The Pennsylvania State University

The Graduate School

Intercollege Graduate Program in Ecology

FINE ROOT PRODUCTION AND LIFESPAN IN ELEVEN TEMPERATE TREE SPECIES GROWING IN A COMMON GARDEN IN POLAND

A Thesis in

Ecology

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2005

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ABSTRACT

Leaf lifespan and structure have been linked to plant competition and nutrient cycling. Analyses of leaf structure and leaf lifespan on global data sets provide strong evidence for long leaf lifespan coupled with low specific leaf area and low mass-based N concentrations. Because fine roots share many characteristics with leaves (e.g. resource acquisition, ephemeral nature), we hypothesized that fine root and leaf lifespan should be correlated, and fine roots lifespan should couple with root traits.

Our common garden in central Poland consisted of replicated, monospecific plots of five hardwood and six conifer species. We used minirhizotrons to observe root production and lifespan over four years.

Minirhizotrons are used to nondestructively observe roots. We tested the important assumption that tube material does not influence root behavior using butyrate and acrylic tubes in plots of three hardwoods and three conifers. Root survivorship near acrylic tubes was shorter for the conifers and longer for the hardwoods, indicating that multi-species lifespan data can be influenced by tube material. Compared to butyrate, acrylic tube standing crop data were more similar to standing crop estimates from soil cores, suggesting acrylic tubes provide the more accurate data in our study systems.

Our species had one peak of root production in summer, while the site experienced no summer drought. Maximum peak production shifted similarly for all species from year to year indicating a strong influence of external factors.

Though the eleven tree species exhibited a wide range in leaf and fine root lifespans, leaf lifespan was not correlated with fine root lifespan. Root nitrogen:carbon ratio was inversely correlated with root lifespan. Other root traits, such as diameter and specific root length, were not correlated with root lifespan. Our studies show that tissue structure and patterns of longevity aboveground can contrast markedly with patterns belowground. To better understand larger-scale nutrient cycling in ecosytems as well as patterns associated with belowground growth strategies, we need to understand similarities of roots across species. We can do this by observing root production and lifespan patterns in multi-year, multi-species studies.

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ACKNOWLEDGEMENTS

I would like to thank my parents, Roy and Jane Page, and my grandmother, Betty Barnes, for all of their support during the many years I have been working towards my degree. I also give thanks to my husband, RP, and my son, Preston, for their encouragement and support. These last few months have been trying on all of us.

To Dave and Peter- I cannot thank you enough for your help and encouragement through my writing process. To Bartek Bułaj and Kuba Oleśinski, thank you so much for taking care of me in Poland and making sure that I stayed out of trouble. Were it not for the both of you, my fieldwork would never have been finished.

Preface

Chapter 2 was previously published as:
Withington, J.M., A.D. Elkin, B.Bułaj, J. Olesiński, K.N. Tracy, T.J. Bouma, J. Oleksyn, L.J. Anderson, J. Modrzyński, P.B. Reich and D.M. Eissenstat. 2003. The impact of material used for minirhizotron tubes for root research. New Phytologist 160:533-544.

Chapter 3 was submitted in March 2005 and has been accepted pending revisions as:
Withington, J.M., P.B. Reich, J. Oleksyn and D.M. Eissenstat. xxxx. Root structure and lifespan are largely independent of leaf structure and lifespan in a common garden comparison of eleven tree species. Ecological Monographs xx:xx-xx.

Chapter 4 will be submitted to Tree Physiology in 2005 as:
Withington, J.M., M. Göbel, J. Oleksyn, P.B. Reich and D.M. Eissenstat. xxxx. Seasonal fine root growth of eleven temperate tree species in a common garden. Tree Physiology xx:xx-xx.

Measuring fine root lifespan is a long and sometimes tedious project. There are few large-scale minirhizotron projects. When I started this project in 1998, it was the largest minirhizotron project in the world. Most of the minirhizotron projects I read about use a maximum of about 36 tubes. We originally installed 140 acrylic (4 per plot * 35 plots) and 54 butyrate tubes (3 per plot*18 plots). We took measurements on 104 acrylic tubes (3 per plot) and all of the butyrate tubes in 1999. At 30 images per tube * 10 sampling dates, that was approximately 47,400 images to analyze from 1999. It took many, many months. Then we collected images on 20 more sampling dates over the next three years. I could not have gotten all of the images taken nor analyzed without the help of many students and friends.

Working in a foreign country is not easy. Knowing how one would accomplish a task in the US, may not necessarily be of use. Other societies, other bureaucracies can run very differently. Poland, like the former German Democratic Republic, is becoming more and more like western Europe every year. I could see the changes in the three years I spent there, like more and more large supermarkets and better phone connections. Let me share a few of the interesting bureaucratic moments:

- We had trouble getting our five huge boxes of minirhizotron tubes out of customs. The Polish customs agents wanted the original receipts for the material proving their worth

and then were upset that we had claimed a value much higher than the receipts showed, having taken into account all of the man hours necessary to etch the windows on the tubes and seal the bottoms. Thank goodness our Polish collaborators knew how to work within the system; they got the boxes released just in time for us to install them.

- In Poland, auto theft is extremely common, and policemen do not need a reason to stop a car and inspect registration and insurance papers. I found cops that were still interested in bribes of 100zl so they wouldn't write us up for tickets worth ~400zl. (Think of that as a $400 ticket.) I also met policemen who were polite and spoke enough English to my Polish that we communicated well.
- The maximum speed in Poland is 100 km/hr. On a four-lane highway I drove frequently, it was not unusual to find construction markers blocking a lane with no prior warning. Nothing like the "Construction 1 mile ahead" signs here. Paying attention to the road ahead was a must. Of course, once I knew this was a possibility, I was always on the lookout for construction signs and immediately adjusted my lane when necessary.
- One year, on a two-lane highway, there was road work being done at a large curve in the road; one lane was blocked. There were no flaggers to help us around. Our only hope was to get there behind a big tuck and let them clear the way. That and just pray. Traffic from the other direction would be coming at 100 km/hr. This spooked my every time I had to pass it.

Ah, but what an experience to work overseas! I spent 15 months over three years in Poland. The last 8 months were on a Fulbright Scholarship. I even got to sing in an *a capella* choir at the medical school in Poznań that year. It was great! Truly some of the best times of my life.

"The fine roots of perennial plants are a royal pain to study. They seem to be everywhere in the soil, penetrating the rotting leaf litter, wedged between stones and proliferating in worm casts."

"One of the most remarkable gaps in our knowledge is that we still do not know which fine roots on the branching root system die and what controls the mortality of individual roots."

Kurt S. Pregitzer 2002
New Phytologist 154:267-270

Chapter 1

Introduction to Fine Roots

Root morphology and anatomy

Root systems

Roots are belowground structures of the sporophytic generation of vascular plants. They undergo apical elongation and respond to gravity (Raven and Edwards 2001). The root system is a network of individual roots arranged in a hierarchical pattern (Fitter 2001) with new roots originating from the pericycle. The number of xylem poles in the stele, or the archy of the root, is important to root architecture and morphology. First and second order laterals of noble fir roots were found to be diarch and triarch while the upper portion of the radicle was pentarch. In general there was no apparent stable pattern in the archy of root orders (Wilcox 1954). Archy is an important consideration in the formation of lateral roots. Laterals form opposite the protoxylem poles, and there appears to be a dominance factor that inhibits the growth of new laterals too close to one another. Angles formed by successive laterals are generally greater than 30º. If the angle is less than 30º, then the average axial distance is greater than the average distance for laterals at wider angles from each other. These factors together influence the possible morphology of a root system (Riopel 1966, 1969).

Roots may be classified by order with primary roots or external links being those roots with no laterals. Higher order roots or internal links have at least one lateral root. Individual roots can be divided into two basic categories: coarse or fine. Coarse roots are larger in diameter, undergo secondary thickening and are important for structural support of the stem as well as providing conduits between the stem and the fine roots. Fine roots are ephemeral and do not

undergo secondary thickening. The fine roots are thinner than the coarse roots, but actual diameters of the fine vs. coarse roots are dependent upon the species; generally, though, fine roots are <1 mm in diameter.

Fine roots are responsible for the bulk of the water and nutrient uptake for the plant. To assist in this task, many roots form symbiotic associations with fungi; these fungi with their thin, extensive network of hyphae are more efficient at taking up immobile nutrients than roots. The fungal hyphae form exchange structures within the root cortex. The nutrients are transported to the roots in exchange for fixed carbon. The nutrients are then transported to the stele and into the xylem for translocation to the rest of the plant. This association can cost the plant a large percentage of its fixed carbon, but if nutrients like phosphorus are scarce, then the plant benefits greatly from its investment (Allen 1991).

Root Anatomy

Anatomy influences physiological processes like water and nutrient uptake through physical resistance to radial movement (McKenzie and Peterson 1995a, Peterson *et al.* 1999). Water and nutrient uptake are the main functions of the fine roots and are the most well-studied aspects of root function with respect to anatomy. Uptake is an important aspect of root efficiency, and efficiency has been linked to root lifespan (Eissenstat and Yanai 1997). The conventional wisdom is that suberin lamellae in the endo- and /or exodermis (Figure **1.1**) cause the greatest radial resistance to water and nutrient uptake (Clarkson 1985). For example, Gierth *et al.* (1999) found that barley roots restricted solute flow into the stele mainly by the Casparian strip of the endodermis, although the cortical cell layers are effective restrictors of apoplastic flow. However, Barrowclaugh *et al*. (2000) found water uptake in onion was least in the region in which the endodermis had only a Casparian strip and the exodermis had no thickenings. It was greatest in the regions where the endodermis and the exodermis had Casparian strips and suberin lamellae. Clarkson *et al*.'s (1987) work with maize also demonstrated that the presence of suberin lamellae in the exodermis does not necessarily indicate low water or solute permeability. Therefore, uptake efficiency changes with anatomy, and the specific anatomical influence will vary with the species of interest.

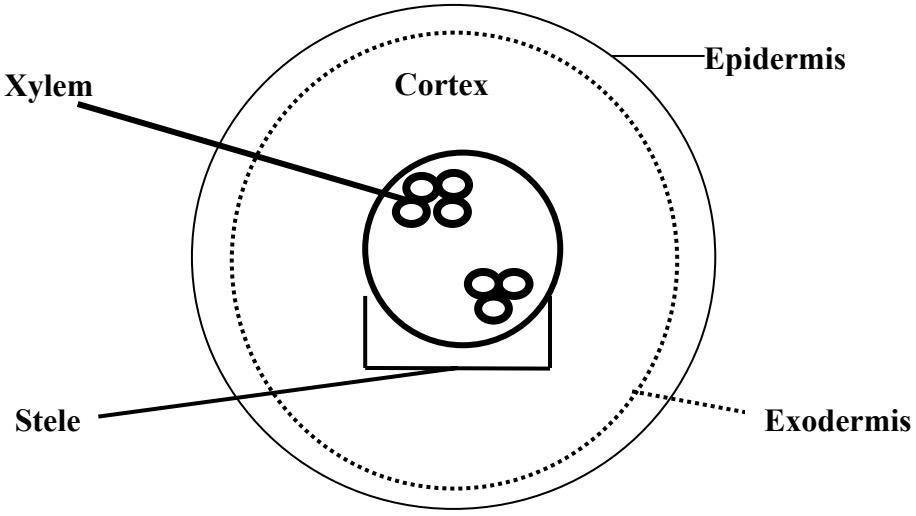

Figure **1.1**: Diagram of the cross-section of a fine root.

Path length, from the root-soil interface to the endodermis, is the most important factor for resistance to water flow into roots without secondary growth. Path length is influenced more by the number of rows of cortical cells than by the size of the cells or whether they are densely or loosely packed (Nardini and Tyree 1999, Rieger and Litvin 1999). Thin roots, which have a shorter radial path, have a higher rate of hydraulic conductivity than thick roots (Rieger and Litvin 1999). Larger diameter roots have lower uptake rates due to increased radial resistance to water and solute movement (Rieger and Litvin 1999), and the largest aspect of resistance of radial water movement was the living cortex regardless of diameter (Peterson and Steudle 1993, Rieger and Litvin 1999).

Root respiration, and therefore daily carbon cost, will vary based on the amount of live roots, and this may be influenced by cortical cell death. When roots turn from white to brown, it is due to the accumulation of tannins in the cortex. Jack pine and eucalyptus (gymnosperm and angiosperm respectively) cortical cells are dead in the tannin zone (McKenzie and Peterson 1995a). In loblolly pine, 84% of the tannin zones had a cortex that was crushed, collapsed or in the process of shedding, while more than 78% of the white roots had an intact and living cortex (McCrady and Comerford 1998). Cortical cell death may also increase susceptibility to pathogens. In a study of cortical senescence in four cereals, Deacon and Mitchell (1985) found that the rate of cortical death was positively related to susceptibility to the wheat take-all fungus.

Root anatomy constrains and enhances root function, which has been linked to root lifespan in terms of root costs and efficiency (e.g. Eissenstat 1992, Eissenstat and Yanai 1997). There are likely, therefore, suites of anatomical traits that together influence root longevity. Though root anatomy with relation to root function has been studied, there is a paucity of work examining patterns of root anatomy among species of contrasting ecology or on the relationship between root anatomy and root lifespan.

One of the few studies that has addressed the relationship between anatomy and root lifespan suggested that there may be trade-offs between longevity and absorption in citrus. Citrus rootstocks with small average diameters and high specific root length (a combination of root diameter and root density) had less thickening of the exodermal cell walls. A well-developed exodermis increases root longevity by maintaining water and ion balance during drought or salt stress and defending against herbivores (Eissenstat and Achor 1999).

At the ecosystem level

Global nutrient cycles

Because of the ephemeral nature of the fine root system, the fine roots are an important part of the terrestrial carbon cycle as well as part of the cycles for other major nutrients like nitrogen and phosphorus. Fine roots can turn over several times a year in some species (Persson 1979, Wells and Eissenstat 2003) and, therefore, can represent more than 50% of total annual biomass production (Vogt *et al.* 1987). That represents a large portion of assimilated carbon for both construction and maintenance of the tissues.

The root system is an important part of carbon and nutrient cycles in the temperate forest. Production, death and decomposition of roots involve a large percentage of the C and N moving through the system. Aerts *et al*. (1992) found N losses in a heathland due to root turnover were 23-87 $g/m^2/yr$ while inputs of organic N were estimated at 1.3-13.8 $g/m^2/yr$. The majority of root turnover and therefore cycling involves the fine roots.

Carbon costs are incurred in building and maintaining the root system such as respiration for growth, ion-uptake and maintenance, producing exudates and maintaining mycorrhizas (Eissenstat and Yanai 1997). As roots age, carbon costs may change. As the ratio of carbon

needed for upkeep to resources gained by the root increases, the root may become less efficient (Eissenstat and Yanai 1997). However, in grape, Volder *et al.* (2005) found that both root N uptake and root respiration (costs) decreased simultaneously in 1st-order roots suggesting little effect of age on root efficiency. Even so, there are trade-offs in the relative costs of maintaining existing roots versus shedding older roots and building new ones with greater uptake capacity (Eissenstat and Yanai 1997, Volder *et al.* 2005). Modeling the cost and benefit of fine roots leads to the prediction that roots are more likely to die when they reach optimal efficiency. However, in citrus Kosola *et al*. (1995) found fairly constant mortality across root ages, while young sugar maple roots tended to have high mortality (Hendrick and Pregitzer 1993, Pregitzer *et al.* 1993). These different lifespan responses suggest that plant strategies to efficiently use carbon are influenced by biological and environmental factors.

Vegetation Dynamics

The biological strategy employed by plants to maintain efficient organs and tissues is thought to be an evolutionary adaptation to the environment where a species evolved, and therefore, these strategies presumably have a genetic basis. Studies on factors that affect leaf lifespan suggest that plant species that evolved on low nutrient sites have developed different life strategies for their ephemeral tissues compared to plant species that evolved on high nutrient sites. Most of the data used to support this theory are based on leaf measurements. In comparing leaf data from a wide range of ecosystems, many patterns have emerged. Plants adapted to low nutrient sites generally exhibit low relative growth rates, photosynthetic rates and tissue turnover rates, and these traits seem to vary together as suites of characters (Chapin *et al.* 1993). Many authors have investigated the idea that increased leaf longevity allows for greater nutrient conservation and nutrient use efficiency (Monk 1966, Chapin 1980, Reich *et al.* 1991). In general, plant species with longer leaf lifespans (e.g. coniferous and hardwood evergreens) also tend to have lower net primary productivity, lower leaf nitrogen content, and lower specific leaf area (leaf area/dry mass) than plants with shorter leaf lifespans (e.g. deciduous trees and shrubs) (Chapin 1980, Reich *et al.* 1991, Reich 1993, Reich *et al.* 1997).

Evergreen and deciduous leaf traits consistently grouped together across biomes, most likely due to compromises on leaf structure and function. For example, higher leaf N content is beneficial because it increases net photosynthesis; however, leaves must be thin enough to allow

sufficient CO_2 diffusion to maintain high photosynthesis, and thin leaves with higher N content are more desirable to herbivores (Reich *et al.* 1997). Even with what is known about leaf lifespan and leaf characteristics, the relationship between growth form and organ-level traits are poorly understood (Reich *et al*. 1998a, 1998b). Very little information exists for root traits of plants with different life strategies, but plant life strategy theories would be more robust if patterns in leaf and root traits concurred.

Fine root lifespan

Factors, such as the soil environment, may affect root lifespan. For example, many plants such as cotton, soybean and big bluestem shed their roots in response to stresses such as drought (Klepper *et al.* 1973, Hayes and Seastedt 1987, Huck *et al*. 1987), while others, like citrus, retain their roots by imposing some type of dormancy such as down-regulating respiration (Kosola and Eissenstat 1994). Soil nutrient status and fertilizer have variable effects on root lifespan. Nitrogen fertilizer decreased root lifespan in Norway spruce, while non-nitrogen fertilizer increased it (Majdi and Kangas 1997). In cherry, white roots had longer lifespans with a low application of nitrogen fertilizer as compared to a high application (Mackie-Dawson *et al.* 1995). Poplar trees were also found to have shorter root lifespans with an increase in soil nitrogen availability (Pregitzer *et al.* 1995). In contrast, Pregitzer *et al.*'s (1993) work in a mixed hardwood forest found that additions of water and nitrogen increased root lifespan, at least for the first 10 days following treatment. There may be multiple factors at work that may explain these seemingly contradictory results for water and nutrient availability.

Root age and time of year when roots are born also influence root lifespan. Hendrick and Pregitzer (1992) found that the time of year a root was born had a significant influence on root lifespan in a northern hardwood forest. Because nutrient absorption decreases as roots age, to maintain high rates of nutrient uptake, Chapin (1993) proposed that a plant must have a high rate of new root production coupled with short lifespans of existing roots.

Root order, root diameter and colonization by mycorrhizal fungi have also been linked with fine root lifespan. Reid *et al*. (1993) found that in kiwi, higher order roots tend to have longer lifespans. Hooker *et al*. (1995) in their work with poplar also found longer lifespans in higher order roots. Often the highest order roots are also the thickest, and order is confounded with diameter (Pregitzer *et al*. 1997). Thinner roots generally have shorter lifespans (desert

shrubs-Caldwell and Camp 1974, kiwi-Reid *et al*. 1993, apple-Wells and Eissenstat 2001). However, other studies have found no relationship between diameter and median root lifespan (perennial grasses-Ryser 1996, citrus-Eissenstat 1991). Hooker *et al*. (1995) found that those roots that were colonized with VA mycorrhizal fungi had significantly shorter lifespans than those not colonized. Majdi and Nylund (1996) found Norway spruce ectomycorrhizal short roots also had decreased longevity compared with nonmycorrhizal roots.

Root chemistry, like leaf chemistry, is important for root function as well as defense against herbivores and pathogens. Higher leaf nitrogen concentrations increase potential herbivory rates while concomitantly increasing potential photosynthetic rates. The finest roots of deciduous trees had higher N concentrations, lower C/N ratios (Pregitzer *et al.*1995, 1997) and higher specific root lengths (mm/mg) (Pregitzer *et al.* 1997). Like thin leaves, thin roots also have higher N concentrations than thick roots (Pregitzer *et al.* 1997) and may be more attractive to herbivores. The same study found that thinner roots were less expensive to build, but the higher N made them more expensive in terms of respiration rates to maintain. Higher N content leads to higher respiration rates (Ryan *et al.* 1996) and therefore, maintenance costs (= fixed carbon needed) (Yanai *et al.* 1995) and is a good indication of tissue activity. Tannin accumulation is also known to be a deterrent to herbivory, which potentially increases root lifespan. However, cortical cells accumulate tannin after they die, and the trade-off is that tannin-filled cells are more resistant to water flow than live cells (McKenzie and Peterson 1995). Therefore, root order, diameter, mycorrhizal colonization and nitrogen concentration must be considered when estimating root lifespan and making comparisons across species.

Relationship among organ traits and lifespan

Roots may be more important than leaves in nutrient cycles in ecosystems, but more work has been done on leaves. Leaves and the finest lateral roots share attributes that suggest that they might have generally similar trait syndromes: both organs are ephemeral, typically exhibit determinant growth, do not undergo secondary thickening and have the primary function of resource acquisition (Eissenstat and Yanai 1997). Trait syndromes are often related to the main processes associated with resource capture: acquisition (photosynthesis, nutrient uptake), interception (specific leaf area=SLA, specific root length=SRL), use (respiration) and turnover (organ lifespan) (Reich *et al.* 2003). Just as thin leaves are less expensive for plants to produce

and use for light interception, long, thin roots are less expensive to produce for nutrient interception (Eissenstat 1992). But fine root lifespan and associated traits are not as well-studied as leaf traits; therefore, root ecologists often consider our collective knowledge base for leaf traits as a starting point for examining root characters for trait syndromes.

Because the primary function of leaves and fine roots is resource uptake and because function and design are often correlated in biological systems, it is reasonable to expect similar relationships among characteristics for roots as have been found on leaves (e.g. high N concentration of a tissue inversely correlated with its lifespan). There have been studies demonstrating correlations among root characteristics that support such an idea, such as the relationship of low SRL with root longevity (Anderson *et al*. 2003, Eissenstat 1991), which mirrors that between low specific leaf area and leaf longevity. Regardless of potential trait syndromes within the leaf and root systems, leaves and roots on the same plant may be influenced by similar pressures resulting in a correlation between leaf and fine root lifespan among species (i.e. if long leaf lifespan helps a plant to conserve a limited supply of N, then extended fine root lifespans would further conserve N) (Monk 1966, Bloom *et al.*1985).

While leaves and roots can be thought to have similar functions, a root system represents a complex network that is rarely characterized with respect to its diverse functions: likely only the finest root orders are similar to leaves in terms of resource acquisition and being ephemeral. Even so, the fine roots are not discrete entities like leaves but the terminal segments in a complex structure. Similar to leaves, it has been assumed that root distribution is optimized (or nearly so) for acquisition of nutrients or for cost-efficient construction (Fitter *et al.* 1988), but root distribution (interception) is frequently less important for acquisition than nutrient availability and mobility (diffusion coefficients) (Marschner 1995). Leaves do not form depletion zones for CO_2 and light, while roots can deplete resources around them. Also, leaf function is not directly dependent on symbionts, while root carbon expenditure, resource acquisition and perhaps defense may be strongly influenced by mycorrhizal fungi (Allen 1991).

Even so, it is not unreasonable to assume that the relationships seen among leaf traits could be present in analogous traits of fine roots. For example, thin leaves have higher rates of photosynthesis, and thin roots have higher rates of hydraulic conductivity (Rieger and Litvin 1999). Based on extensive previous work on leaves (Chapin, Reich, etc.), it can be predicted that roots with longer lifespans will have lower specific root length, lower root nitrogen concentrations, lower uptake rates and lower maintenance respiration than roots with shorter lifespans. There are some data to support this possibility. For example, *Calluna sp.*, a native of

low nutrient heathlands, had a low rate of root turnover while *Molinia sp.*, a productive species from fertile habitats, had a higher rate of turnover (Aerts *et al*. 1992). Aerts *et al.* (1992) found that the rank order of root biomass turnover rates was similar to the rank order of belowground productivity for a group of heathland species. In Ryser and Eek's (2000) study of two *Dactylis* species, the species with the slower relative growth rate had thinner roots with a higher tissue-mass density due to a higher length: volume ratio of roots <0.25 mm diameter. It also had a longer leaf lifespan. There is also indirect evidence suggesting that roots with long lifespans have lower rates of maintenance respiration, lower rates of specific ion absorption and decreased response to increased nutrient availability when comparing roots of different species (Schläpfer and Ryser 1996, Eissenstat 1997). However, no studies have compared root lifespans of species differing in leaf lifespans.

Conifers vs. Hardwoods

Conifers have relatively slower growth rates than hardwoods. Bahaus and Messier (1999) found that conifers had larger average fine root diameters and lower specific root lengths than deciduous species and shrubs in both mineral and organic soils. Data from Chapin (1993) show a deciduous forest with half the aboveground biomass (15 kg m^{-2} vs. 31 kg m^{-2}) but twice the relative growth rate (0.07 yr^{-1} vs. 0.03 yr^{-1}) when compared to an evergreen forest. Slower growth rates can confer stress resistance by reducing nutrient demand for growth and allowing allocation to other processes such as storage or herbivore and pathogen defense. Slow growth can also minimize growth respiration needed to produce new tissues and reduce dependence on new nutrient capture (Chapin *et al.* 1993). Chapin (1993) proposed a feedback loop where slow root growth and slow turnover lead to small nutrient losses and low-nutrient, metabolically inactive roots, which lead to slow nutrient absorption contributing to slow root growth. But, most of Chapin's logic appears to be based upon leaf data. Nadelhoffer *et al*. (1985) found that apparent uptake of N per year, apparent fine root allocation and fine root N turnover was greater in hardwoods such as oaks and maples when compared to conifers such as spruce and pines. They also found that fine root production and estimated fine root turnover was higher for hardwoods compared to conifers. In fact, as fine root production increased, so did the estimates of root turnover.

How to measure root lifespan?

Minirhizotron cameras and tubes were used to collect information on roots for every part of this project. This technology has allowed root researchers wonderful opportunities to follow individual roots over time by taking pictures of roots growing next to clear plastic tubes. Minirhizotron systems have allowed researchers more flexibility in study sites and study species compared with the large, walk-in rhizotrons. Minirhizotron systems are also an improvement over sequential soil cores; data from minirhizotrons are more accurate because there are few assumptions made, while analysis of soil core data requires assumptions about concurrent root birth and death.

One of the basic assumptions of data collection techniques is that observations mimic what occurs in the bulk soil. The first study (Chapter 2) focused on the potential relationship between minirhizotron tubes and root responses. Minirhizotron tubes have been used in root research since the mid 1970's and minirhizotron cameras since 1981 (Bartz Technology Corporation, personal communication). Stiff-walled tubes have been made of glass and various plastics. Glass tubes are considered the best choice but tend to break easily when in a location with cold temperatures or alternating cycles of hot and cold. Plastic tubes are more common, and the type of plastic used by researchers seems to depend on price and availability. We had a unique opportunity to collect similar root data on six species with two commonly-used plastics for tubes made of either polymethylmethacrylate (acrylic) or cellulose acetate butyrate. With three years of data collection, we were surprised to find distinct differences in the root lifespan results collected from the different tube types.

Are fine root and leaf lifespans correlated?

Combining the ideas put forth by Monk (1966), Grime (1977), Chapin (1993) and Reich (1993) on the relationship between leaf lifespan, growth form and plant nutrient use efficiency, we can predict that fine root and leaf lifespan should be positively correlated in tree species of varying leaf lifespan. Chapin (1980, 1993) suggested that long leaf lifespan is an adaptation to low nutrient sites and coupled with slow leaf growth and low photosynthetic rates leading to slow nutrient absorption and therefore slow root growth with long root lifespans. In the same vein,

short leaf lifespan is an adaptation to high nutrient sites and coupled with fast leaf growth, rapid photosynthesis leading to rapid nutrient absorption and fast root growth with short lifespans.

