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Research

Ephemeral root modules in *Fraxinus mandshurica*

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Summary

- Historically, ephemeral roots have been equated with 'fine roots' (i.e. all roots of less than an arbitrary diameter, such as 2 mm), but evidence shows that 'fine roots' in woody species are complex branching systems with both rapid-cycling and slow-cycling components. A precise definition of ephemeral roots is therefore needed.
- Using a branch-order classification, a rhizotron method and sequential sampling of a root cohort, we tested the hypothesis that ephemeral root modules exist within the branching *Fraxinus mandshurica* (Manchurian ash) root system as distal nonwoody lateral branches, which show anatomical, nutritional and physiological patterns distinct from their woody mother roots.
- Our results showed that in *F. mandshurica*, distal nonwoody root branch orders die rapidly as intact lateral branches (or modules). These nonwoody branch orders exhibited highly synchronous changes in tissue nitrogen concentrations and respiration, dominated root turnover, nutrient flux and root respiration, and never underwent secondary development.
- The ephemeral root modules proposed here may provide a functional basis for differentiating and sampling short-lived absorptive roots in woody plants, and represent a conceptual leap over the traditional coarse–fine root dichotomies based on arbitrary size classes.

Introduction

A long-standing question in the biology of woody plants has been to understand which portion of the root system dies relatively rapidly (within 1–2 yr) (Pregitzer, 2002; Trumbore & Gaudinski, 2003; Joslin *et al.*, 2006). While the entire root system dies at the end of the growing season in annual plants, it has been difficult to identify the ephemeral roots in perennial woody plants (e.g. trees). For nearly half a century, ephemeral roots have been defined conveniently, but arbitrarily, as all roots less than a certain diameter, most commonly as those < 2 mm in diameter (Moir & Bachelard, 1969; Vogt *et al.*, 1986; Jackson *et al.*, 1997; Strand *et al.*, 2008). Mounting evidence suggests, however, that fine roots defined in this way probably include both ephemeral roots and perennial roots in all woody species examined thus far (Gaudinski *et al.*, 2001; Wells & Eissenstat, 2001; Matamala *et al.*, 2003; Joslin *et al.*, 2006; Guo *et al.*, 2008a; Strand *et al.*, 2008; Espeleta *et al.*, 2009). The classic single-diameter-class approach fails to precisely define the ephemeral roots on a complex branching perennial root system (Pregitzer, 2002; Högberg & Read, 2006; Joslin *et al.*, 2006; Guo *et al.*, 2008a).

Identifying which roots are short-lived is crucial in quantifying terrestrial carbon (C) and nitrogen (N) cycles at the global scale (Jackson *et al.*, 1997; Matamala *et al.*, 2003; Strand *et al.*, 2008). Assuming that the entire fine-root pool turns over once per year, it has been estimated that > 30% of global annual terrestrial net primary production (NPP) is devoted to fine-root production (Jackson *et al.*, 1997). In forests, the estimate may be as high as 67% (Grier *et al.*, 1981; Santantonio & Grace, 1987). However, if a substantial fraction of fine roots (<2mm) is long-lived, global estimates of root production and turnover will need to be revised. Recently, a few studies have reported evidence for conceiving woody plant root systems as composed of a

two-tier system of root types (short-lived vs long-lived), and have modeled the consequences of such a conception on root dynamics (Riley *et al.*, 2009; Gaudinski *et al.*, 2010). Thus, identifying precisely which roots are ephemeral is a prerequisite for producing accurate estimates of belowground NPP at the global scale.

Previous studies have offered some clues about which roots might be ephemeral. For example, Lyford (1975) and Pregitzer *et al.* (2002) observed densely distributed root scars and 'stumps' on woody roots, implying frequent death of small lateral root branches. Guo *et al.* (2008c) and Valenzuela-Estrada *et al.* (2008) studied the relationship between root anatomy and branch order and found that across all 24 woody species examined, the distal two or three branch orders consistently had a nonwoody structure, an intact cortex and mycorrhizal colonization, whereas the higher-order mother roots showed secondary development, and lacked cortex and mycorrhizal colonization. Based on the anatomical structure of nonwoody roots, and the relationship of this structure to root function, it has been speculated that distal nonwoody branches, which have high metabolic intensity and are not as well-protected as woody roots, might be destined to die rapidly (Wells & Eissenstat, 2003; Guo *et al.*, 2008c). This theory was supported by studies examining root survivorship for each of the distal nonwoody branch orders; these orders were all found to have similarly short life spans (Valenzuela-Estrada *et al.*, 2008; Espeleta *et al.*, 2009). Taken together, these studies suggest that the distal nonwoody branch orders are not only short-lived, but may grow and die simultaneously as intact lateral branches (or modules; *sensu* Harper, 1977).

