Forest Ecology and Management 455 (2020) 117664

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Effects of long-term nitrogen addition and decreased precipitation on the fine root morphology and anatomy of the main tree species in a temperate forest

Xin Zhang[a], Yajuan Xing[a], Qinggui Wang[a,*], Guoyong Yan[a], Miao Wang[b], Guancheng Liu[a], Honglin Wang[a], Binbin Huang[a], Junhui Zhang[c,*]

[a] Heilongjiang Provincial Key Laboratory of Ecological Restoration and Resource Utilization for Cold Region, College of Agricultural Resource and Environment, Heilongjiang University, 74 Xuefu Road, Harbin 150080, China

[b] College of William and Mary, 116 Jamestown Road, Williamsburg, VA 23185, USA

[c] Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang 110016, China

ARTICLE INFO

Keywords:
N addition
Decreased precipitation
Fine root order
Root morphology
Root anatomy

ABSTRACT

Fine root traits vary under increased nitrogen (N) deposition and decreased precipitation across root orders and species, and these differences reflect plant belowground carbon allocation and survival strategies. We aimed to investigate how fine roots respond to N deposition and precipitation changes and provide a database of related information. A long-term platform to explore the changes in fine root morphology and anatomy in response to N addition and decreased precipitation was established in a temperate forest in northern China. The response of *Pinus koraiensis* (PK) fine root diameter, stele diameter and stele-to-root ratio to N deposition differed from that of broadleaf species. Fine roots coordinated nutrient absorption with transportation by changing carbon allocation. Decreased precipitation caused *Phellodendron amurense* (PA) and *Tilia amurensis* (TA) to produce thinner fine roots with fast absorption capacity and PK and *Fraxinus mandshurica* (FM) to produce thicker fine roots with slow absorption capacity. The total absorptive capacity of the fine roots of *Quercus mongolica* (QM) had the smallest change. The interaction of N deposition with decreased precipitation on fine roots depends on tree species and may be inhibited or synergistic. This study provides insight into the variations in fine root dynamics among five tree species that are associated with environmental conditions and forecasts the trend of belowground carbon storage in boreal forests under multifactor climate change.

1. Introduction

In temperate forests, nitrogen (N) is often an important factor limiting plant growth, and precipitation directly affects soil moisture; both N and precipitation may collectively drive global climate change and thereby affect plant growth (De Marco et al., 2014; Valliere and Allen, 2016). Fine roots, with a diameter of less than 2 mm (Helmisaari et al., 2002), play an important role in plant responses to N deposition and decreased precipitation. Fine root branching structures are complex, and many studies have highlighted significant differences in fine root traits in the root branch hierarchy based on root order classification (Pregitzer et al., 2002; McCormack et al., 2015; Iversen et al., 2017). There are two different classes of fine roots, including absorption fine roots at the first two or three (most distal) root orders and transport fine roots at higher root orders (Pregitzer et al., 2002; Guo et al., 2008; McCormack et al., 2015); these classes differ in anatomy and physiology. For example, root traits influencing plant performance (e.g., mycorrhizal colonization, root life span) can vary strongly and nonlinearly with increasing root order, reflecting a shift in function from resource absorption to transport (Pregitzer et al., 2002; McCormack et al., 2015). It is important to explore how the fine roots of each order respond to climate change.

Fine roots have shown diverse plasticity in absorbing soil resources (Joslin et al., 2000; Zhong et al., 2016). Nitrogen deposition and decreased precipitation may increase, decrease or not affect fine root biomass. Nitrogen deposition may decrease the total length and total surface area of fine roots (Herzog et al., 2014), while decreased precipitation may elongate fine roots and decrease their total surface area, which helps fine roots to obtain more resources (Wang et al., 2018). The effects of N deposition and decreased precipitation on root

* Corresponding authors.
E-mail addresses: qgwang1970@163.com (Q. Wang), 676335994@qq.com (J. Zhang).

https://doi.org/10.1016/j.foreco.2019.117664
Received 17 June 2019; Received in revised form 1 October 2019; Accepted 1 October 2019
Available online 12 November 2019

diameter are also diverse; they may not have any effect on fine root diameter (Lü and Tian, 2007) or may increase or decrease fine root diameter, improving nutrient uptake and transport rates (Zhang et al., 2000). Although fine roots have similar diameters, those with a higher proportion of cortex or that are slightly thicker can have an improved absorption capacity due to more opportunities for mycorrhizal colonization (Brundrett, 2002; Valverde-Barrantes et al., 2016); the cortex constitutes a dominant component of the hydraulic resistance of water transportation in absorptive roots (Steudle and Peterson, 1998). Kong et al. (2014) demonstrated that the allometric growth of the cortex and stele could reflect the relative importance of woody plant resource absorption and transport. Many studies have explored the response of fine root diameter and anatomy to N addition and decreased precipitation across tree species, which is helpful for understanding the mechanisms of plant evolution and responses to climate change.

In temperate forests, nitrogen is often an important factor limiting plant growth, and precipitation directly affects soil moisture (De Marco et al., 2014; Valliere and Allen, 2016). These two factors may have potential complex interactions with ecosystems that collectively drive global climate change and thereby affect plant growth (Valliere and Allen, 2016). In most previous studies, the time needed for N fertilization and precipitation to decrease simulation was relatively short (Poorter and Nagel, 2000; Fusaro et al., 2016; Wang et al., 2018), and due to the complexity of the structure of fine roots and the regulation of hidden belowground growth by multiple environmental factors, including soil moisture, temperature, and pH, there are insufficient data on fine root traits, which prevents scientists from determining the dominant dimension of the plant growth strategy (Wang et al., 2006a,b).

In this study, fine roots were divided according to the root order method (Vogt et al., 1996; McCormack et al., 2015). To explore the long-term effects of N deposition, decreased precipitation and their interaction on fine root morphology and anatomy, we also provide supplemental information to predict the dynamics of belowground C pools under global climate change. We proposed the following hypotheses: (1) fine roots in different orders had different sensitivities to N deposition and decreased precipitation; (2) the survival strategies adopted by fine roots to resist environmental stress varied among species; and (3) nitrogen deposition and decreased precipitation might have had synergistic or antagonistic effects on fine root traits.

