Plant species diversity alters fine root traits for higher resource uptake capacity

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Abstract

Fine root traits are critical to the plant's capacity and efficiency to uptake water and nutrients. Although plant diversity is decreasing, our understanding of its effects on fine root traits remains elusive. By synthesizing 103 studies, we found that the effects of plant mixtures were highly dependent on species richness in mixtures, stand age, and soil depth. The positive mixture effects on root biomass increased with species richness, soil depth, and mean annual temperature. Plant mixture effects on root length density shifted from negative to positive, from young to older stands, topsoil to deep soils, and warm to cold climates. The mixture effects on specific root length shifted from positive to negative, from two to higher number species mixtures and topsoil to deep soils, and then negative to positive with increasing stand age. Our results demonstrate the profound plasticity of root traits in response to productivity dynamics in plant mixtures.

Introduction

Plant diversity is decreasing due to ongoing land-use intensification (Newbold *et al.* 2015), which has profound negative impacts on a diverse array of ecological functions that are critical for humanity (Cardinale *et al.* 2012). In terrestrial ecosystems, increased aboveground productivity with plant species diversity is accompanied by greater fine root biomass and productivity (Zhang *et al.* 2012; Ma & Chen 2016). This suggests that plant mixtures with increased aboveground productivity require more water and nutrients in contrast to corresponding monocultures. Although high demands for soil resource uptake might be achieved by increased carbon investment to roots, i.e., large fine root biomass (or root mass per unit soil volume) (Ma & Chen 2016), changes in the architectural, morphological, and chemical traits of fine-roots may also augment soil resource uptake (Bardgett *et al.* 2014; Reich 2014). However, the global effects of plant diversity on fine root traits remain uncertain.

Plant mixtures can increase fine root biomass (Ma & Chen 2016) and simultaneously alter the multiple fine root traits that influence resource uptake capacity and efficiency (Table 1). The high demand for soil resources in plant mixtures was observed to decrease biomass allocation to roots in experiments conducted under optimal soil conditions (Bessler *et al.* 2009; Martin-Guay *et al.* 2019), but increased this allocation to roots in natural forests where water and nutrients are limiting (Ma *et al.* 2019). In the soil profiles of plant mixtures, more fine root biomass or length density might be allocated to the organic horizon where soil nutrient contents are highest, and/or deeper soil layers where few roots compete for nutrients or more water is available for plants when drought occurs (Brassard *et al.* 2013; Oram *et al.* 2018). At the individual root level, specific root length (SRL) may increase in plant mixtures (Shu *et al.* 2018) as higher SRL increases

1

resource uptake efficiency for a given unit of biomass investment (Ostonen *et al.*2007). However, other researchers have reported insignificant (Gould *et al.* 2016), or even negative (Salahuddin *et al.* 2018) effects of plant diversity on SRL. These divergent findings might have resulted from multiple mechanisms involved with root trait changes to meet the resource demands associated with high productivity in plant mixtures (Table 1), including the level of species diversity in plant mixtures, resource availability in different soil layers, changes in resource demands associated with plant development, as well as the background environment.

The effects of plant mixtures on plant productivity increase with plant richness in mixtures (Zhang *et al.*2012). Enhanced plant productivity associated with plant richness in mixtures shall increase the demand for water and nutrients, which leads to greater fine root biomass, as well as changes in the traits of fine roots. Higher species diversity is thought to be associated with a higher complementarity effect, including resource partitioning and abiotic facilitation (Barry *et al.*2019). A higher root length density (RLD) for increased resource uptake capacity is found in more diverse plant communities (Gould *et al.* 2016), which facilitates access to water and nutrients by fine roots. Alternatively, the higher resource demands of species-rich communities might be met by changes in root traits toward higher resource uptake efficiency. Therefore, we expected that a higher specific root length (SRL) and root nitrogen content (RN) and thinner root diameter (RD) increase returns (soil nutrients and water) per carbon investment (Fitter *et al.* 1994; Reich 2014) in species mixtures than their averages in corresponding monocultures.