To test our hypothesis, we needed a study site with many species to compare in monospecific stands (so we could be certain what species' roots we were analyzing). Through our colleagues at the University of Minnesota we were introduced to a wonderful site in central Poland. There were 14 tree species planted in monospecific, replicated plots. The trees were mature (30 yrs old), not seedlings. The plots had rather sandy soil, very little understory and closed-canopy conditions. We were unaware of a comparable site in the US and knew that this would give us a wonderful cross-species comparison to examine our question. We used a minirhizotron camera system to collect four years-worth of data on individual roots of eleven of the species at the site (three of the species had large amounts of understory growth in their plots and so were not used). Our species had large ranges in both leaf and fine root lifespan which provided us with a good possibility of finding a relationship between leaf and fine root lifespan (Chapter 3).

Annual patterns of root production

While understanding root lifespan is of great importance to being able to model belowground carbon storage, root production patterns are another important part of the cycle. The finest roots have the highest productivity and mortality rates (Pregitzer *et al.* 1995). We looked at the patterns of root production in the same eleven species during our four-year study. We asked the questions of whether our species have one or two major peaks in root production each year and if the peaks were consistent from year to year. There are many studies supporting both patterns of root production but very few studies of more than one season addressing the question of consistency. There are even fewer studies which include more than five species. Root phenology is commonly measured with soil cores (Vogt *et al*. 1998) and measured less frequently with the use of a minirhizotron system, though the minirhizotron system can provide very accurate results (Tierney and Fahey 2001). Our use of the minirhizotron system allowed us to observe individual root birth and death in the images and count the number of roots born on each sampling date. Although we did not have as many measurements in the early spring as we would have liked to round out the story, we had very consistent patterns each year in peaks of root production for all eleven species (Chapter 4).

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Chapter 2

The impact of material used for minirhizotron tubes for root research

Abstract

A wide variety of transparent materials are currently used for minirhizotron tubes. We tested the null hypothesis that minirhizotron composition does not influence root morphology and dynamics. We compared minirhizotron data for glass, acrylic and butyrate tubes in apple and acrylic and butyrate tubes in a study with six forest tree species. Root phenology and morphology were generally similar among tubes. Apple root production was greatest against glass; these roots became pigmented later and lived longer than roots near acrylic or butyrate. Roots generally became pigmented faster next to butyrate than acrylic. Root survivorship was shorter near butyrate tubes in three of the four hardwood species; however, survivorship was shorter near acrylic tubes for the three conifer species. Comparison of minirhizotron standing crop data with root standing crop from cores showed that the acrylic data matched more closely than the butyrate data. This study reveals that the transparent material used often has little effect on root production but can substantially influence root survivorship in some plants.

Introduction

Many of the least destructive approaches to observing roots and soil organisms involve transparent materials placed in the soil. Once installed, transparent-wall techniques permit repeated, nondestructive observation of individual roots for growth, phenology and demography (Fahey *et al.* 1999). Traditionally, large underground chambers, often referred to as rhizotrons, were used for root studies. With the development of miniature video cameras, boroscopes and fiberscopes, minirhizotrons are becoming the method of choice to study individual root

demography both in pot studies and in the field (McMichael and Taylor 1987, Hendrick and Pregitzer 1996, Joslin and Wolfe 1999, Johnson *et al.* 2001).

Rigid minirhizotron tubes have been made of materials such as glass (Richards 1984, Eissenstat and Caldwell 1988, Fitter *et al.* 1999), acrylic (polymethylmethacrylate =Perspex® =Plexiglas® =Acrylite®, Vos and Groenwold 1983, Itoh 1985), polycarbonate (=Lexan®, Box and Johnson 1987) and cellulose acetate butyrate (=butyrate =CAB, Box *et al.* 1989, Hendrick and Pregitzer 1992, Wells and Eissenstat 2001). Materials used to make minirhizotrons with flexible walls include: cellulose acetate (Merrill *et al.* 1987), polyvinyl film (Merrill 1992), FEB Teflon film (Kosola 1999) and rubber innertubes used for motorcycle tires (Gijsman *et al*. 1991, López *et al*. 1996). While borosilicate glass (SiO_2) is probably the most similar in chemical composition to elements present in most mineral soils, plastic chemistry is variable and not similar to soil constituents. Plastics are chains of repeating carbon monomers with various backbones and side chains; in some plastics, the side-chains are easily hydrolyzed and released into solution. Plastics have been preferred because plastic minirhizotrons are less prone to breaking or because field conditions or experimental objectives required minirhizotrons with flexible walls to promote good soil contact or allow access to the soil environment (e.g. Kosola 1999). In addition, it is often desirable to scribe or drill holes on the minirhizotron tubes, which is much easier to accomplish with plastics than with glass. The most important assumption regarding the estimation of fine root growth dynamics with minirhizotrons is that roots seen next to the tubes are behaving in a manner similar to those in the bulk soil, but little research has been devoted to potential effects of the minirhizotron material on the data collected. There is also no known standard for material used for rhizotron or minirhizotron tubes. The acceptance that tubes are benign may be why many authors do not indicate the type of material used in their minirhizotron studies.

Tierney and Fahey (2001) compared root production and longevity estimates using butyrate tubes and soil screens in a temperate broadleaf forest. They found similar estimates for root longevity using the two methods and concluded that minirhizotrons do not affect the longevity of fine roots. Johnson *et al.* (2001), in their review of minirhizotron studies, cited unpublished data that showed no difference between fine root biomass density in the bulk soil and either root density against polycarbonate (Mojave Desert) or butyrate (Douglas-fir stands) minirhizotron tubes. They also concluded from this data that minirhizotrons did not affect root production.

However, there is some limited evidence that the type of transparent material may differentially influence root growth and death. The only paper of which we are aware that specifically compares transparent materials was by Taylor and Böhm (1976). They compared acrylic rhizotrons with large windows built in Ames, IA to those made of glass in studies in Auburn, AL. They concluded, based on a variety of field crops in a variety of soils, that soil adhesion tended to be higher against glass than acrylic windows. Root length density against glass was comparable to that in the bulk soil; however, root length density against acrylic was greater than that in the bulk soil. They speculated that the larger air gaps observed to form between soil and acrylic windows tended to cause preferential root growth, leading to artificially increased root length densities by acrylic windows.

We are not aware of any study that has compared different plastics as materials for minirhizotron tubes, despite the wide variety of materials currently in use. We compared three of the most common materials used for minirhizotron field studies: acrylic, butyrate and glass. Our first experiment used all three of these materials for tubes in a project on apple root growth and survivorship. We assumed that glass represented the most natural environment because it is made from silica sand and because of the results of Taylor and Böhm (1976). In a larger experiment comparing root dynamics in six tree species, we compared acrylic and butyrate tubes, which are the most widely used materials for minirhizotron studies, to determine (1) if the tube material affects the number of roots or root mass observed against a minirhizotron tube, (2) if the tube material affects the time to pigmentation, death and disappearance in these roots, and (3) if differences in tube material are found, whether these effects are uniform across tube type for a variety of species and soil types.

Materials and Methods

Experiment 1: Apple

Experiment Site

The first experiment was conducted in an apple orchard at the Russell E. Larson Agricultural Research Center in Rock Springs, Pennsylvania (40.80ºN 77.86ºW, alt: 356 m), using fourteen, 20-year-old *Malus domestica* "Gold Spur delicious"/M26 trees. Trees were about 2.5 m tall and planted at a 2-m spacing in a 'Penn State four-wire low-hedgerow' trellis system with 3.7-m spacing between rows. The soil at this site is a Hagerstown silt loam (Typic Hapludalf) and is characterized by a 20-cm surface layer of dark brown silt and a 93-cm layer of reddish brown silty clay subsoil. The soil is moderately permeable and has a high available water capacity. State College has an average yearly precipitation of 967 mm with the largest amounts falling in May, June and July. The mean annual temperature is 9.3 ºC with average summertime highs of 25-28 ºC.

Fifty-seven minirhizotron tubes (19 of each tube type) were placed in the ground 40 cm from the trunks of the trees, 20 cm apart and inserted at a 30^{o} angle from vertical in May 1997. The design was a completely randomized block with each tree (block) having three tubes placed in the adjacent soil, one of each material: glass, acrylic and butyrate. Plastic tubes were purchased from Pena-Plas (Jessup, PA) three months before installation. Each tube was 38 cm long with an internal diameter of 1.9 cm, an external diameter of 2.2 cm and had two columns of 35, 8 by 8 mm windows scribed on the surface. For plastic tubes, lines were scribed with a soldering iron and then painted black. For glass tubes, black-line decals (0.79 mm) were baked on each tube. Each tube bottom was sealed with a tight fitting rubber stopper to prevent water from entering the tube, and the tops were sealed with a stopper and black tape to prevent the entrance of light. When not in use, tubes were covered with white plastic to minimize solar heating. To encourage root growth, 200 ml of standard-strength Miracle-Gro® nutrient solution were added once to the soil around the tube following tube installation.

Data Collection

An 8 mm rigid, swing-prism boroscope (Olympus America Inc., Lake Success, NY, USA) with a video camera attached to the eyepiece (Bartz Technology, Santa Barbara, CA, USA) and a fiberoptic light source was used to take the videos (a Sony Hi-8 video deck) beginning in June 1998. The one-year lag time between tube installation and video measurement allowed for roots to adjust to the initial soil disturbance (Joslin and Wolfe 1999). The videos were taken two times per week for the first two weeks and then once a week through August 1998. Roots were recorded a total of nine times. Videos were viewed using a Sony Hi-8 video deck and a Macintosh 7500 computer. Roots were tracked from birth to death and then analyzed for survivorship. Number of neighbor roots (number of roots in a frame minus 1) was determined on the last sampling date before a root died or on the last sampling date for roots that were still alive. The date a root became pigmented was recorded in the same fashion. Root diameter was determined on the first date of appearance using RooTracker software (Dave Tremmel, Duke University Phytotron, Durham, NC, USA).

Experiment 2: Six Forest Trees

Experiment Site

This experiment was conducted in a common garden planting at The Morawina Experimental Station in The Siemianice Experimental Forest near Kępno, in central Poland (51°14.87'N, 18°06.35'E, altitude: 150 m). Prior to establishment of the current planting, the vegetation was an 81-yr-old Scots pine (*Pinus sylvestris* L.) stand. Average precipitation for the area is about 580 mm yr^{-1} with most of it falling in June, July and August. Mean annual temperature is 7.5 °C with average summer high temperatures ranging from 18-22 ºC.

The planting consisted of two adjacent sites with three blocks each. There were 14 tree species total in the planting (Szymanski 1982). Within each block there were nine, monospecific 20 x 20 m plots, with a total of nine species per site. Trees were planted in 1970 and in 1971 as one and two-yr-old seedlings respectively at 1 x 1 m spacing; some self-thinning has occurred since planting. Each area had a fairly uniform topography and soil. However, there were

differences in soil properties between the two sites. The first site had a "grey-brown podzolic soil" with a much higher proportion of small fractions (<0.02 mm) and much higher content of macro- and microelements compared with the "brown podzolic soil" of the second site. Soils in both sites are nutrient poor with a plowed A horizon (unpublished data). Average mineral soil pH (in water) ranged from 3.8 to 4.1 in the conifer plots and from 4.1 to 4.4 in the hardwoods. For this experiment we chose three deciduous broad-leaved species in the first site: *Acer pseudoplatanus* L., *Fagus sylvatica* L. and *Quercus robur* L., and three evergreen conifers in the second, adjacent site: *Picea abies* (L.) Karst., *Pinus nigra* Arnold and *Pinus sylvestris* L.

The minirhizotron tubes were made at Penn State and shipped to Poland. The large number of tubes involved, the potential problems with shipping and the earlier difficulty with glass tubes breaking, restricted this experiment to an examination of the two plastics. The acrylic and butyrate tubes were purchased in 1.8 m lengths (manufacturer: Thermoplastic Processes, Stirling, NJ; distributor: Total Plastics/Garron Plastic, Harrisburg, PA) and cut in thirds. The minirhizotron tubes had an inside diameter of 5.2 cm and a wall thickness of 6.4 mm. Using a soldering iron and a guide, they were scribed with a strip of 1 x 1.25 cm windows, and the windows were numbered. Black forester paint was used to fill the indentations and then the excess wiped off to leave clear windows. Tubes were numbered at the top and coded for plastic type to prevent confusion later. Solid PVC rod, cut and lathed to make a bottom plug, was sealed in place with caulk. Tops of the tubes were wrapped in black electrical tape and sealed with a rubber stopper to keep light and rain from entering the tubes; no other covers were used because the tubes were shaded >80% of the time.

In November 1998 (when the acrylic was 3 months old and the butyrate was 6 months old), the 60 cm tubes were installed randomly in the plots at an angle of 30° from vertical. Three tubes of each type were installed per plot, three plots per species. The tubes were at least 3 m from the plot borders, and the butyrate and acrylic were interspersed within each plot.

Data Collection

Minirhizotron images were collected using a minirhizotron camera and associated image capture software (Bartz Technology Corp., Santa Barbara, CA) starting in May 1999, six months after tube installation to allow for the system to recover from the installation disturbance (Joslin and Wolfe 1999). Images were collected 10 times from May through Dec. 1999 at 2-4 week

intervals. They were collected three times each in 2000 and 2001 at four-month intervals because the 1999 data indicated very long-lived roots.

Captured images of the windows in the tubes were later viewed as a time sequence. The date a root was first observed and the date of disappearance were recorded. Root birth and root death were estimated as the day halfway between successive imaging dates (Appendix A, Equation 1). If a root disappeared at some point because another root grew in front of it or if a root was still alive at the end of the data collection period, then it was marked as censored. Individual root lifespan was calculated as the number of days from root birth to root death. In this system, root death was often associated with disappearance; no outward signs of loss of cortical tissue were evident before such disappearances. Only fine roots born in 1999 and 2000 were used in the analyses for lifespan, but followed through 2001. Soil depth was calculated from the position of the window down the tube and the installation angle (Appendix A, Equation 2).

Root production was recorded as the total number of new roots observed per unit area of observation surface per year. Root diameters were determined by direct measurement on a computer screen using the image from the date a root was first observed. This study only examined the production and demography of the finest two orders of roots. Root diameter was used to estimate root order. Using roots collected from the same plots and separated by order, we determined the upper limits of root diameter of the finest two root orders for each species individually using WinRhizo software (Regent Instruments Inc., Quebec, Canada) (unpublished data). The root diameters of the scanned 1^{st} and 2^{nd} order roots were graphed, and the diameter value for the 50^{th} percentile was chosen as the upper limit.

Converting root counts to root length and then dividing by specific root length converted minirhizotron root counts at the end of the study to standing crop in terms of root mass (Appendix A, Equation 3). Root length was calculated using WinRhizoTron (Regent Instruments Inc., Quebec, Canada) for each tube for five dates over the course of the experiment. The regression equation of the relationships within each species (all $R^2>0.92$) was used to convert root number to root length. Specific root length was calculated separately by scanning roots of known order for each species with WinRhizo and dividing sample length by sample dry weight.

Subsamples of 30 roots per plot per species in minirhizotron images from spring and summer 1999 (180 roots total per species) were studied for root pigmentation, which often indicates decrease in root metabolic activity and absorptive capacity (Comas *et al*. 2000, Bouma *et al*. 2001). Pigmentation may also indicate an increase in defensive compounds. The

subsampling of roots was deemed sufficient to cover the range of variability in the pigmentation rates. A random number generator was used to choose tube and window numbers in each season. Then all of the roots seen in that window on that date were noted. This procedure was repeated until enough roots were recorded. Only roots that were white at first appearance were used for this analysis. The date when at least 50% of a root's length was pigmented was recorded as well as the date of death. Roots generally only changed color once before dying. Many roots were pigmented at the time of first appearance and were not included in this analysis.

Root Standing Crop

After three years, estimated root mass visible in minirhizotrons should reflect the balance between fine root production and turnover, in essence an index of standing crop. To better understand the differences in data between the two tube types, we compared minirhizotron root standing crop (SC_{mrt}) with soil fine root standing crop (SC_{cores}). Core samples for root biomass were collected between rows of trees and otherwise randomly located within the plot using a 15-cm long, 4.7 cm diameter soil core sampler (Arts Mfg. and Supply, American Falls, Idaho, USA). Soil cores were taken in July 1999 from three plots per species (three cores per plot) from the same hole at two consecutive depths: 0-15 and 15-30 cm. In addition, eight cores per plot were taken in July 2002 from a depth of 0-15 cm. In this paper we averaged data from both sampling periods.

Following collection, soil core samples with roots were stored at –3 °C for later processing in the laboratory. Soil samples with roots were washed over 1 mm sieves, and roots were manually separated from soil and divided into two categories: < 2 mm and > 2 mm in diameter and oven-dried at 65 °C for one week. In this paper we presented only data on fine roots < 2 mm. Average SC_{cores} was calculated in g m^{-2} of projected surface area of the soil corer used; SC_{mrt} was calculated in g m^{-2} of imaging surface area of the tubes with average viewing area of tubes equalling 37.5 cm^2.

Statistical Methods

Data are reported as significant if $P<0.05$. We examined the possible effect of blocking the tubes by tree in our statistical models for the apple experiment. In no case was this factor found significant ($P>0.4$). Glass tubes were considered the control. Because more than a third of our glass tubes broke during the study, we decided not to include the block effect in the models. A general linear model (one-way ANOVA) was used to analyze root diameter and root number in the apple experiment.

In the forest tree experiment, differences in root production between tube type and year were analyzed using a general linear model with a split-plot design. Wilcoxon tests were performed to compare root lifespans between tube types within each species in the forest tree experiment.

Survivorship curves and root lifespan estimates for each species were calculated using the BASELINE statement of PROC PHREG in SAS (SAS Institute, Cary, NC) (Appendix A, Equation 4). Cox proportional hazards models were used to test for differences within each species for the influence of tube type, diameter, soil depth and time of birth on root lifespan (Allison 1995, Wells and Eissenstat 2001). Separate hazard models were also performed where acrylic and butyrate tubes were analyzed separately to evaluate any possible interactions of a covariate (e.g., diameter) with tube type. The differential risk of white roots browning for the two types of plastics was tested with a Cox proportional hazards model. In the apple experiment, the Cox proportional hazard model was run with comparisons of acrylic and butyrate to glass and then a comparison of the parameter estimates of acrylic and butyrate with a linear hypothesis.

Cox's partial likelihood method (Cox, 1972) estimates regression models of time until "failure" of an individual without specifying an underlying distribution, and the estimate depends only on the ranks of these event times, not their numerical values. It allows for censored data and time dependent covariates, making it very useful for root demography data that is randomly censored. Two kinds of data are presented: hazard functions and survivorship curves. The hazard function estimate quantifies the instantaneous risk that an event (e.g. root death) will occur at time $(t+\Delta t)$ given that the individual (a root) has survived to time (t). For a sample or population, the hazard function reveals overall trends of the individuals over the sampling period. The survivorship curves are calculated from the baseline of the hazard function for an individual whose covariate values (e.g. plastic type, root diameter) are all zero (Allison 1995).

Results

Experiment 1: Apple

The type of transparent material had a significant effect on cumulative apple root production ($P = 0.035$, Figure 2.1). From June through August 1998, cumulative root production was greatest for glass and least for butyrate, with acrylic intermediate. Seasonal patterns of production were similar for the three types of materials (data not shown).

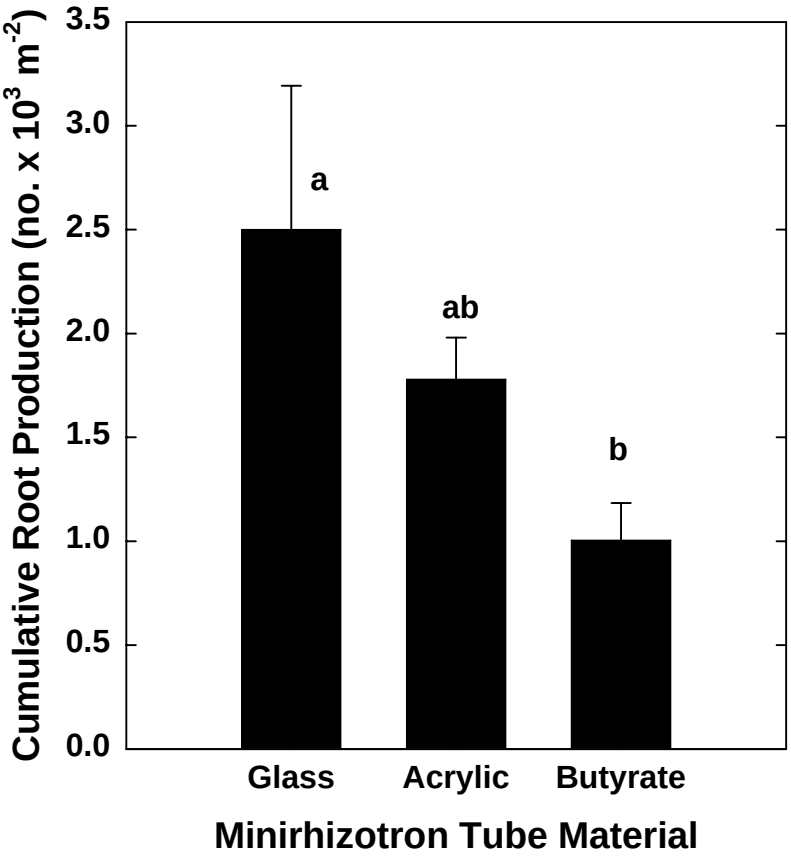

Figure 2.1: Cumulative number of apple roots produced (+SE) per unit area of observation surface for three minirhizotron materials from June-Aug. 1998. Number of tubes of each material were as follows: acrylic, n=19; butyrate, n=19; and glass, n=12. Total surface area on a minirhizotron used for observation was 45 cm^2. Different letters above the bar indicate differences significant at $P < 0.05$ using Duncan's multiple range test.

We also examined whether the type of transparent material might affect root morphology. Mean root diameter ranged from 0.34 to 0.35 mm (Pooled SE=0.20 mm) among the three materials with no evidence that the plastics altered root diameter (data not shown; P >0.60). We did not observe any other features of the roots (e.g. branching) that qualitatively differed among the three transparent materials. Time to root pigmentation was significantly influenced by tube material (Table **2.1**, Figure **2.2a**). Roots became pigmented significantly faster next to butyrate tubes, followed by acrylic and then glass. There was a strong effect of type of transparent material on root survivorship (Table **2.1**, Figure **2.2b**). Roots visible on glass exhibited the highest survivorship, those visible on butyrate the lowest survivorship, with acrylic intermediate.

Table **2.1**: The effects of tube type on risk of pigmentation and lifespan in apple roots adjacent to glass, acrylic and butyrate minirhizotrons. Results of Cox proportional hazards regression are indicated, including hazards ratios (HR, $HR=e^{\beta}$), parameter estimates (ß), standard errors (SE), chi square values (χ^2) and P values. A positive ß indicates an increased risk of mortality with an increase in the parameter. *Risk* is the relative magnitude of the risk in a particular contrast. Degrees of freedom = 1 for all. Abbreviations: acrylic (A), butyrate (B), glass (G). Number of neighboring roots and root diameter were also significant covariates in the model (data not shown). Note: The risk for bivariate, discrete variables is interpreted as the ratio of the risk of one state to another and calculated as [HR*100]; e.g. a HR of 2.11 indicates an increased risk of mortality of 111% in the sampling interval for roots near acrylic vs. those near glass.

	β	**SE**	**χ^2**	***P***	**HR**	**Risk**
Risk of pigmentation						
Acrylic vs. Glass	0.68	0.20	11.18	0.0008	1.97	A > G
Butyrate vs. Glass	1.46	0.21	48.80	0.0001	4.32	B > G
Acrylic vs. Butyrate*	---	---	20.12	0.0001	---	---
Risk of mortality						
Acrylic vs. Glass	0.75	0.29	6.51	0.01	2.11	A > G
Butyrate vs. Glass	1.34	0.30	19.78	0.0001	3.81	B > G
Acrylic vs. Butyrate*	---	---	6.17	0.013	---	----

*This contrast was tested as a linear hypothesis in the proportional hazards model.

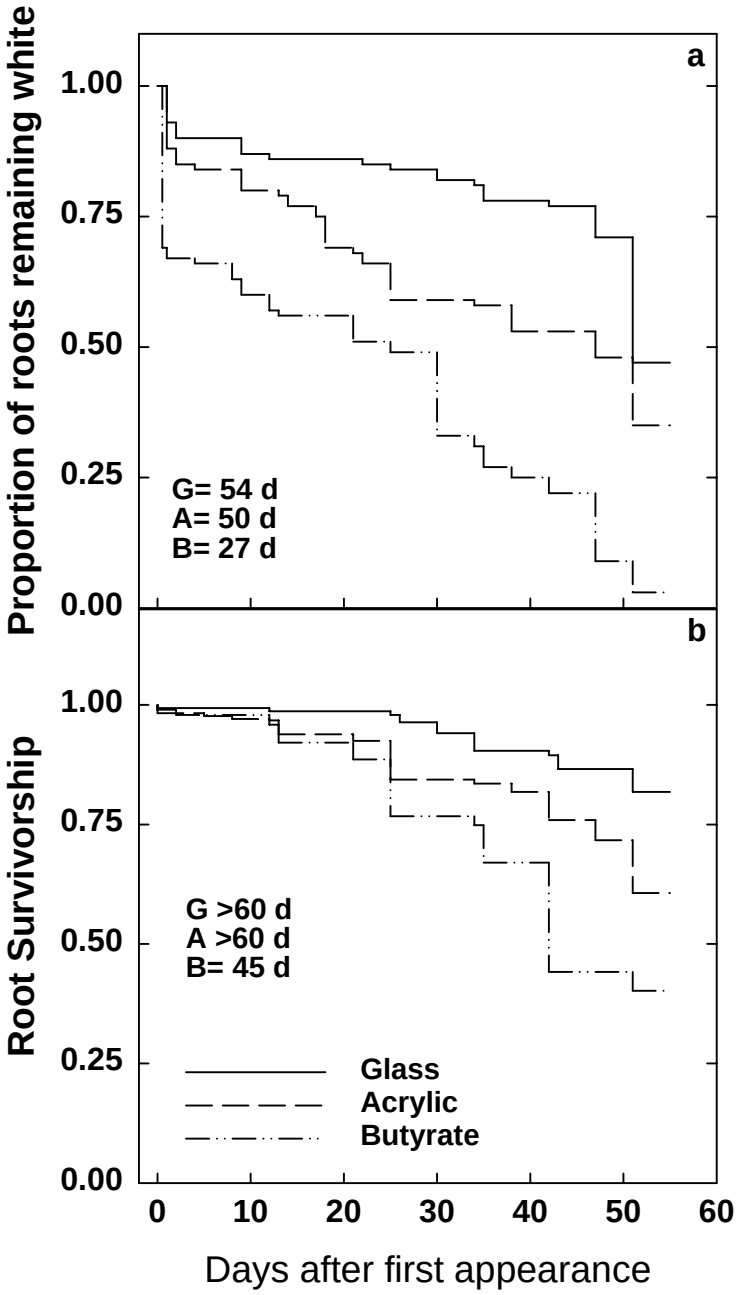

Figure 2.2: (a) Proportion of apple roots not pigmented (still white) growing adjacent to minirhizotron tubes made of three different transparent materials. Curves were generated using the BASELINE statement in PROC PHREG in SAS, which produces the baseline survivor functions for the chosen covariate (plastic) evaluated at the means of the other covariates, in this case birth date. Number of days at which 50% of the roots became pigmented is shown for each material (G= glass, A= acrylic, B= butyrate). (b) Apple root survivorship against minirhizotrons of different transparent materials. Significant differences were found between tube type and the number of roots, tube type and root diameter and tube type and the number of neighbors. Median lifespan estimates in days shown.

Experiment 2: Forest Trees

Root Production

Seasonal root production patterns between acrylic and butyrate tubes did not differ significantly. The largest amount of roots was produced in the summer for all species and tube types. Year had a greater effect on root production than plastic type (Figure **2.3**). The number and mass of roots produced significantly increased from 1999 to 2001 for *Q. robur*, *P. nigra* and *P. sylvestri*s ($P<0.04$). For one species, there was a significant year x plastic interaction (*A. pseudoplatanus*, $P=0.02$) with the production in 1999 and 2000 being similar but the production next to butyrate in 2001 being less than half that next to acrylic. Cumulative root production tended to be greater by acrylic tubes for *P. abies* ($P=0.055$). There was no difference between cumulative production near butyrate and acrylic for the other five species.