2. Materials and methods

2.1. Study site

The experimental field was established in the Changbai Mountains Natural Reserve in Jilin Province, northeastern China (42°24′ N, 128°06′ E, 738 m a.s.l.). The climate is a temperate continental climate affected by the summer monsoon and is dry and windy in spring, short and warm in summer, and long and cold in winter. The climatic data collected at the Changbai Mountains Forest Ecosystem Research Station from 1982 to 2003 showed that the annual average precipitation was approximately 790 mm and that the precipitation was concentrated between May and August. Over the past 50 years, precipitation in North China has decreased, and N deposition has increased significantly (Wang et al., 2006a,b). The vegetation type is a broadleaved Korean pine mixed forest that is over 300 years old. The dominant species are *Pinus koraiensis* (PK), *Phellodendron amurense* (PA), *Quercus mongolica* (QM), *Tilia amurensis* (TA) and *Fraxinus mandshurica* (FM).

2.2. Experimental design

To avoid the influence of hydrology, six 50 × 50 m plots were randomly plotted, surrounded by a > 20 m wide buffer strip, under the same site conditions (slope < 5°). Three of the squares were treated for precipitation reduction (Fig. S1), and the other three were not treated with regard to precipitation. The precipitation transfer apparatus consisted of a highly translucent (95%) polycarbonate V-type translucent plate. To ensure air flow, the translucent plate was fixed to an aluminum frame approximately 1 m from the soil surface. According to Chinese Ecosystem Research Net (CERN) data, in the dry years of 1985, 1997, 1999, 2001 and 2003, the annual precipitation was reduced by approximately 200 mm in comparison to the average recorded over 30 years. The long-term average precipitation decreased by nearly 30% (Wang et al., 2006a,b). Thus, during the growing season, the polycarbonate sheet intercepted approximately 30% of the natural rainfall. In the winter, we removed the polycarbonate sheet and allowed snow to fall naturally on the forest floor. The six squares were separated into two 25 × 50 m subgroups by stainless steel plates, and the stainless-steel plates were inserted 50 cm deep into the soil to prevent the transfer of soil nutrients between adjacent samples. One of the subgroups was subjected to treatment with N addition, and the other was not. Lü and Tian (2007) noted that by 2020, the N deposition rate in the Changbai Mountains will be double than in 2005. The experiment began in May 2009, the amount of N applied in the N addition group was 50 kg N hm^{-2}·a^{-1}, and NH_4NO_3 was selected as the additional N source for simulating N deposition. The study included four treatments, which were control (CK), nitrogen application (+N, +50 kg N·hm^{-2}·a^{-1}), decreased precipitation (−W, −30%, approximately 200 mm·a^{-1} reduction), and interaction of N addition with decreased precipitation (+N−W), and there were 3 replicates per treatment.

2.3. Samples and methods

In the growing season in July 2017, three similarly sized trees of each species (FM, PK, PA, TA, and QM) were randomly selected from each subplot. Around each tree at a depth of 20 cm, 3 points were randomly selected within a diameter of 3 m from the center of the trunk, and the plant roots were excavated using a tablet. The samples from the three points were mixed and randomly placed into two self-sealing pouches, one for root morphology analysis and the other for root anatomy analysis. Because the roots were collected from around each tree species in each plot, it was easy to identify the roots of each species based on branching pattern, color, and texture of the root bark or epidermis. The fine roots of PK, QM and TA were dark brown and black, and the infection of PA fine roots was obvious. The PA fine roots were yellow. The FM yellow fine roots were the most numerous and had the greatest biomass, followed by white roots, and the FM brown shriveled roots have the lowest biomass. The 1st-order roots of FM accounted for 80%-90% of the fine roots of the 1st–5th orders; the branching ratio of the 5th–2nd-order roots was 1:3, while the branching ratio of the 2nd-order roots was 1:10–12 (Wang et al., 2006a,b). After being refrigerated and transported back to the laboratory in an ice box, the root system was immediately washed with running water and placed completely in formalin-acetic acid-alcohol (FAA). Then, it was labeled and placed in a refrigerator at 4 °C.

2.4. Root biomass

Three points were randomly selected in each plot, and 10 cm × 10 cm × 10 cm clods were placed in a self-sealing bag and labeled. After the clods were placed in liquid nitrogen, refrigerated and transported back to the laboratory, they were rinsed with running water. The roots of grasses and shrubs were removed, and as were dead roots. Then, we placed the fine roots in a constant-temperature oven at 65 °C, dried them to a constant weight (48 h), and then weighed them by analytical balance.

2.5. Root classification

The root system was divided as proposed by Fitter and Stickland

(1992). The roots located at the end of the root system and without branches were called 1st-order roots, and the 1st-order roots were attached to the 2nd-order roots. In this study, 150 to 200 1st–3rd-order roots and 20 to 30 4th–5th-order roots were used for root morphology analysis, and 20 1st–5th-order roots were used for anatomical structure analysis. The roots in each order were placed in a vial containing 70% alcohol and then labeled and kept for later use.

2.6. Root morphology

The fine roots from each treatment allocated for use for morphological analysis were removed from the preservation solution, immediately placed on a scanner (CanoScan LiDE 120, DPI = 2400, Canon, Shanghai, China), and imaged. We used WinRHIZO TronMF 2012 software to analyze and process the images to record the length, surface area and diameter of each fine root.

2.7. Root anatomy

The fine roots from each treatment used for the anatomy analysis were removed from the preservation solution. We used paraffin sectioning technology to dehydrate each root of each tree in 70%, 85%, 95% and 100% alcohol and then passed the roots through transparent xylene. Roots prepared in this manner were then randomly selected and embedded in paraffin. From these embedded samples, the 1st–3rd-order fine roots of five tree species were cut into sections 8 μm thick with a microtome, and the 4th–5th-order fine roots were cut into sections 10 μm thick. The prepared slides were then dyed, dewaxed, mounted, and observed with an Olympus BX-51 microscope (Olympus Electronics Inc., Tsukuba, Japan). Photographs were taken with a Motic 3000 CCD digital imaging system. Cortical thickness, root diameter and stele diameter were determined using WinRHIZO TronMF 2012 software (Regent Instrument Inc., Quebec, Canada). Finally, we calculated the ratio of stele diameter to root diameter.

2.8. Data analysis

First, we used the Kolmogorov-Smirnov test to detect whether the data conformed to the normal distribution, and data homogeneity was detected with Levene's test. Second, a general linear model multivariate analysis of variance was used to evaluate the effects of tree species, root order and their interactions on the fine root length, surface area, diameter, cortical thickness, stele diameter and stele-to-diameter ratio. Then, two-way ANOVA was used to analyze the effects of N application, decreased precipitation and their interaction on the root morphology and anatomy of the 1st–5th-order fine roots of each tree species, and multiple comparisons were performed using the LSD method. All data processing was performed using the SPSS 17.0 (SPSS, IBM, USA) data processing software.