The effects of plant mixtures on fine root traits may change with stand development. Underutilized 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, the increasing interspecific complementarity and decreasing functional redundancy increase the positive effects of plant mixtures on standing biomass and productivity (Cardinale *et al.* 2007; Reich 2012), and thus increase water and nutrient demands. Therefore, we expected that the mixture effects on RLD and SRL would be progressively stronger in mixtures over time, due to elevated resource demands. Alternatively, with stand development, the higher fine-root production in mixtures could 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 increase nutrient availability (Fornara *et al.* 2009). Consequently, the high availability of soil nutrients could counteract the high demand in older stands, resulting in no changes in species mixture effects on fine-root traits with stand development.

The mixture effects on fine-root traits may differ between soil layers. High resource demands in mixtures increase rooting depth to satisfy the requirements of water and nutrients, leading to a greater rooting depth (Oram *et al.* 2018). Meanwhile, the mixture effects on RLD may increase with soil depth for a higher resource capacity (Wang *et al.*2014). However, the positive effects of tree species mixtures on root traits, such as SRL and RD were consistent across soil layers to capture soil resources down to 17 meters in tropical plantations (Germon *et al.* 2017). Conversely, high resource demands in mixtures may lead to more fine roots allocated to the surface soil, since it contains the highest nutrient content and water holding capacity, due to the highest content of organic matter (Jobbágy & Jackson 2001; Makita *et al.* 2010; Brassard *et al.* 2013). Moreover, soil depth-dependent responses to species mixtures may increase with stand age, as the positive effects of species mixtures on fine root biomass in mixtures increase over time (Steinbeiss *et al.* 2008; Ma & Chen 2017). The uncertainty of fine-root attributes associated with soil depth in mixtures hampers the appreciation of fine root resource uptake strategy.

Plant mixture effects may be altered through the background environment. Climatic parameters such as temperature and precipitation are crucial factors on fine-root attributes (Freschet*et al.* 2017); however, it remains unclear how the effects of plant mixtures on root attributes change under variable climates. More positive plant-plant interactions have been reported in colder and dryer sites (Armas *et al.* 2011; Paquette & Messier 2011) as facilitative interspecific interactions tend to increase with the reduced availability of resources, as suggested by the stress gradient hypothesis (Maestre *et al.* 2009; Forrester & Bauhus 2016). This interspecific facilitation might be decreased with mean annual temperature (MAT) and mean annual precipitation (MAP) in plant mixtures due to higher soil resource availability, resulting from faster fine-root decay rates in higher MAT and MAP stands (See *et al.* 2019). Therefore, the high resource demands for

fine roots may be amplified to maintain the facilitation in plant mixtures in colder and dryer sites, which could affect fine-root traits in species-rich plant communities. Moreover, plant diversity effects and their temporal trends between forests and grasslands are expected to be different primarily due to variable species or individual recruitment rates (Forrester & Bauhus 2016). Nevertheless, whether plant diversity effects on fine-root traits diverge between ecosystem types remains unclear.

Here we compiled data from 103 studies to examine the effects of plant mixtures on fine-root traits associated with their resource uptake capacity and efficiency. Specifically, we endeavoured to address the following queries: (1) how do fine roots modify their traits in response to plant mixtures? (2) do the responses change with species richness in mixtures, stand age, and soil depth? and (3) do plant-mixture induced responses of root traits change with variable environmental parameters?

Methods

Data collection

Using Google Scholar and Web of Science, we found the peer-reviewed papers through the combination of several search keywords, including (plant diversity OR species diversity OR plant 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 isolate the effects of plant species diversity from other factors, such as water treatment and the nutrition addition; (2) values of fine-root traits could be extracted directly from the text, tables, and figures; (3) papers that focused merely on the effects of diversity on root biomass were excluded; (4) genotype mixtures with species were not included; (5) 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 R/S, RLD, SRL, RD, and RN. For studies that reported root attributes by root order or diameter class, we calculated the community-level means of these values. The plant species richness in mixtures, stand age for forests, or experimental age for grasslands and croplands, and species proportions in mixtures were recorded from the original publications. If different locations, mixture ratios, or abiotic treatments with independent controls were involved in a given publication, we treated them as distinct comparisons (studies) in that publication. In total, 103 studies with 852 paired observations from 64 publications were selected for this meta-analysis.