If the distal nonwoody lateral branches constitute ephemeral modules in woody plants, they should exhibit a certain degree of physiological independence. Previous studies have found that they have higher N concentrations and respiration than higher-order woody roots in a wide range of tree species (Pregitzer *et al.*, 1998, 2002; Valenzuela-Estrada *et al.*, 2008; Li *et al.*, 2010), suggesting that they have greater metabolic intensity. But are their metabolic activities decoupled from their woody mother roots? One way to test this is to see if nonwoody branches exhibit seasonal dynamics of N concentration and respiration that are distinctly different from those of higher-order woody roots.

To test whether nonwoody lateral root branches are ephemeral and physiologically semi-independent modules, it is necessary to track the life history and frequently sample both ephemeral and perennial roots on the same root system. One common method used to study root demography has been minirhizotrons; however, as a result of their small view (2.0 cm × 1.3 cm), minirhizotrons only observe the finest and most short-lived roots (Wells & Eissenstat, 2001; Withington *et al.*, 2006; Valenzuela-Estrada *et al.*, 2008), and infrequently observe thicker and longer-lived perennial fine roots (Guo *et al.*, 2008a). Another technically sophisticated method that has gained prominence in recent years is the use of isotopic tracers, but this method is very expensive and uses a mass-balance approach that may focus more on long-lived coarser roots which dominate total fine-root mass (Gaudinski *et al.*, 2001; Matamala *et al.*, 2003; Joslin *et al.*, 2006; Trumbore *et al.*, 2006). It seems that only rhizotrons (Espeleta *et al.*, 2009), combined with sequential sampling of a root cohort, can quantify the life span of individual roots on the branching root system and at the same time measure tissue N concentrations and respiration rates of all individual roots on the intact fine-root branching systems.

Here, we studied the fine-root system of a common forest tree in northeast China, *Fraxinus mandshurica* (Manchurian ash). The fine-root system of this species has been shown to contain the branch orders that have primary (nonwoody) structure and the branch orders that progress through secondary development and become woody roots (Guo *et al.*, 2008c). Through an 820-d rhizotron observation of both nonwoody and woody branch orders, we tested the hypotheses that nonwoody branch orders, or the first three branch orders in this species, have much shorter life spans than higher-order woody roots, and that these roots grow and die as intact ephemeral units. We grew a root cohort and sampled this cohort by branch order at monthly intervals during two growing seasons to measure the temporal changes of anatomical traits, N concentration and respiration, to test whether ephemeral root modules also exhibit physiological changes over time that are distinct from their woody mother roots.

Materials and Methods

Study site

The study site was located at the Maoershan Research Station (45°21′–45°25′N, 127°30′–127°34′E) of the Northeast Forestry University (Heilongjiang, China). The site has a continental temperate monsoon climate, with mean January, July and annual temperatures of −19.6°C, 20.9°C and 2.8°C, respectively (unpublished data from Maoershan Research Station). The mean annual precipitation is 723 mm, with 477 mm occurring from June to August (Zhou, 1994). The soils are Haplic Luvisols (or Alfisols) with a high organic matter and are well drained (Zhang, 1997; Gong *et al.*, 1999). The soil organic matter was 14.86 ± 0.98, 8.12 ± 0.61 and 5.18 ± 0.58% for soil depths of 0–10, 10–20 and 20–30 cm, respectively. Soil pH was 6.51 ± 0.05, 6.00 ± 0.05 and 5.74 ± 0.06 for soil depths of 0–10, 10–20 and 20–30 cm, respectively. Soil texture was loam, sandy loam and clay loam for soil depths of 0–10, 10–20 and 20–30 cm, respectively. More details of soil characteristics and site information can be found in Wang *et al.* (2006).

Our field experiments were conducted in a 5-hectare 25-yr-old *Fraxinus mandshurica* Rupr. plantation. Three plots, each 25 m × 25 m, were chosen for this study. The site is 490–510 m above sea level, and has an aspect of N10°W and an average slope of *c.* 15°. The mean height of the plantation was 11.55 ± 0.40 m and diameter at breast height was 8.86 ± 0.76 cm at the time of sampling.

Rhizotron and image analysis

In mid-June 2006, we installed 60 rhizotrons in the upper soil layer (0–10 cm), with 20 windows in each plot. Within each plot, 20 trees were randomly selected, and within a 3-m (mostly within 1 m) distance from the tree trunk, a lateral root of fourth to fifth order was identified by tracking it to the base of the tree, and was carefully uncovered. At the mid portion of this root (*c.* 10 cm in length, near the root distal ends), all finer lateral branches were pruned at their bases with scissors. We then carefully placed a Plexiglas acrylic window of 10 cm × 10 cm on the portion of the root where all laterals had been removed. Finally, a black plastic bag containing soil of a weight equal to the soil removed to uncover the roots was placed on the window, to protect rhizotrons from the sunlight, and to press the rhizotrons tightly against the soil. Each rhizotron was installed at an angle of *c.* 30° from horizontal to allow flow of water into the soil beneath the window to minimize changes in soil moisture. During each observation, the black plastic bag was quickly removed from the window, and each window was photographed using a digital camera (G9; Canon, Tokyo, Japan). The plastic bag was replaced once a sequence of images had been collected.

