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Research paper

Relationships between root respiration rate and root morphology, chemistry and anatomy in *Larix gmelinii* and *Fraxinus mandshurica*

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Tree roots are highly heterogeneous in form and function. Previous studies revealed that fine root respiration was related to root morphology, tissue nitrogen (N) concentration and temperature, and varied with both soil depth and season. The underlying mechanisms governing the relationship between root respiration and root morphology, chemistry and anatomy along the root branch order have not been addressed. Here, we examined these relationships of the first- to fifth-order roots for near surface roots (0–10 cm) of 22-year-old larch (*Larix gmelinii* L.) and ash (*Fraxinus mandshurica* L.) plantations. Root respiration rate at 18 °C was measured by gas phase O_2 electrodes across the first five branching order roots (the distal roots numbered as first order) at three times of the year. Root parameters of root diameter, specific root length (SRL), tissue N concentration, total non-structural carbohydrates (starch and soluble sugar) concentration (TNC), cortical thickness and stele diameter were also measured concurrently. With increasing root order, root diameter, TNC and the ratio of root TNC to tissue N concentration increased, while the SRL, tissue N concentration and cortical proportion decreased. Root respiration rate also monotonically decreased with increasing root order in both species. Cortical tissue (including exodermis, cortical parenchyma and endodermis) was present in the first three order roots, and cross sections of the cortex for the first-order root accounted for 68% (larch) and 86% (ash) of the total cross section of the root. Root respiration was closely related to root traits such as diameter, SRL, tissue N concentration, root TNC : tissue N ratio and stele-to-root diameter proportion among the first five orders, which explained up to 81–94% of variation in the rate of root respiration for larch and up to 83–93% for ash. These results suggest that the systematic variations of root respiration rate within tree fine root system are possibly due to the changes of tissue N concentration and anatomical structure along root branch orders in both tree species, which provide deeper understanding in the mechanism of how root traits affect root respiration in woody plants.

Keywords: root cortical proportion, root diameter, root respiration rate, tissue N concentration.

Introduction

Fine roots play an important role in carbon (C) and nutrient dynamics in forest ecosystems (Hendrick and Pregitzer 1993; Gill and Jackson 2000); primary production and respiration of fine root and mycorrhiza determine the total amount of carbon allocated to roots (Haynes and Gower 1995), which may account for nearly 33% of the total net primary production (Jackson et al. 1997). Fine root morphology or architecture influences root turnover, carbon balance and nutrient cycling in forest ecosystems (Eissenstat and Yanai 1997; Norby and Jackson 2000; Pregitzer et al. 2002). For example, Wells et al. (2002) found that thinner roots had a shorter lifespan (or higher turnover rate) in peach (*Prunus persica* L.) trees. Guo et al. (2008*ab*) reported that first-order roots (i.e., the most distal roots or root tips) contributed ~50 and 64% to C and associated nitrogen (N) fluxes to soil with turnover, respectively,

for the first three orders combined in a longleaf pine (*Pinus palustris* Mill.) forest. Despite the general knowledge that fine roots have different morphologies within the complex lateral branching system (Pregitzer et al. 2002; Wang et al. 2006; Guo et al. 2008*b*; Valenzuela-Estrada et al. 2008), little is known about the interrelationship between root morphology and function (Hishi 2007). Understanding the function of different root orders may improve our ability to estimate and model root system respiration, which can account for 40–60% of the total soil respiration in forest ecosystems (Hanson et al. 2000; Högberg et al. 2001; Fahey et al. 2005).

Root respiration provides the driving force for root growth and maintenance and for ion absorption and transport into the xylem (Lambers et al. 2008), which represents the root physiological metabolic capacity (Ryan et al. 1996; Wells and Eissenstat 2003). Root respiration varies with soil depth (Pregitzer et al. 1998), soil N availability (Jia et al. 2010; Burton et al. 2012), temperature (Burton et al. 2002) and CO_2 level (Drake et al. 2008), and it is also closely related to root diameter (Pregitzer et al. 1998; Burton et al. 2012), tissue N concentration (Burton et al. 2002) and total non-structural carbohydrates (TNC) concentration (Xu et al. 2008). Thinner roots have higher tissue N concentration than thicker roots and enhanced respiration rates (Ryan et al. 1996; Pregitzer et al. 1998; Burton et al. 2002; Makita et al. 2009). Most previous studies in root respiration focused on the fine roots less than 1 or 2 mm in diameter (Ryan et al. 1996; Pregitzer et al. 1998; Burton et al. 2002) and were based on the view that all roots of a given size class function in the same way (Pregitzer et al. 2002; Joslin et al. 2006; Guo et al. 2008*b*). However, tree roots branch into different hierarchies (i.e., root orders) from basal to successively more distal roots, which differ markedly in morphology, chemistry and physiology (Majdi et al. 2001; Pregitzer et al. 2002; Wells and Eissenstat 2003). Even fine roots less than 1 or 2 mm in diameter can consist of numerous branching orders (Pregitzer et al. 2002; Wang et al. 2006; Valenzuela-Estrada et al. 2008). Recent studies have shown that the first-order roots exhibit thinner diameter, higher specific root length (SRL) and greater tissue N concentrations than higher order roots in woody plants (Pregitzer et al. 2002; Guo et al. 2004; Withington et al. 2006; Valenzuela-Estrada et al. 2008). More importantly, fine roots within the first five branch orders vary markedly in anatomy in both conifer and hardwood species (Guo et al. 2008*b*). First-order roots with an intact cortex have high mycorrhizal colonization and exhibit typical primary development, while higher order roots (fourth and fifth) show mostly secondary development with a continuous cork layer and no mycorrhizal colonization (Guo et al. 2008*b*). Cortical cells are living parenchyma, and generally have high metabolic rate and require higher N concentration to support their physiological activity (Lux et al. 2004; Hishi 2007). The greatest respiration rates coupled with the highest N concentrations were found in the first-order roots by Jia et al. (2011) and Xia et al. (2010) in larch (*Larix gmelinii* L.) and ash (*Fraxinus mandshurica* L.) plantations. These studies suggest that the respiration of tree roots is not only related to root morphology and chemistry but also potentially related to their anatomical traits (Hishi 2007). Overall, root branch order seems likely to influence root physiological functions within and among species (Pregitzer et al. 2002; Hishi 2007; Guo et al. 2008*b*). Thus, studies linking root respiration to morphology, chemistry and anatomy on the branching fine root systems may more clearly represent the heterogeneity of root respiration, and may deepen the understanding of root form and physiological functions such as C allocation and cycling in forest ecosystems.

