystems, we employed the following model:

$$lnRR = \beta_0 + \beta_1 \cdot FT + \beta_2 \cdot MAT + \beta_3 \cdot AI + \beta_4 \cdot FT \times MAT + \beta_5 \cdot FT \times AI + \pi_{study} + \varepsilon (5)$$

where β_i, π_{study}, ε are same as in equation 4.

We scaled all continuous predictors (species richness, stand age, soil depth, mean annual precipitation and aridity index) in the models during data analysis, i.e., observed value minus mean and divided by one standard deviation. By doing so, β_0 is the overall mean lnRR at the means of all predictors (Cohen *et al.*, 2014). To ease interpretation, we transformed lnRR and its corresponding 95% confidence interval to a percentage change using $(e^{lnRR} - 1) \times 100\%$. If the confidence intervals did not cover zero, then the effects of plant functional trait dissimilarity on FRB or traits were significant at $\alpha = 0.05$. We employed histograms of model residuals and the Shapiro-Wilk test to check the assumption of normality for all models. If normality was violated, bootstrapped estimates were derived by using the *boot* package (Davison & Hinkley, 1997; Canty & Ripley, 2012). All analyses were performed in R 3.6.1 (R Core Team, 2019).

3.4 Results

On average, there were significant effects of plant functional trait dissimilarity on fine root biomass (FRB) and on specific root length (SRL) in plant mixtures (Figs. 3-1a & d), whereas community-weighted mean rooting depth (WRD) and root length density (RLD) did not respond differently to any of plant functional trait dissimilarity (Figs. 3-1b & c). In detail, there was a significantly higher mixture effect on FRB when contrasting shade tolerance was absent among the coexisting species, compared with the contrasting shade tolerance presented in the plant mixtures ($P = 0.004$). However, both the presence of contrasting drought tolerance and plant growth rate in mixtures had a significantly higher effect size on FRB than the absence of contrasting drought tolerance and plant growth rate, respectively (both $P < 0.001$). Moreover, the effect size of mixture effect on SRL was higher in plant mixtures with presence of contrasting drought tolerance ($P = 0.047$), while lower in tree mixtures with high leaf habit dissimilarity than low or medium dissimilarity ($P = 0.009$).

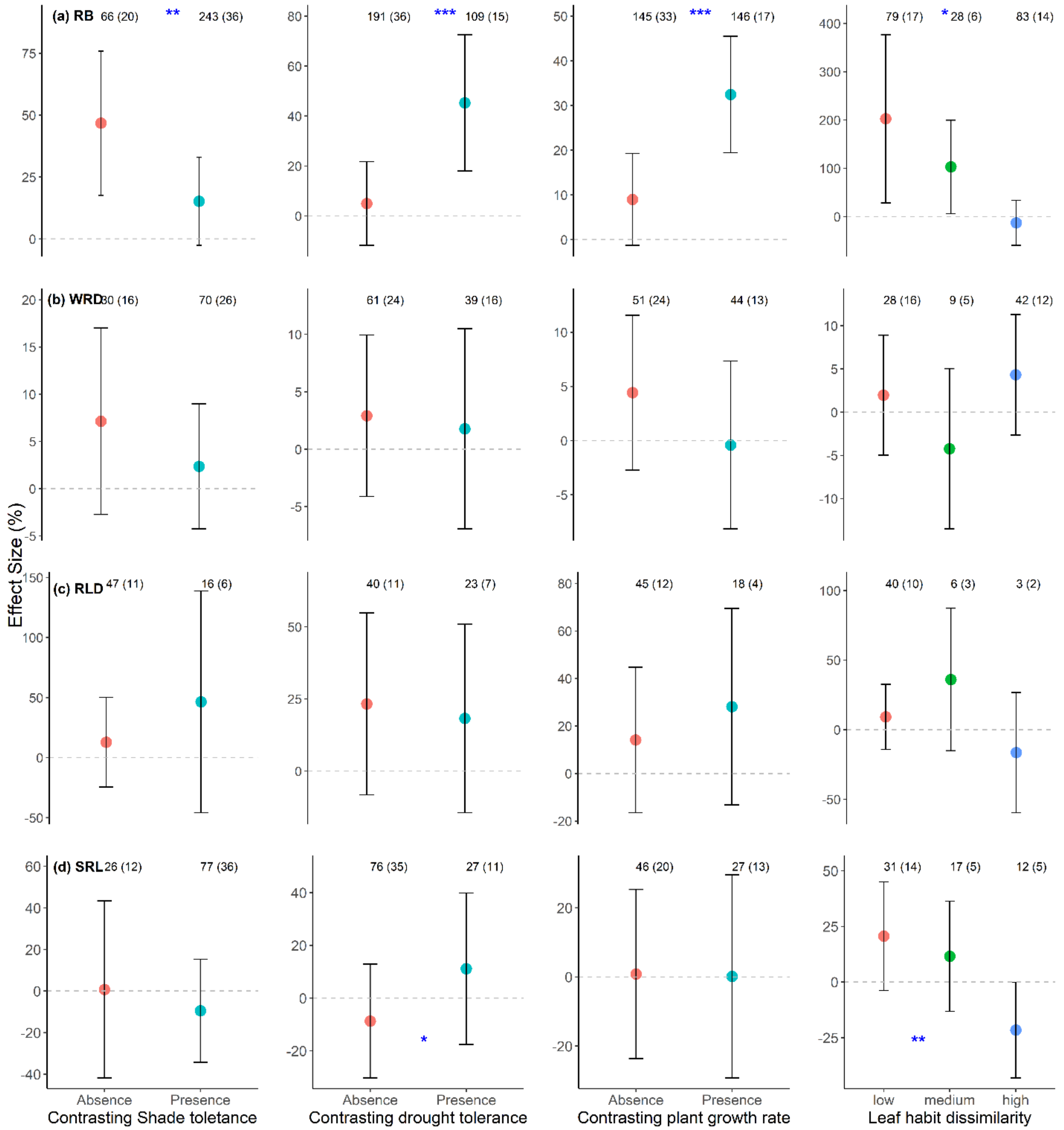

Figure 3-1 Species mixture effects in terms of presence or absence of contrasting shade tolerance, drought tolerance, plant growth rate, and leaf habit dissimilarity on (a) fine-root biomass (FRB), (b) community-weighted mean rooting depth (WRD), (c) root length density (RLD), and (d) specific root length (SRL). Values (estimated β_0 in Equation (4)) are mean ± 95% bootstrapped confidence intervals of the percentage effects between species mixtures and monocultures. The asterisk (*) indicates effects of functional trait dissimilarity is significant (*** means $P < 0.001$, ** means $P < 0.01$, * means $P < 0.05$). The number of observations is shown beside each trait without parentheses, with the number of studies in parentheses.

Among the four root attributes, FRB was the only one that had significant different responses to the impact of contrasting shade tolerance related to stand age (Figs. 3-2a-d). When contrasting shade tolerance was present, the effects of plant mixtures on FRB were shifted from positive to negative over time ($P = 0.004$), but no significant pattern when contrasting shade tolerance was absent ($P = 0.286$). Moreover, there were significant different responses of FRB and RLD to contrasting drought tolerance related to soil depth (Fig. 3-2f, $P = 0.047$ and Fig. 3-2g, $P = 0.048$, respectively). When contrasting drought tolerance was present, the effects of plant mixtures on FRB were increased with soil depth ($P < 0.001$), whereas no response when contrasting shade tolerance was absent ($P = 0.489$). The mixture effect on FRB did not change with species richness in plant mixtures without contrasting drought tolerance (Fig. 3-2e, $P = 0.599$), but that increased in coexisting species with contrasting drought tolerance (Fig. 3-2e, $P < 0.001$). For plant mixtures with contrasting drought tolerance, the effect size of plant mixtures on RLD shifted from negative to positive from surface to deeper soil ($P = 0.009$), but no variation when contrasting drought tolerance was absent ($P = 0.788$). There was no significant difference of mixture effects on SRL with soil depth, no matter whether contrasting drought tolerance was present or not in plant mixtures (Fig. 3-2h, $P = 0.479$). Moreover, the mixture effects on FRB decreased with stand age in mixed-wood forests with low and medium leaf habit dissimilarity but increased with high level (Fig. S3-2, $P < 0.001$).

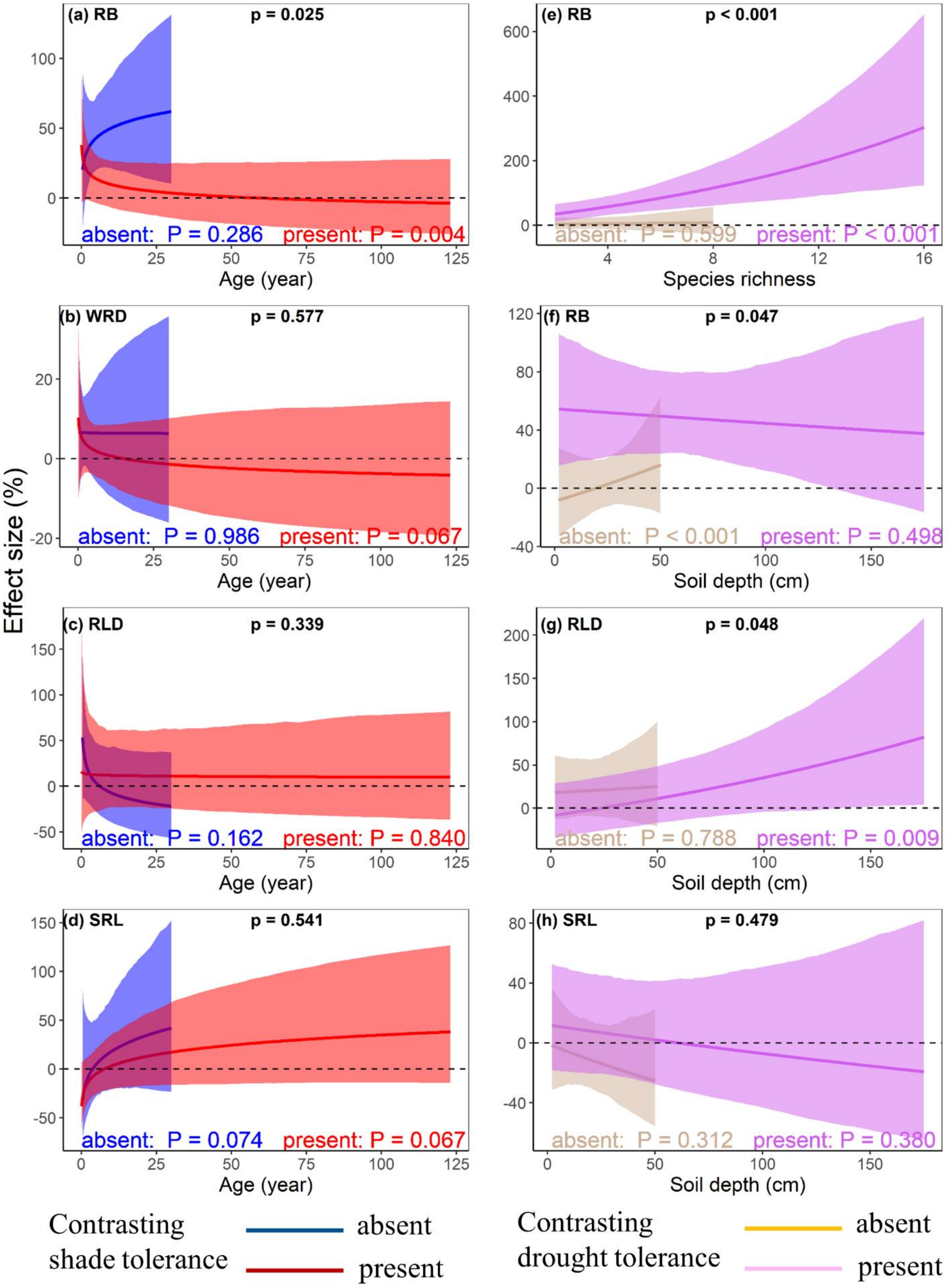
Effect size (%)
(a) RB
p = 0.025
absent: P = 0.286
present: P = 0.004
Age (year)
(b) WRD
p = 0.577
absent: P = 0.986
present: P = 0.067
Age (year)
(c) RLD
p = 0.339
absent: P = 0.162
present: P = 0.840
Age (year)
(d) SRL
p = 0.541
absent: P = 0.074
present: P = 0.067
Age (year)
(e) RB
p < 0.001
absent: P = 0.599
present: P < 0.001
Species richness
(f) RB
p = 0.047
absent: P < 0.001
present: P = 0.498
Soil depth (cm)
(g) RLD
p = 0.048
absent: P = 0.788
present: P = 0.009
Soil depth (cm)
(h) SRL
p = 0.479
absent: P = 0.312
present: P = 0.380
Soil depth (cm)
Contrasting shade tolerance
absent
present
Contrasting drought tolerance
absent
present

Figure 3-2 Effect sizes of species mixture effects on fine-root biomass and traits with age, species richness in mixtures and soil depth with different extent of shade and drought tolerance variations. Species mixture effects on (a) fine-root biomass (FRB), (b) community-weighted mean rooting depth (WRD), (c) root length density (RLD), (d) specific root length (SRL) over time in relation to shade tolerant dissimilarity. Species mixture effects on (e) fine-root biomass (FRB) with species richness in mixtures and (f) fine-root biomass (FRB), (g) root length density (RLD), (h) specific root length (SRL) with soil depth in relation to drought tolerant dissimilarity. The bold black *P*-value text in the upper right corner of each subfigure represents the interactive effect of heterogeneity of shade or drought tolerance with age, species richness in mixtures or soil depth, respectively. The colour of *P*-value text in the bottom left of each subfigure represents the specific heterogeneity of shade or drought tolerance in the coexisting species, respectively, which is corresponding to the same colour line and shadow area. The coloured lines represent the responses of specific heterogeneity of shade or drought tolerance, with their bootstrapped 95% confidence intervals shaded. Figures were plotted based on the selected models from equation (4), the interactive terms species richness*functional trait dissimilarity were excluded in the most parsimonious models for all root attributes except for fine-root biomass with heterogeneity of drought tolerance.

The effects of plant mixtures on FRB, WRD, RLD and SRL in relation to plant functional trait dissimilarity did not differ significantly among different ecosystem types, except for FRB with different leaf habit dissimilarities (Table S3-7, Fig. S3-3). The mixture effect on FRB was higher in natural forests than in planted forests with low leaf habit dissimilarity but was opposite when high leaf habit dissimilarity was present. Moreover, the mixture effects on RLD increased with aridity index in plant mixtures with contrasting shade tolerance but shifted from positive to negative without contrasting shade tolerance (Fig. 3-3a, $P < 0.001$ & $P = 0.022$, respectively). The mixture effects on SRL did not vary significantly with aridity index when contrasting plant growth rate was absent, whereas it shifted from positive to negative when present (Fig. 3-3b, $P = 0.417$ & $P = 0.021$, respectively). The mixture effects on WRD shifted from negative to positive both with mean annual temperature and aridity index in mixed-wood forests with high leaf habit dissimilarity (Fig. 3-3c, $P = 0.009$ & Fig. 3-3d, $P = 0.008$, respectively), but did not change significantly with low and medium levels.

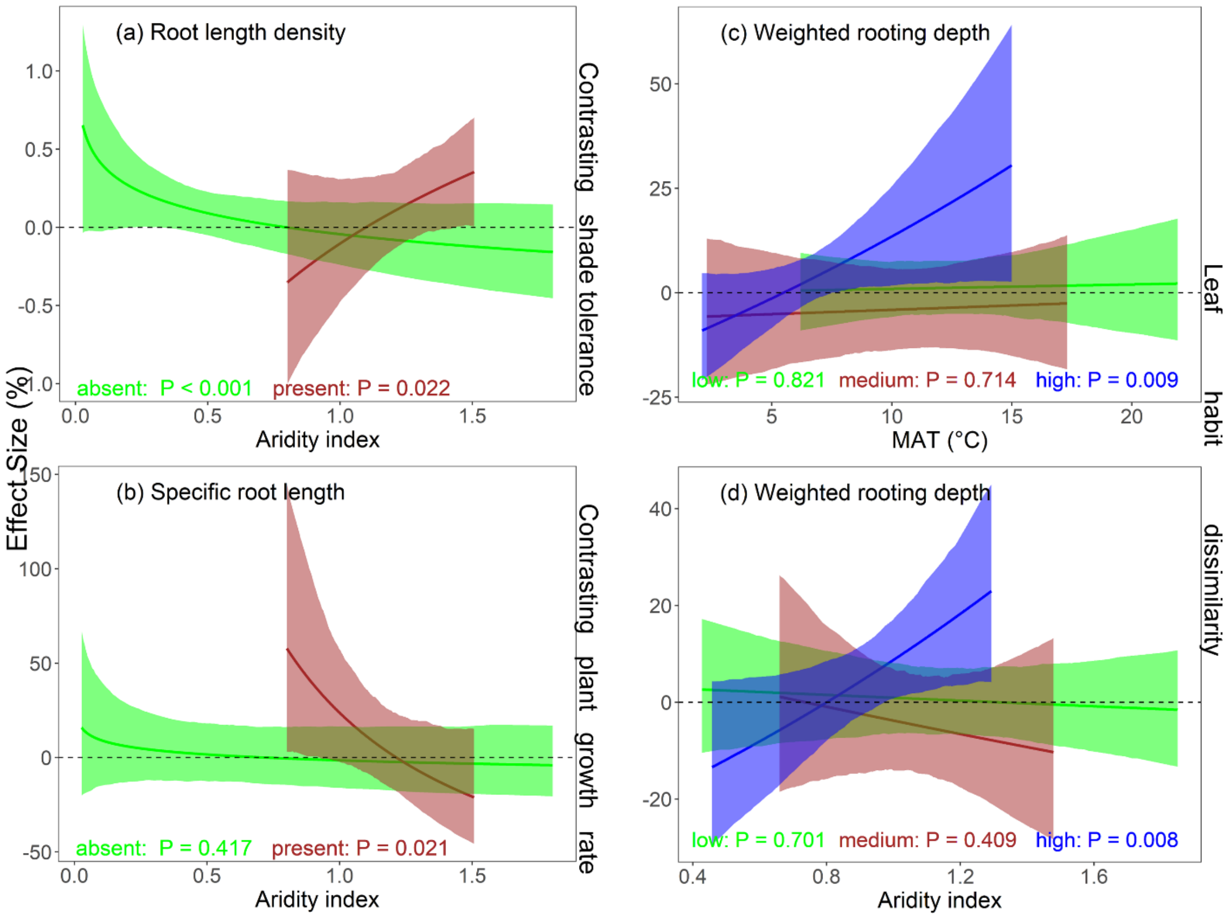

Figure 3-3 Effect sizes of species mixture effects on fine-root traits with aridity index and mean annual temperature (MAT) in relation to functional trait dissimilarity. (a) Species mixture effects on root length density with aridity index with different extent of shade tolerance variations, (b) species mixture effects on specific root length with aridity index with different extent of plant growth rate variations, and species mixture effects on community-weighted mean rooting depth (weighted rooting depth) with (c) MAT and (d) aridity index in related to leaf habit dissimilarity, respectively. The colour of *P*-value text in the bottom left of each subfigure represents the specific functional trait dissimilarity in the coexisting species, respectively, which is corresponding to the same colour line and shadow area. The coloured lines represent the responses of specific functional trait dissimilarity with their bootstrapped 95% confidence intervals shaded.

3.5 Discussion

Analysing 652 paired observations of plant species mixtures with different functional trait dissimilarities and their corresponding monocultures, we discovered that plant functional trait dissimilarity plays a vital role on FRB in plant species mixtures. Moreover, the effects of plant mixtures on FRB changed with shade and drought tolerance dissimilarities, and in relation to stand age and soil depth. However, community-weighted mean rooting depth (WRD) and root length density (RLD)) did not show significant differences to each trait dissimilarity. Also, fine-root traits had negligible responses to stand age and soil depth, no matter contrasting shade and drought tolerance occurred or not in plant mixtures. However, there was a positive mixture effect on RLD with soil depth with heterogeneity of drought tolerance.

We found various responses of plant mixture effects on FRB and traits to plant functional trait dissimilarity. Firstly, the presence of contrasting shade tolerance had a lower FRB than that of absence, also lower when leaf habit dissimilarity was high than low and medium dissimilarities. Heterogeneity in shade tolerance and leaf habit could lead to more efficient light exploitation and utilization in plant species mixtures due to reduced interspecific competition by crown complementarity and various timing for leaf bloom and senescence (Reich *et al.*, 2003; Yachi & Loreau, 2007; Coomes *et al.*, 2009; Williams *et al.*, 2017). Plants in mixtures with contrasting shade tolerance and high leaf habit dissimilarity tend to allocate more carbon to aboveground photosynthesis instead of belowground resource uptake. The mixture effects on FRB were both higher when contrasting drought tolerance or plant growth rate was present than absent, respectively. Plant species mixtures with heterogeneity in drought tolerance and plant growth rate could have a higher FRB to support the high activities of soil exploration due to the larger differences of root distribution, nutrient preference forms and absorbing period for soil resources (Ashton *et al.*, 2010; Ishii & Asano, 2010; Brassard *et al.*, 2013). Secondly, higher SRL for greater resource uptake efficiency would be required to uptake additional soil resources derived from the more water and nutrient sources via fine-root segregation when contrasting drought tolerance was present (Germon *et al.*, 2017; Zwetsloot *et al.*, 2019). Whereas the high possibility of greater aboveground allocation with high leaf habit dissimilarity could enable fine roots uptake the resources more conservatively, i.e., a lower SRL (Reich, 2014). Lastly, we also found there was no significant differences for the effects of species mixture on WRD and RLD whether heterogeneity in plant functional dissimilarity presented or not, which suggest that fine roots do not change the strategy to explore shallow or deep soil layer and capacity of root systems for soil resource uptake (Guderle *et al.*, 2018; Archambault *et al.*, 2019).

We found that, among the 4 fine-root attributes, root biomass was the only one responding differently to stand age in plant species mixtures with or without contrasting shade tolerance. In the older stands, plants could increase the aboveground productivity with closing canopy in plant mixtures with contrasting shade tolerance (Zhang *et al.*, 2012), leading to greater shade and then elevated soil moisture and lower temperature. Therefore, the mixture effects on FRB could decrease over time due to a lower water requirement when contrasting shade tolerance is present. The lacking stand-age variations of plant mixture effects on WRD, RLD and SRL, irrespective of contrasting shade tolerance presented or not, suggests that the heterogeneity of shade tolerance do not play an important role in determining the soil resource uptake over time. Moreover, FRB in plant mixtures with low leaf habit dissimilarity decreased from positive to negative with stand age but increased from negative to positive with high leaf habit dissimilarity. This is expected since for mixed forests with high leaf habit dissimilarity, the competition could override the facilitation in young stands, but the increasing interspecific complementarity in mature stands improve fine root growth (Cardinale *et al.*, 2007; Reich, 2012). However, competition for light interception could be harsher due to the canopy closure with stand development in mixed forests with low leaf habit dissimilarity. Therefore, increasing interspecific competition could weaken the mixture effects on root growth over time.

We also found that the effects of species mixtures on FRB and RLD in relation to soil depth both differed with the extent of drought tolerance variations. The wider range of drought tolerance could have better resource utilization by multiple rooting depths or alleviate the physical stress during water limitation, leading to competitive reduction (Wright *et al.*, 2017). Plant mixtures with contrasting drought tolerance could increase RLD in deeper soil, leaving a stable relationship between species mixture effects on FRB in relation to soil depth. In this way, thinner roots in deeper soil could improve the efficiency of soil exploration (Ma *et al.*, 2018), leading a more acquisitive resource uptake for hydraulic lift (Pretzsch *et al.*, 2013). While plant species mixtures without contrasting drought tolerance could have thicker roots in deeper soil, deriving from positive soil-depth effects on FRB but null effect on RLD. Thus, fine roots with an increasing diameter could have a more conservative strategy to deal with the lower resource availability in deeper soil (Jobbágy & Jackson, 2001). Lastly, the effects of species mixture on FRB increased with species richness significantly in plant mixtures with contrasting drought tolerance, extending the understand of species-richness effects on FRB via specifying the plant functional trait (Peng & Chen, 2021).