3. Results

3.1. Effects of N addition on root morphology and anatomy

Tree species, root order and treatment affected the response of fine root morphology and anatomy to N addition (Table 2). Fine root biomass responded positively to N addition. As shown in Fig. 2, N addition reduced the length of the 1st–2nd-order roots of all tree species except TA. Although the length of the 1st-order roots of FM did not decrease significantly, the length of the 3rd-order roots of TM did (−43%). In this study, the effects of N addition on root diameter showed heterogeneity across species and orders. PA, FM, QM and TA produced thicker absorptive roots (1st-order root) under N addition, while PK produced thinner absorptive roots (Fig. 2).

Nitrogen addition may have promoted the nutrient absorption of the lower order fine roots of PK and FM, as the cortical thickness of the 3rd-order roots decreased (Fig. 3) but that of the 5th-order roots increased (Table 1). Nitrogen addition may have also increased the hydraulic resistance of lower order roots of PA and TA to nutrient uptake, as the cortical thickness of the 2nd-order roots of PA and the 1st–2nd-order roots of TA significantly increased (Fig. 3). However, the stele diameter and the stele-to-diameter ratio of the 1st–2nd-order roots of PA and TA decreased. The nutrient transport capacity of these roots was limited, but those of the 2nd–3rd-order roots of PK, whose nutrient transport capacity improved (Fig. 3), increased. Nitrogen addition decreased the positive correlation between the surface area and length of the fine roots of PK and TA but increased the positive correlation between their surface area and diameter (Table 3).

3.2. Effects of decreased precipitation on root morphology and anatomy

Tree species, root order, and treatment all contributed to the responses of fine root morphology and anatomy to decreased precipitation (Table 2). The length of the fine roots in each order showed different responses to decreased precipitation (Fig. 2). Decreased precipitation decreased the length of the 1st–2nd-order fine roots of all tree species except for the 1st-order fine roots of TA and FM, whose length increased. The effect of decreased precipitation on the diameter of fine roots varied among species. The diameter of the 1st–5th-order roots of PK and the 1st–3rd-order roots of FM increased significantly. The diameter of the 1st-order roots of QM increased by 11%, while that of the 3rd-order roots decreased by 8%. The 2nd–5th-order roots of TA and that of all fine roots of PA were significantly reduced (Fig. 2, Table 1).

The decrease in precipitation resulted in diverse functional adaptation strategies of the fine roots. Decreased precipitation directly promoted absorption and limited transport for the 3rd-order roots of PK, PA, and FM, which showed a decrease in both the cortical thickness and stele diameter (Fig. 3). However, the cortical thickness of the higher order roots increased, which limited nutrient absorption. The stele diameter of the 2nd–5th-order roots of PK and the 4th–5th-order roots of TA decreased, while that of the 4th–5th-order roots of PA and the 5th-order roots of FM increased (Fig. 3, Table 1). The cortical thickness and stele diameter of the 1st–3rd-order fine roots of QM increased significantly (Fig. 3).

3.3. Effects of N addition and decreased precipitation on root morphology and anatomy

Fine root biomass responded strongly to the interaction of N addition with decreased precipitation, showing the synergistic effect of increasing N and decreasing precipitation (Fig. 1). The response of fine root morphology and anatomy to the interaction of N addition with decreased precipitation was influenced by tree species and root order (Table 2). The interaction of N addition with decreased precipitation decreased the length, surface area and diameter of the 1st–2nd-order roots of PK and the 1st–3rd-order roots of PA (Fig. 2, Table 1), and it increased the diameter of the 1st–2nd-order roots of QM but decreased the diameter of the 3rd–5th-order roots of QM. The diameter of the 1st–5th-order roots of FM increased significantly (Fig. 2, Table 1).

The nutrient absorption capacity of the 1st–3rd-order roots of PA and FM improved, and the nutrient transport capacity of the 4th–5th-order roots was enhanced. The cortical thickness of the 1st–3rd-order roots of PA decreased, and the stele diameter of the 3rd–5th-order roots increased. The cortical thickness of the 1st–2nd-order roots of FM decreased, and the stele diameter of the 4th–5th-order roots increased (Fig. 3, Table 1). The function of the lower order roots of TA was limited, increasing nutrients only by enhancing nutrient uptake in 5th-order roots, which reduced the cortical thickness (Fig. 3, Table 1).

Table 1
The effect of N addition and decreased precipitation on the diameter, cortical thickness, stele diameter, stele-to-diameter ratio of 4th–5th-order fine roots of five temperate tree species.

Traits	Root order species	4th-order				5th-order			
		CK	+N	−W	+N−W	CK	+N	−W	+N−W
Diameter	*PK*	0.212^{c}	0.234^{bc}	0.458^{a}	0.241^{b}	0.299^{b}	0.391^{b}	0.507^{a}	0.337^{b}
	PA	0.445^{a}	0.447^{a}	0.224^{c}	0.358^{b}	0.895^{a}	0.841^{a}	0.565^{b}	0.883^{a}
	QM	0.365^{a}	0.229^{b}	0.353^{a}	0.224^{b}	0.589^{a}	0.578^{a}	0.711^{a}	0.425^{b}
	TA	0.256^{c}	0.343^{b}	0.386^{a}	0.307^{b}	0.825^{b}	0.782^{c}	0.979^{a}	0.440^{c}
	FM	0.233^{c}	0.323^{b}	0.192^{d}	0.384^{a}	0.431^{b}	0.546^{b}	0.262^{c}	0.561^{a}
Cortex thickness	*PK*	0.148^{b}	0.152^{b}	0.21^{a}	0.18^{ab}	0.182^{c}	0.226^{b}	0.238^{b}	0.382^{a}
	PA	0.221^{b}	0.336^{a}	0.348^{a}	0.217^{b}	0.581^{a}	0.568^{a}	0.672^{a}	0.934^{a}
	QM	0.086^{bc}	0.167^{a}	0.111^{b}	0.043^{c}	0.364^{a}	0.116^{c}	0.191^{b}	0.112^{c}
	TA	0.247^{a}	0.173^{ab}	0.122^{b}	0.206^{a}	0.341^{a}	0.411^{a}	0.277^{b}	0.171^{b}
	FM	0.256^{a}	0.256^{a}	0.315^{a}	0.171^{b}	0.182^{d}	0.435^{a}	0.307^{b}	0.271^{c}
Stele diameter	*PK*	0.533^{c}	0.884^{a}	0.330^{d}	0.741^{b}	0.970^{c}	1.535^{b}	0.742^{d}	1.839^{a}
	PA	0.238^{d}	0.393^{c}	0.736^{a}	0.555^{b}	1.298^{b}	0.884^{c}	1.678^{a}	1.756^{a}
	QM	0.473^{a}	0.613^{a}	0.549^{a}	0.199^{b}	1.034^{a}	1.014^{a}	1.076^{a}	0.938^{a}
	TA	0.840^{a}	0.430^{c}	0.641^{b}	0.895^{a}	1.413^{a}	1.356^{ab}	1.074^{b}	1.036^{b}
	FM	0.375^{b}	0.723^{a}	0.504^{b}	0.662^{a}	1.183^{b}	1.511^{a}	1.498^{a}	1.510^{a}
Stele-to-diameter ratio	*PK*	0.635^{b}	0.756^{a}	0.438^{c}	0.674^{b}	0.725^{b}	0.771^{a}	0.611^{c}	0.706^{b}
	PA	0.349^{b}	0.369^{b}	0.514^{a}	0.565^{a}	0.528^{a}	0.435^{b}	0.556^{a}	0.542^{a}
	QM	0.735^{a}	0.654^{b}	0.712^{a}	0.700^{a}	0.587^{c}	0.812^{a}	0.738^{b}	0.807^{a}
	TA	0.630^{bc}	0.559^{c}	0.725^{a}	0.697^{ab}	0.673^{b}	0.655^{b}	0.659^{b}	0.752^{a}
	FM	0.423^{c}	0.584^{b}	0.450^{c}	0.654^{a}	0.765^{a}	0.635^{d}	0.709^{c}	0.735^{b}