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

Ecosystem types were categorized as either container, natural forest, planted forest, grassland, or cropland. 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 (MAT) and precipitation (MAP) (when available) conveyed in the original publications or derived based on the geographical location for each site from the WorldClim version 2 Dataset (Fick & Hijmans 2017).

Data analysis

We calculated the community weighted-mean rooting depth (WRD) to compare the fine-root vertical distribution in mixtures with monocultures. WRD was calculated as:

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

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.

Natural log-transformed response ratio (lnRR) (Hedges *et al.* 1999) was employed as the effect size for fine-root biomass and traits (root attributes hereafter). We calculated lnRR as:

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

where X_t is the observed value in the mixture, and X_c is expected value. As Loreau and Hector (2001) recommended, the expected value X_c was calculated as the weighted mean of the corresponding species in monocultures according to the species proportion in mixtures for all root attributes. For root biomass and RLD, X_t is the sum of each constituent species in mixtures. Since R/S, WRD, SRL, RD, and RN 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. Analysis without the data from these three publications yielded quantitatively similar results. For simplicity and inclusivity, we reported the data from all 64 publications.

As relates to the effect size estimates, we employed the number of replications for weighting (Ma & Chen 2016).

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

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

To ensure the assumption of linearity between each trait and the species richness in mixtures (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 attribute, using equation (4):

$$lnRR = \beta_0 + \beta_1 \times X + \pi_{\text{study}} + \varepsilon \text{(4)}$$

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. We conducted our analysis using the restricted maximum likelihood estimation with the *lme4* package with W_r as the weight for each corresponding observation (Bates *et al.* 2015).

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

$$lnRR = \beta_0 + \beta_1 \times R + \beta_2 \times A + \beta_3 \times D + \beta_4 \times R \times A + \beta_5 \times R \times lnD + \beta_6 \times A \times D + \pi_{\text{study}} + \varepsilon \text{(5)}$$

where β is the coefficient 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 attribute (Table S2). We employed the restricted maximum likelihood estimation with the *lme4* package with W_r as the weight for each corresponding observation (Bates *et al.* 2015). The term D in equation (5) was excluded for R/S and WRD since they are trait variables for the entire ecosystem and entire soil profile, respectively. To prevent overfitting, we derived the most parsimonious model, which was selected using the 'dredge' function of the *MuMln* package (Bartoń 2019).

To elucidate whether the species mixture effects changed with the background environment or ecosystem type, we conducted two types of analysis. First, for those studies conducted in natural habitats, we examined whether lnRR was dependent on MAT and MAP. However, these MAT and MAP effects might be confounded with variations in R, A, and D. Second, we added MAT and MAP and their interactions with the predictors to the most parsimonious models derived from equation 5, from which we then obtained the most parsimonious models to determine whether MAT and MAP accounted for additional variations in lnRR. We also substituted MAT and MAP by ecosystem type and then conducted the same analysis as described above. Moreover, we tested whether the effects of plant mixtures on root attributes associated with MAT and MAP differed between ecosystem types through the linear mixed effect model with 'study' as the random effect.

All continuous predictors including R, A, D, MAP, and MAT were scaled to ease the comparisons for fine-root attributes that had variable R, ln(A), ln(D), MAT, and MAP (observed values minus mean and divided by one standard deviation (Cohen *et al.* 2014). In this way, β_0 is the overall mean lnRR at the mean R, mean A, mean D, mean MAP, and mean MAT for each root attribute.

To facilitate interpretation, lnRR and its corresponding 95% confidence interval was transformed to a percentage change using the equation:

$$\left(e^{\text{lnRR}} - 1\right) \times 100\% \ (6)$$

If the CIs did not cover zero, the mixture effect was significant at $a = 0.05$. Histograms of model residues and the Shapiro-Wilk test were employed to check the normality of all models, bootstrapped estimates were derived when the normality was violated by using the *boot* package (Davison & Hinkley 1997; Canty & Ripley 2012). All analyses were performed in R 3.6.1 (R Core Team 2019).