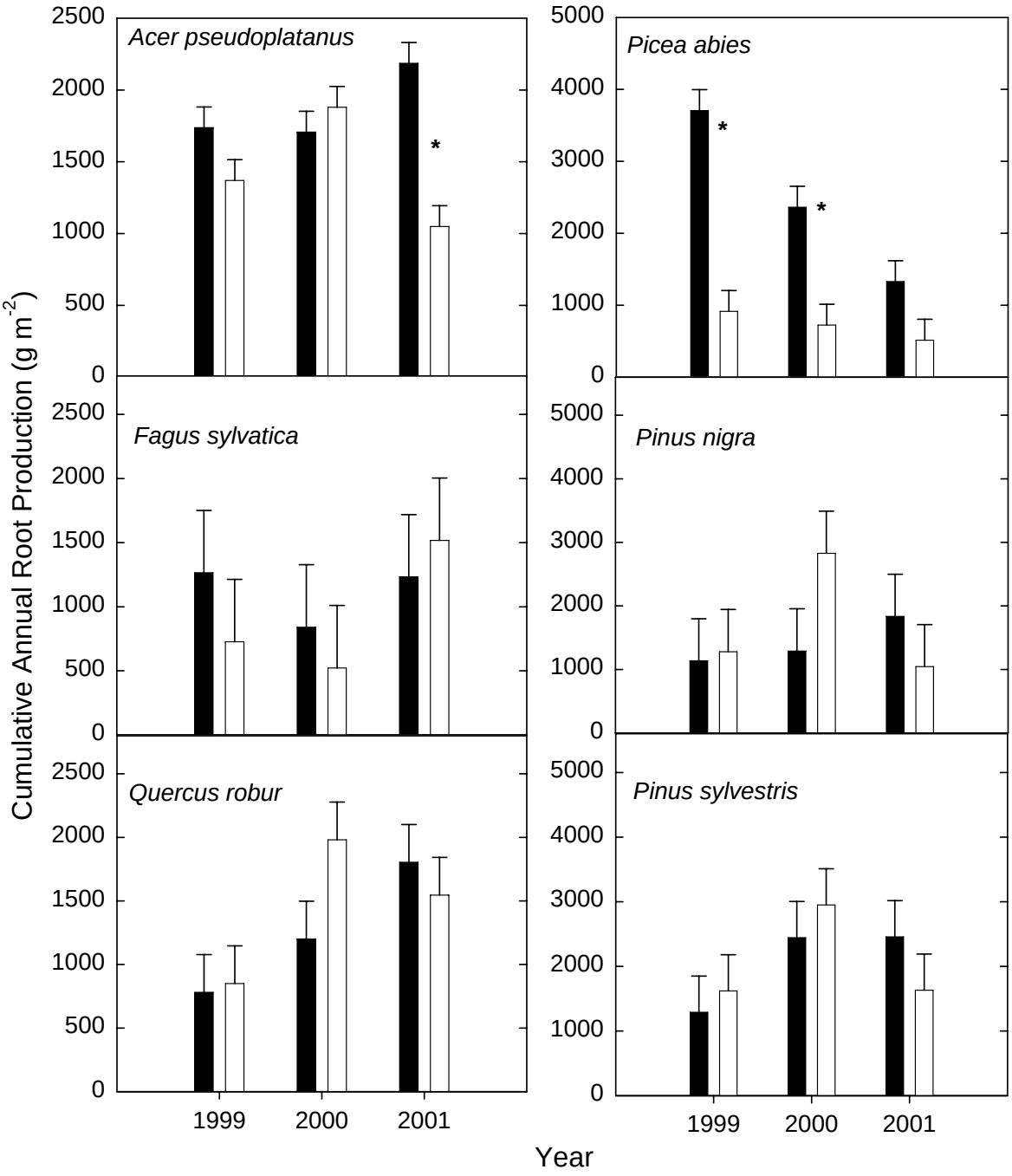

Figure **2.3**: Cumulative annual root production (+SE) for the forest tree experiment in 1999, 2000 and 2001. There were three tubes of acrylic (■) and three tubes of butyrate (□) for each species replicated in three separate plots (9 tubes total of each tube material and for each species). Asterisks indicate a significant difference between acrylic and butyrate production within the marked year ($P<0.05$). Total cumulative production over the experiment was only marginally significant for *P. abies*. Note the different scales of the y-axes.

Morphology

Mean root diameter was significantly influenced in two of the six species. *Fagus sylvatica* roots were significantly thicker near butyrate tubes (0.37 mm vs. 0.27 mm). However, *P. sylvestris* roots were significantly thicker next to acrylic tubes (0.42 mm vs. 0.32 mm). Mean root diameters for the other species were within 0.02 mm for both tube types. Root branching, as indicated by the percentage of first order roots, was not influenced by tube type.

Pigmentation

The risk of pigmentation was significantly influenced by tube type (Table **2.2**). The time from birth (white) to pigmentation was significantly decreased against butyrate tubes for four of six species (Figure **2.4**). Roots remained white about 10 to 42 d (longer by acrylic than butyrate tubes depending on the species. Fine roots that remained white and never became pigmented had a significantly increased risk of dying near butyrate tubes for four of six species (Table **2.2**).

Table **2.2**: The effects of tube type on risk of pigmentation of white roots, the risk of mortality of white roots that never became pigmented, and the overall risk of root mortality for six species using acrylic (A) and butyrate (B) minirhizotrons calculated using Cox proportional hazard regression. Hazard ratios (HR), parameter estimates (β), standard errors (SE), chi square values (χ^2) and *P* values for the effect of tube type are reported. *Risk* is the direction of the risk relationship. Degrees of freedom = 1.

	ß	**SE**	**χ^2**	**P**	**HR**	**Risk**
Pigmentation						
Acer pseudoplatanus	0.072	0.15	0.22	0.64	1.07	----
Fagus sylvatica	0.96	0.29	10.86	0.001	2.6	B > A
Quercus robur	2.30	0.39	33.97	<0.001	9.96	B > A
Picea abies	1.30	0.21	37.23	0.001	3.67	B > A
Pinus nigra	0.62	0.32	3.63	0.056	1.85	(B > A)
Pinus sylvestris	0.47	0.25	3.66	0.05	1.6	B > A
White Root Mortality						
Acer pseudoplatanus	1.77	0.22	64.78	<0.001	5.84	B > A
Fagus sylvatica	0.80	0.30	7.28	0.007	2.23	B > A
Quercus robur	0.82	0.27	9.14	0.003	2.27	B > A
Picea abies	0.26	0.21	1.54	0.21	1.29	---
Pinus nigra	0.77	0.33	5.43	0.02	2.15	B > A
Pinus sylvestris	0.16	0.25	0.42	0.52	1.18	---
Root Mortality						
Acer pseudoplatanus	1.01	0.19	28.22	<0.001	2.74	B > A
Fagus sylvatica	-0.06	0.20	0.08	0.78	0.94	---
Quercus robur	0.53	0.17	9.60	0.002	1.70	B > A
Picea abies	-2.21	0.34	42.46	<0.001	0.11	A > B
Pinus nigra	-3.16	0.58	29.57	<0.001	0.04	A > B
Pinus sylvestris	-2.04	0.31	43.43	<0.001	0.13	A > B

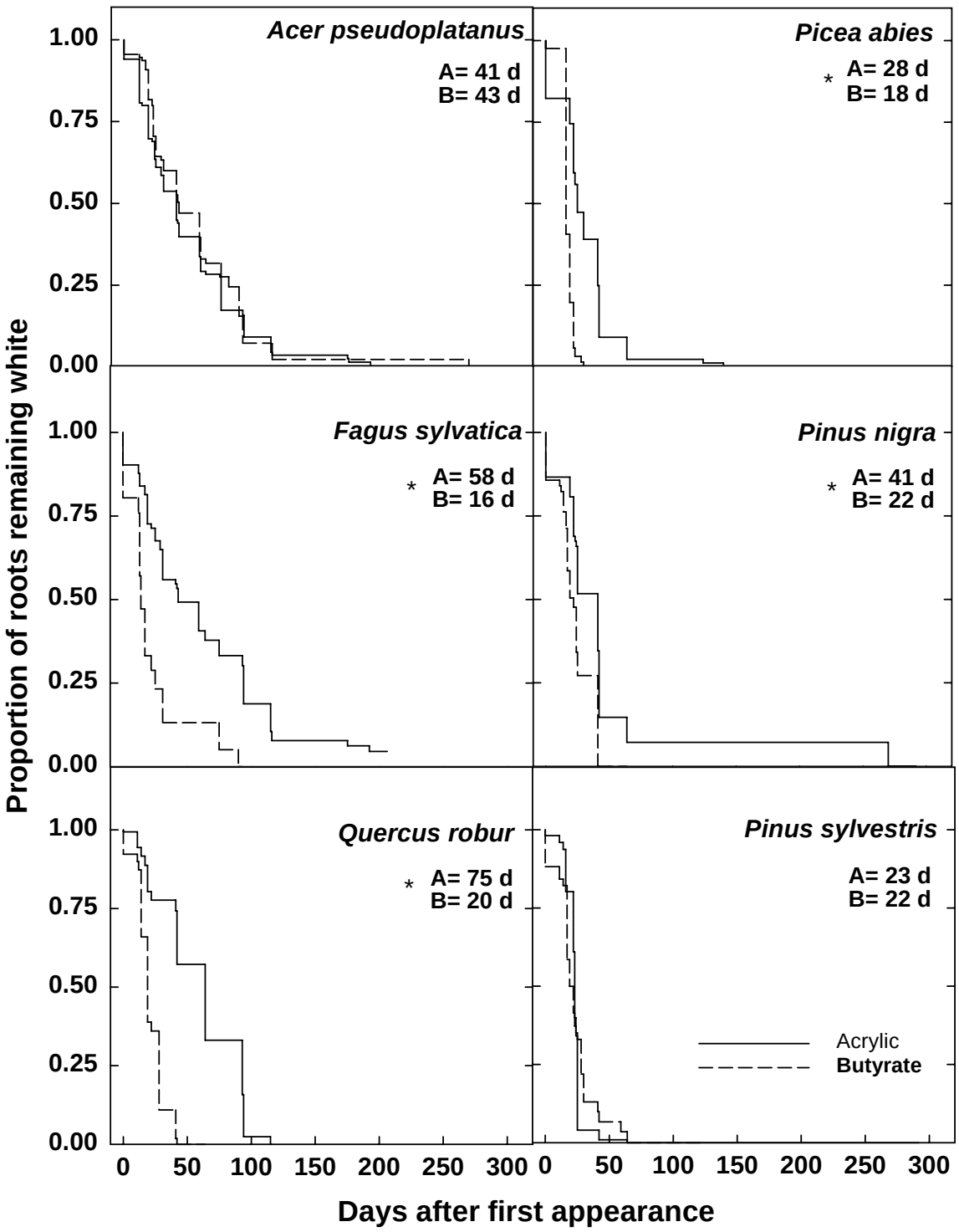

Figure 2.4: Proportion of unpigmented roots (still white) growing next to minirhizotron tubes made of two different plastics, acrylic (—) or butyrate (-). Curves were generated using the BASELINE statement in PROC PHREG in SAS, which produces the baseline survivor functions for the chosen covariate (plastic) evaluated at the means of the other covariates, in this case plot and season of birth. A random subsample of 120 roots of each species born in spring or summer 1999 or 2000 was used for this estimate. Number of days after which 50% of the roots became pigmented is indicated for each material (A= acrylic, B=butyrate). Asterisks indicate significant differences (P<0.05).

Root Lifespan

Tube material affected root survivorship in a species-specific way in the forest trees (Table **2.2**, Figure **2.5**). For butyrate plastic, roots of *A. pseudoplatanus* and *Q. robur* exhibited increased risks of death between sampling dates compared to acrylic (Table **2.2**). Median lifespan of *Q. robur* roots was twice as long against acrylic tubes as against butyrate tubes (580 d vs. 290 d, $P<0.01$). Median lifespan of *A. pseudoplatanus* roots were at least three times longer against acrylic than butyrate (>900 d vs. 300 d, $P<0.01$). In contrast, the three conifer species had decreased risks of root death near butyrate compared to acrylic (Table **2.2**, Figure **2.5**). *Picea abies* and *Pinus* spp. roots lived at least two to three times longer near butyrate tubes than near acrylic tubes (>900 d vs. 340-500 d, $P<0.01$).

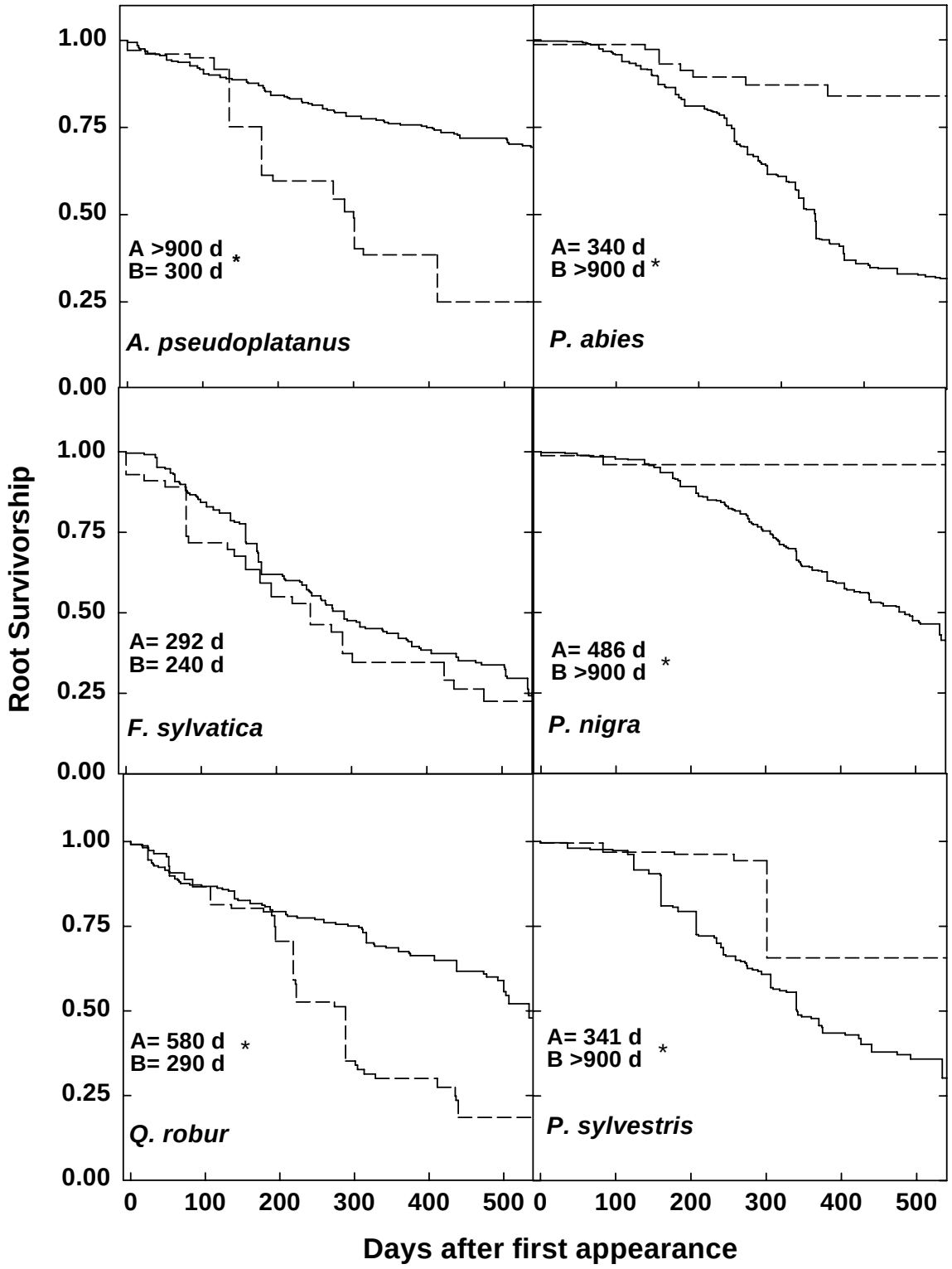

Figure 2.5: Survivorship probabilities for fine roots growing next to minirhizotron tubes made of two different plastics, acrylic (—) or butyrate (--). Curves were generated using the BASELINE statement in PROC PHREG in SAS, which produces the baseline survivor functions for the chosen covariate (plastic) evaluated at the means of the other covariates, in this case soil depth, root diameter and time of birth. Only fine roots born in 1999 and 2000 were used for these estimates. Experiment was run 930 days, but curves are only shown to 500 days so as not to bias for roots born early. Median lifespan estimates in days are indicated (A= acrylic, B=butyrate). Asterisks indicate significant differences (P<0.05).

For individual species, the differential influence of material on patterns of survivorship among individual plots (3 per species) was relatively consistent. Using 900 d as the end-point of the experiment, median root lifespan differences between acrylic and butyrate tubes (lifespan near acrylic minus lifespan near butyrate) among the three plots ranged from 600 d to >700 d for *A. pseudoplatanus*, from 140 d to >600 d for *Q. robur* and from -200 d to 100 d for *F. sylvatica*. For conifers, differences in median root lifespan between butyrate and acrylic tubes among the three plots ranged from 500 d to >550 d for *P. abies*, from 400 d to >500 d for *P. nigra* and from 200 d to >650 d for *P. sylvestris*. Thus, no one plot overly influenced the direction of response, although the magnitude of response was influenced by one plot for *Q. robur* and *P. sylvestris*.

There was no correlation of tube type and root production with root lifespan. Increased root production was coupled with a longer root lifespan (*A. pseudoplatanus*, acrylic), with shorter root lifespan (*P. abies*, butyrate) and with no change in root lifespan (*F. sylvatica*) for different species.

Potential interactions of tube type with root diameter, depth and time of birth were examined for each tube type separately using Cox proportional hazard models. When data were separated by tube type, the relationships with diameter, depth and time of birth were similar to the overall model and significant for four or five out of the six species (data not shown). Most of the significant relationships were for acrylic tubes. Near butyrate tubes, root diameter and soil depth were not risk factors for root mortality, and time of birth was significant for only two of six species (*Q. robur* and *P. sylvestris*).

Standing Crop

We compared the relationship between root standing crop determined by soil coring to a root standing crop index against the tubes at the end of the experiment in order to assess the relative accuracy of the data collected near the different tube types. Standing crop from cores varied 7-fold while SC_{mrt} varied 10-fold near acrylic and 6-fold near butyrate tubes (Figure **2.6**). Standing crop from cores explained 67% of the variation in SC_{mrt} near acrylic tubes ($P<0.0001$, $F_{1,17}= 34.9$), but only 10% of variation near butyrate tubes ($P=0.11$, $F_{1,17}= 2.81$). The lower adjusted R^2 value for the butyrate tubes was in part a result of the standing crop indexes of *P. nigra* and *F. sylvatica* being similar in magnitude to those near *A. pseudoplatanus* even though the SC_{cores} of *P. nigra* and *F. sylvatica* were 3-6 fold lower.

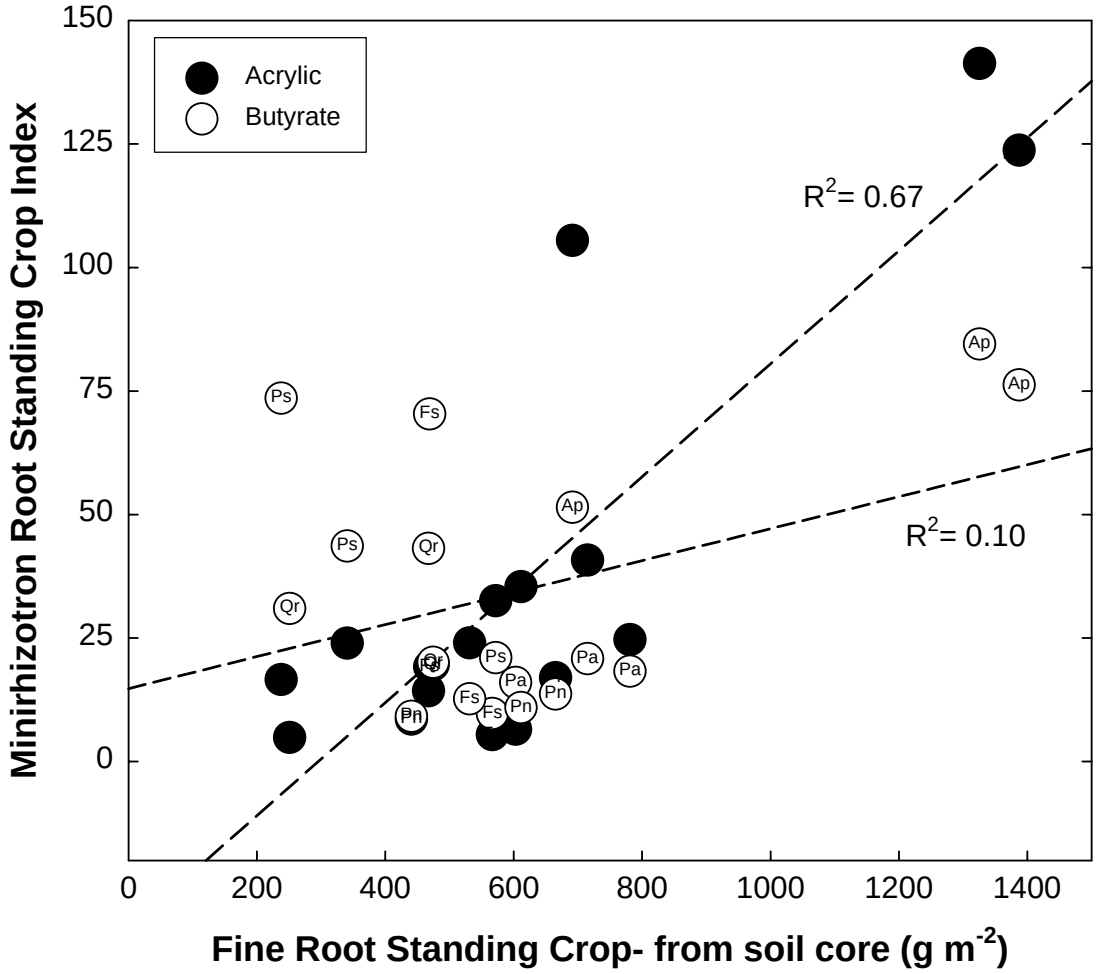

Figure **2.6**: Relationship between standing fine root (< 2 mm) biomass from soil cores taken to 45 cm depth in 1999 and an index of standing fine root (< 1 mm) crop against the acrylic and butyrate tubes, also 0-45 cm, in the same plots at the end of the experiment in 2001. Each data point represents either the acrylic or butyrate standing crop index (average of three tubes, calculated in g m^{-2} of imaging surface area of the tubes) with the soil core standing crop estimate of the plot (average of 11 cores, calculated in g m^{-2} of projected surface area of the soil corer used). Species labels are shown for butyrate tubes (e.g. Fs= *Fagus sylvatica*). Plant species for corresponding acrylic point will be the same as that for butyrate for a given soil standing crop.

Discussion

Minirhizotrons are currently one of the most commonly used methods in field root research. Our results provide an unpleasant reminder of the potentially adverse effects that an

observer may have on organism behavior. In particular, root pigmentation and root survivorship were often strongly affected by the type of tube material. Roots adjacent to butyrate tubes usually had a greater risk of becoming pigmented in both experiments (Table **2.2**, Figures **2.2**a and **2.4**). Also, those roots born next to butyrate tubes that did not become pigmented usually had a greater risk of dying (Table **2.2**). Root pigmentation has been observed in minirhizotron and developmental studies to be due to an accumulation of phenolic compounds (e.g. McKenzie and Peterson 1995, Comas *et al*. 2000) as well as associated with a marked decrease in root respiration rates and metabolic activity (Comas *et al*. 2000). If pigmentation represents a condition of reduced root absorptive capacity (Comas *et al*. 2000, Volder *et al*. 2005), then roots, whether they became pigmented or remained white, had a greater risk of lost absorptive function and/or death near butyrate tubes.

Root lifespan was also strongly influenced by tube type, but the specific response differed among species. In Experiment 1, apple root survival was longest by glass and shortest by butyrate with acrylic intermediate (Table **2.1**, Figure **2.2b**). In the forest tree experiment, mortality of white roots that never became pigmented was higher by butyrate than acrylic in three hardwood species and one of the conifer species (Table **2.2**). The remaining two conifer species exhibited no difference between the two tube types. In terms of total root survivorship, two of the three hardwoods exhibited longer survival by acrylic than butyrate (*F. sylvatica* exhibited no difference), whereas the three conifers exhibited much longer survival by butyrate than acrylic. These species-specific responses make generalizations about tube influence difficult.

Although for the forest tree experiment we did not have a specific control, we could compare a minirhizotron standing crop index to soil core standing crop as a direct assessment of differences in results among tube types. Standing crop from cores exhibited a much stronger positive correlation with biomass of roots observed at the end of the experiment by acrylic than by butyrate tubes (Figure **2.6**); however, SC_{mrt} for a number of species was not influenced by tube type. The unusually high SC_{mrt} near butyrate tubes for some species appears to be an artifact of the tubes.

As a second assessment of differences, we assumed that basic tree physiology requires that annual fine root production and foliage production should be in some type of rough balance. For instance, for 20 grassland, mixed and forested plots in North America, fine root production represented from 40-70% of total fine tissue (fine roots plus foliage) production (Reich *et al*. 2001). For the six forest species in Poland, we estimated the fraction of total fine tissue production (fine root plus leaf production) contributed by fine roots, using the fine root turnover

estimates calculated for both tube types (see median root lifespans in Figure **2.5**) and the SC_{cores} (Figure **2.6**) and litterfall production data (J. Oleksyn, unpublished data) (Appendix A, Equation 5). Using the acrylic tube data, percent fine root production of total fine tissue production varied 3.5-fold across the six species, from 12% for *A. pseudoplatanus* to 44% for *P. abies*. The percent estimate for *A. pseudoplatanus* was low compared to the rest of the species whose percent production estimates ranged closer to 2-fold, from 20% to 44%. Using the butyrate data, percent fine root production of total fine tissue production ranged 94-fold, from 0.5% for *P. nigra* to 47% for *A. pseudoplatanus* and *Q. robur*. While some of the butyrate percent production estimates fall within the range of other reported estimates, some species were very low. The data from butyrate tubes indicate that for *P. nigra*, *P. abies* and *P. sylvestris*, only 0.5, 5 and 6% respectively of total fine tissue production was root production. It is difficult to envision how these estimates could be accurate. Summarizing, by using total tissue production to establish boundary conditions on the overall productivity of the trees, more species exhibited dubious estimated root production estimates near butyrate than acrylic.

For some species and some factors, there were no significant differences between minirhizotron tube types. Intrinsic properties such as diameter and morphology were not affected by tube type. Mean root diameter was generally very similar among tube types for a given species. In only two forest trees were differences observed, and the results were mixed. Root branching was also unaffected in both experiments. Extrinsic properties like time of birth and soil depth were similarly unaffected.

New root production was also only modestly affected by tube material. Seasonal root production patterns were unaffected by tube type. Total new root production was only affected by tube type consistently in two of seven species. Apple roots were most numerous near glass followed by acrylic and butyrate tubes, but there were no seasonal differences (Figure **2.1**). In Experiment 2, only *P. abies* exhibited fairly consistent higher root production by acrylic than by butyrate over the three years of the study (Figure **2.4**).

Although they never did a comparison of the two materials at the same time and in the same place, Taylor and Böhm (1976) predicted higher root production against acrylic windows than glass windows due to greater amounts of soil gaps by acrylic tubes. We did not observe different size soil gaps among glass, acrylic and butyrate minirhizotrons. With the small-diameter minirhizotron tubes used in our studies, we had good soil contact with the minirhizotron surface regardless of the transparent material used. In addition, the sandy soil at the forest site enhanced tube-soil contact.

