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REGULAR ARTICLE

Lower order roots more palatable to herbivores: a case study with two temperate tree species

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Abstract Determining which kinds of roots are likely to be consumed by root herbivores may improve our understanding of the mechanistic control on fine root dynamics. Here, we tested the hypothesis that root herbivores prefer to consume the distal lower order roots in their branching networks. Insecticide was applied to soil to quantify effects of root herbivores on root biomass and production in the first five orders (the distal roots numbered as first-order) in *Fraxinus mandshurica* and *Larix gmelinii* plantations from May 2008 to July 2009. Root morphology, chemistry, anatomy and physiology were measured simultaneously across branching orders. Among the first five order roots, significant consumptions by herbivores were found only for the two distal lower order roots throughout growing seasons, with 62% of biomass and 57% of production for *F. mandshurica*, and 71% and 79% for *L. gmelinii*, respectively. Our results suggest that the distal lower order roots are more palatable and attractive to root herbivores in both plantations, probably because they have higher tissue N, greater respiration rates and lower cellulose. Thus, overlooking herbivore consumption may lead to large underestimation in root biomass and production, which are critical in determining C budget and nutrient cycles in forest ecosystems.

Keywords Fine root biomass and production · Ingrowth core · Insecticide · Root branch order · Root feeders · Soil sequential core

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Introduction

During their development, tree roots branch into different hierarchies (i.e. root orders) from basal to successively more distal roots, which differ markedly in forms and functions (Robinson et al. 2003). Even fine roots (diameter <2 mm) can consist of numerous branching orders (Pregitzer et al. 2002; Guo et al. 2008a; Valenzuela-Estrada et al. 2008). In contrast to higher order roots, the distal lower order roots are generally nonwoody (Guo et al. 2008a;

Valenzuela-Estrada et al. 2008), and have smaller diameter, higher nitrogen (N) concentrations (Pregitzer et al. 2002; Li et al. 2010) and lower cellulose (Guo et al. 2004), and shorter longevity (Wells et al. 2002a; Guo et al. 2008b). Such suites of traits enable distal lower order roots to explore soil resources efficiently, but may also make them more susceptible to root herbivores. For example, greater N concentrations in roots may enhance their respiration rates (Pregitzer et al. 1998; Burton et al. 2002; Makita et al. 2009), providing attractants (i.e. CO_2) for soil insects (Johnson and Gregory 2006). In addition, lower cellulose concentrations may be more digestible to root-feeders (Brown and Gange 1990). Despite the general knowledge that root-feeding insects graze on fine roots (Eissenstat and Yanai 1997; Hunter 2008), little is known about which kinds of roots are more susceptible to root herbivory within the branching network, because the relationships between root orders and herbivory in woody plants have generally been overlooked.

In forest ecosystems, herbivores affect root growth through their consumption of root tissues and mycorrhizal fungi (Brown and Gange 1990). Fine root biomass and production may be underestimated due to such consumptions. For example, experimental insecticide treatments significantly reduced herbivore density and consequently increased fine root biomass in a regenerated pine stand (Stevens and Jones 2006) and a natural cypress forest (Hishi and Takeda 2008). Stevens et al. (2002) predicted that about 10% of total net primary productivity (NPP) may be consumed by root herbivores in temperate forests. Such a large proportion of the carbon (C) removal by herbivory suggests that the influence of root herbivory on C and nutrient cycles is crucial in forest ecosystems (Hunter 2008). However, there may be a conceptual problem associated with these studies, i.e., the treatment of fine roots (diameter <2 mm) as a homogeneous mass compartment. This implies that all individual fine roots suffer similar stress from herbivory, or that root-feeders graze on fine roots without feeding preference. However, fine roots as defined probably include both nonwoody and woody roots in most woody plants (Guo et al. 2008a; Valenzuela-Estrada et al. 2008), and may have differences in palatability and attractiveness to root-feeding insects. Brown and Gange (1990) and Price (1991) indicated that root-feeding insects prefer to graze on young and fresh palatable roots, suggesting that only one portion of fine roots may be consumed and participate in the cycles of C and nutrients in soil food webs. Thus, using the diameter-based approach may have hindered the understanding of the role of root herbivores in fine-root dynamics at the individual tree level or ecosystem scale. The root order-based approach may provide a better alternative in defining the role of root herbivores in fine-root dynamics by separating palatable roots from unpalatable roots based on root morphology, anatomy and chemistry related to root branch order in woody plants (Pregitzer et al. 2002; Guo et al. 2008a; Valenzuela-Estrada et al. 2008; Li et al. 2010). Nevertheless, we are not aware of any experimental study that has explored the interrelationship between fine root biomass production and herbivore feeding based on root branching order classification.

