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Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest

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Abstract Fine roots are a key component of carbon (C) flow and nitrogen (N) cycling in forest ecosystems. However, the complexity and heterogeneity of the fine root branching system have hampered the assessment and prediction of C and N dynamics at ecosystem scales. We examined how root morphology, biomass, and chemistry differed with root branch orders (1–5 with root tips classified as first order roots) and how different root orders responded to increased C sink strength (via N fertilization) and reduced carbon source strength (via canopy scorching) in a longleaf pine (*Pinus palustris* L.) ecosystem. With increasing root order, the diameter and length of individual roots increased, whereas the specific root length decreased. Total root biomass on an areal basis was similar among the first four orders but increased for the fifth order roots. Consequently, total root length and total root surface area decreased systematically with increasing root order. Fine root N and lignin concentrations decreased, while total non-structural carbohydrate (TNC) and cellulose concentrations increased with increasing root order. N addition and canopy disturbance did not alter root morphology, but they did influence root chemistry. N fertilization increased fine root N concentration and content per unit area in all five orders, while canopy scorching decreased root N concentration. Moreover, TNC concentration and content in fifth order roots were also reduced by canopy scorching. Our results indicate that the small, fragile, and more easily overlooked first and second order roots may be disproportionately important in ecosystem scale C and N fluxes due to their large proportions of fine root biomass, high N concentrations, relatively short lifespans, and potentially high decomposition rates.

Keywords Nitrogen · *Pinus palustris* · Root architecture · Root biomass · Total non-structural carbohydrates

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Introduction

Over the past three decades, studies of terrestrial ecosystem structure and function have focused increasingly on the role of fine roots in carbon (C) and nutrient cycling dynamics (Edwards and Harris 1977; Vogt et al. 1986; Hendricks et al. 1993; Fahey and Arthur 1994; Jackson et al. 1997). Comprehensive reviews have revealed that fine roots may account for nearly 40% of total net primary production (Vogt et al. 1996; Jackson et al. 1997). While most investigators have viewed fine roots ≤2 mm in diameter as homogenous units, recent findings indicate that substantial variability exists within roots ≤2 mm with respect to their form and function (Pregitzer et al. 1998, 2002; Wells and Eissenstat 2001). Consequently, accurate assessments and predictions of C and nutrient cycling dynamics at the ecosystem scale are contingent upon an improved understanding of the variation in form and function within the fine root guild (Norby and Jackson 2000; Wells and Eissenstat 2001; Tierney and Fahey 2002; Waisel et al. 2002; De Kroon and Visser 2003).

Root branching structure is a fundamental characteristic of form that can be intimately linked to functions that control fine root C allocation and fates (Fitter 2002). Pregitzer et al. (1998, 2002) reported that root nitrogen (N) concentrations and corresponding respiration decreased predictably from the distal first order roots to the third order roots in nine North American tree species. Also, Wells et al. (2002) reported that root mortality rates varied consistently with branching order in peach (*Prunus persica* Batsch) roots with median life-spans ranging

from 3 to 4 months for first order roots compared to 7 to 8 months for higher order roots. Moreover, Pregitzer et al. (1997) hypothesized that root C-fractions varied by order with lower order roots exhibiting relatively high concentrations of labile organic compounds compared to higher order roots that have greater concentrations of structural compounds such as cellulose and lignin. If correct, this would be consistent with the general hypothesis that lower order fine roots have relatively low construction costs yet higher respiration, mortality, and decomposition rates than higher order fine roots (Eissenstat and Yanai 1997; Pregitzer et al. 2002).

Notwithstanding the recent advances, evaluating the role of different root orders in C and N cycling at the ecosystem scale remains difficult. In addition to nutrient concentrations and turnover rates, the biomass of the various fine root orders is necessary for area estimates of C and N pools and fluxes, and the distribution of biomass by order has not been adequately assessed. Moreover, the manner in which different root orders respond to changes in C source and sink relations has received little research attention. Pregitzer et al. (2002) reported that greater soil N availability led to increases in root N concentrations and consequently respiration rates (and hence C sink strength) in the first three orders of roots, but this response was not consistent across species or over time. The responses of fine root branches to alterations in canopy carbohydrate supply (C source strength) have been largely unexplored. Canopy assimilation and soil (root) respiration are tightly coupled in some systems (Högberg et al. 2001), but may be decoupled by stored C for up to 3 years in others (Langley et al. 2002). Studies linking C assimilation and root C utilization will likely improve the mechanistic understanding of the belowground C allocation and fates at the whole-plant and ecosystem scales.