We considered other possible reasons for the observed tube effects and tube differences. The composition of the plastics was a possible explanation, although we can only speculate on the influences. Butyrate and acrylic plastics used for the tubes are similar in physical properties, such as hardness, specific gravity and tensile strength (data sheets from Thermoplastic Processes, Inc. of Stirling NJ and CYRO Industries of Rockaway NJ). However, from personal experience, we know that acrylic is more likely to shatter and crack than butyrate and must be handled more carefully. Acrylic (polymethylmethacrylate) has a polyethylene backbone with methyl ester groups present as side chains, and these groups can hydrolyze to produce methanol. Cellulose acetate butyrate is a polyester with a cellulose backbone. During production, the hydroxyl groups on the glucose molecules are replaced with esters of acetic acid and butyric acid. These esters readily hydrolyze and degrade to their alcohol and acid constituents and may be responsible for the characteristic smell associated with butyrate. Some microorganisms, like saprophytic fungi, release cellulases which would breakdown CAB and can release organic acids which can breakdown acrylic. Therefore, for both plastics, chemical reactions on the surfaces and the release of small molecular weight chemicals are possible, but probably more common for butyrate (Robert Minard, personal communication). Consequently, chemicals being released at the surface of the butyrate and/or acrylic tubes may interact with the soil solutes and microfauna to influence root pigmentation and survival.

Conclusions

Because minirhizotron studies are so labor intensive, there has been a lack of investigation of such basic questions as what is the best type of transparent material to use in minirhizotron research. We feel that there is need for more study in this area to explain the reasons behind the differential responses that our study found and to determine if there is a best material for rigid minirhizotron tubes. Of the material we tested, we assumed glass to be the most inert, but it is difficult to use. In our study in Pennsylvania, about a third of our tubes broke after only one winter.

How can we evaluate the reliability of the two plastic tube materials? In the forest tree experiment, acrylic was less detrimental than butyrate in terms of survivorship of white roots and time to root pigmentation, which is associated with reduced root function. Although the contrasts of SC_{mrt} with SC_{cores} provides only an indirect means of gauging the reliability of the

minirhizotron data, they clearly suggest that data obtained using butyrate tubes is more problematic than that near acrylic for some species. Standing crop adjacent to butyrate minirhizotron tubes did not correlate as well as expected with SC_{cores}. Also, a comparison of root productivity with total fine tissue productivity suggests that data for butyrate tubes yielded dubious estimates of root production for some species. Finally, differences in plastic chemistry also suggest that butyrate tubes were more likely than acrylic tubes to chemically influence the rhizosphere. Although each of these lines of evidence is circumstantial, they collectively suggest there is a differential response of roots of different tree species near minirhizotron tubes of different materials. Hence, we suggest the use of glass tubes whenever conditions allow, and we emphasize the importance of researchers reporting the type of minirhizotron tube material used. Tube type probably does not affect relative differences in root survivorship within a species, such as the consistent evidence that finer roots are shorter lived than coarse roots (e.g. Wells and Eissenstat 2001). Minirhizotron researchers, however, should be aware of the potential problems when comparisons are made across species. In our study, the root standing crop index across all species appeared less problematic for acrylic than butyrate tubes.

Researchers are continually frustrated by the lack of good methods for understanding root dynamics. Although there have been great technological improvements in minirhizotron cameras and analyzing software, there are still some artifacts as identified in this study. We feel minirhizotrons are still the preferred method for observing roots *in situ*. However, our results suggest more research should be conducted to refine this important tool for studying belowground dynamics.

Acknowledgements

The authors would like to thank Robert Minard, Dept. of Chemistry, The Pennsylvania State University, for his helpful consultation on plastic chemistry. We also acknowledge the very useful comments of two anonymous reviewers and thank them for their time. We appreciate the technical assistance of Dora E. Flores-Alva, the assistance of Sheila Sanabria who was supported on a Root Biology Training Grant (NSF DBI 96002255) and the diligent efforts of Mariellen Withers who helped process the images. J. Withington was supported by an NSF Root Biology Training Grant (NSF DBI 96002255), The William J. Fulbright Foundation and The Women's Welsh Clubs of America. A. Elkin was supported by Women in Science and Engineering

Research (NATO Program) and the National Science Foundation Research Experience for Undergraduates program. This work was also supported by an Eastern European International Supplement to an NSF grant (IBN 9596050). NSF also contributed to the work through the following grants: DEB 0128944 (PSU), DEB 0090069 (UMN) and DEB 0128958 (UMN).

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Chapter 3

Root structure and lifespan are largely independent of leaf structure and lifespan in a common garden comparison of eleven tree species

Abstract

Global data sets provide strong evidence of convergence for leaf structure with leaf longevity such that species having thick leaves, low specific leaf area, low mass-based nitrogen concentrations and low photosynthetic rates typically exhibit long leaf lifespan. Leaf longevity and corresponding leaf structure have also been widely linked to plant potential growth rate, plant competition and nutrient cycling. We hypothesized that selection forces leading to variation in leaf longevity and leaf structure have acted simultaneously and in similar directions on the longevity and structure of the finest root orders. Our four-year study investigated the links between root and leaf lifespan and root and leaf structure among eleven north-temperate tree species in a common garden in central Poland. Study species included the hardwoods: *Acer pseudoplatanus* L., *Acer platanoides* L., *Fagus sylvatica* L., *Quercus robur* L., and *Tilia cordata* Mill., and the conifers: *Abies alba* Mill., *Larix decidua* Mill., *Picea abies* (L.) Karst., *Pinus nigra* Arnold, *Pinus sylvestris* L. and *Pseudotsuga menziesii* (Mirbel) Franco. Leaf lifespan, estimated by leaf fall and needle cohort measurements, ranged from 0.5 yr to 8 yr. Median fine root lifespan, estimated using minirhizotron images of individual roots, ranged from 0.5 yr to 2.5 yr and was not correlated with leaf lifespan. Root nitrogen: carbon ratio was negatively correlated with root longevity, which corroborates previous research that has suggested a trade-off between organ lifespan and higher organ N concentrations. Specific traits such as thickened outer tangential walls of the exodermis were better predictors of long-lived roots than tissue density or specific root length, which have been correlated with lifespan in previous studies. Although leaf lifespan has been repeatedly linked to structure and chemistry, our results suggest that tissue structure and longevity aboveground (leaves) can contrast markedly with that belowground (roots).

Introduction

The longevity of plant organs is an important factor in plant growth strategies (Grime 1977, Chapin *et al.* 1987, Reich *et al*. 1992), plant competition (Aerts and Berendse 1989), nutrient cycling (Berendse and Aerts 1987, Aerts *et al*. 1992) and responses to global carbon change (Pregitzer *et al*. 1995, Reich *et al*. 1997). Many important ecological factors have been associated with long tissue and organ lifespan. These include low potential growth rate (Chapin *et al*. 1993), superior sustained growth in areas with low resource availability (Aerts and Berendse 1989, Schläpfer and Ryser 1996), high shade tolerance (Reich *et al*. 2003) and long nutrient retention times (Grime 1977, Chapin 1980, Aerts 1995). Although root systems can represent more than 50% of total net primary productivity (Caldwell 1987), the majority of the studies providing evidence for growth strategies have focused on leaves (Monk 1966, Chabot and Hicks 1982, Williams *et al*. 1989, Reich *et al*. 1992).

The collective data on leaves have indicated that plants have trade-offs in terms of energy requirements and physical constraints, such that general patterns have emerged as successful trait syndromes (*sensu* Reich *et al*. 2003) or suites of traits (*sensu* Chapin *et al*. 1993). For example, plant species with longer leaf lifespans also tend to have lower potential growth rates, specific leaf areas (leaf area/dry mass, SLA), leaf nitrogen concentrations and mass-based photosynthetic rates (Reich 1993, Reich *et al.* 1991, 1992, 1997, Wright *et al*. 2004).

Trait syndromes are often related to the main processes associated with resource capture: acquisition (photosynthesis, nutrient uptake), interception (specific leaf area, specific root length), use (respiration) and turnover (organ lifespan) (Reich *et al*. 2003). Leaves and the finest lateral roots share attributes that suggest that they might have generally similar trait syndromes: both organs are ephemeral, typically exhibit determinant growth, do not undergo secondary thickening, have the primary function of resource acquisition and use resources for respiration (Eissenstat and Yanai 1997). Just as thin leaves are less expensive for plants to produce and use for light acquisition, long, thin roots are less expensive to produce for nutrient acquisition. Because many of the functions of roots are similar to leaves, a reasonable starting point for hypothesizing the root characters associated with trait syndromes could stem from the existing knowledge of leaf traits.

Although root trait syndromes may be linked to leaves, leaf and root traits have rarely been investigated together with regard to longevity, though some information in available for grassland species. For example, studies in Minnesota at Cedar Creek examined root and leaf lifespan in more than 30 grassland species with respect to various traits and found that across the forbs, grasses and legumes, long root lifespan was significantly associated with slow root respiration, low specific root length (SRL) and low nitrogen to carbon (N/C) ratios (Craine et al. 2002, Tjoelker *et al*. 2005). They also found long leaf lifespan associated with slow photosynthetic rates, slow leaf respiration, low SLA and low N:C

ratios. With measurements of root lifespan based on the ratio of standing fine root (<2 mm) biomass to fine root production into ingrowth cores, both studies also found long root lifespan correlated with low N concentrations as well as low respiration rates. Ryser and colleagues (Ryser 1996, Schläpfer and Ryser 1996), who examined root and leaf turnover in grass species with respect to their growth rate and nutrient availability, found that slower-growing species had longer-lived leaves and roots when leaf and root turnover were calculated as the ratio of necromass to total root biomass.

We examined root and leaf traits of 11 woody species selected to represent a wide range in leaf lifespan. Six of the eleven species were conifers in the *Pinaceae*; the others were hardwoods in diverse families. There have been anecdotal observations that conifer roots are longer-lived than hardwood roots of temperate forest trees (Vogt and Bloomfield 1991). The observations are also consistent with the findings that roots of more primitive families, such as the *Magnoliaceae* and *Pinaceae*, generally have thicker roots than species in more evolutionarily advanced families, such as the *Aceraceae*, *Fagaceae* and *Tiliaceae* (e.g. Baylis 1975, Comas and Eissenstat 2004). These observations, combined with the typically longer-lived leaves of the conifers, suggest that the putative differences in root lifespans between hardwoods and conifers may simply reflect longer leaf and root lifespans of the conifers.

We included the coniferous species *Larix decidua* (deciduous larch) in our study to examine the interaction of the conifer-hardwood relationship and the deciduous-evergreen habit. If larch root characteristics were more similar to the roots of the other conifer species, this would indicate a stronger phylogenetic influence on the roots; however, if they were more similar to the roots of the hardwood species, this would indicate a closer coupling of leaf function with root function.

To test the potential link between root and leaf longevity, we estimated root lifespan using minirhizotron tubes. We first hypothesized that fine root lifespan would be positively correlated with leaf lifespan among diverse tree species that vary widely in leaf lifespan. Second, we hypothesized that root structural characteristics and root chemistry would be related to root longevity in a manner analogous to that typically found in leaves. Third, we hypothesized that conifer root longevity would be longer than those of hardwood roots based on reviews of limited past research.

Methods

Field Site

Our field site was a common garden planting in the Siemianice Experimental Forest in central Poland (51°14.87'N, 18°06.35'E, altitude: 150 m). Climate of the region is transitional between

maritime and continental, and the average annual precipitation was 591 mm with about half falling from May to August (weather data recorded 300 m from the field site from 1968-1997). Average temperature was 8.24°C with a mean growing season of about 213 d, calculated as the number of days with an average temperature ≥5°C (Szmanski and Ceitel 1989, Ceitel and Wawro 1999a, 1999b).

The site consisted of two adjacent plantings separated by 20 m. There were 14 tree species total, nine species per planting, with some species duplicated between plantings (Szymanski 1982). Each planting had three replicate blocks; species were planted in nine, monospecific 20 x 20 m plots in each block, for a total of 27 plots per planting. Trees were planted in 1970 and in 1971 as one- and two-yr-old seedlings respectively at 1 x 1 m spacing, although the spacing has changed somewhat due to some prescribed and self-thinning. Each planting had a fairly uniform topography and soil with very few understory plants present due to the high tree density (Withington *et al*. 2003). The soil in all the plots was nutrient poor with a plowed A-horizon; soil texture averaged 80% sand and 15% silt. Soils were generally loamy sands and classified as fine-loamy, mixed, Mesic Kanhaplic Haplustalfs and sandy, mixed, Mesic Typic Ustipsamments. For this experiment, we sampled all nine species in the first planting: five deciduous broad-leaved species *Acer pseudoplatanus* L., *Acer platanoides* L., *Fagus sylvatica* L., *Quercus robur* L., and *Tilia cordata* Mill., a deciduous conifer, *Larix decidua* Mill., and four evergreen conifers *Abies alba* Mill., *Picea abies* (L.) Karst., and *Pseudotsuga menziesii* (Mirbel) Franco. In the second planting we sampled three species: the evergreen conifers *Pinus nigra* Arnold, *Pinus sylvestris* L. and *Picea abies*.

To assess potential differences between the plantings, we analyzed *P. abies* in both plantings, and we also used % clay (soil texture) in the plots as a covariate. We used a general linear model to test differences in five measured variables of *P. abies* both above- and belowground. Trees in 1999 were significantly taller in the first planting than the second ($F_{1,5}$=8.07, P=0.05; 11.4 m vs. 7.3 m). The dbh (diameter at breast height) after 30 years was also significantly greater in planting 1 than planting 2 ($F_{1,5}$=11.05, P=0.03). When significantly different, aboveground measurements of *P. abies* were kept separate by planting for analyses. Variables associated with the finest root orders of *P. abies* (e.g. diameter, production, lifespan) were not significantly different between plantings (all P>0.27).

Given that the two plantings were adjacent in a flat area, the only likely differences between the plantings would be in the soil characteristics. Therefore, we looked for correlations between soil texture, measured as percent clay, and root characteristics. Percent clay varied from 1.2 to 13.5% with all but one plot having less than 9% clay. At the plot level, there were no significant correlations of percent clay with root N/C ratio, SRL, root tissue density or diameter (data not shown). Clay did not explain a significant amount of the variation in median root lifespan across species. Therefore, we were satisfied that soil texture was not a significant source of variation in our study. All root-related measurements on

the six plots of *P. abies* were combined for analyses, and we included the *Pinus* spp. in the analyses with no adjustments for their location in the second planting.

Root lifespan estimates

Owing to a previous study at this field site which found acrylic plastic minirhizotron tubes provided root standing biomass estimates more consistent with those estimated by soil cores than cellulose acetate butyrate tubes (Withington *et al*. 2003), only acrylic tubes were used in this study. The minirhizotron tubes had an inside diameter of 5.2 cm, a wall thickness of 6.4 mm and a length of 60 cm. Tubes were sealed with a rubber stopper and wrapped in black electrical tape to keep light and rain from entering the tubes; no other covers were used because the tubes were shaded most of the time. In November 1998, the tubes were installed randomly in the plots at an angle of 30° from vertical and at least 3 m from the plot borders with the top 10 cm of the tube above the soil surface. Three tubes were installed per plot, three plots per species. We used only two *A. alba* plots (six tubes total) because the third was overgrown with a different tree species. One tube in *A. pseudoplatanus* and one tube in a *T. cordata* plot were lost over the course of the experiment due to vandalism, so these species had eight tubes total.

Minirhizotron images were collected using a minirhizotron camera and associated image capture software (Bartz Technology Corp., Santa Barbara, CA) starting in May 1999, six months after tube installation to reduce problems associated with installation disturbance on subsequent root dynamics (Joslin and Wolfe 1999). Images were collected from May through Dec. 1999 at 2-4 week intervals. Because the 1999 data indicated very long-lived roots, sampling intervals were lengthened in 2000-2002 to monthly intervals from April through Nov.

Images of the windows in the tubes were viewed as a time sequence. Roots observed on the first sampling date were not used, as their birth dates were unknown. The date a root was first observed and the date of disappearance were recorded. If the observation of a root became obscured because another root grew in front of it, or if a root was still alive at the end of the data collection period, then that root was recorded as statistically censored (i.e. time to root death was greater than the observation time, Allison 1995). Root birth and root death were estimated as the day midway between successive imaging dates (Appendix A, Equation 1). Individual root lifespan was calculated as the number of days from root birth to root death.

Determining the vitality of roots from external appearance alone is difficult. Our first step was to correlate appearance of roots in the minirhizotron windows to root vitality, we used a modified version of the vital stain procedure of Comas *et al*. (2000). In June 2000, we collected roots of all species in the top

15 cm, two locations per plot. Dyed root cross-sections were observed under a dissecting microscope; red cells exhibited active cellular function and were considered alive. Notes were collected on the location of the red color in the root cross-sections and the external appearance of the roots.

Although the vital stain/root function correlated well with the coloration of grape roots for Comas *et al*. (2000), for our species, appearance alone was not enough to evaluate root vitality. Loose mycelium and loss of cortical tissue observed in the vital stain sections were the two best characteristics for all species in classifying roots as dead; however, these characteristics were only visible in cross-section and not apparent in the minirhizotron images. Roots with a dark-brown to black coloration were not consistently dead or dying. For some species, like *P. menziesii*, some dark brown roots were found to be dead while others had at least 80% functional stele and cortex. If a root exhibited signs of cortical breakdown and wrinkling it was marked as dead. However, aging roots were the exception. For >95% of the roots, we relied on the date of disappearance as the date of death; no outward signs of loss of cortical tissue and hyphal mantle deterioration were evident before such disappearances. Thus, root lifespan and root persistence are essentially synonymous in this study.

Often, root lifespan (inverse of root turnover) is estimated by methods that only can estimate root production and root standing crop (Gill and Jackson 2000). Minirhizotron production data were used to estimate average root lifespan as an alternative to median lifespan based on survivorship analysis. Minirhizotron root production (number of roots born per sampling date) was converted to root length using a subset of images distributed across the study period, including each year and representing each season. From this subset of images, root length was determined for each species using the program WinRHIZO Tron (Regent Instruments Inc., Quebec, Canada), and correlated with total numbers of roots (for all 11 species, r^2>0.88).

We calculated the differences in five dates on which cumulative root length mortality equaled cumulative root length production (Figure **3.4**). We then calculated the harmonic mean of the times to get an estimate of root lifespan (Table 3.3) (Appendix A, Equation 6). A second estimate of average fine root lifespan was calculated as one-half the ratio of the maximum standing root crop to the annual root length production (SC/Prod) from the last two years of the study (Appendix A, Equation 7).

Survivorship curves and root lifespan estimates for each species were calculated using the BASELINE statement of PROC PHREG in SAS v8.02 (SAS Institute Inc., Cary, NC). Pearson product moment correlations were used to test for correlations between tissue lifespan and other characteristics such as N/C ratio and SRL. Root and leaf lifespans were log transformed before data analyses. Cox proportional hazards models were used to test for differences within each species for the influence of diameter, soil depth and time to pigmentation on root lifespan (Cox 1972, Allison 1995, Wells and Eissenstat 2001).

Root Order and Root Pigmentation

We were interested in comparing only 1st and 2nd order roots of each species, but root order is frequently difficult to determine definitively in minirhizotron windows because the visible portion of the root is usually too small to observe the different levels of branching. Root diameter is more easily measured, so we looked for a correlation between diameter and root order that would allow us to only analyze the finest root orders. We looked at the distributions of root diameters from the 1st and 2nd-order scanned roots, separated into groups at 0.1 mm intervals (WinRhizo, Regents Instruments Inc., Quebec, Canada). We compared these to the distribution of minirhizotron root diameters, which were determined by direct measurement on a computer screen using the image from the date a root was first observed (Figures **3.1** and **3.2**). By comparing the two distributions, we were able to select a maximum root diameter for each species that would allow us to use roots of each species which were 1st-order and possibly 2nd-order but unlikely to be 3rd-order or higher (see vertical lines on Figures 3.1 and 3.2). Unless otherwise noted, all results for roots are for the finest two orders of roots.

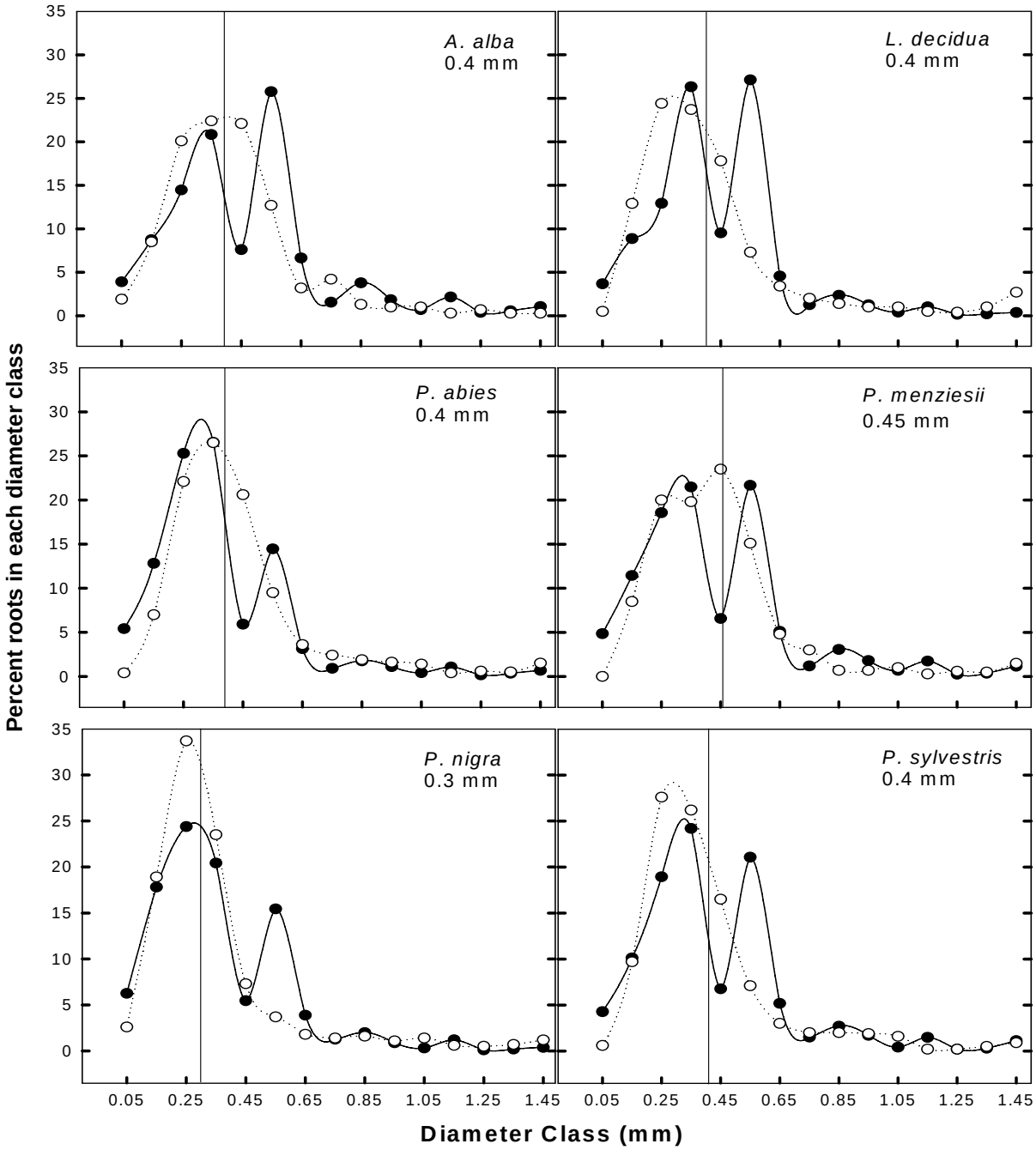

Figure **3.1**: Percentage of 1st- and 2nd-order root tips in 10 µm diameter classes for the six conifer species. Classes range from <0.1 mm to 1.4-1.5 mm. Solid circles and solid lines are the diameters of roots known to be 1st- and 2nd-order scanned with WinRhizo (0-30 cm soil cores in each plot; 2, 3 or 6 plots per species; n= 5000-90000 root tips). Open circles and dashed lines are for all roots seen in minirhizotron windows from 1999-2002 (8, 9 or 18 tubes per species; n= 400-2800 roots). Maximum diameter used as a cut-off to indicate mostly 1st-order roots in the minirhizotron samples are written below the species names and indicated with a vertical line.

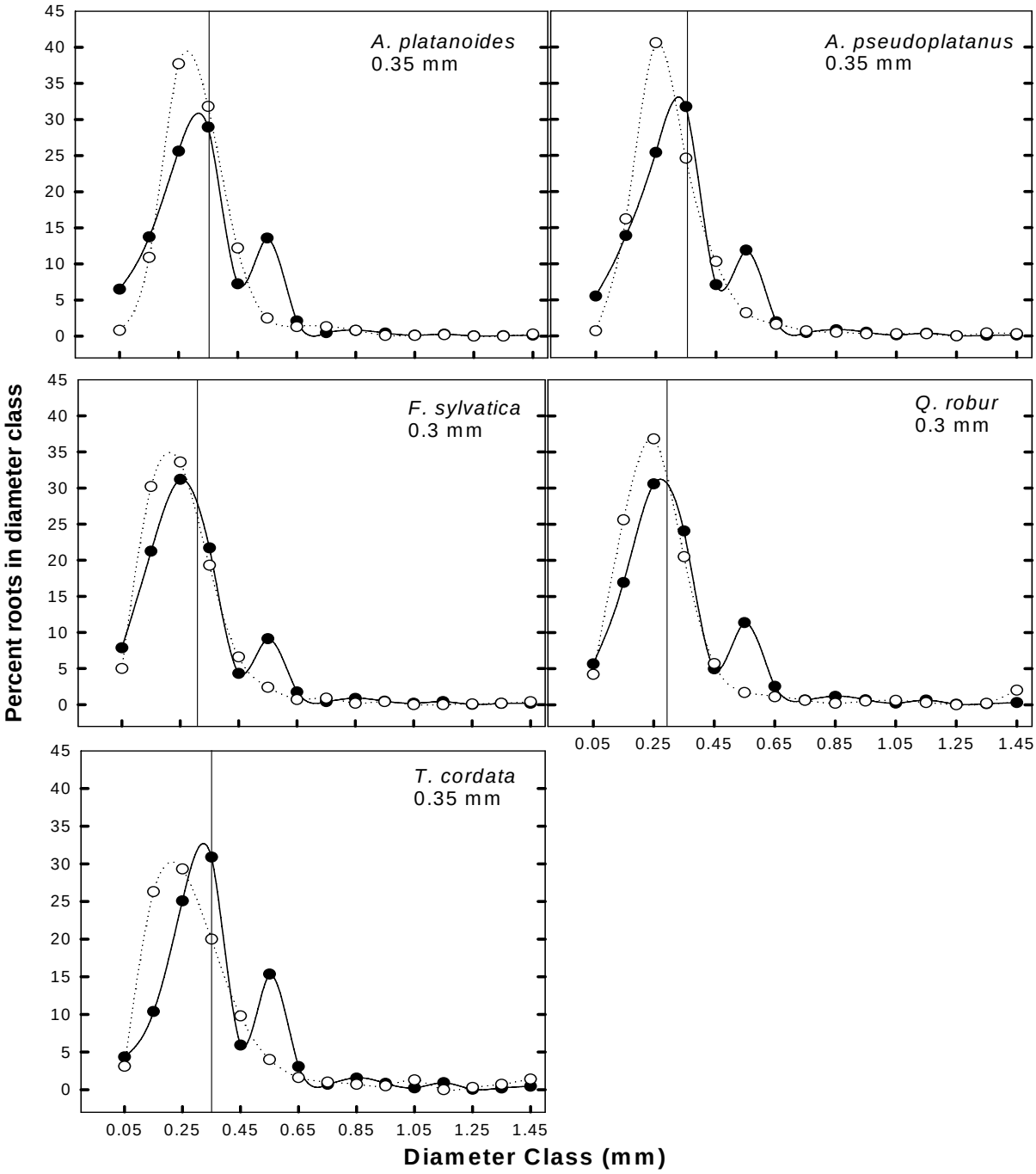

Figure **3.2**: Percentage of 1st- and 2nd-order root tips in 10 μm diameter classes for the five hardwood species. Classes range from <0.1 mm to 1.4-1.5 mm. Solid circles and solid lines are the diameters of roots known to be 1st- and 2nd-order scanned with WinRhizo (0-30 cm soil cores in each plot; 2, 3 or 6 plots per species; n= 5000-90000 root tips). Open circles and dashed lines are for all roots seen in minirhizotron windows from 1999-2002 (8, 9 or 18 tubes per species; n= 400-2800 roots). Maximum diameter used as a cut-off to indicate mostly 1st-order roots in the minirhizotron samples are written below the species names and indicated with a vertical line.