Despite the wide range of variations in ecosystem types, the responses of the four root attributes did not differ with the extent of variations in plant functional traits, except for FRB in mixed-wood forests with different leaf habit dissimilarities. FRB was higher in mixed natural forests with low leaf habit dissimilarity but lower with high leaf habit dissimilarity than mixed plantations, respectively. Planted forests typically have a lower FRB and soil water content, a lower concentration of soil nutrients such as nitrogen and phosphorus than natural forests (Liao *et al.*, 2012; Cai *et al.*, 2019). With increasing leaf habit dissimilarity, the higher plant mixture effects on FRB in planted forests than natural forests could increase the ability to gain larger quantity of soil water and nutrients to meet the higher plant metabolisms due to the more complete light utilization.

We found the effects of plant mixtures on RLD, WRD and SRL changed with aridity index and mean annual temperature within plant functional trait dissimilarity. Firstly, the mixture effects on RLD decreased with aridity index in plant mixtures with contrasting shade tolerance but increased without contrasting shade tolerance. The shade-intolerant species could benefit from the shade-tolerant species through microclimatic amelioration (Wright *et al.*, 2017; Barry *et al.*, 2019), which ensure the plant community functioning well by higher light utilization. In humid area with rich soil nutrients due to fast litter decay rate (Liu *et al.*, 2020), this beneficial relationship could be strengthened by resource partitioning. In this way, plants may call for higher RLD to uptake soil nutrients to maintain the plant community functioning. However, lack of microclimatic alleviation in plant mixtures without contrasting shade tolerance results in a more intense competition in drier area due to poorer resource availability. Therefore, enhanced interspecific facilitation under harsher environment could promote a higher mixture effect on RLD in drier areas without contrasting shade tolerance (Forrester & Bauhus, 2016). Secondly, the presence of contrasting plant growth rate decreased SRL with aridity index in plant mixtures, suggesting a lower resource uptake efficiency in wetter areas. Plants with different growth rates could use the soil nutrients more completely, which alleviates the nutrient limitations for plant growth during nutrient deficiency. The mitigation of nutrient limitations could be weakened because of the higher resource availability in wetter areas. Thus, fine roots of plant mixtures with contrasting plant growth rate could have a more conservative nutrient uptake with aridity index. Lastly, fine roots developed in deeper soil with both elevated mean annual temperature and aridity index in mixed-wood forests with high leaf habit dissimilarity. The deeper soil penetration for resource demands is required to meet the more complete light utilization and greater photosynthesis in warmer and wetter areas.

In conclusion, our results demonstrate higher FRB in plant mixtures containing contrasting drought tolerance or plant growth rate than those with homogeneous functional traits, respectively. However, FRB was lower in mixtures with contrasting shade tolerance than without or in mixed-wood forests with high leaf habit dissimilarity than lower levels, respectively. We further reveal that the mixture effects on FRB decreased over time with contrasting shade tolerance and increased with species richness in plant mixtures with contrasting drought tolerance, respectively. These divergent responses of FRB to various plant functional trait dissimilarity indicate different ratios of aboveground and belowground biomass allocation for better utilization on light vs water and nutrients. We further reveal that the mixture effects on FRB decreased over time with contrasting shade tolerance and increased with species richness with contrasting drought tolerance, respectively. Also, the mixture effects on FRB increased with soil depth when contrasting drought tolerance was absent. These findings provoke us to take plant functional trait dissimilarity into account when interpreting the effects of plant mixtures on FRB. Moreover, the mixture effects on SRL were higher with contrasting drought tolerance than without, but lower in mixtures with high leaf habit dissimilarity than lower levels. The mixture effects on RLD increased with soil depth when contrasting drought tolerance was present. In addition, the effects of species mixtures on RLD, SRL and WRD changed differently with aridity index or mean annul temperature with plant functional trait dissimilarity. Collectively, our study suggests that plant functional trait dissimilarity drives the variations in plant mixture effects on FRB and traits. Given that the key role of FRB and traits in mediating the soil water and nutrients (Bardgett *et al.*, 2014; McCormack *et al.*, 2017), we should pay more attention to plant functional trait dissimilarity in future study.

Chapter 4. ENHANCED MIXTURE EFFECTS ON SOIL RESOURCE UPTAKE CAPACITY UNDER ALTERED PRECIPITATION IN YOUNG BOREAL FORESTS

4.1 Summary

- Fine roots underpin plant water and nutrient uptake and regulate the terrestrial biogeochemical cycles. Both plant diversity loss and precipitation changes are known to influence fine-root biomass (FRB) and root functional traits, but how changes in precipitation influence the effects of plant diversity on FRB and root functional traits remain unclear.
- We manipulated the precipitation changes in young natural boreal forests dominated by *Populus tremuloides, Pinus banksiana*, and their relatively even mixtures under 25% throughfall addition, ambient water and 25% throughfall reduction during the growing season (May to October). We collected soil samples to measure FRB and root functional traits including root surface area, fine-root volume, root length and tissue density, root mean diameter, length ratio (ratio of root length with Ø < 0.5 mm to the total fine root length), and specific root length and area. We hypothesized that the effects of species mixtures on FRB and root functional traits are more pronounced under water reduction and addition than under ambient precipitation.
- We found that FRB in mid-summer (August) was higher in evenly mixed- than single-species-dominated stands under ambient precipitation (41%), with more pronounced increases under water reduction (89%) and under water addition (71%), but species mixtures had no effects on FRB in fall (October). Root surface area, fine-root volume, and root length density responded to species mixtures similarly to those of FRB. However, the responses of other root functional traits were weak.

- Our results provide the first evidence that the positive effects of tree species mixtures on fine root traits of soil resource uptake capacity are more pronounced under reduced and increased water availability than under ambient precipitation in natural boreal forests. However, tree species mixtures have limited effects on fine root resource uptake efficiency.

Keyword: plant mixtures, root biomass, root functional traits, water addition, water reduction

4.2 Introduction

Plant fine roots (Ø < 2 mm) play an important role in regulating terrestrial carbon and nutrient cycling (Hendricks *et al.*, 1993; Clemmensen, 2013). Although fine-root biomass (FRB) constitutes only a small proportion of whole plant biomass, fine-root production represents around 22% of the global terrestrial net primary production because of its high turnover rate and subsequent decay (McCormack *et al.*, 2015). In response to changes in biotic interactions and abiotic environment, plants regulate soil resource uptake capacity and efficiency by altering FRB and root functional traits (Gould *et al.*, 2016; McCormack *et al.*, 2017; Carmona *et al.*, 2021; Freschet *et al.*, 2021). Root surface area (RSA, total root surface area per soil volume), fine-root volume (FRV, total root volume per soil volume), root length density (RLD, length of root per soil volume), root tissue density (RTD, root dry mass per root volume), and root mean diameter (RMD, average diameter of roots) are positively related to the resource uptake capacity of the plant root system (Bardgett *et al.*, 2014; Weemstra *et al.*, 2016; Li *et al.*, 2017; Wambsganss *et al.*, 2021). A higher fraction of very fine root (Ø < 0.5 mm) means less carbon invested for the given absorptive capacity via root length, which could be used as a proxy for the resource uptake efficiency of the root system (Bergmann *et al.*, 2017; Altinalmazis-Kondylis *et al.*, 2020). Moreover, as indices of root investment benefit to cost, specific root length (SRL) and area

(SRA), as length/mass and area/mass ratios, respectively, are positively related to resource uptake efficiency per unit carbon investment to fine roots (Eissenstat *et al.*, 2000; Ostonen *et al.*, 2007; McCormack *et al.*, 2012). Root trait-based methods can help us to better understand plant function and strategies in response to global changes such as altered precipitation and biodiversity loss.

Plants adjust FRB and root functional traits to acclimate to the altered water availability. More frequent and intense drought and heavy rain events have been threatening stability in ecological communities (Wright *et al.*, 2015; Donat *et al.*, 2017), and further affect water and nutrient cycling and plant survival and productivity (McDowell *et al.*, 2011; Wilcox *et al.*, 2017; Choat *et al.*, 2018). Increasing precipitation generally drives more fine-root investment, i.e., increasing FRB, to uptake soil resources derived from elevated water availability (Zhang *et al.*, 2019; Wang *et al.*, 2020), whereas decreasing precipitation has the opposite effect because of reduced transpiration and respiration rates (Zang *et al.*, 2014; Brunner *et al.*, 2015; Zhou *et al.*, 2018). Meanwhile, fine roots could adjust toward a more acquisitive strategy with elevated water availability: a greater RLD, SRL and SRA to meet the higher water and nutrient demands to support increased aboveground productivity (Ryser, 2006; Comas *et al.*, 2013; Zhang *et al.*, 2019). Also, fine roots could increase resource uptake capacity and efficiency to alleviate stress from limited water: thinner fine roots with greater RLD, SRL and SRA to improve the hydraulic conductance (Comas *et al.*, 2012; 2013; Olmo *et al.*, 2014; Ma *et al.*, 2018). However, the different responses of FRB and root functional traits have been reported in response to changes in precipitation. For instance, decreased FRB and SRL have been reported under reduced precipitation (Brunner *et al.*, 2015; Zhou *et al.*, 2018), but others found no changes in FRB and SRL under either increased (Zhang *et al.*, 2019) or decreased precipitation (Zhang *et al.*, 2019;

Wang *et al.*, 2020). Yet, how FRB and functional traits respond to altered water availability in natural forests remains virtually unknown, although their responses can strongly determine the fate of terrestrial ecosystems under ongoing climate change.

Plant species composition and diversity could also influence FRB and root functional traits (Peng & Chen, 2021). Coniferous forests usually invest more in fine roots (Yuan & Chen, 2010; Domisch *et al.*, 2015; Finér *et al.*, 2017), which have a slow litter decomposition and thus generally have low soil nutrient availability indicated by a lower soil carbon: nitrogen ratio (Cools *et al.*, 2014; See *et al.*, 2019). In the meantime, species mixtures can increase FRB and alter root functional traits to enhance resource uptake efficiency and capacity in response to their greater demands for water and nutrients (Peng & Chen, 2021). The higher FRB and changes in root functional traits in species mixtures can result from higher soil volume filling and niche partitioning among component species since different plant species may prefer different sources of water and nutrients among soil depths and different forms of nutrients (Ashton *et al.*, 2010; Brassard *et al.*, 2013; Silvertown *et al.*, 2015). We therefore expected that species mixture of broadleaf and conifer tree species could have a higher FRB and root functional traits with higher resource uptake efficiency and/or capacity than the average of corresponding monocultures.

Recent conceptual syntheses suggest that plant diversity can help mitigate the negative effects of climate change (e.g., reduced water availability) and enhance the positive effects of climate change (e.g., increased water availability) on ecosystem functioning (Hisano *et al.*, 2018; Mori *et al.*, 2021). Previous experiments have reported increasing average tree growth, aboveground productivity and ecosystem resistance to various natural climate events such as wet or dry, mild or extreme in more diverse grasslands and forests (Isbell *et al.*, 2015; Klaus *et al.*, 2016; O'Brien *et al.*, 2017; Wagg *et al.*, 2017; Chen *et al.*, 2022). However, knowledge about the acclimating strategies of fine roots with altered precipitation in species mixtures remains in its infancy, particularly in natural forests. The difference in nutrient niches and water lifting from the deep-rooted species to shallow-rooted species in mixtures may increase water uptake per root length and area (Guderle *et al.*, 2018), and further, strengthen the higher water uptake induced by greater aboveground productivity under increased precipitation and weaken water stress under reduced precipitation, respectively (Wu *et al.*, 2011). Moreover, the effects of plant mixtures on Arbuscular mycorrhizal (AM) fungi biomass have been shown to increase under both water addition and reduction (Chen *et al.*, 2019). Trees with higher AM fungi biomass could proliferate greater fine roots, and the colonized fine roots with AM fungi could uptake nutrients more efficiently via mediating root functional traits and mycorrhizal hyphae (Zangaro *et al.*, 2008; Chen, WL *et al.*, 2016; Cheng *et al.*, 2016). Therefore, we expected that the positive effects of species mixtures on FRB are more pronounced with both water addition and reduction conditions; meanwhile, root functional traits should shift toward a higher resource uptake capacity and/or efficiency to maintain the elevated resource demands from the increasing aboveground productivity.

A better understanding of the interactive effects of species mixtures and precipitation changes on fine roots will deepen the knowledge about belowground processes, and further help us to manage plant communities under global climate change. To explore whether water availability would affect the effects of species mixtures on FRB and root functional traits, we manipulated the precipitation changes in young natural boreal forest stands dominated by *Populus tremuloides* (broadleaved species), *Pinus banksiana* (coniferous species), and their relatively even mixtures in 2016. The precipitation change treatment included 25% throughfall addition, ambient water and 25% throughfall reduction during the growing season. After three-year treatments, we collected soil samples to examine the interactive effects of species mixtures and precipitation changes on fine roots. We hypothesized that (1) on average, mixtures would have a higher FRB and root functional traits with a higher resource uptake capacity and/or efficiency (i.e., greater RSA, FRV, RLD but lower RTD, and/or greater very fine root fraction, SRL and SRA) than those of corresponding monocultures; alternatively, FRB and root functional traits of resource uptake capacity and efficiency increase with increasing tree species diversity; (2) the effects of species mixtures and tree species diversity would be more pronounced under both water addition and reduction. In our young boreal stands, the soil temperature peaks in August and falls to the lowest in October during the growing season (Chen *et al.*, 2021). As FRB and functional traits are related to temperatures (Pregitzer *et al.*, 2000; Wang *et al.*, 2021), we also tested the above hypotheses between the two sampling months, August and October.

4.3 Materials and Methods

4.3.1 Site description

The study was conducted in the central boreal forests of Canada, located north of Lake Superior and west of Lake Nipigon, ca. 150 km north of Thunder Bay, Ontario, Canada (49°27' N–49°38' N, 89°29' W–89°54' W). The study area falls within the Moist Mid-Boreal (MBX) ecoclimatic region, and is characterized by warm summers and cold, snowy winters (Ecoregions Working Group, 1989). The mean annual temperature is 2.8 °C, and annual precipitation is 670 mm. The soils of the upland sites are relatively deep glacial tills of the Brunisolic order (Group *et al.*, 1998). The forests in the study area are natural fire-origin stands with vertical structures (overstory trees, understory trees, and other woody and non-woody plants) (Kumar *et al.*, 2018). The dominant overstory tree species include jack pine (*Pinus banksiana* Lamb.) and trembling aspen (*Populus tremuloides* Michx.) (Table 4-1). Common understorey shrub species in the area include mountain maple (*Acer spicatum* Lam.), alder (*Alnus* spp.), and beaked hazel (*Corylus cornuta* Marsh.).

Table 4-0-1 Characteristics (mean and 1 s.e.m., n = 3) of the study stands in Northwestern Ontario, Canada. Stand types are single-species *Populus tremuloides* dominated (broadleaf), single-species *Pinus banksiana* dominated (conifer), and their mixtures (mixed wood).

Stand type	*Populus*	*Pinus*	*Populus+Pinus*
Height (m)	3.02 ± 1.22	3.49 ± 1.43	3.70 ± 2.13
Stand basal area (m^2 ha^{-1})	1.55 ± 0.35	0.93 ± 0.33	1.39 ± 0.24
Stand density (trees ha^{-1})	5933 ± 1790	11600 ± 4148	9200 ± 1301
Tree species composition (% of stand basal area)			
Populus tremuloides	92 ± 2	1 ± 1	28 ± 3
Pinus banksiana	3 ± 2	98 ± 1	48 ± 7
Betula papyrifera	3 ± 2	1 ± 1	15 ± 4
Other species	2 ± 1	1 ± 0	4 ± 1
Soil carbon concentration (0-15cm, g kg^{-1})	20.41 ± 1.65	14.24 ± 2.39	17.15 ± 3.86
Soil nitrogen concentration (0-15cm, g kg^{-1})	1.20 ± 0.12	0.93 ± 0.12	1.06 ± 0.21
Soil pH	5.22 ± 0.24	5.21 ± 0.29	5.21 ± 0.30
Forest floor depth (cm)	1.89 ± 0.75	2.76 ± 0.17	1.52 ± 0.35

4.3.2 Experimental design

Young forests represent a dominant component of boreal forests because of short fire return intervals, which are expected to be further shortened under climate change (Flannigan *et al.*, 2009). Moreover, mortality and growth of young forests are more sensitive to climate change (Chen, HYH *et al.*, 2016), and smaller individuals with high stem densities make the experiment of limited treatment areas feasible to cover a large number of stems, which are essential to examine community-level processes. In this study, nine 11-year-old spatially interspersed (distance > 1 km between the nearest) stands (each with an area > 1 ha), three each for pure jack pine, pure trembling aspen, and their even mixtures were selected from our long-term plot network in 2016. Similar to our previous study (Ma & Chen, 2018), single- and mixed-species stands were defined as stands containing a single species with over 80% stand basal area and stands in which any of the component species had less than 80% stand basal area, respectively.

Within each stand, we applied three split-plot water availability treatments: the ambient, 25% growing season (May to October) throughfall water reduction under the canopy, and 25% throughfall water addition, which are the medians of the expected water availability variability during the 21st century for Canada's boreal forests (Pachauri *et al.*, 2014). Each of the 27 treatment plots consisted of an area of 6 × 6 m (36 m^2), including a mean tree density of 98 stems per plot (range =33 to 416 stems), which is similar to those observed in a mature boreal forest plot of an area of 0.2 ha (Chen & Luo, 2015).

For each nine water reduction treatment plots, we built rain shelters under the canopy, consisting of four shelters (3 m × 3 m), which were held in place by metal stakes and wires. Each metal frame supported 4 U-shaped clear acrylic troughs (3 m long, 20 cm wide) that were spaced 35 cm apart. The U-shaped troughs were oriented at a 10° angle, with the high end positioned at 1.8 m and the low end at 1.35 m above ground. The rain shelters funnelled water into two 8-cm (inner diameter) polyvinyl chloride (PVC) pipes, each with 6 different holes of size (diameter: 0.64, 1.93, 3.18, 4.45, 5.72 and 6.99 cm), arranged at the intervals of 46 cm, to distribute the collected water evenly over the adjacent water addition plot. Between treatment plots, a 5 m buffer zone was established (Chen *et al.*, 2019). To monitor soil water availability, we measured the volumetric soil water content using a Decagon soil moisture sensor biweekly, at a depth of 5 cm below the soil surface during the snow-free period.

4.3.3 Data Collection

For each treatment plot, we recorded the plant species, and the diameter at breast height (DBH), taken at 1.3 m above root collar, for all live trees in 2017 and 2020. Stand basal areas by tree species were summed to the plot level and used for assigning stand type classification (Table 1). Coniferous tree proportion for each plot was the ratio of the basal area of coniferous tree species to the total tree basal area in that plot. Tree species richness was the number of tree species in the plot. We calculated Shannon's index using the tree species proportion based on the relative basal area by *Vegan* package (Oksanen *et al.*, 2013). Tree species evenness was calculated as the ratio of Shannon's index to the natural logarithm of species richness (Pielou, 1969). Stem density and basal area were based on the inventory data of 2017.

To sample fine roots, we extracted three soil cores (6.6 cm diameter) in each plot from the mineral soil at random locations to a depth of 15 cm after removing the litter layer gently, on August 9th and October 30th, 2019 by using a power auger, that is, three years after the beginning of our water alteration treatments. We mixed the three soil-core samples into one sample evenly for each treatment plot in the field. In total, we extracted 162 cores for roots, resulting in 54 samples for laboratory analysis.

Soil samples were transported in an ice-filled cooler from the field to the lab. We stored the samples in a freezer at – 18 ºC until they were processed. These samples were initially sieved gently through a sieve with a 2 mm mesh size, rocks and woody debris were removed, and visible roots were hand-sorted. Then the soil samples went through a 1-mm-mesh-size sieve, where visible roots were also hand-sorted. The remaining soil samples were gently washed over a sieve (0.085 mm mesh size), and fine roots with lengths over 1 cm were collected with tweezers. Fine roots (< 2 mm in diameter) were selected and further sorted to live or dead status. Live roots were pale-coloured on the exterior, elastic and flexible, free of decay and had a whitish cortex, whereas dead roots were brown or black, rigid and inflexible, were in various stages of decay and had a darker cortex (Persson, 1983).

Live roots were scanned at a resolution of 600 dpi (EPSON EXPRESSION 10000XL), then analysed by WinRhizo Software (version 2022c Pro, Regents Instruments Inc. Canada). Fine-root length, surface area (RSA) and volume (FRV) for each figure were measured automatically by the software and were then summed up to plot level for per soil volume (RSA, $cm^2\ cm^{-3}$; FRV, $cm^3\ dm^{-3}$), and mean diameter (RMD; mm) for each plot was weighted by the root length proportion of each figure. The scanned fine roots were oven-dried to a constant mass at 65 ºC and weighed, then root length density (fine-root length/ soil volume, RLD, $m\ cm^{-3}$), root tissue density (root dry weight/ fine-root volume, RTD, $g\ cm^{-3}$), specific root length (fine-root length/ dry root weight, SRL, $m\ g^{-1}$) and specific root area (fine-root surface area/ dry root weight, SRA, $cm^2\ g^{-1}$) were calculated. Then FRB for each plot was scaled up from per soil auger (34.82 cm^2) to per m^2. The distributions 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) were also extracted through the scanning process. The very fine root fraction (diameter class of 0.0–0.5 mm) is usually occupied by 1st and 2nd root orders (most distal parts of the root system). In total, we derived eight root traits, RSA, FRV, RLD, RTD, RMD, length ratio (LR, ratio of root length with diameter less than 0.5 mm to the total root length, %), SRL and SRA.

4.3.4 Statistical analysis

Firstly, to examine the effects of species composition (overstory type, T), water treatment (W) and sampling month (M) on FRB and root functional traits, the following linear mixed-effect model was employed, and we conducted post hoc comparisons with *lsmeans* and *emmeans* packages (Lenth, 2016):

$$Y_{ijklm} = T_i + W_{j(l)} + M_k + T_i \times W_{j(l)} + T_j \times M_k + W_{j(l)} \times M_k + \pi_l + \varepsilon_{m(ijkl)} \qquad (1)$$

where Y_{ijklm} is FRB, RSA, FRV, RLD, RTD, RMD, LR, SRL and SRA; T_i is the stand type (i = broadleaf, conifer and mixture); $W_{j(l)}$ is the water treatment (j = 25% rainfall addition, 25% rainfall reduction or ambient) nested within each whole plot l (l = 1, 2, …9); and M_k is the sampling month (k = August or October); π_l is the random effect of plot; $\varepsilon_{m(ijkl)}$ is the sampling error. We used the restricted maximum likelihood estimation with the package *lme4* to conduct the analysis (Bates *et al.*, 2015).

Secondly, we used the response ratio (RR) to represent the effects of species mixtures on FRB and root functional traits:

$$RR = \frac{X_{observed}}{X_{expected}} \tag{2}$$

where $X_{observed}$ is the observed value in mixtures, and $X_{expected}$ is the expected value in mixtures. $X_{expected}$ was calculated as the weighted mean of the corresponding species in monocultures according to the proportion of species basal area in mixtures (Loreau *et al.*, 2001).

Then we used the linear mixed-effect model to test whether the effects of species mixtures on FRB and root functional traits changed with sampling month (M) and water treatment (W):

$$RR_{ijkl} = W_{i(k)} + M_j + W_{i(k)} \times M_j + \pi_k + \varepsilon_{l(ijk)} \tag{3}$$

where RR_{ijkl} is the calculated mixture effects on FRB, RSA, FRV, RLD, RTD, RMD, LR, SRL and SRA; $W_{j(k)}$ is the water treatment (j = 25% rainfall addition, 25% rainfall reduction or ambient) nested within each whole plot k (k = 1, 2, …9); and Mj is the sampling month (j = August or October); π_k is the random effect of plot; $\varepsilon_{l(ijk)}$ is the sampling error. We conducted the analysis using the restricted maximum likelihood estimation with the *lme4* package (Bates *et al.*, 2015). The effects of species mixtures were significant at $\alpha = 0.05$ if the 95% confidence intervals of estimated RR did not cover 1. Values of RR on FRB above 1 or below 1 indicate that the FRB is overyielding or under-yielding, respectively. The difference between groups was significant if 95% confidence intervals of their coefficients did not overlap the other's mean.

To simultaneously account for the influences of species identity (or composition) and diversity on fine-root attributes, we tested the effects of tree species diversity (D) and coniferous tree proportions (C) on FRB and root functional traits, and whether the effects of species richness differed with water availability (W) and sampling month (M) using the following model:

$$Y_{ijklmn} = D_i + W_{j(m)} + M_k + C_l + D_i \times W_{j(m)} + D_i \times M_k + W_{j(l)} \times C_l + M_k \times C_l + \pi_m + \varepsilon_{n(ijklm)} \quad (4)$$

where Y_{ijklmn} is the value of FRB, RSA, FRV, RLD, RTD, RMD, LR, SRL and SRA; D_i is the tree species diversity (either species richness (R), Shannon diversity index (H), or species evenness (J)), C_l is the coniferous tree proportion; $W_{j(m)}$, M_k, π_m, and $\varepsilon_{n(ijklm)}$ are the same as in eqn. 1. The models with species evenness consistently had the lowest values of Akaike information criterion (AIC) among three diversity metrics (Table S4-1). We thus focus our reporting on the species evenness models.