Each value is the mean (SE) of nine replications, and the statistical analysis unit is each plot. Significant differences ($P < 0.05$) are indicated by different lowercase letters. CK represents the control group, +N represents N addition, −W represents decreased precipitation, and +N−W represents interaction of N addition and decreased precipitation. *PK* represents *Pinus koraiensis*; *PA* represents *Phellodendron amurense*; *QM* represents *Quercus mongolica*; *TA* represents *Tilia amurensis*; and *FM* represents *Fraxinus mandshurica*.

4. Discussion

4.1. Comparison of the response of fine roots of different tree species to N addition

The response of fine roots to atmospheric N deposition is related to their nutrient and water resource acquisition, energy allocation strategy and own growth (Eissenstat et al., 2015; Bowsher et al., 2016; Wang et al., 2016a,b). Our results showed that N addition increased fine root biomass. According to the theory of minimum limiting factors, excess N addition may exacerbate the restriction of other nutrients in the soil. Fine roots need to increase root growth to obtain more restricted nutrients (Wang et al., 2013). Yan (2017) proposed that N addition might result in a decrease in the total fine root surface area but an increase in the thickness of a single fine root, which may eventually result in an increase in the total biomass of the root system.

Plants also undergo morphological plasticity adjustment (Wang et al., 2018). Previous studies found differences in nutrient content, physiological function and turnover metabolism among different orders of fine roots (Pregitzer et al., 2002). In this study, lower order roots were more sensitive to soil N availability than higher order roots. Nitrogen application significantly reduced the length of the 1st–2nd-order roots of PK, PA, QM and FM, which was consistent with previous research results (Yu et al., 2007). This might be because the N resources in the soil were sufficient under N addition, and plants constructed coarser roots to achieve optimal resource delivery strategies and enhance the N-resorption rate and nutrient transport rate rather than invest more C to build longer fine roots to explore resources (Wang et al.,

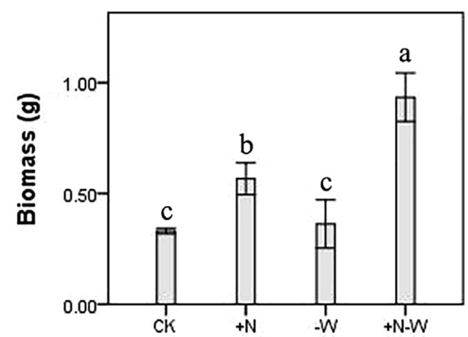

Fig. 1. Fine root biomass in the control (CK), nitrogen addition (+N), decreased precipitation (−W), and interaction of N addition and decreased precipitation (+N−W) treatments in the broad-leaved Korean pine forest. Error bars represent the SE of the mean. Significant differences ($P < 0.05$) between treatments are indicated by different lowercase letters.

Table 2
ANOVA results of the effects of tree species, treatment and root order on fine root morphology and anatomy.

Source of variation	df	*P* values: Length	Surface area	Average diameter	Cortical thickness	Stele diameter	Stele-to-root diameter
Species (S)	4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment (T)	3	< 0.001	< 0.001	< 0.001	0.004	< 0.001	< 0.001
Root order (R)	4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
S × T	12	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
S × R	16	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
T × R	12	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
S × T × R	48	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

ANOVA: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, NS not significant.