Results

On average, root biomass was significantly greater in mixtures than the average of corresponding monocultures; however, R/S, WRD, RLD, SRL, MRD, and RN did not vary significantly (Fig. 1). The mixture effects on root biomass increased significantly with species richness in mixtures (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). Plant mixture effects on RLD were not altered, while those on SRL decreased significantly with higher levels of species richness in mixtures (Fig. 2). Both the effects of plant mixtures on RLD and SRL increased with stand age and increased with soil depth for RLD, but decreased for SRL (Fig. 2). The mixture effects on R/S, WRD, MRD, and RN did not change significantly in response to the species richness in mixtures, stand age, or soil depth, as the null models (with intercept only) were those that were most parsimonious (Table S3).

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

The mixture effect on RLD decreased significantly, changing from positive to negative with MAT ($P < 0.001$, Fig. 4a), while, on average, mixture effects on root biomass increased with MAP ($P = 0.008$, Fig. 4b), with more pronounced increases in species-rich mixtures (Fig. 4b, $P < 0.001$). Moreover, plant mixture effect did not differ between different ecosystem types for root attributes except root biomass, with positive effects of species mixtures on root biomass in containers, grasslands, and planted forests, but not in natural forests (Fig. 5, Table S3). The mixture effects on WRD both marginally decreased with MAT and increased with MAP in natural forests, but not in other ecosystems (Figs. S2a, S2b). The negative mixture effects with MAT on RLD was found only in croplands ($P = 0.001$, Fig. S2c). The mixture effects on R/S, SRL, and RN

did not change significantly with the interactive effects between ecosystem type and MAP or MAT (Tables S4, S5).

Discussion

To the best of our knowledge, our meta-analysis has provided the first global evidence that belowground fine-root attributes could be modified to meet elevated resource demands in species-rich plant communities. We demonstrated that species mixtures increased fine root biomass, with more pronounced increases observed in older stands and deeper soil layers. Although on average, plant mixtures did not alter other fine root traits, the effects of species 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 plant mixtures on root length density shifted from positive to negative, mostly in croplands, with increasing mean annual temperature. Plant mixtures had no effects on weighted rooting depth in grasslands or planted forests but had a positive effect under cold and moist climates, and a negative effect under warm and dry climates in natural forests.

Unsurprisingly, we found that species mixtures, on average, increased fine root biomass, and the positive mixture effects increased with species richness and soil depth with more pronounced species richness effects in both older stands and deeper soil layers. Our results extended the aboveground overyielding to belowground (Cardinale *et al.* 2007; Zhang *et al.* 2012; Liang *et al.* 2016), particularly, the pronounced diversity effects in more species-rich and older stands and in deep soils (Ma & Chen 2016). This result supported the notion that complementary effects increase with species richness in mixtures (Barry *et al.* 2019), and functional redundancy decreases with stand age, while interspecific facilitation increases with soil depths (Jobbágy & Jackson 2001; Makita *et al.* 2010; Reich 2012; Forrester & Bauhus 2016). This finding suggests elevated water and nutrient demands in species-rich and old mixtures, leading to deeper soil exploration. Moreover, the mixture effects on root biomass were significantly different between ecosystem types, which might have resulted from differences in the average species richness of mixtures between ecosystem types (Table S3).

However, contrary to the prediction of the stress gradient hypothesis, we found that the mixture effects on root biomass increased with mean annual precipitation, particularly in species-rich mixtures. Although interspecific facilitation may be enhanced through resource limitations (Maestre *et al.* 2009; Forrester & Bauhus 2016), it is possible that increased water and nutrient availability augmented niche differentiation, which consequently leads to stronger diversity effects on productivity (Searle & Chen 2019). Further, in dry climates, species mixtures can increase soil moisture content, which alleviates heightened water requirements for roots (Lange *et al.* 2014). The enhanced mixture effects on root biomass with water availability in more diverse communities might be attributable to stronger positive resource partitioning in species-rich mixtures (Barry *et al.* 2019).