The objectives of this study were to determine how fine root morphology, biomass, and chemistry varied with branching order, and responded to C source (via foliage removal) and sink (via N fertilization) strength manipulations in a longleaf pine (*Pinus palustris* L.) ecosystem. We tested the following hypotheses: (1) specific root length, total root length, and total root surface area decrease as root order increases, but total mass increases with ascending root order; (2) root N concentration decreases as root order increases, but total fine root N content is relatively constant across root orders due to the increase in mass with ascending root order; (3) root total non-structural carbohydrate (TNC) concentration increases with increasing order, and the total TNC content is disproportionately greater in higher order roots due to increasing biomass with increasing root order; (4) N fertilization will increase fine root N concentration in all fine root orders, but most markedly in the first order roots which have the most intimate soil contact and greatest proportion of total root length; (5) canopy scorching will decrease TNC concentrations most significantly in the first order roots because these roots have the least amount of carbon storage (Eissenstat and Yanai 1997), are most distal from the photosynthate source (Kosola et al. 2002), and have the highest maintenance respiration rates among all root orders (Pregitzer et al. 2002); and (6) lower order roots have lower lignin concentrations, due to their ephemeral nature, and, thus, are less expensive to construct.

Materials and methods

Study site and experimental treatments

This study was conducted in a 50 ha longleaf pine plantation located at the Joseph W. Jones Ecological Research Center in Newton, Georgia. The plantation was established in 1980 using an approximate 1.5×2.0 m planting grid. The soil has been classified as Typic Quartzipsamment characterized by coarse sand that exceeds 2.5 m in depth, weak development of soil horizons due to frequent fire and mixing by soil fauna, low organic matter content, and lack of silt and clay (Goebel et al. 2001).

Sixteen 20×20 m study plots were established in randomized factorial design consisting of two fertilization (control and 50 kg N ha^{-1} $year^{-1}$) and two foliar removal (control and 80% plus needle scorch) treatments, with each treatment combination replicated four times. To reduce potential edge effects, sample collection was confined to the central 15×15 m subplot within each plot, and treatment plots were separated by at least 20 m.

N fertilization was initiated in January 2001. Ammonium nitrate was added at a rate of 50 kg N ha^{-1} $year^{-1}$, which is two-fold increase above the N mineralization rates of this ecosystem (Wilson et al. 1999). Nitrogen additions tracked natural temporal patterns of N mineralization; the N was applied on a monthly basis and the proportion of the 50 kg N $ha^{-1}year^{-1}$ added each month was based on the percentage of annual N mineralization occurring during that particular month in comparable stands (Wilson et al. 1999; Carter et al. 2004).

In June 2002, following the initiation of current-year needle production, canopy scorching was conducted in four fertilized and four non-fertilized plots. The scorch treatment removed 80±3% of the foliage starting from the bottom of the canopy (quantified by destructively harvesting six scorched and six non-scorched trees outside of the study plots). Needle scorch was accomplished using a portable propane torch that propelled hot air to the canopy sufficient to kill foliage without damaging branches and terminal buds (Carter et al. 2004). Longleaf pine ecosystems are fire dependent and trees frequently experience 100% crown scorching with no apparent effects on survival.

Root sampling, dissection, and morphology assessments

Root sampling was conducted in late August 2002. Fine roots were collected using an approach similar to that described by Pregitzer et al. (2002). In each plot, one block of soil (30 cm l ×20 cm w ×10 cm d) was removed from a randomly selected location using machetes and small knives, placed in a plastic bag on ice, and transported to the laboratory within 10 min for processing. In the laboratory, the fine roots in each soil block were sorted in two steps.

In step 1, large intact root branch networks were carefully removed from the soil with metal probes, placed in deionized water (1°C), and gently stirred to dislodge the soil from the roots. These root networks were then placed under 10× magnification where residual soil, organic matter particles, and dead root fragments (based on criteria described by Vogt and Persson 1991) were carefully removed using forceps. Following cleaning, the root networks were kept moist with deionized water (1°C) and dissected into branch orders following the protocols described by Fitter (1982, 1987), Berntson (1997), and Pregitzer et al. (2002). Distal roots were classified as first order, the root from which two first order roots branched was classified as second order, and so on (Fig. 1). The

diameter of each root section and the length of relatively short root sections were measured using a 40× stereomicroscope with an ocular micrometer (±0.025 mm), while the length of relatively long root sections (e.g., fourth and fifth order roots) was assessed using a measuring tape to the nearest 0.5 mm.

