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ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Temporal dynamics of fine root production, mortality and turnover deviate across branch orders in a larch stand

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Abstract

Fine roots play a key role in carbon, nutrient, and water biogeochemical cycles in forest ecosystems. However, inter-annual dynamics of fine root production, mortality, and turnover on the basis of long-term measurement have been less studied. Here, field scanning rhizotrons were employed for tracking fine root by branch order over a 6 years period in a larch plantation. For total fine roots, from the first- to the fifth-order roots, annual root length production, length mortality, standing crops, and turnover rate varied up to 3.4, 2.3, 1.5, and 2.3-folds during the study period, respectively. The inter-annual variability of those roots indices in the first-order and the second-order roots were greater than that of the higher order (third- to fifth-order) roots. The turnover rate was markedly larger for the first-order roots than for the higher order roots, showing the greatest variability up to 20 times. Seasonal dynamics of root length production followed a general concentrated pattern with peak typically occurring in June or July, whereas root length mortality followed a general bimodal mortality pattern with the dominant peak in May and the secondary peak in August or October. Furthermore, the seasonal patterns of root length production and mortality were similar across years, especially for the first-order and the second-order roots. These results from long-term observation were beneficial for reducing uncertainty of characterizing fine root demography in consideration of large variation among years. Our findings highlight it is important for better understanding of fine root dynamics and determining root demography through distinguishing observation years and root branch orders.

Keywords Root phenology · Seasonal pattern · Rhizotron · Root window · Inter-annual variation · *Larix principis-rupprechtii*

Introduction

Drawing a holistic picture of fine root demography through time is critical for simulating and evaluating belowground carbon (C) and nutrients cycles. Although fine roots account for less than 2% of the total ecosystem biomass, they may contribute up to 40% of the total ecosystem production through their fast turnover (Vogt et al. 1995; Jackson et al. 1997). However, substantial uncertainties remain for the estimation of fine root production and turnover rate (Strand et al. 2008; Neumann and Godbold 2020).To date, our understanding of fine root dynamics is mainly based on short-term (less than 3 years) studies (Freschet et al. 2021; Iversen et al. 2021). Therefore, we need to explore temporal variability in fine root annual demographics.

Long-term research is an available way to reveal the variability of fine roots dynamics (Strand et al. 2008). The inter-annual fluctuation and seasonal pattern of fine root demographics are the two main sources of the variability (Krasowski et al. 2010; McCormack et al. 2014). Few previous studies have reported that fine root production, mortality and turnover rate showed widely inter-annual variation (Joslin et al. 2000; Iversen et al. 2008; Strand et al. 2008; Day et al. 2013; Krasowski et al. 2018). For instance, fine root production and mortality varied more than 4-folds in subtropical forests (Joslin et al. 2000; Kou et al. 2018), and

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up to 30-folds in a temperate forest (Krasowski et al. 2010). The variability in fine root annual demographics may depend on climatic factors (Day et al. 2013; Kou et al. 2018), or closely linked to study sites and tree species (Krasowski et al. 2010, 2018). Therefore, more studies on fine root inter-annual variation are needed in other sites and forest types. In addition to the inter-annual variation, fine root production also showed seasonal patterns including the concentrated, bimodal, multiple, and equally distributed patterns (McCormack et al. 2014, 2015b; Radville et al. 2016; Iversen et al. 2018). Compared with root production, the seasonal pattern of fine root mortality was less studied. Some studies reported that fine root mortality flush occurred in spring and late fall (Ruess et al. 2003; Wang et al. 2020), while other studies suggested that the most of fine roots died during winter season (Tierney et al. 2001; Cleavitt et al. 2008). Thus far, our understanding of the seasonal pattern of fine roots is still limited, and whether these patterns are stable across years remain unclear.

The fine root population is composed of roots with different branching levels and functions (Pregitzer et al. 1997; McCormack et al. 2015a). Numerous studies have shown that fine root morphology, stoichiometry and function systemically changed with the root branch order (Doi et al. 2017; Mucha et al. 2020; Freschet et al. 2021). Moreover, root branch order was the key predictor of turnover rate (Guo et al. 2008b; Huo and Cheng 2019). Recently, Kou et al. (2018) reported that nitrogen (N) addition increased the production, mortality, and turnover rate of the lower order fine roots but not the higher order fine roots. Thus, the lower order roots, as mainly responsible for nutrient and water absorption, and the higher order roots, as mainly responsible for transportation and storage, maybe show different temporal behaviors to the changing environmental factors (McCormack et al. 2015a). Distinguishing root branch order will greatly improve our understanding of fine roots dynamics (Huo and Cheng 2019).

Rhizotron and minirhizotron are non-destructive methods that are well suited to observe root dynamics for long-term studies (Taylor et al. 1990; Dannoura et al. 2008). However, the small viewing window (e.g., 1.4 × 1.8 cm) of the traditional minirhizotron cannot enable us to categorize roots by branch order precisely, in particular for the higher order roots (Wells and Eissenstat 2001). Compared with minirhizotron, the most advantage of field rhizotron is with the larger viewing widow (e.g., 20 × 30 cm) continuous observation of root branching (Nakahata and Osawa 2017; Huo and Cheng 2019). Therefore, field rhizotron approach provides an opportunity for us to study the fine root dynamics by distinguishing root branch orders. In this study, the field rhizotron method was employed to observe root dynamics during a 6 years study period in a *Larix principis-rupprechtii* Mayr (larch) plantation, which is either a key plantation species in China or the dominant species in boreal and temperate forests on the globe. Our objectives were (1) to reveal the inter-annual variability in fine root length production, mortality, standing crops and turnover rate across root branch orders; and (2) to identify the seasonal pattern of root length production and mortality across years. We hypothesized that the inter-annual and seasonal fluctuations of lower order roots are greater than those of higher order roots, as lower order roots are likely more sensitive to environmental and tree physiological changes (McCormack et al. 2015a; Kou et al. 2018).

Materials and methods

Study site

This study was conducted at the Guandishan Longxing Forestry Farm which is located in the middle part of Lvliang Mountains, in Shanxi Province, China (37° 30′ N, 111° 36′ E). The region belongs to the eastern part of Loess Plateau, and has a temperate continental monsoon climate (Sun et al. 2015). The mean January, July and annual air temperature are − 8.9 °C, 17.0 °C, and 4.7 °C averaged from 2012 to 2017, respectively (Fig. S1). The mean annual precipitation is 851–1068 mm, and 85% of the total amount falls in growing season (May–October) (Fig. S1).