In this study, we focused on the relationship between root branch order and herbivore feeding of two temperate tree species, *Fraxinus mandshurica* Rupr. and *Larix gmelinii* Rupr., which are commercial tree species extensively used in plantations in northeastern China. We applied insecticide (chlorpyrifos) to the soil in controlled experiments to determine the effects of grazing by root herbivores on fine root standing biomass and production across the first five branching orders (the distal roots numbered as first-order) in tree plantations of each species. We measured root morphology (diameter), chemistry (tissue N, total nonstructural carbohydrates (TNC), cellulose and lignin concentrations), anatomy (cortical proportions) and physiology (respiration rates) within each fine root order. We tried to address the following questions: (1) How do fine root biomass and production change among branching orders after insecticide applications to soil? (2) Do these changes consistently occur in the distal lower order roots throughout growing seasons? (3) Does biomass of distal low order roots have a stronger correlation with root herbivore density than that of higher order roots? We hypothesize that herbivores should have strong preference to consume the distal low order roots among roots along the branching network, because these distal roots are generally nonwoody, and contain high level of nutritions (e.g. higher tissue N content and lower cellulose).

Materials and methods

Study site and plot establishment

The study site was located at the Maoershan Forest Research Station (127°30′–127°34′E,45°21′–45°25′ N) of the Northeast Forestry University, in Heilongjiang, China. The site has a continental temperate monsoon climate with mean January, July and annual temperatures at −19.6°C, 20.9°C and 2.8°C, and with annual precipitation ranging from 600 to 800 mm, of which 80% falls in June, July and August (Wang 2006). The growing season ranges from 120 to 140 days. Soils are Hap-Boric Luvisols (Gong et al. 1999) with high organic matter content, well-developed horizons, and well drained (Wang et al. 2006).

The study was conducted in *F. mandshurica* and *L. gmelinii* plantations located on a southwest-facing slope of approximately 13°, with elevation ranging from 450 to 500 m above sea level. The stand properties and soil characteristics of the two plantations are shown in Supplementary Table 1. Both plantations were established in 1986 by planting nursery-raised 2-year-old bare root seedlings using a 1.5×2.0 m planting grid. In each plantation, three 20×30 m study plots were established in a randomized factorial design in the October of 2007. In each study plot, a pair of two 6×6 m subplots were established: one randomly assigned to the insecticide treatment (insecticide applied in mid May 2008) and the other treated as the control. The two subplots were kept at 6–8 m apart to minimize potential edge effects.

Insecticide treatment

Chlorpyrifos, a broad-spectrum organophosphate insecticide, is generally used to eliminate soil insects in the field (Wells et al. 2002b; Stevens and Jones 2006). In this study, chlorpyrifos EC (a commercial insecticide containing 40% chlorpyrifos; Dongfeng Chem. Co., Zhejiang, China) was used to remove soil herbivores. The insecticide was diluted by 100-fold with tap water and applied to the insecticide treatment subplots every 4 weeks from May of 2008 to September of 2009. The insecticide solution was sprinkled on the soil surface at a rate of 0.5 Lm^{-2}, and an equivalent volume of tap water was applied to the control subplots at the same time.

Root biomass and root herbivore sampling

Root sampling was conducted four times between mid July and October of 2008 and between May and July of 2009. Six soil cores (60.4 mm in inner diameter) were taken at two depths (i.e. 0–10 and 10–20 cm) from random locations within each subplot at each sampling time. The samples were placed in the plastic bags on ice, transported to the laboratory within 15 min for processing, and sorted in two steps.

In step 1, roots and soil macrofauna in both treatments were removed from the samples by hand in laboratory. All roots were carefully separated from the soil cores with metal probes and placed in a refrigerator (4°C) until dissected into different branch orders. Root segments were cleaned of residual soil particles with forceps in deionized water. Cleaned root segments were then dissected into different branch orders following the procedure described in Pregitzer et al. (2002), i.e., the distal nonwoody branch order as the first order. Some but not all soil cores contained up to six root orders, and we included only the first five orders in our analysis. Roots of different branch orders were dried (65°C) to a constant mass and then weighed to determine fine root ash-free standing biomass ($g\ m^{-2}$).

In step 2, after removing roots and soil macrofauna, the microfauna from each soil core were extracted using a modified Tullgren funnel at a constant temperature of 35°C in a cabinet with a white light for 72 h (Hishi and Takeda 2008). Organisms from each soil core were stored in 75% ethanol until identified to order or family using the key of Stehr (1987). Averages of total soil fauna density (0–20 cm) were 31600 m^{-2} in *F. mandshurica* and 26100 m^{-2} in *L. gmelinii*. Most organisms were Acari, Collembola and Colepotera (see Supplementary Tables 2 and 3), which accounted for 85% in *F. mandshurica* and 89% in *L. gmelinii*. However, putative root herbivores only accounted for 10% in *F. mandshurica* and 9% in *L. gmelinii*. At each sampling time, root herbivore density for each order or family in each soil cores was summed for 0–10 cm and 10–20 cm depth, and expressed per unit ground area (see Supplementary Table 2). At each sampling time, we spent about 10 days to process all the soil cores.