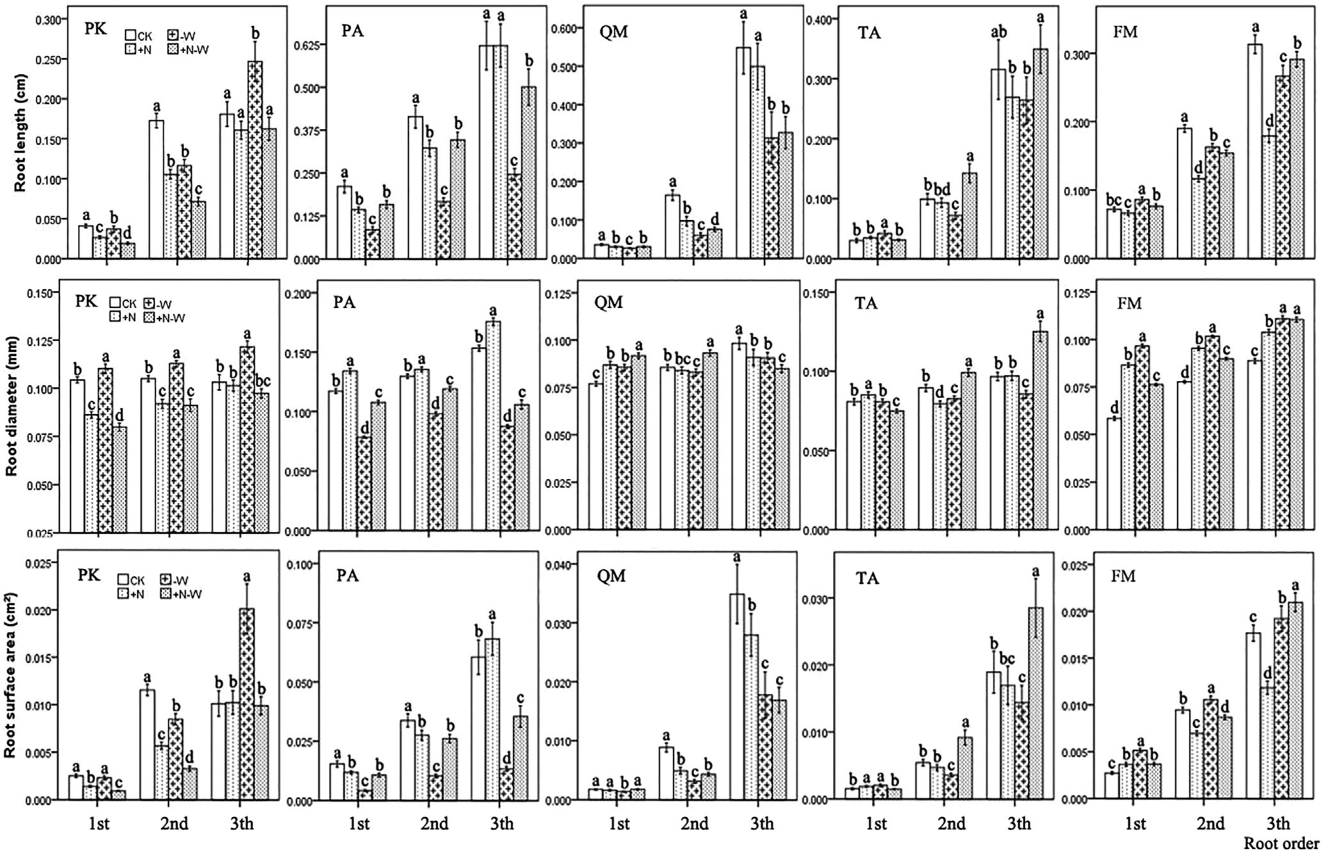

Fig. 2. Mean length, diameter and surface area of fine roots in the control (CK), nitrogen addition (+N), decreased precipitation (−W), and interaction of N addition and decreased precipitation (+N−W) treatments in five temperate tree species. *PK*, *Pinus koraiensis*; *PA*, *Phellodendron amurense*; *QM*, *Quercus mongolica*; *TA*, *Tilia amurensis*; *FA*, *Fraxinus mandshurica*. Error bars represent the SE of the mean. Significant differences ($P < 0.05$) between treatments are indicated by different lowercase letters.

2013). The results also showed that the diameter of the 1st–2nd-order roots of four broadleaf species increased, but that of PK significantly decreased, likely because the fine root production of conifer species was higher than that of broadleaf species. At the same time, N addition increased the N content of the fine roots, resulting in increased respiratory capacity and thus more photosynthesis, but the photosynthetic carbon assimilation of PK was basically unaffected by N addition (Noguchi et al., 2013). Therefore, insufficient assimilate products allocated to the end of the root system of PK, and N input cannot meet respiratory consumption, leading to the reduction of fine root diameter (Noguchi et al., 2013).

In this study, although the diameter of the 1st–3rd-order roots of PK was reduced, the stele diameter and the stele-to-root ratio were significantly increased, and the thickness of the cortex of the 3rd-order roots decreased significantly, which optimized fine root nutrient transportation. In contrast, the stele-to-root ratios of 1st–3rd-order fine roots of the four broadleaved species except TA's 2nd-order and FM's 3rd-order root were reduced. The return rate of C input by PK's fine roots was higher than that of other broadleaved tree species. The heterogeneity of the response of fine roots to N addition may be related to leaf morphology. However, due to the limited conditions of the experimental site, more coniferous broadleaf species need to be studied later.

4.2. Comparison of the response of fine roots of different tree species to decreased precipitation

Decreased precipitation led to changes in the correlations of fine root morphology depending on the species. Previous studies have suggested that there might be two different strategies used by fine roots to cope with decreased precipitation: the rapid absorption strategy with the relatively thinner roots that have a high absorption rate and short lifespan. The second is the slow absorption strategy with thicker roots that have a lower absorption rate and long lifespan (Eissenstat et al., 2000; Bouma et al., 2001). Under the conditions of decreased precipitation, the PA and TA fine roots adopted the fast strategy, and the PK and FM fine roots adopted the slow strategy, while the QM fine roots adopted both strategies. Previous studies suggested that fine roots show heterogeneity in their response to decreased precipitation, but the total nutrient recovery and water uptake efficiency might be the same between thin roots and thick roots relative to root life (Eissenstat and Yanai, 1997; Kong et al., 2014).

The decrease in the cortical thickness and stele diameter of the 3rd-order roots of PK and FM indicated the occurrence of improved absorptive function under the condition of soil water deficit, which was beneficial to the survival of absorptive roots with slow absorption strategy (Kong et al., 2017; Ivano et al., 2015). The increase in the cortical thickness and stele diameter of the 4th–5th-order roots of PK and FM helped to enhance transport ability. Kong et al. (2017) proposed the nutrient absorption-transportation hypothesis, speculating that fine roots would adapt to changing climates by optimizing their anatomy and adopting different nutrient absorption-transport strategies (Kong et al., 2017). Decreased precipitation changed the positive correlations between the fine root diameter, cortical thickness, and stele diameter among all tree species (Table 3), which indicated that the anatomical structure of the root system was closely related to its physiological function.