Study plots were established on a north-facing slope of approximately 5°, with an elevation ranging from 1700 to 1800 m above the sea level. The larch (*Larix principis-rupprechtii* Mayr) plantation was established in 1990 by planting 2-year-old seedlings using an approximate 2 × 2 m planting grid system. Larch was the exclusive overstorey species (mean diameter at breast height = 12.1 cm; mean height = 11.5 m; mean stem density = 1158 N/ha at the beginning of the experiment in 2012), and the understorey vegetation was largely absent. Three 20 × 20 m plots were established with 50 m spacing of neighboring plots in the plantation. The soil in the plantation is a mountain brown earth characterized by well-developed horizons and well drained. The thickness of the forest floor is approximately 5 cm, whereas mineral soil depth could exceed 50 cm. At the beginning of the experiment, we collected a mixed soil sample (soil depth, 0–30 cm) by drilling 5 points with a soil core (inner diameter = 2.5 cm) in each plot. The soils were used to analyze soil chemical properties (pH, total C and total N) after air drying. Soil pH (H_2O) was determined on air-dried subsamples of soil using a 2:1 (weight:volume) slurry. Soil total C and total N content were determined with an elemental analyzer (Elementar Vario EL III, Hanau, Germany).

Rhizotrons' installation and image processing

In May 2012, ten rhizotrons (available viewing window is 20 cm × 30 cm, width × depth) were installed adjacent (about 1 m from the center of trunk) to each of 10 measurement trees (randomly selected) in each plot, for a total of 30 rhizotrons. A maximum observation depth is 30 cm, beyond which few roots occurred in the site. The field rhizotron method had been introduced in detail in our previous study (Huo and Cheng 2019). Briefly, the installation process can be divided into three steps (Fig. S2). First, we cut a soil profile by inserting the sharp flat chisel (25 cm × 35 cm, width × depth) vertically into the soil. Take one side close to the measurement tree of the chisel as the undisturbed side, and then dug out the soil from the other side (disturbed side) of the chisel until the chisel is removed. Secondly, glass plate (thickness 5 mm) tightly faced to the soil profile by four stainless long nails in each corner. Finally, the pit was backfilled with excavated soil and litter. After installation, mark the location of the rhizotrons on the ground by using small red flags. The glass plates were securely buried in soil during the study periods, only except for sampling dates. Thus, the temperature of the glass surface is the same as that of the bulk soil.

Images were collected from the field rhizotrons approximately monthly throughout the growing season. At each sampling date, we carefully dug out the soil from the disturbed side until the glass plate was entirely emerged. Then, root image was recorded using a flat scanner (Epson Perfection V33, Japan) (see detail in Huo and Cheng 2019). The pit was backfilled with the excavated soil and litter after the observation within few minutes. Image recording was suspended during the winter (November–April) when the soil was frozen. Sampling dates are shown in Table S1. Note that there were only two sampling times for 2012 and 2014 due to the current year of installation and graduate year of students for image collecting, respectively. Roots first appeared on August 2012, which was three months after the installation. The dataset of 2012 and 2014 were likely unreliable because of the limited sampling times. Therefore, the results and analyses of the present study were based on the rest 4 years of reliable observations (2013, 2015, 2016, and 2017).

A total of 973 root images were digitized by the same laboratory technician using the software Rootfly (Version 2.0.2, General Public License, Clemson, South Carolina, USA). The image size was 20 cm × 30 cm (24 bit color, 600 dpi) which permit us to categorize individual root by branch order (Huo and Cheng 2019). Root branch order was assigned according to a topological classification scheme in which roots with no visible dependent laterals were considered to be order 1, roots with a single visible set of dependent laterals were considered to be order 2, and so on (Wang et al. 2006). We successfully sampled the fourth-order and the fifth-order roots. However, there are too few fourth-order and fifth-order roots to be figured out alone. Thus, we grouped these third-order, fourth-order, and fifth-order roots into the higher order roots according to their anatomical characteristics from previous study, which reported that first-order and second-order roots are absorptive roots, and third-order, fourth-order and fifth-order roots are mainly transport roots of larch species (Guo et al. 2008c; Gu et al. 2014). Data recorded for each root included the identification number, diameter, length, soil depth of birth, date of birth, date of death, date of disappear, and branch order. Roots were identified as being born when they first appeared on the surface of the glass plate. Death was defined as when roots turned black from brown or white of living roots, shriveled, and produced no new roots in subsequent viewings. In addition, some roots suddenly disappeared rather than death following slow senescence and were classified as dead roots.

Data analysis

Root production, mortality and standing crops were calculated based on the length of all roots and separated by root branch order, observation date and year. These measurements were expressed as length of roots per surface area of glass plate (i.e., root length m m^{-2}). Annual root production is the sum of the length of new born roots, living roots elongation, and maturation from lower to higher order at each observing date of a year. Similarly, annual root mortality is the sum of the length of dead roots at each observing date of a year. Annual mean standing crops were averaged of live roots at each observing date of a year. Root turnover rate of each year was estimated by annual root length production dividing annual mean standing crops (Burton et al. 2000; Strand et al. 2008).

Seasonal patterns of fine root length production and mortality were determined for each year (exclude 2012 and 2014). For a given observation date, the root length data were divided by the number of days between the current and previous observation dates, and then the calendar monthly data were calculated. Estimates of the calendar monthly production and mortality were used rather than point measurements from each observation date as the length of time between observations varied. The calendar monthly data were normalized by dividing the total annual root production and mortality for the year. Thus, the proportion distribution of root production and mortality allowed us to compare across years with different absolute values. Root production was assumed to be zero for the months of winter (November to April), and overwinter mortality was included into the early spring dataset. This simplifying assumption was made as it was not possible to sample image over winter months due to soil freezing. For each year, smooth spline curves were employed to link the adjacent points of monthly root length production and root length mortality.

Three plots were treated as replication ($n=3$), and data of ten rhizotrons in each plot were averaged. To examine inter-annual effects on annual root length production, annual mortality, and mean annual standing crops, we used a linear mixed-effects model with year as a fixed effect and plots as a random effect. For the seasonal patterns, the differences in peak time of root length production and mortality among years were also tested by using a linear mixed-effects model (year as a fixed effect and plot as a random effect) for each root order. When appropriate, we compared means between treatments by post hoc Turkey HSD tests. We also calculated the coefficients of variation (CV) among years of root indices for each root order. The CVs were used to evaluate the extent of the inter-annual variation. The relationships between climatic factors (temperature and precipitation) and root indices, and root production versus root mortality were analyzed using Pearson's correlation. All the statistical analyses were considered as significant at the 0.05 level. In some instances, data were log transformed to normalize the distribution of residuals. All statistical analyses were performed with JMP (version 8; SAS Institute, Cary, NC, USA).