During the data analysis, assumptions of normality were examined by Shapiro-Wilk's, and RLD, LR were log-transformed to meet the normality assumption. All statistical analyses were performed in R 4.2.1 (R Core Team, 2022).

4.4 Results

Stand type analysis showed that water treatment and sampling month had significant effects on FRB while the effects of stand type on FRB differed with sampling month (Table 4-2). FRB was higher under water addition and lower under reduction than under ambient precipitation in August, but was higher under ambient precipitation in October, and it was on average higher in

August than in October (Fig. 4-1A, Table 4-2). Moreover, FRB was highest in broadleaf stands and lowest in conifer stands with the mixtures being in the intermediate under all water treatment in August, there was no significant difference among stand types in October (Fig. 4-1A). Both RSA and FRV differed significantly with stand type and sampling month (Table 4-2). Mixtures had higher RSA and FRV than monocultures in August but not in October (Figs. 4-1B & C). RTD was lower under water addition and reduction than ambient precipitation in August but no differences in October (Fig. 4-1E, Table 4-2). Stand type, water treatment and sampling month did not affect other root traits, including RLD, RMD, LR, SRL and SRA, except for a marginal higher SRA in August than in October (Fig. 4-1, Table 4-2).

Table 4-0-2 Effects of water treatment (W), stand type (T), sampling month (M) on fine-root biomass, root surface area, fine-root volume, root length density, root tissue density, mean root diameter and length ratio (0-0.5mm diameter), specific root length, specific root area. Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). *P* and R^2 are the significance of the model and explained variance by the model, respectively.

Source	df	F	*P*
Fine-root biomass ($R^2_{marginal}$ = 0.368, $R^2_{conditional}$ = 0.711)			
W	2,34	5.02	**0.012**
T	2,6	1.42	0.312
M	1,34	5.39	**0.026**
W × M	2,34	0.98	0.386
W × T	4,34	1.43	0.246
T × M	2,34	10.63	**< 0.001**
Root surface area ($R^2_{marginal}$ = 0.304, $R^2_{conditional}$ = 0.619)			
W	2,34	2.07	0.140
T	2,6	1.25	0.351
M	1,34	6.04	**0.019**
W × M	2,34	0.58	0.564
W × T	4,34	0.97	0.424
T × M	2,34	6.36	**0.004**
Fine-root volume ($R^2_{marginal}$ = 0.460, $R^2_{conditional}$ = 0.720)			
W	2,34	0.55	0.580
T	2,6	1.94	0.224

M	1,34	19.33	**< 0.001**
W × M	2,34	1.42	0.255
W × T	4,34	0.88	0.485
T × M	2,34	17.41	**< 0.001**
Root length density ($R^2_{marginal}$ = 0.226, $R^2_{conditional}$ = 0.546)			
W	2,34	1.37	0.268
T	2,6	0.90	0.454
M	1,34	0.88	0.355
W × M	2,34	0.65	0.528
W × T	4,34	1.35	0.272
T × M	2,34	1.98	*0.154*
Root tissue density ($R^2_{marginal}$ = 0.353, $R^2_{conditional}$ = 0.413)			
W	2,34	4.43	**0.019**
T	2,6	0.64	0.558
M	1,34	10.54	**0.003**
W × M	2,34	1.30	0.287
W × T	4,34	0.53	0.714
T × M	2,34	2.83	*0.073*
Mean root diameter ($R^2_{marginal}$ = 0.149, $R^2_{conditional}$ = 0.617)			
W	2,34	0.59	0.561
T	2,6	0.50	0.632
M	1,34	0.67	0.419
W × M	2,34	0.02	0.982
W × T	4,34	1.81	0.149
T × M	2,34	1.58	0.221
Length ratio (0-0.5mm diameter) ($R^2_{marginal}$ = 0.176, $R^2_{conditional}$ = 0.616)			
W	2,34	0.56	0.576
T	2,6	0.31	0.745
M	1,34	1.50	*0.230*
W × M	2,34	0.26	0.770
W × T	4,34	1.04	0.402
T × M	2,34	1.05	0.362
Specific root length ($R^2_{marginal}$ = 0.175, $R^2_{conditional}$ = 0.450)			
W	2,34	0.74	0.484
T	2,6	0.58	0.589
M	1,34	0.10	0.758
W × M	2,34	0.17	0.844
W × T	4,34	2.54	*0.058*
T × M	2,34	0.07	0.935
Specific root area ($R^2_{marginal}$ = 0.177, $R^2_{conditional}$ = 0.362)			
W	2,34	1.44	0.244
T	2,6	0.32	0.741
M	1,34	4.11	**0.050**
W × M	2,34	0.15	0.497
W × T	4,34	1.36	0.269

T × M	2,34	0.71	0.858

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). **Bold** numbers of *P* values represent significant ($P < 0.05$) and *Italic* numbers marginally significant ($P < 0.1$) effects of each term, respectively. *P* and R^2 are the significance of the model and explained variance by the model, respectively.

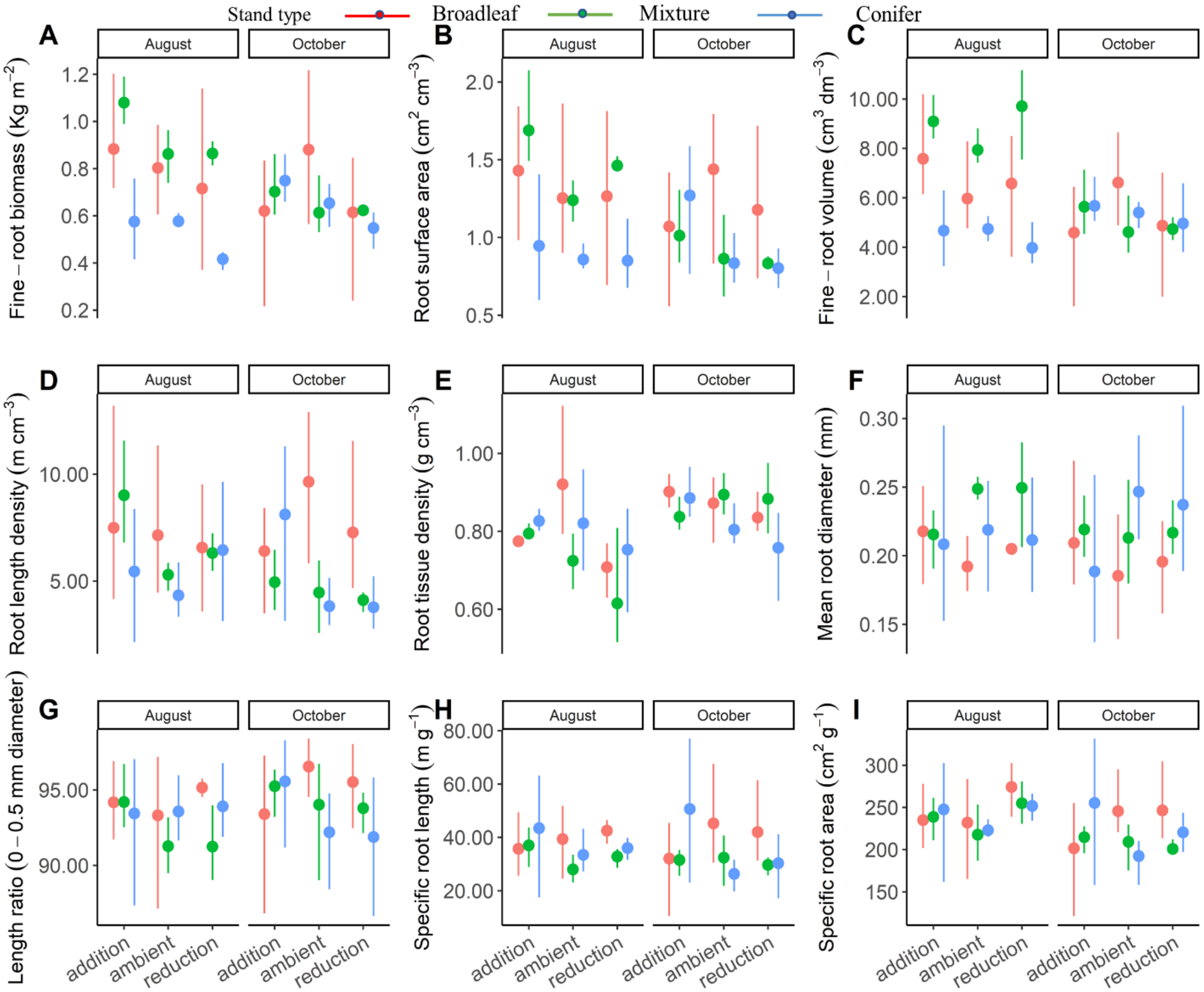

Figure 4-1 The effects of stand type, water alteration and sampling months on (A) fine-root biomass, (B) root surface area, (C) fine-root volume, (D) root length density, (E) root tissue density, (F) root mean diameter, (G) root length ratio of diameter < 0.5 mm, (H) specific root length, (I) specific root area, respectively. Values are mean and 95% confidence intervals.

The mixture effects on FRB differed with water treatment and sampling month (Table 4-3). There were consistent positive mixture effects with greater magnitudes under water reduction and addition than under ambient precipitation in August, but the mixtures had no or weak effects under any water treatments in October (Fig. 4-2). In August, FRB in species mixtures was on average 41%, 71% and 89% higher than the averages of corresponding single-species dominated stands under ambient, water addition, and water reduction, respectively.

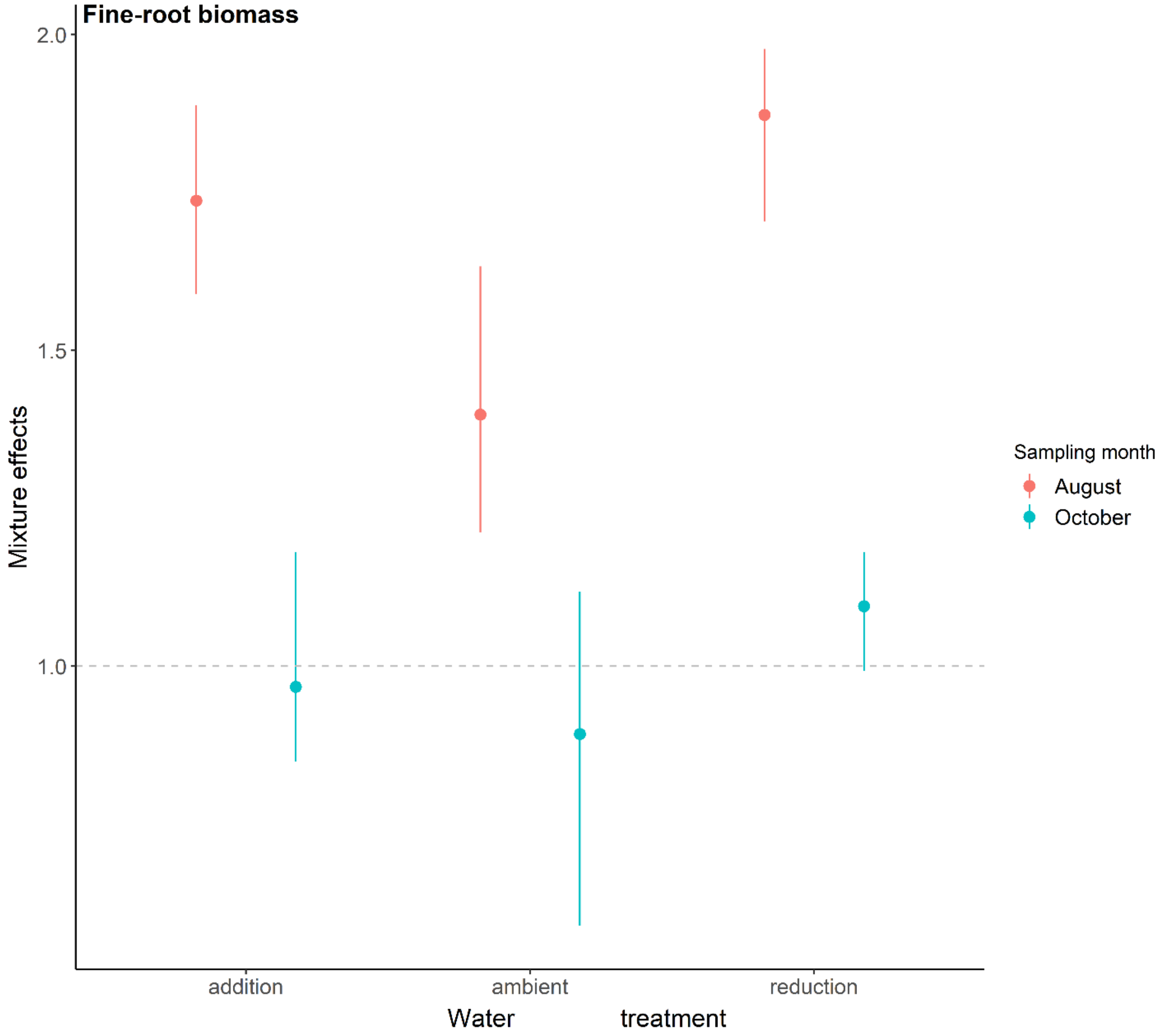

Figure 4-2 The effects of species mixtures on fine-root biomass in relation to water alteration treatments and sampling months, respectively. The effects represent the response ratio compared to the monocultures and the mixtures (see Materials and Methods). Values are mean ± 95% confidence intervals. The significance (P) is represented for each term tested. The difference between groups was significant if 95% Cis of their coefficients did not overlap the other's mean.

The effects of mixtures on RSA, FRV, RLD, and RTD differed with sampling month, and the effects also interacted with water treatment for RLD, RTD, RMD, and SRL with marginal significance ($0.1 > P \geq 0.05$), while the mixture effects on LR and SRA did not differ with either sampling month or water treatment (Table 4-3). RSA and FRV were significantly higher in mixtures than the average of monocultures under all water treatments in August but not in October (Figs. 4-3A & B). The effect of mixtures on RLD was significantly positive under water addition in August but not in other water treatments nor any water treatments in October, whereas the effect on RTD was significantly positive under water reduction in October (Figs. 4-3C & D). Moreover, there were positive mixture effects on RMD and negative mixture effects on SRL under both ambient and water reduction in August but not in water addition nor any water treatments in October (Figs. 4-3E & G).

Table 4-0-3 Effects of water treatment (W), sampling months (M) on the effects of species mixtures of fine-root biomass, root surface area, fine-root volume, root length density, root tissue density, mean root diameter and length ratio (0-0.5mm diameter), specific root length, specific root area.

Source	df	F	*P*
Fine-root biomass			
W	2,12	4.84	**0.029**
M	1,12	58.50	**< 0.001**
M×W	2,12	1.00	0.397
Root surface area			
W	2,10	0.50	0.623
M	1,10	31.37	**< 0.001**
M×W	2,10	1.59	0.252
Fine-root volume			
W	2,9	2.52	0.130
M	1,10	51.48	**< 0.001**
M×W	2,10	1.60	0.249
Root length density			
W	2,10	0.53	0.603
M	1,10	14.66	**0.003**
M×W	2,10	4.11	**0.050**
Root tissue density			
W	2,12	0.25	0.780
M	1,12	8.92	**0.011**

M×W	2,12	3.63	*0.058*
Mean root diameter			
W	2,10	0.07	0.931
M	1,10	2.88	0.120
M×W	2,10	3.34	*0.077*
Length ratio (0-0.5mm diameter)			
W	2,10	0.47	0.637
M	1,10	2.58	0.139
M×W	2,10	1.30	0.315
Specific root length			
M	2,10	0.41	0.676
W	1,10	0.37	0.556
M×W	2,10	3.29	*0.080*
Specific root area			
W	2,10	0.90	0.438
M	1,10	0.43	0.526
M×W	2,10	1.20	0.341

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). **Bold** and *Italic* numbers of P values represent significant ($P < 0.05$) and marginal ($P < 0.1$) effects of each term, respectively.

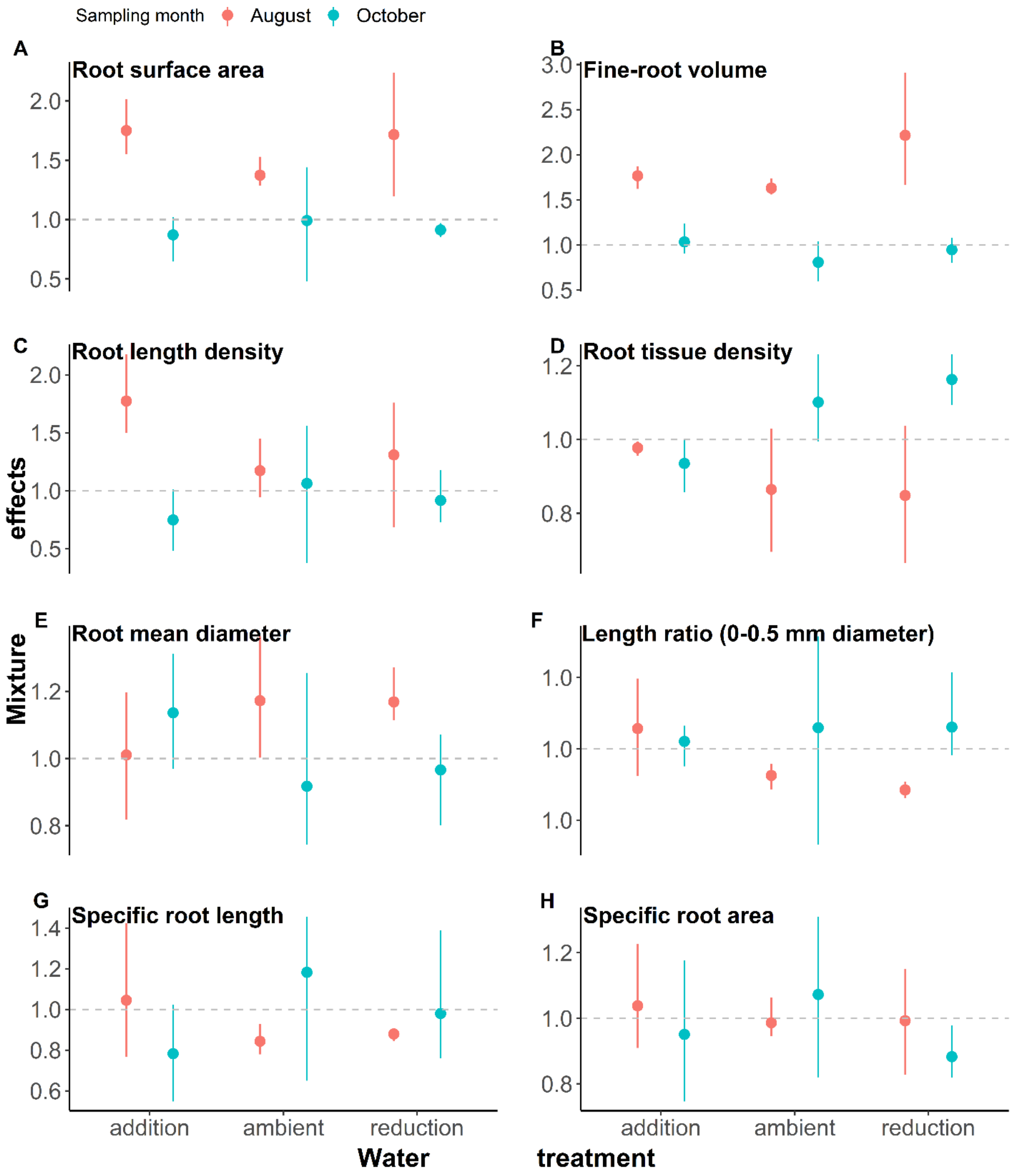

Figure 4-3 The effects of tree mixtures on (A) fine-root biomass, (B) fine-root volume, (C) specific root length, (D) specific root area, (E) root length density, (F) root tissue density, (G) root surface area, (H) root mean diameter and (I) root length ratio of diameter < 0.5 mm in relation to water alteration treatments. The effects represent the response ratio of a given root attribute compared to the monocultures and the mixtures (*see* Methods). Values are mean ± 95% confidence intervals. The significance (P) is represented for each term tested. The difference between groups was significant if 95% Cis of their coefficients did not overlap the other's mean.

The analysis based on species composition and diversity indicated that the effects of species evenness on FRB, RSA, FRV, and RLD also depended on sampling month (Table 4-4). FRB, RSA, FRV, and RLD increased with tree species evenness in August, but those root attributes decreased in October (Figs. 4-4A-D).

Table 4-0-4 Effects of water treatment (W), overstory plant evenness (J), proportion of conifer trees (C) and sampling month (M) on fine-root biomass, root surface area, fine-root volume, root length density, root tissue density, mean root diameter and length ratio (0-0.5mm diameter), specific root length, specific root area.

Source	df	F	*P*
Fine-root biomass			
W	2,36	0.96	0.391
J	1,6	0.10	0.763
C	1,6	3.26	0.121
M	1,36	10.07	**0.003**
J × W	2,36	0.31	0.732
C × W	2,36	1.81	0.179
J × M	1,36	24.72	**< 0.001**
C × M	1,36	1.38	0.248
Root surface area			
W	2,36	0.70	0.504
J	1,6	0.01	0.919
C	1,6	4.03	0.092
M	1,36	5.65	**0.023**
J × W	2,36	0.87	0.429
C × W	2,36	1.79	0.182
J × M	1,36	11.50	**0.002**
C × M	1,36	2.79	0.103
Fine-root volume			
W	2,36	0.27	0.762
J	1,6	0.02	0.881
C	1,6	1.00	0.355
M	1,36	3.75	*0.061*

J × W	2,36	0.44	0.644
C × W	2,36	0.25	0.781
J × M	1,36	14.02	**0.001**
C × M	1,36	0.99	0.325
Root length density			
W	2,36	0.65	0.530
J	1,6	0.01	0.909
C	1,6	3.99	*0.093*
M	1,36	3.89	*0.056*
J × W	2,36	1.17	0.323
C × W	2,36	1.77	0.184
J × M	1,36	4.02	*0.052*
C × M	1,36	3.31	*0.077*
Root tissue density			
W	2,36	3.46	**0.042**
J	1,6	0.11	0.748
C	1,6	3.36	0.116
M	1,36	0.32	0.573
J × W	2,36	1.63	0.210
C × W	2,36	1.83	0.176
J × M	1,36	0.07	0.788
C × M	1,36	0.07	0.799
Mean root diameter			
W	2,36	0.07	0.930
J	1,6	0.07	0.796
C	1,6	1.15	0.325
M	1,36	3.81	*0.059*
J × W	2,36	0.36	0.698
C × W	2,36	1.08	0.351
J × M	1,36	1.66	0.206
C × M	1,36	2.99	*0.092*
Length ratio (0-0.5mm diameter)			
W	2,36	1.52	0.232
J	1,6	0.21	0.662
C	1,6	0.72	0.428
M	1,36	5.36	**0.026**
J × W	2,36	1.13	0.335

C × W	2,36	1.44	0.251
J × M	1,36	2.36	0.134
C × M	1,36	3.49	*0.070*
Specific root length			
W	2,36	0.93	0.405
J	1,6	0.01	0.947
C	1,6	1.08	0.339
M	1,36	3.79	*0.059*
J × W	2,36	1.55	0.227
C × W	2,36	1.49	0.238
J × M	1,36	2.83	0.101
C × M	1,36	3.35	*0.076*
Specific root area			
W	2,36	2.63	*0.086*
J	2,36	0.01	0.972
C	2,36	0.56	0.482
M	1,36	1.39	0.247
J × W	2,36	2.18	0.128
C × W	2,36	0.81	0.453
J × M	1,36	1.89	0.178
C × M	1,36	2.85	0.100

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom (df). **Bold** numbers of P values represent significant ($P < 0.05$) and *Italic* numbers marginally significant ($0.05 < P < 0.1$) effects of each term.

