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FINE ROOT ARCHITECTURE OF NINE NORTH AMERICAN TREES

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Abstract. The fine roots of trees are concentrated on lateral branches that arise from perennial roots. They are important in the acquisition of water and essential nutrients, and at the ecosystem level, they make a significant contribution to biogeochemical cycling. Fine roots have often been studied according to arbitrary size classes, e.g., all roots less than 1 or 2 mm in diameter. Because of the size class approach, the position of an individual root on the complex lateral branching system has often been ignored, and relationships between the form of the branching root system and its function are poorly understood.

The fine roots of both gymnosperms and angiosperms, which formed ectomycorrhizae (EM) and arbuscular mycorrhizae (AM) fungal associations, were sampled in 1998 and 1999. Study sites were chosen to encompass a wide variety of environments in four regions of North America. Intact lateral branches were collected from each species and 18 561 individual roots were dissected by order, with distal roots numbered as first-order roots. This scheme is similar to the one commonly used to number the order of streams. Fine root diameter, length, specific root length (SRL; m/g), and nitrogen (N) concentration of nine North American tree species (*Acer saccharum, Juniperus monosperma, Liriodendron tulipifera, Picea glauca, Pinus edulis, Pinus elliottii, Pinus resinosa, Populus balsamifera,* and *Quercus alba*) were then compared and contrasted.

Lateral roots <0.5 mm in diameter accounted for >75% of the total number and length of individual roots sampled in all species except *Liriodendron tulipifera*. Both SRL and N concentration decreased with increasing root order in all nine species, and this pattern appears to be universal in all temperate and boreal trees. Nitrogen concentrations ranged from 8.5 to 30.9 g/kg and were highest in the first-order "root tips." On a mass basis, first-order roots are expensive to maintain per unit time (high tissue N concentration). Tissue N appears to be a key factor in understanding the C cost of maintaining first- and second-order roots, which dominate the display of absorbing root length. There were many significant differences among species in diameter, length, SRL, and N concentration. For example, two different species can have similar SRL but very different tissue N concentrations. Our findings run contrary to the common idea that all roots of a given size class function the same way and that a common size class for fine roots works well for all species. Interestingly, fine root lateral branches are apparently deciduous, with a distinct lateral branch scar. The position of an individual root on the branching root system appears to be important in understanding the function of fine roots.

Key words: Acer saccharum; *carbon;* Juniperus monosperma; Liriodendron tulipifera; *nitrogen;* Picea glauca; Pinus edulis; Pinus elliottii; Pinus resinosa; Populus balsamifera; Quercus alba; *roots.*

INTRODUCTION

At the level of the individual plant, the recognition that essential soil resources are highly variable in space and time has resulted in research designed to understand how fine roots respond to altered resource availability. Plants are remarkably capable of utilizing pulses of limiting nutrients (Bilbrough and Caldwell 1997), down-regulate root respiration when and where the soil is dry (Kosola and Eissenstat 1994, Burton et al. 1998), and rapidly increase the rate of nutrient uptake per unit root length when localized pulses of nutrients are added to soil (Jackson et al. 1990, Jackson and Caldwell 1991, Ryel et al. 1996, Cui and Caldwell 1998, Ryel and Caldwell 1998). Physiological adjustment of fine root function is, therefore, an important response to soil heterogeneity. Roots also rapidly proliferate in zones where limiting nutrients are made available (Drew et al. 1973, Drew and Saker 1975, Eissenstat and Caldwell 1988, Pregitzer et al. 1993, Bilbrough and Caldwell 1995, Bilbrough and Caldwell 1997). It is clear that individual plants can rapidly adjust the structure and function of their root system to compete for limiting soil resources.

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Even though we understand that fine roots play an important role in the function of individual plants, it is remarkable how little we know about the linkages between root system structure and function. For example, all of the studies cited in the paragraph above have looked at only a portion of the root system in order to quantify root responses to altered resource availability. Some studies of root growth utilize line intersection methods to quantify root response, some trace root length on glass walls or plates, and others utilize minirhizotrons or washed roots and image analysis. Physiological studies often work with arbitrary portions of intact or excised roots of a given size. None of these studies relate the structure of the intact root system to resource acquisition; position and form of the individual root on the branching fine root system are typically ignored. An appropriate analogy would be trying to understand leaf-level photosynthesis without knowing leaf age, leaf thickness (mass per unit area), leaf N concentration, position of the leaf on the shoot, or position of the shoot in the canopy, all of which influence C assimilation at the individual leaf level (Field and Mooney 1983, 1986, Reich et al. 1992). In other words, we know that fine roots are important in the acquisition of essential soil resources and that root growth and physiology are highly plastic, but we do not really understand how to describe the relationship between root form and root function.

From a forest ecosystem perspective, the situation is no better in terms of understanding relationships among root system structure and ecosystem function. At the ecosystem level, the definition of a fine root has historically been arbitrary. Roots have traditionally been sampled destructively, separated from the soil, sorted into arbitrary size classes (e.g., roots 0–1 or 0–2 mm in diameter), dried, and then weighed to determine root biomass. Arbitrary size classes seem reasonable because the objective at the ecosystem scale is to understand the contribution of roots to the cycling of energy and nutrients and this approach has documented that fine roots and mycorrhizae are an important and dynamic component of all terrestrial ecosystems. In many instances, fine roots account for a significant portion of ecosystem net primary productivity. In cold, nutrient deficient, or droughty situations carbon (C) allocation to fine roots has often been estimated to be more than half of whole-system net primary production (NPP; Vogt et al. 1986), and it is now recognized that fine roots are important in understanding global primary productivity and C cycling (Jackson et al. 1997). The death and decay of fine roots is also an important component of terrestrial nutrient cycles (Fahey and Arthur 1994). Despite the widespread recognition that fine roots play key roles in ecosystem function, it is not clear which roots die and decay in response to altered resource availability. The arbitrary size class approach to quantifying fine root standing crop sheds little light on how essential soil resources alter belowground biomass and biogeochemistry through time.

In order to predict how the fine roots of trees respond to the altered availability of growth-limiting soil resources, it is critical to understand the form (morphology and anatomy) of the root system. The woody roots of mature trees are widely distributed with horizontal roots routinely exploring the soil for distances greater than 20 m from the tree trunk; woody tap roots typically reach soil depths that exceed 10 m and roots can explore the subsoil up to 30 m below the surface (Lyr and Hoffmann 1967, Lyford 1975). These woody roots primarily serve anchorage, transport, and storage functions. In both angiosperms and gymnosperms, lateral "fine roots" arise at variable distances from the apical meristem (i.e., root tip) in the pericycle of the parent root and subsequently grow through the cortex (Charlton 1996). These lateral roots are sometimes called "branch roots" because they can exhibit a complex branching structure (Esau 1965). The majority of these lateral roots remain as smaller diameter, nonwoody roots, and there are literally millions of individual root tips per hectare concentrated on these fine lateral branches (Lyford 1975). Lateral "fine root" branches are the focus of this study.

Pregitzer and colleagues carefully dissected portions of the absorbing root systems of plants in forests (Pregitzer et al. 1997, 1998). They found that most of the absorbing root length in sugar maple (*Acer saccharum*) and white ash (*Fraxinus americana*) seedlings was concentrated in the form of short, very fine (diameter <0.5 mm) lateral branches and the N concentration of these small, distal roots was high (Pregitzer et al. 1997). The respiration rate of plant tissues is strongly correlated with N concentration (Ryan 1991, Reich et al. 1992) and Pregitzer et al. (1998) confirmed that fine root respiration rates were linearly related to root N concentration, implying that the small, distal lateral branches have the highest rates of metabolism. We hypothesized that nonwoody, lateral fine root branches of trees account for the vast majority of fine root length in all North American trees and we predicted the pattern observed for sugar maple and white ash seedlings would be universal in mature temperate and boreal trees, regardless of climate, soil type, tree taxonomic rank (angiosperm vs. gymnosperm), or stage of root development. We also predicted that most fine roots of mature trees are much smaller than typically assumed, with fine root diameters rarely >0.5 mm regardless of the position of an individual root on the lateral branch.

Specific root length (SRL, m/g) is the ratio of root length to mass. It has been used as a simple index of root benefit to cost, assuming that resource acquisition is proportional to length, and root cost (construction and maintenance) is proportional to mass (Fitter 1991, Eissenstat 1992, Eissenstat and Yanai 1997). We hypothesized that distal roots have the highest SRL and N concentrations, regardless of soil conditions, tree

species, or climate. In other words, the position of a root on the branching lateral fine root system is an important predictor of SRL and N concentration. Because there is such a strong relationship between tissue N concentration and specific respiration rate in all plant tissues (Ryan 1991, Ryan et al. 1996), it is important to understand if SRL and N concentration vary systematically depending on the position of a root on the branching root system.

Many of the fine roots of trees are heavily infected by mycorrhizae, which can influence C allocation to fine roots and rates of nutrient acquisition by plants (Reid et al. 1983, Allen 1992, Rygiewicz and Andersen 1994, Smith et al. 1997). Mycorrhizae also sometimes alter the morphology and longevity of fine roots (Hooker et al. 1995, Norman et al. 1996). Trees are infected largely with either ectomycorrhizae (EM) or arbuscular mycorrhizae (AM).