Root production in ingrowth cores

The ingrowth core method was used to quantify root biomass production via measure root ingrowth into root-free soil core (Vogt et al. 1998). In June 2008, 40 soil cores with an inner diameter of 100 mm were sampled adjacent to the treatment plots at the same depth as soil cores for each plantation. All visible living and dead root materials were carefully removed, and the remaining soil material was placed back into the same hole. Twenty soil cores were randomly selected and treated with chlorpyrifos similar to the insecticide-treated subplots, and the other 20 remained treated as controls. These ingrowth cores were harvested with a soil core of 60.4 mm internal diameter 12 months later. In the laboratory, fine roots in the ingrowth cores were sorted by different branch orders and weighed following the same procedure described for soil sequential cores. Biomass in different order roots from the ingrowth cores was calculated as root production and expressed as g m^{-2} $year^{-1}$ (Vogt et al. 1998).

Root respiration measurements

In July, 2008, three soil cores at 0–10 cm depth were randomly sampled in each plot (but outside subplots), and placed in a cooler on ice and transported to the laboratory. In the laboratory, roots were separated into three parts used to analyze for root respiration, chemistry and anatomy, respectively. The first sub-samples for respiration measurements were quickly sorted by branch order. The second sub-samples for anatomy were cleaned and immediately fixed in Formalin-Acero-Alcohol (FAA) solution (90 ml 50% ethanol, 5 ml 100% glacial acetic acid, 5 ml 37% methanol) and stored in a refrigerator (4°C). The third sub-samples for chemical analysis were also sorted by branch orders and stored in a refrigerator (4°C).

Three sub-samples of cleaned root segments for each branch order (about 0.5 g fresh weight) were wrapped in moistened tissue paper for use in respiration measurements (Pregitzer et al. 1998). Root respiration of different branch orders (O_2 consumption at 18°C) were measured with temperature controlled O_2 electrodes (model LD 2/3, Hansatech, England) connected to constant temperature circulating water baths (Pregitzer et al. 1998). Following respiration measurement, the sub-samples were analyzed with root-analyzer software (WinRhizo Pro (S) v. 2004b, Regent Instruments Inc. Canada) for determining morphology, and then, were dried (65°C) to a constant mass and then weighed to determine specific respiration rate (nmol O_2 g^{-1} s^{-1}).

Root chemical analysis

Fine roots from the third sub-samples of different branch orders were cleaned, dried (at 65°C to a constant mass), weighted, ground, and homogenized for chemical analysis. Total N and C were determined using a Macro Elemental Analyzer (vario MACRO, Elementar Co. Germany). Root soluble sugar and starch were analyzed by the Buysse & Merckx (1993) method, and summed as total nonstructural carbohydrate. Root cellulose and lignin were determined by the method used by Guo et al. (2004). All root chemistry indices were expressed on an ash-free, dry mass basis (Guo et al. 2004).

Root anatomy

Fine roots fixed in FAA solution were dissected into different orders in laboratory. The procedure of root anatomy was the same as that described in Guo et al. (2008a). For each species, 20 segments were randomly chosen per order for first to third orders, and 15 segments per order for fourth and fifth orders (Guo et al. 2008a). After the dissection, individual root segments were stained with safranine-fast green, dehydrated in a set of alcohol solution, and embedded in paraffin; and sections of 8 μm thick were prepared (de Neergaard et al. 2000; Guo et al. 2008a). These sections were measured for anatomical features, and photographed under a compound microscope (BH1, Olympus). For each root segment, three cross-sections were chosen to measured root diameter, cortex thickness, and stele diameter to the nearest 1 μm, and the proportions of cortical cross-sectional area in the root cross-section were determined.

Data analysis

Means and standard deviations of root herbivore density, biomass and production in different orders at each sampling time were calculated in the three control and three insecticide-treated plots. Fisher's LSD test (P=0.05) was used to test the differences in herbivore density, root biomass and production of each order between control and treatment plots (n=3)

within each species at each sampling time. Within each species, a mixed-level (2×2×3) three-way (insecticide treatment, soil depth, sampling time) factorial ANOVA was used to determine the effects of insecticide treatment, soil depth and sampling time on fine root biomass among five branch orders (PROC GLM procedures, SAS Institute). The correlations between standing biomass and herbivore density or other soil fauna (excluding herbivores) were analyzed by linear regression (PROC GLM procedures, SAS Institute). For each species, root morphology, chemistry, anatomy and respiration rates of each order from three soil cores were averaged and then the means at plot level calculated, and the differences in these root traits among five branch orders were identified by Tukey's HSD test.