From July 2006 to October 2008, all 60 rhizotrons were photographed at intervals of 10 d to 1 month during the growing season. Photographing usually began at *c.* 06:00 h and lasted for *c.* 3 h, which helped to reduce the impact of intensive sunlight on the roots below the rhizotrons. The roots on the collected images were classified into different orders, as described by Pregitzer *et al.* (2002), and analyzed using RooTracker Ver.2.6.2 (Duke University Phytotron, Durham, NC, USA) to track the life history of individual roots of the first five orders. The life span of individual roots was defined as the time elapsed from the first appearance to death. A root was considered dead when it turned black and shriveled or disappeared (Wells & Eissenstat, 2001), and these visual cues were confirmed by root anatomy and vital staining (de Neergaard *et al.*, 2000).

Our rhizotron method allows the use of a larger observation frame (e.g. 10 cm × 10 cm in this study) compared with minirhizotrons (e.g. 2 cm × 1.3 cm frame-size), but might result in greater disturbance of the soil environment. We tested this disturbance effect 3 months after rhizotron installation and found that the differences in inorganic N concentrations and soil water contents between the soil under the root windows and the adjacent undisturbed soil were all within 10% (Supporting Information Table S1), suggesting that disturbance of the soil environment by the root windows was limited.

Establishment of a root cohort and measurements of root diameter, anatomical traits, N concentrations and respiration

In July 2007, we established a root cohort and sampled this cohort over the next 2 yr. A total of 445 fourth to fifth roots were randomly selected. As described earlier, under the heading 'Rhizotron and image analysis', all lateral branches along the mid portion of these roots were pruned. The bare portion of each root was marked with two long, thin, bright-red plastic threads at each end, and this root was then put back to its original position and buried in the soil. One month later, the soil around these roots was carefully brushed off and all new lateral branches initiated on the bare portion of each root were exposed. These new lateral branches were considered as a root cohort born within 1 month. Each root (previously selected) and its newly initiated lateral branches were photographed at the first observation, and the position of each new lateral branch on the photograph was carefully marked, in order to identify the roots belong to this cohort in future sampling. After photographing, all roots were covered again with soil and great care was taken to minimize disturbance of the roots.

The new lateral branches and their mother roots that we initially selected were sampled 12 times during the growing seasons of the next 2 yr. The specific months of sampling were August, September and October of 2007, May, June, July, August, September and October of 2008, and May, June and July of 2009. At each sampling, a total of 30 root-branching systems, including new lateral branches and the mother roots we initially selected, were sampled from all three plots (10 per plot). The remaining roots were left to grow in the soil until being sampled. Sampling was terminated after July 2009 because the remaining roots were insufficient for the mass required for respiration studies and tissue chemistry analysis.

Once sampled, each root sample was divided into two subsamples. One was gently washed in deionized water and immediately fixed in Formalin-Aceto-Alcohol (FAA) solution (90 ml of 50% ethanol, 5 ml of 100% glacial acetic acid and 5 ml of 37% methanol) for anatomical analyses. The other was immediately put in a cooler and transported to the laboratory, where respiration measurements were performed within 5 h (Pregitzer *et al.*, 1998). In the laboratory, roots were dissected into different orders, as described in Pregitzer *et al.* (2002), in deionized water at *c.* 1°C. Excess water was blotted and subsamples of at least 0.5 g (FW) were prepared for respiration measurements.

Respiration, determined as O_2 consumption, was measured at 24°C using gas-phase O_2 electrodes (Model LD 2/2; Hansatech, Norfolk, UK) connected to constant-temperature circulating water baths, as described in Burton *et al.* (2002). Once measured for respiration, root subsamples were frozen and transported to the Elementary Analysis Laboratory at Peking University where measurements of N concentration were carried out using an elemental analyzer (Vario Micro cube; Elementar, Hanau, Germany).

Subsamples fixed in FAA solution were transported to the laboratory for anatomical analyses. In each sampling, at least 15 individual root segments for first-, second- and third-order (respectively) root-anatomical analyses, and five segments for fourth- and fifth-order (respectively) root-anatomical analyses, were randomly chosen. Individual root segments were stained with Safranine-fast green, dehydrated in a set of alcohol solutions, embedded in paraffin and 8-μm-thick sections were prepared (de Neergaard *et al.*, 2000). These sections were photographed under a compound microscope (BH1; Olympus, Tokyo, Japan), measured to determine root diameter to the nearest 1 μm and analyzed to establish the degree of mycorrhizal colonization and the anatomical structure. The rates of mycorrhizal colonization (represented by the appearances of hypha coils or arbuscules on any section of a root segment; see Brundrett, 2004; Vierheilig *et al.*, 2005) and secondary growth (represented by the appearance of both secondary xylem and a continuous cork layer on any section of the root segment; see Guo *et al.*, 2008c) were calculated based on the frequency of occurrence across individual root segments for each root dissected by order.