Conifers in the Pinales and many angiosperms form EM. Read (1992) contends that the primary function of EM-dominated roots is not to absorb nutrients from the soil, but rather to provide a food base from which the fungus can extend into the soil. EM infections are potentially long-lived [≤5 yr (Orlov 1957, 1960)], which presumably allows the EM-infected root to live as long as C is translocated to the fungus. On the other hand, AM fungi, which predominate in more primitive conifers and many angiosperms, infect only newly developing fine roots, which apparently turn over rapidly (Friese and Allen 1991). In support of Friese and Allen's (1991) observations, Hooker et al. (1995) experimentally demonstrated that AM-colonized roots of *Populus* had shorter life spans than uncolonized roots. AM infections are apparently transient. The fungus invades a root and sequesters C until changes in root ontogeny (e.g., deposition of condensed tannins and phenolic compounds) precludes continued infection (Allen et al. 1989, 1992). AM fungi are always searching for a new, uninfected root tip at the appropriate stage of development and the infection lasts a relatively short period of time. Given that EM and AM fungi have quite different life histories, how are the lateral fine roots of trees influenced by the dominant type of mycorrhizal infection? We hypothesized that EM-dominated fine roots from several contrasting tree taxa, when compared to AM-dominated fine roots, would exhibit larger mean diameters and lower SRL, and therefore greater construction and maintenance costs per unit length (Eissenstat and Yanai 1997).

Finally, there has been considerable debate about the influence of soil N availability on C allocation to fine roots in forests (Hendricks et al. 1993). Although there have been several field experiments designed to quantify C allocation to fine roots at different levels of N availability using traditional destructive sampling techniques, there are few descriptions of the influence of soil N on fine root architecture in forests based on experimental investigations in the field.

To test our hypotheses and predictions, we designed a field experiment which ranged across different biomes and deliberately compared and contrasted angiosperms and gymnosperms and EM- and AM-dominated roots in control and fertilized treatments. The overall objective of our research was a better fundamental understanding of the branching fine root systems of North American trees.

Methods

Study sites

The study sites are located in four different regions of the United States (Fig. 1). Each region represents a different set of environmental conditions (Table 1). Nine tree species were chosen for study. The roots of two angiosperms form AM fungi: *Acer saccharum* Marsh. (sugar maple) and *Liriodendron tulipifera* L. (tulip poplar). Two other angiosperms, *Populus balsamifera* L. (balsam poplar) and *Quercus alba* L. (white oak) were infected with EM. Conifers in the Pinales are largely infected with EM, and this was the case for *Picea glauca* (Moench) Voss, (white spruce), *Pinus edulis* Engelm. (pinyon pine), *Pinus elliotti* Engelm. (slash pine), and *Pinus resinosa* Ait. (red pine). However, the fine roots of *Juniperus monosperma* (Engelm.) Sarg. (one-seed juniper) formed AM. The type of mycorrhizal infection for all tree species was verified by staining and direct microscopic observation.

We chose study sites and species–mycorrhizal contrasts to encompass the wide range of vegetation types and environments represented in the forests of North America. *Picea glauca* and *Populus balsamifera* were sampled at the Bonanza Creek Long Term Ecological Research Site (LTER) in Alaska. Stands of both species were growing on alluvial loess within the floodplain of the Tanana River, and in both forest types the upper 10 cm of the soil was dominated by organic matter (O horizons). Annual precipitation at Bonanza Creek is relatively low (Table 1), however, low soil temperatures and low evapotranspiration, frozen soil, and ground water complicate moisture relations at Bonanza Creek.

The fine roots of *Juniperus monosperma* and *Pinus edulis* were excavated in a pinyon–juniper woodland at the Sevilleta LTER site in New Mexico. This forest is open, and grows on the semi-arid north-facing slope of a draw. The soils are circum-neutral, rocky, sandy loams (Table 1), with virtually no litter layers except under individual pinyon and juniper trees. Soils in the interspaces (between the trees) are often bare and there is obviously considerable natural movement of soil after thunderstorms.

Quercus alba was sampled in Georgia from a mesic mixed-hardwood ecosystem. *Pinus elliottii* was growing in a 21-yr-old managed plantation in northern Florida and *Liriodendron tulipifera* roots were excavated at the USDA Forest Service Coweeta Research Station in North Carolina in the foothills of the Appalachians;

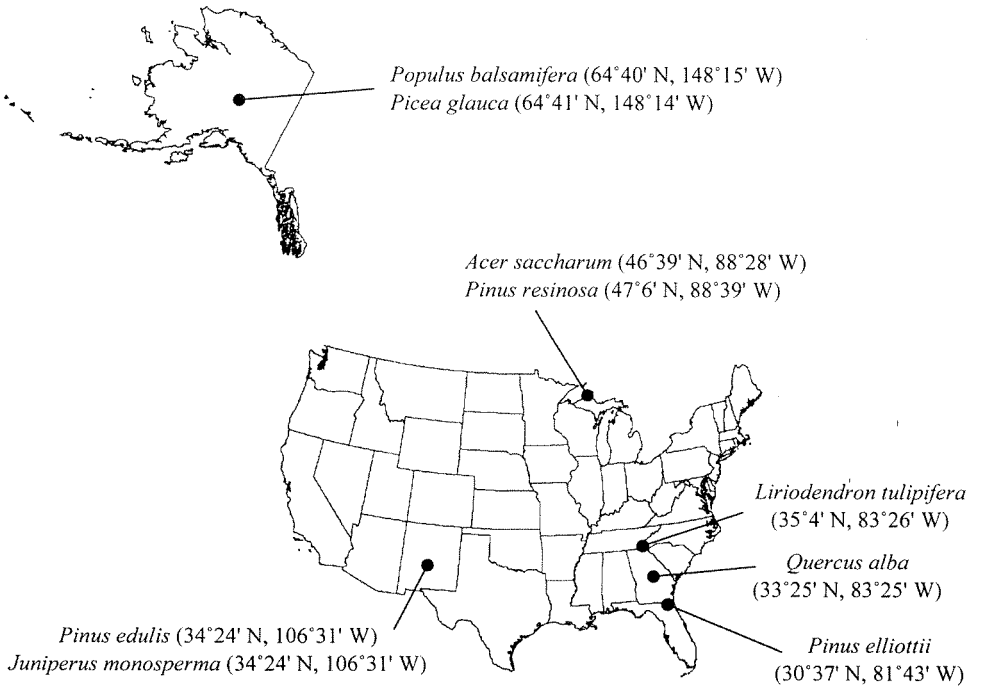

FIG. 1. Study site locations. All sites are in the United States.

Coweeta is also an LTER site. The soils from these three different forests in the southeastern United States varied considerably in texture and pH (Table 1) and all of these stands are managed. The *Pinus elliottii* plots were a part of a long-term fertilizer and competition control study (University of Georgia Plantation Management Research Cooperative) and there was no understory vegetation in these stands.

Acer saccharum roots were sampled from a mesic northern hardwood forest growing on loamy sand soil in the western Upper Peninsula of Michigan. *Pinus resinosa* roots were collected from a nearby 50-yr-old plantation, which had been experimentally thinned almost 30 yr ago (Liechty et al. 1986). The *Pinus resinosa* roots were growing in a dry–mesic sandy soil (Table 1).

In each of the eight different forest ecosystem types, we established six 30 × 30-m plots. Three plots were fertilized and the remaining three plots were used as controls. The two treatments were assigned at random to each of the six replicate plots. Ten g $N \cdot m^{-2} \cdot yr^{-1}$ of NH_4^+–NO_3^- were applied to each of the three fertilized plots in both 1998 and 1999, except for the *Pinus elliottii* study sites, which were a portion of a long-term silvicultural experiment (Table 1).

Small, intact segments of the distal portion of the branching fine root system were sampled (Fig. 2). At Sevilleta, the *Pinus edulis* and *Juniperus monosperma* root segments were collected from the same plots, but from trees that were widely spaced. One species was sampled from each of the remaining seven study sites, where each of the stands was dominated by a single species (Table 1). Two intact segments of the fine root system of each species were collected from each of the six plots in 1998 and again in 1999. Insects destroyed one of the *Pinus elliotti* control plots in 1998 before the initial samples were collected and wildfire destroyed four additional plots in 1998 after they were sampled, so only the two remaining plots (one control and one fertilized) of *Pinus elliotti* were sampled in 1999.

Fine root excavation

Small, intact segments of the fine root system were very carefully excavated during the summers of 1998 and 1999 at approximately the time of maximum root growth for each of the different ecosystem types. The southeastern sites were sampled in May, while Michigan and Alaska were sampled in mid- or late June. Samples from the New Mexico site were excavated in late August, just after the beginning of the annual monsoon season (Table 1).

A random location was chosen in each plot under a randomly chosen individual tree and a shovel was used to remove a soil block ~20 × 10 × 10 cm deep, from which the intact root segments were collected. The segments were carefully gathered from the overturned soil block. Each individual segment was usually attached to a small woody fine root <1 mm in diameter. It was not difficult to identify the roots of each species based on their location (attached to larger roots and under the canopy of the species of interest) and the general ap-

TABLE 1. Summary of site properties, dates of root excavation, and selected soil properties.