In the present study, we examined the relationships between root respiration and root morphology (diameter and SRL), tissue chemistry (total N, soluble N and TNC concentrations) and anatomy (cortical thickness, stele diameter) among the first five root orders in the near surface soil (0–10 cm) in larch and ash plantations. The following questions were addressed: (1) How does fine root respiration rate change along the branching order? (2) Are root respiration rates correlated with root morphology, chemical compositions and anatomical traits?

Materials and methods

Study site

Larch and ash are commercial tree species extensively used in plantations in northeastern China. The study was conducted on plantations at the Maoershan Experimental Station (127°30′–127°34′E, 45°21′–45°25′N) of Northeast Forestry University, Heilongjiang province, China. This region is characterized by a cold, temperate-zone, continental, monsoon climate with an average annual temperature of 2.8 °C and average daily air temperatures ranging from –19.6 °C in January to 20.9 °C in July. The mean annual precipitation is 723 mm, of which 477 mm occurs in June, July and August, and the mean annual evapotranspiration is 1094 mm. The soil is loam (Hap-Boric Luvisol) with a medium organic matter content of 13.6 and 14.9% at 0–10 cm layer in larch and ash plantations, respectively; the average soil temperature at 10 cm depth is 1.5, 14.2 and 5.8 °C in May, July and October for larch plots, and 2.0, 16.0 and 4.5 °C for ash plots; more details about the soil were given by Wang et al. (2006). During winter (December–April), the soil is usually frozen to a depth of 1 m. Both plantations were established in 1986 by planting 2-year-old seedlings on a 1.5–2.0 m planting grid. In 2007, the larch and ash trees had average trunk diameters of 13.9 and 10.3 cm at breast height, and the heights of the trees were 15.3 and 15.4 m, respectively.

Experimental design

The experiment was a subset of a larger parent experiment designed to test the effect of N fertilizer on different aspects of

stand performance (Jia et al. 2010). The two species were in separate plantations at separate experimental sites; the experimental design and sampling and measurement protocol described below were the same for both species. The parent experiment for each species was a split-split-plot randomized complete block (RCB) experimental design with three replicates and two levels of fertilization (since 2002) with six 20 × 30 m plots. In the present experiment, only the three control or unfertilized plots were sampled in each species at three times, October 2007, May 2008 and July 2008. Roots for soluble N measurements were sampled later in May, July and October 2009. Roots from each sampling date were partitioned into five root orders for subsequent measurements. The present experimental design was thus a split-plot RCB with three replicates; the whole plot treatment was sampling date with three levels as given above, and the sub-plot treatment was root order with five levels, 1–5. Root respiration, chemical and anatomical parameters were measured for samples from each plot, each sampling date and each root order for a total of 45 measurements for each root parameter.

Root sample collection

Root sampling was done thrice each day, 6:00, 13:00 and 17:00 h, and preparation of roots and respiration measurements were completed within 4 h of sample collection. A crew of four people was assigned the task of preparing the root samples so that the respiration measurements could be completed within the 4 h time limit.

Three soil blocks were collected at random from each control plot (no fertilizer) in both plantations with a specially constructed 20 × 20 cm rectangular soil corer with sharpened edges. Each soil block was sampled at a depth of 0–10 cm, and all three block samples from each plot were mixed by hand to form a composite sample. Several large roots with all five branch orders were removed and placed in formalin-aceto-alcohol solution (90 ml 50% ethanol, 5 ml 100% glacial acetic acid and 5 ml 37% methanol) to stabilize for subsequent anatomy analyses; the remaining root samples were placed in a cooler and immediately transported to the experiment station laboratory near the plantations. Each composite sample was divided into sub-samples while submersed in deionized water. Roots for TNC analysis were killed in a microwave and stored in a refrigerator for later analysis.

Root respiration, root morphology and root tissue N concentration

Root respiration measurements were made as soon as possible after sampling and were completed within 4 h of sampling. The roots were incised with scissors at branch nodes and separated into five root branch orders (with root tips designated as first-order roots) following the procedure described in Pregitzer et al. (2002). Root samples (0.5 g fresh weight) were immersed in a constant temperature circulating water bath at 18 °C and allowed to equilibrate for 30 min. Root respiration was then measured at 18 °C by measuring O_2 consumption using gas-phase O_2 electrodes (Model LD 2/2, Hansatech Instruments Ltd, King's Lynn, UK) connected to the circulating water baths (Burton et al. 2002). Two complete O_2 electrode systems and water baths were used allowing simultaneous respiration measurements to be performed on separate root samples.