Data analysis

A Kaplan–Meier model was used for survivorship analysis, from which the median life span was estimated (Kaplan & Meier, 1958; Majdi *et al.*, 2001). The log-rank test was used to compare the survival curves of different branch orders. Differences in root diameter, secondary growth rate, mycorrhizal colonization and standing biomass among different orders were analyzed using one-way ANOVA with Dunnett's test for multiple comparisons, and by a nonparametric test (Kruskal–Wallis test) when the normal assumption was not met (but the results from this method were not shown when they yielded the same results as those from ANOVA). Pearson correlation coefficients were calculated for N concentration and respiration among different branch orders.

Root production per year, N input per year and total respiration for each order were calculated based on the life span estimates, mean N concentration and respiration (on a mass basis) in this study, and on the standing biomass reported in the previously published study of the same plantation (Wang *et al.*, 2006). Because of their low mortality rate, the median life spans of the fourth- and fifth-order roots could not be estimated directly, and were assumed to be 8 yr. The justification for this assumption was the reported woody-root life span of 5–13 yr (Matamala *et al.*, 2003; Gaudinski *et al.*, 2010). All statistical analyses were performed using SPSS software (2001, version 13.0; SPSS Inc., Chicago, IL, USA).

Results

Root life-history

Through rhizotron windows, we tracked 95 lateral root clusters produced from the fourth- and fifth-order roots that were initially selected. Some roots observed but lacking clear visual images were not included in the analysis. Most root clusters consisted of the first three orders: among 95 clusters, 70 included the first three orders, 17 included the first two orders and eight were individual first-order roots. In total, 679 roots of the first order, 168 of the second order, 70 of the third order, 52 of the fourth order and 52 of the fifth order were included in the analysis (Table 1).

The first three orders of roots were similar in their mortality patterns (Fig. 1). The survivorship of first-, second- and third-order roots decreased rapidly with time, and by the end of the study, > 90% of these roots had died (Fig. 1, Table 1). The median life spans (represented by the age at which 50% of roots of a given order had died) for first-, second- and third-order roots were 510, 666 and 692 d, respectively (Table 1). By contrast, the fourth- and fifth-order roots consistently showed a much lower mortality rate than roots of the first three orders (Fig. 1). By the end of the study, only five out of 52 fourth-order roots and four out of 52 fifth-order roots had died (Fig. 1; Table 1). Because of such a low mortality rate (< 10%), the median life span could not be estimated for these two orders (Table 1).

Table 1 Survivorship of *Fraxinus mandshurica* roots by branch order from July 2006 to October 2008

Branch order	Sample size	Mortality (d)[1]			Final survivorship (%)[2]
		25%	50%	75%	
1	679	391	510	697	1.73 ± 0.21[a]
2	168	391	666	731	2.74 ± 1.38[a]
3	70	457	692	731	5.42 ± 2.72[a]
4	52	> 820	> 820	> 820	90.41 ± 1.86[b]
5	52	> 820	> 820	> 820	92.27 ± 2.01[b]

[1]Number of days until cumulative mortality rates reach 25, 50 and 75%, estimated by survival analysis (Kaplan–Meier).
[2]Percentage of roots still alive when the 820-d study ended.
Different superscript letters indicate significant differences among values for Dunnett's *t*-test at $P < 0.05$.

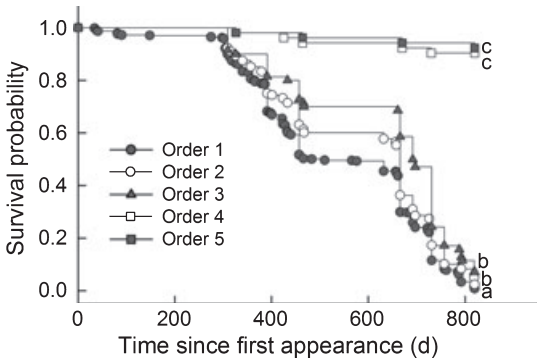

Fig. 1 Root survivorship of the first five branch orders of *Fraxinus mandshurica* during the study period from July 2006 to October 2008. Different letters indicate significant differences for the log-rank test at $P < 0.01$.

In most cases, first-, second- and third-order roots died together within a 1-month observation period (Fig. 2). The majority, or 70.1%, of the first-order roots died together with their third-order mother roots within a 1-month observation period, increasing to 81.9% in 2 months (Fig. 2). For the second-order roots, 71.4% died together with their third-order mother roots within a 1-month observation period, increasing to 86.5% in 2 months (Fig. 2).

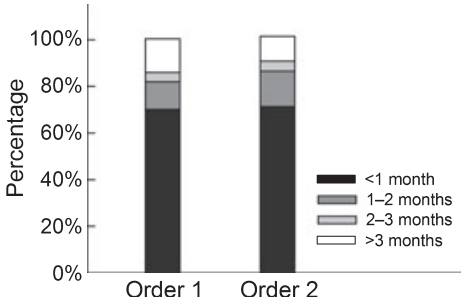

Fig. 2 Percentage of first-order and second-order roots of *Fraxinus mandshurica* that died with their third-order mother roots within different observation periods.