Property	*Acer saccharum*	*Liriodendron tulipifera*	*Populus balsamifera*	*Quercus alba*	*Juniperus monosperma*	*Picea glauca*	*Pinus edulis*	*Pinus elliottii*
Taxonomic rank	A	A	A	A	G	G	G	G
Dominant mycorrhizae	AM	AM	EM	EM	AM	EM	EM	EM
Stand age (yr)	uneven	40	≥100	>60	uneven	≥100	uneven	21
Basal area (m^2/ha)	34	34	37	26	...	34	...	24
Overstory (%)	92	85	70	68	...	98	...	100
Mean annual precipitation (mm)	883	1816	287	1263	388	287	388	1303
Mean annual temperature (°C)	3.8	12.7	−3.3	16.5	12.7	−3.3	12.7	20.0
Fertilization (NH_4NO_3; $gN \cdot m^{-2} \cdot yr^{-1}$)	10	10	10	10	10	10	10	†
Total N applied (1998 and 1999; g/m^2)	20	20	20	20	20	20	20	†
Soil carbon (gC/kg; 0–20 cm)	21.03	84.28	34.64	65.22	39.21	29.20	39.21	13.59
Soil nitrogen (gN/kg; 0–20 cm)	1.86	5.76	2.10	3.73	2.96	1.44	2.96	0.50
Soil texture (0–20 cm)	sandy loam	sandy loam	organic	clay loam	sandy loam	organic	sandy loam	sand
Soil pH (0–20 cm)	4.4	5.7	5.9	5.2	6.9	5.5	6.9	5.2
1998 root excavation date	10 Jun	21 May	22 Jun	22 May	22 Aug	22 Jun	22 Aug	23 May
1999 root excavation date	12 Jun	20 May	24 Jun	21 May	27 Aug	24 Jun	27 Aug	22 May
Number of root segments dissected	24	24	24	24	24	24	24	14
Mean root axis diameter (mm)	0.5	1.2	0.9	0.9	0.8	0.9	0.9	0.7
Total number of roots dissected	2532	870	2443	2444	2025	2436	2096	1328
Total root length dissected (m)	8.2	8.4	6.1	5.7	10.7	7.1	9.1	3.4
Total over dried root mass (g)	0.2	1.0	0.1	0.2	0.7	0.3	0.6	0.1

Notes: Overstory is measured as a percentage of basal area. Abbreviations: G, Gymnosperm; A, Angiosperm; EM, Ectomycorrhizae; AM, Arbuscular mycorrhizae.

† The *P. elliottii* plantation was fertilized several times: 1979, P (22 g/m^2); 1991, NPK (22 g/m^2 of each element); 1997, NPK (22 gN/m^2, 0.3 gP/m^2, 0.5 gK/m^2).

pearance of the lateral branches (diameter, branching pattern, color, and texture of the root bark or epidermis). Great care was taken to extract segments that were intact with minimal breakage. The samples were teased out of the soil and tapped to remove loose organic matter and mineral soil. They were immediately placed in a Whirl-pac bag on ice in a cooler and then transported within minutes to a few hours to the lab and frozen for dissection at a later date.

Root dissection techniques

A total of 18 561 individual roots were dissected from the 206 intact root segments, which were collected from the nine species over two field seasons (Table 1). Individual roots were dissected by order, with the distal roots numbered as first-order roots (Fig. 2). This system is analogous to one commonly used to number the order of streams (Horton 1945), and our approach was modeled after the root architecture work of Fitter (1982, 1985). Each root segment averaged ~100 individual roots and the total length of roots sampled was 65.4 m (Table 1). The total number of roots dissected per segment was driven by the mass needed for C:N analysis. For example, a greater number of individual *Quercus alba* and *Populus balsamifera* roots were needed to provide ~2 mg dry mass for each root order than were needed for *Liriodendron tulipifera,* which has a much lower SRL. Roots were carefully dissected under a 10× stereomicroscope (Semi 2000-C, Carl Zeiss, Thornwood, New York, USA). An ocular micrometer was

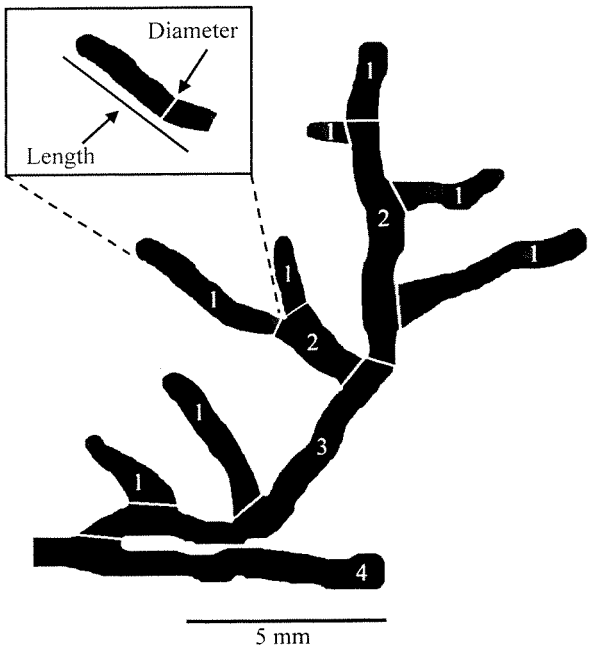

FIG. 2. Digitized image of a small portion of an intact segment of the branching fine root system of *Juniperus monosperma* collected from the Sevilleta Long Term Ecological Research study site. The numbers represent the order of each individual root. The white lines illustrate where the individual roots were separated from one another. The inset illustrates how the length and diameter of each root were measured.

used for length and diameter measurements, which were recorded by 0.1-mm diameter and length classes to speed the measurement process. Roots were kept moist at 1°C with deionized water during the dissection process (Pregitzer et al. 2000). Any soil particles adhering to the roots were very carefully removed with forceps and the root was rinsed clean with deionized water. We did not make a point to remove fungal hyphae from the roots unless they encapsulated an aggregrate of soil particles or dead organic matter that was obviously not a part of the root. Only live roots were measured; dead roots were removed and discarded. Live roots were distinguished from dead roots using methods described by Vogt and Perrson (1991). After dissection, individual roots from a segment were composited by order, oven dried (65°C for 24 h), and stored in sealed glass vials.

Using the 10× stereomicroscope, a video image was also taken of portions of representative intact root segments before they were dissected to record the general appearance of the branching fine root system. A scale was included in each of the video images. We did not record root branch angles or note the branching scheme (herringbone, dichotomous, etc.).

Root soil content and carbon and nitrogen concentrations

Root mass was corrected for ash content following the procedures described by Karam (1993). Subsamples for each species, treatment, plot, segment, and root order were analyzed for C and N concentrations with a Carlo Erba NA 1500 NC elemental analyzer (Elantech, Lakewood, New Jersey, USA). The elemental analyzer was calibrated with an atropine standard, and every 10th sample was a pine needle reference-tissue sample (No. 1575) available from the National Institute of Standards and Technology (NIST; Gaithersburg, Maryland, USA). The mean total N recovery rate for nutrient analysis of NIST standard pine needles across both years (1998 and 1999) was 98.3% (1 SE = 0.5%).

Statistics

Length and diameter measurements were rank-transformed in order to meet the assumptions of normality (Zar 1984). It was not necessary to transform the SRL, C:N ratios, and C and N tissue concentration measurements because they met the assumptions of normality. Differences in length, diameter, SRL, C:N ratio, and C and N concentration among year of collection, species, fertilizer treatment, and root order were then analyzed using a mixed-level (2 × 9 × 2 × 3) four-way (year, species, fertilizer treatment, root order) factorial ANOVA (PROC GLM Procedures, SAS Institute 1996). Tukey's hsd test was performed on length, diameter, SRL, and N concentrations for species within a root order. Only the first three orders of roots were analyzed statistically because the sample sizes for the fourth order roots were highly variable. In many instances, the fourth order roots were the main axis and were woody. Sample sizes ranged from 2056 to 54 across root order (1–3) for length and diameter data, and from 24 to 13 for the composite SRL and chemical analyses. Pearson's product–moment correlation coefficients were calculated between the diameter of each species and length, SRL, C and N concentrations, and C:N ratio.

RESULTS

Root length, diameter, and SRL

The lateral fine root branches of the nine different species varied in their overall architecture. For example, *Liriodendron tulipifera* roots were relatively thick and unbranched, while the fine roots of *Acer saccharum* were much thinner and the lateral branches were sometimes intricate, with many short individual roots (Fig. 3). The pines, especially *Pinus elliotti* and *Pinus edulis,* exhibited short lateral branches with bifurcation typical of EM dominated roots (Fig. 3). The overall appearance of the lateral fine root branches is quite variable among the nine different taxa, although it is clear from general inspection the fine roots of all nine species are dominated by short, thin individual roots (Fig. 3). We observed what appear to be lateral branch scars on all nine species, representative examples of which are illustrated in Fig. 4. It is not clear if these are abscission or bundle-sheath scars, but our