Results

Annual fine root length production, mortality, standing crops and turnover rate

The soil total C, total N, and pH (H_2O) of the top mineral layer (0–30 cm) were 2.36 ± 0.035 g kg^{-1} (mean ± standard error), 0.13 ± 0.004 g kg^{-1}, and 7.1 ± 0.41, respectively. During the entire study period (2012 – 2017), the total number of roots being observed was 6309, which composed of 5355 first-order roots, 777 s-order roots, 138 third-order roots, 29 fourth-order roots, and 10 fifth-order roots (Table S2). The mean diameters were 0.41, 0.46, 0.95, 1.37, and 2.57 mm for the first-order to the fifth-order roots, respectively (Table S2). As mentioned above (see detail in "Materials and methods"), these roots were separated into three groups, first-order, second-order and higher order (≥ 3 order) roots in the subsequent results. In addition, we totally sampled 34 times during the 6 years study period, but only two times per year in 2012 and 2014 (Table S1; Fig. S3). Therefore, we only reported the reliable dataset of the rest 4 years (2013, 2015, 2016, and 2017) in the following results.

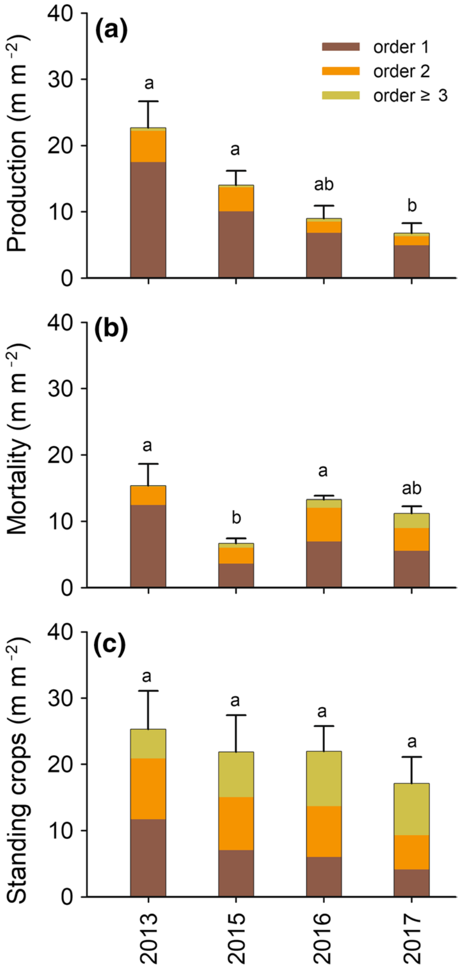

Fig. 1 Annual fine root length production (**a**), mortality (**b**) and standing crops (**c**) with branch orders measured in 4 years in a *Larix principis-rupprechtii* plantation. Error bars are total standard errors ($n=3$). Bar with different letters are significantly different among the total amount of the years in multiple comparisons by the Tukey's honestly significant difference test ($\alpha=0.05$)

Table 1 The results of linear mixed-effects model analysis for annual root length production, mortality and standing crops among 4 years (2013, 2015, 2016, and 2017)

Fine root index	df	*F*	*P* value
Production	3	8.133	**0.008**
Mortality	3	7.338	**0.043**
Standing crops	3	0.483	0.704

The values in bold type indicate that it is statistically significant at the 0.05 level

Annual root length production and mortality of fine roots varied significantly among years (Fig. 1, Table 1). Annual root length production, mortality, and standing crops varied 3.4, 2.3, and 1.5 folds between the highest and lowest years, respectively (Table 2). Root length production was highest (22.67 m m^{-2}) in 2013 and lowest (6.76 m m^{-2}) in 2017 (Fig. 1a). Furthermore, the CVs for root production among years were 56%, 55%, and 9% for the first-order, the second-order, and the higher orders (≥ 3

Table 2 The coefficients of variation (CV) and the maximum folds (Max/Min) of variation for root length production, mortality, turnover rate among 4 years

Fine root index	Root branch order	CV%	Max/Min
Production	First-order	56	3.5
	Second-order	55	3.4
	Higher-order	9	1.2
	All order	54	3.4
Mortality	First-order	53	3.4
	Second-order	35	2.1
	Higher-order	88	26.7
	All order	32	2.3
Standing crops	First-order	45	2.8
	Second-order	22	1.8
	Higher-order	25	1.9
	All order	16	1.5
Turnover rate	First-order	13	1.3
	Second-order	35	1.9
	Higher-order	25	1.7
	All order	38	2.3

orders) roots, respectively (Table 2). The value of root length mortality was the highest (15.35 m m^{-2}) in 2013 and the lowest (6.67 m m^{-2}) in 2015 (Fig. 1b). It is important to note that the CV (88%) of higher order roots was likely overestimated due to the extremely low mortality (0.005 m m^{-2}) in 2013 (Fig. 1b; Table 2). The long-lived nature of higher order roots lead to the low mortality in the first year (2013) after rhizotrons' installation. The CV of root length mortality markedly declined from 88 to 47% when excluding the extreme data of 2013. The mean standing crops of root length was 21.55 m m^{-2} across years, and did not differ significantly among years (Fig. 1c, Table 1).

Root order distribution of annual root length production, mortality, and standing crops clearly changed with observation years (Fig. 2). For the annual root production across years, the contribution of the first-order, the second-order and the higher order roots were 75%, 22%, and 3%, respectively (Fig. 2a). Furthermore, the root order distribution of root production tended to be quickly stable since the first year (2013) after the rhizotron installation. The root order distribution of root length mortality across years, were on average 60%, 31%, and 9% for the first-order, the second-order, and the higher order roots, respectively (Fig. 2b). Furthermore, the higher order roots accounted for only 0.5% of total root mortality in 2013, but increased rapidly to 9% in 2015 (Fig. 2b). Compared with root length production and mortality, the standing crops had a higher proportion of the higher order roots, which accounted for 33% across years. Specially, the proportion of the higher order roots monotonically increased from 17 to 45% during the study period (Fig. 2c).