Results

Root herbivore densities were reduced by 95% in *F. mandshurica* and 98% in *L. gmelinii* plantations in the insecticide-treated plots as compared to the control plots (P values<0.01, Fig. 1; Supplementary Table 2). Insecticide application resulted in greater total living root biomass (sum of the first five orders). The average biomass among the four sampling times was 193 gm^{-2} for *F. mandshurica* and 112 gm^{-2} for *L. gmelinii* in the insecticide-treat plots, while the corresponding values in the control plots were 145 gm^{-2} and 77 gm^{-2}. For both species, however, significant increases in biomass were observed only in the first two order roots in the insecticide-treated plots (P values<0.01, Fig. 2; Supplementary Table 4). From July 2008 to July 2009, the average root biomass in insecticide-treated plots was increased by 44% in the first order and 42% in the second order for *F. mandshurica*, and by 79% and 53% for *L. gmelinii*, respectively (Fig. 2). Effects of herbivory on the biomass of the third order roots changed with sampling time (Fig. 2). In contrast to higher order roots, the first two order roots with thinner diameter had greater proportional cortex, greater tissue N, higher respiration rates (at 18°C) and lignin concentrations, but lower TNC and cellulose concentrations (Table 1).

Density of root herbivores was negatively correlated with fine root biomass of the first two orders in *F. mandshurica* (R^2=0.77, P<0.01) and in *L. gmelinii* (R^2=0.66, P<0.01) (Fig. 3a), but not with that of higher order (third to fifth) roots (Fig. 3b). In contrast, density of other soil fauna (excluding root herbivores) was uncorrelated with either lower or higher order roots (Fig. 3c and d). Compared with the control plots, fine root biomass production over 1 year in ingrowth cores increased by 12 gm^{-2} yr^{-1} (50% increase) for *F. mandshurica* and 5 gm^{-2} yr^{-1} (36% increase) for *L. gmelinii* in the insecticide-treated plots (Fig. 4), with the first two order roots accounting for 57% (*F. mandshurica*) and 79% (*L. gmelinii*) of the increase, respectively.

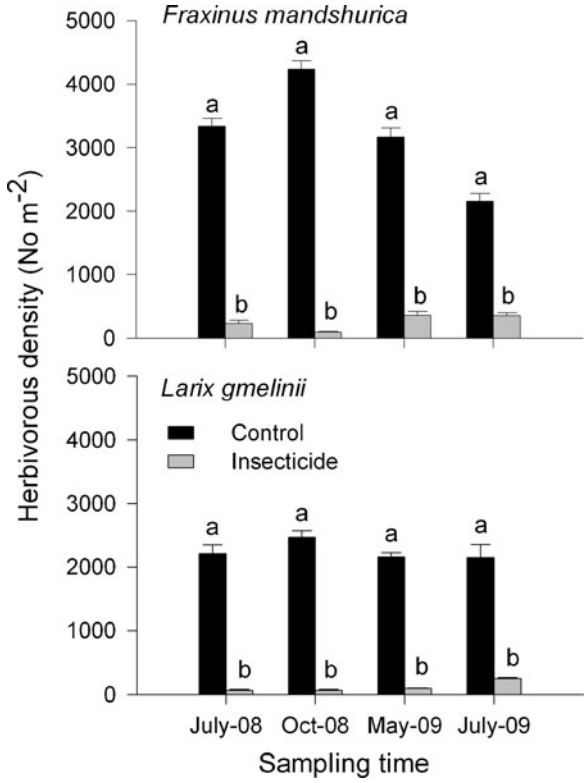

Fig. 1 Mean root herbivores density from four sampling times in *Fraxinus mandshurica* and *Larix gmelinii* plantations under two experimental treatments (insecticide vs. control). Soil samples were taken from soil depth of 0–20 cm. *Error bar* represents one standard deviation of the mean (n=3). Within panels, different letters indicate that the density values show significant differences according to Fisher's LSD test at P=0.05

Discussion

Effects of root herbivory on fine root biomass

Root herbivores may influence plant growth directly via consumption of root tissues in terrestrial ecosystems