In step 2, the remainder of the soil block was sieved (0.5 mm mesh) to collect smaller root segments. Following sieving, the residual soil was placed in deionized water (1°C) and stirred repeatedly to float tiny root segments to the surface for collection. The root segments collected by sieving and floating in step 2 were sometimes difficult to assign to a particular root order, and the following procedure was used to minimize potential errors. When a white, light brown, or black mycorrhizal root tip could be identified using 10× magnification, the root orders were conclusively classified using the root tip as a first order root. In cases where a root tip was not present, a root branch was assigned to an order by comparing its diameter and length to those of more conclusively identified root orders isolated from the larger root networks during step 1 of sorting (i.e., a root branch was assigned to a specific root order when its diameter and length were within one standard deviation of the mean diameter and length of the more conclusively identified root orders). While this method undoubtedly led to some inaccurate classifications, we feel that the experimental error attributed to this sorting approach was minimal for several reasons. First, the ambiguity in assigning orders to roots collected in step 2 occurred primarily among roots from the third to fifth orders, and the mean diameter and length of roots in these three orders differed significantly from each other (Table 1). Second, the contribution of roots collected in step 2 to the total biomass of each order was relatively small (2, 13, 24, 21, and 33% of the total biomass for orders 1–5, respectively). Finally, the roots collected in step 2 were not used in root chemistry assessments and in the calculation of specific root length (i.e., only the more conclusively identified root orders collected during step 1 of sorting were used in the root chemistry assessments and specific root length calculations).

In addition to collecting fine root tissues, samples of large lateral roots (LLR) were collected from three randomly selected trees in each plot. For each tree, one LLR attached directly to the tap root was excavated to approximately 20 cm from the tree base, measured for diameter using a digital caliper, and cored using an increment borer. The three LLR cores from each plot were composited in a Whirl-pac bag, placed on ice, and transported to the laboratory.

Root biomass and chemical analyses

The LLR and fine root orders collected during step 1 that were used for biomass and chemistry assessments were oven-dried at 100°C for 1 h (to denature enzymes and reduce the loss of TNC via respiration during drying) followed by drying at 70°C to a constant mass (Smith 1969). Fine root orders collected during step 2 that were used for biomass estimates only were directly dried at 70°C to constant mass. Root samples were then weighed, ground, and homogenized using SPEX 8000-D mixer mill (SPEX, Edison, NJ), and subsampled for ash determination (550°C for 4 h). Root biomass estimates have been expressed on an ash-free, dry mass basis (Bledsoe et al. 1999). In turn, biomass estimates were used in conjunction with diameter and length estimates to calculate specific root length, total length, and total surface area for each order as described by Jackson et al. (1997).

The ground and homogenized LLR and fine root tissues collected during step 1 were subsampled for chemical analyses. Total C and N were analyzed using a Perkin Elmer Model 2100 CHN analyzer (Perkin-Elmer, Norwalk, CT). TNC concentrations were analyzed in a 2-step process in which sugar and starch concentrations were determined separately with a modified phenol-sulfuric acid method (Buysse and Merckx 1993). Root C-fraction concentrations including extractives (removed using a 2-stage extraction in dichloromethane and boiling water), cellulose (i.e., acid-soluble structural components removed using a 2-stage digestion in 72 and 2.5% sulfuric acid), and lignin (i.e., acid-insoluble structural components which are the residual of the 2-stage sulfuric acid digestion minus ash mass) were assessed using the forest products serial digestion technique (Ryan et al. 1990; Hendricks et al. 2000). All root chemistry indices have also been expressed on an ash-free, dry mass basis.

Data analysis

Differences in morphology, biomass, and chemistry among the root order, N fertilization, and foliage scorch treatments were analyzed as a multifactorial, repeated measures experiment using PROC MIXED, an iterative method that allows testing of both fixed effects and covariance components (Littell et al. 1996). The fixed effect factors were root order, N fertilization, and foliar scorching. Measurements on different root orders were considered as repeated measures from a spatial and a temporal perspective: sampling roots of different orders is similar to sampling roots that were located at different distances from the tree base or that were initiated at different times.