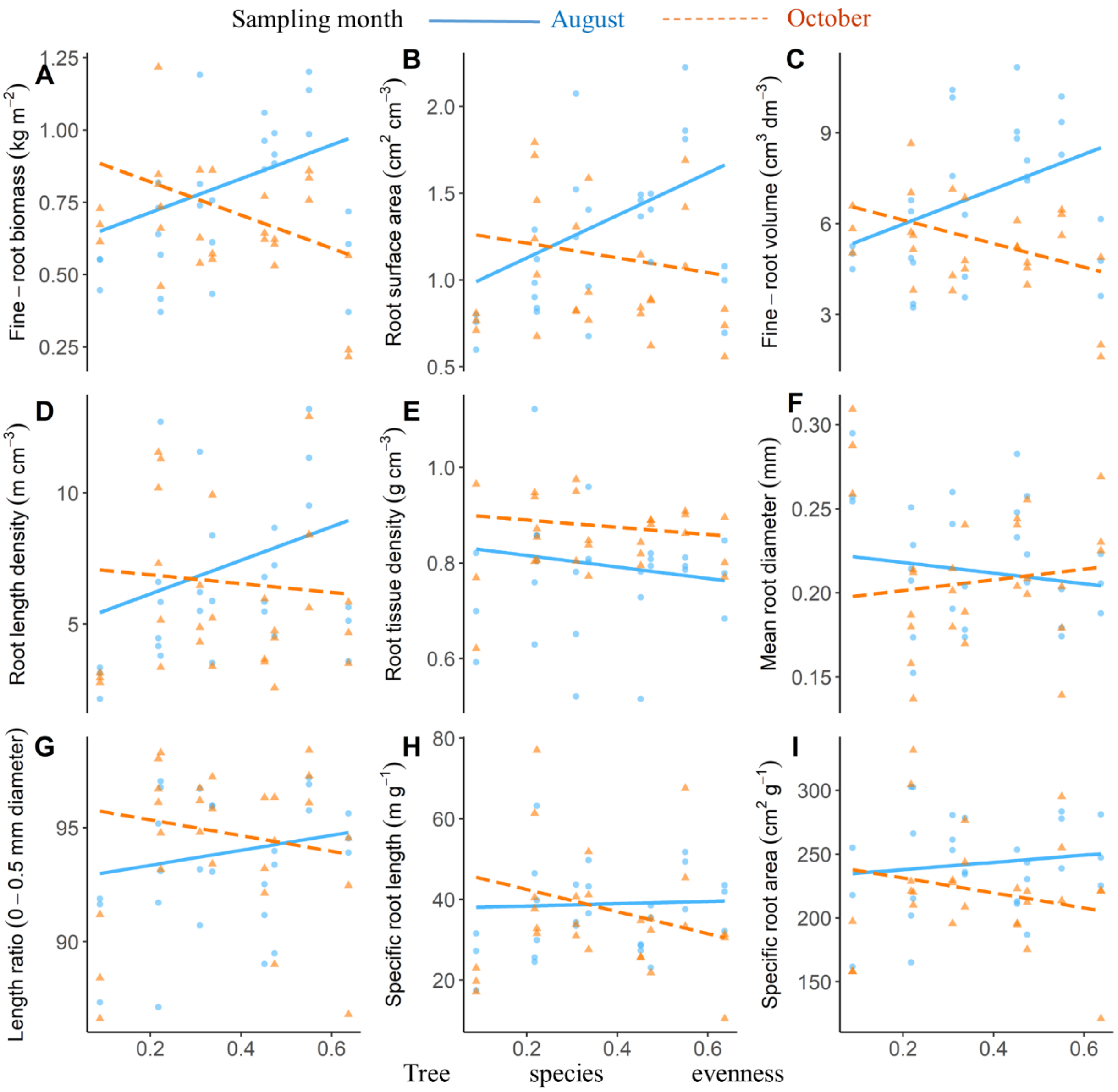

Figure 4-4 The interactive effects of tree species evenness with sampling month on (A) fine-root biomass, (B) root surface area, (C) fine-root volume, (D) root length density, (E) root tissue density, (F) root mean diameter, (G) root length ratio of diameter < 0.5 mm, (H) specific root length, (I) specific root area. Lines and grey areas represent the fitted regression with 95% confidence interval for each root attribute.

4.5 Discussion

Our findings demonstrated one of the first evidence that plant species mixtures and water availability interactively influenced fine-root biomass and root functional traits in natural forests.

Firstly, our results showed there were positive effects of mixtures on fine-root biomass (FRB), root surface area (RSA), and fine-root volume (FRV) in ambient water stands in August. The positive effects of species mixtures on FRB were more pronounced under both water addition and reduction, but those effects on RSA and FRV did not differ among water treatments. Secondly, we found the mixture effect on root length density (RLD) was significantly positive under water addition in August but not in other water treatments nor any water treatments in October, whereas the effect on root tissue density (RTD) was significantly positive under water reduction in October. Lastly, our experiment revealed positive mixture effects on root mean diameter (RMD) and negative mixture effects on specific root length (SRL) under both ambient and water reduction in August but not in water addition nor any water treatments in October. Additionally, the species mixtures had no or weak effects on FRB and all root functional traits under any water treatments in October.

We found on average, FRB and root functional traits responded differently to sampling month and water treatment, and the effects of stand types were depended on sampling month. Firstly, the greater FRB, RSA, FRV, and lower RTD in August than in October were consistent with the changes in soil temperature in our plots (Chen *et al.*, 2021). The different resource uptake capacities between mid summer and fall could result from high demands for water and nutrients during foliage production in August and low demands during foliage senescence (Brassard *et al.*, 2009). Secondly, compared with ambient water condition, water addition increased and water reduction decreased FRB in August, corroborating to previous findings (Wang *et al.*, 2020; Asefa *et al.*, 2022). Accompanied with the lower RTD with a faster root decay rate , forest stands could meet the higher resource demands for greater aboveground productivity under water addition and alleviate stress under reduction, respectively (Stape *et al.*, 2010; Freschet *et al.*, 2017). Lastly, the highest FRB in broadleaf stands and lowest in conifer stands under all water treatment in August could be due to the nutrient availability. Broadleaf stands have a greater nutrient availability due to a faster decay rate, leading more fine-root investment (See *et al.*, 2019). Moreover, mixtures had a greater RSA and FRV than monocultures in August, which could be due to the drought stress buffering. The drought-tolerant species (*Pinus banksiana*) could benefit the species of low drought-tolerant (*Populus tremuloides*) via microclimate amelioration in August of critical water deficit (Niinemets & Valladares, 2006; Chen *et al.*, 2021).

Our results showed significant positive effects of species mixtures on FRB, RSA, and FRV under ambient precipitation in August, but the other traits had no or weak responses to species mixtures, which partly support our first hypothesis. Fine roots have greater biomass for higher soil resources in plant mixtures due to increased soil volume filling and positive interspecific facilitation, such as lifting water via deep-rooted species to favour the shallow-rooted species (Silvertown *et al.*, 2015; Zwetsloot *et al.*, 2019). This water lifting accompanied with greater root surface area and volume per soil volume favours fine roots with a higher resource uptake capacity to absorb the soil resources. Compared with the corresponding monocultures, resource partitioning among the coexisting species also requires a greater FRB, RSA, and FRV to contain and uptake the elevated soil resources (Barry *et al.*, 2019). The different effects of species mixtures on RSA, FRV vs LR, SRL, SRA might suggest plants tends to share strategy of greater root surface area and volume per soil volume but stable uptake efficiency in young forests where soil space and resources are utilized incompletely (de Kroon *et al.*, 2012; Ma & Chen, 2017). Additionally, mixture effects on FRB and root functional traits were strong under physiologically active summer month (August) but not in the fall (October).

This could be attributable to the higher resource requirements derived from the greater plant metabolisms in August of a higher temperature with an exaggerated water requirement due to the lower water availability.

Importantly, our findings revealed that the positive effect of species mixtures on FRB were more pronounced under water addition and reduction in August, and those effects on root functional traits were less sensitive with water treatment, which partly acknowledged our second hypothesis. The shifted effects of species mixtures on FRB and RLD are anticipated since increased FRB and root length per soil volume are required to absorb increased water availability by precipitation addition (Wright *et al.*, 2015; Fischer *et al.*, 2016). The positive mixture effects on FRB with water addition could be a solid explanation for the stimulated soil respiration in our previous study since FRB is positive related to soil respiration (Pregitzer *et al.*, 2008; Chen *et al.*, 2021). The different mixture effects on FRV, RSA, RLD vs LR, SRL, SRA under water addition in August suggest plants appears to be higher resource uptake capacity but stable efficiency when confronting increased water availability. It has been also reported a noninteractive effect of irrigation and species mixtures since coexisting trees share similar ecological strategies, occupy and compete for the same soil depth (Altinalmazis-Kondylis *et al.*, 2020). Meanwhile, the enhanced species mixture effects on FRB with water reduction are aligned with the stress gradient hypothesis, i.e., the interspecific facilitation could be increased in hash environment under low resource availability (Maestre *et al.*, 2009; Forrester & Bauhus, 2016). The stronger mixture effects on FRB with water reduction could ascribe to the higher water and nutrient demand induced by greater aboveground productivity, via fine-root segregation (Brassard *et al.*, 2013; Ammer, 2019; Zwetsloot *et al.*, 2019). While, by conducting a 4-year throughfall-exclusion experiment, Zwetsloot and Bauerle (2021) found a species-specific response of fine-root production (root tips m^{-2}) to drought in plant mixtures: the negative effects of throughfall exclusion on root production were dampened for Norway Spruce when grown intermixed with European beech, but it turned unchanged when scaled at the community level. Moreover, the weak interactive effects of species mixtures and water reduction on FRB and root functional traits in October could be the compromise between higher nutrient availability from fast litter decay of species mixtures and low water availability from water reduction (Liu *et al.*, 2020; Xiao *et al.*, 2020).

Our results about species composition and diversity showed that the effects of species evenness on FRB, RSA, FRV, and RLD also depended on sampling month, which partly refused our hypothesis. By sampling soil from 209 plots across the European forest, Finér *et al.* (2017) found the conifer proportion has a positive effect on FRB due to the lower soil nutrient availability in coniferous forests (Augusto *et al.*, 2015; Dawud *et al.*, 2017). However, the stable FRB and root functional traits with increased coniferous tree proportion could be attributable to the fact that the decreases in soil resource availability did not differ significantly because of the young stand age. While, leaf decay rate and fine-root productivity, turnover rate have been reported to increase with plant species evenness (Ward *et al.*, 2010; Lin *et al.*, 2013; Ma & Chen, 2018), leading to a higher nutrient availability. Therefore, fine roots with greater FRB, RSA, FRV, and RLD in August enable a better absorption on elevated soil resources to meet the elevated resource demands derived from the higher aboveground productivity in forests with higher species evenness (Zhang *et al.*, 2012). Additionally, the decreased FRB, RSA, FRV, and RLD in October could ascribe to the lower nutrient availability. The stable LR, SRL, and SRA might suggest plants tends to share strategy of greater resource uptake capacity but stable efficiency with higher evenness in young stands where soil resources are limited. We found the effects of coniferous tree proportion and species evenness on all root attributes did not significantly change with water treatment. These negligible interactive effects could result from the lower variations of soil resource induced by the small magnitudes of altered precipitation, or other root functional traits such as community-weighted rooting depth, mycorrhizal colonisation intensity play a more related role in mediating the resource changes in stands of different coniferous tree proportions (Archambault *et al.*, 2019; Freschet *et al.*, 2021).

In conclusion, our results showed overyielding belowground in broadleaf-coniferous mixtures under ambient water and further revealed the more pronounced positive mixture effects on resource uptake capacity under both water addition and reduction. Our findings highlight the regulating role of precipitation changes in FRB in plant mixtures. Whereas the resource uptake efficiency (morphological traits as indices) had weak or no responses to mixture effects across water treatments in our young boreal stands under mild precipitation alterations. Additionally, the resource uptake capacity was higher under physiologically active summer than in the fall. We also unveiled that species evenness had positive effects on resource uptake capacity in August, but all shifted into negative in October. The unchanged evenness effects on root traits represented resource uptake efficiency suggests fine roots tend to change the uptake capacity rather than efficiency in young forests with different species abundance distributions. Collectively, our study raises the alarming clock to pay more attention to FRB and root functional traits in the face of concurrent changes of plant diversity and frequent precipitation events.

4.6 Acknowledgements

We appreciate the field assistance from our summer student assistants. This study was funded by the Natural Sciences and Engineering Research Council of Canada (RGPIN-2014–04181, RTI-2017–00358, and STPGP506284) and the Canadian Foundation for Innovation (36014).

4.7 Authorship

SP and HYHC designed the study; SP collected the data; SP and HYHC discussed and interpreted the data; SP performed the analysis and wrote the first draft of the manuscript; and all authors wrote interactively through multiple rounds of revisions.

4.8 Conflict of interest

The authors declare no competing interests.

Chapter 5. GENERAL CONCLUSION

The findings of this dissertation show global-scale evidence that the effects of plant species richness had a positive effect on fine-root biomass, extending the aboveground overyielding to belowground (Zhang *et al.*, 2012; Liang *et al.*, 2016). Moreover, my findings extend our understanding of the important role of plant species mixtures on fine-root biomass (FRB). The positive effects of species mixtures not only increased with species richness (Ma & Chen, 2016), but were also more pronounce in older stands and deeper soil. The effects of species mixture on FRB and root functional traits shifted with functional trait dissimilarity and background environment. Lastly, our experiment demonstrated the mixture effects on FRB could be both promoted with precipitation addition and reduction. A summary of the key findings is as follows:

- The effects of species mixtures on root functional traits were highly dependent on species richness in plant mixtures, stand age, soil depth, or environmental stress. Our results suggest the water and nutrient demands were elevated in species-rich and old mixtures, led to deeper soil exploration. Because of the important role of fine roots in soil water, carbon and nutrient cycling, our results suggest that increased FRB, with shifts in fine root traits, could be one of the drivers for the positive diversity-productivity relationship in species mixtures.
- Our results demonstrated the opposite responses of FRB to the mixture effects with heterogeneity of shade tolerance, leaf habit vs drought tolerance, plant growth rate. The greater FRB in plant mixtures with contrasting drought tolerance and plant growth rate suggests that more fine roots for water and nutrient uptake are allocated, whereas the lower fine-root biomass in plant mixtures with contrasting shade tolerance and high leaf habit dissimilarity indicates that plants allocate more carbon

for aboveground to utilize light. These findings provoke us to pay more attention to the functional trait dissimilarity when interpreting the effects of plant mixtures on FRB, especially for those who aim to the belowground productivity and carbon sequestration

- We found a positive effect of species mixtures on FRB in August under ambient water condition, and more pronounced with both water addition and reduction. Root surface area, fine-root volume, and root length density responded to species mixtures similarly to those of FRB. Species mixtures had no effects on FRB, root surface area, fine-root volume, and root length density in October. Root tissue density, root mean diameter, length ratio (ratio of root length with Ø < 0.5 mm to the total root length), specific root length and specific root area had weak responses to plant mixtures and water treatment. Our findings demonstrate that plant mixtures improve the fine-root investment with changing water availability and indicate that both mild water addition and reduction enhance soil resource uptake capacity in the young boreal mixed-wood forests.

My thesis elucidated why the diversity effects on fine-root biomass and root functional traits were inconsistent in the published literatures. The divergent findings mainly result from the differences in species richness level, plant functional dissimilarity, stand age, soil depth, and the mean annual temperature or precipitation. Although my findings enable us a better understand of the biodiversity-ecosystem functioning from belowground perspective, the root functional traits do not link to the root function well. There are several issues we need to tackle with.

There is a highly urgent to reveal the root economic spectrum (RES). A well-know leaf economic spectrum (LES) has been established (Wright et al., 2004): leaf with a high specific lead area, high nutrient concentrations, high assimilation and respiration rates would promote light interception and carbon fixation. Unlike one dimensional LES, previous studies report inconsistent results of RES. There is one axis of root traits variation (resource acquisitive vs conservative syndrome) existing by using 18 field-grown herbaceous species in central Argentina, or root respiration dimension by 74 species (31 graminoids and 43 herbaceous and dwarf shrub eudicots) collected in three biomes (Roumet *et al.* 2006; Roumet *et al.* 2016). Also, root traits could be linked to two contrasting strategies of root life: a conseravtive strategy for thick roots cooperated with mycorrihizal fungi for resource uptake and a aquisitive strategy for thin roots to use the phototynthetic carbon more efficiently for soil exploration (Ma *et al.* 2018b). Kong *et al.* 2014 proposed the two axes of trait variations of 96 angiosperm woody species from subtropical China: a diameter-related dimension and a branching architecture dimension. Or a dimension related to root foraging capacity and resource conservation and a dimension related to root respiration and metabolism along a succession gradient (6 - 69 years) in south France (Erktan *et al.* 2018). What's more, a multidimensional root trait of woody species for RES prevails recently (Weemstra *et al.* 2016; de la Riva *et al.* 2018). The explanations for different dimensions between leaf and root traits could result from inconsistent functional analogues and different evolutionary pressures, and/ or the much more environmental constraints and mycorrhizal interactions offset selection belowground (Weemstra *et al.* 2016; Bergmann *et al.* 2017; Ma *et al.* 2018b) . Moreover, compared to LES, lacking sufficient data pool of root traits (e.g. root length/ hair density, branching) and their spatial and temporal distribution hamper the solid conclusion for RES. Recent endeavour is being made to collect more root traits contributing to trait datasets (Freschet et al., 2021).

My thesis used the resource uptake capacity and efficiency to classified serveral root traits. A more comprehensive root-trait category should be employed when we are unveiling the diverisyt effects on fine roots (McCormack et al., 2015; 2017). The traits I selected in my thesis were mainly belong to morphological traits, which should expand to traits including chemistry (e.g., celluose, secondary compound), physiology (e.g., exudation, uptake water, respiration rate), root dynamics (production, mortality, life span and decomposition), anatomy (e.g., stele diameter, vessel, exodemis) and microbial assocications (e.g., colonization and foraging strategy of mycorrhizal fungi). Moreover, water and nutrient uptake rate could be measured via isotopic tracer to better explained the resource uptake capacity and efficiency (Kulmatiski et al., 2017). In this way, we can know how the resource uptake by fine roots responds to effects of species diversity and precipitation changes. More importantly, we should get the root traits more functional. Functional traits have been used to better understand the ecosystem processes several decades ago (Díaz & Marcelo 2001), but how the root traits connect to the specific functions is still merged under water. Recent workshops held by New Phytologist improved our understanding about the functional of root traits. Frameworks about the belowground traits with plant functioning is bulding (Freschet et al., 2021). But root ecologist should have on the same page about standardizing root classification, sampling, processing and trait measurements (Freschet et al., 2020).

Fine roots is in the ‘big black box”, i.e., soil. Tedious and careful works should always be paid, but the nature is beautiful. One day, I dropped an apple on the soil. There was no need to clean it again, since it is not dirty, it is life.

REFERENCES

Altinalmazis-Kondylis A, Muessig K, Meredieu C, Jactel H, Augusto L, Fanin N, Bakker MR. 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): 437-455.

Ammer C. 2019. Diversity and forest productivity in a changing climate. *New phytologist* **221**(1): 50-66.

Archambault C, Paquette A, Messier C, Khlifa R, Munson AD, Handa IT. 2019. Evergreenness influences fine root growth more than tree diversity in a common garden experiment. *Oecologia* **189**(4): 1027-1039.

Asefa M, Samantha JW, Cao M, Xiaoyang S, Yudi ML, Jie Y. 2022. Above and belowground plant traits are not consistent in response to drought and competition treatments. *Ann Bot.*

Ashton IW, Miller AE, Bowman WD, Suding KN. 2010. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* **91**(11): 3252-3260.

Augusto L, De Schrijver A, Vesterdal L, Smolander A, Prescott C, Ranger J. 2015. Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. *Biological Review* **90**(2): 444-466.

Bardgett RD, Mommer L, De Vries FT. 2014. Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution* **29**(12): 692-699.

Barry KE, Mommer L, van Ruijven J, Wirth C, Wright AJ, Bai Y, Connolly J, De Deyn GB, de Kroon H, Isbell F, et al. 2019. The Future of Complementarity: Disentangling Causes from Consequences. *Trends in Ecology & Evolution* **34**(2): 167-180.

Bartoń K. 2019. Multi-Model Inference, R package version 1.43.6.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Usinglme4. *Journal of Statistical Software* **67**(1): 1-48.

Bauhus J, Khanna PK, Menden N. 2000. Aboveground and belowground interactions in mixed plantations of Eucalyptus globulus and Acacia mearnsii. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **30**(12): 1886-1894.

Baxendale C, Orwin KH, Poly F, Pommier T, Bardgett RD. 2014. Are plant-soil feedback responses explained by plant traits? *New Phytol* **204**(2): 408-423.

Bergmann J, Ryo M, Prati D, Hempel S, Rillig MC. 2017. Root traits are more than analogues of leaf traits: the case for diaspore mass. *New phytologist* **216**(4): 1130-1139.

Bessler H, Temperton VM, Roscher C, Buchmann N, Schmid B, Schulze ED, Weisser WW, Engels C. 2009. Aboveground overyielding in grassland mixtures is associated with reduced biomass partitioning to belowground organs. *Ecology* **90**(6): 1520-1530.

Beyer F, Hertel D, Jung K, Fender AC, Leuschner C. 2013. Competition effects on fine root survival of Fagus sylvatica and Fraxinus excelsior. *Forest Ecology and Management* **302**: 14-22.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource Limitation in Plants - an Economic Analogy. *Annual Review of Ecology and Systematics* **16**: 363-392.

Brassard BW, Chen HYH, Bergeron Y. 2009. Influence of Environmental Variability on Root Dynamics in Northern Forests. *Critical Reviews in Plant Sciences* **28**(3): 179-197.

Brassard BW, Chen HYH, Cavard X, Laganière J, Reich PB, Bergeron Y, Paré D, Yuan Z, Chen H. 2013. Tree species diversity increases fine root productivity through increased soil volume filling. *Journal of Ecology* **101**(1): 210-219.

Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C. 2015. How tree roots respond to drought. *Frontier in Plant Science* **6**: 547.

Cai H, Li F, Jin G. 2019. Fine root biomass, production and turnover rates in plantations versus natural forests: effects of stand characteristics and soil properties. *Plant and Soil*.

Callaway JC, Sullivan G, Zedler JB. 2003. Species-rich plantings increase biomass and nitrogen accumulation in a wetland restoration experiment. *Ecol Appl* **13**(6): 1626-1639.

Canty A, Ripley B. 2012. boot: Bootstrap R (S-Plus) functions. *R package version* **1**(7).

Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 6.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, et al. 2012. Biodiversity loss and its impact on humanity. *Nature* **486**(7401): 59-67.

Carmona CP, Bueno CG, Toussaint A, Trager S, Diaz S, Moora M, Munson AD, Partel M, Zobel M, Tamme R. 2021. Fine-root traits in the global spectrum of plant form and function. *Nature* **597**(7878): 683-687.

Chen C, Chen X, Penga S, Xiao W, Guo Y, Zhang Y, Zhu Y, McLaren B, Chen HYH. 2022. Neighborhood dissimilarity consistently attenuates competition stress on tree growth under altered water availability in a natural boreal forest. *Agricultural and Forest Meteorology* **324**: 109101.

Chen HY, Popadiouk RV. 2002. Dynamics of North American boreal mixedwoods. *Environmental Reviews* **10**(3): 137-166.

Chen HYH, Brassard BW. 2013. Intrinsic and Extrinsic Controls of Fine Root Life Span. *Critical Reviews in Plant Sciences* **32**(3): 151-161.

Chen HYH, Luo Y. 2015. Net aboveground biomass declines of four major forest types with forest ageing and climate change in western Canada's boreal forests. *Global Change Biology* **21**(10): 3675-3684.

Chen HYH, Luo Y, Reich PB, Searle EB, Biswas SR. 2016. Climate change-associated trends in net biomass change are age dependent in western boreal forests of Canada. *Ecology Letters* **19**(9): 1150-1158.

Chen WL, Koide RT, Adams TS, DeForest JL, Cheng L, Eissenstat DM. 2016. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proceedings of the National Academy of Sciences* **113**(31): 8741-8746.

Chen X, Chen HYH. 2018. Global effects of plant litter alterations on soil CO2 to the atmosphere. *Glob Chang Biol* **24**(8): 3462-3471.

Chen X, Chen HYH, Chen C, Peng S. 2019. Water availability regulates negative effects of species mixture on soil microbial biomass in boreal forests. *Soil Biology and Biochemistry* **139**.