On average, 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, did not differ between species mixtures and the mean of corresponding monocultures. The lack of a mixture effect on the root:shoot ratio suggested that fine root overyielding was of the same magnitude as its aboveground counterpart on a global scale. Furthermore, the variations of mixture effects on root biomass were synchronous with those of root length density and specific root length (Fig. S2). The neutral mixture effects may have been attributable to the fact that the majority of the original studies consisted of two species mixtures with short experimental durations, in which mixture effects are expected to be minimal due to limited interspecific interactions between individual plants (Lei *et al.* 2012; Beyer *et al.* 2013; Siebenkäs & Roscher 2016). Nevertheless, the mixture effects on several functional traits were highly dependant on the species richness in mixtures, stand age, soil depth, or environmental stress.

We found that the effects of species mixtures on root length density shifted from negative to positive from young to old stands, topsoil to deep soils, and warm to cold climates. Firstly, the increasing plant mixture

effects on root length density with stand age were anticipated, since diversity effects should facilitate fine roots to have a high resource uptake capacity to satisfy the elevated water and nutrient demands (Cardinale *et al.* 2007; Zhang *et al.* 2012), which could be achieved by the higher horizontal soil volume utilization of fine roots in older stands (Brassard *et al.*2013; Ma & Chen 2017). Secondly, increased root length density with soil depth implied that fine roots penetrated deeper into the soil to uptake additional soil resources to compensate for the overyielding of plant mixtures (Zhang *et al.* 2012; Ma & Chen 2017; Oram *et al.* 2018). Lastly, in colder climates where fine roots are likely to face lower resource availability due to the slower fine root decay rate (See *et al.* 2019), the increased interspecific facilitation in mixtures (Forrester & Bauhus 2016) might increase root length density for an improved resource capacity. The shifted mixture effects on root length density suggested that the intense competition for resources overridden interspecific facilitation in young stands, shallow soil, and colder sites.

We also found that the mixture effects on specific root length shifted from positive to negative from two to higher numbers of species in mixtures, topsoil to deep soils, and from negative to positive with increasing stand age. Firstly, the decreased specific root length with the species richness in mixtures implied that fine roots reduced resource uptake efficiency in more diverse communities (Ostonen *et al.* 2007), which might have resulted from more intense resource competition. A recent meta-analysis demonstrated that the soil organic carbon content exhibited small variations with increasing species richness globally (Chen *et al.* 2019), which suggested a stable soil nutrient pool regardless of species richness. Therefore, increased soil nutrient competition leads to a decreased specific root length for a more conservative strategy with lower carbon costs (Reich 2014). Secondly, in alignment with the notion that interspecific facilitation increases with soil depth (Forrester & Bauhus 2016), we found that the mixture effect on specific root length decreased in deeper soils for lower resource availability. To support aboveground progressive overyielding with species richness (Liang *et al.* 2016), the high diversity effects on specific root length in surface soil might be a compensating strategy of resource uptake in this root-rich soil layer (Yuan & Chen 2010). Lastly, the increased mixture effects on specific root length with stand age were attributable to elevated water and nutrient demands in older stands, which resulted in a high resource uptake efficiency. Moreover, we found that the negative diversity effects on specific root length shifted to neutral in older stands (> 5 years, Fig. 3b). Due to the positive mixture effects on soil organic carbon content with stand age (> 5 years) (Chen *et al.* 2019), the intense competition for nutrients in more diverse communities might be counteracted in older stands.

We found that plant mixture effects on root attributes were highly dependant on the species richness in mixtures, stand age, soil depth, or environmental stress. To address the high water and nutrient demands in the support of greater aboveground productivity in plant species mixtures, fine roots increased the root biomass and/or root length density, but decreased the specific root length, in relation to both the species richness in mixtures and soil depth. We also found that the plant mixture effects on root biomass, root length density, and specific root length increased with stand development. Across global climatic variations, the mixture effects on root biomass increased with the mean annual precipitation, and the increased trends were more pronounced in more diverse plant communities, while the mixture effects 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 examining mixture effects on root attributes. Because of the dominant role of fine roots in soil resource exploration, our results suggest that increased fine root biomass with shifts in fine root traits enhanced soil resource uptake to support high primary production in mixtures.