Fig. 3 Average life span, from emergence to death, for the *Fraxinus mandshurica* roots of the first three orders. Closed circles, mean ± SE of the emergence date; open circles, mean ± SE of the death date. Different lower-case letters indicate significant differences among the values for Dunnett's *t*-test at $P < 0.05$ (a, b and c indicate the differences in emergence date among the three orders; x and y indicate the differences in death date among the three orders).

On average, third-order roots emerged earlier, yet died later, than first- and second-order roots (Fig. 3). Third-order roots were born significantly earlier than first- and second-order roots, and second-order roots were born significantly earlier than first-order roots ($P < 0.05$, Fig. 3). During the mortality process, first-order roots died significantly earlier than third-order roots ($P < 0.05$, Fig. 3). However, no significant difference in the death rate was found between first- and second-order roots ($P > 0.05$, Fig. 3), or between second- and third-order roots ($P > 0.05$, Fig. 3).

Root diameter and anatomical traits

The mean root diameter increased significantly as the root order increased (Table 2), from 0.21 ± 0.01 mm in first-order roots to 1.32 ± 0.05 mm in fifth-order roots. The root anatomy of the first three orders of roots was similar (Table 2). All lacked secondary growth, had an intact cortex and were substantially colonized by mycorrhizal fungi, with a colonization rate of 46.2, 40.5 and 34.6%, respectively (Table 2). By contrast, fourth- and fifth-order roots showed a completely different anatomical structure. Both orders had a well-developed secondary xylem and a continuous cork layer (indicators of secondary growth), and lacked cortex and mycorrhizal colonization (Table 2).

Root N concentrations

Generally, the mean N concentration declined significantly as the branch order increased (Fig. 4) but the rate of decline across successive root orders varied. The first root order had the highest mean N concentration, of 2.54%. The mean N concentration declined moderately from first-order to second-order roots, and from second-order to third-order roots (Fig. 4). By contrast, the mean N concentration decreased sharply from third-order to fourth-order roots, but again moderately from fourth-order to fifth-order roots, to a mean concentration of 0.91% in fifth-order roots (Fig. 4).

Although first-, second- and third-order roots differed in mean N concentration, their seasonal variation in N concentration was almost identical (Fig. 4). Consequently, these distal roots exhibited a very strong correlation with each other in terms of N concentration (correlation coefficients ranging from 0.98 to 0.86, $P < 0.01$) (Fig. 4; Table 3). By contrast, fourth- and fifth-order roots lacked notable changes in N concentration with season (Fig. 4), and showed no correlation with the N concentrations found in the first, second and third root orders (Fig. 4; Table 3).

Table 2 Root traits of different branch orders of *Fraxinus mandshurica* in the upper soil (0–10 cm)

Root order	Root diameter (mm)[1]	Secondary growth rate (%)[1]	Mycorrhizal colonization (%)[1]	Standing biomass (g m^{-2}) [%][2]	Median life span (yr)	Root production per year (g m^{-2}) [%][2]	Nitrogen input per year (g m^{-2}) [%][2]	Respiration rate (nmol s^{-1} m^{-2}) [%][2]
1	0.21 ± 0.01[a]	0.0 ± 0.0	46.2 ± 4.3[d]	44.5 ± 5.0[b] [32.1]	1.4	31.9 [54.7]	0.85 [63.6]	906.0 [46.6]
2	0.24 ± 0.01[b]	0.0 ± 0.0	40.5 ± 5.7[c]	19.2 ± 2.3[a] [13.8]	1.8	10.5 [18.1]	0.25 [18.7]	352.9 [18.1]
3	0.43 ± 0.02[c]	0.0 ± 0.0	34.6 ± 3.1[b]	16.3 ± 1.1[a] [11.8]	1.9	8.6 [14.7]	0.17 [12.5]	271.3 [13.9]
4	0.83 ± 0.05[d]	100.0 ± 0.0	0.0 ± 0.0[a]	18.5 ± 3.3[a] [13.3]	8.0*	2.3 [3.9]	0.02 [1.7]	154.5 [7.9]
5	1.32 ± 0.05[e]	100.0 ± 0.0	0.0 ± 0.0[a]	40.2 ± 3.2[b] [29.0]	8.0*	5.0 [8.6]	0.04 [3.4]	261.1 [13.4]

Mean values ± SE are shown; different superscript letters indicate a significant difference among values for Dunnett's *t*-test at $P < 0.05$. Values in '[ ]' show the percentages of the first five root orders.

[1]Root diameter, secondary growth rate and mycorrhizal colonization are shown as mean ± SE across all 12 sample dates during the study period from August 2007 to July 2009.

[2]Root production per year, nitrogen (N) input per year and respiration (on an area basis) were estimated based on the standing biomass data from the published study of the same plantation (Wang *et al.*, 2006), and the life span, mean [N] and respiration rate (on a mass basis) obtained in this study. Median life spans of the root orders 4 and 5 orders (marked with *) lacked direct measurement and were assumed to be 8 yr (details on the assumption are given in the Materials and Methods section).