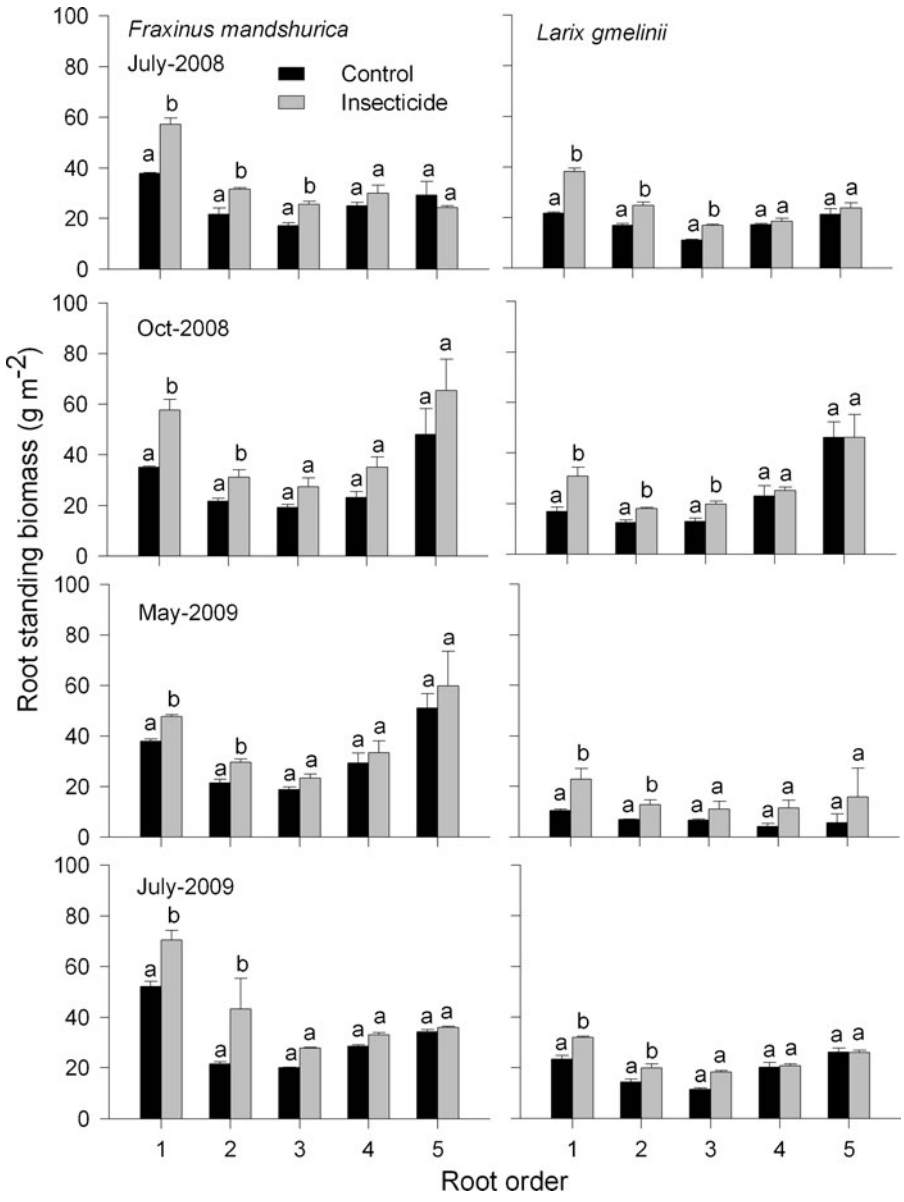

Fig. 2 Mean root standing biomass among the five orders of fine roots at four sampling times in *Fraxinus mandshurica* and *Larix gmelinii* plantations under two experimental treatments. The statistical analysis was the same as those in Fig. 1

(Brown and Gange 1990; Hunter 2008). However, the impacts of herbivory on fine root biomass and production in forest ecosystems are less studied (Hunter 2001, 2008; Stevens et al. 2002; Bauerle et al. 2007). In an experiment using root ingrowth cores to assess the effects of herbivores on fine root production, Stevens and Jones (2006) found that fine root biomass (<1.0 mm in diameter in this case) nearly doubled when root herbivores were excluded by insecticide in a regenerated pine stand in South Carolina, USA. Our study was based on branch-order and showed that the increases in biomass were primarily observed in the first and second order roots in both a hardwood (*F. mandshurica*) and a coniferous (*L. gmelinii*) species (Fig. 2). This result supports our hypothesis that herbivores prefer to graze on the distal lower order roots in the branching network. To our knowledge, this is the first study to clearly reveal the interrelations between root branch order and root-feeding insects in woody plants. Because application of the insecticide (chlorpyrifos) should have limited effects on soil fertility (Wells et al. 2002b; Supplementary Table 1) and plant growth (Stevens and Jones 2006), it is highly likely that the

Table 1 Diameter, tissue N, total nonstructural carbohydrates (TNC), cellulose, lignin, respiration rate (at 18°C) and cortical cross-section proportion among the five order roots in *Fraxinus mandshurica* and *Larix gmelinii* plantations (mean±standard error, n=3)