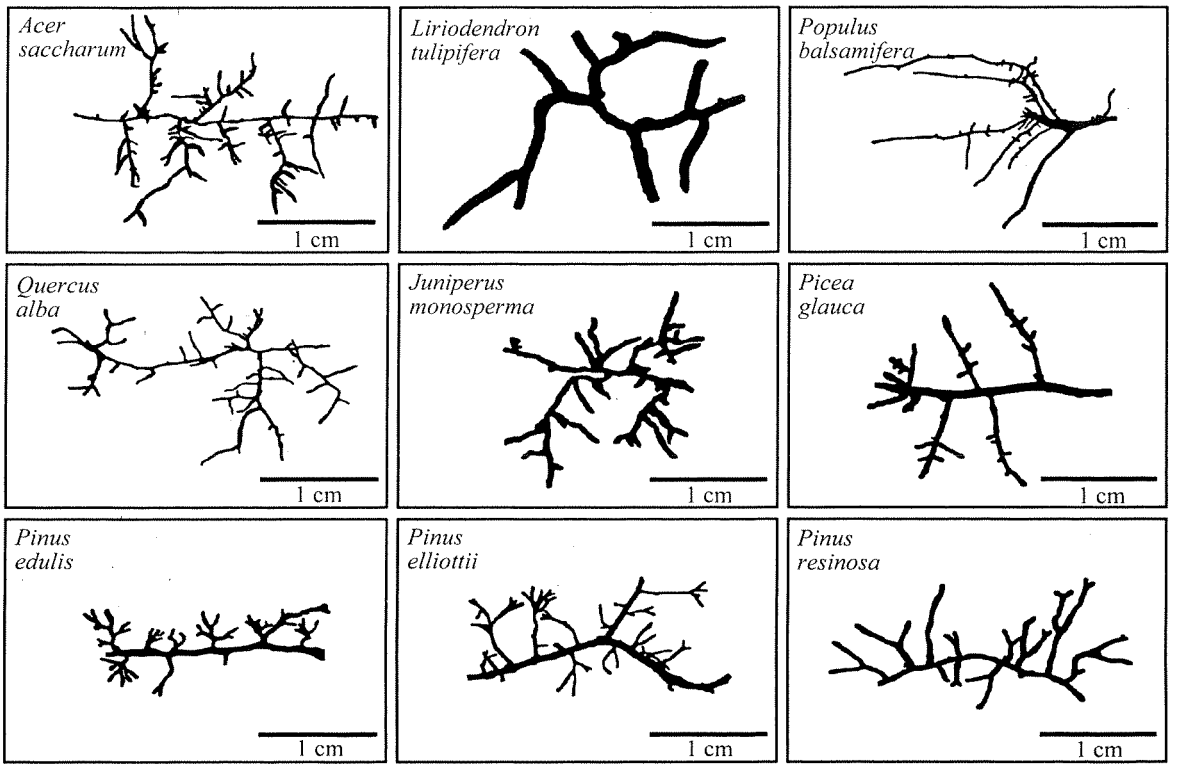

FIG. 3. Representative examples of the branching fine root systems of nine North American tree species. These are intact segments of the fine root systems similar to those segments that were dissected. The examples shown here were washed free of soil, their images digitized on a flatbed scanner, and the images converted to black and white.

deduction is that lateral roots are deciduous in the same sense that leaves, needles and some branches are deciduous.

For a given species, the mean length of an individual root significantly increased with order (Table 2), but within an order, root length was not particularly variable (note error bars for each root order in Fig. 5). Across all nine species, the mean length of a first-order root was ~2 mm, second order roots averaged ~6.5 mm, and third order roots averaged 15 mm. Individual roots of *Liriodendron tulipifera* and *Juniperus monosperma* were the longest (Fig. 5). Root length varied more between the two collection dates than among the fertilizer treatments (Table 2). Significant statistical interactions occurred between year and order; species and order; and year, species, and order (Table 2). These interactions may be partly due to inherent differences in the way lateral fine roots are constructed among species.

Mean root diameters increased with order (Fig. 5) and these increases were highly significant, as were mean differences among species (Table 2). *Liriodendron tulipifera* had the thickest root systems, while *Quercus alba* and *Populus balsamifera* had the thinnest roots (Fig. 5). Fertilizer treatments had little effect on root diameter (Table 2). Significant interactions among year, species, and order may be due to variation in the diameter of *Pinus edulis and Juniperus monosperma* roots, which were smaller in diameter in 1999 compared to 1998 in the control plots (*data not shown*). Root length and diameter were poorly correlated, with Pearson's correlation coefficients ranging from a low of $r = 0.14$ for *Acer saccharum* to a high of $r = 0.43$ for *Picea glauca*; most coefficients were less than $r = 0.3$. Diameter was also poorly correlated with C concentrations and C:N ratios. For some species, such as *Acer saccharum,* root diameter varied little among first, second, and third order roots, while individual root length almost doubled with each order (Fig. 5). On the other hand, species such as *Liriodendron tulipifera* and *Picea glauca* exhibit a more substantial increase in both root length and diameter with order (Fig. 5).

The distribution of relative cumulative root length by diameter class illustrates several interesting points. First, with the exception of *Liriodendron tulipifera,* ≥75% of the total fine root length sampled can be accounted for by roots that are <0.5 mm in diameter (Fig. 6). In some species, for example *Acer saccharum* and *Populus balsamifera,* virtually all of the total length of fine roots can be accounted for by roots <0.3 mm in diameter. The roots of *Liriodendron tulipifera* were much thicker than most of the other taxa sampled, with >75% of total root length having diameters >0.5 mm (Fig. 6).

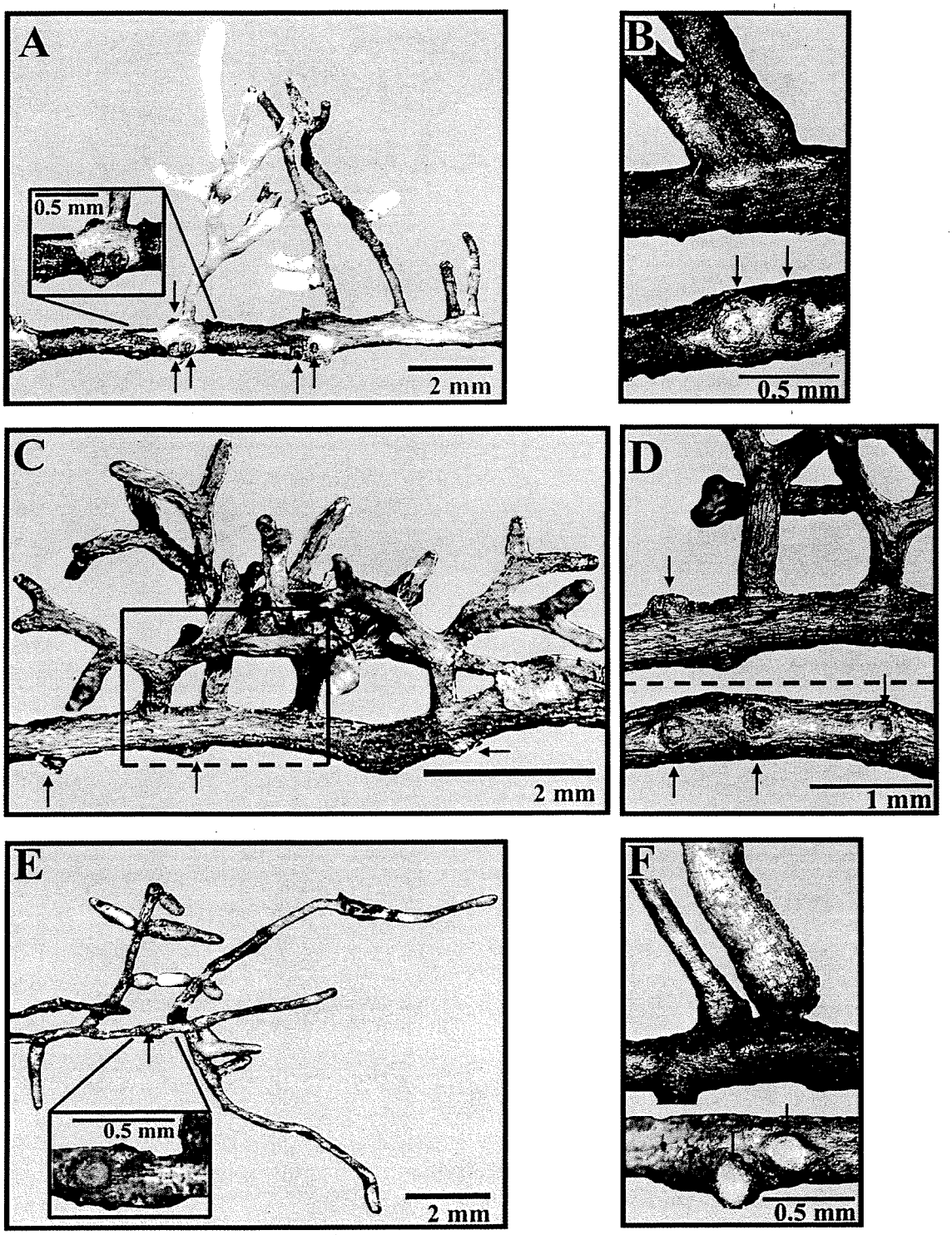

FIG. 4. Examples of lateral fine root branches illustrating lateral branch scars. All roots were collected in 1999 from the top 10 cm of the soil (Table 1). (A) *Pinus resinosa*: Arrows identify selected root-branch scars, and the inset enlarges a small portion of the root. (B) *Pinus resinosa*: The upper portion of the panel depicts two lateral roots; the lower portion of the image shows the branch scars after the roots were removed. (C) *Pinus elliottii*: lateral roots exhibit bifurcation typical of EM-dominated lateral branches. (D) *Pinus elliottii*: The upper portion of the image shows the exact inset from panel C at a higher magnification (22×). Notice that one of the lateral roots in panel C has died, leaving a branch scar (the single arrow above the dashed line locates the scar). The lower portion of panel D is a top view showing the branch scars (three arrows) left after all three of the lateral branches shown in the inset of panel C died. (E) *Acer saccharum*: The inset enlarges the faint branch scar identified by the arrow. (F) *Acer saccharum*: The upper portion of the image shows two lateral roots; the lower portion of the image is a top view showing two branch scars (arrows) left by the removal of the two roots in the upper portion of the image.