For the analysis of repeated measures data with the MIXED procedure, an appropriate covariance structure must be identified which best characterizes the covariance estimates of the data with the smallest number of parameters (Littell 2000). The most commonly used covariance structures include Simple (SIM), Compound Symmetric (CS), Autoregressive, order 1 [AR(1)], and Unstructured (UN). Following the procedure described by Littell (2000), we first used UN to generate REML correlation and covariance estimates of our data, and then identified AR(1) as the best fit model with the smallest number of parameters. This covariance structure was used in the tests of all root variables. When

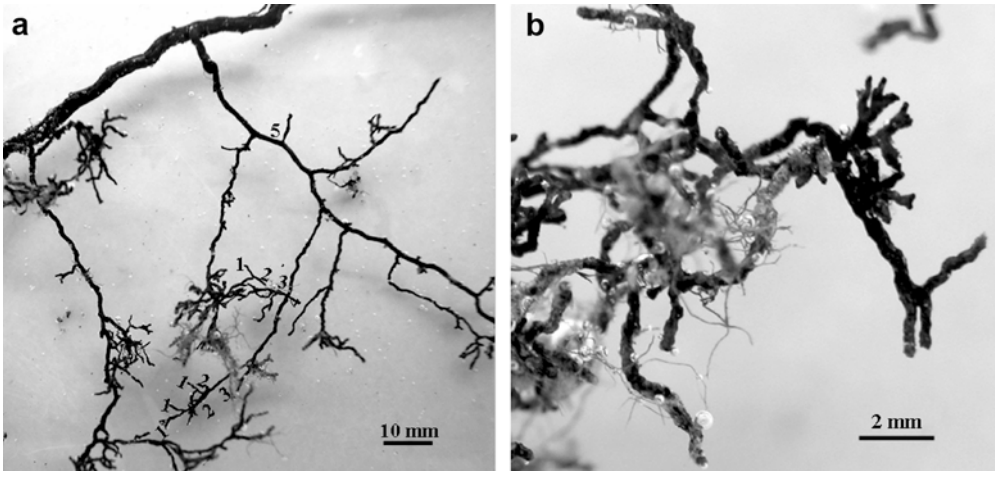

Fig. 1 **a**. A fine root branch network of *P. palustris* with representative root orders (1–5) labeled in black; **b**. A fine root branch network with numerous root tips

Table 1 *P. palustris* fine root morphological and biomass estimates by order. Data were pooled across treatments and values represent means with 1 SE in parentheses. Different superscript letters within each column indicate significant differences ($P<0.05$) among root orders 1–5. *Dashes* indicate unmeasured parameters. *LLR*: large lateral roots

Order	Diameter (mm)	Length (mm)	Specific root length (m g^{-1})	Biomass (g m^{-2})	Total length (m m^{-2})	Total surface area (m^2 m^{-2})
1	0.35^{a}(0.02)	3.2^{a}(0.2)	32.9^{a}(4.2)	13.3^{a}(2.2)	436.4^{a}(75.5)	0.48^{a}(0.07)
2	0.34^{a}(0.03)	3.5^{a}(0.2)	22.1^{b}(2.9)	9.3^{a}(1.2)	205.7^{b}(28.3)	0.22^{b}(0.03)
3	0.51^{b}(0.03)	12.2^{b}(0.8)	10.6^{c}(0.8)	15.1^{a}(1.8)	159.8^{b}(18.7)	0.26^{b}(0.04)
4	0.79^{c}(0.07)	57.1^{c}(4.4)	2.5^{d}(0.5)	14.3^{a}(2.1)	35.0^{c}(5.5)	0.09^{c}(0.01)
5	1.56^{d}(0.15)	115.3^{d}(12.7)	0.7^{e}(0.2)	26.4^{b}(4.2)	19.3^{c}(3.4)	0.09^{c}(0.01)
LLR	33.40 (3.30)	–	–	–	–	–

a variable had no significant response to fertilization and scorching treatments in the mixed models analysis, data were pooled across treatments and paired comparisons among root orders were made in a simple ANOVA (Tukey's HSD test) treating root order as the only response variable.

Results

Root morphology and biomass indices

The first and second root orders had similar mean diameters and lengths; however, beyond the second order mean diameter and length increased significantly with order (Table 1). Specific root length decreased significantly from first to fifth order roots (Table 1). Mean diameter and length, and specific root length did not respond to the fertilization or scorching treatments (data not shown).

Root biomass varied significantly with root order, primarily because fifth order roots had significantly higher biomass than the first four orders which did not differ significantly from each other (Table 1). When only the biomass estimates from the more conclusively determined root orders (i.e., branches collected during step 1 of sorting) were analyzed, the results were similar with the exception that the second order roots had significantly lower biomass than orders 1, 3, and 4. For both the more conclusively determined (i.e., step 1) and total (i.e., steps 1 +2) root order biomass estimates, no significant fertilization or scorching effects were detected.