Root order	Diameter (mm)	Tissue N (mg g^{-1})	TNC (mg g^{-1})	Cellulose (mg g^{-1})	Lignin (mg g^{-1})	Respiration rate (nmol O_2 g^{-1} s^{-1})	Cortical cross-section (%)
Fraxinus mandshurica							
1	0.26^{a}±0.01	27.54^{a}±0.15	144.83^{a}±1.99	190.16^{a}±8.67	187.62^{a}±11.85	19.20^{a}±0.22	84.9^{a}±9.0
2	0.30^{a}±0.01	22.90^{b}±0.10	150.96^{a}±5.18	224.78^{b}±3.59	161.43^{b}±4.59	17.66^{b}±0.27	81.1^{a}±6.1
3	0.33^{a}±0.04	19.52^{c}±0.52	167.02^{b}±2.16	253.77^{c}±5.22	142.81^{c}±5.88	14.42^{c}±0.15	60.2^{b}±7.2
4	0.46^{b}±0.05	13.69^{d}±0.15	188.75^{c}±2.26	295.67^{d}±1.35	127.62^{d}±8.15	10.67^{d}±0.23	1.0^{c}±0.1
5	0.90^{c}±0.10	10.77^{d}±0.21	211.16^{d}±2.19	330.39^{e}±3.99	99.09^{e}±2.70	9.41^{d}±0.22	1.0^{c}±0.1
Larix gmelinii							
1	0.34^{a}±0.02	20.02^{a}±0.04	97.99^{a}±0.65	206.32^{a}±4.54	220.01^{a}±18.82	18.37^{a}±0.21	66.6^{a}±7.1
2	0.38^{a}±0.05	17.62^{b}±0.07	102.75^{a}±0.84	228.04^{b}±5.80	196.88^{b}±20.44	16.43^{b}±0.19	55.1^{a}±7.2
3	0.42^{a}±0.02	15.48^{c}±0.16	122.65^{b}±0.79	250.61^{b}±6.84	160.42^{c}±10.70	14.38^{c}±0.20	48.6^{a}±7.2
4	0.51^{b}±0.04	12.68^{d}±0.09	140.31^{c}±1.55	273.85^{c}±11.20	137.94^{d}±8.72	9.21^{d}±0.18	1.0^{b}±0.0
5	0.86^{c}±0.05	9.82^{e}±0.15	166.75^{d}±2.80	313.84^{d}±8.18	111.95^{e}±8.21	7.58^{e}±0.21	1.0^{b}±0.0

Different superscript letters within a species indicate significant differences (P<0.05) among the five order roots.

biomass increases in the first two order roots after the insecticide treatment were due primarily to the large reduction in herbivore densities.

Why do the herbivores prefer to consume the first and second order roots? There are several possible reasons. First, the survival and growth of herbivores are often limited by N availability in their diets (Mattson 1980; Hunter 2008). Because low order roots have higher N concentrations (Table 1) and may supply greater protein and amino acids, they are more

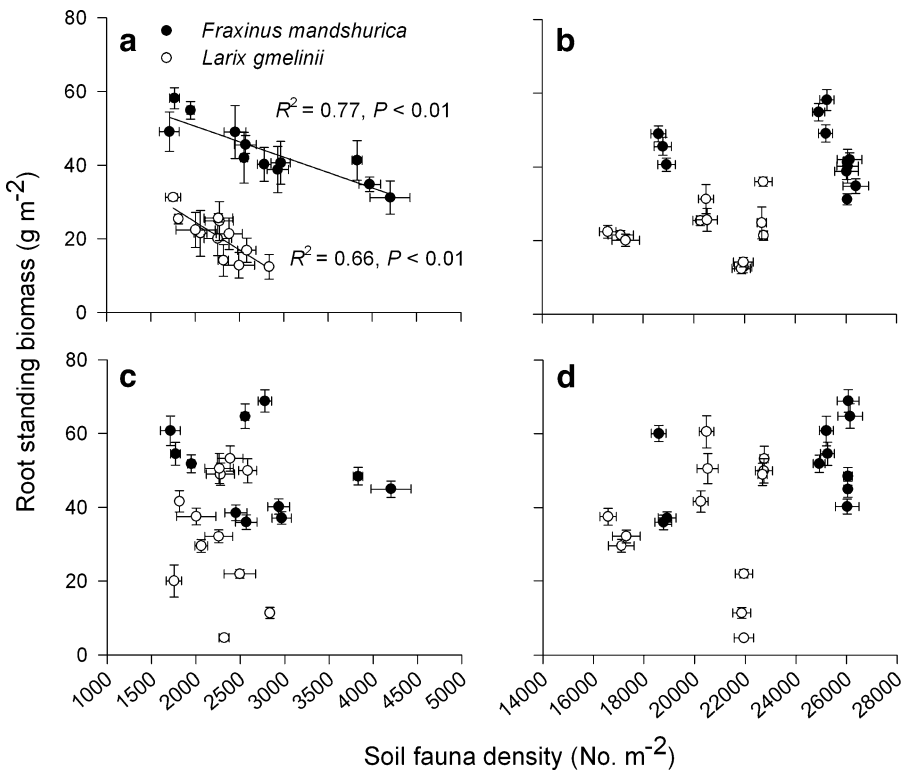

Fig. 3 Relationships between root standing biomass and soil fauna density at topsoil layer (0–10 cm). **a** The first two order roots vs. root herbivory. **b** The first two order roots vs. other soil faunas. **c** The third to fifth order roots vs. root herbivory. **d** The third to fifth order roots vs. other soil faunas. The biomass data for the five order roots were from three control subplots in *Fraxinus mandshurica* and *Larix gmelinii* plantations during four sampling times from July of 2008 to July of 2009. The *error bar* represents standard deviation (n=3)