Besides lifespan, time to root pigmentation was also determined. The transition of roots from white to pigmented usually indicates a reduction in root metabolic activity (Comas *et al*. 2000, Bouma *et al*. 2001). Roots generally changed color only once before dying. The date when at least 50% of a root's length was pigmented was noted. The relationship between time to pigmentation and median root lifespan among the species was tested with a general linear model. The influence of the time to pigmentation on root mortality within each species was tested with a Cox proportional hazards model (Cox 1972, Allison 1995, Wells and Eissenstat 2001).

Root Morphology, Anatomy and Nitrogen Concentrations

Root diameter, tissue density, SRL (Table **3.1**) and nitrogen concentration are important traits hypothesized to be correlated with root lifespan of the finest root orders. Because the fungal ectomycorrhizal mantle appreciably affects root diameter, we also made anatomical sections of roots to distinguish plant root diameter from the entire mycorrhizal root diameter. Non-mycorrhizal white roots were examined separately from the mycorrhizal, pigmented roots.

Table 3.1: Definitions and descriptions of abbreviations used in the manuscript.

Abbreviation	Description
Dia-T_R	Total root diameter
Dia-P_R	Diameter of plant tissue only, fungal mantles excluded
LS_L	Average leaf lifespan
LS_{25R}	First quartile estimate of root lifespan
LS_{50R}	Second quartile (median) estimate of root lifespan
LS_{SCRoot}	Mean estimated root lifespan (max standing crop/annual root production)
LS_{PMRoot}	Root lifespan estimated from the amount of time required for cumulative root mortality to equal cumulative root production
N/C_R	Ratio of root Nitrogen to root Carbon (w/w)
$\%N_L$	Leaf percent nitrogen (w/w)
$Prod_R$	Root production against the minirhizotron tubes
RLD	Root length density, total amount of root length of all root orders per unit soil volume (length/soil volume)
RLD_{1+2}	Root length density of 1st and 2nd order roots
SLA	Specific leaf area (area/unit mass)
SRL	Specific Root Length (length/unit mass)
TissDen	Root tissue density (mass/volume)

Soil cores (4.8 cm dia) from previously undisturbed soil were randomly collected in July 1999 in each plot to a depth of 15 cm (Arts Mfg. & Supply, American Falls, Idaho, USA). Roots were cleaned from the cores by hand in plastic tubs and sorted into three root order groups: (a) 1st, (b) 2nd and (c) 3rd and 4th. For our purposes, 1st-order roots are all external links with no daughter roots; second-order roots have only 1st-order daughter roots, and so on up the hierarchy. Additional soil cores were collected in July 2001 in each plot at two depths: 0-15 cm and 16-30 cm. These roots were cleaned and sorted into three groups: the combined orders (a) 1 and 2, (b) 3 and 4, and (c) 5 and higher. All roots were scanned on a desktop scanner using WinRHIZO software at 400DPI (Regent Instruments Inc., Quebec, Canada) to obtain diameter distributions for roots of known order. After scanning, root samples were dried for 48 h at 50ºC and weighed. Root length density (L_v, length*soil volume^{-1}, cm cm^{-3}) was calculated for each root-order class, and total L_v represents the sum of the three classes. Specific root length, which is inversely proportional to the square of root diameter, assuming cylindrical geometry (SRL= (length* volume^{-1}) x (volume*mass^{-1})), was calculated as the ratio of the root length in the sample to the dry

weight. Dried roots were pulverized using a SPEX mixer/mill (SPEX Industries Inc., Metuchen, NJ), and 15 mg subsamples were ashed in a muffle furnace at 500°C for 6 h so that dry weight could be expressed on an ash-free basis.

In June 2001, roots were excavated in each plot at two locations to a depth of 15 cm from an area of about 2800 cm^2. Roots were gently washed by hand and then preserved in 60% ethanol for later anatomical observations by light microscopy. Cross-sections were made by hand under a dissecting scope. A minimum of 36 roots of each species was selected, and sections were made 1 mm behind the root cap for each root. The finest root orders were pooled. Sections were stained with 0.05% toluidine blue (in acetate buffer pH 4.5) to color the cell walls. We measured 25-30 cross sections from different roots for total diameter and ectomycorrhizal mantle thickness (where present).

Root tissue density was calculated as the ratio of the ash-free dry weight of the sample to root volume. Root volume was estimated using root diameter and root length and assuming cylindrical geometry. Using the values for mantle thickness, we calculated the average diameter of the scanned roots without the hyphal mantle for the nine ectomycorrhizal species (all but the *Acer* spp.). We then used these diameter estimates to calculate a second estimate of SRL that excluded fungal mantles and was based only on ash-free dry weight of plant material, assuming tissue densities of the mantle and plant root as well as percent ash weights were the same.

Because minirhizotron observations indicated that the white roots of ectomycorrhizal species lived a long time, we also looked for specific anatomical differences between mycorrhizal and white roots. Non-ectomycorrhizal roots were collected in the top 10 cm at three random locations in the plots using hand-trowels. These roots were generally white to beige with root hairs and exhibited longer lengths (>2 cm) than ECM roots. Seven to twelve non-mycorrhizal roots of each species were hand-sectioned at the beginning of the maturation zone and stained for suberin and lignin with 0.05% phloroglucinol followed by two drops of 36% HCl (Jensen 1962). We sampled few roots because this type of root was difficult to find. The presence or absence of a thickened hypodermal layer (exodermis) in the sections was noted. No nonmycorrhizal roots could be found for the *Pinus* spp.

Nitrogen concentration in roots was estimated in relatively young roots of known maximum age from ingrowth tubes. In May 2001, five ingrowth tubes were installed per plot (11 species, 3 plots each). Ingrowth tubes were made from plastic screen (1.5 x 1.5 mm holes) rolled to make tubes 3 cm in diameter and 25 cm long. The organic layer, if present, was pulled back from an area between two trees about 1 m apart. The tubes were installed horizontally in the mineral soil just under the organic layer. Prior to installation, mineral soil, sifted to remove roots and large organic debris, was added to each ingrowth tube.

Ingrowth tubes were excavated after 90 d. Cores were pooled per plot and roots extracted. Roots were immediately hand-washed and sorted into two classes: (a) root orders 1 and 2 (finest two orders) and

(b) orders 3 and 4. No roots of higher order were present. The roots were dried at 50°C for 36-48 hrs. We determined carbon and nitrogen concentrations of the samples for each plot using an elemental analyzer (model EA1108, Fisons Instruments, Pt. Pleasant, NJ, USA).

Leaf and tree biometric data

Foliar lifespan of deciduous species was determined using a combination of phenological observations (spring leaf unfolding) and data on leaf shedding obtained from litter traps (see below). For evergreen conifers, foliage age was assessed by counting needle cohorts in four branches (two high and two low light) per plot.

The projected leaf area was determined using an image analysis system and the WinFOLIA Pro for broadleaved species and WinSEEDLE Software for conifers (Regent Instruments Inc., Quebec, Canada). Specific leaf area (SLA, defined as the projected leaf area divided by leaf dry mass) was calculated for the current-year leaves used for determination of foliar lifespan.

Foliar nitrogen concentration was measured on dried (65°C for 48h) tissue ground in a mill (Kikro-Feinmühle Culatti , IKA Labortechnik Staufen, Germany). Tissue samples were digested by the micro-Kjeldahl method and processed using a BÜCHI Distillation Unit B-322 (BÜCHI Analytical Inc., Switzerland). Data are means of equally weighted composite samples of mature current-year foliage obtained from the individual branches (four per plot, two plots per species).

Total foliar and other nonfoliar miscellaneous litterfall (woody litter, debris, seeds, etc.) were collected monthly from 31 May 1996 through 30 April 1997 using 0.38m^2 litter traps. To the bottom of each trap under coniferous species, 1-mm mesh plastic window screen was attached. Eight litter traps per plot were placed directly on the forest floor. Litter was oven dried at 65°C, and estimates of the annual production of litter biomass were calculated.

Results

Defining first- and second-order roots

To compare roots with similar putative functions among 11 species, we needed to compare roots of the same order. Order is relatively easy to determine with excavated roots but is problematic in

minirhizotron images with only portions of roots visible. We defined a maximum root diameter for first-order roots of each species. Average fine root diameters from intact cores were similar among the species and ranged from 0.36 mm in *F. sylvatica* to 0.62 mm in *A. alba* (Table **3.2**). Average root diameters from excavated 1st- and 2nd-order roots were moderately larger than the root diameters collected from the minirhizotron images (Figure **3.1**, Table **3.2**). This was due to the greater amount of 2nd-order roots in the scanned samples compared to the minirhizotron samples (branching was often outside the surface of the tube-soil interface). Maximum diameters used as a cut-off for 1st-order roots in the minirhizotron images were selected for each species (Figures **3.1** and **3.2**).

Table **3.2**: Root morphological characteristics determined from mixed-age, 1st- and 2nd-order roots observed in minirhizotron tubes or collected from soil cores. SRL was corrected for average hyphal mantle thickness. TissDen was calculated as the ratio of root mass to root volume assuming a cylindrical geometry. Dia-T_R was the average of scanned roots from soil cores. $Prod_R$ was the average length produced per year against the minirhizotrons. RLD, RLD_{1+2} was calculated from intact-soil core data. N/C ratio was determined for young (<90d), 1st- and 2nd-order roots collected in August 2000. Standard errors are noted in parentheses.

Species	SRL ($m\ g^{-1}$)	TissDen ($g\ cm^{-3}$)	Dia-T_R (mm)	$Prod_R$ ($cm\ yr^{-1}$)	RLD ($cm\ cm^{-3}$)	RLD_{1+2} (%)	N/C (w/w)
Hardwoods							
Acpl	52.1 (5.2)	0.143 (0.01)	0.41 (0.02)	127.1 (40)	9.91 (1.2)	56.8	0.024 (0.001)
Acps	49.1 (2.6)	0.133 (0.01)	0.46 (0.06)	86.8 (19)	11.7 (1.2)	51.0	0.031 (0.002)
Fasy	90.7 (13)	0.173 (0.03)	0.36 (0.03)	33.3 (13)	7.88 (0.93)	52.9	0.044 (0.007)
Quro	68.1 (9.8)	0.133 (0.01)	0.46 (0.04)	22.1 (6.5)	8.64 (1.0)	59.8	0.036 (0.003)
Tico	45.8 (7.3)	0.214 (0.07)	0.43 (0.03)	42.1 (13)	7.02 (1.1)	48.8	0.047 (0.004)
Averages	61.1 (4.7)	0.159 (0.02)	0.42 (0.02)	62.3 (19)	9.05 (0.83)	53.8 (2.0)	0.036 (0.004)
Conifers							
Abal	26.0 (4.2)	0.153 (0.01)	0.62 (0.05)	20.9 (8.2)	5.92 (0.39)	49.4	0.041 (0.008)
Lade	40.9 (2.7)	0.155 (0.01)	0.53 (0.01)	18.4 (5.5)	7.14 (0.61)	66.0	0.051 (0.009)
Piab	33.4 (2.5)	0.196 (0.02)	0.54 (0.03)	31.1 (4.9)	6.14 (0.75)	58.8	0.053 (0.004)
Pini	39.9 (8.4)	0.287 (0.04)	0.45 (0.05)	38.0 (4.8)	6.81 (1.7)	66.7	0.035 (0.003)
Pisy	24.7 (2.3)	0.248 (0.04)	0.58 (0.05)	25.2 (4.3)	3.47 (0.80)	68.1	0.050 (0.007)
Psme	27.2 (3.7)	0.188 (0.01)	0.57 (0.05)	26.9 (17)	6.02 (1.8)	56.1	0.034 (0.010)
Averages	32.0 (2.9)	0.204 (0.02)	0.55 (0.02)	26.8 (2.9)	5.91 (0.53)	60.9 (3.0)	0.044 (0.003)

Correlation of Root Lifespan with Leaf Lifespan

Average leaf lifespans of the deciduous hardwood species were approximately 0.45 yr and had a range of only 20 d. *Larix decidua*, a deciduous conifer, had only a slightly longer average leaf lifespan at 0.51 yr. Leaf lifespan of the five evergreen conifers had a much larger range in leaf lifespan, from 2.44 yr for *P. sylvestris* to 8.77 yr for *P. abies* (Table **3.3**).

Table **3.3**: Fine root and leaf lifespan estimates. Fine roots are the smallest two orders for each species. Median fine root lifespan (LS_{50Root}) is shown with first and third quartiles as indicators of variability below and is based on roots born from May 1999 - Nov 2001 and followed through Nov 2002. Root lifespan was also estimated as the geometric mean of the number of days between two equal values of cumulative production and cumulative mortality (LS_{PMRoot}) from minirhizotron data. A third estimate of root lifespan was estimated as the ratio of the maximum standing crop against the minirhizotron tubes in 2002 to the average of root production against the tubes in 2001 and 2002 (LS_{SCRoot}). Average leaf lifespan (LS_{Leaf}) is based on 11 months of leaf fall data for deciduous species and on needle cohorts for evergreen species.

Species	LS_{50Root} (years)	LS_{PMRoot} (years)	LS_{SCRoot} (years)	LS_{Leaf} (years)
A. platanoides	1.62 0.73, 3.01	2.00	1.80	0.46
A. pseudoplatanus	2.47 0.65, undef.	1.83	1.69	0.46
F. sylvatica	0.57 0.33, 1.38	0.80	0.55	0.45
Q. robur	0.98 0.45, 1.49	1.22	0.69	0.47
T. cordata	0.64 0.49, 1.10	0.46	1.87	0.43
A. alba	1.13 0.45, 1.93	2.50	2.00	8.22
L. decidua	1.10 0.54, 2.50	1.88	1.48	0.51
P. abies	0.70 0.42, 1.10	1.17	0.97	8.77
P. nigra	0.77 0.48, 1.46	1.41	0.83	3.84
P. sylvestris	0.67 0.44, 1.11	1.48	1.18	2.44
P. menziesii	1.62 0.64, undef.	1.67	3.27	5.48

The median lifespan of the fine roots (LS_{50R}) was not correlated with leaf lifespan, either across all species (P= 0.73) or within the hardwood (P= 0.32, data not shown) or conifer groups (P= 0.93) (Tables **3.4** and **3.5**, Figure **3.3**). Conifers exhibited a similar range in LS_{50R} (0.67 yr to 1.62 yr) as that of hardwoods (0.57 yr to 2.47 yr) (Table **3.3**). Leaf lifespan was longer for the conifers (0.51 yr to 8.77 yr) than for the hardwoods (0.43 yr to 0.47 yr) (Table **3.3**).

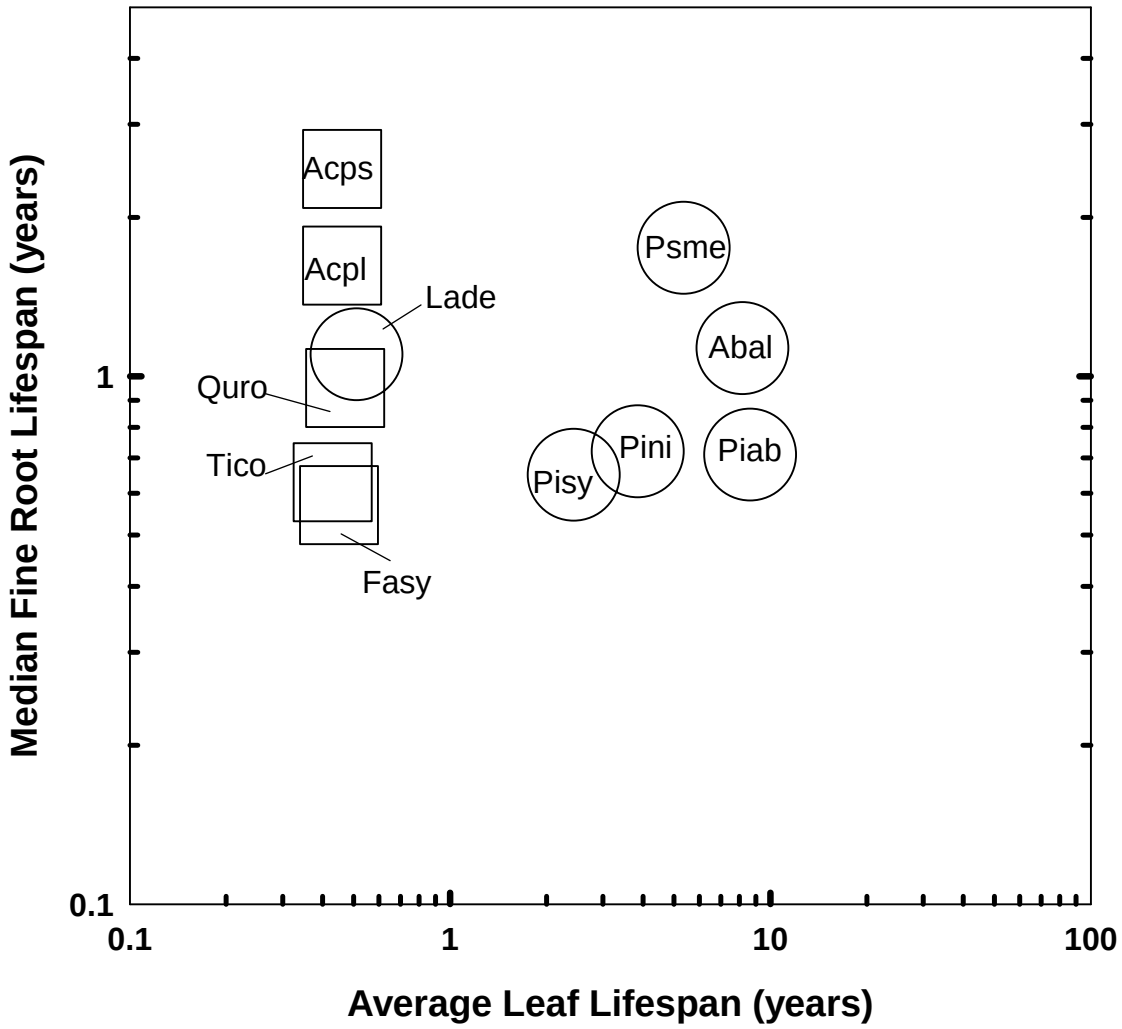

Figure **3.3**: Relationship of leaf lifespan to fine root lifespan on a log-log scale. Hardwoods are denoted by squares and conifers by circles. Species abbreviations are given with the first two letters of the genus and species. Note: *P. abies* is plotted as the average of the two sites for leaf lifespan.

Because many studies investigating root turnover use methods that assume steady-state conditions in order to correlate root production with root lifespan, we examined our data for correlation between root production and root lifespan. Root production was much greater in the first year when the roots were exploring disturbed soil near the tubes (Figure **3.4**). Root production and mortality were roughly equal for most species by the end of the second year (Figure **3.5**).

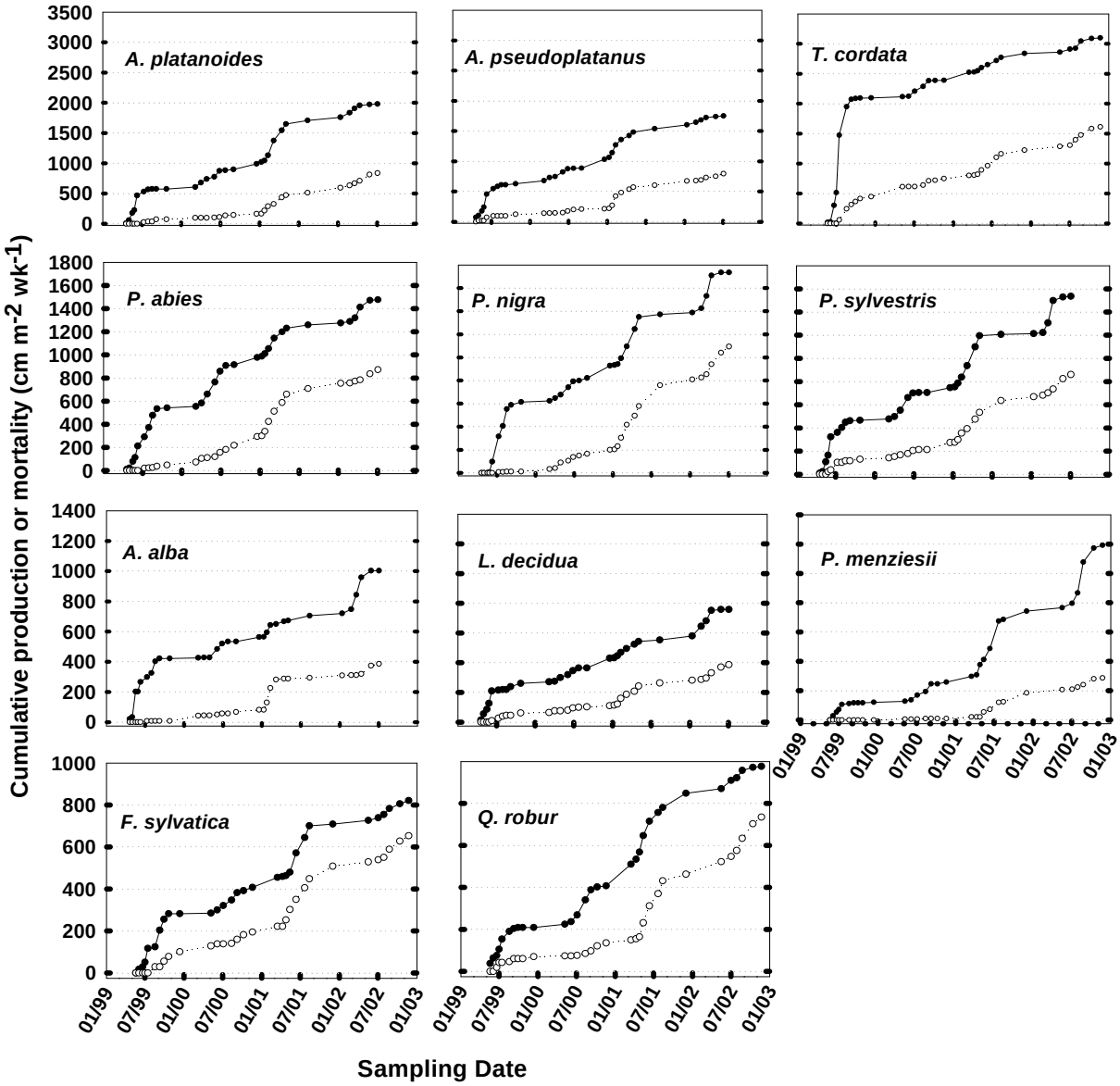

Figure **3.4**: Cumulative production (closed circles) and cumulative mortality (open circles) of fine roots (1st and 2nd order roots only) against minirhizotron tubes from May 1999 to November 2002 averaged across plots (2, 3 or 6 plots per species). Species abbreviations are noted on each graph. The average difference between production and mortality can be found in Table 3. Note: the y-axes scales are different.

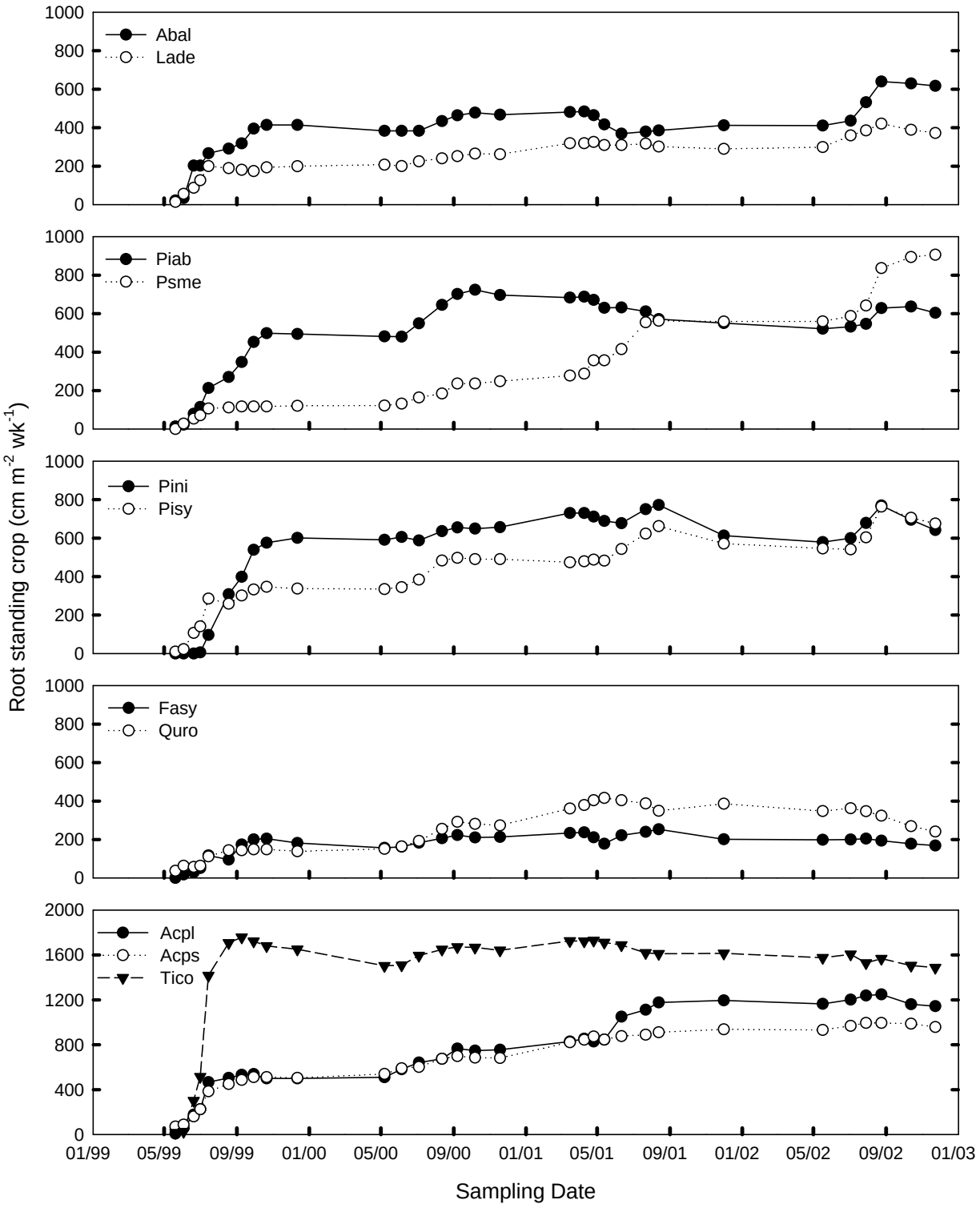

Figure **3.5**: The standing crop of roots against minirhizotron tubes from May 1999 to November 2002 averaged across plots (2, 3 or 6 plots per species). Root standing crop was estimated by the difference in cumulative production and cumulative mortality of fine roots (1st and 2nd order roots only). Species abbreviations are noted on each graph. Note that the y-axes scales differ among species.

Root lifespan based on the differences in time for equivalent values of cumulative fine root length production and mortality (LS_{PMRoot}) was correlated with median root lifespan (r=0.56, P=0.038). LS_{PMRoot} was generally longer than the median lifespans (Table 3.3). For *A. alba* (1.13 yr vs. 2.5 yr) and for *P. sylvestris* (0.67 y vs. 1.48 y), LS_{PMRoot} was more than twice as long.

Root lifespan based on root production and standing crop (LS_{SCRoot}) was not correlated with median fine root lifespan (r=0.10, P=0.767) nor was it correlated with any other root or leaf characteristics (Table 3.4, Table 3.5). Standing-crop-based estimates of root lifespan ranged from 0.83 yr (*P. nigra)* to 3.27 yr (*P. menziesii*) in the conifers and from 0.55 yr (*F. sylvatica*) to 1.87 yr (*T. cordata*) in the hardwoods; generally longer than the median lifespans based on individual root cohort analyses (LS_{50R})(Table 3.3). The range of root lifespans for the cohort-based estimates and the standing-crop-based estimates were similar for the hardwoods, but whereas LS_{50R} had *A. pseudoplatanus* roots living the longest by far, the LS_{SCRoot} estimate indicated that *T. cordata* roots lived longest. For the conifers, the estimated values by the two approaches were similar.

Table 3.4: Pearson product moment correlation coefficients of root characteristics across all 11 species. All comparisons are on a log-log basis. See Table 3.1 for descriptions of abbreviations.