TABLE 2. Results of mixed-level four-way factorial ANOVA.

Source of variation	ANOVA			ANOVA				
		P values			*P* values			
	df	Root length	Root diameter	df	Specific root length	Root C	Root N	Root L:N	Root C:N
Year	1	0.35	0.29	1	<0.01	0.18	<0.01	0.08	<0.01
Species	8	<0.01	<0.01	8	<0.01	<0.01	<0.01	<0.01	<0.01
Fertilizer treatment	1	0.85	0.83	1	0.94	0.07	<0.01	0.12	<0.01
Order	3	<0.01	<0.01	2	<0.01	<0.01	<0.01	<0.01	<0.01
Year × species	8	<0.01	<0.01	8	<0.01	<0.01	<0.01	<0.01	<0.01
Year × trt	1	0.83	0.81	1	0.31	0.14	0.15	0.13	0.73
Year × order	3	0.01	<0.01	2	0.88	0.13	0.03	0.10	0.98
Species × trt	8	0.99	0.99	8	0.14	0.03	<0.01	0.04	<0.01
Species × order	21	<0.01	<0.01	16	<0.01	<0.01	<0.01	<0.01	<0.01
Trt × order	3	0.99	0.98	2	0.82	0.62	0.54	0.75	0.16
Year × species × trt	8	0.99	0.99	8	0.01	0.05	<0.01	<0.01	<0.01
Year × species × order	21	<0.01	<0.01	16	1.00	0.90	0.62	0.85	0.72
Year × trt × order	3	0.97	0.96	2	0.63	0.45	0.58	0.73	0.96
Species × trt × order	21	0.99	0.99	16	0.97	0.97	0.98	0.98	0.99
Year × species × trt × order	21	0.99	0.97	16	0.99	0.89	0.99	0.99	0.92

Notes: Root L:N = length per unit N. Length and diameters were rank-transformed. *P* values are from a Type-III sums-of-squares analysis.

Another way to examine the distribution of root numbers and root length is to summarize the proportions accounted for by each root order. In this case, it is clear that first-order roots account for ~75% of the total number of roots (Fig. 7A). Because mean length increases with order, the distribution of total length is more evenly distributed across orders (Fig. 7B). However, the length distribution by order also illustrates that the short lateral first and second order roots account for ~75% of the total length of those roots sampled in most species (Fig. 7B).

Root carbon and nitrogen

Mean carbon concentrations ranged from 445 to 555 g/kg and increased slightly with root order (Fig. 8). Concentrations varied from one year to the next, but the direction and response was species specific (*data not shown*). In general, C concentrations were often ~50% of dry mass as is often assumed, but there were large deviations from this expected value (Fig. 8). For example, the mean C concentration of first-order roots could differ by 70–80 g/kg when contrasting some of the species studied (Fig. 8). It would be interesting to know if these differences were related to the concentration of nonstructural carbohydrates or other labile microbial substrates.

Unlike C, which typically increased slightly with order, N concentrations decreased significantly as root order increased (Table 2, Fig. 8). Mean concentrations ranged from 8.5 to 30.9 g/kg, averaging ~16 g/kg across all three orders and species. Nitrogen concentrations also varied significantly among species. Gymnosperms tended to have lower fine root N concentrations than angiosperms (Fig. 8). Nitrogen concentrations also varied between years, but the response was species specific (*data not shown*), resulting in a significant year by species interaction (Table 2).

C:N ratio, specific root length, and root length per unit nitrogen

Carbon to nitrogen (C:N) ratios increased, often dramatically, with increasing root order across all nine species (Fig. 9), driven mostly by the decrease in N concentration in higher order roots (Fig. 8). The C:N ratio is a good way to understand the investment of C and N to individual roots of a given order on a mass basis because both C and N concentration are important in understanding root construction and maintenance costs (see *Discussion* below).

Specific root length (SRL; m/g) decreased, again sometimes dramatically, with increasing root order (Fig. 9). Significant differences occurred among order, species, and year, but once again, the fertilizer treatment was not significant (Table 2). Significant interactions between year and species illustrate how the time of collection may influence SRL, and this statistical interaction may be due to ontogenetic or environmental variation (*data not shown*). Some of the angiosperms such as *Acer saccharum, Populus balsamifera,* and *Quercus alba* had much higher SRL than the gymnosperms, which exhibited relatively low SRL (Fig. 9). However, *Liriodendron tulipifera* had the lowest SRL, even lower than all of the gymnosperms (Fig. 9). Year to year variability in SRL was higher in the angio-

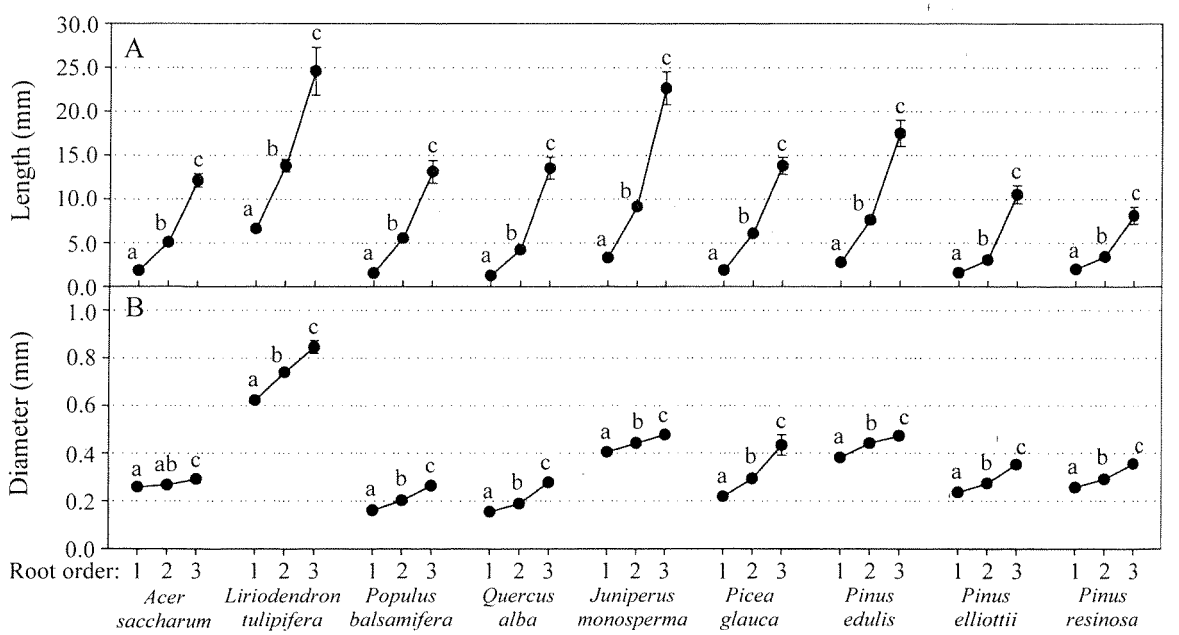

FIG. 5. Mean root length and diameter for the first three orders of roots from nine North American trees. Data are pooled across treatments and years. Error bars represent one standard error of the mean. Lowercase letters that differ within a species indicate significant ($P < 0.05$) differences among individual root orders.

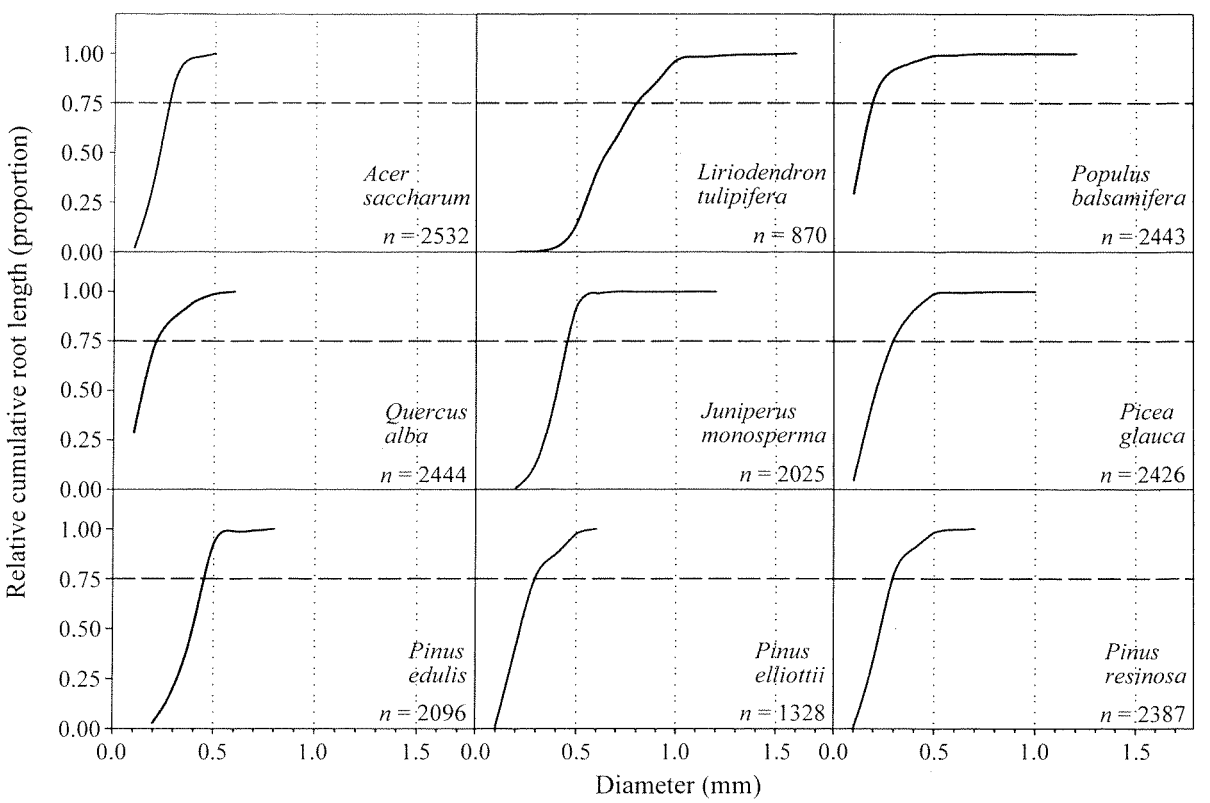

FIG. 6. Relative cumulative root length (proportion of total length dissected) by root diameter class. Dashed lines indicate 75% of the total absorbing root length sampled.