Table 1 Fine-root traits and resource uptake strategies.

Trait	Definition and comments	Resource uptake strategy*	Mixture effects	Mixture effects	Mixture effects
			positive	negative	null
Root system					

7

Root/shoot (R/S)	Root biomass divided by shoot biomass, the strategy for plants to adjust the investment between above- and below-ground with changing in the environment and life stage (Bessler *et al.* 2009; Ma *et al.* 2019).	Capacity	(Baxendale *et al.* 2014)	(Bessler *et al.* 2009)	(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	(Oram *et al.* 2018)	(Shu *et al.* 2018)	(Siebenkäs & Roscher 2016)
Root length density (RLD, m m^{-3})	Total fine root length per unit soil volume, which reflects the ability of species to compete for resources (Guderle *et al.* 2018).	Capacity	(Gould *et al.* 2016)		(Bauhus 2000; Siebenkäs & Roscher 2016)
Morphology					
Specific root length (SRL, m g^{-1})	Root length per root dry mass, absorptive capability 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)

8

Mean root diameter (RD, mm)	Mean diameter of fine root (< 2 mm) components. Thicker RD with a slow root growth rate facilitates root penetration of denser soil; roots with thinner RD reduces dependence on mycorrhizal symbiosis (Ma *et al.* 2018).	Efficiency	(Salahuddin *et al.* 2018)	(Gould *et al.* 2016)	(Beyer *et al.* 2013)
Chemistry					
Root N 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 *et al.* 2014).	Efficiency	(Bauhus 2000)	(Hajek *et al.* 2014; Salahuddin *et al.* 2018)	(Callaway *et al.* 2003)

*Fine roots alter root traits to meet high resource demands by augmenting soil exploration **capacity** (high RLD and R/S, deep WRD) and/or **efficiency** (high SRL and RN, thin MRD).
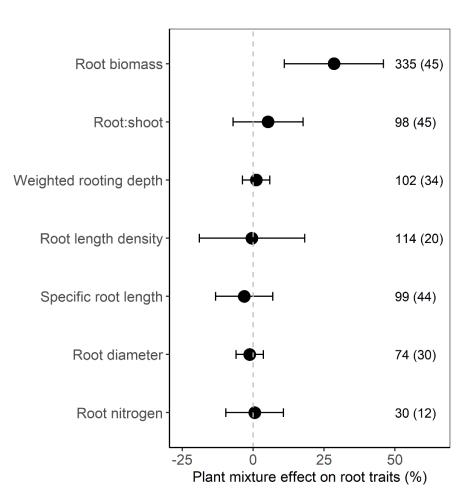
9

Figure captions

Fig. 1 Comparison of fine-root attributes in plant mixtures and monocultures at the community level. Values (estimated β 0 in Equation (5)) are mean ± 95% confidence intervals of the percentage effects between plant mixtures and monocultures. The number of observations is shown beside each attribute without parentheses, with the number of studies in parentheses.
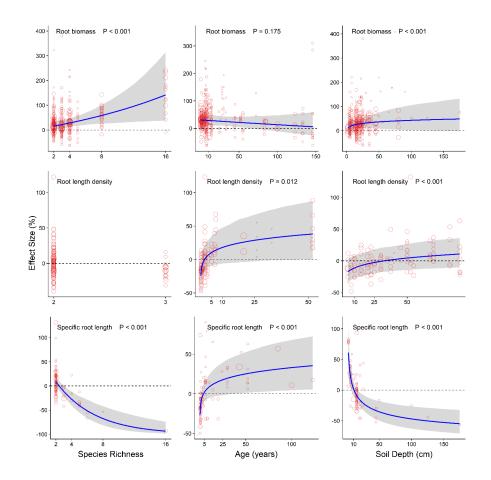
Fig. 2. Plant mixture effects on fine-root attributes in relation to species richness in the mixtures, stand age, and soil depth at the community level. Plant mixture effects on (A) fine-root biomass (root biomass), (B) root length density (RLD) and (C) specific root length (SRL) in relation to the species richness in mixtures, stand age, and soil depth. The blue lines for each attribute were estimated β 1, β 2, and β 3 in Equation (5), respectively, with bootstrapped 95% confidence intervals shaded in gray. The sizes of red circles represent the relative weights of corresponding observations