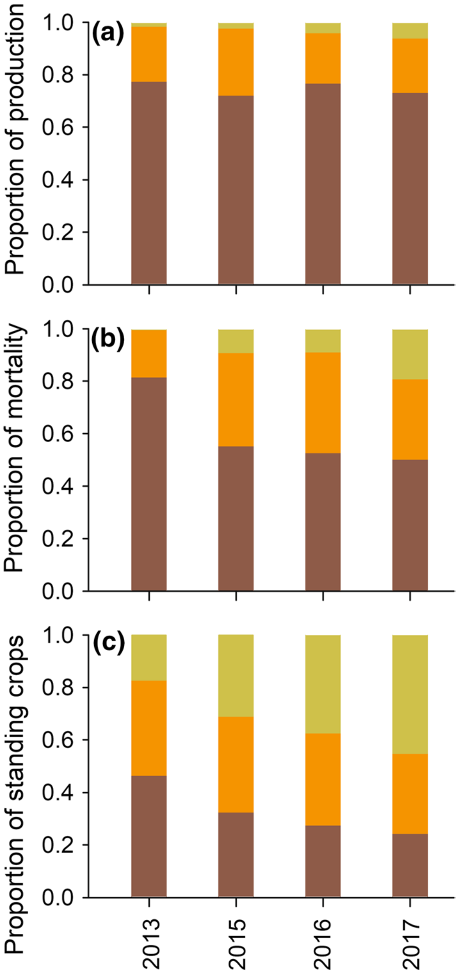

Fig. 2 Proportional contributions of root branch orders to annual root length production (**a**), annual mortality (**b**) and annual mean standing crops (**c**) of fine roots appearing in 4 years. The bar colors are the same as those shown in Fig. 1

The average root turnover rate of fine roots guild (all orders together) was 0.63 $year^{-1}$ across years, and varied 2.3-folds between the highest and the lowest years (Fig. 3). Moreover, root turnover rates across years were 1.41, 0.39, and 0.07 $year^{-1}$ for the first-order, the second-order, and the higher order roots, respectively (Fig. 3). The variation of root turnover rates is much higher among root orders (21.6-folds) than among years (2.3 folds). Overall, root turnover rates decreased with observation year increase, and the CVs of root turnover rates among years were 13%, 35%, and 25% for the first-order, the second-order, and the higher order roots, respectively (Fig. 3).

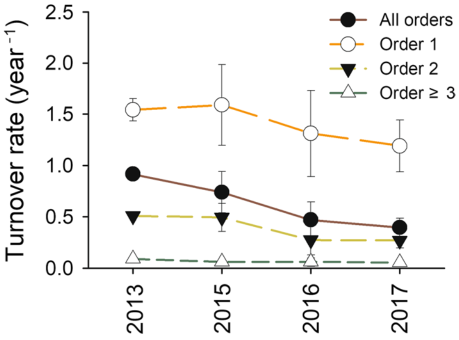

Fig. 3 Turnover rates (T = annual length production/annual mean standing crops) for first-order roots, second-order roots and higher order (order ≥ 3) roots during 4 years

Seasonal dynamics of fine root length production and mortality

Root length production followed concentrated patterns across root orders and observation years (Fig. 4a, b, c). For the first-order and the second-order roots, the peak production typically occurred in June (Fig. 4a, b), whereas the peak production for the higher order roots occurred in different month (from May to August) during the 4 years observation (Fig. 4c). Based on the peak times (month), the CVs of root production were lower for the first-order (14%) and the second-order (8%) roots than for the higher order (20%) roots across years. Root length mortality generally followed bimodal patterns across root orders and observation years (Fig. 4d, e, f). The dominant peak typically occurred in May, and the secondary peak commonly occurred in August or October (Fig. 4d, e, f). It is noteworthy that the peak times of the higher order roots mortality among years shifted wider than that of the first-order and the second-order roots. Overall, the seasonal patterns of root length production and mortality appeared to be stable across years (Fig. 4).

There were significantly positive relationships between monthly mean air temperature and monthly root length production of the first-order ($r = 0.586$, $P < 0.01$), the second-order ($r = 0.445$, $P < 0.05$), and the fine roots guild (all orders together, $r = 0.534$, $P < 0.01$) during growing seasons (Table 3). However, monthly root length mortality did not show any correlation with monthly air temperature and

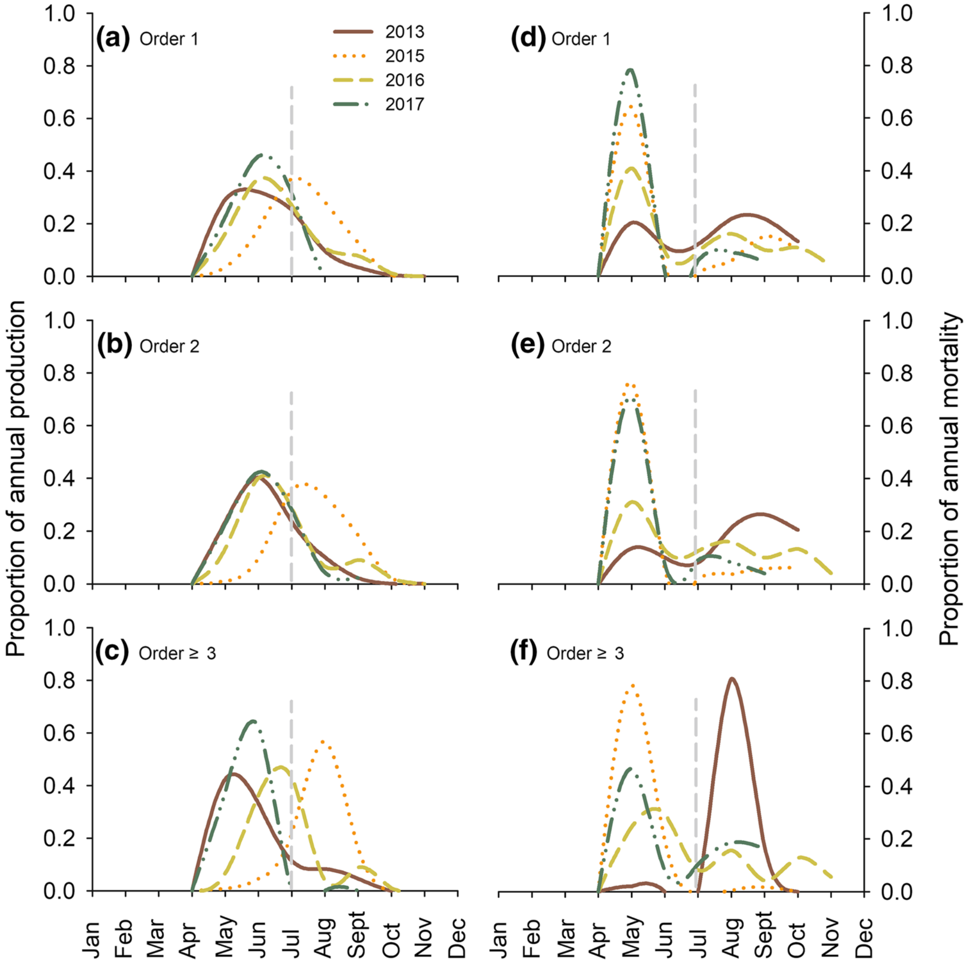

Fig. 4 Patterns of root monthly length production (left column) and mortality (right column) for first-order roots (**a**, **d**), second-order roots (**b**, **e**) and higher order (order ≥ 3) roots (**c**, **f**) during 4 years. The grey vertical lines indicate July