	ROOTS								
	LS_{50R}	**LS_{SCRoot}**	**LS_{PMRoot}**	**N/C_R**	**SRL_R**	**$TissDens_R$**	**Dia-T_R**	**Dia-P_R**	**$Prod_R$**
LS_{25R}	**0.845**	0.346	0.466	**-0.698**	-0.204	-0.323	0.110	0.348	**0.654**
LS_{50R}		0.101	**0.659**	**-0.704**	-0.103	**-0.619**	0.156	0.446	0.406
LS_{SCRoot}			-0.204	0.101	-0.368	0.032	0.222	0.391	0.156
LS_{PMRoot}				-0.390	0.428	-0.351	*0.534*	**0.616**	0.126
N/C_R					-0.238	0.380	0.328	0.067	**-0.652**
SRL_R						-0.393	**-0.912**	**-0.835**	-0.243
$TissDens_R$							0.124	-0.167	0.264
Dia-T_R								**0.906**	-0.040
Dia-P_R									-0.064

Coefficients in italics are significant at α=0.10. Coefficients in bold face are significant at α=0.05

Table 3.5: Pearson product moment correlation coefficients of root characteristics across the six conifer species. All comparisons are on a log-log basis. See Table 3.1 for descriptions of abbreviations.

	ROOTS								
	LS_{50R}	**LS_{SCRoot}**	**LS_{PMRoot}**	**N/C_R**	**SRL_R**	**$TissDens_R$**	**Dia-T_R**	**Dia-P_R**	**$Prod_R$**
LS_{25R}	**0.857**	-0.246	0.250	-0.563	0.111	-0.245	-0.035	0.075	-0.204
LS_{50R}		0.253	0.608	-0.531	-0.142	-0.606	0.355	0.508	-0.432
LS_{SCRoot}			0.586	0.303	-0.261	**-0.834**	0.682	*0.755*	*-0.763*
LS_{PMRoot}				-0.216	0.267	-0.662	0.545	0.629	-0.465
N/C_R					0.065	-0.246	0.225	0.067	-0.598
SRL_R						0.092	**-0.814**	*-0.752*	0.267
$TissDens_R$							-0.636	-0.719	*0.793*
Dia-T_R								**0.971**	-0.305
Dia-P_R									-0.326

Coefficients in italics are significant at α=0.10. Coefficients in bold face are significant at α=0.05

Correlation of Leaf Lifespan with Leaf Characteristics

We examined leaf traits that previously have been correlated with leaf lifespan across a wide range of species. Leaf N concentrations ranged from a low of 1.08% in *P. nigra* to a high of 2.2% in *A. pseudoplatanus* (Table 3.6). In our study, leaf N concentration was inversely correlated with leaf lifespan across all eleven species (P= 0.01) and was also positively correlated with specific leaf area (P=0.01) (Table 3.6). Specific leaf area was inversely correlated with leaf lifespan across all species (P=0.002).

Table 3.6: Leaf morphology (Specific Leaf Area, SLA), chemistry (percent nitrogen, %N) and production ($Prod_L$). Standard errors are shown in parentheses. Values for *Picea abies* are the averages of the two plantings.

Species	SLA ($cm^2\ g^{-1}$)	$Prod_L$ ($kg\ ha^{-1}$)	%N (w/w)
A. platanoides	272 (8)	2953	1.62
A. pseudoplatanus	213 (35)	3827	2.20
F. sylvatica	287 (5)	3305	2.18
Q. robur	172 (1)	2225	1.86
T. cordata	309 (31)	2957	1.87
A. alba	58.5 (4)	908	1.72
L. decidua	103 (5)	1183	1.81
P. abies	54.4 (9)	1770	1.20
P. nigra	38.6 (0.4)	3047	1.08
P. sylvestris	44.8 (0.3)	3165	1.18
P. menziesii	74.1 (2)	1400	1.32

Correlation of Root Lifespan with Root Characteristics Across Species

Overall, there was a tendency for longer-lived roots to have lower N/C ratios. For the finest roots, the N/C ratio was negatively correlated with LS_{50R} across all 11 species (P=0.02, Figure 3.6, Table 3.4). Although LS_{PMRoot} was correlated with median fine root lifespan, LS_{PMRoot} only tended towards negative correlation with N/C ratio (r= -0.37, P=0.13).

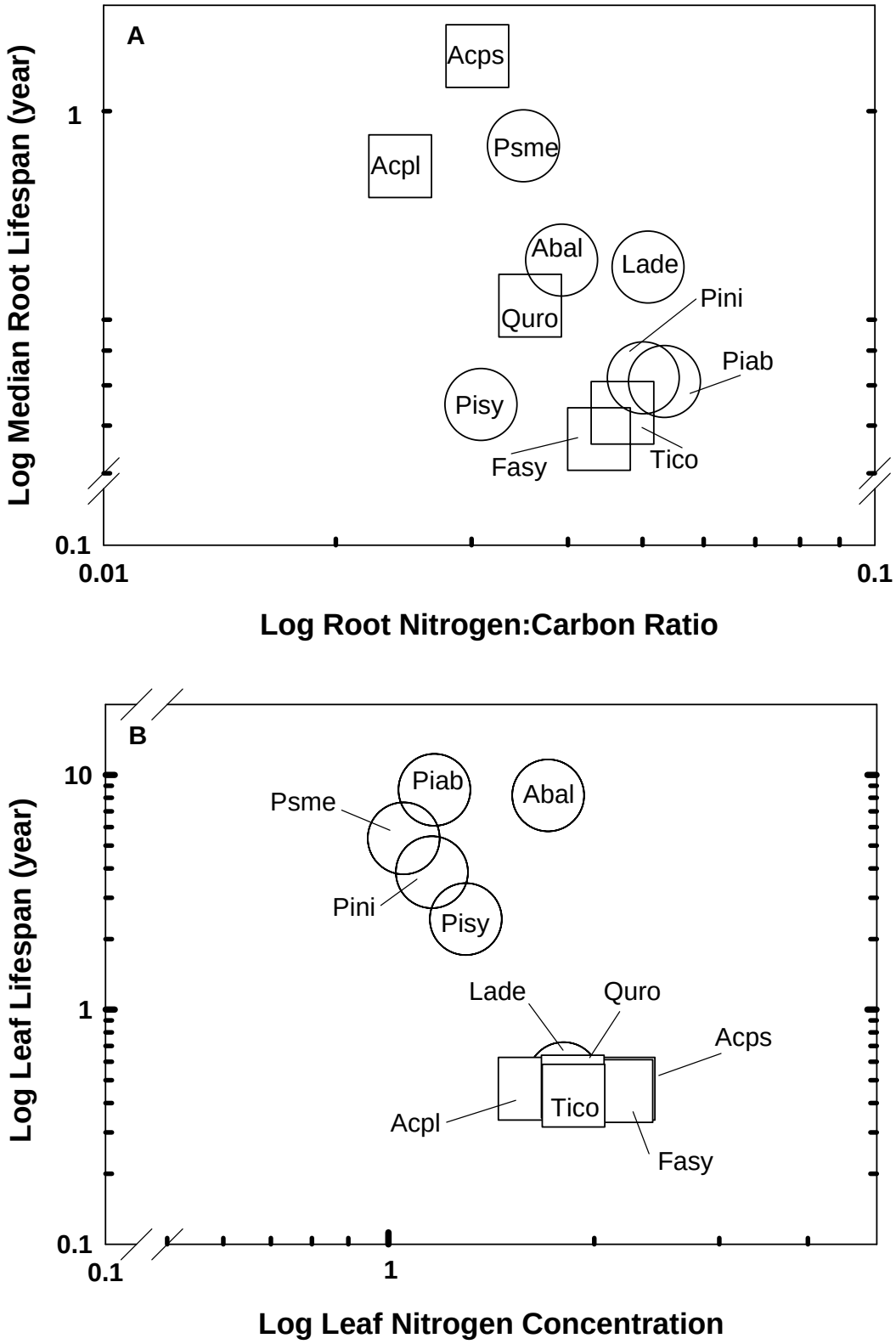

Figure **3.6**: The relationship between nitrogen-carbon ratio and longevity in roots (A) and nitrogen concentration and longevity in leaves (B). Traits are on a log scale. Hardwoods are denoted by squares and conifers by circles. Species abbreviations are given with the first two letters of the genus and species.

In contrast to general plant growth strategies, new root production tended to be positively correlated with LS_{50R} (P=0.08, Table **3.4**). The *Acer* spp. had a large effect on the correlation. *Acer platanoides*, for example, exhibited root growth of more than 4 m m^{-2} yr^{-1}, yet had one of the longest median fine root lifespans. If the two *Acer* spp. were omitted, there was little evidence of a relationship between median root lifespan and root production (P=0.12).

Contrary to expectation, we found that denser root tissue tended to be associated with shorter median root lifespan (r= -0.582, P=0.06), but specific root length was not correlated with LS_{50R} (P=0.59) (Table **3.4**). Average fine root diameters calculated with or without hyphal mantles were not correlated with root lifespan, perhaps because fine root diameters were quite variable within a species, even for only the first two root orders (Figures **3.1** and **3.2**).

Correlation of Root Lifespan with Root Characteristics Within a Species.

Within each species, we examined three characteristics of individual roots observed in the minirhizotron windows: diameter, depth in soil and time to pigmentation. Fine root lifespan was significantly associated with root depth in soil in seven species (Table **3.7**); deeper roots lived longer than shallower roots (Figures **3.7** and **3.8**). In four species, larger diameter roots had a lower risk of death than thinner roots (Table **3.7**).

Table 3.7: The effects of diameter, soil depth and number of days until a white root turned brown (pigmentation) on the risk of fine root mortality for the eleven species calculated using Cox proportional hazard regression. The value of the hazard ratio (HR) is less than one when the parameter estimate (β) is negative. This indicates an inverse relationship between the variable (e.g. diameter) and the risk of death. When the Chi-Square test is significant then the description of the risk is indicated in the Risk column.

	β	SE	χ^2	*P*	HR	Risk
Diameter						
Acpl	-1.85	1.38	1.79	0.181	0.157	---
Acps	-1.97	1.73	1.30	0.254	0.139	---
Fasy	-2.06	1.08	3.64	0.057	0.128	Thin>Coarse
Quro	-2.39	1.01	5.51	0.019	0.092	Thin>Coarse
Tico	-0.60	0.94	0.41	0.523	0.548	---
Abal	-6.73	2.16	9.69	0.002	0.001	Thin>Coarse
Lade	1.95	2.00	0.96	0.328	7.057	---
Piab	-2.57	1.25	4.22	0.040	0.076	Thin>Coarse
Pini	-0.75	0.88	0.73	0.393	0.470	---
Pisy	-0.41	1.24	0.11	0.742	0.665	---
Psme	-1.69	1.41	1.43	0.231	0.184	---
Soil Depth						
Acpl	-0.03	0.01	27.73	<0.0001	0.969	Shallow>Deep
Acps	-0.03	0.01	11.44	0.0007	0.975	Shallow>Deep
Fasy	-0.01	0.01	4.74	0.0295	0.988	Shallow>Deep
Quro	-0.02	0.004	24.83	<0.0001	0.976	Shallow>Deep
Tico	-0.01	0.004	11.04	0.0009	0.986	Shallow>Deep
Abal	0.01	0.01	0.93	0.335	1.01	---
Lade	-0.01	0.01	2.51	0.113	0.985	---
Piab	-0.03	0.01	18.93	<0.0001	0.973	Shallow>Deep
Pini	-0.02	0.004	18.70	<0.0001	0.981	Shallow>Deep
Pisy	-0.01	0.01	4.51	0.0336	0.989	Shallow>Deep
Psme	-0.03	0.01	12.20	0.0005	0.972	Shallow>Deep
Pigmentation						
Acpl	-0.01	0.004	13.00	0.0003	0.987	Fast>Slow
Acps	-0.01	0.002	4.18	0.041	0.995	Fast>Slow
Fasy	-0.01	0.002	8.71	0.003	0.994	Fast>Slow
Quro	-0.003	0.001	11.22	0.001	0.997	Fast>Slow
Tico	0.001	0.002	0.59	0.444	1.001	---
Abal	-0.004	0.01	0.61	0.435	0.996	---
Lade	-0.03	0.01	3.75	0.052	0.975	Fast>Slow
Piab	-0.002	0.001	5.42	0.020	0.998	Fast>Slow
Pini	-0.01	0.002	11.02	0.001	0.994	Fast>Slow
Pisy	-0.01	0.002	7.10	0.008	0.995	Fast>Slow
Psme	-0.001	0.002	0.17	0.682	0.999	---

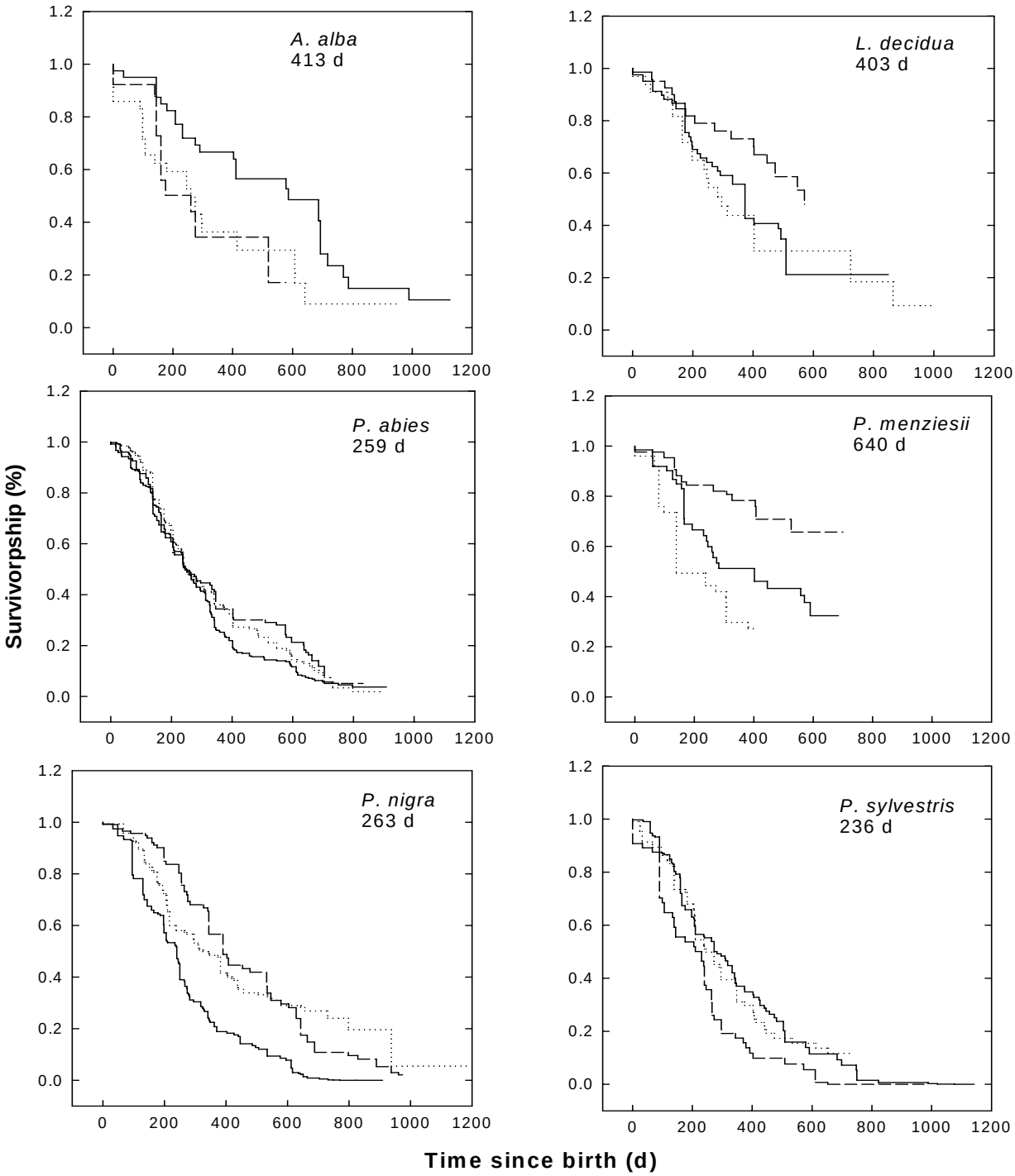

Figure **3.7**: Root survivorship curves from minirhizotron data for the conifer species with roots segregated into three depth classes (0-15 cm, 16-30 cm and 31-45 cm). Shallowest roots (0-15 cm) are denoted with solid lines, deepest roots (31-45 cm) with dotted lines and roots 16-30 cm with dashed lines. Root birth and death were estimated as halfway between successive sampling dates from when a root was not present to the date it first appeared. Proportional hazards models were run with depth as a continuous variable; depth classes were chosen to simplify the presentation. Median root survivorship (days) indicated is for all roots regardless of soil depth.

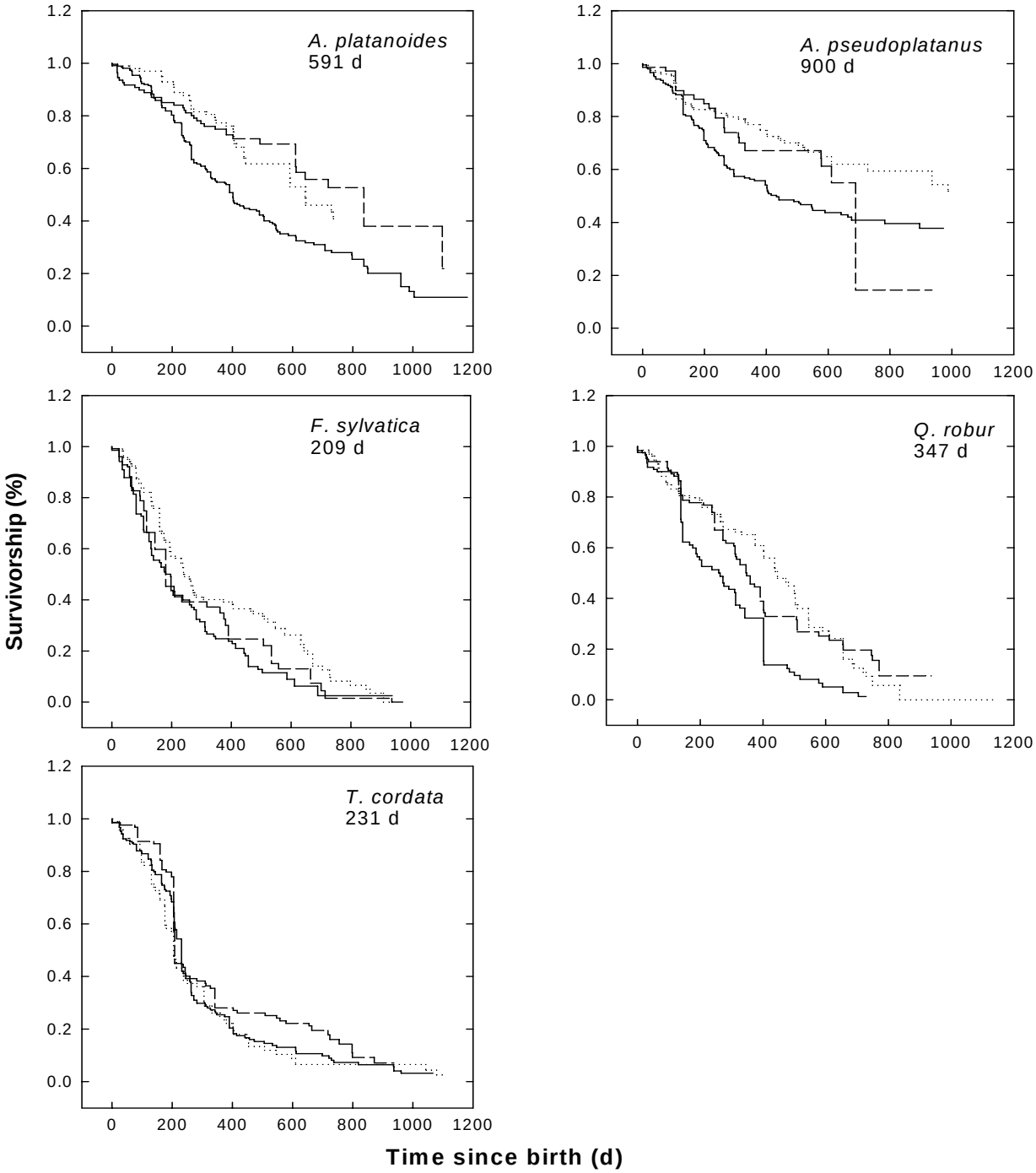

Figure **3.8**: Root survivorship curves from minirhizotron data for the hardwood species with roots segregated into three depth classes (0-15 cm, 16-30 cm and 31-45 cm). Shallowest roots (0-15 cm) are denoted with solid lines, deepest roots (31-45 cm) with dotted lines and roots 16-30 cm with dashed lines. Root birth and death were estimated as halfway between successive sampling dates from when a root was not present to the date it first appeared. Proportional hazards models were run with depth as a continuous variable; depth classes were chosen to simplify the presentation. Median root survivorship (days) indicated is for all roots regardless of soil depth.

Within each species, the longer a root remained white, the lower its risk of mortality, and the longer its lifespan (Table **3.7**). All fine roots are white when they are born. Most fine roots in this study became pigmented very quickly, within approximately 14 days after first appearance in the minirhizotron windows. In the six conifer species, 60-90% of the roots were brown in less than two weeks. For hardwoods, the amounts were similar with 70-88% of the fine roots becoming pigmented in less than 14 days.

Mycorrhizas and Root Anatomy

All fine roots observed in cross section were mycorrhizal, consistent with observations of roots in the minirhizotron windows. The two *Acer* spp. were predominantly colonized with AM mycorrhizal fungi. However, about 5% of the *Acer* roots collected from cores and excavations exhibited typical ECM mantles and morphotypes. None of the *Acer* roots visible in the minirhizotron windows exhibited ECM morphotypes. The other nine species were predominantly colonized with ECM fungi and frequently exhibited multiple morphotypes in close proximity. Average mantle thickness for the ectomycorrhizal species ranged from 0.03 mm to 0.10 mm with the thickest mantles appearing on the conifer species; however, in terms of thickness relative to the length of the root diameter, the largest mantles were observed on *F. sylvatica* (Table **3.8**).

Table 3.8: Anatomical characteristics observed and measured on mixed-age, first-order roots. Average cross-sectional area of the plant part of the mycorrhizal root (XS-area, mm^2). Average percent of the root diameter contributed by ectomycorrhizal mantle (Mantle). Whether or not the epidermis (hardwoods) or the outermost layer of cortex (conifers) was largely intact [wh= white roots, myc= mycorrhizal roots; mostly intact (>90%) = +, some degradation (<90%) = 0]. Presence of root hairs [many (>16 hairs on circumference) = ++, some (~10-14 hairs on circumference) = +, none= 0]. Presence of exodermis in white root samples [always= ++, not in every cross section= +, never= 0]. An exodermis was never observed in ectomycorrhizal roots.

Species	XS-area	Mantle	Epidermis	Root hairs	Exodermis
Acpl	0.135	--	myc +	myc ++	++
Acps	0.164	--	myc +	myc ++	++
Fasy	0.080	14.0	myc 0/ wh +	myc 0/ wh ++	0/ ++
Quro	0.119	9.4	myc 0/ wh +	myc 0/ wh +	0/ +
Tico	0.126	5.1	myc 0/ wh +	myc 0/ wh ++	0/ ++
Abal	0.273	4.1	myc 0/ wh +	myc 0/ wh ++	0/ 0
Lade	0.159	6.3	myc 0/ wh +	myc 0/ wh ++	0/ ++
Piab	0.166	6.3	myc 0/ wh +	myc 0/ wh +	0/ ++
Pini	0.108	9.4	myc 0	myc 0	0
Pisy	0.181	9.2	myc 0	myc 0	0
Psme	0.212	4.3	myc 0/ wh +	myc 0/ wh ++	0/ ++

The 1st-order roots of both *Acer* spp. had a pronounced exodermis (Figure 3.9). Wall thickening (approximately 20 μm thick) was only on the outer tangential walls of the exodermis (the cell layer just interior to the epidermis). Passage cells, which do not have thickened walls, were also observed in the exodermis in some sections. These would allow water, nutrients and arbuscular mycorrhizal hyphae to enter the root cortex.

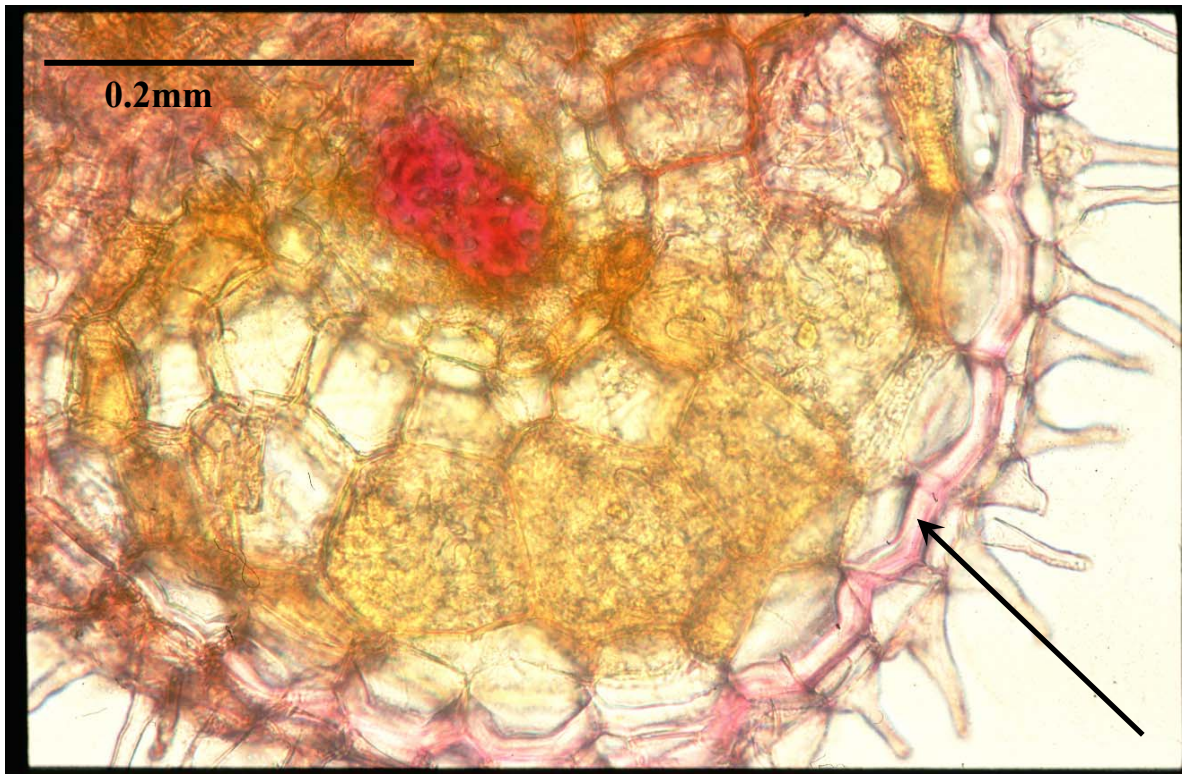

Figure **3.9**: Cross-section of a 1st-order *Acer pseudoplatanus* root stained with 0.05% phloroglucinol. The xylem and the thickened walls of the exodermis are stained red, and the latter is marked with an arrow.

The lower risk of mortality of white roots observed with the minirhizotrons led us to try to collect some of these roots of each species for anatomical investigation. A small percentage (<2%) of the finest order roots collected for *A. alba*, *L. decidua*, *P. menziesii*, *P. abies*, *F. sylvestris*, *Q. robur* and *T. cordata* were white; these roots had root hairs and were >2 cm long (Table 8). We examined 5-10 white, non-ectomycorrhizal roots of each of the ectomycorrhizal species to note how they differed from roots with distinct mantles. A very similar, thickened exodermis was noted in the non-ectomycorrhizal white roots of *L. decidua*, *P. abies*, *P. menziesii*, *F. sylvestris*, *Q. robur* and *T. cordata*. The exodermis of these six species was not as thick as that of the *Acer* spp., but it was present in all white root sections (Table **3.8**, Figure **3.10**).