Following respiration measurements, the samples were immediately placed in a refrigerator at 4 °C, and later transported to the university laboratory in a cooler. The same samples from each root order were scanned using an Expression 10000XL 1.0 scanner (Epson Telford Ltd, Telford, UK). Root images were analyzed by WinRHIZO (Pro2004b) software (Regent Instruments Company, Canada) for the diameter and length of each individual root. The same root samples were then oven-dried at 75 °C for 24 h and weighed. Root respiration was calculated as nmol $O_2 \cdot g^{-1} \cdot s^{-1}$ (dry weight) and SRL ($m \cdot g^{-1}$) was calculated by dividing total root length of each branch order by the corresponding dry weight. The dried root samples were then ground and the total N concentration of each of the five root orders was measured with a Kjeltec 2300 Analyzer (Foss Tecator AB, Höganäs, Sweden).

Soluble sugar and starch content

Total sugar and starch concentrations were measured by the modified phenol–sulfuric acid method (Buysse and Merckx 1993), and TNC concentrations were calculated from the sugar and starch concentrations. Roots which had previously been killed in a microwave oven were separated into branch orders, dried and ground. Soluble sugar was extracted by steeping 40 mg of dried ground roots in 10 ml of 80% ethanol overnight, and then centrifuging at 2200–5000 rpm for 15 min. The extraction process was repeated three times, and the supernatants of the three extractions were combined for sugar analysis. The residue was prepared for the analysis of starch concentration by drying at 100 °C for 3 h, hydrolyzing with 3 ml 3% HCl in a boiling water bath for 0.5 h and then filtering. Supernatants and filtrates were analyzed for glucose as soluble sugar and starch concentration, using phenol–sulfuric acid against a glucose standard; the absorbance was read at 490 nm by a UV–VIS spectrophotometer.

Soluble N concentration

Roots for soluble N concentration were cut with scissors at the branch nodes, separated into branch orders in the ice water as soon as possible after sampling and then stored in liquid nitrogen. First- and second-order roots were ground with a mortar and pestle in liquid nitrogen. Soluble protein was extracted from 100 mg of ground root powder with distilled water at 4 °C for 30 min, and then centrifuged for 10 min at 10,000 rpm.

Soluble protein concentration was determined with Coomassie Blue reagent by a spectrophotometer at 595 nm. Bovine albumin was used as the standard (Bradford, 1976).

Anatomical analysis

Fifteen roots from each branch order were stained with safranin-fast green, and then dehydrated in 70, 85, 95 and 100% alcohol, embedded in paraffin and slides of 8-μm thick root sections were prepared with a microtome for determination of anatomical characteristics. The slides were photographed under a compound microscope (BX-51, Olympus Corporation, Tokyo, Japan) and cortex thickness and stele diameter were measured for each root cross section (Guo et al. 2008*b*). The cortex % was calculated as ratio of cortex cross section to total root cross section. The ratio of stele diameter to root diameter was also calculated. Root anatomical analysis was a labor-intensive, time-consuming task and was done for all five root orders sampled in July 2008, but only for the first two root orders at the other two sampling dates.

Data analysis

Separate split-plot analyses of variance were done for each of the measured factors as the dependent variable with season as the main plot treatment and root order as the sub-plot treatment (Table S1 available as Supplementary Data at *Tree Physiology* Online). Significant differences among the means for the root orders were tested with the least significant difference test (Figure 1). Data for the three seasons (sampling