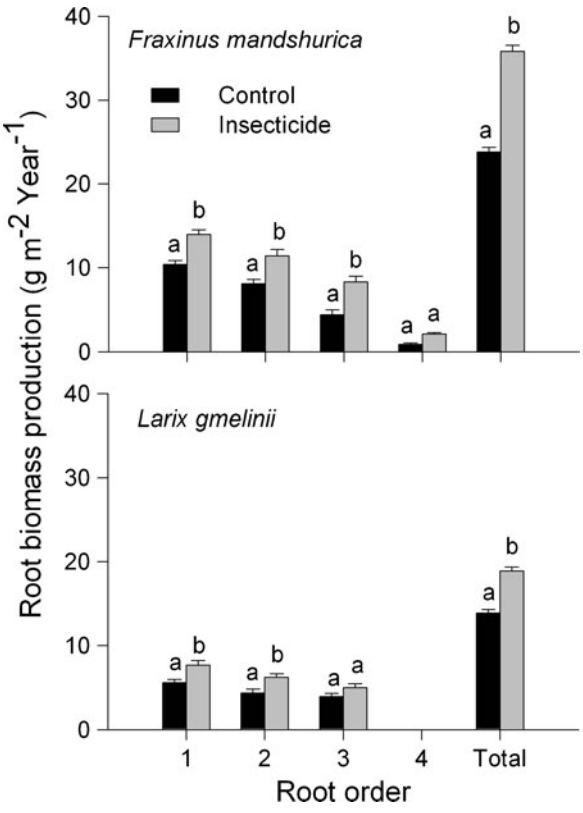

Fig. 4 Fine root biomass production among the first three or four orders in *Fraxinus mandshurica* and *Larix gmelinii* plantations under two experimental treatments measured by one-year in-growth cores. The data were from 20 soil cores of 0–20 cm soil depth. The statistical analysis was the same as those in Fig. 1

palatable to root-feeding insects (Brown and Gange 1990; Brussaard 1998). For example, Dawson et al. (2002) reported that the larvae of cranefly (*Tipula paludosa*) fed voraciously on white clover (*Trifolium repens*) roots with higher N concentration rather than perennial ryegrass (*Lolium perenne*) roots with lower N concentration in upland grassland. Second, chemicals released from the roots appear to be a critical factor orienting soil insect herbivores to locate roots (Johnson and Gregory 2006). Lower order roots are generally absorptive roots (Guo et al. 2008a), have high metabolic and respiration rates (Hishi 2007; Xia et al. 2010), and thus exude more chemical cues such as carbon dioxide (CO_2), amino acids and sugars into the rhizosphere (Brown and Gange 1990). Higher respiration rates, together with more exudates, enable root feeders to distinguish lower order roots from higher orders. Third, higher order roots are comprised mainly of perennial woody tissues (Guo et al. 2008a), generally with higher cellulose concentrations (Guo et al. 2004), and may be indigestible to many root-feeding insects (Brown and Gange 1990; Hunter 2008). Finally, lower order roots have an intact cortex (Guo et al. 2008a), which consists of parenchyma (Hishi 2007), resulting in softer and more palatable tissues in lower order roots. For example, Bauerle et al. (2007) found that the sucking insect, grape phylloxera (*Daktulosphaira vitifoliae*), often attracted root tips of young age to create a feeding site (gall) in vineyard of California, USA. Thus, the distal lower order roots may be preferred by herbivores because they have greater tissue N, higher respiration rates, greater proportional cortex, and lower cellulose concentrations.

In this study, we did find that lower order roots had higher lignin concentrations, which may enhance defensive functions (Eissenstat and Yanai 1997; Hunter 2008). For example, Johnson et al. (2010) reported that lignin may increase root toughness in tobacco (*Nicoiana tabacum*). However, lignin did not appear to hinder herbivorous consumptions in this study. This may be due to the trade-off in herbivores grazing strategies between root nutrition and defense in that lower order roots with higher tissue N and lower cellulose concentrations are preferentially grazed, even when they have higher levels of defensive secondary compounds (Johnson et al. 2010). Given the widespread linkages between root anatomy, tissue N concentration and branch orders across temperate tree species (*see* Pregitzer et al. 2002; Guo et al. 2008a), the decreasing susceptibility of roots to herbivores with ascending root order may be a common phenomenon in woody plants.

Effects of root herbivory on fine root production

Even though root herbivores may significantly alter fine root longevity or turnover as reported in peach trees (*Prunus persica*) by Wells et al. (2002a) and in longleaf pine stand (*Pinus palustris*) by Stevens et al. (2002), little is known about the effects of root herbivory on fine root production in forest ecosystems (Stevens and Jones 2006; Hunter 2008; Coyle et al. 2008). Our results from the ingrowth method showed that root herbivory may be a critical factor in estimating fine root biomass production because root herbivores could consume a large amount of root biomass, e.g., 36% of *L. gmelinii* and 50% of *F*