Total root length and surface area decreased significantly with increasing root order. Notably, the first and second order roots combined accounted for more than 75% of total root length and more than 60% of total root surface area among the first five root orders (Table 1).

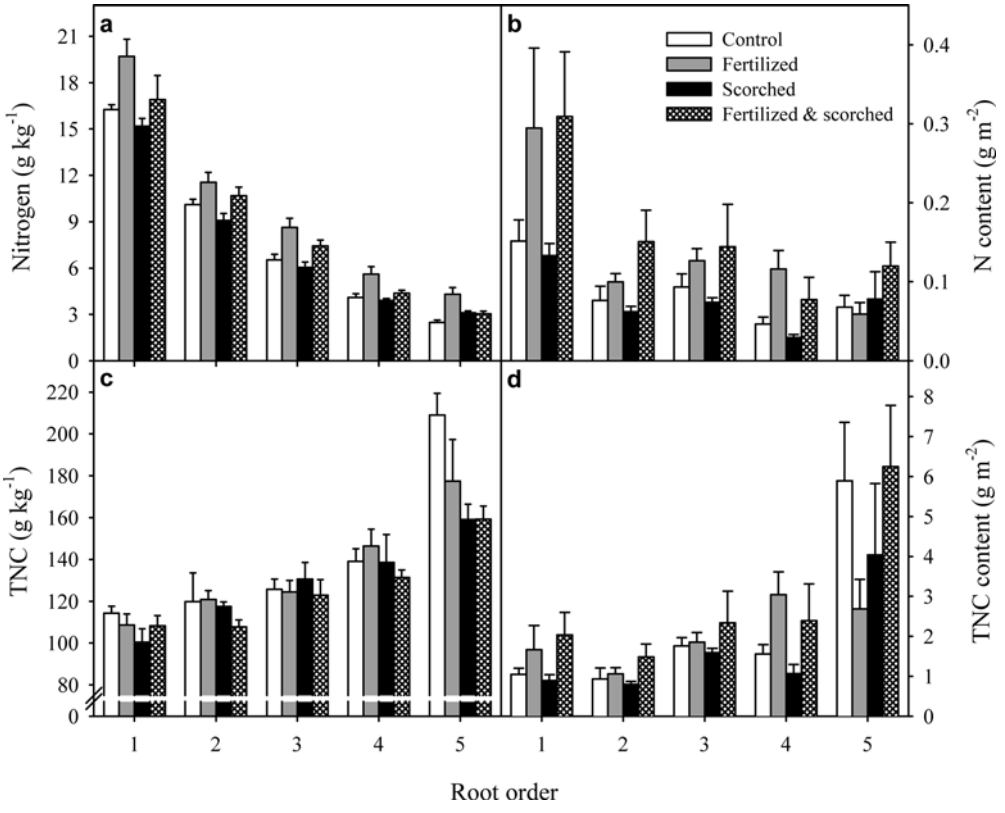

Fig. 2a–d Effects of N fertilization and canopy scorching on *P. palustris* root order. **a** N concentration, **b** N content, **c** TNC concentration, and **d** TNC content. *Error bars* represent one standard error of the mean

Root N and TNC

Root N concentrations decreased significantly from the first to the fifth order ($P<0.0001$; Fig. 2a). For the control plots, first order roots had a mean N concentration of 16.3 ±0.3 g kg^{-1} compared to 2.5±0.2 g kg^{-1} for fifth order roots. LLR had a mean N concentration of 3.2±0.2 g kg^{-1}. Root N content also differed significantly by order ($P<0.0001$); lower order roots generally exhibited higher N content values (Fig. 2b) driven largely by the decrease in root N concentrations with increasing root order (Fig. 2a).

In contrast to N, root TNC concentration increased significantly with order ($P<0.0001$; Fig. 2c). For the control plots, first order roots had mean TNC concentration of 114.3±3.2 g kg^{-1} compared to 209.1±10.3 g kg^{-1} for fifth order roots. LLR had mean TNC concentration of 105.7±5.6 g kg^{-1}, comparable to the concentration in the first order roots. Root TNC content also differed significantly with order as the higher order roots generally exhibited higher TNC contents (Fig. 2d).