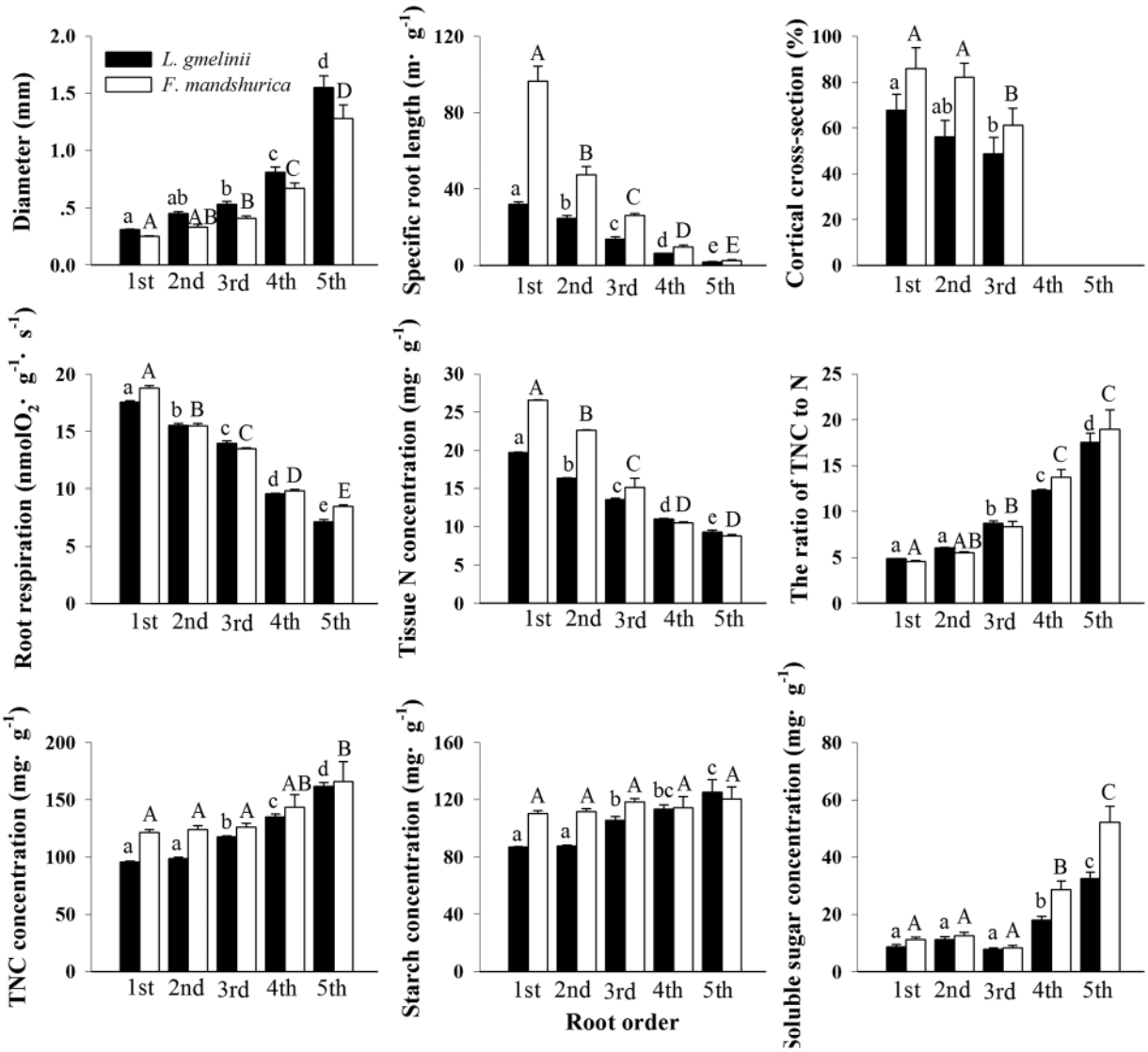

Figure 1. Root morphology, anatomy and chemical composition and root respiration (18 °C) at different branch orders in summer (July) for larch and ash (mean ± standard error). TNC represents total non-structural carbohydrates concentration, similar for all figures. Different lower- and upper-case letters indicate significant ($P < 0.05$, Tukey's honestly significant difference test) differences among individual root orders within the same parameter for larch and ash respectively.

dates) were pooled to examine the relationships between root respiration and root morphological and chemical properties. Linear regression models were developed with root respiration as the dependent variable, and the other measured parameters (root N concentration and root TNC : tissue N ratio, root starch : tissue N ratio, root sugar : tissue N ratio and stele-to-root diameter ratio) as independent variables. Respiration, root diameter and SRL data were log transformed and separate linear regression models were developed with log respiration as the dependent variable, and log root diameter and log SRL as the independent variable. Data analyses were done with SAS software (SAS 2009, SAS Institute Inc., Cary, USA).

Results

Root morphology, anatomy and chemical composition at different branch orders

Measured attributes for the near surface roots (0–10 cm) for larch and ash are plotted against root order in Figure 1. Many of the root attributes show similar or opposite monotonic trends over root order indicating a high degree of correlation among the attributes. Within the five branch orders and in both species, first-order roots had the highest SRL, root tissue N concentration, respiration and cortical cross section and the smallest diameter, the lowest starch and soluble sugar concentration and the lowest root TNC to tissue N ratio (Figure 1). With ascending root order in both species, root diameter, and soluble sugar and starch concentration all increased, while SRL, tissue N concentration, cortical cross section and respiration decreased (Figure 1). Cortical tissue was observed only in the first three order roots, and was not present in fourth- and fifth-order roots (Figure 2), resulting in a stele-to-root diameter ratio increase with ascending branch order in both species (data not shown). Cross sections of the cortex for the first-order root accounted for 68% (larch) and 86% (ash) of the total cross section of the root (Figure 1).

Root branch order had highly significant effects ($P < 0.01$) on root traits of diameter, SRL, respiration, tissue N concentration and TNC concentration (including both starch and soluble sugar; Table S1 available as Supplementary Data at *Tree Physiology* Online). The root attributes measured also differed seasonally, with the exception of SRL in the larch roots (Table S1 available as Supplementary Data at *Tree Physiology* Online). Root respiration rate for all branch orders was highest in July. Root diameter, tissue N concentration and soluble sugar concentration were highest in May, and starch concentrations were highest in October in both species. The seasonal dynamics of TNC and SRL varied with species (Table S2 available as Supplementary Data at *Tree Physiology* Online). The interactions between root branch order and season also had significant effects on root traits ($P < 0.01$), except for either SRL or root respiration in larch ($P > 0.05$; Table S1 available as Supplementary Data at *Tree Physiology* Online).