Chen X, Peng S, Chen C, Chen HYH. 2021. Water availability regulates tree mixture effects on total and heterotrophic soil respiration: A three-year field experiment. *Geoderma* **402**.

Cheng L, Weile Chen, Thomas S. Adams, Xing Wei, Le Li, Michael Luke McCormack, Jared L. DeForest, Roger T. Koide aDME. 2016. Mycorrhizal fungi and roots are complementary in foraging within nutrient patches. *Ecology* **10**(97): 2815-2823.

Choat B, Brodribb TJ, Brodersen CR, Duursma RA, Lopez R, Medlyn BE. 2018. Triggers of tree mortality under drought. *Nature* **558**(7711): 531-539.

Clemmensen KE, Adam Bahr, O. Ovaskainen, A. Dahlberg, Alf Ekblad, Håkan Wallander, J. Stenlid, R. D. Finlay, D. A. Wardle, and B. D. Lindahl. 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**(6127): 1615-1618.

Cohen P, West SG, Aiken LS. 2014. *Applied multiple regression/correlation analysis for the behavioral sciences*: Psychology Press.**Comas LH, Becker SR, Cruz VM, Byrne PF, Dierig DA. 2013.** Root traits contributing to plant productivity under drought. *Frontier in Plant Science* **4**: 442.

Comas LH, Mueller KE, Taylor LL, Midford PE, Callahan HS, Beerling DJ. 2012. Evolutionary Patterns and Biogeochemical Significance of Angiosperm Root Traits. *International Journal of Plant Sciences* **173**(6): 584-595.

Cools N, Vesterdal L, De Vos B, Vanguelova E, Hansen K. 2014. Tree species is the major factor explaining C:N ratios in European forest soils. *Forest Ecology and Management* **311**: 3-16.

Coomes DA, Kunstler G, Canham CD, Wright E. 2009. A greater range of shade-tolerance niches in nutrient-rich forests: an explanation for positive richness-productivity relationships? *Journal of Ecology* **97**(4): 705-717.

Davison AC, Hinkley DV. 1997. *Bootstrap methods and their application*: Cambridge university press.

Dawud SM, Raulund-Rasmussen K, Ratcliffe S, Domisch T, Finér L, Joly F-X, Hättenschwiler S, Vesterdal L, Ostertag R. 2017. Tree species functional group is a more important driver of soil properties than tree species diversity across major European forest types. *Functional Ecology* **31**(5): 1153-1162.

Davison AC, Hinkley DV. 1997. *Bootstrap methods and their application*: Cambridge university press.

Ding Y, Nie Y, Chen H, Wang K, Querejeta JI. 2021. Water uptake depth is coordinated with leaf water potential, water-use efficiency and drought vulnerability in karst vegetation. *New Phytologist* **229**(3): 1339-1353.

de Kroon H, Hendriks M, van Ruijven J, Ravenek J, Padilla FM, Jongejans E, Visser EJW, Mommer L. 2012. Root responses to nutrients and soil biota: drivers of species coexistence and ecosystem productivity. *Journal of Ecology* **100**(1): 6-15.

Díaz, Sandra, and Marcelo Cabido. 2001. Vive la différence: plant functional diversity matters to ecosystem processes.*Trends in ecology & evolution* **16**(11): 646-655.

Domisch T, Finer L, Dawud SM, Vesterdal L, Raulund-Rasmussen K. 2015. Does species richness affect fine root biomass and production in young forest plantations? *Oecologia* **177**(2): 581-594.

Donat MG, Lowry AL, Alexander LV, O'Gorman PA, Maher N. 2017. Addendum: More extreme precipitation in the world's dry and wet regions. *Nature Climate Change* **7**(2): 154-158.

Ecoregions Working Group. 1989. Ecoclimatic regions of Canada, first approximation. *Ecological land classification series* **23**: 118.

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL. 2000. Building roots in a changing environment: implications for root longevity. *New phytologist* **147**: 33-42.

Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37**(12): 4302-4315.

Finér L, Domisch T, Dawud SM, Raulund-Rasmussen K, Vesterdal L, Bouriaud O, Bruelheide H, Jaroszewicz B, Selvi F, Valladares F. 2017. Conifer proportion explains fine root biomass more than tree species diversity and site factors in major European forest types. *Forest Ecology and Management* **406**: 330-350.

Fischer FM, Wright AJ, Eisenhauer N, Ebeling A, Roscher C, Wagg C, Weigelt A, Weisser WW, Pillar VD. 2016. Plant species richness and functional traits affect community stability after a flood event. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**(1694): 20150276.

Fitter A, Caldwell M, Pearcy R. 1994. Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. *Exploitation of environmental heterogeneity by plants: ecophysiological processes above-and belowground*: 305-323.

Flannigan M, Stocks B, Turetsky M, Wotton M. 2009. Impacts of climate change on fire activity and fire management in the circumboreal forest. *Global Change Biology* **15**(3): 549-560.

Fornara DA, Tilman D, Hobbie SE. 2009. Linkages between plant functional composition, fine root processes and potential soil N mineralization rates. *Journal of Ecology* **97**(1): 48-56.

Forrester DI, Bauhus J. 2016. A Review of Processes Behind Diversity—Productivity Relationships in Forests. *Current Forestry Reports* **2**(1): 45-61.

FreschetGT, Pages L, IversenCM, ComasLH, Rewald B, Roumet C, Klimesova J, ZadwornyM, Poorter H, Postma JA etal. 2020. A startingguide to rootecology: strengthening ecologicalconcepts andstandardizing root classification, sampling, processingandtrait measurements. URL:https://hal.archives-ouvertes.fr/hal-02918834.

Freschet GT, Roumet C, Comas LH, Weemstra M, Bengough AG, Rewald B, Bardgett RD, De Deyn GB, Johnson D, Klimešová J. 2021. Root traits as drivers of plant and ecosystem functioning current understanding, pitfalls and future research needs. *New phytologist* **3**(223): 1123-1158.

Freschet GT, Valverde-Barrantes OJ, Tucker CM, Craine JM, McCormack ML, Violle C, Fort F, Blackwood CB, Urban-Mead KR, Iversen CM, et al. 2017. Climate, soil and plant functional types as drivers of global fine-root trait variation. *Journal of Ecology* **105**(5): 1182-1196.

Germon A, Guerrini IA, Bordron B, Bouillet J-P, Nouvellon Y, de Moraes Gonçalves JL, Jourdan C, Paula RR, Laclau J-P. 2017. Consequences of mixing Acacia mangium and Eucalyptus grandis trees on soil exploration by fine-roots down to a depth of 17 m. *Plant and Soil* **424**(1-2): 203-220.

Gould IJ, Quinton JN, Weigelt A, De Deyn GB, Bardgett RD. 2016. Plant diversity and root traits benefit physical properties key to soil function in grasslands. *Ecology Letters* **19**(9): 1140-1149.

Group SCW, Canadian Agricultural Services Coordinating Committee., National Research Council Canada., Canada. Agriculture., Branch. A-FCR. 1998. *The Canadian system of soil classification*: NRC Research Press.

Guderle M, Bachmann D, Milcu A, Gockele A, Bechmann M, Fischer C, Roscher C, Landais D, Ravel O, Devidal S, et al. 2018. Dynamic niche partitioning in root water uptake facilitates efficient water use in more diverse grassland plant communities. *Functional Ecology* **32**(1): 214-227.

Hajek P, Hertel D, Leuschner C. 2014. Root order- and root age-dependent response of two poplar species to belowground competition. *Plant and Soil* **377**(1-2): 337-355.

Hedges LV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* **80**: 1150-1156.

Hendricks JJ, Nadelhoffer KJ, Aber JD. 1993. Assessing the role of fine roots in carbon and nutrient cycling. *Trends in Ecology and Evolution* **8**: 174-178.

Hisano M, Searle EB, Chen HYH. 2018. Biodiversity as a solution to mitigate climate change impacts on the functioning of forest ecosystems. *Biological Review* **93**(1): 439-456.

Hisano M, Chen HYH. 2020. Spatial variation in climate modifies effects of functional diversity on biomass dynamics in natural forests across Canada. *Global Ecology and Biogeography* **doi**: 10.1111/geb.13060.

Hisano M, Chen HYH, Searle EB, Reich PB. 2019. Species-rich boreal forests grew more and suffered less mortality than species-poor forests under the environmental change of the past half-century. *Ecol Lett* **22**(6): 999-1008.

Hooper DU, Adair EC, Cardinale BJ, Byrnes JE, Hungate BA, Matulich KL, Gonzalez A, Duffy JE, Gamfeldt L, O'Connor MI. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* **486**(7401): 105-108.

Isbell F, Craven D, Connolly J, Loreau M, Schmid B, Beierkuhnlein C, Bezemer TM, Bonin C, Bruelheide H, de Luca E, et al. 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature* **526**(7574): 574-577.

IPBES. 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, unedited version of 6 May 2019.

Isbell F, Craven D, Connolly J, Loreau M, Schmid B, Beierkuhnlein C, Bezemer TM, Bonin C, Bruelheide H, de Luca E, et al. 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature* **526**(7574): 574-577.

Ishii H, Asano S. 2010. The role of crown architecture, leaf phenology and photosynthetic activity in promoting complementary use of light among coexisting species in temperate forests. *Eco Res* **25**: 715-722.

Jobbágy EG, Jackson RB. 2001. The distribution of soil nutrients with depth: Global patterns and the imprint of plants. *Biogeochemistry* **53**: 51-77.

Kattge J, DÍAz S, Lavorel S, Prentice IC, Leadley P, BÖNisch G, Garnier E, Westoby M, Reich PB, Wright IJ, et al. 2011. TRY - a global database of plant traits. *Global Change Biology* **17**(9): 2905-2935.

Klaus VH, Holzel N, Prati D, Schmitt B, Schoning I, Schrumpf M, Solly EF, Hansel F, Fischer M, Kleinebecker T. 2016. Plant diversity moderates drought stress in grasslands: Implications from a large real-world study on (13)C natural abundances. *Science of The Total Environment* **566**: 215-222.

Kulmatiski A, Peter B. Adler, John M. Stark, and Andrew T. Tredennick. 2017. Water and nitrogen uptake are better associated with resource availability than root biomass. *Ecosphere* **8**(3).

Kumar P, Chen HYH, Thomas SC, Shahi C, Gilliam F. 2018. Linking resource availability and heterogeneity to understorey species diversity through succession in boreal forest of Canada. *Journal of Ecology* **106**(3): 1266-1276.

Lange M, Habekost M, Eisenhauer N, Roscher C, Bessler H, Engels C, Oelmann Y, Scheu S, Wilcke W, Schulze ED, et al. 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. *Plos One* **9**(5): e96182.

Lei P, Scherer-Lorenzen M, Bauhus J. 2012. The effect of tree species diversity on fine-root production in a young temperate forest. *Oecologia* **169**(4): 1105-1115.

Lenth RV. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software* **69**: 1-33.

Li H, Liu B, McCormack ML, Ma Z, Guo D. 2017. Diverse belowground resource strategies underlie plant species coexistence and spatial distribution in three grasslands along a precipitation gradient. *New phytologist* **216**(4): 1140-1150.

Liang J, Crowther TW, Picard N, Wiser S, Zhou M, Alberti G, Schulze ED, McGuire AD, Bozzato F, Pretzsch H, et al. 2016. Positive biodiversity-productivity relationship predominant in global forests. *Science* **354**(6309): 10.1126/science.aaf8957.

Liao C, Luo Y, Fang C, Chen J, Li B. 2012. The effects of plantation practice on soil properties based on the comparison between natural and planted forests: a meta-analysis. *Global Ecology and Biogeography* **21**(3): 318-327.

Lin G-G, Mao R, Zhao L, Zeng D-H. 2013. Litter decomposition of a pine plantation is affected by species evenness and soil nitrogen availability. *Plant and Soil* **373**(1-2): 649-657.

Liu J, Liu X, Song Q, Compson ZG, LeRoy CJ, Luan F, Wang H, Hu Y, Yang Q. 2020. Synergistic effects: a common theme in mixed-species litter decomposition. *New phytologist* **227**(3): 757-765.

Loreau M, Naeem, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, et al. 2001. Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. *Science* **294**: 804-808.

Ma Z, Chen HYH. 2016. Effects of species diversity on fine root productivity in diverse ecosystems: a global meta-analysis. *Global Ecology and Biogeography* **25**(11): 1387-1396.

Ma Z, Chen HYH. 2017. Effects of species diversity on fine root productivity increase with stand development and associated mechanisms in a boreal forest. *Journal of Ecology* **105**(1): 237-245.

Ma Z, Chen HYH. 2018a. Positive species mixture effects on fine root turnover and mortality in natural boreal forests. *Soil Biology and Biochemistry* **121**: 130-137.

Ma Z, Guo D, Xu X, Lu M, Bardgett RD, Eissenstat DM, McCormack ML, Hedin LO. 2018b. Evolutionary history resolves global organization of root functional traits. *Nature* **555**(7694): 94-97.

Maestre FT, Callaway RM, Valladares F, Lortie CJ. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* **97**: 199-205.

Makita N, Hirano Y, Mizoguchi T, Kominami Y, Dannoura M, Ishii H, Finér L, Kanazawa Y. 2010. Very fine roots respond to soil depth: biomass allocation, morphology, and physiology in a broad-leaved temperate forest. *Ecological Research* **26**(1): 95-104.

Mallon EE, Turetsky MR, Thompson ID, Fryxell JM, Wiebe PA. 2016. Effects of disturbance on understory succession in upland and lowland boreal forests and implications for woodland caribou (Rangifer tarandus caribou). *Forest Ecology and Management* **364**: 17-26.

Martin-Guay MO, Paquette A, Reich PB, Messier C. 2019. Implications of contrasted above- and below-ground biomass responses in a diversity experiment with trees. *Journal of Ecology* **doi**: 10.1111/1365-2745.13265.

McCormack ML, Adams TS, Smithwick EA, Eissenstat DM. 2012. Predicting fine root lifespan from plant functional traits in temperate trees. *New phytologist* **195**(4): 823-831.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, et al. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New phytologist* **207**(3): 505-518.

McCormack ML, Guo D, Iversen CM, Chen W, Eissenstat DM, Fernandez CW, LeLi, Ma C, Ma Z, Poorter H, et al. 2017. Building a better foundation: improving root-trait measurements to understand and model plant and ecosystem processes. *New phytologist* **215**(1): 27-37.

McDowell NG, Beerling DJ, Breshears DD, Fisher RA, Raffa KF, Stitt M. 2011. The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends in Ecology and Evolution* **26**(10): 523-532.

Mori AS, Dee LE, Gonzalez A, Ohashi H, Cowles J, Wright AJ, Loreau M, Hautier Y, Newbold T, Reich PB, et al. 2021. Biodiversity–productivity relationships are key to nature-based climate solutions. *Nature Climate Change* **11**(6): 543-550.

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**(4): 787-793.

Niinemets Ü, Valladares F. 2006. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. *Ecological Monographs* **4**(76): 521-547.

O'Brien MJ, Ong R, Reynolds G. 2017. Intra-annual plasticity of growth mediates drought resilience over multiple years in tropical seedling communities. *Global Change Biology* **23**(10): 4235-4244.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara R, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. Package 'vegan'. *Community ecology package, version* **2**(9): 1-295.

Olmo M, Lopez-Iglesias B, Villar R. 2014. Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate. *Plant and Soil* **384**(1-2): 113-129.

Oram NJ, Ravenek JM, Barry KE, Weigelt A, Chen HM, Gessler A, Gockele A, de Kroon H, van der Paauw JW, Scherer-Lorenzen M, et al. 2018. Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. *Journal of Ecology* **106**(1): 265-277.

Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, et al. 2007. Specific root length as an indicator of environmental change. *Plant Biosystems* **141**(3): 426-442.

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P. 2014. *Climate change 2014: synthesis report. Contribution of*

Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change: Ipcc.

Pellegrini AFA, Ahlstrom A, Hobbie SE, Reich PB, Nieradzik LP, Staver AC, Scharenbroch BC, Jumpponen A, Anderegg WRL, Randerson JT, et al. 2018. Fire frequency drives decadal changes in soil carbon and nitrogen and ecosystem productivity. *Nature* **553**(7687): 194-198.

Peng S, Chen HYH. 2021. Global responses of fine root biomass and traits to plant species mixtures in terrestrial ecosystems. *Global Ecology and Biogeography* **30**(1): 289-304.

Persson HÅ. 1983. The distribution and productivity of fine roots in boreal forests. *Plant and Soil* **71**: 87-101.

Peters JL, Sutton, A. J., Jones, D. R., Abrams, K. R., & Rushton, L. 2006. Comparison of Two Methods to Detect Publication Bias in Meta-analysis. *Jama* **295**(6): 676-680.

Pielou EC. 1969. *An introduction to mathematical ecology*: New York, USA, Wiley-Inter-science.

Pittelkow CM, Liang X, Linquist BA, van Groenigen KJ, Lee J, Lundy ME, van Gestel N, Six J, Venterea RT, van Kessel C. 2015. Productivity limits and potentials of the principles of conservation agriculture. *Nature* **517**(7534): 365-368.

KS, Burton AJ, King JS, Zak DR. 2008. Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO2 and tropospheric O3. *New phytologist* **180**(1): 153-161.

Pregitzer KS, King JS, Burton AJ, Brown SE. 2000. Responses of tree fine roots to temperature. *New phytologist* **147**(1): 105-115.

Pretzsch H, Schutze 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 (Stuttg)* **15**(3): 483-495.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*.

R Core Team. 2022. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*.

Reich PB. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology* **102**(2): 275-301.

Reich PB, David Tilman, Forest Isbell, Kevin Mueller, Sarah E. Hobbie, Dan FB Flynn, and Nico Eisenhauer. 2012. Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. *Science* **336**(6081): 589-592.

Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB. 2003. The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* **164**(S3): S143-S164.

Ryser P. 2006. The mysterious root length. *Plant and Soil* **286**(1): 1-6.

Salahuddin, Rewald B, Razaq M, Lixue Y, Li J, Khan F, Jie Z. 2018. Root order-based traits of Manchurian walnut & larch and their plasticity under interspecific competition. *Sci Rep* **8**(1): 9815.

Searle EB, Chen HYH. 2019. Complementarity effects are strengthened by competition intensity and global environmental change in the central boreal forests of Canada. *Ecology Letters*.

See CR, Luke McCormack M, Hobbie SE, Flores-Moreno H, Silver WL, Kennedy PG. 2019. Global patterns in fine root decomposition: climate, chemistry, mycorrhizal association and woodiness. *Ecology Letters* **22**(6): 946-953.

Shu W, Shen X, Lei P, Xiang W, Ouyang S, Yan W. 2018. Temporal changes of fine root overyielding and foraging strategies in planted monoculture and mixed forests. *BMC Ecol* **18**(1): 9.

Siebenkäs A, Roscher C. 2016. Functional composition rather than species richness determines root characteristics of experimental grasslands grown at different light and nutrient availability. *Plant and Soil* **404**(1-2): 399-412.

Silvertown J. 2004. Plant coexistence and the niche. *Trends in Ecology & Evolution* **19**(11): 605-611.

Silvertown J, Araya Y, Gowing D. 2015. Hydrological niches in terrestrial plant communities: a review. *Journal of Ecology* **103**: 93-108.

Stape JL, Binkley D, Ryan MG, Fonseca S, Loos RA, Takahashi EN, Silva CR, Silva SR, Hakamada RE, Ferreira JMdA, et al. 2010. The Brazil Eucalyptus Potential Productivity Project: Influence of water, nutrients and stand uniformity on wood production. *Forest Ecology and Management* **259**(9): 1684-1694.

Steinbeiss S, Bessler H, Engels C, Temperton VM, Buchmann N, Roscher C, Kreutziger Y, Baade J, Habekost M, Gleixner G. 2008. Plant diversity positively affects short-term soil carbon storage in experimental grasslands. *Global Change Biology* **14**(12): 2937-2949.

Toïgo M, Perot T, Courbaud B, Castagneyrol B, Gégout J-C, Longuetaud F, Jactel H, Vallet P, Hector A. 2018. Difference in shade tolerance drives the mixture effect on oak productivity. *Journal of Ecology* **106**(3): 1073-1082.

Wagg C, O'brien MJ, Vogel A, Scherer-lorenzen M, Eisenhauer N, Schmid B, Weigelt A. 2017. Plant diversity maintains long-term ecosystem productivity under frequent drought by increasing short-term variation. *Ecology* **98**: 2952-2961.

Wambsganss J, Beyer F, Freschet GT, Scherer-Lorenzen M, Bauhus J. 2021. Tree species mixing reduces biomass but increases length of absorptive fine roots in European forests. *Journal of Ecology* **109**(7): 2678-2691.

Wang J, Defrenne C, McCormack ML, Yang L, Tian D, Luo Y, Hou E, Yan T, Li Z, Bu W, et al. 2021. Fine-root functional trait responses to experimental warming: a global meta-analysis. *New phytologist* **230**(5): 1856-1867.

Wang P, Huang K, Hu S. 2020. Distinct fine-root responses to precipitation changes in herbaceous and woody plants: a meta-analysis. *New Phytol* **225**(4): 1491-1499.

Ward SE, Ostle NJ, McNamara NP, Bardgett RD. 2010. Litter evenness influences short-term peatland decomposition processes. *Oecologia* **164**(2): 511-520.

Weemstra M, Mommer L, Visser EJ, van Ruijven J, Kuyper TW, Mohren GM, Sterck FJ. 2016. Towards a multidimensional root trait framework: a tree root review. *New phytologist* **211**(4): 1159-1169.

Wilcox KR, Shi Z, Gherardi LA, Lemoine NP, Koerner SE, Hoover DL, Bork E, Byrne KM, Cahill J, Jr., Collins SL, et al. 2017. Asymmetric responses of primary productivity to precipitation extremes: A synthesis of grassland precipitation manipulation experiments. *Global Change Biology* **23**(10): 4376-4385.

Williams LJ, Paquette A, Cavender-Bares J, Messier C, Reich PB. 2017. Spatial complementarity in tree crowns explains overyielding in species mixtures. *Nature Ecology & Evolution* **1**(4): 63.

Wright AJ, Ebeling A, de Kroon H, Roscher C, Weigelt A, Buchmann N, Buchmann T, Fischer C, Hacker N, Hildebrandt A, et al. 2015. Flooding disturbances increase resource availability and productivity but reduce stability in diverse plant communities. *Nature Communications* **6**(1): 1-6.

Wright A, Schnitzer SA, Reich PB. 2015. Daily environmental conditions determine the competition–facilitation balance for plant water status. *Journal of Ecology* **103**: 648-656.

Wright AJ, Wardle DA, Callaway R, Gaxiola A. 2017. The Overlooked Role of Facilitation in Biodiversity Experiments. *Trends Ecol Evol* **32**(5): 383-390.

Wu Z, Dijkstra P, Koch GW, PeÑUelas J, Hungate BA. 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology* **17**(2): 927-942.

Xiao W, Chen C, Chen X, Huang Z, Chen HYH, Soininen J. 2020. Functional and phylogenetic diversity promote litter decomposition across terrestrial ecosystems. *Global Ecology and Biogeography* **29**(12): 2261-2272.

Yachi S, Loreau M. 2007. Does complementary resource use enhance ecosystem functioning? A model of light competition in plant communities. *Ecology Letters* **10**(1): 54-62.

Yuan ZY, Chen HYH. 2010. Fine Root Biomass, Production, Turnover Rates, and Nutrient Contents in Boreal Forest Ecosystems in Relation to Species, Climate, Fertility, and Stand Age: Literature Review and Meta-Analyses. *Critical Reviews in Plant Sciences* **29**(4): 204-221.

Zang U, Goisser M, Häberle K-H, Matyssek R, Matzner E, Borken W. 2014. Effects of drought stress on photosynthesis, rhizosphere respiration, and fine-root characteristics of beech saplings: A rhizotron field study. *Journal of Plant Nutrition and Soil Science* **177**(2): 168-177.

Zangaro W, de Assis RL, Rostirola LV, de Souza PB, Goncalves MC, Andrade G, Nogueira MA. 2008. Changes in arbuscular mycorrhizal associations and fine root traits in sites under different plant successional phases in southern Brazil. *Mycorrhiza* **19**(1): 37-45.

Zhang X, Xing Y, Yan G, Han S, Wang Q, Lupwayi N. 2019. Effects of precipitation change on fine root morphology and dynamics at a global scale: a meta-analysis. *Canadian Journal of Soil Science* **99**(1): 1-11.

Zhang Y, Chen HYH, Reich PB. 2012. Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. *Journal of Ecology* **100**(3): 742-749.