Nitrogen fertilization significantly increased root N concentrations in all five fine root orders ($P<0.05$; Fig. 2a), but not in the LLR ($P=0.53$). In the fertilized plots, mean N concentrations were 21, 14, 32, 37, and 74% higher for orders 1–5, respectively than corresponding control values. In contrast, scorching significantly reduced root N concentrations ($P=0.001$). N concentrations averaged across the five fine root orders were 6% lower in scorched plots compared to the control plots, and 15% lower in fertilized and scorched plots compared to the fertilized plots (Fig. 2a). The interactions among root order, fertilization, and scorching were not significant for root N concentration ($P=0.69$). Root N contents also increased in response to fertilization ($P=0.02$), but were not affected by scorching ($P=0.86$) (Fig. 2b). (More details on statistical tests can be found in electronic supplementary material.)

Nitrogen fertilization did not significantly affect root TNC concentrations ($P=0.33$) but scorching did ($P=0.02$), and the impact of scorching was most significant in the fifth order roots (Fig. 2c). The mean TNC concentration of fifth order roots in the scorched plots (159.1±7.3 g kg^{-1}) was 24% lower than that in corresponding control plots (Fig. 2c). The interaction between scorching and root order was marginally significant ($P=0.05$). TNC concentrations in LLR were not significantly influenced by scorching ($P=0.81$). In addition, scorching did not significantly impact root TNC content ($P=0.78$). (More details on statistical tests can be found in electronic supplementary material.)

Since the root biomass estimates contained a degree of error, we compared the effects of conclusively determined (i.e., step 1) versus total (i.e., steps 1+2) biomass estimates on the statistical test results involving N and TNC contents. In all cases, the two estimates of biomass yielded the same results.

Root C-fractions

Total C varied significantly with root order, but the differences were relatively small (values ranged from 510.2 to 545.1 g kg^{-1} with an overall mean of 528.0 g kg^{-1} and coefficient of variation of 1.7% across all orders and treatments) and did not change systematically with order (data not shown). Also, LLR had similar C concentrations (mean = 525.0 g kg^{-1}, coefficient of variation = 2.3%).

Similar to total C, extractives and lignin concentrations varied only slightly across the first five root orders and generally decreased with increasing order (Fig. 3). In contrast, the cellulose increased systematically with root order, ranging from 185.3 ± 7.5 g kg^{-1} to 284.7 ± 7.5 g kg^{-1} in first and fifth order roots, respectively. LLR exhibited higher cellulose concentrations (386.4 ± 21.7 g kg^{-1}) and lower lignin concentrations (254.6 ± 8.0 g kg^{-1}) than the five fine root orders (Fig. 3).

Discussion

As predicted in hypothesis 1, the specific root length, total length, and total surface area of *P. palustris* fine roots decreased, often dramatically, with increasing order (Table 1). Consistent with the fine root branch characteristics described by Pregitzer et al. (2002) for other *Pinus* species, the first two orders of *P. palustris* roots were short and thin, yet abundant, such that these two orders comprised the majority of the total length and surface area of fine roots. In contrast, fourth and fifth order roots were individually longer and larger in diameter than the lower order roots, but collectively these roots constituted a small proportion of the total length and surface area

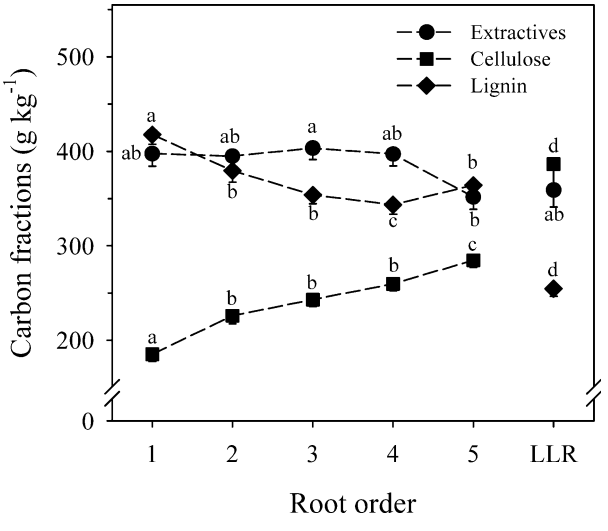

Fig. 3 *P. palustris* root C-fraction concentrations. Data were pooled across treatments, and *error bars* represent one standard error of mean. Different lower-case letters within each C-fraction indicate significant ($P<0.05$, Tukey's HSD test) differences among individual root orders

perhaps reflecting their importance primarily in resource transport and storage rather than uptake.

Contrary to the prediction in hypothesis 1, total root mass did not systematically increase with order (Table 1). Root standing biomass was similar among the first four orders but increased significantly with the fifth order (Table 1). While few data are available for comparisons, Pregitzer et al. (2002) reported specific root length and total root length by order for nine North American tree species. Multiplying these two values for each order revealed that the total mass of first order roots tended to be slightly greater than higher order roots. Since first order roots are likely to have relatively high turnover rates (see discussion below) due to their small diameter and/or branch position (i.e., if a higher order branch dies the root tips must also die), the finding that root tips have an equal or greater total mass than higher root orders is noteworthy from C and nutrient cycling perspectives.