Relationship between root respiration and root form, chemical composition

There were significant negative correlations between log fine root respiration rate and log root diameter (Figure 3a), and positive correlations between log respiration rate and log SRL for surface roots (Figure 3b, $P < 0.001$). Significant positive relationships were exhibited between fine root respiration and root tissue N concentration (Figure 4a), and negative correlations occurred between root respiration and root TNC : tissue N ratio (Figure 4b, $P < 0.001$). Significant negative correlations were also found between root respiration rate and the ratios of root starch and soluble sugar to tissue N concentrations (Figure S1 available as

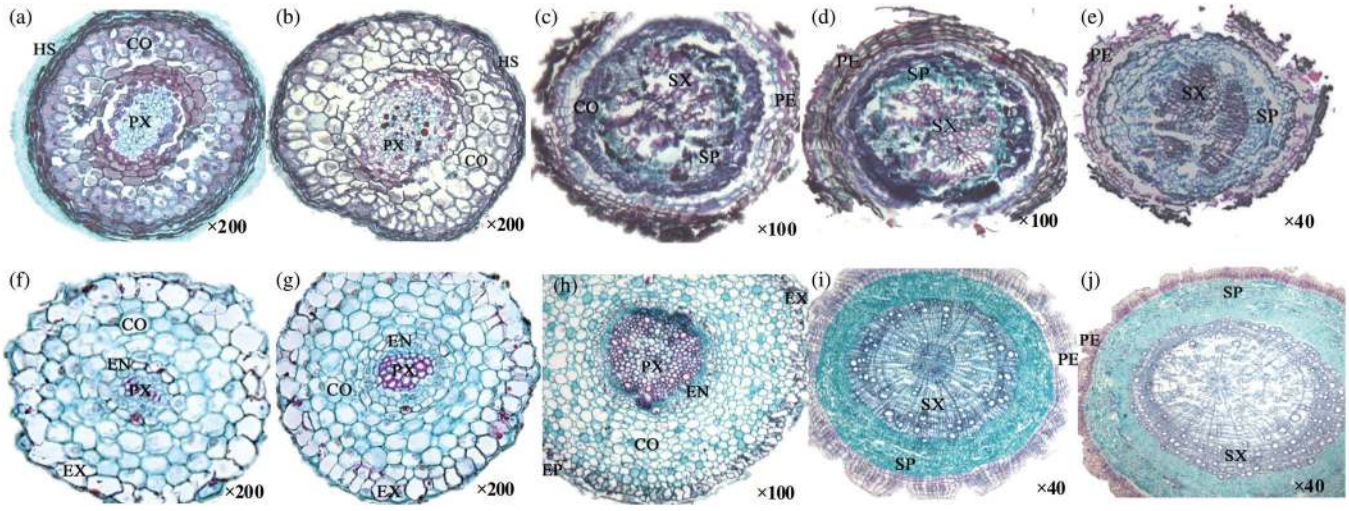

Figure 2. Typical anatomical structures of roots among the first five orders in larch (*L. gmelinii*) and ash (*F. mandshurica*). From the first- to the fifth-order roots in larch are a–e, and in ash are f–j. CO, cortex; EN, endodermis; EP, epidermis; EX, exodermis; HS, hyphal sheath; PE, periderm; PX, primary xylem; SP, secondary phloem; SX, secondary xylem.

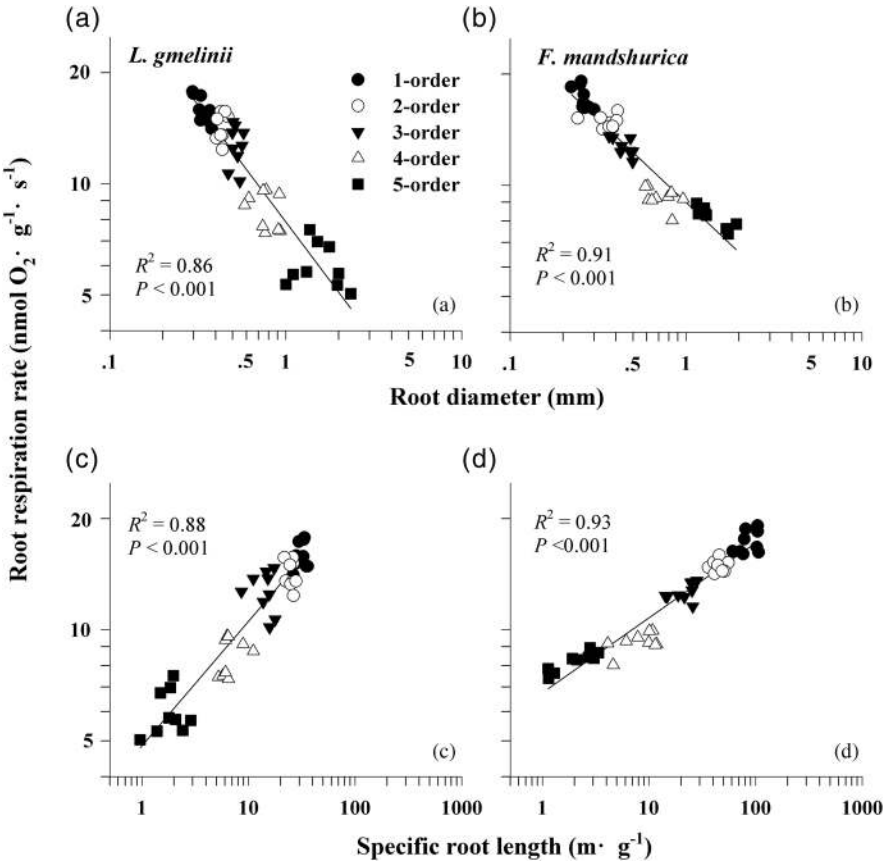

Figure 3. Root respiration and root morphology (diameter and SRL) plotted on a log–log scale for larch (*L. gmelinii*) and ash (*F. mandshurica*) plantations. Data were taken from three sampling seasons (October, 2007, May 2008 and July 2008) in the three no fertilizer plots for each species.