Zhou G, Zhou X, Nie Y, Bai SH, Zhou L, Shao J, Cheng W, Wang J, Hu F, Fu Y. 2018. Drought-induced changes in root biomass largely result from altered root morphological traits: Evidence from a synthesis of global field trials. *Plant, cell & environment* **41**(11): 2589-2599.

Zwetsloot MJ, Goebel M, Paya A, Grams TEE, Bauerle TL. 2019. Specific spatio-temporal dynamics of absorptive fine roots in response to neighbor species identity in a mixed beech-spruce forest. *Tree Physiol* **39**(11): 1867-1879.

Zwetsloot MJ, Bauerle TL. 2021. Repetitive seasonal drought causes substantial species-specific shifts in fine-root longevity and spatio-temporal production patterns in mature temperate forest trees. *New phytologist* **231**(3): 974-986.

Zwetsloot MJ, Goebel M, Paya A, Grams TEE, Bauerle TL. 2019. Specific spatio-temporal dynamics of absorptive fine roots in response to neighbor species identity in a mixed beech-spruce forest. *Tree Physiology* **39**(11): 1867-1879.

APPENDIX I: SUPPLEMENTAL INFORMATION FOR CHAPTER 2

Table S 2-1 Reviewed references of the responses of fine-root traits to species mixtures in this meta-analysis.

Reference	**RB**	**R/S**	**WRD**	**RLD**	**SRL**	**RD**	**RN**
(Archambault *et al.* 2019)			Yes				
(Bakker *et al.* 2016)	Yes				Yes		
(Bauhus 2000)	Yes		Yes	Yes			Yes
(Baxendale *et al.* 2014)		Yes			Yes	Yes	
(Bennett *et al.* 2016)		Yes			Yes	Yes	
(Bessler *et al.* 2009)		Yes					
(Beyer *et al.* 2013a)	Yes				Yes	Yes	
(Beyer *et al.* 2013b)	Yes	Yes			Yes	Yes	
(Bolte & Villanueva 2005)	Yes		Yes		Yes		
(Bu *et al.* 2017)					Yes		
(Callaway *et al.* 2003)	Yes	Yes					Yes
(Cesarz *et al.* 2013)		Yes			Yes		
(Dai *et al.* 2018)	Yes		Yes				
(Domisch *et al.* 2015)	Yes		Yes		Yes		
(Fang *et al.* 2014)	Yes	Yes	Yes				
(Fender *et al.* 2013)	Yes		Yes				
(Finér *et al.* 2017)	Yes		Yes				
(Fredericksen & Zedaker 1995)	Yes		Yes				
(Gao *et al.* 2013)	Yes		Yes				
(Germon *et al.* 2017)	Yes				Yes	Yes	
(Göransson *et al.* 2016)	Yes		Yes				
(Hajek *et al.* 2014)	Yes	Yes			Yes	Yes	
(Hendriks & Bianchi 1995)					Yes		
(Jacob *et al.* 2014)	Yes				Yes	Yes	

(Jesch *et al.* 2018)	Yes	Yes	Yes				
(Lang'at *et al.* 2013)	Yes	Yes					
(Lei *et al.* 2012a)					Yes	Yes	
(Lei *et al.* 2012b)	Yes		Yes				
(Li *et al.* 2006)				Yes			
(Liu *et al.* 1985)	Yes		Yes				
(Ma & Chen 2017, 2018)	Yes						
(Maestre & Reynolds 2007)		Yes					
(Meinen *et al.* 2009)	Yes		Yes			Yes	
(Mommer *et al.* 2010)	Yes	Yes					
(Mommer *et al.* 2015)	Yes						
(Mueller *et al.* 2013)	Yes		Yes				
(Oram *et al.* 2018)	Yes		Yes				
(Pausch *et al.* 2013)		Yes					Yes
(Postma & Lynch 2012)				Yes			
(Qin 2006)	Yes			Yes			
(Qin 2009)		Yes			Yes	Yes	Yes
(Ravenek *et al.* 2014)	Yes						
(Reich *et al.* 2004)		Yes					
(Rivest *et al.* 2015)					Yes	Yes	
(Salahuddin *et al.* 2018)	Yes				Yes	Yes	
(Sanaullah *et al.* 2011)		Yes					
(Schmid & Kazda 2002)	Yes						
(Shu *et al.* 2018)	Yes		Yes		Yes		
(Siebenkäs & Roscher 2016)			Yes	Yes	Yes	Yes	
(Smith *et al.* 2013)	Yes		Yes		Yes		
(van Eekeren *et al.* 2010)	Yes		Yes				
(Wang *et al.* 2014)				Yes	Yes	Yes	

(Wang *et al.* 2018)	Yes	Yes			Yes	Yes	
(Xiang *et al.* 2015)	Yes		Yes	Yes	Yes		
(Xie *et al.* 1995)	Yes		Yes				
(Xu *et al.* 2010)		Yes					
(Yan *et al.* 2014)	Yes			Yes	Yes	Yes	
(Zhai *et al.* 2002)	Yes		Yes	Yes			
(Zhang *et al.* 2001)	Yes	Yes	Yes				
(Zhang *et al.* 2007)		Yes					Yes
(Zhang *et al.* 2014)				Yes		Yes	
(Zhang *et al.* 2015)					Yes	Yes	
(Zutter *et al.* 1999)			Yes	Yes	Yes		

Note: RB: fine-root biomass (g m^2); R/S: ratio of root biomass to shoot biomass; WRD: community-weighted mean rooting depth (cm); RLD: root length density (m dm^{-3}); SRL: specific root length (m g^{-1}); RD: mean root diameter (mm); RN: root nitrogen content (%)

Table S2-2 Values of Akaike information criterion of species richness, stand age, and soil depth for each root trait (see Materials and Methods).

Root traits	R			A			D		
	X	$X+X^2$	Log(X)	X	$X+X^2$	Log(X)	X	$X+X^2$	Log(X)
RB	**372.6**	377.9	380.1	**392.2**	395.4	394.8	393.3	397.8	**390.0**
R/S	66.03	68.40	**65.04**	**62.86**	67.32	63.14			
WRD	**-141.97**	-137.81	-136.05	-134.83	-128.77	**-139.43**			
RLD	**55.00**	55.00	55.00	52.58	53.08	**51.04**	47.76	49.48	**46.51**
SRL	122.98	**116.21**	119.44	122.14	126.04	**118.18**	123.02	126.12	**122.74**
RD	**-70.34**	-66.76	-70.05	-68.57	-63.47	**-68.94**	**-69.47**	-64.33	-69.11
RN	-26.07	-21.98	**-26.36**	-26.76	-27.17	**-27.24**	**-26.53**	-24.73	-26.50

Note: R, A, and D are species richness, stand age or experimental age, and soil depth, respectively. An exponential function was not included since the dependent variable contained negative values. Abbreviations for root traits are the same as in Table S2-1.

Table S2-3 Akaike information criterion (AIC) values of full model (Eq. 5 in Material and Methods) and the most parsimonious model for each root trait with species richness (R), stand age (A), and soil depth (D) at the community level.

Root trait	Full model		The most parsimonious model	
	AIC	Terms	AIC	Terms
RB	365.3	R + A + ln(D) + R × A + R × ln(D) + A × ln(D)	358.1	R + A + ln(D) + R × A + R × ln(D)
R/S	72.1	ln(R) + A + ln(R) × A	55.9	NULL
WRD	-129.7	R + ln(A) + R × ln(A)	-142.1	NULL
RLD	69.4	R + A + ln(D) + R × A + R × ln(D) + A × ln(D)	45.4	ln(D) + ln(A)
SRL	87.0	R + ln(A) + ln(D) + R × ln(A) + R × ln(D) + ln(A) × ln(D)	81.6	R + ln(A) + ln(D) +R × ln(A) + R × ln(D)
RD	-38.9	R + A + D + R × A + R × D + A × D	-77.2	NULL
RN	-9.6	R + A + D + R × A + R × D + A × D	-32.6	NULL

Note: Abbreviations for root traits are the same as in Table S2-1.

Table S2-4 The values of Akaike information criterion for mean annual temperature (MAT) and mean annual precipitation (MAP) (see Materials and Methods).

Root trait	MAT			MAP		
	X	X+X^2	Log(X)	X	X+X^2	Log(X)
RB	**375.51**	377.55	375.28	**375.67**	378.02	375.74
R/S	**8.54**	10.33	10.01	**9.34**	10.41	10.47
WRD	**-133.51**	-128.30	-133.43	**-133.51**	-128.73	-133.58
RLD	**43.40**	44.84	43.81	**53.75**	55.19	53.41
SRL	**10.99**	11.16	11.08	**10.89**	12.63	10.99
RD	**-48.54**	-45.17	-48.59	**-49.05**	-47.59	-48.77
RN	**-17.79**	-17.40	-18.03	**-22.77**	-18.27	-21.96

Note: Numbers in bold were selected into the full models. Abbreviations for root traits are the same as in Table S2-1.

Table S2-5 Results of the most parsimonious models: Effects of species richness (R), stand age (A), soil depth (D), mean annual precipitation (MAP), or mean annual temperature (MAT) on the ln*RRs* of root biomass and root length density in natural habitats.

Root trait	Model	AIC	Source	Coefficient	df	t value	P value
Root biomass	1	358.1	intercept	0.234	1, 31	4.9	**< 0.001**
			R	0.147	1, 313	5.0	**< 0.001**
			A	-0.058	1, 30	-1.4	0.176
			ln(D)	0.049	1, 316	3.3	**< 0.001**
			R × A	0.203	1, 307	3.7	**< 0.001**
			R × ln(D)	0.051	1, 324	3.6	**< 0.001**
	2	319.7	intercept	0.231	1, 28	4.9	**< 0.001**
			R	0.186	1, 298	6.1	**< 0.001**
			A	-0.055	1, 29	-1.4	0.182
			ln(D)	0.049	1, 300	3.4	**< 0.001**
			R × A	0.107	1, 280	1.9	0.060
			R × ln(D)	0.004	1, 300	0.2	0.823
			MAP	0.231	1, 62	2.8	**0.007**
			R × MAP	0.269	1, 280	5.6	**< 0.001**
Root length density	1	45.4	intercept	-0.015	1, 19	-0.2	0.874
			ln(D)	0.086	1, 96	3.8	**< 0.001**
			ln(A)	0.183	1, 48	2.6	**0.012**
	2	41.4	intercept	0.056	1, 14	0.7	0.466
			ln(D)	0.094	1, 79	3.6	**< 0.001**
			ln(A)	0.120	1, 33	2.2	**0.039**
			MAT	-0.279	1, 15	-3.9	**0.001**

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom. Model 1 was the most parsimonious model selected from equation 5, model 2 was selected from Eq. 7 (see Material and Methods).

Table S2-6 Results from the tests of publication bias by funnel tests of asymmetry with the sample size as the predictor. In all cases, we analyzed the overall response ratio across the entire dataset followed by the most parsimonious covariate (species richness, stand age, and soil depth) models we selected (see Materials and Methods for the most parsimonious model selection).

Root trait	z	p
RB	-1.039	0.299
RB + covariates	-0.942	0.346
RS	0.185	0.853
RS + covariates	NA	
WRD	-0.042	0.967
WRD + covariates	NA	
RLD	0.052	0.958
RLD + covariates	0.240	0.810
SRL	-0.256	0.798
SRL + covariates	-0.081	0.936
RD	-0.129	0.898
RD + covariates	NA	
RN	-0.225	0.822
RN + covariates	NA	

Note: Abbreviation for root traits is the same as in Table S2-1.

Table S2-7 Effects (*P* values) of mean annual temperature (MAT) and mean annual precipitation (MAP) on effect sizes of root traits in species mixtures.

Root trait	MAT				MAP			
	Estimate	df	F value	*P*	Estimate	df	F value	*P*
RB					0.132	1, 56	7.5	**0.008**
R/S	0.081	1, 6	1.0	0.365	0.031	1, 5	0.4	0.727
WRD	0.003	1, 27	0.02	0.896	0.003	1, 22	0.0003	0.988
RLD	-0.279	1, 17	11.6	< **0.001**				
SRL	0.002	1, 20	0.0006	0.980	0.047	1, 21	0.2	0.658
RD	0.017	1, 7	0.2	0.709	-0.036	1, 7	0.7	0.437
RN	0.040	1, 1	3.3	0.406	0.082	1, 1	23.1	0.079

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom. The most parsimonious models for RB and RLD did not contain MAP term. Abbreviation for root traits is the same as in Table S2-1.

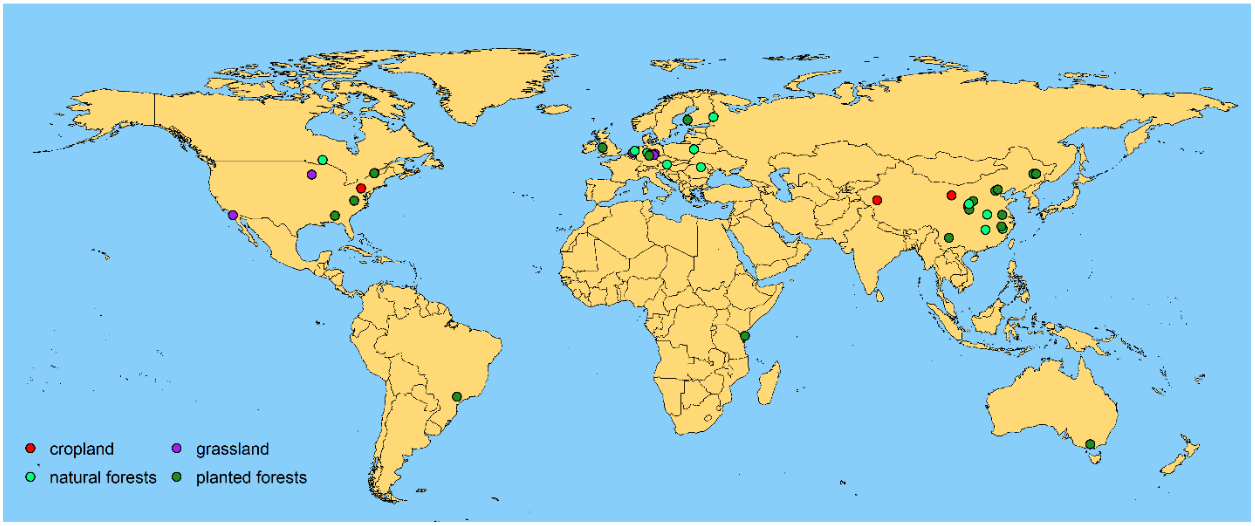

Fig. S2-1 Global distribution of plant diversity experiments focusing on diversity effects on fine-root traits in this meta-analysis. Experiments conducted in containers were excluded in this Figure.

Appendix reference 2-1 – Data Sources

Archambault, C., Paquette, A., Messier, C., Khlifa, R., Munson, A.D. & Handa, I.T. (2019). Evergreenness influences fine root growth more than tree diversity in a common garden experiment. *Oecologia*, **189**, 1027-1039. http://doi.org/10.1007/s00442-019-04373-5

Bakker, L.M., Mommer, L. & van Ruijven, J. (2016). Can root trait diversity explain complementarity effects in a grassland biodiversity experiment? *Journal of Plant Ecology*, **11**, 73-84.

Bauhus, J., Khanna, P.K. and Menden, N. (2000). Aboveground and belowground interactions in mixed plantations of Eucalyptus globulus and Acacia mearnsii. *Canadian Journal of Forest Research*, **30**, 1886-1894.

Baxendale, C., Orwin, K.H., Poly, F., Pommier, T. & Bardgett, R.D. (2014). Are plant-soil feedback responses explained by plant traits? *New Phytologist*, **204**, 408-423.

Bennett, J.A., Riibak, K., Tamme, R., Lewis, R.J., Pärtel, M. & Semchenko, M. (2016). The reciprocal relationship between competition and intraspecific trait variation. *Journal of Ecol.*, **104**, 1410-1420.

Bessler, H., Temperton, V.M., Rpscher, C., Buchmann, N., Schmid, B., Schilze, E. *et al.* (2009). Aboveground overyielding in grassland mixtures is associated with reduced biomass partitioning to belowground organs. *Ecology*, **90**, 1520-1530.

Beyer, F., Hertel, D., Jung, K., Fender, A.-C. & Leuschner, C. (2013a). Competition effects on fine root survival of Fagus sylvatica and Fraxinus excelsior. *Forest Ecology and Management*, **302**, 14-22.

Beyer, F., Hertel, D. & Leuschner, C. (2013b). Fine root morphological and functional traits in Fagus sylvatica and Fraxinus excelsior saplings as dependent on species, root order and competition. *Plant and Soil*, **373**, 143-156.

Bolte, A. & Villanueva, I. (2005). Interspecific competition impacts on the morphology and distribution of fine roots in European beech (Fagus sylvatica L.) and Norway spruce (Picea abies (L.) Karst.). *Eurean Journal of Forest Reserach*, **125**, 15-26.

Bu, W., Schmid, B., Liu, X., Li, Y., Härdtle, W., von Oheimb, G. *et al.* (2017). Interspecific and intraspecific variation in specific root length drives aboveground biodiversity effects in young experimental forest stands. *Journal of Plant Ecology*, **10**, 158-169.

Callaway, J.C., Sullivan, G. & Zedler, J.B. (2003). Species-rich plantings increase biomass and nitrogen accumulation in a wetland restoration experiment. *Ecology Application*, **13**, 1626-1639.

Cesarz, S., Fender, A.-C., Beyer, F., Valtanen, K., Pfeiffer, B., Gansert, D. *et al.* (2013). Roots from beech (Fagus sylvatica L.) and ash (Fraxinus excelsior L.) differentially affect soil microorganisms and carbon dynamics. *Soil Biology and Biochemistry*, **61**, 23-32.

Dai, Y., Sun, P., Kang, D., Deng, J., Ren, C., Yang, G. *et al.* (2018). Fine root biomass of artificial forests in loess hilly region and its influencing factors (in Chinese with English abstract). *Chinese Journal of Ecology*, **37**, 2229-2236.

Domisch, T., Finer, L., Dawud, S.M., Vesterdal, L. & Raulund-Rasmussen, K. (2015). Does species richness affect fine root biomass and production in young forest plantations? *Oecologia*, **177**, 581-594.

Fang, Y., Xu, B., Liu, L., Gu, Y., Liu, Q., Turner, N.C. *et al.* (2014). Does a mixture of old and modern winter wheat cultivars increase yield and water use efficiency in water-limited environments? *Feild Crop Research*, **156**, 12-21.

Fender, A.-C., Gansert, D., Jungkunst, H.F., Fiedler, S., Beyer, F., Schützenmeister, K. *et al.* (2013). Root-induced tree species effects on the source/sink strength for greenhouse gases (CH4, N2O and CO2) of a temperate deciduous forest soil. *Soil Biology and Biochemistry*, **57**, 587-597.

Finér, L., Domisch, T., Dawud, S.M., Raulund-Rasmussen, K., Vesterdal, L., Bouriaud, O. *et al.* (2017). Conifer proportion explains fine root biomass more than tree species diversity and site factors in major European forest types. *Forest Ecology and Management*, **406**, 330-350.

Fredericksen, T.S. & Zedaker, S.M. (1995). Fine root biomass, distribution, and production in young pine-hardwood stands. *New Forests*, **10**, 99-110.

Gao, C., Tang, G., Li, K. & Xie, Q. (2013). Root biomass and its distribution of Azadirachta indica and Acacia auriculiformis plantations in the Dry-hot Valley (in Chinese with English abstract). *Acta Ecology Sinica*, **33**, 1964-1972.

Germon, A., Guerrini, I.A., Bordron, B., Bouillet, J.-P., Nouvellon, Y., de Moraes Gonçalves, J.L. *et al.* (2017). Consequences of mixing Acacia mangium and Eucalyptus grandis trees on soil exploration by fine-roots down to a depth of 17 m. *Plant and Soil*, **424**, 203-220.

Göransson, H., Bambrick, M.T. & Godbold, D.L. (2016). Overyielding of temperate deciduous tree mixtures is maintained under throughfall reduction. *Plant and Soil*, **408**, 285-298.

Hajek, P., Hertel, D. & Leuschner, C. (2014). Root order- and root age-dependent response of two poplar species to belowground competition. *Plant and Soil*, **377**, 337-355.

Hendriks, C.M.A. & Bianchi, F.J.J.A. (1995). Root density and root biomass in pure and mixed forest stands of Douglas-fir and beech. *NJAS-Wagen. Journal of Life Science.*, **43**, 321-331.

Jacob, A., Hertel, D. & Leuschner, C. (2014). Diversity and species identity effects on fine root productivity and turnover in a species-rich temperate broad-leaved forest. *Functional Plant Biology*, **41**. http://dx.doi.org/10.1071/FP13195

Jesch, A., Barry, K.E., Ravenek, J.M., Bachmann, D., Strecker, T., Weigelt, A. *et al.* (2018). Below-ground resource partitioning alone cannot explain the biodiversity-ecosystem function relationship: A field test using multiple tracers. *Journal of Ecology,* **106**, 2002-2018. http://doi.org/10.1111/1365-2745.12947

Lang'at, J.K., Kirui, B.K., Skov, M.W., Kairo, J.G., Mencuccini, M. & Huxham, M. (2013). Species mixing boosts root yield in mangrove trees. *Oecologia*, **172**, 271-278.

Lei, P., Scherer-Lorenzen, M. & Bauhus, J. (2012a). Belowground facilitation and competition in young tree species mixtures. *Forest Ecology and Management*, **265**, 191-200.

Lei, P., Scherer-Lorenzen, M. & Bauhus, J. (2012b). The effect of tree species diversity on fine-root production in a young temperate forest. *Oecologia*, **169**, 1105-1115.

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F.A. *et al.* (2006). Root distribution and interactions between intercropped species. *Oecologia*, **147**, 280-290.

Liu, C., Guo, X. & Xu, Z. (1985). A prelimnary study on root system of the artificially mixed stand of *Pinus tabuliformis* and *Quercus Varibilis* in Xishan Region, Beijing (in Chinese). *Journal of Beijing Forest College*, 77-84.

Ma, Z. & Chen, H.Y.H. (2017). Effects of species diversity on fine root productivity increase with stand development and associated mechanisms in a boreal forest. *Journal of Ecology*, **105**, 237-245.

Ma, Z. & Chen, H.Y.H. (2018). Positive species mixture effects on fine root turnover and mortality in natural boreal forests. *Soil Biology and Biochemistry*, **121**, 130-137.

Maestre, F.T. & Reynolds, J.F. (2007). Biomass responses to elevated CO2, soil heterogeneity and diversity: an experimental assessment with grassland assemblages. *Oecologia*, **151**, 512-520.

Meinen, C., Hertel, D. & Leuschner, C. (2009). 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.

Mommer, L., Padilla, F.M., van Ruijven, J., de Caluwe, H., Smit-Tiekstra, A., Berendse, F. *et al.* (2015). Diversity effects on root length production and loss in an experimental grassland community. *Functinal of Ecology*, **29**, 1560-1568.

Mommer, L., Van Ruijven, J., De Caluwe, H., Smit-Tiekstra, A.E., Wagemaker, C.A.M., Joop Ouborg, N. *et al.* (2010). Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. *Journal of Ecology*, **98**, 1117-1127.

Mueller, K.E., Tilman, D., Fornara, D.A. & Hobbie, S.E. (2013). Root depth distribution and the diversity–productivity relationship in a long-term grassland experiment. *Ecology*, **94**, 787-793.

Oram, N.J., Ravenek, J.M., Barry, K.E., Weigelt, A., Chen, H., Gessler, A. *et al.* (2018). Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. *Journal of Ecology*, **106**, 265-277.

Pausch, J., Zhu, B., Kuzyakov, Y. & Cheng, W. (2013). Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biology and Biochemistry*, **57**, 91-99.

Postma, J.A. & Lynch, J.P. (2012). Complementarity in root architecture for nutrient uptake in ancient maize/bean and maize/bean/squash polycultures. *Annals of Botany*, **110**, 521-534.

Qin, J. (2006). Eco-physiological effect of *Populus davidiana-Quercus liaotungensis* mixed forest in Ziwuling forest area (in Chinese with English abstract). *Northwest A & F University*

Qin, J. (2009). Study on growth dynamics and ecological function of *Robinia pseudoacacia* and *Ulmus pumila* mixed forest in the Loess Plateau (in Chinese with English abstract). *Northwest A & F University.*

Ravenek, J.M., Bessler, H., Engels, C., Scherer-Lorenzen, M., Gessler, A., Gockele, A. *et al.* (2014). Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. *Oikos*, **123**, 1528-1536.