Fig. 3 Temporal and spatial trends of root biomass (root biomass) and specific root length (SRL) associated with plant species richness (SR) in mixtures. Temporal trends of the effect size of root biomass and SRL corresponding to SR in mixtures (a, b) and spatial trends of the effect size of root biomass and SRL corresponding to SR in mixtures (c, d). Coloured and black lines refer to the specific age or soil depth responses and their average responses, respectively, with their bootstrapped 95% confidence intervals shaded in gray. *P* values are the interactive terms tested.

Fig. 4 Plant mixture effects on root length density (RLD) and root biomass (RB) in relation to MAT and MAP, respectively. The effect sizes of (a) RLD between species mixtures and monocultures in relation to mean annual temperature (MAT). The effect sizes of plant mixtures on (b) root biomass with MAP in relation to species richness. In Fig. 4a, the sizes of the red circles represent the relative weights of corresponding observations, whereas the blue lines are the estimated coefficient, with bootstrapped 95% confidence intervals shaded in gray. In Fig. 4b, the coloured and black lines represent specific species richness and their average responses, respectively, with their bootstrapped 95% confidence intervals shaded in gray

Fig. 5 Plant mixture effects on ecosystem types with (a) root biomass, (b) root/shoot ratio, (c) community-weighted mean rooting depth, (d) root length density, (e) specific root length, (f) mean root diameter, (g) root nitrogen between ecosystem types. Values are mean ± 95% confidence intervals of the percentage effects between the plant mixtures and monocultures. The numbers of observations are shown beside each attribute without parentheses, with the number of studies in parentheses
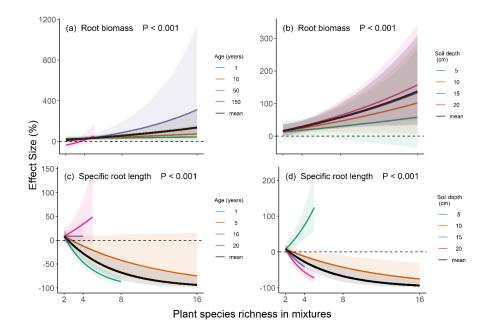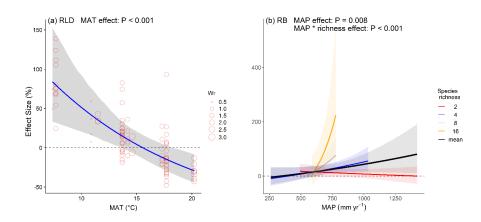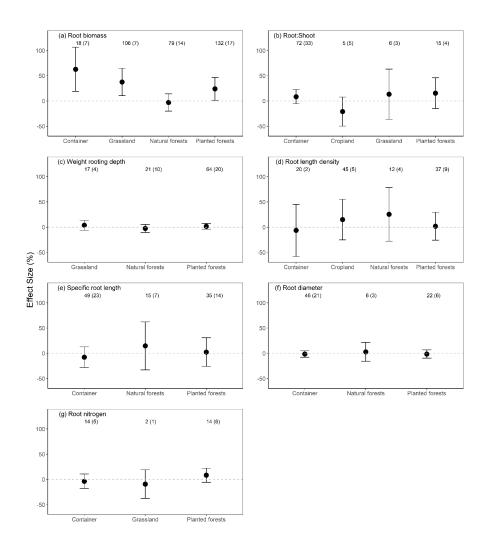
Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

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Conflict of interest

The authors declare no competing interests.