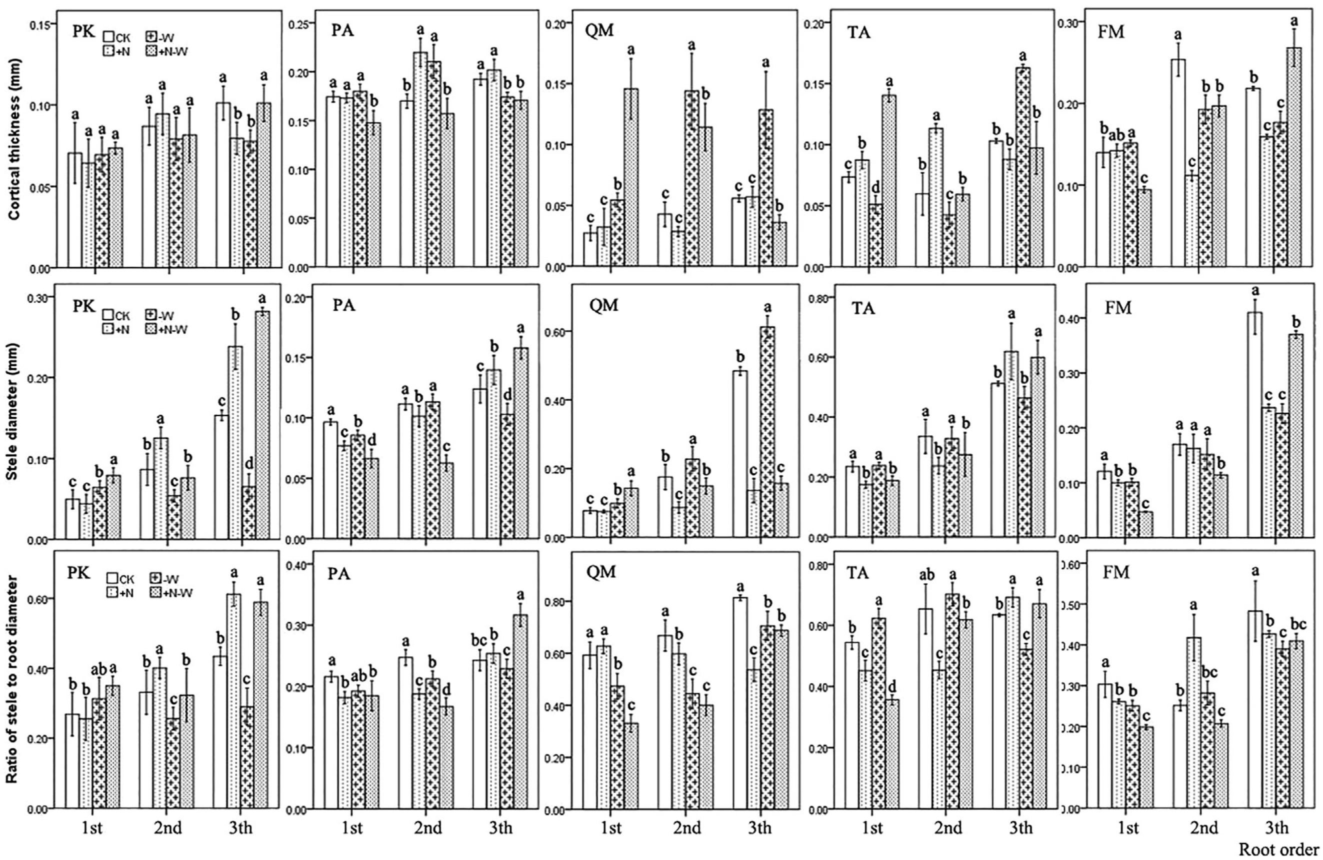

Fig. 3. Mean cortical thickness, stele diameter and stele-to-diameter ratio of the fine roots in the control (CK), nitrogen addition (+N), decreased precipitation (−W), and interaction of N addition and decreased precipitation (+N−W) treatments in five temperate tree species. *PK*, *Pinus koraiensis*; *PA*, *Phellodendron amurense*; *QM*, *Quercus mongolica*; *TA*, *Tilia amurensis*; *FA*, *Fraxinus mandshurica*. Error bars represent the SE of the mean. Significant differences ($P < 0.05$) between treatments are indicated by different lowercase letters.

4.3. Comparison of the response of fine roots of different tree species to the interaction of N addition with decreased precipitation

Fine root growth, morphology and activity are easily affected by environmental factors (Zhang et al., 2000; Liu et al., 2013). Our results indicated that fine root biomass was more responsive to the interaction of N addition with decreased precipitation and that N addition was the dominant factor. Nitrogen addition increased the soil N availability but changed the soil environment and microbial communities, which decreased the water uptake efficiency of the fine roots with lower mycorrhizal colonization rates (Li et al., 2015; Zhou et al., 2019), and decreased precipitation decreased soil water availability. These changes resulted in plants allocating more belowground biomass to ensure nutrient uptake. Valliere and Allen (2016) also proposed that N addition may intensify the effect of decreased precipitation on fine roots.

The interaction of N addition with decreased precipitation led to diverse changes in diameter and anatomy across species. This may be related to the drought resistance, acid tolerance and photosynthetic ability of different tree species. Nitrogen addition may increase fine root diameter by intensifying the acidity of the soil (Zang et al., 2011), but decreased precipitation may weaken the effects of soil acidification on PA. The interaction of N addition with decreased precipitation caused TA and QM to produce thicker fine roots with longer lifespans that could obtain more nutrients to compensate for the nutrients lost as a result of decreased fine root nutrient absorption per unit time (Kong et al., 2014). For FM, the interaction of N addition with decreased precipitation gradually weakened this increase in nutrients. It has been speculated that the effect of N addition and decreased precipitation on the diameter of the fine roots of FM may be antagonistic.

Different root orders of each tree species showed obvious functional heterogeneity. The absorption capacity of the 1st-order roots of PA and FM was significantly improved, and the transport capacity of the 4th–5th-order roots was enhanced. The absorption and transport capacities of the 1st–2nd-order roots of TA were limited, and the absorption and transport capacities of the 5th-order roots were enhanced. This indicated that fine roots redistribute carbon and maintain plant growth by adjusting the structure of transport and absorption roots.

5. Conclusion

(1) Fine roots adjusted their morphology in response to N addition, decreased precipitation and their interaction, and they showed strong cross-species response signals. (2) The inter- and intraspecific response heterogeneity of fine roots with N addition may be derived from root order and leaf morphology, respectively. (3) The anatomical structure of the root system was closely related to its physiological function. Decreased precipitation caused fine roots to balance nutrient absorption with transportation, which may have important implications for our understanding of ecology and plant evolution. (4) The influence of the interaction of N addition with decreased precipitation on the fine root biomass was stronger than a single factor. Decreased precipitation might weaken the toxicity of soil acidification to the fine roots of PA. Nitrogen addition may increase the drought resistance of the fine roots of TA and QM. Assessing variation and trade-offs in relation to plant traits facilitates the understanding and prediction of alterations to ecosystem processes under changing global climatic conditions.