Table 3 The results of Pearson's correlation analysis between root length production and length mortality and climatic factors

Fine root index	Root order	*n*	Temperature		Precipitation		Monthly mortality	
			r	*P* value	*r*	*P* value	*r*	*P* value
Monthly production	First-order	24	**0.586**	**0.004**	0.377	0.084	− 0.201	0.927
	Second-order	24	**0.445**	**0.029**	0.287	0.174	− 0.331	0.114
	Higher-order	24	0.374	0.072	0.302	0.151	0.064	0.766
	All orders	24	**0.534**	**0.007**	0.362	0.082	− 0.107	0.617
Monthly mortality	First-order	24	0.050	0.816	− 0.229	0.283		
	Second-order	24	0.052	0.808	− 0.230	0.331		
	Higher-order	24	0.117	0.587	− 0.098	0.649		
	All orders	24	0.050	0.816	− 0.207	0.331		

Temperature and precipitation are monthly means during growth season from May to October. $n = 24$ (6 months per year × 4 years)

The values in bold type indicate that it is statistically significant at the 0.05 level

precipitation across all root orders (Table 3). Root monthly length production was not clearly correlated with root length mortality (Table 3), and both seasonal patterns were in asynchronous times (Fig. 4).

Discussion

Inter-annual fluctuations of fine root production, mortality and turnover by root branch order

We found that the inter-annual fluctuations of root indices were greater for the first-order and the second-order roots than for the higher order roots in larch (Fig. 1, Table 2), which supported our hypothesis. The manner in which fine roots of the different branch orders respond to changes in environmental conditions has received little attention. Previous studies with minirhizotrons reported that annual fine root production and mortality could vary 2 to 30-folds among years in temperate forests (Iversen et al. 2008; Krasowski et al. 2010; Fukuzawa et al. 2013). However, the previous studies commonly oversimplified the fine roots as a single pool, which may mask the direction and magnitude of inter-annual variation of different branch orders roots. In the current study, rhizotrons with large viewing area provide us an opportunity to classify the fine roots into different root order pools. Our results suggest that the first-order and the second-order roots are more sensitive to changes in exogenous and endogenous factors than the higher order roots. This finding is similar to the results by Kou et al. (2018) who showed that N deposition concurrently increased the production and mortality of the first two orders roots (absorptive roots) but not the higher order roots (transport roots). Absorptive fine roots involve primarily in the uptake of soil water and nutrient, whereas transport fine roots serve primarily transport and structural functions (McCormack et al. 2015a). Given that the different functions, the mechanisms for regulating root dynamics of distinct fine root pools may be different.

Several potential factors may contribute to the inter-annual variations of fine roots across branch orders. First, environmental factors (e.g., temperature, precipitation, and soil N) affect the variability of annual root production and mortality (Finér et al. 2011). Few studies reported that the responses of the thinner roots (diameter < 1 mm) production to soil warming (Shi et al. 2017; Jarvi and Burton 2020) and drying (Rytter 2013) were greater than that of thicker roots (1–2 mm). In addition, soil N-enriched patches can stimulate the proliferation of absorptive roots but not transport roots (Hodge 2006). At present, very few data are available to distinguish the response of different orders (or functional types) roots to environmental change. In the current study, the climate is relatively mild during 6 years study period (Fig. S1), but annual root length production and mortality of the first-order roots changed by up to 56% or 3.5-folds (Table 2). Therefore, the weak year to year fluctuations in climate potentially lead to strong variations in annual root production and mortality. Second, tree physiological processes may drive the inter-annual variation. The first two order roots have higher nutrient uptake and metabolic activity (e.g., respiration) compared with higher order roots (Guo et al. 2004; Jia et al. 2011). With photosynthate sufficient, plants favor to produce lower order roots to improve the ability for acquiring soil resources, whereas C limitation likely promotes low-order roots mortality to reduce maintaining respiration cost (Eissenstat and Duncan 1992; Eissenstat and Yanai 1997; Xia et al. 2010). Here, we speculate that lower order roots are more closely related to tree physiological processes than higher order roots resulting in larger inter-annual fluctuations. Third, root herbivores prefer the first-order and the second-order roots to the higher order roots (Sun et al. 2011), which may intensify the inter-annual variations in fine roots. Moreover, the materials of root windows affected fine root growth and behavior (Withington et al. 2003), this effect on young first-order and second-order roots may be greater than that on old higher order roots. Collectively, given that the multiple impacts of environment changes, tree physiology, root herbivores, and other factors, the larger inter-annual variation could exhibit on lower order roots than on higher order roots.

Moreover, our results showed that the turnover rate decreased with the increase of observation years, and the highest variation was 2.3-folds among years (Fig. 3; Table 2). This finding was partly consistent with that reported in Krasowski et al. (2018), the turnover rates varied more than 3 times during multi-years observation in two balsam fir stands. Another previous study also reported that root turnover rates decreased with years since initiation of minirhizotron study (Strand et al. 2008), which was in line with our results. The estimates of fine root turnover rate remain highly variability among different methods (Hendricks et al. 2006; Strand et al. 2008) and different pools of roots being observed or measured (Guo et al. 2008a; Gaudinski et al. 2010). Our results showed that root turnover rates markedly decreased with the increase of root order, and the highest variation was up to 21.6-folds between the higher order and the first-order roots across years (Fig. 3; Table 2). At present, little is known about the turnover rates (or lifespan) of higher order roots due to methodological limitation (Huo and Cheng 2019). Using C isotope approach, the turnover rates were up to ten times slower for the higher order roots (thicker roots with diameter 0.5–2.0 mm) than for the lower order roots (thinner roots with diameter $<$ 0.5 mm) in temperate forests (Matamala et al. 2003; Riley et al. 2009). Overall, our findings highlighted the need to estimate root turnover rate not only by separating different root branch orders but also by multiple years of measurement.