Consistent with hypothesis 2 and previous reports (Pregitzer et al. 2002), root N concentration decreased with increasing root order (Fig. 2a). However, total fine root N was not relatively constant across root orders as hypothesized. Due to the unexpected even distribution of root biomass across the first four orders, root total N content also decreased with increasing root order (Table 1, Fig. 2b). While the fifth order roots were significantly greater in biomass than the lower orders (Table 1), the low N concentration of the fifth order roots caused their total N content to be relatively low. The inverse correlation between root order and N content has important C flux implications since N may serve as an index of root enzyme content, and, hence, metabolic activity (Ryan 1991).

While N content signifies root metabolic activity or C sink strength, root TNC content represents locally available carbohydrates for metabolic activities or C source strength. TNC content increased with increasing root order (Fig. 2d) due largely to the increase in TNC concentration with order (Fig. 2c) as predicted in hypothesis 3. The positive correlation between TNC content and root order provides additional support for the view that high order fine roots with relatively low N contents and metabolic activities serve important C transport and storage functions (Esau 1965; Eissenstat 1997). Furthermore, the branching structure, short and thin low order branches (i.e., orders 1–3) arising from longer and thicker high order roots (Fig. 1), provides a short route for TNC transport from higher order roots to the more metabolically active lower order roots.

Assessing the patterns and controls of biomass, N, and TNC distribution across root orders may provide valuable insights into belowground C allocation and fates. Fine root respiration is the dominant component of total soil respiration; roots may account for up to 65% of total soil CO_2 efflux (Boone et al. 1998; Högberg et al. 2001; Bhupinderpal-Singh et al. 2003). Furthermore, root mortality represents a major flux of C and nutrients to the soil organic matter pool (Vogt et al. 1986; Eissenstat and Yanai 1997). Our results indicate that first order roots have relatively high N contents due to their high N concentration and relatively high biomass. These findings coupled with reports of relatively high respiration (Pregitzer et al. 1998) and turnover (Wells et al. 2002) rates for lower order roots, suggest a predominant role of first order roots in ecosystem-scale C and N cycling. However, due to their small size and fragile nature, these roots may be easily missed in biomass sorting (Caldwell and Virginia 1989) and under-represented in process level studies of C flow and nutrient cycling at the ecosystem scale (Dornbush et al. 2002).

The manner in which fine roots of the different branching orders respond to changes in C source and sink relations has received little research attention (Pregitzer et al. 2002). N fertilization increased N concentrations (and consequently C sink strength) in all fine root orders consistent with hypothesis 4 (Fig. 2a); however, the proportional increase in N concentration was similar among the five orders in contrast to hypothesis 4. Pregitzer et al. (2002) reported increases in root N concentration after fertilization in the first three root orders, but in only three out of nine tree species. The strong response to N fertilization across all five fine root orders in our study was probably related to the low soil N availability of the study site and low pre-fertilization root tissue N concentrations (Lambers et al. 1998). The potential relationship between pre-treatment N concentration and root order responses to fertilization may explain the differential results reported by Pregitzer et al. (2002). In contrast to the fine root orders, fertilization did not increase the N concentration of LLR, probably due to the short-term nature of the fertilization treatment (i.e., 1.5 years) and/or the large biomass of these roots which may buffer changes in N concentration.

Although fine root biomass did not increase significantly after N fertilization, the first four orders exhibited a trend of increasing biomass which contributed to the increases in fine root N content in fertilized plots (Fig. 2b). The increase in N content following fertilization may lead to greater C consumption in the fine root branches. However, this greater C consumption does not necessarily translate into greater proportions of carbohydrate allocation to roots because the total amount of C fixed may also increase with increasing soil N availability (Hendricks et al. 1993; Nadelhoffer 2000). Further work linking C supply to demand at whole tree and ecosystem scales is needed.