Supplementary Data at *Tree Physiology* Online), and between root TNC and tissue N concentrations in both species (Figure S2 available as Supplementary Data at *Tree Physiology* Online). Additionally, cortical thickness of the first- and second-order roots was positively related to soluble N concentration (Figure 5a and b, $P < 0.01$), and root respiration rate was negatively related to stele-to-root diameter proportion for all of the first five root orders (Figure 5c and d, $P < 0.01$) in both species.

Discussion

Root respiration is a major source of carbon dioxide (CO_2) efflux from forest soils and plays an important role in C cycling at ecosystem levels (Atkin et al. 2000; Högberg et al. 2001; Litton et al. 2007). Assessing the patterns of root respiration over a range of root morphology, chemistry and anatomy may provide valuable insights into belowground C allocation and fates. The objective of this study was to examine how root respiration is related to root morphology, chemistry and anatomy along the root branch order hierarchy in larch and ash plantations. We found that root respiration rate exhibited systematic changes from the first- to the fifth-order roots, and that the first-order roots have the highest respiration rate in both tree species, suggesting a predominant role of first-order roots in ecosystem-scale C and N cycling. Root respiration rates strongly correlated with traits of root morphology, chemistry and anatomy. These results are for near-surface roots (0–10 cm), and they may help us to precisely estimate and model root respiration in woody plants.

Root diameter and SRL are commonly used parameters of fine root (<2 mm) morphology and have been studied as indicators of environmental changes such as soil nutrient availability (Eissenstat and Yanai 1997; Ostonen et al. 2007). The effects of fine root diameter on root respiration have been reported by previous studies in forests of sugar maple (*Acer saccharum* Marshall) (Pregitzer et al. 1998; Burton et al. 2012), aspen (*Populus tremuloides* Michx.) (Desrochers et al. 2002) and eucalypti (*Eucalyptus*) (Marsden et al. 2008; Chen et al. 2010). These researchers found that thinner roots had higher respirations. A closely related parameter, SRL, also strongly affected root respiration rate in mature oak (*Quercus serrata* Thunb.) forest (Makita et al. 2009), because it is correlated with root diameter within species (Eissenstat and Yanai 1997). Several studies have shown that fine root respiration differed widely over a relatively narrow range of root diameter (Pregitzer et al. 1998; Makita et al. 2009; Chen et al. 2010).

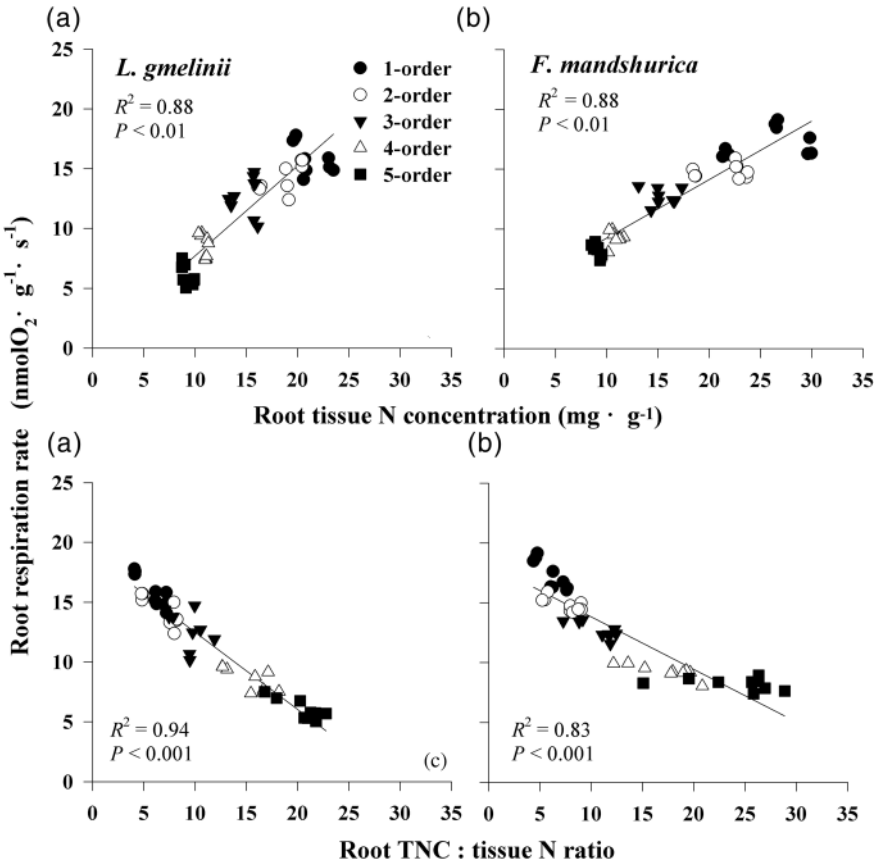

Figure 4. Root respiration and root chemistry (tissue N concentration and TNC : tissue N ratio) for larch (*L. gmelinii*) and ash (*F. mandshurica*) plantations. Data were taken from three sampling seasons (October 2007, May 2008 and July 2008) in the three no-fertilizer plots for each species.