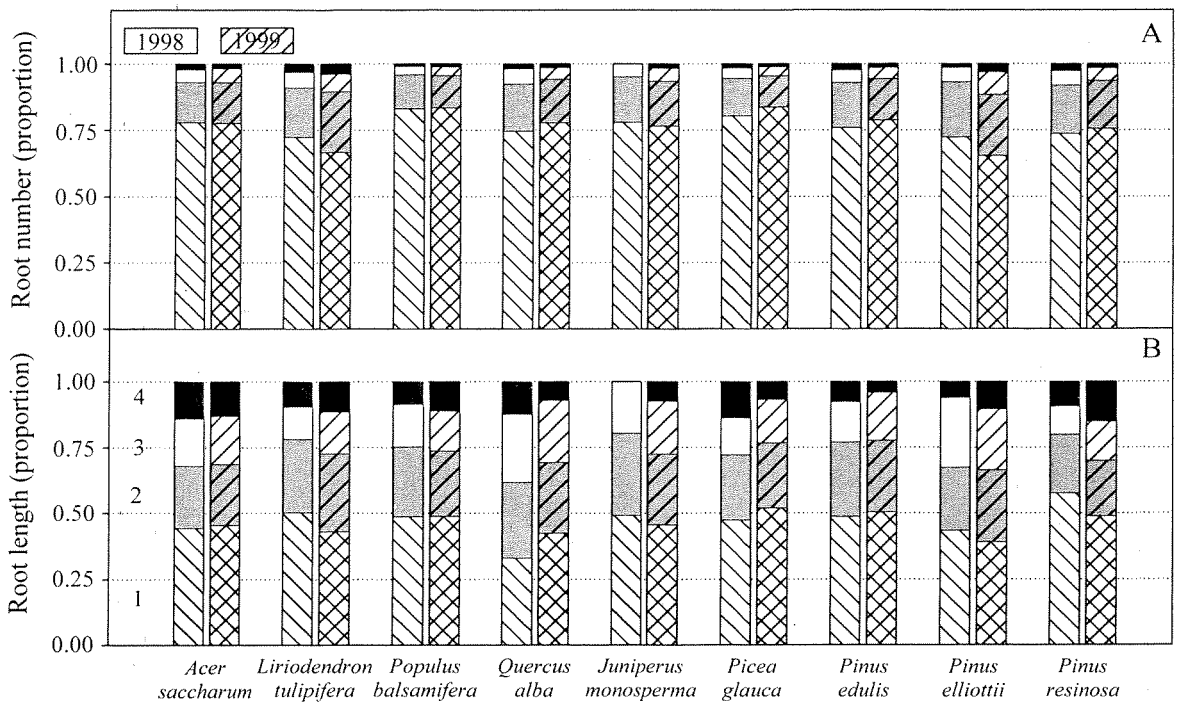

FIG. 7. Mean proportion of (A) the total number of roots for each root order and (B) the total root length for each root order, within sampling year and species. Each shading value represents a different root order, as labeled next to the bar for *Acer saccharum* in panel (B).

sperms compared to the gymnosperms, but this may be due to the fact that mean SRL is greater in angiosperms than gymnosperms. In terms of SRL, *L. tulipifera* and *J. monosperma* are similar, but when comparing N tissue concentrations, these two species are significantly different (compare Figs. 8 and 9).

Root length per gram of N invested also tended to decline with increasing root order, but there were ex-

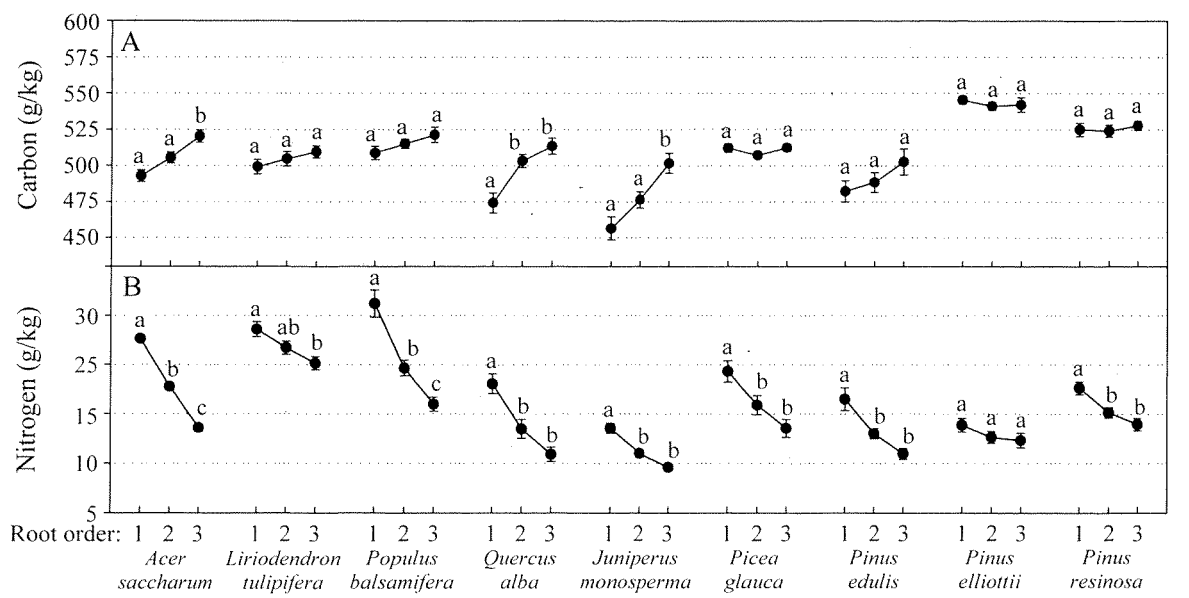

FIG. 8. Mean root carbon (C) and nitrogen (N) concentrations (g/kg) for the first three orders of roots from nine North American trees. Data are pooled across treatments and years. Error bars represent one standard error of the mean. Lowercase letters that differ within a species indicate significant ($P < 0.05$) differences among individual root orders.