To the best of our knowledge, few studies with rhizotron or minirhizotron have lasted over 5 years, thus our results provided a rare opportunity to evaluate the equilibrium time of soil disturbance after the rhizotron installation (Strand et al. 2008; Nakahata and Osawa 2017). We found that the equilibrium time based on the distribution of root branch orders was longer than that based on total fine roots as a single pool (Figs. 1, 2). Specifically, the root length density recovered rapidly after a growing season in the year of installation (Fig. S3). Extensive studies using minirhizotrons reported that root proliferation phenomena resulted from root pruning and soil nutrient increasing near the tube surface (Joslin and Wolfe 1999; Johnson et al. 2001; Krasowski et al. 2010). However, the distribution of root branch orders of standing crops may not represent those in bulk soil in the first year of installation. Because the newly born roots are young, it takes time to grow into higher order (old) roots, so the equilibrium of root population needs longer time (Krasowski et al. 2018). The root population data of soil block (or soil core) is an important reference for evaluating the artificial effects of rhizotron (tube) installation. The higher order roots accounted for one-third of the total fine root length standing crops in bulk soil according to a previous study (Wang et al. 2006), which measured standing crops using soil monolith in a larch plantation. Our results suggested that the proportion of the higher order roots did not reach the above value (approximate 33%) until the third year (2015) after the rhizotron installation (Fig. 2). Moreover, this equilibrium time was partly supported by the minirhizotron study of Strand et al. (2008) that observed root mean lifespan return to equilibrium by third year in a total 8 years observation. Therefore, we recommend the optimal equilibrium time after the rhizotrons' installation was at least 3 years for examining different root orders especially higher order roots dynamics in temperate forests.

Seasonal patterns of fine root production and mortality

Our results revealed that root length production followed a general concentrated seasonal pattern with the peak production typically occurring between late spring and early summer for all root branch orders (Fig. 4a, b, c). The concentrated pattern of root length production is in line with those of other deciduous species in the temperate forests (McCormack et al. 2014, 2015b; Withington et al. 2021). The seasonal patterns of fine root production possibly were regulated by tree species strategies and regional climate (McCormack et al. 2014). The larch is an early leaf expansion and fast stems growth strategies species (Wang et al. 2006), which may lead to fine roots flush in the early days of the growing season. The region of the current study belongs to a typical continental monsoon climate with rain and heat corresponding period (Sun et al. 2015). Fine roots, as soil resource acquisition organs, the timing of root production was likely linked to favorable environmental (e.g., temperature, soil moisture, and nutrient) conditions (McCormack et al. 2015b; Iversen et al. 2018). This is supported by the positive relationships between monthly root length production and monthly mean air temperature for absorptive roots (the first-order and the second-order roots) (Table 3). There was also noticeable inter-annual variation of peak times among different root orders (Fig. 4a, b, c). For the first-order and the second-order roots, their production could use the nonstructural carbohydrate stored in trees (Luo 2003). This could buffer the impact of climatic fluctuation on their production thereby resulting in the relatively stable peak times across years. In contrast, the peak times of the higher order roots were varied greatly in different years (Fig. 4c, Table 4). One possible cause is that the C required for the higher order roots growth mainly comes from photosynthate of the current year. Another possible cause is that the relatively small sample size of the higher order roots might result in the higher uncertainty to estimate peak times (Table S2). Overall, the concentrated seasonal patterns of fine root length production were consistent across years and orders.

Table 4 The results of linear mixed-effects model analysis for the peak times of root length production and root mortality among 4 years

Fine root index	Root order	df	*F*	*P* value
Peak time of production	Order 1	3	2.286	0.156
	Order 2	3	1.844	0.217
	Order ≥ 3	3	14.533	**0.001**
First peak time of mortality	Order 1	3	1.500	0.287
	Order 2	3	2.133	0.174
	Order ≥ 3	3	2.667	0.119
Second peak time of mortality	Order 1	3	2.148	0.172
	Order 2	3	3.529	0.068
	Order ≥ 3	3	0.515	0.683

The values in bold type indicate that it is statistically significant at the 0.05 level

Fine root length mortality followed a general bimodal pattern with primary pulse in early spring and secondary pulse in fall for all root branch orders (Fig. 4d, e, f). For the primary pulse, it may be due to the including of overwinter mortality in the current study (see detail in "Materials and methods"). Tierney et al. (2001) reported considerable overwinter mortality in a northern hardwood forest. Soil freezing may cause non-woody fine roots either directly cellular injury or indirectly physical damage (Cleavitt et al. 2008). For the secondary pulse, previous studies also reported that high fine root mortality occurred in the late growing season in temperate forests (Ruess et al. 2003; Wang et al. 2020). Though the factors controlling latter root mortality are unclear, it is possible that trees could actively regulate root mortality for reducing the cost of maintains respiration during non-growing season. In addition, our results also showed that the mortality seasonal patterns (the peak time) of the first-order roots were always synchronous with that of the second-order roots in each year (Fig. 4d, e), indicating that the first-order and second-order roots of larch form a "root module" as found in other tree species (e.g., Xia et al. 2010). However, the mortality seasonal patterns between the first-order roots and the higher order roots exhibited asynchronously in two (2013 and 2016) out of four observation years (Fig. 4d, f). It is currently unclear what factors regulate root mortality especially for higher order roots, which is worth investigating in future studies.

Conclusions

Our results based on a 6 years rhizotron observation demonstrated that annual fine root length production, mortality, and turnover rate were greatly fluctuated among years. The data of single year or even short-term observations may lead to biased estimates of fine root dynamics compared with multiyear or long-term observation. Importantly, we found that the inter-annual fluctuations were greater for the first-order and the second-order roots than for the higher order roots. The first two order roots, as absorptive fine roots, sensitively suffer more from climatic fluctuation and tree physiological status. Across years and root branch orders, fine root length production and mortality generally followed concentrated seasonal pattern and bimodal seasonal pattern, respectively. Furthermore, the seasonal patterns of the first-order roots were commonly synchronous with that of the second-order roots but not the higher order roots, supporting the previous "root module" hypothesis. In addition, our results showed turnover rates could differ more than 20 times between the first-order and the higher order roots, thus it is very important to distinguish root branch orders for obtaining robust estimates of root dynamics. Our findings emphasize that long-term study in fine root dynamics particularly including higher order roots would deepen our mechanistic understanding of inter-annual and intra-annual variations of fine root demography and belowground carbon cycle.