One possible reason is that the position of an individual root on the complex lateral branching system (i.e., root order) has often been ignored (Pregitzer et al. 2002). Our results on the relationship between root respiration and root diameter in both species were consistent with previous studies, but the respiration rate differed greatly according to root branching order. Fine roots <2 mm in diameter can include all the first five orders on the root branching system, and each root order had a significantly different respiration rate (Figure 1). The first-order roots with the smallest diameter and highest SRL showed greatest respiration rate in both species, and in contrast, the fifth-order roots with the largest diameter and smallest SRL displayed lowest respiration rate (Figures 1 and 3). Lower order roots are physiologically very active (Pregitzer et al. 2002; Wells and Eissenstat 2003), and can provide greater effective absorbing surface (on a per unit root mass basis) for nutrient and water uptake in a highly heterogeneous soil (Eissenstat and Yanai 1997; Comas et al. 2002; Guo et al. 2004), leading to the higher respiration rate. Thus, a more detailed classification of fine root diameter <2 mm, such as determining root diameter and SRL on the basis of root order, can aid in the exploration of the heterogeneity of root function, and may enhance our understanding of the mechanism of root morphological effects on root respiration in woody plants.

In the present study, we found a significant positive relationship between root respiration rate and root tissue N concentration, which was consistent with many previous studies (Ryan et al. 1996; Pregitzer et al. 1998; Burton et al. 2000, 2002; Makita et al. 2009; Chen et al. 2010). The tissue N explained 88% of the variation in root respiration rate for both larch and ash (Figure 4), which was higher than that in other reports. For example, tissue N could explain 70% of the observed variation in root respiration rate for sugar maple (Pregitzer et al. 1998), 63% for mature oak (Makita et al. 2009), and 52% for acacia (*Acacia crassicarpa* Cunn. ex Benth.) (Chen et al. 2010). We observed that fine roots <2 mm contained five branch orders, each with a significantly different respiration rate in both species (Figure 1). With ascending root orders, both root tissue N concentration and respiration rate declined systematically (Figure 4). The first-order roots are typical absorptive roots (Guo et al. 2008*b*; Valenzuela-Estrada et al. 2008), with the highest tissue N concentration indicating that the root cells contain greater amount of proteins, which are tightly linked with higher cellular activities including respiration, nutrient

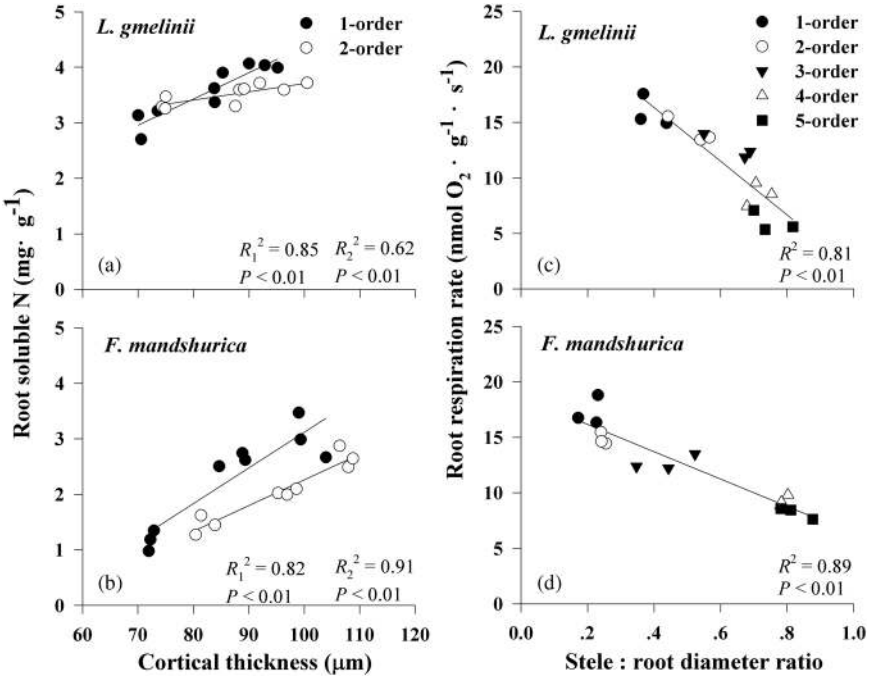

Figure 5. Soluble N concentration and cortical thickness, root respiration and stele : root diameter ratio in larch (*L. gmelinii*) and ash (*F. mandshurica*) roots. Data were taken from three soil blocks in the three no-fertilizer plots for each species.

assimilation and transportation (Ryan et al. 1996; Pregitzer et al. 1998). Previous studies on root morphology and chemistry have shown that root tissue N concentration decreased systematically with ascending root branch orders in nine North American trees (Pregitzer et al. 2002) and 49 Chinese temperate and tropical species (Li et al. 2010). The inverse correlation between root order and N concentration has important C flux implications since tissue N may serve as an index of root respiration (Pregitzer et al. 1998, 2002; Valenzuela-Estrada et al. 2008; Li et al. 2010). Nevertheless, Burton et al. (2012) found that roots within the same diameter range showed declining respiration rates and tissue N concentration with increasing soil depth. Combining results by Burton et al. (2012) for deeper roots with our results for near surface (0–10 cm) roots suggests that although respiration and tissue N concentration might be lower for deeper roots, the deeper roots may also show a relationship between root order, tissue N concentration and respiration. More investigations are required to determine how the relationship between respiration and tissue N for the same root order changes from surface to deep soil layers.