Reich, P.B., Tilman, D., Naeem, S., Ellsworth, D.S., Knops, J., Craine, J. *et al.* (2004). Species and functional group diversity independently influence biomass accumulation and its response to CO2 and N. *Proceeding of the National Academy of Sciences of the United States of Amerian*, **101**, 10101-10106.

Rivest, D., Paquette, A., Shipley, B., Reich, P.B., Messier, C. & Sayer, E. (2015). Tree communities rapidly alter soil microbial resistance and resilience to drought. *Functional Ecology*, **29**, 570-578.

Salahuddin, Rewald, B., Razaq, M., Lixue, Y., Li, J., Khan, F. *et al.* (2018). Root order-based traits of Manchurian walnut & larch and their plasticity under interspecific competition. *Scientific Report*, **8**, 9815.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C. & Kuzyakov, Y. (2011). Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. *Applied of Soil Ecology*, **48**, 38-44.

Schmid, I. & Kazda, M. (2002). Root distribution of Norway spruce in monospecific and mixed stands on different soils. *Forest Ecology and Management*, **159**, 37-47.

Shu, W., Shen, X., Lei, P., Xiang, W., Ouyang, S. & Yan, W. (2018). Temporal changes of fine root overyielding and foraging strategies in planted monoculture and mixed forests. *BMC Ecology*, **18**, 9. https://doi.org/10.1186/s12898-018-0166-z

Siebenkäs, A. & Roscher, C. (2016). Functional composition rather than species richness determines root characteristics of experimental grasslands grown at different light and nutrient availability. *Plant and Soil*, **404**, 399-412.

Smith, A.R., Lukac, M., Bambrick, M., Miglietta, F. & Godbold, D.L. (2013). Tree species diversity interacts with elevated CO2 to induce a greater root system response. *Global Change and Biology*, **19**, 217-228.

van Eekeren, N., Bos, M., de Wit, J., Keidel, H. & Bloem, J. (2010). Effect of individual grass species and grass species mixtures on soil quality as related to root biomass and grass yield. *Applied Soil Ecology*, **45**, 275-283.

Wang, B.J., Zhang, W., Ahanbieke, P., Gan, Y.W., Xu, W.L., Li, L.H. *et al.* (2014). Interspecific interactions alter root length density, root diameter and specific root length in jujube/wheat agroforestry systems. *Agroforestry Systems*, **88**, 835-850.

Wang, Z., Xu, W., Chen, Z., Jia, Z., Huang, J., Wen, Z. *et al.* (2018). Soil Moisture Availability at Early Growth Stages Strongly Affected Root Growth of Bothriochloa ischaemum When Mixed With Lespedeza davurica. *Frontiers in Plant Science*, **9**, 1050. https://doi.org/10.3389/fpls.2018.01050

Xiang, W., Fan, G., Lei, P., Zeng, Y., Tong, J., Fang, X. *et al.* (2015). Fine root interactions in subtropical mixed forests in China depend on tree species composition. *Plant and Soil*, **395**, 335-349.

Xie, W., Xu, X., Li, H., Zhang, D. & Tang, H. (1995). Studies on the characteristics of root system in Pine-Eucommia mixed stand (in Chinese with English abstract). *Journal of Anhui Agricultural University*, **22**, 256-261.

Xu, B., Deng, X., Zhang, S. & Shan, L. (2010). Seedling biomass partition and water use efficiency of switchgrass and milkvetch in monocultures and mixtures in response to various water availabilities. *Environmental Management*, **46**, 599-609.

Yan, D., Zhang, Z. & Yang, X. (2014). Root Structures and Its Relation with Top Soil Properties of Typical Forest in Southern Mountains in Henan Province. *Journal of Northeast Forest University*, **42**, 30-36.

Zhai, M., Jiang, S. & Jia, L. (2002). Fine-root dynamics in mixed plantation of poplar and black locust (in Chinese with English abstract). *Jorunal of Beijing Forest University*, **24**, 39-44.

Zhang, C., Postma, J.A., York, L.M. & Lynch, J.P. (2014). Root foraging elicits niche complementarity-dependent yield advantage in the ancient 'three sisters' (maize/bean/squash) polyculture. *Annals of Botany*, **114**, 1719-1733.

Zhang, W., Ahanbieke, P., Wang, B., Gan, Y., Li, L., Christie, P. *et al.* (2015). Temporal and spatial distribution of roots as affected by interspecific interactions in a young walnut/wheat alley cropping system in northwest China. *Agroforestry Systems*, **89**, 327-343.

Zhang, Y., Shen, Y., Bai, S. & Wang, Z. (2001). Effects of the mixed on root growth and sidtribution of Fraxinus mandsjurica and Larix gmelinii (in Chinese with English abstract). *Scientia Silvae Simicae*, **37**, 16-23.

Zhang, Z., Rengel, Z. & Meney, K. (2007). Growth and resource allocation of Canna indica and Schoenoplectus validus as affected by interspecific competition and nutrient availability. *Hydrobiologia*, **589**, 235-248.

Zutter, B.R., Mitchell, R.J., Glover, G.R. & Gjerstad, D.H. (1999). Root length and biomass in mixtures of broomsedge with loblolly pine or sweetgum. *Canadian Journal of Forest Research*, **29**, 926-933.

APPENDIX II: SUPPLEMENTAL INFORMATION FOR CHAPTER 3

Table S3-1 Reviewed references analyzed in this meta-analysis

Reference	**RB**	**WRD**	**RLD**	**SRL**
(Archambault *et al.* 2019)		Yes		
(Bauhus 2000)	Yes	Yes	Yes	
(Baxendale *et al.* 2014)				Yes
(Bennett *et al.* 2016)				Yes
(Beyer *et al.* 2013a)	Yes			Yes
(Beyer *et al.* 2013b)	Yes			Yes
(Bolte & Villanueva 2005)	Yes	Yes		Yes
(Brassard *et al.* 2011)	Yes			
(Bu *et al.* 2017)				Yes
(Cesarz *et al.* 2013)				Yes
(Corre-Hellou & Crozat 2005)	Yes			
(Dai *et al.* 2018)	Yes	Yes		
(Dimitrakopoulos & Schmid 2004)	Yes			
(Domisch *et al.* 2015)	Yes	Yes		Yes
(Fang *et al.* 2014)	Yes	Yes		
(Fender *et al.* 2013)	Yes	Yes		
(Finér *et al.* 2017)	Yes	Yes		
(Fredericksen & Zedaker 1995)	Yes	Yes		
(Gao *et al.* 2013)	Yes	Yes		
(Germon *et al.* 2017)	Yes			Yes

(Göransson *et al.* 2016)	Yes	Yes		
(Hajek *et al.* 2014)	Yes			Yes
(Hendriks & Bianchi 1995)				Yes
(Jacob *et al.* 2014)	Yes			Yes
(Lang'at *et al.* 2013)	Yes			
(Laossi *et al.* 2008)	Yes			
(Lei *et al.* 2012a)				Yes
(Lei *et al.* 2012b)	Yes	Yes		
(Leuschner *et al.* 2001)	Yes			
(Li *et al.* 2006)			Yes	
(Liu *et al.* 1985)	Yes	Yes		
(Ma & Chen 2017)	Yes			
(Meinen *et al.* 2009)	Yes	Yes		
(Mommer *et al.* 2015)	Yes			
(Mueller *et al.* 2013)	Yes	Yes		
(Oram *et al.* 2018)	Yes	Yes		
(Qin 2006)	Yes		Yes	
(Qin 2009)				Yes
(Rivest *et al.* 2015)				Yes
(Salahuddin *et al.* 2018)	Yes			Yes
(Schmid & Kazda 2002)	Yes			
(Shu *et al.* 2018)	Yes	Yes		Yes

(Siebenkäs & Roscher 2016)		Yes	Yes	Yes
(Smith *et al.* 2013)	Yes	Yes		Yes
(van Eekeren *et al.* 2010)	Yes	Yes		
(Wang *et al.* 2014)			Yes	Yes
(Wang *et al.* 2018)	Yes			Yes
(Xiang *et al.* 2015)	Yes	Yes	Yes	Yes
(Xie *et al.* 1995)	Yes	Yes		
(Xu *et al.* 2008)	Yes			
(Xu *et al.* 2010)	Yes			
(Yan *et al.* 2014)	Yes		Yes	Yes
(Zhai *et al.* 2002)	Yes	Yes	Yes	
(Zhang *et al.* 2001)	Yes	Yes		
(Zhang *et al.* 2015)				Yes
(Zutter *et al.* 1999)		Yes	Yes	Yes

Note: RB: fine-root biomass (g m2); WRD: community-weighted mean rooting depth (cm); RLD: root length density (m dm-3); SRL: specific root length (m g-1).

Table S3-2 List of References cited in this meta-analysis for the functional trait dissimilarity, which
were not available in the original publications, TRY Plant Trait Database and Plants For A Future (PFAF) Database.

Authors	Year	Published source	Title
Allen & Duke	2006	*Species Profiles for Pacific Island Agroforestry (www.traditionaltree.org)*	Bruguiera gymnorrhiza: large-leafed mangrove
Crous et al.	2012	*Biological Invasions*	Drought-tolerance of an invasive alien tree, *Acacia mearnsii* and two native competitors in fynbos riparian ecotones
Du et al.	2018	*Forests*	The Transcriptomic Responses of *Pinus massoniana* to Drought Stress
Gilman & Watson	1993	*Fact Sheet ST-4 University of Florida*	Acacia auriculiformis Earleaf Acacia
Gilman & Watson	1993	*Fact Sheet ST-264 University of Florida*	Fraxinus excelsior Common Ash
Gilman & Watson	1993	*Fact Sheet ST-473 University of Florida*	Pinus strobus Eastern White Pine
Hillebrand & Matthiessen	2009	*Ecology Letters*	Biodiversity in a complex world: consolidation and progress in functional biodiversity research
Kangas & Martian		*2013North Dakota Forest Service*	*Siberian Larch*: An Underutilized Species
Lin et al.	2001	*Agroforesry Systems*	Nutritive quality and morphological development under partial shade of some forage species with agroforestry potential
Peltier & Marigo	1999	*Journal of Plant Physiology*	Drought Adaptation in *Fraxinus excelsior* L.: Physiological Basis of the Elastic Adjustment
Piškur et al.	2010	*European Journal of Forest Research*	Diversity and pathogenicity of *Botryosphaeriaceae* on declining in Slovenia and Italy following extreme weather conditions
Rigo & Caudullo	2016	*European Atlas of Forest Tree Species*	*Quercus ilex* in Europe: distribution, habitat, usage and threats
Shi	2006	*Dissertation for the Degree of Doctor*	Effects of fertilization on fine root dynamics in *Manchurian Ash* and *Davurian Larch* plantations
Shi et al.	2007	*Journal of Plant Ecology (in Chinese)*	Estimating fine root production, mortality and turnover with

			minirhizotrons in *Larix Gmelinii* and *Fraxinus Mandshurica* plantations
Smith	1987	*Estuarine, Coastal and Shelf Science*	Effects of Seed Predators and Light Level on the Distribution of *Avicennia Marina* (Forsk.) Vierh. in Tropical, Tidal Forests
Wyka et al.	2007	*Tree Physiology*	Acclimation of leaves to contrasting irradiance in juvenile trees differing in shade tolerance
Yin & Shen	2016	*Chinese Journal of Applied Ecology (in Chinese)*	Shade tolerance and the adaptability of forest plants in morphology and physilology: A revie
Zhang et al.	2005	*Acta Phamaceutica Sinica (in Chinese)*	Chemical constituents from mangrove plant *Ceriops tagal*
Zhang et al.	2013	*Plos One*	Structure and Composition of Natural Gmelin Larch (*Larix gmelinii var. gmelinii*) Forests in Response to Spatial Climatic Changes
Zhao et.	2017	*Environmental Science and Pollution Research*	Do shallow soil, low water availability, or their combination increase the competition between grasses with different root systems in karst soil?

Table S3-3 Dissimilarity of leaf habit and the corresponding ranking value in plant mixtures at the community level in collected publications.

	Ranking value
Deciduous broadleaf	low
Deciduous conifer	low
Evergreen broadleaf	low
Evergreen conifer	low
Conifer-broadleaf deciduous	medium
Conifer-broadleaf evergreen	medium
Evergreen-deciduous conifer	medium
Evergreen-deciduous broadleaf	medium
Evergreen conifer-deciduous broadleaf	high
Deciduous conifer-evergreen broadleaf	high

Table S3-4 The values of Akaike information criterion of species richness, stand age and soil depth for each root attributes (see Materials and Methods).

Attribute	R			A			D		
	X	$X+X^2$	Log(X)	X	$X+X^2$	Log(X)	X	$X+X^2$	Log(X)
Root biomass	278.9	**275.2**	308.2	344.3	346.3	**343.0**	343.9	349.2	**342.6**
Weighted rooting depth	**-110.4**	-107.2	-103.8	-103.8	-98.1	**-109.1**			
Root length density	**76.6**	77.1	76.5	73.7	74.0	**72.6**	**71.9**	75.4	72.1
Specific root length	**80.9**	84.1	81.7	80.9	82.7	**75.9**	**81.8**	85.1	81.8

Note: R, A and D are species richness, stand age or experimental age, and soil depth, respectively. An exponential function was not included since the dependent variable contains negative values. For each predictor (R, A and D), the functions were chosen as shown in the bold numbers.

Table S3-5 The values of Akaike information criterion of mean annual precipitation (MAP), mean annual temperature (MAT) and aridity index (AI) for each root attributes (see Materials and Methods).

Attribute	MAP			MAT			AI		
	X	X+X^2	Log(X)	X	X+X^2	Log(X)	X	X+X^2	Log(X)
Root biomass									
	338.3	338.0	338.7	**337.9**	337.2	339.4	**339.1**	342.5	339.1
Weighted rooting depth									
	-100.2	-100.2	-100.2	**-100.3**	-100.3	-100.0	**-101.5**	-101.4	-101.5
Root length density									
	71.9	72.7	**70.3**	**72.2**	72.0	72.5	73.7	74.0	**71.7**
Specific root length									
	11.9	11.8	**11.3**	**11.7**	11.2	11.7	11.9	11.9	**11.5**

Note: For each predictor (MAP, MAT and AI), the functions were chosen as shown in the bold numbers.

Table S3-6. The Akaike information criterion (AIC) values for the full model (Eq. 3 in Materials and Methods) and the most parsimonious model, and the effects (*P* value) of the species richness in mixtures (R), stand age (A), soil depth and functional trait dissimilarity (FT) of the most parsimonious models.

Attribute	Full model	Most parsimonious model							
	AIC	AIC	$R^{\#}$	D*	ln(A)	FT	FT × R	FT × D	FT × ln(A)
Contrasting shade tolerance									
RB	277.9	268.9	< **0.001**	**0.003**	0.737	**0.004**	–	–	**0.025**
WRD	-93.8	-99.9	< **0.001**	–	0.582	0.309	–	–	0.577
RLD	93.9	78.5	–	–	0.246	0.503	–	–	0.339
SRL	100.2	83.5	–	–	**0.029**	0.621	–	–	0.541
Contrasting drought tolerance									
RB	250.1	241.0	**< 0.001**	0.459	**< 0.001**	**< 0.001**	**< 0.001**	**0.047**	–
WRD	-94.3	-110.7	–	–	–	0.652	–	–	–
RLD	96.3	77.7	–	**0.044**	–	0.687	–	**0.048**	–
SRL	102.3	86.8	–	0.241	–	**0.047**	–	0.479	–
Contrasting plant growth rate									
RB	269.4	243.2	**< 0.001**	**< 0.001**	**0.003**	**< 0.001**	–	–	–
WRD	-114.9	-122.6	**< 0.001**	–	–	0.111	–	–	–
RLD	92.1	72.9	–	–	–	0.404	–	–	–
SRL	105.8	84.0	–	–	**0.031**	0.739	–	–	–

Leaf habit dissimilarity									
RB	227.2	202.6	–	–	0.291	0.182	–	–	**0.016**
WRD	-65.8	-98.5	–	–	–	0.102	–	–	–
RLD	74.8	59.3	–	–	–	0.116	–	–	–
SRL	80.4	46.3	–	–	–	**0.009**	–	–	–

1. Abbreviations for root attributes are the same as in Table S3-1.

2. # The species richness function is R^2 instead of linear R in the most parsimonious models when FT is shade tolerance or plant growth rate for root biomass; * the function of soil depth is log-linear D in the most parsimonious models for root biomass.

3. According to our core hypotheses, when FT is shade tolerance (ST), the most parsimonious model for each root attribute is keeping the interaction term of ST × A with the lowest AIC value; When FT is drought tolerance (DT), the most parsimonious model for each root attribute is keeping the interaction term of DT × A with the lowest AIC value (except WRD since there is no soil depth by definition); When FT is plant growth rate or leaf habit dissimilarity, the most parsimonious model for each root attribute is keeping FT with the lowest AIC value.

Table S3-7 The values of Akaike information criterion (AIC) for the most parsimonious models with or without ecosystem type as a predictor variable (see Materials and Methods).

Attribute	Term	AIC	Term	AIC
Contrasting shade tolerance				
RB	R^2+ ln(D)+ST+ln(A)+ln(A) ×ST	**268.8**	R^2+ ln(D)+E+ST+ ln(A)+ln(A) ×ST+ST×E	274.7
WRD	R+ST+ ln(A)+ln(A) ×ST	**-99.9**	R+ST+ ln(A)+ln(A) ×ST+ST×E	-96.4
RLD	ST+ln(A)+ln(A) ×ST	78.5	ST+ ln(A)+ln(A) ×ST+ST×E	**78.1**
SRL	ST+ln(A)+ln(A) ×ST	**83.5**	ST+ ln(A)+ln(A) ×ST+ ln(A) ×E+ST×E	89.9
Contrasting drought tolerance				
RB	R+ln(A)+ln(D)+DT+R×DT + DT×ln(D)	**241.0**	R+ln(A)+ln(D)+DT+E+R×DT +DT×ln(D)+ E×DT	249.9
WRD	DT	**-110.7**	DT+E+E×DT	-99.4
RLD	DT+D+DT×D	**77.7**	DT+E+E×DT+E×DT	84.5
SRL	DT+D+DT×D	**86.8**	DT+E+E×DT	92.3
Contrasting plant growth rate				
RB	R^2+ ln(D)+GR	**244.7**	R^2+ ln(D)+E+GR+GR×E	257.5
WRD	R+GR	**-122.7**	R+GR+E+ E×GR	-101.0
RLD	GR	**72.9**	GR+E+ E×GR	78.5
SRL	ln(A)+GR	**84.0**	ln(A)+GR+E+ E×GR	93.5
Leaf habit dissimilarity				

RB	LHD+ln(A)+LHD×ln(A)	202.5	LHD+ln(A)+E+LHD×ln(A)+E×LHD+E×ln(A)	**201.3**
WRD	LHD	**-98.6**	E+LHD+E×LHF	-78.7
RLD	LHD	**59.3**	E+LHD+E×LHF	65.5
SRL	LHD	**46.3**	E+LHD+E×LHF	59.2

Note: R, A, D, E, ST, DT, GR and LHD are species richness, stand age or experimental age, soil depth, ecosystem type, contrasting shade tolerance, contrasting drought tolerance, contrasting plant growth rate and leaf habitat dissimilarity, respectively. Numbers in bold indicate lower AIC values. Abbreviations for root traits are the same as in Table S3-1

Table S3-8 The effects (P values) of mean annual temperature (MAT) and Aridity index (AI) on effect sizes of root attributes in relation to functional trait dissimilarity in plant mixtures.

Attribute		MAT			AI		
		Estimate	df	*P*	Estimate	df	*P*
Contrasting shade tolerance							
RB	absent	-0.026	1, 124	0.745	0.136	1, 105	0.305
	present	0.116	1, 48	0.061	-0.003	1, 54	0.969
WRD	absent	-0.014	1, 37	0.602	-0.043	1, 70	0.309
	present	-0.001	1, 32	0.966	-0.013	1, 30	0.506
RLD	absent	-0.014	1, 57	0.826	-0.327	1, 57	**< 0.001**
	present	-0.120	1, 57	0.168	0.489	1, 57	**0.022**
SRL	absent	0.077	1, 16	0.374	0.508	1, 49	0.077
	present	-0.009	1, 17	0.918	-0.068	1, 15	0.279
Contrasting drought tolerance							
RB	absent	0.069	1, 110	0.309	-0.067	1, 98	0.434
	present	-0.062	1, 42	0.365	-0.049	1, 33	0.505
WRD	absent	0.006	1, 39	0.788	0.004	1, 33	0.896
	present	-0.015	1, 30	0.551	-0.039	1, 27	0.276
RLD	absent	-0.019	1, 2	0.880	-0.222	1, 2	0.243
	present	-0.099	1, 5	0.658	0.242	1, 12	0.430
SRL	absent	-0.033	1, 16	0.709	-0.085	1, 15	0.229
	present	-0.028	1, 29	0.749	-0.261	1, 28	0.485
Contrasting plant growth rate							
RB	absent	0.054	1, 63	0.402	0.039	1, 45	0.526
	present	0.077	1, 63	0.254	-0.023	1, 128	0.794
WRD	absent	-0.001	1, 51	0.911	-0.001	1, 36	0.729
	present	0.001	1, 43	0.621	-0.001	1, 31	0.635

RLD	absent	-0.098	1, 4	0.512	-0.121	1, 4	0.445
	present	-0.254	1, 33	0.427	0.287	1, 49	0.633
SRL	absent	0.059	1, 19	0.448	-0.048	1, 16	0.417
	present	0.155	1, 38	0.108	-1.212	1, 47	**0.021**
Leaf habit dissimilarity							
RB	Low	0.091	1, 14	0.297	0.033	1, 16	0.658
	Medium	-0.077	1, 38	0.618	0.128	1, 94	0.509
	high	0.002	1, 33	0.989	0.081	1, 40	0.602
WRD	Low	0.004	1, 15	0.821	-0.007	1, 38	0.701
	Medium	0.011	1, 39	0.714	-0.045	1, 64	0.409
	high	0.126	1, 9	**0.009**	0.124	1, 8	**0.008**
RLD	Low	-0.164	1, 42	**0.015**	–	–	–
	Medium	0.192	1, 42	0.521	–	–	–
	high	-0.142	1, 42	0.121	–	–	–
SRL	Low	0.096	1, 6	0.548	-0.046	1, 7	0.596
	Medium	0.114	1, 7	0.641	-0.227	1, 7	0.534
	high	-0.203	1, 6	0.552	0.226	1, 13	0.346

Note: Linear mixed-effects model fit tests used Satterthwaite approximations for denominator degrees of freedom. The interaction effect of leaf habit dissimilarity and AI is unavailable due to the insufficient data. Abbreviations for root traits are the same as in Table S3-1.