Table 3
Pearson's correlations of the fine root surface area with length and diameter and of fine root diameter with cortical thickness and stele diameter in each treatment.

Species	Treatment	Surface area		Diameter	
		length	diameter	Cortical thickness	Stele diameter
PK	CK	0.918*	0.037	0.456*	0.421*
	+N	0.823*	0.390*	0.663*	0.678*
	−W	0.924*	0.724*	0.840*	0.704*
	+N−W	0.914*	0.507*	0.772*	0.790*
PA	CK	0.872*	0.678*	0.849*	0.863*
	+N	0.882*	0.775*	0.765*	0.784*
	−W	0.841*	0.730*	0.906*	0.900*
	+N−W	0.909*	0.569*	0.916*	0.969*
QM	CK	0.916*	0.697*	0.902*	0.910*
	+N	0.885*	0.561*	0.402*	0.715*
	−W	0.818*	0.870*	0.634*	0.811*
	+N−W	0.879*	0.553*	0.064	0.812*
TA	CK	0.916*	0.641*	0.910*	0.883*
	+N	0.909*	0.724*	0.498*	0.515*
	−W	0.951*	0.511*	0.618*	0.793*
	+N−W	0.896*	0.799*	0.351	0.869*
FM	CK	0.773*	0.783*	0.026	0.968*
	+N	0.911*	0.686*	0.891*	0.910*
	−W	0.829*	0.787*	0.689*	0.941*
	+N−W	0.929*	0.655*	0.504*	0.855*

P values for significant effects and interactions are reported at $p < 0.05$ (*) level. CK represents the control group, +N represents N addition, −W represents decreased precipitation, and +N−W represents interaction of N addition and decreased precipitation. *PK* represents *Pinus koraiensis*; *PA* represents *Phellodendron amurense*; *QM* represents *Quercus mongolica*; *TA* represents *Tilia amurensis*; and *FM* represents *Fraxinus mandshurica*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This research was supported by grants from the National Natural Science Foundation of China, China (41430639, 41773075, 41575137, 31370494, 31170421).

Author contribution statement

QW and JZ designed the study, were awarded funding, supervised data collection and contributed to and edited manuscripts. QW, XZ, YX, GY, MW and JZ contributed the whole manuscript preparation and design and wrote the main manuscript text. QW, XZ, YX, GY, MW and JZ prepared all Figures, YX, XZ, GL, HW, BH, JZ and QW prepared field experiments, prepared tables and collected literatures. All authors reviewed the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2019.117664.

References

Bouma, T.J., Yanai, R.D., Elkin, A.D., Hartmond, U., Flores-Alva, D.E., Eissenstat, D.M., 2001. Estimating age-dependent costs and benefits of roots with contrasting life span: comparing apples and oranges. New Phytol. 150, 685–695. https://doi.org/10.2307/1353673.

Bowsher, A.W., Miller, B.J., Donovan, L.A., 2016. Evolutionary divergences in root system morphology, allocation, and nitrogen uptake in species from high- versus low-fertility soils. Funct. Plant Biol. 43, 129–140. https://doi.org/10.1071/FP15162.

Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154, 275–304. https://doi.org/10.1046/j.1469-8137.2002.00397.x.

De Marco, A., Proietti, C., Cionni, I., Fischer, R., Screpanti, A., Vitale, M., 2014. Future impacts of nitrogen deposition and climate change scenarios on forest crown defoliation. Environ. Pollut. 194, 171–180. https://doi.org/10.1016/j.envpol.2014.07.027.

Eissenstat, D.M., Kucharski, J.M., Zadworny, M., Adams, T.S., Koide, R.T., 2015. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytol. 208, 114–124. https://doi.org/10.1111/nph.13451.

Eissenstat, D.M., Wells, C.E., Yanai, R.D., Whitbeck, J.L., 2000. Building roots in a changing environment: implications for root longevity. New Phytol. 147, 33–42. https://doi.org/10.1046/j.1469-8137.2000.00686.x.

Eissenstat, D.M., Yanai, R.D., 1997. The ecology of root lifespan. Adv. Ecol. Res. 27, 1–60. https://doi.org/10.1016/S0065-2504(08)60005-7.

Fusaro, L., Salvatori, E., Manes, F., 2016. Effects of nitrogen deposition, drought and their interaction, on functional and structural traits of *fraxinus ornus* L. and *quercus ilex* L. Giornale Botanico Italiano 151, 174–189. https://doi.org/10.1080/11263504.2016.1193070.

Fitter, A.H., Stickland, T.R., 1992. Architectural analysis of plant root systems: III. Studies on plants under field conditions. New Phytol. 121, 243–248. https://doi.org/10.1111/j.14698137.1992.tb01110.

Helmisaari, H.S., Makkonen, K., Kellomäki, S., Valtonen, E., Mälkönen, E., 2002. Below- and aboveground biomass, production, and nitrogen use in Scots pine stands in eastern Finland. For. Ecol. Manage. 165, 317–326. https://doi.org/10.1016/S0378-1127(01)00648-X.

Herzog, C., Steffen, J., Pannatier, E.G., Hajdas, I., Brunner, I., 2014. Nine years of irrigation cause vegetation and fine root shifts in a water-limited pine forest. PLoS One 9. https://doi.org/10.1371/journal.pone.0096321.

Ivano, B., Claude, H., Dawes, M.A., Matthias, A., Christoph, S., 2015. How tree roots respond to drought. Front. Plant Sci. 6. https://doi.org/10.3389/fpls.2015.00547.

Iversen, C.M., Mccormack, M.L., Powell, A.S., Blackwood, C.B., Freschet, Grégoire T., Kattge, J., et al., 2017. A global fine-root ecology database to address below-ground challenges in plant ecology. New Phytol. 215, 15–26. https://doi.org/10.1111/nph.14486.

Joslin, J.D., Wolfe, M.H., Hanson, P.J., 2000. Effects of altered water regimes on forest root systems. New Phytol. 147, 117–129. https://doi.org/10.1046/j.1469-8137.2000.00692.x.

Kong, D., Ma, C., Zhang, Q., Li, L., Chen, X., Zeng, H., Guo, D., 2014. Leading dimensions in absorptive root trait variation across 96 subtropical forest species. New Phytol. 203, 863–872. https://doi.org/10.1111/nph.12842.

Kong, D., Wang, J., Zeng, H., Liu, M., Miao, Y., Wu, H., Kardol, P., 2017. The nutrient absorption–transportation hypothesis: optimizing structural traits in absorptive roots. New Phytol. 213, 1569–1572. https://doi.org/10.1111/nph.14344.