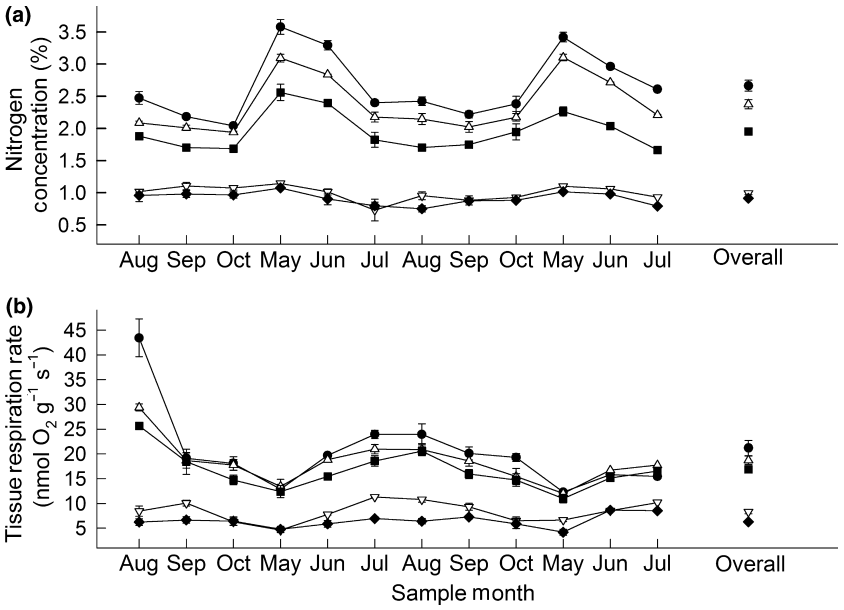

Fig. 4 Nitrogen concentration (a) and respiration (b) of first-order (circles), second-order (triangles, apex up), third-order (squares), fourth-order (triangles, apex down) and fifth-order (diamonds) roots of *Fraxinus mandshurica* over time. Each point connected by a line represents the mean ± SE of three replicate plots sampled in a given month. The separated points on the right show the mean ± SE of 12 means of three replicate plots sampled during the entire study period from August 2007 to July 2009.

Root respiration

Similarly to N concentration, respiration decreased significantly with increasing branch order (Fig. 4). The first-order roots had the highest mean respiration, reaching 20.7 nmol O_2 g^{-1} s^{-1}. The mean respiration value declined moderately from first-order roots to third-order roots, but sharply from third-order to fourth-order roots, with fourth-order roots having a mean respiration rate of 8.1 nmol O_2 g^{-1} s^{-1} (Fig. 4). The mean respiration rate for fifth-order roots was even lower (6.2 nmol O_2 g^{-1} s^{-1}) (Fig. 4). Mean respiration in the first three orders of roots showed similar temporal patterns (Fig. 4), and there was a strong correlation among the first three ephemeral branch orders, but not between these orders and the fourth and fifth orders (Table 3).

Annual root production and N input

For the first five root orders, nonwoody roots accounted for 32.1, 13.8 and 11.8% of standing root biomass (orders 1, 2 and 3, respectively), 54.7, 18.1 and 14.7% of root production per year, 63.6, 18.7 and 12.5% of N input via root mortality per year, and 46.6, 18.1 and 13.9% of total respiration (Table 2). By contrast, fourth- and fifth-order roots represented 13.3 and 29.0% of standing biomass, but both orders contributed < 9% of root production and N input per year for all five root orders combined (Table 2).

Table 3 Pearson correlations for nitrogen (N) concentrations and respiration rates (in parentheses) among different root branch orders of *Fraxinus mandshurica*

	Order 1	Order 2	Order 3	Order 4
Order 2	0.98* (0.88*)			
Order 3	0.88* (0.84*)	0.86* (0.90*)		
Order 4	0.14 (0.28)	0.16 (0.42*)	0.17 (0.53*)	
Order 5	0.24 (0.10)	0.26 (0.26)	0.29 (0.35)	0.49* (0.49*)

*Correlations are significant at $P < 0.05$.

Because of the lack of direct mean (median) life span estimates of fourth- and fifth-order roots, these estimates were approximate.

Discussion

Test of the central hypothesis

The central hypothesis of this study was that ephemeral modules exist within the branching *F. mandshurica* fine-root system in the form of distal nonwoody lateral branches, which were found to be composed of roots from first to third branch order in this study. We also tested the hypothesis that these nonwoody lateral branches show anatomical, nutritional and physiological patterns that are distinct from their higher-order woody mother roots. Our hypotheses were strongly supported by three lines of evidence.