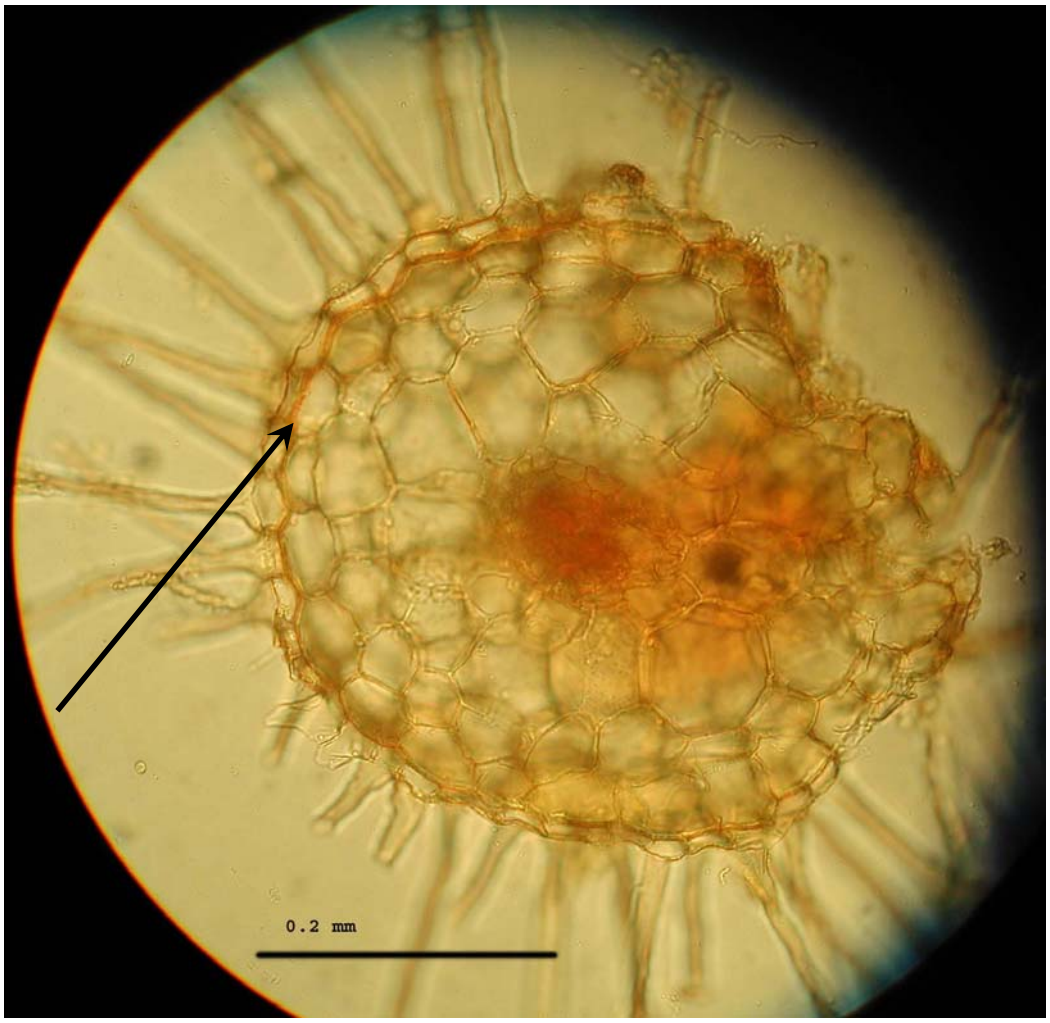

Figure **3.10**: Cross-section of a white 1st-order *Picea abies* root stained with 0.05% phloroglucinol. The exodermis is stained red and marked with and arrow.

Conifers vs. Hardwoods

The average root diameters of the five hardwood species with mantles, if present, were about 23% thinner than the conifer roots (r=0.81, P=0.002). The average root diameters of the hardwoods were only marginally thinner than the conifers (r=0.57, P=0.066) when considering only the plant part of the roots (Table **3.2**).

Specific root length was significantly longer in the hardwood roots (45.8 m g^{-1} to 90.7 m g^{-1}) than in the conifer roots (24.7 m g^{-1} to 40.9 m g^{-1}) (P=0.01) (Table **3.2**). Because tissue density is often correlated with SRL, we predicted a similar relationship between the hardwoods and conifers for root

tissue density. Root tissue density was insignificantly greater in the conifers (0.153 g cm^{-3} to 0.287 g cm^{-3}) than in the hardwoods (0.133 g cm^{-3} to 0.214 g cm^{-3}) and was not correlated with SRL (P=0.29) (Tables 3.4 and **3.5**).

Total root length density (root length in a volume of soil, L_v), including roots of all orders, was 53% greater in the hardwood plots than in the conifer plots (P=0.01), but L_v of the finest order roots was not significantly (21%) greater in the hardwood plots than the conifer plots (P=0.86) (Table **3.2**). All species had 50-60% of the total root length composed of 1st- and 2nd-order roots; the average percentage of the finest order roots was 13% greater in the conifer plots (P=0.10) reflecting the differences in root architecture between the two groups (Table **3.2**).

The range in N/C ratios was greater in the hardwood fine roots than in the conifer roots, and the overall average value was not significantly different between the hardwood and conifer species (Table **3.2**). Average root lifespan based on number of days for root mortality to equal root production was negatively correlated with N/C ratios of the hardwood species (r= -0.98, P<0.0001) but was not correlated with the N/C ratios of the conifer species (r= -0.22, P<0.10).

Root growth rate was determined by fine root length production visible in the minirhizotrons over the four years of the study. Hardwood roots tended to grow faster than conifer roots. Although variability was high, estimated root length production was approximately 50% greater for the hardwoods than for the conifers (P=0.08).

The relationship among leaf N concentrations, SLA and leaf production was similar to previous reports in the literature. Leaf N concentrations were significantly greater in the hardwoods than in the conifers (P=0.01) with values ranging from 1.62 % to 2.20% in the hardwoods and from 1.08 % to 1.81 % in the conifers. Also, the SLA of the deciduous hardwood leaves was significantly greater than that of conifer leaves (P<0.0001) (Tables **3.6** and **3.7**). Average leaf production estimated using litterfall traps was significantly greater in the hardwoods than in the conifers (P=0.047).

Relationships among Root Traits and Leaf Traits of all Eleven Species

Across all 11 species, SLA was inversely correlated with root total diameter (P=0.002) and marginally correlated with average root diameter excluding the mantle (P=0.07). This relationship was mainly driven by the phylogenetic differences between the *Pinaceae* and the other tree families. Trees in the pine family tended to have leaves with lower SLA and coarser roots than those of hardwoods, which corresponds to the *Pinaceae* being an evolutionarily more primitive plant family than the hardwood families examined. Within the *Pinaceae*, there was no significant correlation between SLA and root diameter (Table 3.4, r<0.21).

Root diameter and root tissue density are components of SRL. For our species, SRL was inversely correlated with average root diameter excluding the hyphal mantle (P= 0.004) and to total root diameter (P=0.001) (Table **3.4**). Therefore, thinner roots had more length per unit mass. Root tissue density was not correlated with SRL for our species.

While SRL was not correlated with root N/C ratio, it was positively correlated with leaf N concentration (P=0.02); species with leaves with larger N concentrations had roots with a greater length to mass ratio. New root length production was inversely correlated with N/C ratio (P=0.02) (Table **3.4**). However, this relationship was driven by the two *Acer* spp. and their large root production values but relatively low N concentrations. If the *Acer* spp. were excluded, the correlation was not significant (P=0.91).

Discussion

Root lifespan research lags behind leaf lifespan research, not due to the lack of important questions, but due to the extensive labor required to characterize root longevity, the need for special technical equipment, and the lack of suitable field sites. Our study is the first of its kind, to our knowledge, to use minirhizotrons to assess such a relatively large number of tree species growing in replicated, monoculture plots. However, even 11 species provided relatively small statistical power. With so many species and such long root lifespans, data collection and analysis took four years; this is in sharp contrast to the single summer of leaf data collection and analysis in this study (though, we acknowledge that other studies of leaf longevity have taken as many as 8-10 yr of censusing).

Various studies have found relationships among root traits and lifespan in grasses, forbs, lianas and trees, but many of these looked at only one or two species or made comparisons among roots on the same individuals. Studies of variation in roots within a root system indicate that thick roots with low SRL, low N concentrations and slow respiration live longer (*Vitis labruscana*: Anderson *et al*. 2003, *Bouteloua gracilis*: Gill *et al*. 2002, *Malus domestica*: Wells and Eissenstat 2001, *Prunus persica*: Wells *et al*. 2002). Indirect evidence in comparisons across species suggest that roots with long lifespans have suites of traits such as thick root diameter, high tissue density, slow maintenance respiration, slow ion absorption and decreased response to increased nutrient availability (Eissenstat 1997, Eissenstat *et al*. 2000, Ryser 1996, Schläpfer and Ryser 1996), similar to the syndromes of multiple leaf traits.

There are also reasons why roots may not have trait syndromes that are similar to leaves. While leaves and roots can be thought to have similar functions, a root system represents a complex network that is rarely characterized with respect to its diverse functions: likely only the finest root orders are similar to leaves in terms of resource acquisition and being ephemeral (Wells and Eissenstat 2001, Pregitzer *et al*.

2002). Even so, the fine roots are not discrete entities like leaves but represent a network with a complex structure. Also, leaf function is not directly dependent on symbionts, while root carbon expenditure, resource acquisition and perhaps defense may be strongly influenced by mycorrhizal fungi and other rhizospheric organisms. Roots also have characteristics without parallels in leaves that correlate with longevity; long-lived roots on the same root system tend to be deeper in the soil and are of higher order (*B. gracilis*: Gill *et al*. 2002, *M. domestica*: Wells and Eissenstat 2001).

Is Fine Root Lifespan Correlated with Leaf Lifespan?

Fine root and leaf lifespan were not correlated (Figure **3.3**, Table **3.4**). The different methods of estimating root lifespan (median, residence time based on production-mortality, residence time based on standing crop) resulted in similar relationships. The species with the longest-lived leaves (*P. abies*) did not have the longest-lived fine roots, nor did the species with the shortest-lived leaves (the hardwoods) have the shortest-lived roots. Similarly, Ruess *et al.* (2003) found *Picea mariana* to have long-lived leaves but short-lived fine roots (~0.3 yr). There was also no correlation of root lifespan with leaf lifespan among the six *Pinaceae* species, although they had a large range in both leaf and root lifespan.

The best estimator of root lifespan was median lifespan (LS_{50R}) based on data compiled from individual roots: no assumptions about steady-state birth and mortality rates were required and median tends to be the best estimator of central tendency for distributions which tend to be skewed (e.g. survivorship). In our study, estimates of survivorship based on the medians (LS_{50R}) were so long for some species that many of the roots born later in the study were not observed until death (censored); thus, estimates like LS_{PMRoot} can be a useful alternative. Root lifespan based on standing crop (LS_{SCRoot}) is a common way to estimate root lifespan when sequential coring or ingrowth cores are used. Most species achieved a fairly consistent standing crop soon after the 1st year (Figure **3.5**), but LS_{SCRoot} calculates a mean residence time, and the values for lifespan were therefore inflated by the few very long-lived roots of each species compared to the median values. Lack of steady-state conditions where production does not equal mortality also can cause this approach to provide inaccurate estimates of lifespan, as was the case in *Tilia cordata* and *Pseudotsuga menziesii.* Relationships between traits which were significant for the median were not significant for LS_{SCRoot}. We also calculated an average lifespan (LS_{PRRoot}) based on the time required for cumulative root mortality to equal cumulative root production (Figure **3.4**). This estimator was also a mean value for root lifespan (residence time); the values were still longer than our median estimates but were correlated with them. Lifespan estimates based on LS_{PRRoot} were potentially more accurate than the median estimate for species with long root lifespans where many of the roots were censored and roots produced early in the study did not exhibit "typical" lifespans.

Are Root Characteristics or Chemistry Correlated with Fine Root Lifespan Across Species?

Root nitrogen:carbon ratio was the only root characteristic significantly correlated with fine root lifespan. Other studies with herbaceous plants have also found significant correlations of N concentration with root lifespan (Craine *et al.* 2002, Tjoelker *et al*. 2005). Because N is more directly correlated to root metabolic activity and palatability, it is likely more directly linked to root longevity than characteristics such as SRL and root production.

The means to deter herbivory are often in opposition to the means to take up resources efficiently. For example, thin leaves and roots can have higher maximum photosynthetic or nutrient absorption rates by allocating more N to those tissues, but this is at the risk of greater susceptibility to herbivory. For leaves the relationship between N concentration and herbivore palatability has been documented (e.g. Fox and Macauley 1977, Cooke *et al*. 1984). For roots, the relationship is not well-studied, but there are data to suggest that the relationship should be the same. Wells (1999) and Eissenstat *et al.* (2000), for example, found that within *Acer saccharum* root systems, the finest order roots had the highest N concentrations and shortest lifespans. Application of fungicide and insecticide to the root zone significantly increased their lifespans, suggesting herbivory/parasitism was an important determinant of lifespan in this system for the fine roots.

Specific root length is a measure of how biomass is distributed to produce absorptive surface, similar conceptually to specific leaf area. It has been hypothesized that SRL is linked with root uptake ability and nutrient depletion zones and therefore with root efficiency and lifespan (Eissenstat 1992). However, even when we corrected for the presence of mycorrhizal hyphal mantles on the nine ECM species, our results did not reveal a relationship between SRL and root lifespan (Table **3.10**). Species with similar SRL values often had very different root lifespans (e.g. *P. abies* and *P. menziesii*). Tjoelker *et al*. (2005) also found no relationship between SRL and root lifespan.

Tissue density has been correlated with relative growth rate in grasses (Schläpfer and Ryser 1996, Ryser 1996), root proliferation in citrus (Eissenstat 1991), as well as with root lifespan (Ryser 1998). However in our study, tissue density, one component of SRL, was only marginally correlated with median fine root lifespan (r= -0.582, P=0.06, Table **3.4**). Roots with higher tissue densities tended to have shorter root lifespans. This pattern was opposite than was expected because, as with leaves, root toughness is thought to be positively correlated with lifespan. However, since we measured tissue density on only one date, we suspect this correlation may be a reflection of other factors associated with plastic tissue density changes with soil dryness rather than fundamental genetic differences in tissue density (Comas and Eissenstat 2004).

Numerous studies have revealed a tight linkage of leaf structure with leaf lifespan (e.g. Reich 1993, Reich *et al*. 1991, 1997). In our study, shorter leaf lifespan was significantly correlated with high

leaf N concentration and with high specific leaf area. Although we predicted the latter relationship based on increased appeal of thinner leaves to herbivores, the relationship between SLA and leaf lifespan could be a result of the difference in SLA between the hardwood and conifer species. Leaves with high N concentrations also had high SLA (Tables **3.6** and **3.7**), which is consistent with previous results linking thin leaves with a higher maximum photosynthetic rate (indicated by leaf N concentration). Leaf N concentration and SLA have been shown repeatedly to be part of a suite of traits associated with relative growth rate and shade tolerance (Reich *et al*. 2003 and citations therein). Similar relationships have been found across a wide range of species and from a wide range of habitats.

Are Root Characteristics Correlated with Fine Root Lifespan Within Species?

Of the traits examined within species, the discovery of an exodermis in the fine roots of many species was the most significant with relation to root lifespan. Soil depth of the roots and rate of pigmentation also significantly influenced root lifespan.

The extremely long median root lifespans of *A. platanoides* and *A. pseudoplatanus* were unexpected based on their high rate of root production and short leaf lifespan. Our observation of a very thick, pronounced exodermis may explain their long-lived roots. The exodermis can decrease the rate of ion uptake in the root, but more importantly it can provide a protective layer against herbivory and desiccation even after the epidermis has broken down and sloughed off (Kamula *et al*. 1994).

We observed distinct exodermis in the fine roots of both *Acer* species. Brundrett and Kendrick (1988) reported a similar type of exodermis in *Acer saccharum*. White, non-ectomycorrhizal roots with exodermis were also observed in many of the conifers as well as *F. sylvestris*, *Q. robur* and *T. cordata*. These kinds of roots have been reported before for *P. menziesii* (Bogar and Smith 1965). However, we could find no report of *Pinaceae* roots having an exodermis. The exodermis found in the white, non-ectomycorrhizal roots, though thinner than the exodermis of the *Acer* species, helps explain our finding that roots that remained white longer tended to have longer root lifespans (Table **3.8**). The exodermis in these species should provide similar protection and increased root longevity as previously suggested in *Acer*.

When we examined other root traits within each species that could influence root lifespan, root depth and rate of pigmentation were the most closely associated with risk of root mortality (Table **3.7**). Compared to roots in the surface soil layers, roots deeper in the soil tend to have lower N concentrations (Kimmins and Hawkes 1978, Pregitzer et al. 1998). Deeper roots also tend to experience less variable temperatures, less variable moisture and possibly fewer herbivores, all of which could result in the longer root lifespans we observed. Soil depth has previously been found to have mixed-effects on root

longevity. Depth has been positively correlated with root lifespan in peach (Wells *et al*. 2002) and grape (Anderson *et al*. 2003) but not in perennial bunchgrass (Gill *et al*. 2002).

Pigmentation is often an indication of tannin accumulation for defense as well as decreased physiological activity (Comas *et al*. 2000). In cross-sections, we observed tannins associated with the Hartig net of ectomycorrhizal fungi. If pigmentation in our system was an indication of decreased physiological activity, then roots that pigmented faster might also have shorter lifespans. Indeed, in our system, faster rates of pigmentation were associated with decreased root longevity within each species (Table **3.9**). This could indicate that pigmentation in our system was more a sign of decreased root physiological activity associated with aging and was less an indicator of accumulated root defenses.

Within the root system of a plant, diameter has often been an important correlate of root lifespan (*Vitus labrusca* 'Concord', Anderson *et al*. 2003; *B. gracilis*, Gill *et al.* 2002; *M. domestica*, Wells *et al*. 2002); however, it was not an important characteristic in our study. This was most likely due to the limited range of root diameters we included for each species to be certain we included only the finest root orders (see Methods, Figures **3.1** and **3.2**). When we included all of the roots seen in the minirhizotron windows in the analysis, increases in diameter significantly reduced the risk of mortality for all but *A. pseudoplatanus* and *P. nigra* (data not shown).

Do the Roots of Conifers Life Longer than the Roots of Hardwoods?

A couple of studies have found hardwood species to have shorter root lifespans than those of conifers (*Liquidambar styraciflua* vs. *Pinus taeda*, Matamala *et al*. 2003; Vogt and Bloomfield 1991). However, in our study, we found the two groups had similar root lifespans, no matter how root lifespan was estimated.

There has been some previous indication that hardwood roots have smaller diameters than conifer roots (Comas and Eissenstat 2004). While evolutionarily primitive hardwood families, like the *Magnoliaceae*, tend to have thicker roots (Baylis 1975), our hardwood species were all from more advanced families. We predicted that for our species the hardwood fine roots would be thinner than conifer fine roots, but we found substantial overlap in the root diameter values. In our study, average fine root diameters were not significantly different between the hardwoods and conifers. Not finding hardwood root diameters to be significantly thinner than conifer root diameters may be due to the inclusion of only 1st and 2nd order roots in our study with a very narrow range in root diameters (e.g. 0.20 mm to 0.35 mm). Alternatively, averaging all the root diameters to one value per species for the correlation matrix might have obscured potential relationships.

Although diameters were not significantly different between the hardwoods and conifers, hardwood roots had significantly longer SRL than the conifers. Thus, hardwood roots did exhibit more root length per unit mass invested. With the potentially larger amounts of root surface area per gram and greater leaf N concentration, it was unexpected to find hardwood roots had significantly smaller (-27%) N/C ratios than the conifers. Although we did not separate fungal mass from plant tissue, this was most likely due to the high N content of the chitin in the larger ECM fungal masses associated with the conifer roots.

Larix decidua is an interesting case since it has characteristics of both a coniferous and deciduous species. Aboveground, *L. decidua* is a fast-growing species with high SLA, high leaf nitrogen concentration and short leaf lifespan (Tables **3.6** and **3.7**). Like the leaves, *L. decidua* roots had low root tissue density and had the highest SRL among the *Pinaceae* species. In these aspects, it was very much like the deciduous hardwoods in the study, as our hypothesis on parallel root and leaf traits predicted. However, larch root production was the slowest of the studied species, its N/C ratio and average root diameter were large and its roots lived for a long time (Tables **3.3** and **3.6**), making larch roots very similar to other *Pinaceae* roots. *Larix* is a good example of how plants, especially trees, can have very different characteristics above- and belowground.

Conclusions

We could not support the hypothesis that fine root lifespan and leaf lifespan are linked. Some species (e.g. *Acer pseudoplatanus*) had short-lived leaves (0.5 y) but very long-lived fine roots (2.5 y) while other species (e.g. *Picea abies*) had very long-lived leaves (8.8 y) but short-lived fine roots (0.7 y). The roots of the *Acer* spp. with their thick exodermis were built to survive a long time, their lifespan controlled to a great extent by their anatomy instead of their physiology. Of the rest of the root traits we measured, only root nitrogen:carbon ratio was inversely correlated with fine root lifespan. Even though roots and leaves are on the same individual, they can be under very different environmental pressures, which may cause them to be uncoupled. With more research, including careful attention to the measurement of root lifespan, we hope that the interrelationship among root traits and root lifespan will eventually be as well understood as those of leaves.

Acknowledgements

The authors would like to thank Bartosz Bułaj and Jakub Olesiński for their invaluable help organizing activities in Poland, arranging for technical help when required and collecting minirhizotron images. We also thank Jerzy Modrzyński of the Agricultural University of Poznań for his work on the tree height, dbh and leaf collection data and the critical comments of earlier drafts of this manuscript by Tracy Gartner and Ruth Yanai. We appreciate the diligent efforts of Mariellen Withers who helped process the images. JMW was supported by an NSF Root Biology Training Grant (NSF DBI 9602255), the William J. Fulbright Foundation and The Women's Welsh Clubs of America. This research was supported by an Eastern European International Supplement to an NSF grant (IBN 9596050). NSF also contributed to the work through the following grants: DEB 01298944 (PSU), DEB 0090069 (UMN) and DEB 0128958 (UMN).

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Chapter 4

Seasonal patterns of fine root growth of eleven temperate tree species in a common garden

Abstract

Fine roots play an important role in global carbon and nutrient cycling, but we still do not understand enough about belowground processes and patterns to create accurate growth models concerning the fate of carbon in ecosystems. We studied annual root production patterns of 11 tree species growing in a common garden to examine whether annual root production is similar from year to year for each species and how consistent is the timing of the root production peaks. Our three-year study used minirhizotron tubes to record the births of individual roots. Total annual fine root production of each species was not similar from year to year. Peaks of root production each year were at similar times across all eleven species: Aug.-Sept. in 2000 and 2002 and about two months earlier in 2001. This is the first study to observe not only similar timing of fine root production across many species but also to observe the peaks of eleven species adjusting synchronously from year to year, suggesting a large influence of environmental cues on fine root production in this system. Our research also emphasizes the importance of long-term, multi-species studies for broadening our understanding of forest ecosystems.

Introduction

Being ephemeral and living only three months to three years, fine roots can annually return as much as four to five times more organic matter to the soil than aboveground litter in a range of ecosystems. In forest ecosystems in particular, 50% of net primary production can be used to produce and maintain fine roots (Vogt *et al*. 1996), even though fine roots do not make up a large percentage of belowground biomass at any one time (Lyr and Hoffman 1967). Thus, roots

play an important role in global carbon cycling (Fogel 1983, Caldwell *et al*. 1987, Vogt *et al*. 1996).

Growth periodicity of shoots and roots indicates patterns of partitioning of resources in the plant, patterns which have been of interest to scientists for centuries. The Ancient Greeks, for example Aristotle, Theophrastus and Democritus, described root and shoot phenology more than 2300 years ago (Einarson and Link 1976). Leaf patterns of production are fairly predictable in temperate systems, while root patterns are not so, and yet there are still many fewer studies on root phenology.

One of the oldest records of the relationship between shoot and root growth was by Theophrastus of Lesbos (372-287 BC) (cited in Einarson & Link 1976). He noted that plants are adjusted to the seasons and season was more important than plant characteristics such as size and plant water relations in determining timing of plant growth. Focusing on agricultural species including grasses, herbs and trees, Theophrastus did not note peaks of growth through the year but did say that he observed simultaneous growth of roots and shoots in all species.

Historically, the pattern of annual root growth has been described as having two peaks, one in the spring/summer and one in the fall (Table 1; Resa 1877, Buesgen 1901, Engler 1903, Lyr and Hoffman 1967, Puhe 2003). Fine root growth may compete with shoots for carbohydrates (Webb 1976, Bloom *et al.* 1985) and may be linked with low soil moisture availability in summer plus increased soil moisture in the fall leading to a pattern of two peaks of growth (Merritt 1968, Puhe 2003). Even with seemingly sufficient summer precipitation, high air temperatures and specific soil characteristics can lead to decreased soil water availability in the summer (Lowry 1962, Weber and Nkedirim 1998). Sampling techniques and the age of the trees under study may also affect the results; soil core data require many assumptions about root birth and death, and seedlings are more likely to have two or more flushes of root growth during the growing season than mature trees (Reich *et al.* 1980). In a very detailed, 2.5-year study on the root growth of seedlings (age 0-2.5 y) of 14 tree species, Engler (1903) observed root production to exhibit a main peak starting in the late spring with a second peak in September-October of about 20 and 60% of the main peak. For three out of nine species, Lyr and Hoffman (1967) reported two peaks of growth with the second in the late summer at depths of about 1 m.

But two peaks of growth are not necessarily the rule. In many situations, authors have reported only one peak in root growth in the spring/summer (Table 4.1; Wieler 1894, Brundrett and Kendrick 1988, Kern *et al*. 2004), a pattern often linked to warm temperatures and moist soil conditions during the summer.

Table 4.1: Literature survey of fine root production in trees. We report patterns of root production using timing of major peaks (Main, Secondary = 2°) given as months or seasons of the year. Also included are the number of years of data reported (# Yrs), the species studied, the age of the trees (Age) and research location. We also note the researcher's observation method (Method) when possible.

Main	2° Peak	# Yrs	Method	Age	Species	Location	Citation
Feb-Mar		2	excavation	mature	*Aesculus hippocastanum*	Bonn, Germany	Resa 1877
Jul		1	soil cores	11 y	*Picea sitchensis*	Dumfriesshire, Scotland	Ford & Deans 1977
Aug		1	IG cores	15-20 y	*Pinus sylvestris*	Jadraas, Sweden	Ericsson & Persson 1980
Jul		1	observ. lab	0-10 y	*Malus domesticus*	Kent, UK	Atkinson 1983
Sp-Fa		1	excavation	ng	mixed forest	Waterloo, Ontario, Canada	Brundrett & Kendrick 1988
Jun		1	MR tubes	74 y (st)	mixed hardwoods	Michigan, USA	Hendrick & Pregitzer 1992
Jun-Jul		1	MR tubes	26 y	*Picea abies*	Skogaby, Sweden	Majdi & Nylund 1996
Jun-Jul		3	MR tubes	15-18 y	*Picea mariana*	Bonanza Creek, AK, USA	Reuss *et al.* 1998
Jun-Jul		2	MR tubes	MF	mixed hardwoods	Michigan, USA	Burton *et al*. 2000
Sp-Su		5	MR tubes	ng	5 deciduous broadleaf	Oak Ridge, TN, USA	Joslin *et al.* 2001
Wi		3	MR tubes	35 y (st)	*Quercus ilex*	Spain	Lopez *et al*. 2001
July		2	MR tubes	MF	3 deciduous broadleaf	Hubbard-Brook Forest, NH, USA	Tierney *et al*. 2003
Su		var.	various	various	*Picea abies*	various- areas with no summer drought	Puhe 2003
Su		2	MR tubes	1-2 y	*Populus deltoides*	Rhinelander, WI, USA	Kern *et al*. 2004
Aug-Oct	Apr	2	excavation	ng	*Quercus robur*	Bonn, Germany	Resa 1877
Jun-Jul	Oct	2	excavation	ng	*Alnus glutinosa*	Bonn, Germany	Resa 1877
Wi-Sp	Fa	2	excavation	ng	*Pinus pinea*	Bonn, Germany	Resa 1877

Apr	Aug-Oct	2	excavation	ng	*Tilia europaea*	Bonn, Germany	Resa 1877
Su	Wi-Sp	2	pots	2-4 y	*Populus canadensis*	Braunschweig, Germany	Wieler 1894
Su	Wi-Sp	2	pots	2-4 y	*Betula alba*	Braunschweig, Germany	Wieler 1894
Su	Su-Fa	2	pots	2-4 y	*Quercus robur*	Braunschweig, Germany	Wieler 1894
Apr-Jul	Sep-Nov	2	ng	1-8 y	6 conifers + 7 hardwoods	Zurich, Switzerland	Engler 1903
Su	Wi-Sp	2	excavation	2-4 y	*Pseudotsuga menziesii* (Wenatchee source)	Oregon, USA	Krueger & Trappe 1967
Sp	Su	2	excavation	2-4 y	*Pseudotsuga menziesii* (Willamette source)	Oregon, USA	Krueger & Trappe 1967
Jun	Oct	1	observ. box	2 y	*Pinus resinosa*	Indiana, USA	Merritt 1968
Sp-Su	Fa	1	MR tubes	10-11 y	*Pinus taeda*	NC, USA	King *et al.* 2002
Sp	Fa	var.	various	various	*Picea abies*	various- areas with summer drought	Puhe 2003

Abbreviations (Peaks): Sp= Spring, Su= Summer, Fa= Fall, Wi= Winter

Other abbreviations: IG= ingrowth, MF= mature forest, MR= minirhizotron, ng= not given, st= stand age

In this study, we had a unique opportunity to examine root production across many species for four years. We predicted species would have similar total annual production from year to year. If conditions were poor for a year (e.g. lower than average precipitation, high temperatures), we expected root production for all species to decrease. We also predicted that the pattern of annual production (i.e. number and timing of peaks) for each species would be consistent from year to year.