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Author contribution statement CH and WC conceived and designed the experiments. CH and LY performed the experiments. CH, JG, LY analyzed the data. CH, PW, JG, WC wrote the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Burton AJ, Pregitzer KS, Hendrick RL (2000) Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia 125:389–399. https://doi.org/10.1007/s004420000455

Cleavitt NL, Fahey TJ, Groffman PM, Hardy JP, Henry KS, Driscoll CT (2008) Effects of soil freezing on fine roots in a northern hardwood forest. Can J Forest Res 38:82–91. https://doi.org/10.1139/X07-133

Dannoura M, Kominami Y, Oguma H, Kanazawa Y (2008) The development of an optical scanner method for observation of plant root dynamics. Plant Root 2:14–18. https://doi.org/10.3117/plantroot.2.14

Day FP, Schroeder RE, Stover DB, Brown ALP, Butnor JR, Dilustro J, Hungate BA, Dijkstra P, Duval BD, Seiler TJ, Drake BG, Hinkle CR (2013) The effects of 11 yr of CO_2 enrichment on roots in a Florida scrub-oak ecosystem. New Phytol 200:778–787. https://doi.org/10.1111/nph.12246

Doi R, Tanikawa T, Miyatani K, Hirano Y (2017) Intraspecific variation in morphological traits of root branch orders in *Chamaecyparis obtuse*. Plant Soil 416:503–513. https://doi.org/10.1007/s11104-017-3230-0

Eissenstat DM, Duncan LW (1992) Root growth and carbohydrate responses in bearing citrus trees following partial canopy removal. Tree Physiol 10:245–257. https://doi.org/10.1093/treephys/10.3.245

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

Finér L, Ohashi M, Noguchi K, Hirano Y (2011) Factors causing variation in fine root biomass in forest ecosystems. For Ecol Manage 261:265–277. https://doi.org/10.1016/j.foreco.2010.10.016

Freschet GT, Roumet C, Comas LH, Weemstra M, Bengough AG, Rewald B, Bardgett RD, DeDeyn GB, Johnson D, Klimešová J, Lukac M, McCormack ML, Meier IC, Pagès L, Poorter H, Prieto I, Wurzburger N, Zadworny M, Bagniewska-Zadworna A, Blancaflor EB, Brunner I, Gessler A, Hobbie SE, Iversen CM, Mommer L, Picon-Cochard C, Postma JA, Rose L, Ryser P, Scherer-Lorenzen M, Soudzilovskaia NA, Sun T, Valverde-Barrantes OJ, Weigelt A, York LM, Stokes A (2021) Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. New Phytol 232:1123–1158. https://doi.org/10.1111/nph.17072

Fukuzawa K, Shibata H, Takagi K, Satoh F, Koike T, Sasa K (2013) Temporal variation in fine-root biomass, production and mortality in a cool temperate forest covered with dense understory vegetation in northern Japan. For Ecol Manage 310:700–710. https://doi.org/10.1016/j.foreco.2013.09.015

Gaudinski JB, Torn MS, Riley WJ, Dawson TE, Joslin JD, Majdi H (2010) Measuring and modeling the spectrum of fine-root turnover times in three forests using isotopes, minirhizotrons, and the Radix model. Global Biogeochem. https://doi.org/10.1029/2009GB003649

Gu JC, Xu Y, Dong X, Wang H, Wang Z (2014) Root diameter variations explained by anatomy and phylogeny of 50 tropical and temperate tree species. Tree Physiol 34:415–425. https://doi.org/10.1093/treephys/tpu019

Guo DL, Mitchell RJ, Hendricks JJ (2004) Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. Oecologia 140:450–457. https://doi.org/10.1007/s00442-004-1596-1

Guo DL, Li H, Mitchell RJ, Han W, Hendricks JJ, Fahey TJ, Hendrick RL (2008a) Fine root heterogeneity by branch order: exploring the discrepancy in root turnover estimates between minirhizotron and carbon isotopic methods. New Phytol 177:443–456. https://doi.org/10.1111/j.1469-8137.2007.02242.x

Guo DL, Mitchell RJ, Withington JM, Fan P, Hendricks JJ (2008b) Endogenous and exogenous controls of root life span, mortality and nitrogen flux in a longleaf pine forest: root branch order predominates. J Ecol 96:737–745. https://doi.org/10.1111/j.1365-2745.2008.01385.x

Guo DL, Xia M, Wei X, Chang W, Liu Y, Wang Z (2008c) Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. New Phytol 180:673–683. https://doi.org/10.1111/j.1469-8137.2008.02573.x

Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecot SD, Guo DL (2006) Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. J Ecol 94:40–57. https://doi.org/10.1111/j.1365-2745.2005.01067.x

Hodge A (2006) Plastic plants and patchy soils. J Exp Bot 57:401–411. https://doi.org/10.1093/jxb/eri280

Huo CF, Cheng WX (2019) Improved root turnover assessment using field scanning rhizotrons with branch order analysis. Ecosphere 10:e02793. https://doi.org/10.1002/ecs2.2793

Iversen CM, Ledford J, Norby RJ (2008) CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. New Phytol 179:837–847. https://doi.org/10.1111/j.1469-8137.2008.02516.x

Iversen CM, Childs J, Norby RJ, Ontl TA, Kolka RK, Brice DJ, McFarlane KJ, Hanson PJ (2018) Fine-root growth in a forested bog is seasonally dynamic, but shallowly distributed in nutrient-poor peat. Plant Soil 424:123–143. https://doi.org/10.1007/s11104-017-3231-z

Iversen CM, McCormack ML, Baer JK, Powell AS, Chen W, Collins C, Fan Y, Fanin N, Freschet GT, Guo D, Hogan JA, Kou L, Laughlin DC, Lavely E, Liese R, Lin D, Meier IC, Montagnoli A, Roumet C, See CR, Soper F, Terzaghi M, Valverde-Barrantes OJ, Wang C, Wright SJ, Wurzburger N, Zadworny M (2021) Fine-root ecology database (FRED): a global collection of root trait data with coincident site, vegetation, edaphic, and climatic data, version 3. United States. https://doi.org/10.25581/ornlsfa.014/1459186

Jackson RB, Mooney HA, Schulze ED (1997) A global budget for fine root biomass, surface area, and nutrient contents. Proc Natl Acad Sci 94:7362–7366. https://doi.org/10.1073/pnas.94.14.7362

Jarvi MP, Burton AJ (2020) Root respiration and biomass responses to experimental soil warming vary with root diameter and soil depth. Plant Soil 451:435–446. https://doi.org/10.1007/s11104-020-04540-1

Jia S, Wang Z, Li X, Zhang X, Mclaughlin NB (2011) Effect of nitrogen fertilizer, root branch order and temperature on respiration and tissue N concentration of fine roots in *Larix gmelinii* and *Fraxinus mandshurica*. Tree Physiol 31:718–726. https://doi.org/10.1093/treephys/tpr057

Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. Environ Exp Bot 45:263–289. https://doi.org/10.1016/S0098-8472(01)00077-6

Joslin JD, Wolfe MH (1999) Disturbances during minirhizotron installation can affect root observation data. Soil Sci Soc Am J 63:218–221. https://doi.org/10.2136/sssaj1999.03615995006300010031x