In addition, root respiration is considered to be fuelled by non-structural carbohydrates rather than structural C fixed in cell walls of roots. TNC (mainly starch and soluble sugar) in plant tissues are important substrates for root respiration. For example, respiratory utilization of carbohydrates in roots provides the essential energy for nutrient uptake and transportation, root growth and maintenance and symbiotic processes in the rhizosphere (Bouma et al. 1996; Farrar and Jones 2000; Martínez et al. 2002; George et al. 2003; Xu et al. 2008). In our study, the starch and soluble sugar or TNC in the first-order roots was the lowest among the five root orders in both species, but respiration rate was the highest (Figure 1), suggesting that the first-order roots spend much energy for root physiological processes such as growth and maintenance (Guo et al. 2004). In contrast, higher order roots serving as the storage of carbohydrates have higher TNC concentration, and show lower metabolic activity. TNC concentrations of first-order roots (121 mg·g^{-1}) in our study were similar to longleaf pine (114 mg·g^{-1}) reported by Guo et al. (2004), and starch concentrations (110 mg·g^{-1}) were similar to two *Actinidia* species (*polygama* and *arguta*) fine roots (<2 mm diameter) (Boldingh et al. 2000), but they were higher than some fine roots (<1 mm diameter) in a temperate forest (Zhou et al. 2011).

However, in contrast to root tissue N, few studies focused on the relationship between root respiration and TNC in woody plants. Desrochers et al. (2002) reported that TNC concentration in aspen seedling roots was positively correlated with respiration rate measured at 15 and 25 °C. In our study, we found that root TNC concentration was negatively related to root tissue N concentration in both species (Figure S2 available as Supplementary Data at *Tree Physiology* Online), leading to the concomitant negative correlation between root respiration rate and root TNC : root tissue N ratio (Figure 4). The conflicting results between Desrochers et al. (2002) and our study were perhaps because (i) they used seedling roots and did not find the correlation between N concentration and respiration rate, whereas we used roots from established (22-year-old)

plantations and (ii) they used an entirety (bulk) of seedling roots <2 mm in diameter as the sample in the respiration chamber, and did not separate the fine roots into different orders or perform separate measurements of TNC for each root order. Separation of roots into branch orders requires excising, which will lead to the wound respiration for a very brief period (several minutes) after excision (Rakonczay et al. 1997). We allowed our root samples to equilibrate to the measurement temperature (18 °C) for 30 min before we measured respiration.

Moreover, we also acknowledge that without knowing how much of the measured TNC is stored or utilized by root respiration, it is difficult to tease apart the mechanisms that can lead to both positive and negative correlations between TNC and respiration rate. Root TNC : N ratio may serve as an alternative indicator of root respiration, because TNC is an important substrate for tissue respiration, and tissue N is closely associated with maintenance respiration. The TNC : N ratio increased from the distal root tip toward the higher order roots potentially indicating TNC allocated to the first-order roots will be depleted more rapidly by higher respiration than the fifth-order roots.

Different branch orders showed marked differences in anatomy in temperate conifer and hardwood trees (Guo et al. 2008*b*), yet the relationship between root respiration and anatomy is unclear. In the present study, the first-order roots exhibited primary development traits with an intact cortex in both larch and ash (Figure 2), which generally have high metabolic rate (e.g., respiration) and high nutrient content (e.g., tissue N) (Esau 1977; Eissenstat and Achor 1999; Hishi 2007; Guo et al. 2008*b*). In contrast, the fourth- and fifth-order roots showed mostly secondary development traits such as secondary xylem and cork layers (Figure 1), which are generally metabolically inert and nutrient-poor (Esau 1977; Hishi 2007). As the root order increased, the proportion of cortex in total cross section declined, and cortex tissue disappeared in the fourth- and fifth-order roots (Figure 1), whereas the stele-to-root diameter ratio increased. Strong correlations between root respiration rates and stele-to-root diameter ratio were found in both species (Figure 5c and d). In addition, we found that the soluble N concentration positively correlated with cortical thickness in the first two order roots (Figure 5a and b). The active ion uptake usually occurs in cortical cells, and the ions can be taken into the symplast in any of the cortical cells (Peterson et al. 1999), and thus larger cortical thickness can provide a greater potential for ion uptake (Kumar et al. 2007). On the other hand, the majority of tissue N in cortical parenchyma cell is soluble N (such as proteins), which may serve as an indicator of N uptake activity due to their main physiological functions of ion uptake, assimilation and transportation (Ryan et al. 1996; Pregitzer et al. 1998; Lambers et al. 2008). Both larger cortical thickness and higher soluble N concentration in the first two order roots may enhance their metabolic activities such as respiration rate.

Conclusion

Our results in both larch and ash showed that the order of a root in the branching system may indicate its form and function. From the first to fifth order, all of root diameter, TNC and the ratio of root TNC to tissue N concentration increased, whereas SRL, tissue N concentration and the proportion of cortical cross section decreased. The first-order roots had the highest respiration rate and the fifth-order roots had the lowest. The rate of root respiration systematically decreased with ascending branch orders. The monotonic shifts of root respiration rate along the five branch order roots closely related to root diameter, SRL, tissue N concentration, root TNC : tissue N ratio and stele proportion because root morphology, chemistry and anatomy concomitantly shift with branch orders. It should be noted that our results are restricted to surface roots (0–10 cm depth). Given the widespread linkage between root anatomy, tissue chemistry (e.g., tissue N) and branch orders across temperate and tropical tree species (Pregitzer et al. 2002; Valenzuela-Estrada et al. 2008; Guo et al. 2008*b*; Li et al. 2010), our findings here may be a common phenomenon in terrestrial woody plants.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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