mandshurica with the majority being the two distal order roots (57% for *F. mandshurica* and 79% for *L. gmelinii*). The potential underestimation of fine root production due to herbivory is supported by the consistent and significant decreases in the standing biomass of the first two order roots in the controlled experiments of our study. In addition, we found, in another study at the same sites, that fine root production (<1 mm in diameter) were 241 and 158 $g m^{-2} yr^{-1}$ in *F. mandshurica* and *L. gmelinii* plantations, respectively, based on the sequential core with decision-matrix method (Mei et al. 2010). According to our estimates, herbivore consumption of the first two order roots could be 58 $g m^{-2} yr^{-1}$ (*F. mandshurica*) and 37 $g m^{-2} yr^{-1}$ (*L. gmelinii*), respectively, accounting for underestimates of 20% and 19% of the biomass production by the decision-matrix method. Accordingly, at least about 20% of fine root production may be consumed by root herbivores in both plantations, regardless of the approaches used. Because of the widespread distribution of root herbivores in forest ecosystems, the fine root production is highly likely to be underestimated in many previous studies.

Effects of root herbivory on fine root dynamics

Root herbivory is the direct cause of fine root mortality and turnover. Our results showed that the first two order roots were consumed by insects throughout the growing seasons (Fig. 2), with the consumption peak occurring in summer for *F. mandshurica* (44%) and in autumn for *L. gmelinii* (38%). Fine root biomass of different orders varied with sampling time; however, there was no interaction between insecticide treatments and sampling time in both species (see Supplementary Table 4), which indicates the constant herbivory effects on root dynamics over the growing season. Besides root herbivores, other soil faunal species (e.g. detritivores, predators) may indirectly influence root dynamics either through predation on soil insects or through enhancing litter decomposition and soil organic matter turnover (Brown and Gange 1990; Brussaard 1998; Gange 2000). Thus, the patterns of root dynamics (Fig. 2) may reflect the overall effects of all soil organisms in response to insecticide applications. However, the indirect contributions of these other soil fauna to root dynamics may be limited, because no correlation was found between standing biomass of the first two order roots and the other soil faunal densities (Fig. 3b). In contrast, standing biomass of the first two order roots displayed a negative correlation with herbivore densities (Fig. 3a), with up to 77% (in *F. mandshurica*) and 66% (in *L. gmelinii*) of the biomass variations explained by the changes in herbivore density. These results suggest that root herbivores play a key role in affecting the dynamics of the two distal lower order roots in both plantations.

Implications

The findings of this study may have important implications to understanding of how root herbivory affect root growth and functions, as well as C budget and nutrient cycles in forest ecosystems. First, there is good evidence that the first and second order roots are the main body of absorptive roots, and their length account for >75% of the total root length in the first five orders in many woody plants (Guo et al. 2004; Wang et al. 2006; Valenzuela-Estrada et al. 2008). The herbivores mainly consume the distal lower order roots, including infected mycorrhizal fungi, may greatly reduce root functions such as nutrients and water uptake (Gange 2000; Hunter 2008). Our study demonstrated that over half of root biomass in the two distal lower orders may be consumed in both *F. mandshurica* and *L. gmelinii*, implying a huge loss of absorptive root length. This may further weaken the physiological functions such as capture, allocation and reserve of C and nutrients at the whole-tree level (Hunter 2008). Second, overlooking root herbivory effects not only underestimates root biomass and production, but also underestimates the flux of N derived from roots in biogeochemical cycles. Given the higher tissue N contents of the two distal lower order roots, N transferred from roots to soil may be missed by up to 0.73 $g m^{-2} yr^{-1}$(32%) in *F. mandshurica* and 0.34 $g m^{-2} yr^{-1}$ (38%) in *L. gmelinii*. In addition, the damaged roots together with deposition of frass and cadavers are more easily decomposed, which can accelerate C and nutrients mineralization (Brussaard 1998; Hunter 2001; Stevens et al. 2008). Thus, the contribution of root N returned to soil may be much higher than previously thought in biogeochemical cycles (Vogt et al. 1986; Jackson et al. 1997). Finally, root herbivores grazing primarily on

the first and second order roots observed in this study supports the view of 'root modules' in woody plants (Pregitzer et al. 2002; Xia et al. 2010), i.e. roots of the distal low orders may function as a module with similar longevities in their life cycles. Some recent studies found that there were similar root longevities between the first and second orders (Valenzuela-Estrada et al. 2008; Espeleta et al. 2009) or among the first three orders (Xia et al. 2010). These similarities were attributed to the similar anatomical structures (nonwoody) and physiological functions (absorptions) among these roots (Guo et al. 2008a). Our data suggest that it is highly likely that similar grazing stress by herbivores may also be a critical factor that causes the lower order roots to die together with similar estimated longevities. The analogy may be valid between these distal lower order roots and leaves on shoot system, i.e., both are palatable and attractive to herbivores. Although our findings are limited to *F. mandshurica* and *L. gmelinii*, the same may be for other woody plants, due to the consistent linkages between root anatomy, chemistry and root branch orders across all 24 woody species examined thus far (Guo et al. 2008a; Valenzuela-Estrada et al. 2008). Thus, the distal two order roots should be considered as a functional basis for determining the interactions between roots and herbivores in forest ecosystems.

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