Li, W., Jin, C., Guan, D., Wang, Q.K., Wang, A.Z., Yuan, F.H., Wu, J.B., 2015. The effects of simulated nitrogen deposition on plant root traits: a meta-analysis. Soil Biol. Biochem. 82, 112–118. https://doi.org/10.1016/j.soilbio.2015.01.001.

Liu, J., Feng, A.N., Kun, Y., Qiu, B.C., Wang, Z.H., 2013. Application of smart root system for determining morphological parameters of fine roots of *Hevea brasiliensis*. Chinese J. Plant Ecol. 37, 786–792. https://doi.org/10.3724/SP.J.1258.2013.00082.

Lü, C., Tian, H., 2007. Spatial and temporal patterns of nitrogen deposition in China: synthesis of observational data. J. Geophys. Res.: Atmos. 112, D22. https://doi.org/10.1029/2006JD007990.

McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Helmisaari, H.S., Hobbie, E.A., Iversen, C.M., Jackson, R.B., 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytol. 207, 505–518. https://doi.org/10.1111/nph.13363.

Noguchi, K., Nagakura, J., Kaneko, S., 2013. Biomass and morphology of fine roots of sugi (*Cryptomeria japonica*) after 3 years of nitrogen fertilization. Front. Plant Sci. 4, 1–7. https://doi.org/10.3389/fpls.2013.00347.

Poorter, H., Nagel, O., 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Australian J. Plant Physiol. 27https://doi.org/10.1071/PP99173_CO. 1191–1191.

Pregitzer, K.S., Deforest, J.L., Burton, A.J., Allen, M.F., Hendrick, R.R.L., 2002. Fine root architecture of nine north American trees. Ecol. Monogr. 72, 293–309. https://doi.org/10.2307/3100029.

Steudle, E., Peterson, C.A., 1998. How does water get through roots? J. Exp. Bot. 49, 775–788. https://doi.org/10.1093/jexbot/49.322.775.

Valliere, J.M., Allen, E.B., 2016. Interactive effects of nitrogen deposition and drought-stress on plant-soil feedbacks of *Artemisia californica* seedlings. Plant Soil 403, 277–290. https://doi.org/10.1007/s11104-015-2776-y.

Valverde-Barrantes, O.J., Horning, A.L., Smemo, K.A., Blackwood, C.B., 2016. Phylogenetically structured traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms. Plant Soil 404, 1–12. https://doi.org/10.1007/s11104-016-2820-6.

Vogt, K.A., Vogt, D.J., Palmiotto, P.A., BoonJOH, P., Asbjornsen, H., 1996. Review of root dynamics in forest ecosystems grouped by climate, climatic forest type, and species. Plant Soil 187, 159–219. https://doi.org/10.1007/bf00017088.

Wang, G.L., Fahey, T.J., Xue, S., Liu, F., 2013. Root morphology and architecture respond to N addition in *Pinus tabuliformis*, west China. Oecologia 171, 583–590. https://doi.org/10.1007/s00442-012-2441-6.

Wang, W., Wang, Y., Hoch, G., Wang, Z., Gu, J., 2018. Linkage of root morphology to anatomy with increasing nitrogen availability in six temperate tree species. Plant Soil 425, 189–200. https://doi.org/10.1007/s11104-018-3563-3.

Wang, W.N., Wang, Y., Wang, S.Z., Wang, Z.Q., Gu, J.C., 2016a. Effects of elevated N availability on anatomy, morphology and mycorrhizal colonization of fine roots: a review. J. Appl. Ecol. 27, 1294–1302. https://doi.org/10.13287/j.1001-9332.

201604.032.
Wang, X.R., Gu, J.C., Mei, L., Han, Y.Z., Yu, S.Q., Shi, J.W., Yu, L.Z., 2006a. Fine root order morphology and proportion between mother roots and daughter roots in *Fraxinus mandshurica* and *Larix gmelinii* plantations. Acta Ecol. Sinica 26, 1686–1692. https://doi.org/10.3321/j.issn:1000-0933.2006.06.007.
Wang, Y., Cao, M.K., Tao, B., Ke rang, L.I., 2006b. The characteristics of spatio-temporal patterns in precipitation in China under the background of global climate change. Geogr. Res. 25, 1031–1040. https://doi.org/10.1007/s11442-006-0415-5.
Wang, Y., Dong, X., Wang, Z., Gu, J., Epron, D., 2016b. Root tip morphology, anatomy, chemistry and potential hydraulic conductivity vary with soil depth in three temperate hardwood species. Tree Physiol. 36, 99–108. https://doi.org/10.1093/treephys/tpv094.
Yan, G.Y., 2017. Effected of simulated nitrogen deposition on fine root dynamics and morphological structure of *Larix gmelinii*. Northeast Forestry University CDMD: 2.1018.811194.
Yu, L.Z., Ding, G.Q., Shi, J.W., Yu, S.Q., Zhu, J.J., Zhao, L.F., 2007. Effect of fertilization on fine root diameter, root length and specific root length in *Larix kaempferi* plantation. J. Appl. Ecol. 5, 957–962. https://doi.org/10.13287/j.1001-9332.2007.0160.
Zang, U., Lamersdorf, N., Borken, W., 2011. Response of the fine root system in a Norway spruce stand to 13 years of reduced atmospheric nitrogen and acidity input. Plant Soil 339, 435–445. https://doi.org/10.1007/s11104-010-0598-5.
Zhang, X.Q., Wu, K.H., Murach, D., 2000. A review of methods or fine-root production and turnover of trees. Acta Ecol. Sinica 20, 875–883 doi: 1000-0933(2000) 05-0875-09.
Zhong, B.Y., Xiong, D.C., Shi, S.Z., Feng, J.X., Xu, C.S., Deng, F., Chen, Y.Y., Chen, G.S., 2016. Effects of precipitation exclusion on fine-root biomass and functional traits of *Cunninghamia lanceolata* seedlings. J. Appl. Ecol. 27, 2807–2814. https://doi.org/10.13287/j.1001-9332.201609.023.
Zhou, M.X., Yan, G.Y., Xing, Y.J., Chen, F., Zhang, X., Wang, J.Y., Zhang, J.H., Dai, G.H., et al., 2019. Nitrogen deposition and decreased precipitation does not change total nitrogen uptake in a temperate forest. Sci. Total Environ. 651, 32–41. https://doi.org/10.1016/j.scitotenv.2018.09.166.