In contrast to the fertilization effects, canopy scorching had a negative impact on fine root N concentrations (Fig. 2a). If root N concentration is a reflection of N uptake rate, the relatively low N concentrations in scorched plots indicates that N uptake is sensitive to the reduction in current photosynthetic supply which is consistent with the findings of a defoliation study conducted by Kosola et al. (2001). In contrast, root biomass was not negatively influenced by scorching, a pattern also similar to that reported in Kosola et al. (2001). Kosola et al. (2001) suggested that the physiological capacity to acquire nutrients may be more sensitive to a reduction in current photosynthate than root demography

(i.e., production and mortality). Collectively, these results suggest that mild C limitations caused by temporary defoliation may not have a large impact on root production and mortality, especially when alternative sources of C (e.g., storage) are available and when foliage recovery is rapid (3–4 months in this study). In systems that have adapted to frequent disturbance (e.g., frequent fire), C storage may be particularly large and capable of maintaining fine roots and mycorrhizae for extended periods (e.g., 3 years; Langley et al. 2002). Longer-term removal of photosynthetic capacity along with limited C storage, however, clearly leads to increased root mortality (Eissenstat and Duncan 1992; Goins and Russelle 1996; Ruess et al. 1998).

As predicted in hypothesis 5, root TNC concentrations were reduced by scorching (Fig. 2c). However, contrary to this hypothesis and previous postulations (e.g., Lyford 1975; Kosola et al. 2002), the greatest reduction was not observed in distal first order roots, but rather in the fifth order roots. An earlier study by Kosola et al. (2001) showed that root TNC decreased in fine roots (0.5–2 mm) but not in very fine roots (<0.5 mm) as a result of defoliation. The reason for the differential responses in TNC to defoliation between different root size classes was not discussed by Kosola et al. (2001). Here we speculate that TNC in the lower order (and small-diameter) roots may be preferably maintained since these roots are critical for resource uptake, based on their dominance in total root length (Pregitzer et al. 2002) and total surface area (Table 1). Furthermore, N concentrations are low in the fifth order roots, and consequently the demand for carbohydrates to support metabolic activities in these roots may also be low, making it possible for these roots to export stored non-structural carbohydrates to meet the C demand of lower order roots that have greater metabolic activities.

The results of this study, combined with the findings of Kosola et al. (2001) and Langley et al. (2002), suggest that C storage may provide a strong and lasting buffer between fluctuations of current photosynthate and belowground C use in ecosystems that have adapted to frequent disturbance (e.g., fire and insect herbivory) where adults survive and recover from disturbance. With sufficient C storage, both root demography and respiration may be maintained. In contrast, in ecosystems where disturbance is infrequent and catastrophic, C storage may be much smaller and serve as only a short-term buffer (Högberg et al. 2001). How C storage affects the C economy at the whole plant level in different ecosystems merits further investigation.

Our hypothesis 6 predicted that roots of lower order would be less lignified, and, thus, have lower construction costs. However, lignin concentrations decreased slightly yet significantly with increasing root order (Fig. 3). Other studies have reported that root lignin concentrations were inversely correlated with root diameter (Hendricks et al. 2000; Dornbush et al. 2002; Fenandez et al. 2003), and since root diameter is positively correlated with root order (Pregitzer et al. 2002 and this study), these results indirectly support the root order-lignin patterns observed in this study. Consequently, these findings suggest that root tips do not have lower construction costs.

Interestingly, root order was strongly related to cellulose concentration, with the higher order roots containing greater cellulose concentrations consistent with the pattern hypothesized by Pregitzer et al. (1997). Chapin et al. (1986) suggested that cellulose was positively correlated with tissue age for stems and leaves. Our results suggest that the same may be true for roots as cellulose concentration and presumably age increased with branch order. The high cellulose concentration in LLR (386.4 ± 21.7 g kg^{-1}, which is 10% higher than fifth order roots, Fig. 3) probably reflects markedly greater age of LLR relative to fifth order roots. Direct measurements of age for roots of different branching orders are difficult to obtain with minirhizotron cameras (Wells et al. 2002). Root cellulose concentrations may serve as an alternative indicator of age for the different branching orders.

In conclusion, as suggested previously (e.g., Pregitzer et al. 2002), the order of a root in the branching system may dictate its form and function. Lower order, smaller diameter roots dominate total root length and surface area and are therefore important for resource uptake, whereas higher order, larger diameter roots are more important in C storage. Also, lower order roots constitute a large proportion of total fine root biomass, which coupled with their high N concentrations, leads to a commanding role of these roots in total root N content and possibly root respiration at the ecosystem scale. Furthermore, lower order roots are strongly responsive to environmental alterations such as changes in soil fertility and availability of canopy assimilates. These factors, along with their small size, fragile nature, and short lifespan (e.g.,Wells et al. 2002), point to the vital but understudied role of the smallest of the fine roots in ecosystem scale C and N fluxes in a changing global environment.

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