First, we found that in *F. mandshurica*, lateral branches composed of the first three orders have a much shorter life span than their woody mother roots. During the 820-d experiment, > 90% of these nonwoody roots (orders 1–3) died, in sharp contrast to the mortality rate of < 10% in their woody mother roots (i.e. fourth- and fifth-order roots; Fig. 1; Table 1). Moreover, the median life span among the first three orders was so similar (Table 1) that the turnover (i.e. repeated growth and mortality cycle) of the low orders (e.g. first order) within the intact lateral branching systems of first-order to third-order roots would be unlikely, otherwise first- and second-order roots should have much shorter life spans than the third-order roots. Most of the first- and second-order roots (> 70%) died together with their third-order mother roots within a 1-month observation period, further supporting the conclusion that first three root orders comprised a growth-and-death unit (or a module) in the root system of *F. mandshurica* (Fig. 2). The differences in root life span among the first three branch orders were mainly caused by the differences in the date of initiation: third-order roots initiated earlier than their daughter roots (Fig. 3). By comparison, the date of death was more similar among the first three orders of roots (Fig. 3). Based on these findings, we conclude that the distal three orders turn over frequently as intact lateral branches on their higher-order mother roots, and thus form ephemeral modules in the root system of this species.

Although our study is the first to directly estimate the life span of both ephemeral and perennial branch orders (first- to fifth-order roots), our results are consistent with previous reports on root life span in woody species. Several studies using minirhizotrons have reported that root life span was generally < 2 yr for first- to third-order roots and that the difference in root life span among these orders was small (Wells & Eissenstat, 2001; Withington *et al.*, 2006; Valenzuela-Estrada *et al.*, 2008). Studies using ^{13}C tracers in elevated CO_2 experiments, by contrast, showed that pine roots < 1 mm in diameter (probably including some fourth- and fifth-order roots) had mean resident C times of 4.0–4.6 yr, and roots between 1 and 2 mm (probably mainly consisting of fifth- or higher-order roots) had mean resident C times of 5.1–6.6 yr (Matamala *et al.*, 2003), suggesting that some fine roots may live for > 5 yr. Studies using bomb ^{14}C showed a mean C age of 3–11 yr or 4–11 yr for live fine-roots as a whole (< 2 mm) (Gaudinski *et al.*, 2001; Joslin *et al.*, 2006), also suggesting long-lived fine roots. These studies almost certainly confound the life span of perennial roots with the life span of ephemeral distal branches, and their results show that a portion of fine roots (which are probably perennial woody roots) must be very long-lived. A recent study, based on model simulations, clearly showed that in fine roots of trees, a short-lived group may live for < 1 yr but a long-lived root group can live for > 10 yr (Gaudinski *et al.*, 2010).

Second, the ephemeral lateral root branches maintained distinct anatomical traits throughout their life span. Our previous study (Guo *et al.*, 2008c) demonstrated that in 23 temperate woody species, including *F. mandshurica*, the first two to three orders were nonwoody. However, because samples were taken only once by Guo *et al.* (2008c), it was unclear whether the primary structure of these nonwoody roots would be maintained over time. Here, we found that during their entire life, the first three root orders were restricted to primary structure, containing a living cortex and being frequently mycorrhizal. Because these anatomical traits have been associated with absorptive capacity (Pregitzer *et al.*, 1998; Wells & Eissenstat, 2003; Guo *et al.*, 2008c; Valenzuela-Estrada *et al.*, 2008), we conclude that these nonwoody roots serve resource-absorption functions and never go through secondary growth, and were therefore unlikely to become part of the perennial root system. As such, these nonwoody roots are analogus to leaves on the shoot system, in that they are destined for resource acquisition and do not experience a shift in function during their life history. By comparison, all fourth- and fifth-order roots sampled during this study lacked cortex and mycorrhizal symbiosis, and showed well-developed secondary vascular bundles and continuous cork layers. The anatomical structure of woody roots suggests that they have very limited

capacity for resource uptake (Guo *et al.*, 2008c; Valenzuela-Estrada *et al.*, 2008), but may be resistant to environmental stress (Wells & Eissenstat, 2003; Guo *et al.*, 2008c), which may, in part, explain their low mortality rates and long life span.

It should be noted that the nonwoody roots studied here belong to the lateral branches that have determinate growth, analogus to the 'short roots' in the pine root system, as proposed by Wilcox (1964). Lyford (1975) and Eissenstat & Achor (1999) also recognized that these distal fibrous roots differ fundamentally from pioneer roots (or 'long roots' in the terminology of Wilcox's pine root classification), may often grow into longer roots, produce many 'short' determinate laterals and eventually go through secondary development. Fourth- or higher-order woody roots may belong to the latter type.