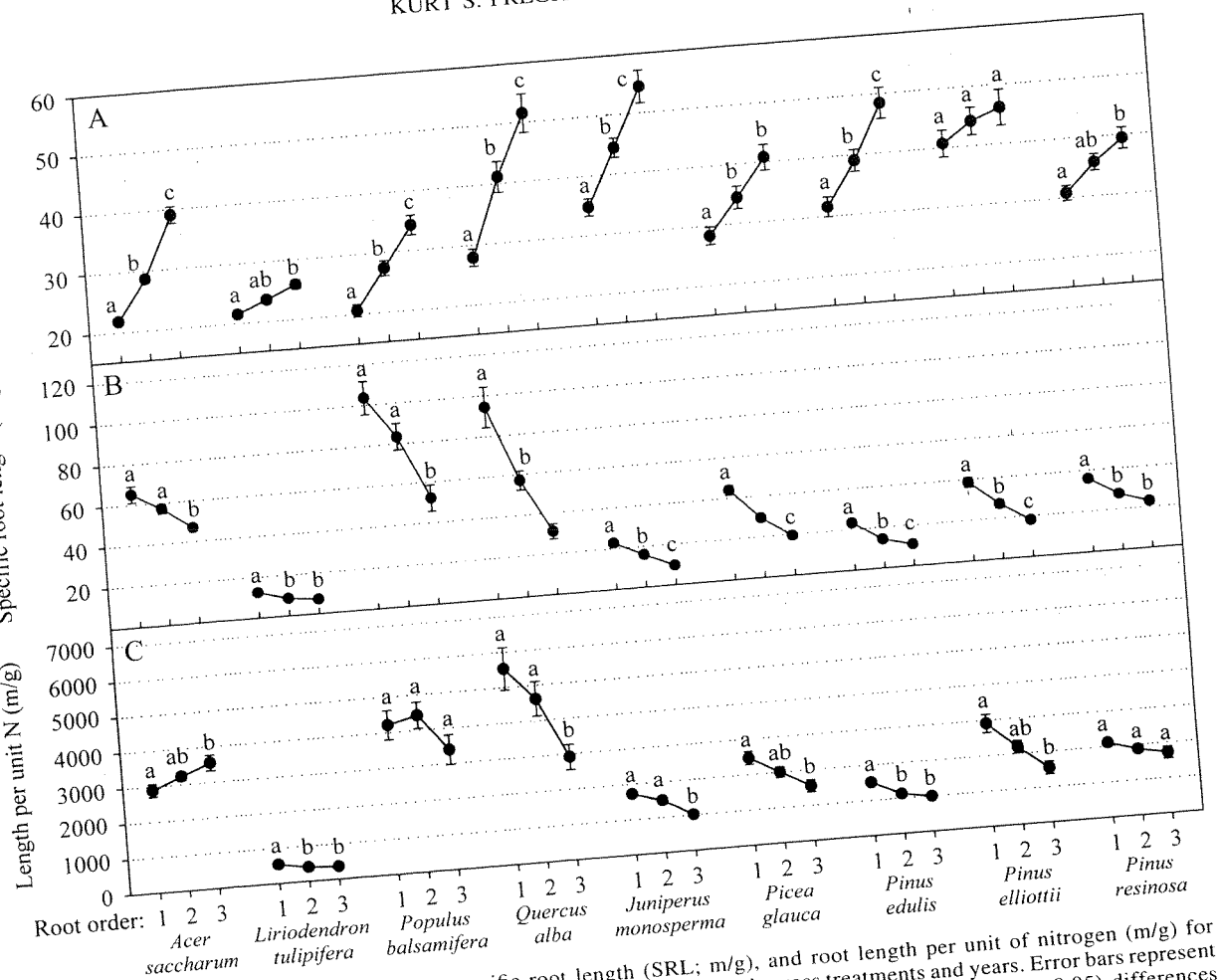

FIG. 9. Carbon to nitrogen ratio (C:N), specific root length (SRL; m/g), and root length per unit of nitrogen (m/g) for the first three orders of roots from nine North American trees. Data are pooled across treatments and years. Error bars represent one standard error of the mean. Lowercase letters that differ within a species indicate significant ($P < 0.05$) differences among individual root orders.

ceptions (e.g., *Acer saccharum* and *Populus balsamifera*; Fig. 9). As with SRL, there were large and significant differences in the length of roots per gram of N invested among some of the species (Table 2, Fig. 9).

Fertilizer effects

The fertilizer treatments resulted in a significant increase in root N concentration in some species, a significant treatment by species interaction, and a significant year by species by treatment interaction (Table 2, Fig. 10). For example, N concentration in the first-order roots of *Populus balsamifera, Picea glauca* and *Pinus edulis* increased substantially from 1998 to 1999 in the fertilized plots, while the N concentration of the first-order roots of *Acer saccharum* and *Liriodendron tulipifera* did not vary from 1998 to 1999 in the fertilized plots (Fig. 10). Species responded in different ways to fertilization in terms of altered root tissue N concentrations. The fertilizer treatments decreased C:N ratios in some instances, driven largely by species-specific changes in N concentration. The variable response associated with the fertilizer treatments appears to be primarily related to the variable tissue N concentrations.

DISCUSSION

Test of original hypotheses

Our first hypothesis was that nonwoody, lateral roots <0.5 mm in diameter would account for the majority of the fine roots in all nine species regardless of climate, soil type, tree taxonomic rank, or type of mycorrhizal infection. This proved to be true, except for *Liriodendron tulipifera* (Figs. 6 and 7). Our second hypothesis was that the position of an individual root on the branching root system was an important factor in understanding SRL and N concentration. In other words it is misleading to assume that all roots in an arbitrary fine root size class function the same way. This also proved to be true. First-order roots had much higher SRL and N concentration, and SRL and N concentration systematically decreased with order in all nine

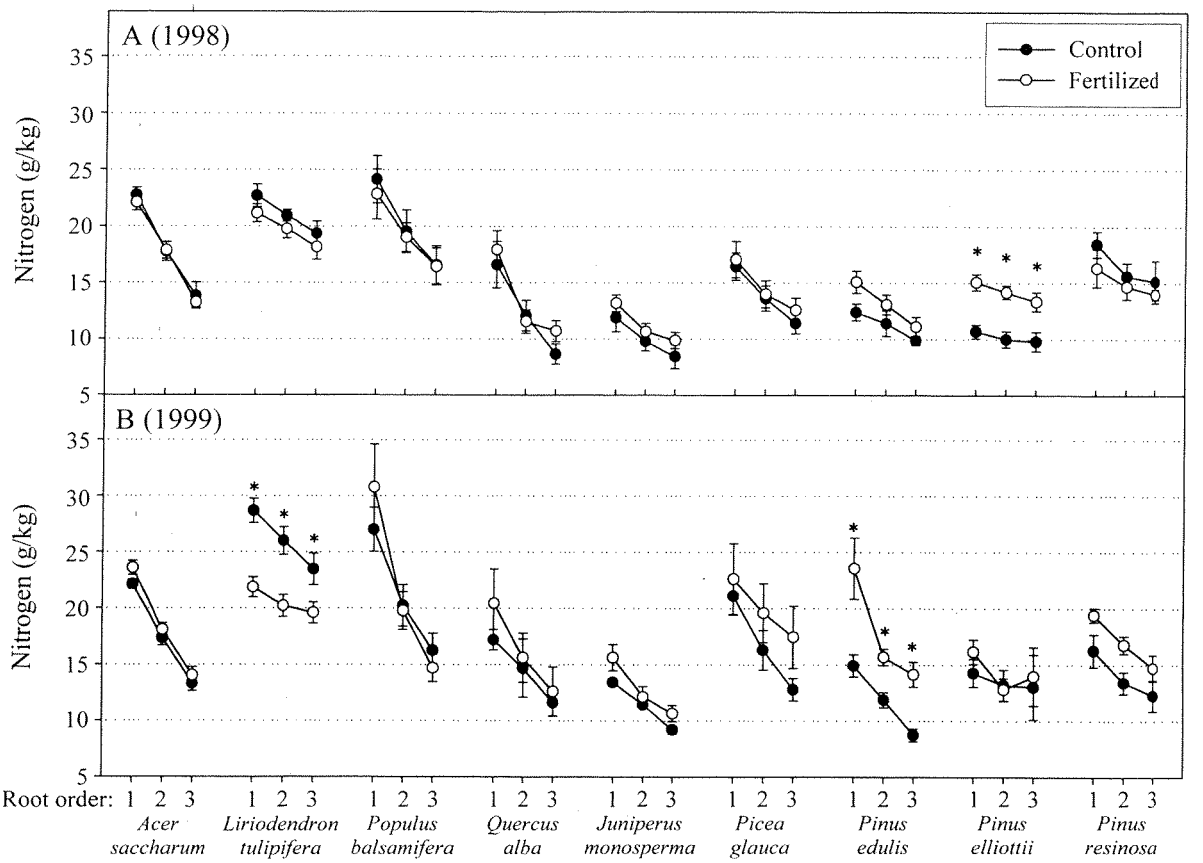

FIG. 10. Mean nitrogen concentration (g/kg) of the first three orders of roots from nine North American trees in control and N-fertilized plots. Roots were collected during (A) 1998 and (B) 1999. Error bars represent one standard error of the mean. Asterisks indicate that N concentrations for a given root order are significantly different ($P < 0.05$) when comparing roots collected from control and fertilized plots.

species (Figs. 8 and 9), resulting in a dramatic increase in the C:N ratio as root order increased (Fig. 9). Our third hypothesis was that EM roots would be larger, have lower SRL and lower N concentrations than AM roots across all habitats and species. We conducted these a priori planned statistical contrasts and rejected this hypothesis. There were large differences in SRL and N concentration among species regardless of the type of mycorrhizal infection. The ecological significance of differences among species are discussed below.

General conclusions across species and study sites

Our study was designed to encompass a wide variety of climatic and soil conditions, and we sampled fine roots of both gymnosperms and angiosperms that formed both EM and AM. In addition to variability induced by climate, soil, and type of mycorrhizae, other factors which we did not control for, such as the age and physiological stage of root development, can also influence SRL and other important attributes of fine roots (McKenzie and Peterson 1995, Schreiber 1996, Eissenstat and Achor 1999). In spite of all of this ontogenetic and ecological variability, several general conclusions are apparent.

First, the convenient assumption that all roots of a given size class, e.g., all fine roots <2 mm in diameter, function the same way is flawed. In all nine tree species we studied, and apparently in all perennial plants (Fitter 1996), a single lateral branch emerges at any point along a larger perennial root, as clearly illustrated in Fig. 2. The position of an individual root on the lateral branch is important. The lateral branches typically do not have >3–4 orders of roots (Lyford 1975, Figs. 3 and 4). First- and second-order roots dominate the total number of roots and total root length in all the trees we studied (Fig. 7). First-order roots consistently have the highest SRL and N concentration in all the species we studied, and the lowest C:N ratio (Figs. 8 and 9). With the exception of *Liriodrendron tulipifera,* the mean diameter of all first-, second-, and third-order roots was <0.5 mm (Fig. 5).

Yanai et al. (1995) simulated the efficiency of nutrient acquisition by fine roots and concluded the most efficient root would be "infinitely fine." Their conclusion, based on theoretical calculations, appears to be

correct in that the majority of the fine roots deployed by the trees in this study are thin, and compared to higher order roots, are less expensive to construct from a C cost perspective (Eissenstat and Yanai 1997). The concept of efficiency includes rates of nutrient acquisition per gram of C expended to construct and maintain absorbing root length. We did not measure rates of nutrient acquisition and so the true efficiency of individual roots on the branching root system has yet to be determined. Nonetheless, first- and second-order roots clearly have significantly lower SRL (Fig. 9).