Appendix S3-1 List of plant species of relative shade tolerance, relative drought tolerance, relative plant growth rate and leaf habit cited in this meta-analysis

Species name	relative shade tolerance	relative drought tolerance	relative growth rate
Abies alba Mill	tolerant	low	rapid
Abies balsamea	tolerant	low	slow
Acacia auriculiformis	intolerant	intermediate	rapid
Acacia mangium	intolerant	high	intermediate
Acacia mearnsii	tolerant	intermediate	rapid
Acer pseudoplatanus L.	Intolerant	intermediate	intermediate
Acer Rubrum L.	tolerant	low	rapid
Acer saccharum Marsh	tolerant	intermediate	slow
Alnus glutinosa	intolerant	low	rapid
Alnus glutinosa (L.) Gaertn.	Intolerant	high	rapid
Azadirachta indica	Intermediate	intermediate	rapid
Betula alleghaniensis	Intermediate	intermediate	rapid
Betula papyrifera	intolerant	low	rapid
Betula pendula	intolerant	low	rapid
Betula pendula Roth	Intolerant	low	rapid
Carpinus betulus L.	intolerant	intermediate	slow
Castanea henryi	Intermediate	Intermediate	
Castanea sativa Mill	Intolerant	high	intermediate
Castanopsis sclerophylla	Intermediate	low	
Cinnamomum camphora	tolerant	low	slow
Cyclobalanopsis glauca	Intermediate	low	slow
Eucalyptus globulus	intolerant	low	rapid
Eucalyptus grandis	intolerant	low	rapid
Eucommia ulmoides	Intolerant	low	rapid
Fagus sylvatica	tolerant	intermediate	intermediate
Fraxinus excelsior	intolerant	intermediate	rapid
Fraxinus mandshurica	intolerant	low	intermediate
Juglans mandshurica	intolerant	low	
Koelreuteria bipinnata	intolerant	Intermediate	rapid
Larix gmelinii	Intolerant	low	slow
Larix laricina	intolerant	low	rapid
Larix laricina (Duroi) K. Koch	Intolerant	low	rapid
Larix sibirica Ledeb.	intolerant	high	rapid
Liquidambar formosana	Intermediate	low	intermediate
Liquidambar styraciflua L.	intolerant	low	rapid
Lithocarpus glaber	tolerant	low	slow
Nyssa sinensis	intolerant	Intermediate	

Ostrya carpinifolia Scop	Intermediate	high	
Picea abies	intolerant	intermediate	rapid
Picea abies (L.) Karst	intolerant	intermediate	rapid
Picea glauca	Intermediate	high	slow
Picea rubens	tolerant	intermediate	intermediate
Pinus banksiana Lamb	intolerant	low	rapid
Pinus massoniana	Intolerant	high	rapid
Pinus nigra Arnold	Intolerant	intermediate	intermediate
Pinus resinosa	intolerant	low	rapid
Pinus strobus	Intermediate	high	rapid
Pinus strobus L.	Intolerant	high	rapid
Pinus sylvestris L.	Intolerant	intermediate	rapid
Pinus tabuliformis	intolerant	intermediate	intermediate
Pinus taeda L.	intolerant	low	rapid
Platycladus	Intermediate	high	slow
Populus canadensis	intolerant	intermediate	rapid
Populus davidiana	intolerant	high	rapid
Populus tremula	intolerant	low	rapid
Populus tremuloides Michx.	intolerant	low	rapid
Populus trichocarpa	intolerant	low	rapid
Pseudotsuga menziesii	Intermediate	low	intermediate
Pseudotsuga menziesii (Mirb.) Franco	intolerant	low	rapid
Quercus acutissima	tolerant	low	intermediate
Quercus cerris L	Intermediate	high	intermediate
Quercus ilex L	tolerant	high	slow
Quercus liaotungensis	Intermediate	high	slow
Quercus petraea	intolerant	high	intermediate
Quercus petraea (Matt.) Liebl	Intermediate	high	slow
Quercus robur L.	tolerant	intermediate	slow
Quercus rubra	Intermediate	intermediate	intermediate
Quercus serrata	Intermediate	low	
Quercus variabilis	tolerant	low	
Rhus chinensis	intolerant	low	
Robinia Pseudoacacia L.	intolerant	high	rapid
Sapindus saponaria	intolerant	Intermediate	intermediate
Tilia cordata Mill.	Intermediate	intermediate	intermediate
Tilia platyphyllos Scop	Intermediate	low	intermediate
Thuja occidentalis	Intermediate	low	slow
Triadica sebifera	intolerant	low	rapid
Ulmus Pumila L.	intolerant	high	rapid

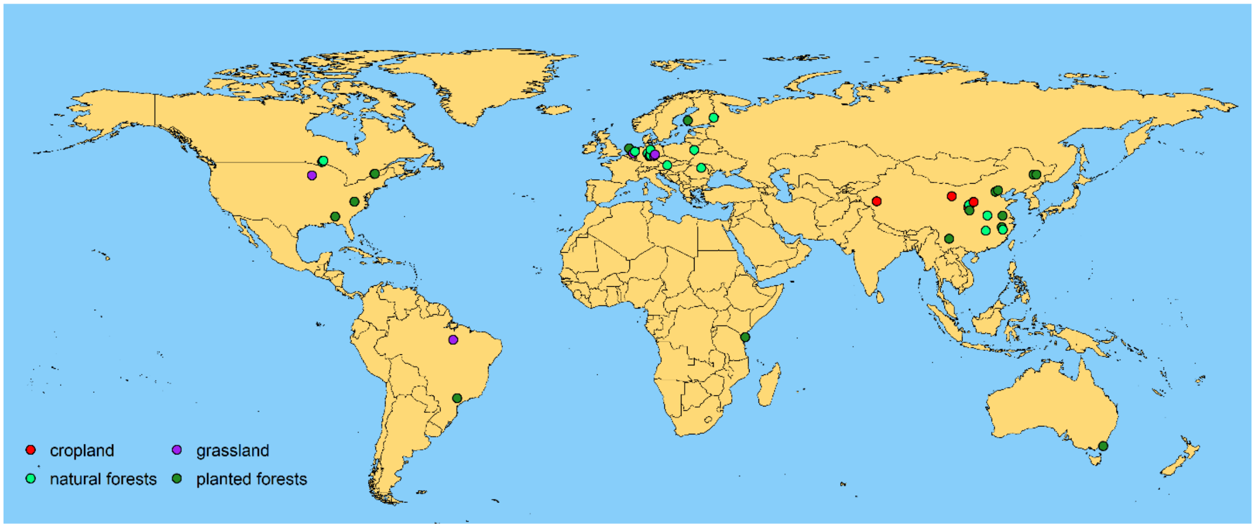

Fig. S3-1 Global distribution of plant diversity experiments focusing on diversity effects on fine-root attributes in this meta-analysis. Experiments conducted in containers were excluded in this figure.

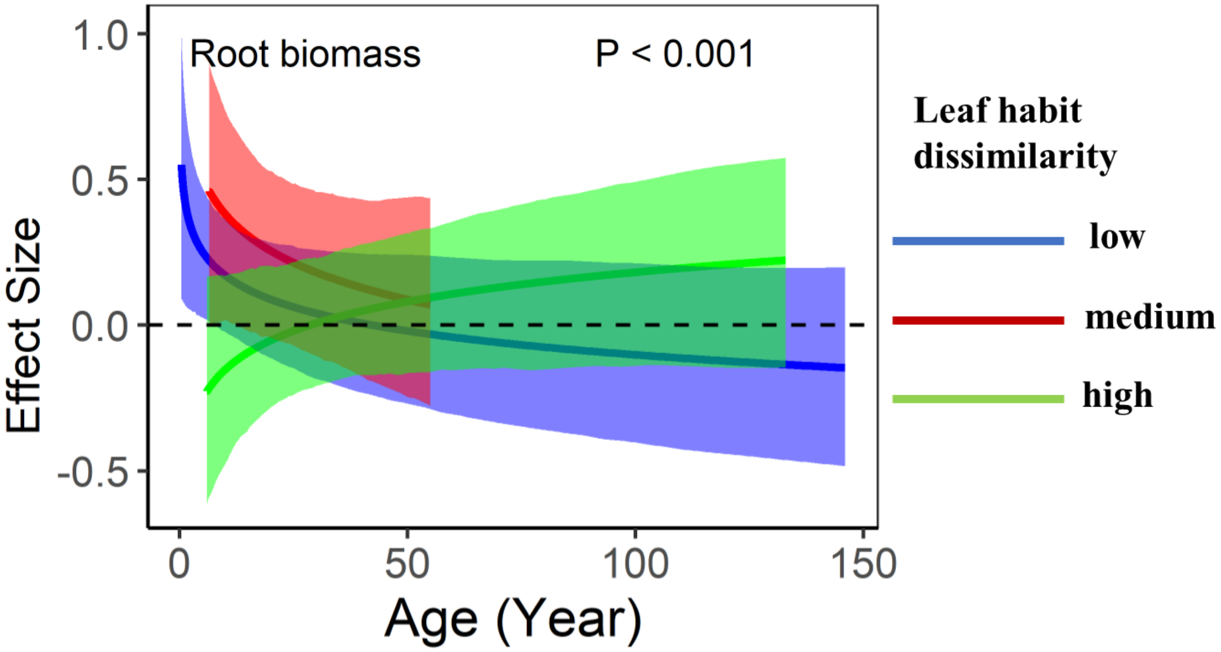

Fig. S3-2 Effect sizes of stand-age effects on fine-root biomass in forest mixtures with different tree leaf habit dissimilarity. The coloured lines represent the specific leaf habit dissimilarity responses, with their bootstrapped 95% confidence intervals shaded.

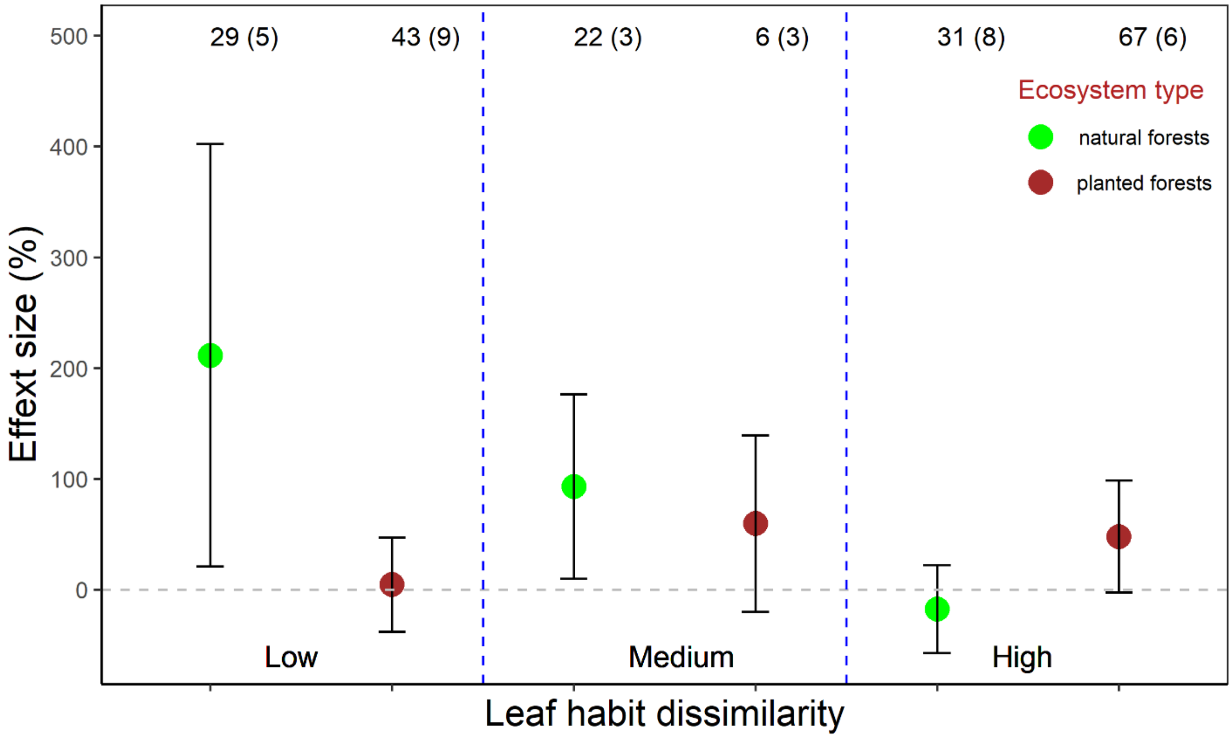

Fig. S3-3 Comparison of mixture effects on fine-root biomass between natural forest mixtures and planted forest mixtures with different tree leaf habit dissimilarity. The number of observations is shown without parentheses, with the number of studies in parentheses.

Appendix references 3-1-Data sources:

Archambault, C., Paquette, A., Messier, C., Khlifa, R., Munson, A.D. & Handa, I.T. (2019). Evergreenness influences fine root growth more than tree diversity in a common garden experiment. *Oecologia*, 189, 1027-1039.

Bauhus, J., Khanna, P.K. and Menden, N. (2000). Aboveground and belowground interactions in mixed plantations of Eucalyptus globulus and Acacia mearnsii. *Can. J. For. Res.*, 30, 1886-1894.

Baxendale, C., Orwin, K.H., Poly, F., Pommier, T. & Bardgett, R.D. (2014). Are plant-soil feedback responses explained by plant traits? *New Phytol.*, 204, 408-423.

Bennett, J.A., Riibak, K., Tamme, R., Lewis, R.J., Pärtel, M. & Semchenko, M. (2016). The reciprocal relationship between competition and intraspecific trait variation. *J. Ecol.*, 104, 1410-1420.

Beyer, F., Hertel, D., Jung, K., Fender, A.-C. & Leuschner, C. (2013a). Competition effects on fine root survival of Fagus sylvatica and Fraxinus excelsior. *Forest Ecol. Manag.*, 302, 14-22.

Beyer, F., Hertel, D. & Leuschner, C. (2013b). Fine root morphological and functional traits in Fagus sylvatica and Fraxinus excelsior saplings as dependent on species, root order and competition. *Plant Soil*, 373, 143-156.

Bolte, A. & Villanueva, I. (2005). 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. Forest Res.*, 125, 15-26.

Brassard, B.W., Chen, H.Y.H., Bergeron, Y. & Pare, D. (2011). Differences in fine root productivity between mixed- and single-species stands. *Funct. Ecol.*, 25, 238-246.

Bu, W., Schmid, B., Liu, X., Li, Y., Härdtle, W., von Oheimb, G. *et al.* (2017). Interspecific and intraspecific variation in specific root length drives aboveground biodiversity effects in young experimental forest stands. *J. Plant Ecol.*, 10, 158-169.

Cesarz, S., Fender, A.-C., Beyer, F., Valtanen, K., Pfeiffer, B., Gansert, D. *et al.* (2013). Roots from beech (Fagus sylvatica L.) and ash (Fraxinus excelsior L.) differentially affect soil microorganisms and carbon dynamics. *Soil Biol. Biochem.*, 61, 23-32.

Corre-Hellou, G. & Crozat, Y. (2005). Assessment of Root System Dynamics of Species Grown in Mixtures under Field Conditions using Herbicide Injection and 15N Natural Abundance Methods: A Case Study with Pea, Barley and Mustard. *Plant Soil*, 276, 177-192.

Dai, Y., Sun, P., Kang, D., Deng, J., Ren, C., Yang, G. *et al.* (2018). Fine root biomass of artificial forests in loess hilly region and its influencing factors (in Chinese with English abstract). *Chin. J. Ecol.*, 37, 2229-2236.

Dimitrakopoulos, P.G. & Schmid, B. (2004). Biodiversity effects increase linearly with biotope space. *Eco. Lett.*, 7, 574-583.

Domisch, T., Finer, L., Dawud, S.M., Vesterdal, L. & Raulund-Rasmussen, K. (2015). Does species richness affect fine root biomass and production in young forest plantations? *Oecologia*, 177, 581-594.

Fang, Y., Xu, B., Liu, L., Gu, Y., Liu, Q., Turner, N.C. *et al.* (2014). Does a mixture of old and modern winter wheat cultivars increase yield and water use efficiency in water-limited environments? *Feild Crop Res.*, 156, 12-21.

Fender, A.-C., Gansert, D., Jungkunst, H.F., Fiedler, S., Beyer, F., Schützenmeister, K. *et al.* (2013). Root-induced tree species effects on the source/sink strength for greenhouse gases (CH4, N2O and CO2) of a temperate deciduous forest soil. *Soil Biol. Biochem.*, 57, 587-597.

Finér, L., Domisch, T., Dawud, S.M., Raulund-Rasmussen, K., Vesterdal, L., Bouriaud, O. *et al.* (2017). Conifer proportion explains fine root biomass more than tree species diversity and site factors in major European forest types. *Forest Ecol. Manag.*, 406, 330-350.

Fredericksen, T.S. & Zedaker, S.M. (1995). Fine root biomass, distribution, and production in young pine-hardwood stands. *New Forests*, 10, 99-110.

Gao, C., Tang, G., Li, K. & Xie, Q. (2013). Root biomass and its distribution of Azadirachta indica and Acacia auriculiformis plantations in the Dry-hot Valley (in Chinese with English abstract). *Acta Ecol. Sinica*, 33, 1964-1972.

Germon, A., Guerrini, I.A., Bordron, B., Bouillet, J.-P., Nouvellon, Y., de Moraes Gonçalves, J.L. *et al.* (2017). Consequences of mixing Acacia mangium and Eucalyptus grandis trees on soil exploration by fine-roots down to a depth of 17 m. *Plant Soil*, 424, 203-220.

Göransson, H., Bambrick, M.T. & Godbold, D.L. (2016). Overyielding of temperate deciduous tree mixtures is maintained under throughfall reduction. *Plant Soil*, 408, 285-298.

Hajek, P., Hertel, D. & Leuschner, C. (2014). Root order- and root age-dependent response of two poplar species to belowground competition. *Plant Soil*, 377, 337-355.

Hendriks, C.M.A. & Bianchi, F.J.J.A. (1995). Root density and root biomass in pure and mixed forest stands of Douglas-fir and beech. *NJAS-Wagen. J. Life Sc.*, 43, 321-331.

Jacob, A., Hertel, D. & Leuschner, C. (2014). Diversity and species identity effects on fine root productivity and turnover in a species-rich temperate broad-leaved forest. *Funct. Plant Biol.*, 41.

Lang'at, J.K., Kirui, B.K., Skov, M.W., Kairo, J.G., Mencuccini, M. & Huxham, M. (2013). Species mixing boosts root yield in mangrove trees. *Oecologia*, 172, 271-278.

Laossi, K.-R., Barot, S., Carvalho, D., Desjardins, T., Lavelle, P., Martins, M. *et al.* (2008). Effects of plant diversity on plant biomass production and soil macrofauna in Amazonian pastures. *Pedobiologia*, 51, 397-407.

Lei, P., Scherer-Lorenzen, M. & Bauhus, J. (2012a). Belowground facilitation and competition in young tree species mixtures. *Forest Ecol. Manag.*, 265, 191-200.

Lei, P., Scherer-Lorenzen, M. & Bauhus, J. (2012b). The effect of tree species diversity on fine-root production in a young temperate forest. *Oecologia*, 169, 1105-1115.

Leuschner, C., Hertel, D., Coners, H. & Buttner, V. (2001). Root competition between beech and oak: a hypothesis. *Oecologia*, 126, 276-284.

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F.A. *et al.* (2006). Root distribution and interactions between intercropped species. *Oecologia*, 147, 280-290.

Liu, C., Guo, X. & Xu, Z. (1985). A prelimnary study on root system of the artificially mixed stand of *Pinus tabuliformis* and *Quercus Varibilis* in Xishan Region, Beijing (in Chinese). *J. Beijing For. Colleg.*, 77-84.

Ma, Z. & Chen, H.Y.H. (2017). Effects of species diversity on fine root productivity increase with stand development and associated mechanisms in a boreal forest. *J. Ecol.*, 105, 237-245.

Meinen, C., Hertel, D. & Leuschner, C. (2009). 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.

Mommer, L., Padilla, F.M., van Ruijven, J., de Caluwe, H., Smit-Tiekstra, A., Berendse, F. *et al.* (2015). Diversity effects on root length production and loss in an experimental grassland community. *Funct. Ecol.*, 29, 1560-1568.

Mueller, K.E., Tilman, D., Fornara, D.A. & Hobbie, S.E. (2013). Root depth distribution and the diversity–productivity relationship in a long-term grassland experiment. *Ecology*, 94, 787-793.

Oram, N.J., Ravenek, J.M., Barry, K.E., Weigelt, A., Chen, H., Gessler, A. *et al.* (2018). Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. *J. Ecol.*, 106, 265-277.

Qin, J. (2006). Eco-physiological effect of *Populus davidiana-Quercus liaotungensis* mixed forest in Ziwuling forest area (in Chinese with English abstract). *Northwest Agricultural and Forestry University*.

Qin, J. (2009). Study on growth dynamics and ecological function of *Robinia pseudoacacia* and *Ulmus pumila* mixed forest in the Loess Plateau (in Chinese with English abstract). *Northwest Agricultural and Forestry University*.

Rivest, D., Paquette, A., Shipley, B., Reich, P.B., Messier, C. & Sayer, E. (2015). Tree communities rapidly alter soil microbial resistance and resilience to drought. *Funct. Ecol.*, 29, 570-578.

Salahuddin, Rewald, B., Razaq, M., Lixue, Y., Li, J., Khan, F. *et al.* (2018). Root order-based traits of Manchurian walnut & larch and their plasticity under interspecific competition. *Scientific Reports*, 8, 9815.

Schmid, I. & Kazda, M. (2002). Root distribution of Norway spruce in monospecific and mixed stands on different soils. *Forest Ecol. Manag.*, 159, 37-47.

Shu, W., Shen, X., Lei, P., Xiang, W., Ouyang, S. & Yan, W. (2018). Temporal changes of fine root overyielding and foraging strategies in planted monoculture and mixed forests. *BMC Ecology*, 18, 9.

Siebenkäs, A. & Roscher, C. (2016). Functional composition rather than species richness determines root characteristics of experimental grasslands grown at different light and nutrient availability. *Plant Soil*, 404, 399-412.

Smith, A.R., Lukac, M., Bambrick, M., Miglietta, F. & Godbold, D.L. (2013). Tree species diversity interacts with elevated CO2 to induce a greater root system response. *Glob. Chang. Biol.*, 19, 217-228.

van Eekeren, N., Bos, M., de Wit, J., Keidel, H. & Bloem, J. (2010). Effect of individual grass species and grass species mixtures on soil quality as related to root biomass and grass yield. *Appl. Soil Ecol.*, 45, 275-283.

Wang, B.J., Zhang, W., Ahanbieke, P., Gan, Y.W., Xu, W.L., Li, L.H. *et al.* (2014). Interspecific interactions alter root length density, root diameter and specific root length in jujube/wheat agroforestry systems. *Agroforest. Syst.*, 88, 835-850.

Wang, Z., Xu, W., Chen, Z., Jia, Z., Huang, J., Wen, Z. *et al.* (2018). Soil Moisture Availability at Early Growth Stages Strongly Affected Root Growth of Bothriochloa ischaemum When Mixed With Lespedeza davurica. *Frontier in Plant Science*, 9, 1050.

Xiang, W., Fan, G., Lei, P., Zeng, Y., Tong, J., Fang, X. *et al.* (2015). Fine root interactions in subtropical mixed forests in China depend on tree species composition. *Plant Soil*, 395, 335-349.

Xie, W., Xu, X., Li, H., Zhang, D. & Tang, H. (1995). Studies on the characteristics of root system in Pine-Eucommia mixed stand (in Chinese with English abstract). *J. Anhui Agric. Univ.*, 22, 256-261.

Xu, B., Deng, X., Zhang, S. & Shan, L. (2010). Seedling biomass partition and water use efficiency of switchgrass and milkvetch in monocultures and mixtures in response to various water availabilities. *Environ. Manag.*, 46, 599-609.

Xu, B., Shan, L., Zhang, S., Deng, X. & Li, F. (2008). Evaluation of switchgrass and sainfoin intercropping under 2:1 row-replacement in semiarid region, northwest China. *African Journal of Biotechnology*, 7, 4056-4067.

Yan, D., Zhang, Z. & Yang, X. (2014). Root Structures and Its Relation with Top Soil Properties of Typical Forest in Southern Mountains in Henan Province. *J. Northeast For. Univ.*, 42, 30-36.

Zhai, M., Jiang, S. & Jia, L. (2002). Fine-root dynamics in mixed plantation of poplar and black locust (in Chinese with English abstract). *J. Beijing For. Univ.*, 24, 39-44.

Zhang, W., Ahanbieke, P., Wang, B., Gan, Y., Li, L., Christie, P. *et al.* (2015). Temporal and spatial distribution of roots as affected by interspecific interactions in a young walnut/wheat alley cropping system in northwest China. *Agroforest. Syst.*, 89, 327-343.

Zhang, Y., Shen, Y., Bai, S. & Wang, Z. (2001). Effects of the mixed on root growth and sidtribution of Fraxinus mandsjurica and Larix gmelinii (in Chinese with English abstract). *Scientia Silvae Simicae*, 37, 16-23.

Zutter, B.R., Mitchell, R.J., Glover, G.R. & Gjerstad, D.H. (1999). Root length and biomass in mixtures of broomsedge with loblolly pine or sweetgum. *Candian Journal of Forest Reaseach*, 29, 926-933.

APPENDIX III: SUPPLEMENTAL INFORMATION FOR CHAPTER 4

Table S4-1 The values of Akaike information criterion (AIC) of models with either plant species richness (R), Shannon diversity index (H) and plant species evenness (J) (see Materials and Methods).

Root attribute	R	H	J
Fine-root biomass	604.72	605.96	**599.80**
Root surface area	607.54	600.06	**591.86**
Fine-root volume	170.25	166.21	**157.79**
Root length density	252.71	243.83	**234.54**
Root tissue density	-24.27	-31.46	**-41.72**
Root mean diameter	-117.68	-122.30	**-134.48**
Length ratio (0-0.5mm diameter)	261.42	253.66	**239.30**
Specific root length	372.90	372.78	**357.29**
Specific root area	479.40	476.40	**457.77**

Note: Numbers in bold indicate lower AIC values.