Third, the ephemeral lateral branches as a whole showed distinct nutritional and physiological patterns throughout their life history. The first three root orders, which had high N concentrations and a high degree of respiration, exhibited marked cyclic temporal patterns tuned to seasonality in these two physiology-related parameters (Fig. 4). This contrasts sharply with the lack of any appreciable temporal pattern in the fourth- and fifth-order roots, which had low N concentrations and a low degree of respiration (Fig. 4). Even more striking was the tight coupling among the first three orders to temporal patterns of N concentration and respiration (Fig. 4). Consequently, the first three orders showed strong correlation amongst themselves in terms of N concentration and respiration, but only weak or no correlation with fourth- and fifth-order roots (Table 3). These findings suggest that nonwoody lateral branches act as semi-independent physiological units. Previous studies were unable to recognize this phenomenon, mainly because roots were sampled only once in their life span (e.g. Guo *et al.*, 2008c; Li *et al.*, 2010) or during the same season (e.g. Pregitzer *et al.*, 2002).

Implications and uncertainties

The findings of this study demonstrate that root systems of perennial plants may be organized like shoot systems, with rapidly cycling modular units devoted to energy-expensive resource-acquisition processes located at the distal ends of the branching perennial woody shoot or root systems. Although we have only demonstrated that this is the case for *F. mandshurica*, similarities in the relationship between anatomy and branching architecture across all 24 woody species examined thus far (Guo *et al.*, 2008c; Valenzuela-Estrada *et al.*, 2008) suggest that the same functional organization may be widespread in woody plants. This would dramatically change our view of root structure and function compared with the traditional coarse–fine root dichotomies based on arbitrary size classes.

Another important implication of this work is that if ephemeral root modules are in the form of lateral nonwoody branches, sampling these lateral branches would save a tremendous amount of resources (e.g. time) in comparison with separating roots by branch order. Taking a more functional definition of the diameter cut-off between the roots of only primary growth and those with secondary growth (e.g. 0.5 mm in *F. mandshurica*, Table 2) would be also far superior to an arbitrarily chosen diameter and still capture much of the variation between absorptive and structural roots.

It should be emphasized that not all root 'modules' are necessarily of three orders, even in the species studied here. In *F. mandshurica*, most 'modules' were composed of the first three orders of roots, but some also consisted of the first two, or just first-order roots. This variability within species and potential variability across different species should be investigated further.

We also recognize that the rhizotron method used here may have resulted in the identification of roots that are not identical to naturally grown roots. We examined this possibility and found the following: rhizotron-induced changes to the soil mineral N content and moisture were minimal (Table S1); the morphology and chemistry (tissue N content) of roots in the current study were comparable to those of naturally growing roots from the same sites (see Wang *et al.*, 2006); and life span estimates of roots in the present study were comparable to those from minirhizotrons at the same site (see Yu, 2006, who showed that the median life span for first-order roots of *F. mandshurica* was 350 ± 12 d, and for second- and third-order roots combined (termed higher orders) was > 500 d). We therefore conclude that our results may not deviate considerably from the natural root life cycle for *F. mandshurica* at this study site. However, the potential artifacts of the rhizotron method deserve further investigation given that this method may have to be applied if ephemeral modules are to be observed among diverse plant taxa.

We found that the ephemeral nonwoody lateral branches, though representing only 57.7% of the standing biomass out of all fine roots, contributed 87.4% of the biomass turnover, 94.8% of the N input to the soil annually and 78.6% of total fine-root respiration (Table 2). Therefore, these ephemeral 'modules' seem to be the primary driver of root turnover in the *F. mandshurica* root system. However, these estimates were only approximate because the life spans of fourth- and fifth-order roots were not directly measured as a result of their very low mortality rate (< 10%) during our study period. If these woody roots actually turn over faster than we assumed (once every 8 yr), their contribution to root turnover would be greater than our estimates.

Despite the uncertainty in turnover estimates of higher-order woody roots, our results clearly showed that root-turnover estimates based on the fine-root concept (i.e. all

roots of < 2 mm cycle rapidly) would significantly overestimate actual root turnover, mainly because the ephemeral root modules identified here, and possibly in other trees (Wang *et al.*, 2006; Guo *et al.*, 2008b), have a much smaller mass than fine-root pools identified using single-diameter class (0–2 mm). Moreover, evidence shows that the lateral root modules, once dead, may decompose slowly (Fan & Guo, 2010), a concept quite at odds with the existing literature. As such, the view that C cycling in the soil system is dominated by the decomposition of older detrital matter should also be reconsidered, as suggested by Högberg & Read (2006).

Conclusion

The conventional single-diameter classification of fine roots has failed to differentiate short-lived roots from long-lived roots because arbitrary diameter classes lack a functional basis. Here, we provide evidence that in the *F. mandshurica* fine-root system, the short-lived roots are nonwoody lateral branches (termed 'ephemeral root modules' here). These modules have a primary structure and mycorrhizal colonization throughout their life span. This novel finding provides a basis for separating roots of different life span and function. Within the ephemeral root module, the differences among roots in age, mycorrhizal colonization and initial growth rates that probably have important consequences for their respiration, nutrient uptake capacity and longevity, can be further characterized by branch order and root age-cohorts, as shown in this study.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Characteristics (mean ± SE) of the soil under the root windows (disturbed) and the adjacent natural conditions (undisturbed) in 0–2 cm soil layer on September 15th, 2006

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