Although first-order roots are relatively inexpensive to construct, they will be more expensive to maintain per unit time on a mass basis than higher order roots. Pregitzer et al. (1997) reported that first-order roots had the highest tissue N concentrations and speculated that these roots would have the highest respiration rates based on the observation that plant tissue N concentration is directly related to respiration rate (Ryan 1991, Ryan et al. 1996). Later, this hypothesis was confirmed for the fine roots of *Acer saccharum* (Pregitzer et al. 1998), and Burton et al. report similar results for all nine species in this study (A. J. Burton, K. S. Pregitzer, R. W. Ruess, R. L. Hendrick, and M. F. Allen, *unpublished manuscript*). Leaf tissue N concentration and leaf respiration are also directly related (Reich et al. 1992, 1997). The present study confirms that the first-order roots of all the species we studied had the highest tissue N concentrations (Fig. 8), and it now appears that this relationship may be general for all temperate and boreal trees.

In all the species studied, the steep increase in the C:N ratio with increasing root order is perhaps the best indication of the relative investment decline of N vs. C as you move from the distal root tip toward the larger woody roots. The C:N ratios (Fig. 9) clearly demonstrate that a gram of C invested in first-order roots will be more expensive to maintain per unit time than a gram invested in third-order roots. Fine root construction and maintenance costs are inversely related, which is a very efficient C allocation strategy for building and maintaining ephemeral absorbing root length along a perennial network of highly lignified, mostly woody and dead, expensive to construct, but cheap to maintain, structural roots. Tissue N concentration is a key factor in understanding the C cost of maintaining first- and second-order roots, which dominate the display of absorbing root length, and there are large differences in tissue N among species (Fig. 8). Our understanding of N investment in first- and second-order roots still suffers for a lack of convincing evidence concerning whether N is retranslocated from these roots before they die.

Fine root lateral branches are apparently deciduous, with a distinct abscission or bundle sheath scar, in the same sense that aboveground plant modules (leaves, needles, and branches) are deciduous (Fig. 4). The fact that nonwoody lateral roots arise from the woody roots of trees is well known, but it is quite remarkable that the concept of root mortality has not been linked to the differentiation and mortality of lateral roots. Lyford (1975) states: "that a high proportion of small-diameter laterals on tree roots disappear is indisputable because there are many bare spaces or stumps of former laterals along woody roots in places where laterals must have been numerous just after they developed. Nevertheless, there have been few direct studies of individual roots to determine exactly what happens to them over time and whether, in fact, ageing alone is involved or whether disappearance is due primarily to injury by pathogens, to root consumers, or to starvation." Wells and Eissenstat (2001) recently demonstrated marked differences in survivorship among apple (*Malus domestica*) roots of different diameters, with smaller diameter roots living shorter life-spans than larger diameter roots. Do entire lateral branches die? Our observations (Fig. 4) and those of Lyford (1975) clearly suggest this is the case. We postulate that lateral branches composed primarily of first-, second- and third-order roots are the basic fine root "modular unit" (sensu Harper 1977) in all temperate and boreal trees. We have observed more complex branches (i.e., branches with more root orders) and these seem to be associated with places in the soil where nutrients or water are more abundant. Clearly, we must now study the branch structure in more detail and determine which roots actually die and if branch architecture and the point of abscission are plastic in response to soil resource heterogeneity.

The concept of "fine roots" in trees

The structural portion of the root system cannot move without great C cost to the plant and it takes time to deploy new woody roots. Trees must then respond to rapid changes in soil resource availability by physiological and structural adjustments to the fine absorbing roots. Structural adjustments in all the trees we studied apparently happen through the growth and mortality of lateral branches, whose form is variable among taxa (Fig. 3). We must now refine our understanding of fine roots as modular units designed for water and nutrient acquisition, whose life expectancy and form is ecologically relevant and directly related to resource acquisition. We predict the life expectancy of a lateral fine root is inversely related to SRL and N concentration, and we are currently working to test this hypothesis. The issue of whether lateral roots die as whole, integrated branching units must also still be resolved. There are clear alternatives to this hypothesis. For example, it is possible that first-order roots represent the majority of new fine root production and individual root mortality. It is also possible that the point of root abscission on a lateral branch is plastic and varies from one circumstance to another, but this alternative does not seem very plausible based on what we understand about shoot ontogeny.

Typical fine root size classes, e.g., all roots 0–1 or

0–2 mm in diameter, are much too large in most instances (Figs. 5 and 6) and include many roots which have relatively long life expectancies: roots that serve primarily transport and storage functions. With the exception of *Liriodendron tulipifera,* the most reasonable fine root size class for the nine species we studied would consist of all roots <0.5 mm in diameter, and perhaps an even smaller size class in some instances, e.g., *Acer saccharum* and *Populus balsamifera* (Fig. 6).

Differences among species

Although the general architecture of the fine roots of all nine species was similar, with short lateral branches and lateral branch scars, obvious differences in length, diameter, SRL and N concentration occurred among species. The angiosperms, *Acer saccharum, Populus balsamifera,* and *Quercus alba* displayed individual roots with smaller mean diameters (Fig. 5). The SRL of the first- and second-order roots of these three angiosperms was often more than double that of the conifers we studied (Fig. 9). Obviously, the C cost of constructing individual absorbing roots of these angiosperms is much less than, for example, the roots of the three pines (Fig. 9). The angiosperm *Liriodendron tulipifera* was a major exception in terms of individual root diameter and SRL compared to the other angiosperms. Plants in the Magnoliaceae are considered part of the ancestral angiosperm complex (Judd et al. 1999). The primitive angiosperm and all the gymnosperms had short, thick fine roots, which had lower SRL.

During both 1998 and 1999, root samples <0.5 mm in diameter were examined to determine mycorrhizal infection. The percentage of roots infected with AM varied widely among species and sample dates (mean percentage infected ranged from 7 to 41%), while almost all the first-order EM roots (root tips) were infected (mean infection rate >90% for all EM taxa; J. Lansing and M. Allen, *unpublished data*). The dominant type of mycorrhizal infection, however, does not appear to be correlated with the length, diameter, or SRL of individual roots. *Liriodendron tulipifera* (angiosperm) and *Juniperus monosperma* (gymnosperm) are both dominated by AM fungi and their roots are thick and exhibit low SRL. In contrast, *Acer saccharum* and *Quercus alba* are dominated by AM and EM fungi, respectively, and both of these angiosperms have thin roots and high SRL.

Significant differences in N concentration also occurred among species (pairwise comparisons not shown, but see Fig. 8). One interesting finding was the fact that species can have similar SRL, e.g., *Liriodendron tulipifera* and *Juniperus monosperma* (Fig. 9), but significantly different N concentrations (Fig. 8). Burton et al. found that tissue N concentration explains much of the variability in specific rates of root respiration for all these same species and study sites (A. J. Burton, K. S. Pregitzer, R. W. Ruess, R. L. Hendrick, and M. F. Allen, *unpublished manuscript*). From a construction cost perspective (C cost of building a new root; Eissenstat and Yanai 1997), both *Liriodendron tulipifera* and *Juniperus monosperma* deploy relatively expensive roots (low SRL). However, the roots of *Liriodendron tulipifera* have high concentrations of N, while those of *Juniperus monosperma* are the lowest we report (Fig. 8). Obviously, the C cost of constructing a new root is not necessarily related to the cost of maintaining that same root per unit time. Another interesting observation is that individual roots from warm, dry environments such as Sevilleta have low tissue N. In general, is fine root tissue N lower in habitats where the cost of maintenance respiration is potentially high?

Nitrogen additions

Nitrogen fertilizer treatment did not significantly alter individual root length, diameter, SRL or C concentration (Table 2). The N additions did significantly increase root tissue N concentration in *Pinus elliottii* in 1998 and *Pinus edulis* in 1999 (Fig. 10). We expected the fertilizer additions to increase root tissue N in most instances, but this was often not the case (Fig. 10). These results suggest that the response to altered N availability at the individual root level may be complex. Species-specific responses may be the rule rather than the exception. These results are short-term (two years of treatment), and N availability following fertilization was probably complicated by microbial demand for N as well as differences in moisture availability among the study sites.

It is important to understand whether increasing soil N availability increases root tissue N in the long term, because much of the total cumulative flux of CO_2 from the soil is dominated by root construction and maintenance respiration (Boone et al. 1998). Fine root maintenance respiration apparently dominates the C cost of deploying fine roots (Eissenstat and Yanai 1997). Therefore, we might not expect an increase in soil respiration following fertilization if root tissue N does not increase with increasing soil N availability. Obviously, changes in fine root biomass would also factor into understanding how soil N availability alters soil respiration.

This study raises many unresolved and potentially significant ecological issues. Are species-level differences in the C cost of deploying and maintaining absorbing roots through time directly related to rates of nutrient uptake? Eissenstat and Yanai (1997) report that much of the C cost of ion uptake is related to N acquisition. Do individual roots with high N concentrations exhibit greater rates of N or P acquisition integrated over their life-span? Do roots from dry environments always have lower tissue N and therefore lower rates of respiration at high soil temperatures? Are root life-span and nutrient acquisition inversely

related to tissue N as is the case for leaves (Reich et al. 1997)?

We have much to learn about the linkages between the structural attributes of fine roots and how they function. The acquisition of water and limiting nutrients from the soil is a key ecological challenge for plants. This study demonstrates that we need to begin to focus on understanding how differences in root structure are related to the acquisition of essential soil resources. The SRL and N concentration of individual roots differ significantly among nine species of North American trees, and therefore, the C cost of constructing and maintaining fine roots also differs significantly among species. Additional investigations may prove these differences to be directly related to water and nutrient uptake and fine root life-span.

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