Joslin JD, Wolfe MH, Hanson PJ (2000) Effects of altered water regimes on forest root systems. New Phytol 147:117–129. https://doi.org/10.1046/j.1469-8137.2000.00692.x

Kou L, Jiang L, Fu X, Dai X, Wang H, Li S (2018) Nitrogen deposition increases root production and turnover but slows root decomposition in *Pinus elliottii* plantations. New Phytol 218:1450–1461. https://doi.org/10.1111/nph.15066

Krasowski MJ, Lavigne MB, Olesinski J, Bernier PY (2010) Advantages of long-term measurement of fine root demographics with a minirhizotron at two balsam fir sites. Can J for Res 40:1128–1135. https://doi.org/10.1139/X10-068

Krasowski MJ, Lavigne MB, Szuter MA, Olesinski J, Kershaw JA, McGarrigle E (2018) Age-related changes in survival and turnover rates of balsam fir (*Abies balsamea* (L.) Mill.) fine roots. Tree Physiol 38:865–876. https://doi.org/10.1093/treephys/tpy010

Luo Y (2003) Uncertainties in interpretation of isotope signals for estimation of fine root longevity: theoretical considerations. Global Change Biol 9:1118–1129. https://doi.org/10.1046/j.1365-2486.2003.00642.x

Matamala R, Gonzàlez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH (2003) Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science 302:1385–1387. https://doi.org/10.1126/science.1089543

McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM (2014) Variability in root production, phenology, and turnover rate among 12 temperate tree species. Ecology 95:2224–2235. https://doi.org/10.1890/13-1942.1

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo DL, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB, Leppälammi-Kujansuu J, Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Rewald B, Zadworny M (2015a) 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

McCormack ML, Gaines KP, Pastore M, Eissenstat DM (2015b) Early season root production in relation to leaf production among six diverse temperate tree species. Plant Soil 389:121–129. https://doi.org/10.1007/s11104-014-2347-7

Mucha J, Zadworny M, Helmisaari HS, Nihlgård B, Repo T, ŻytkowiakM MS, Reich PB, Oleksyn J (2020) Fine root classification matters: nutrient levels in different functional categories, orders and diameters of roots in boreal *Pinus sylvestris* across a latitudinal gradient. Plant Soil 447:507–520. https://doi.org/10.1007/s11104-019-04395-1

Nakahata R, Osawa A (2017) Fine root dynamics after soil disturbance evaluated with a root scanner method. Plant Soil 419:467–487. https://doi.org/10.1007/s11104-017-3361-3

Neumann M, Godbold DL (2020) Improving models of fine root carbon stocks and fluxes in European forests. J Ecol 108:496–514. https://doi.org/10.1111/1365-2745.13328

Pregitzer KS, Kubiske ME, Yu CK, Hendrick RL (1997) Relationships among root branch order, carbon, and nitrogen in four temperate species. Oecologia 111:302–308. https://doi.org/10.1007/s004420050239

Radville L, McCormack ML, Post E, Eissenstat DM (2016) Root phenology in a changing climate. J Exp Bot 67:3617–3628. https://doi.org/10.1093/jxb/erw062

Riley WJ, Gaudinski JB, Torn MS, Joslin JD, Hanson PJ (2009) Fine-root mortality rates in a temperate forest: estimates using radiocarbon data and numerical modeling. New Phytol 184:387–398. https://doi.org/10.1111/j.1469-8137.2009.02980.x

Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornssön B, Allen MF, Maurer GE (2003) Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. Ecol Monogr 73:643–662. https://doi.org/10.1890/02-4032

Rytter RM (2013) The effect of limited availability of N or water on C allocation to fine roots and annual fine root turnover in *Alnus incana* and *Salix viminalis*. Tree Physiol 33:924–939. https://doi.org/10.1093/treephys/tpt060

Shi SZ, Xiong DC, Deng F, Feng JX, Xu CS, Zhong BY, Chen YY, Chen GS, Yang YS (2017) Interactive effects of soil warming and nitrogen addition on fine root production of Chinese fir seedlings. Chin J Plant Ecol 41:186–195. https://doi.org/10.17521/cjpe.2016.0274 (**In Chinese**)

Strand AE, Pritchard SG, McCormack ML, Davis MA, Oren R (2008) Irreconcilable differences: Fine-root life spans and soil carbon persistence. Science 319:456–458. https://doi.org/10.1126/science.1151382

Sun Y, Gu J, Zhuang H, Guo D, Wang Z (2011) Lower order roots more palatable to herbivores: a case study with two temperate tree species. Plant Soil 347:351–361. https://doi.org/10.1007/s11104-011-0854-3

Sun W, Song X, Mu X, Gao P, Wang F, Zhao G (2015) Spatiotemporal vegetation cover variations associated with climate change and ecological restoration in the Loess Plateau. Agric for Meteorol 209–210:87–99. https://doi.org/10.1016/j.agrformet.2015.05.002

Taylor HM, Upchurch DR, McMichael BL (1990) Applications and limitations of rhizotrons and minirhizotrons for root studies. Plant Soil 129:29–35. https://doi.org/10.1007/BF00011688

Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD, Driscoll CT (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. Biogeochemistry 56:175–190. https://doi.org/10.1023/A:1013072519889

Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H (1995) 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 Z, Guo D, Wang X, Gu J, Mei L (2006) Fine root architecture, morphology, and biomass of different branch orders of two Chinese temperate tree species. Plant Soil 288:155–171. https://doi.org/10.1007/s11104-006-9101-8

Wang N, Wang C, Quan X (2020) Variations in fine root dynamics and turnover rates in five forest types in northeastern China. J for Res 31:871–884. https://doi.org/10.1007/s11676-019-01065-x

Wells CE, Eissenstat DM (2001) Marked differences in survivorship among apple roots of different diameters. Ecology 82:882–892. https://doi.org/10.1890/0012-9658

Withington JM, Elkin A, Bułaj B, Olesiński J, Tracy KN, Bouma TJ, Oleksyn J, Anderson LJ, Modrzyński J, Reich PB, Eissenstat DM (2003) The impact of material used for minirhizotron tubes for root research. New Phytol 160:533–544. https://doi.org/10.1046/j.1469-8137.2003.00903.x

Withington JM, Goebel M, Bułaj B, Oleksyn J, Reich PB, Eissenstat DM (2021) Remarkable similarity in timing of absorptive fine-root production across 11 diverse temperate tree species in a common garden. Front Plant Sci. https://doi.org/10.3389/fpls.2020.623722

Xia M, Guo DL, Pregitzer KS (2010) Ephemeral root modules in *Fraxinus mandshurica*. New Phytol 188:1065–1074. https://doi.org/10.1111/j.1469-8137.2010.03423.x