Methods

Field Site

Our field site was a common garden planting in the Siemianice Experimental Research Forest in central Poland, near the village of Biadaszki (51°14.87'N, 18°06.35'E, altitude: 150 m). Climate of the region is transitional between maritime and continental. From 1968-1997, the average annual precipitation was 591 mm, with about half falling from May to August. Average temperature was 8.24°C with a mean growing season of about 213 d, calculated as the number of days with an average temperature ≥5°C (Szmański and Ceitel 1989, Ceitel and Wawro 1999a, 1999b). Rainfall and air temperatures during our study period, from 1999-2002, were typical (**Figures 4.1 and 4.2**).

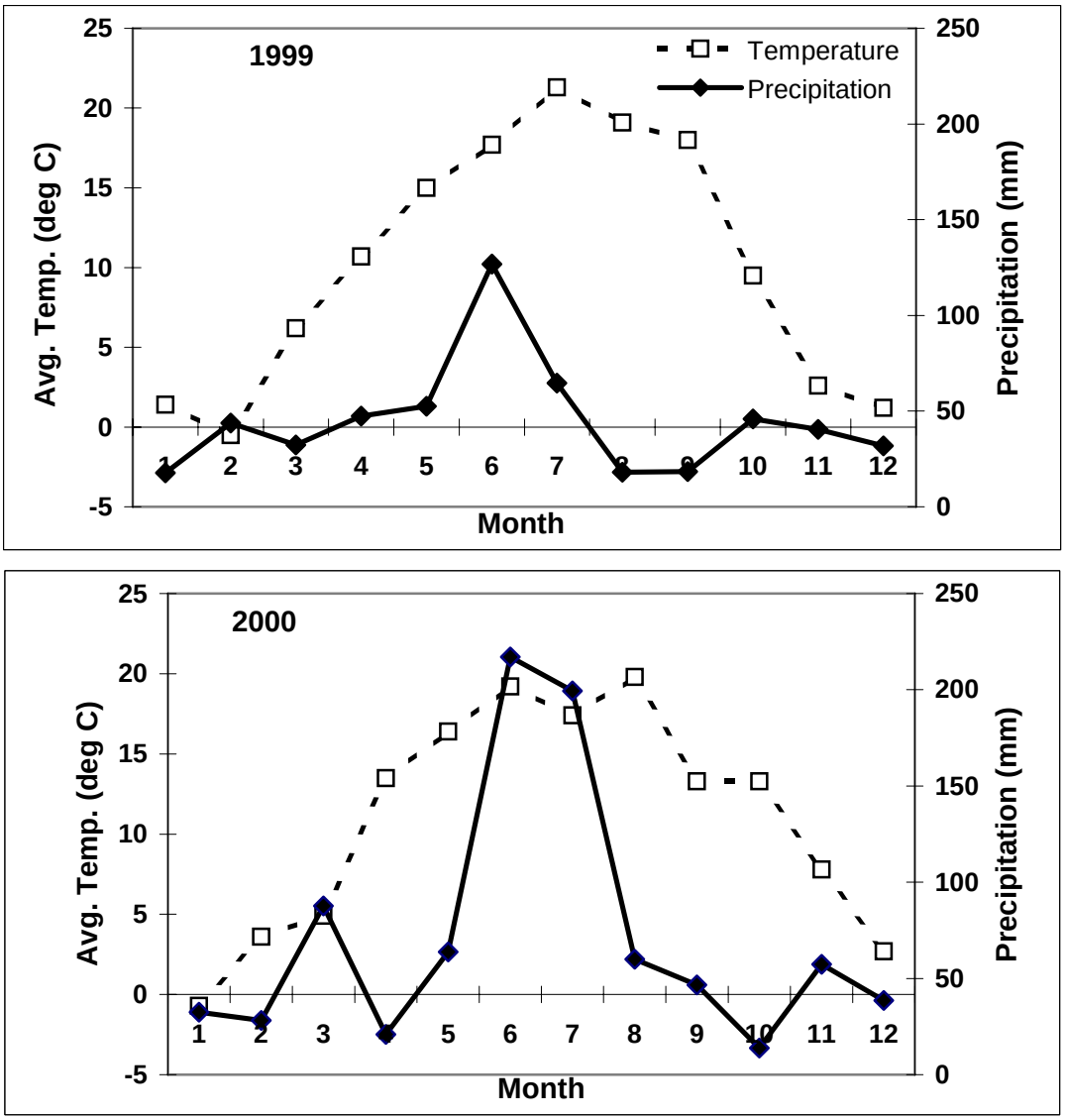

Figure **4.1**: Average monthly temperatures and total monthly precipitation in Biadaszki, Poland from 1999-2000.

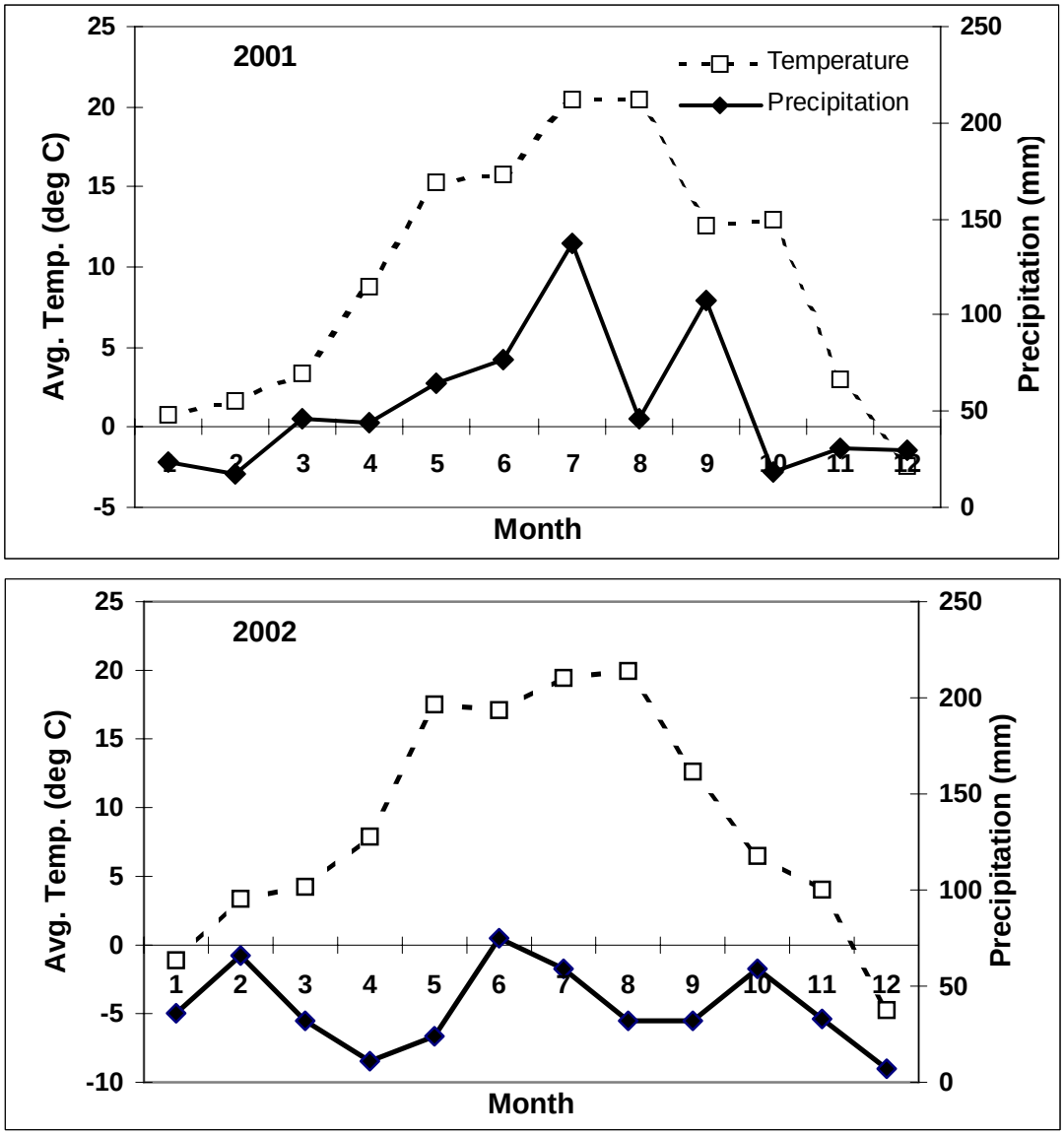

Figure **4.2**: Average monthly temperatures and total monthly precipitation in Biadaszki, Poland from 2001-2002.

The site consisted of two adjacent plantings with 14 species total, nine species per planting, and with some species duplicated between plantings (Szymański 1982). Species were planted in nine, monospecific 20 x 20 m plots in each of three blocks, for a total of 27 plots per planting. Tree seedlings were planted in 1970 and in 1971 at 1 x 1 m spacing. Each planting had a fairly uniform topography with very few understory plants due to the high tree density (Withington *et al*. 2003). Soils were generally nutrient poor loamy sands (average 80% sand and

15% silt) and classified as fine-loamy, mixed, Mesic Kanhaplic Haplustalfs and sandy, mixed, Mesic Typic Ustipsamments (O. Chadwick and J. Chorover, unpbl. data). We used % clay in the plots as a covariate with some of the root and leaf traits measured to assess potential differences based on soil texture. For this experiment, we sampled five deciduous broad-leaved species: *Acer pseudoplatanus* L., *Acer platanoides* L., *Fagus sylvatica* L., *Quercus robur* L., and *Tilia cordata* Mill. We also sampled one deciduous conifer, *Larix decidua* Mill., and five evergreen conifers: *Abies alba* Mill., *Picea abies* (L.) Karst., *Pinus nigra* Arnold, *Pinus sylvestris* L. and *Pseudotsuga menziesii* (Mirbel) Franco.

Fine Root Production

In a previous study at this field site, we found acrylic plastic provided root standing biomass estimates more consistent with those estimated by soil cores than cellulose acetate butyrate plastic (Withington *et al.* 2003); therefore, only observations from acrylic tubes were used in this study. Three tubes were installed per plot, three plots per species in Nov. 1998 with care to keep them at least 3 m from the plot borders. We used only two *A. alba* plots (six tubes total) because the third was overgrown with a different tree species. One tube in an *A. pseudoplatanus* and one tube in a *T. cordata* plot were lost over the course of the experiment due to vandalism, so these species had eight tubes total. The minirhizotron tubes were 0.65 m long and had an inside diameter of 5.2 cm and a wall thickness of 6.4 mm. The tubes were installed at an angle of 30º from vertical and were scribed with a strip of 1 x 1.25 cm windows down the upper side. Tops of the tubes were wrapped in black electrical tape and sealed with a rubber stopper to keep light and rain from entering the tubes.

Minirhizotron images were collected using a minirhizotron camera and associated image capture software (Bartz Technology Corp., Santa Barbara, CA) starting in May 1999, six months after tube installation. Images were collected in 1999 at 2-4 week intervals, but sampling intervals were lengthened in 2000-2002 to monthly intervals from April through Nov. because initial data indicated very long-lived fine roots.

This study examined the production of the finest two orders of roots. Order is relatively easy to determine with excavated roots but is problematic when looking in minirhizotron images with only portions of roots visible. For a given species in a particular location, root diameter can be used to indicate root order. To choose a maximum diameter for 1st order roots (finest order),

we compared the diameter distributions of excavated and scanned roots of only the first two orders from soil cores with the diameter distributions of the roots in the minirhizotron images (Chapter 3).

Root production, in terms of numbers of roots per unit viewing area (cm m^{-2} $month^{-1}$), was determined by counting the roots born on each sampling date and summing within plots. These values were converted to root length using regression equations of the relationship between number of roots and root length for each species (Equation 12, Appendix A). To obtain these equations, we used a subset of images that included all fine roots present in all tubes for five dates distributed across the study period including each year and representing each season. From this subset of images, root length was determined for each species using the program WinRHIZO Tron (Regent Instruments Inc., Quebec, Canada), and correlated with total numbers of roots present in the tubes (for each of the 11 species, all r^2>0.88).

After 1999, the time interval for the first sampling date was adjusted to assume all new roots were born on Feb 1 or later because roots can grow at temperatures of only 2-3ºC, temperatures which were found in February each year. We calculated our production averages based on percentages so that each plot was similarly weighted. To calculate average production by species, we converted the plot production values on each date to percent of annual production (Equation 8, Appendix A). Plots with annual production of less than or equal to five roots were dropped from the analysis because production was too low to accurately assess seasonality. Additionally, plots that had low (≤15%) annual root production relative to that of other plots of that species and also produced relatively few roots for calculating percentages (<15 roots) were not included in the analysis. We calculated the standard error based on the percent annual production for each sampling date for all of the plots of that species. The averages and standard errors were then converted back to length per $meter^2$ per month for graphical illustration using the average annual root production of each species multiplied by the percentile data (expressed as a fraction).

Results

Data are only presented for three years of the study (data from 1999 were not used). The first year, production was very high for all species, most likely a result of disturbance from tube installation (Joslin and Wolfe 1999). Although monthly average air temperatures were above 5°C

by March each year (Figures **4.1 and 4.2**) indicating plants could be physiologically active, leaf expansion at the site occurred around the middle to end of April each year (personal observations) when monthly average temperatures were above 10°C. Leaf fall in the deciduous species occurred about October each year, again when air temperatures were around 10°C. Needle fall in the evergreens was generally continuous during the year.

On average root production for each species differed greatly from year to year (Table 4.2). Variation across plots was also very high, as reflected in the large standard errors. For 10 of the 11 species, the largest annual production per plot was in 2001 (Table **4.2**). The lowest production year was 2002 for 4/5 of the hardwoods and 2000 for 5/6 of the conifers.

Table **4.2**: Total annual fine root production per plot (cm m^{-2} $month^{-1}$) for each species from 2000-2002 plus the average production for the three years. There were 2, 3 or 6 plots per species with 2 or 3 tubes per plot. Information was gathered on numbers of new roots for each sampling day and converted to root length. Data are presented as average root length per viewing area on the tubes in a plot per month. Standard errors of the mean are given in parentheses.

	2000	**2001**	**2002**	**3-yr avg.**
Acer platanoides	1919 (626)	4175 (1490)	1638 (847)	2577 (662)
Acer pseudoplatanus	1523 (36)	3516 (202)	1124 (269)	2054 (383)
Fagus sylvatica	897 (12)	1601 (717)	565 (212)	1037 (299)
Quercus robur	919 (351)	2734 (961)	777 (320)	1477 (442)
Tilia cordata	1241 (209)	2651 (971)	1360 (496)	1751 (392)
Hardwood Avg.	1329 (172)	2935 (432)	1093 (211)	
Abies alba	805 *	921 (67)	1378 (886)	1081 (280)
Larix decidua	466 (105)	1276 (282)	1236 (613)	993 (237)
Picea abies	1738 (431)	1935 (555)	983 (341)	1552 (264)
Pinus nigra	1139 (335)	3550 (1152)	2177 (1104)	2289 (586)
Pinus sylvestris	1130 (237)	2397 (788)	1379 (772)	1635 (379)
Pseudotsuga menziesii	1636 *	2317 (1486)	2029 (1128)	2096 (627)
Conifer Avg.	1240 (195)	2104 (349)	1456 (281)	

* = only one plot produced roots this year

In 2000 and 2002, all eleven species had one major peak of fine root growth in the late spring to summer (**Figures 4.3** and **4.4**). For some species the peaks were distinct (e.g. *P. sylvestris*), while for others the summer peak was part of a pattern of high growth from spring to

summer (e.g. *L. decidua*). In 2001, there was evidence of two peaks, one in early spring and one in mid-summer. Root production was low each year in April-May during leaf expansion (Figures 4.3 and 4.4). This pattern was the same for the six deciduous species as well as the five evergreens, which added a new cohort of needles at this time.

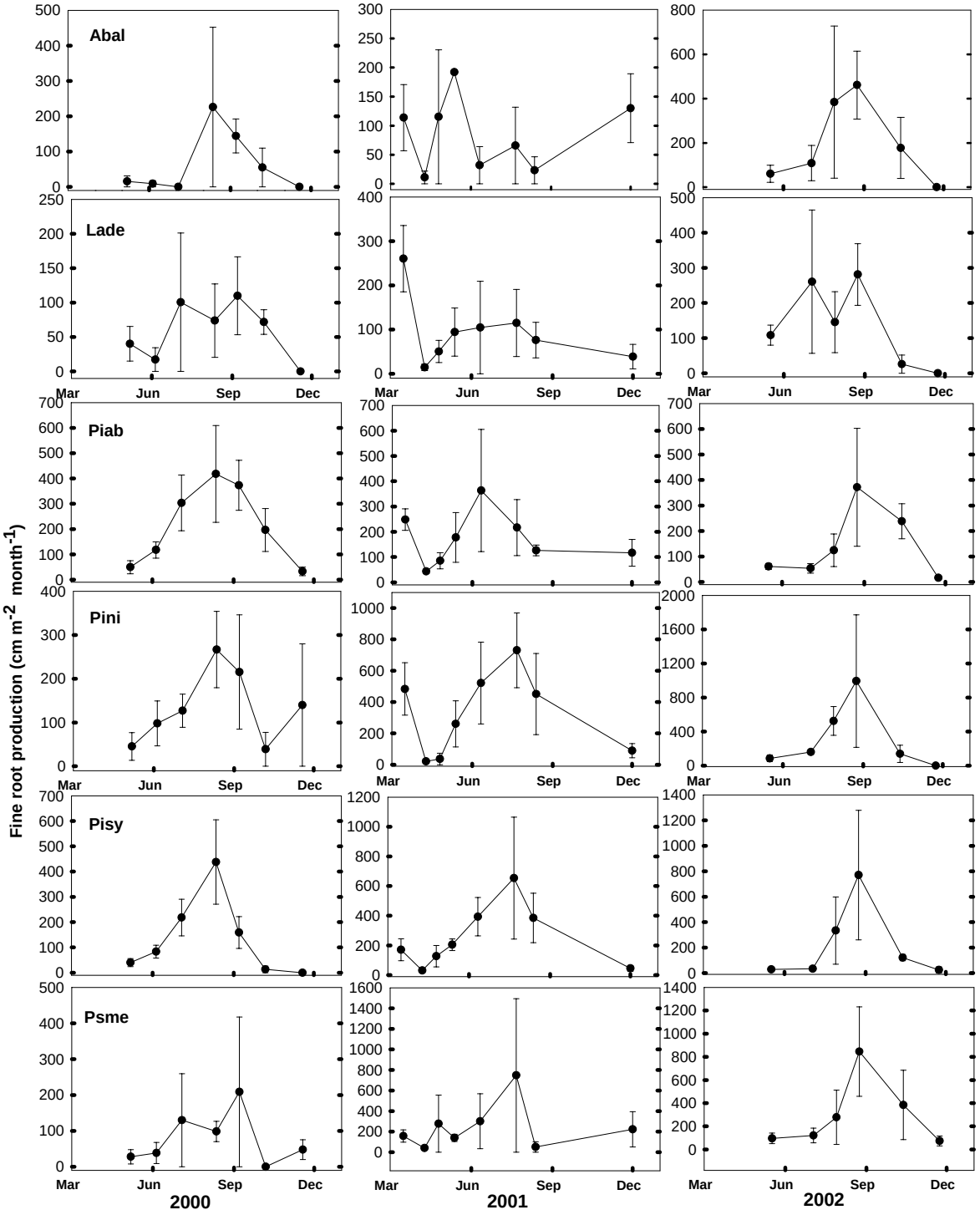

Figure 4.3: Fine root production (± SE) of six conifer species growing in a common garden for the years 2000, 2001 and 2002. Each plot was weighted equally in calculating the seasonal patterns (2-6 plots per species; see text for details). Species are abbreviated with the first two letters of their genus and species name. The first point in each year assumed that root growth did not occur in December or January; calculations were made assuming 1 Feb was the previous sampling date.

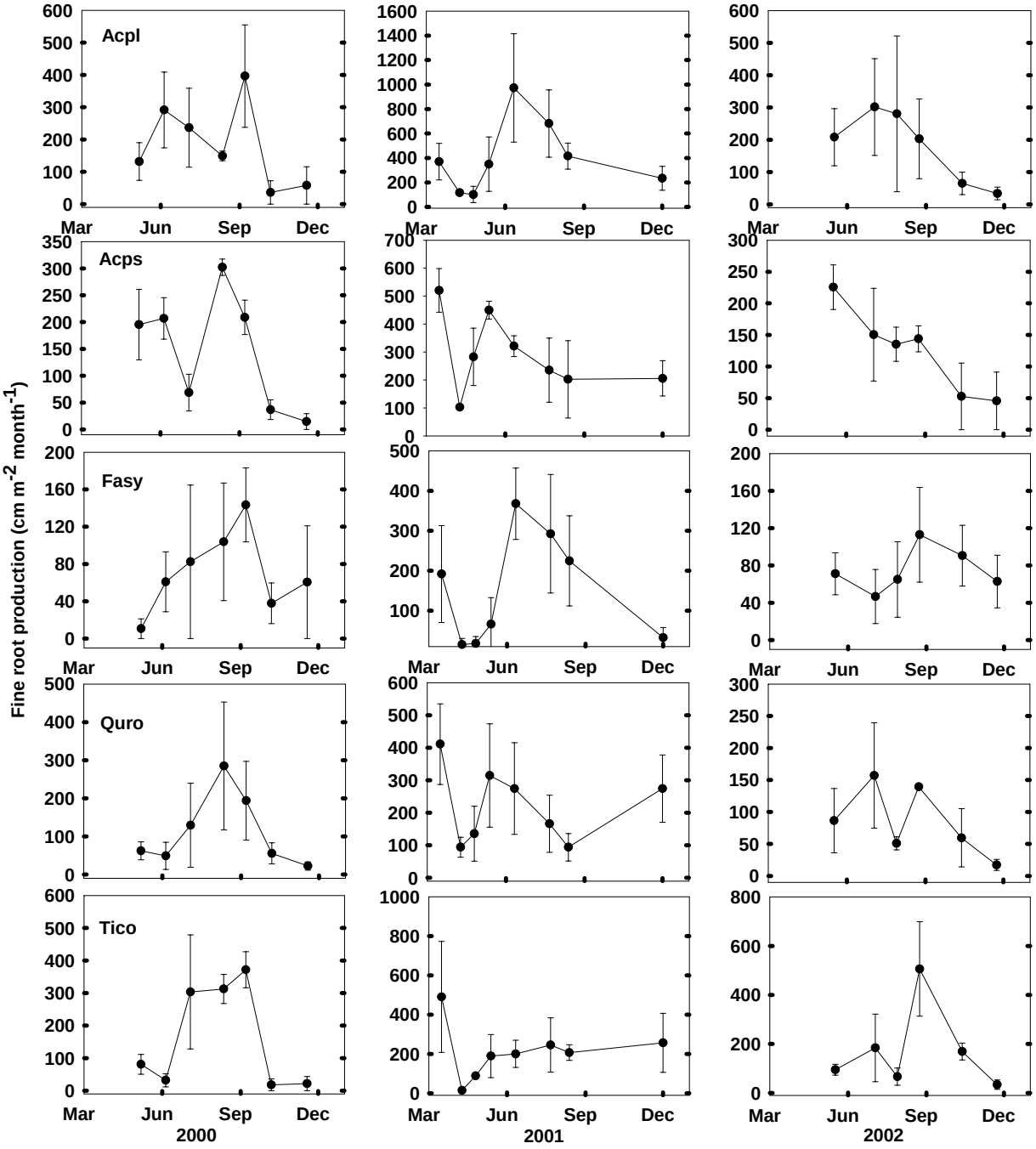

Figure 4.4: Fine root production (± SE) of five deciduous broadleaf species growing in a common garden for the years 2000, 2001 and 2002. Each plot was weighted equally in calculating the seasonal patterns (2-6 plots per species; see text for details). Species are abbreviated with the first two letters of their genus and species name. The first point in each year assumed that root growth did not occur in December or January; calculations were made assuming 1 Feb was the previous sampling date.

Root growth generally peaked during the summer (Figures 4.3 and 4.4). One peak was the rule, but there were a few exceptions. Eight of the eleven species had large amounts of root production between February and March 2001 (Figures 4.3 and 4.4). For four of the species, this was the largest peak of 2001. Peaks of growth were generally not observed in the fall (Figures 4.3 and 4.4). Exceptions to this pattern were *P. nigra* in 2000 and *A. alba* and *Q. robur* in 2001.

The timing and magnitude of root growth peaks varied by year (Figures 4.3 and 4.4). However, the timing of summer peaks within each year was quite similar for all species. In 2000, the peak was around the end of August-beginning of September. In 2001, the peak was earlier, around June-July. In 2002, the peak was again generally around the beginning of September, although a few species had earlier peaks (*Acer* species and *Q. robur*).

Discussion

In this study, we used the minirhizotron technique to observe growth periodicity of roots in six conifer and five broadleaved trees in a common garden for three years. We examined patterns of root production for timing of peaks and the relation of root production to leaf production. While there was substantial year-to-year variation, patterns of root production were quite consistent across species. We found that the commonly held notion of two peaks of root growth during the growing season was often not supported. In addition, conifers and broadleaved trees had similar annual root production patterns. Year-to-year variation in timing of peak fine root growth was also consistent across species.

Compared to sequential soil cores, non-destructive technology, such as minirhizotron camera systems, provide better data on timing of root growth and do not have to correct for over or underestimating production due to simultaneous root growth and mortality (Vogt *et al*. 1998, Tierney and Fahey 2001). With nondestructive observations, we could determine when root growth resumed in the spring and stopped in the fall for many years at the same location. Because of the long root lifespans of these species (Chapter 3), a one-month sampling interval was sufficient to accurately estimate the total number of roots born during that interval.

Total fine root production was quite variable from year to year for all eleven species (Table 4.2). Atkinson (1983) reported differences of 6-20-fold in yearly maximum and minimum root length production of *Malus domesticus* over 10 years. He ascribed the differences he

observed to planting response and to seedling physiology. In the current study, our trees were all at least 30 years old and no longer seedlings.

The greatest total production was in 2001, the same year that we measured a fairly large early spring peak in many species. Hartig (1863) observed a peak in early February, and Engler (1903) reported a similar spring peak for his 14 species. Krueger and Trappe (1967) reported an early spring and a summer peak for two provenances of *Pseudotsuga menziesii* seedlings, and Carter *et al.* (2004) found a small March peak followed by a larger summer peak for *Pinus palustris* in a one-year study in Georgia (Table 4.1). Although the air temperature readings were only 1-2°C during these spring peaks, Krueger and Trappe (1967) found significant root growth for one of their provenances of *P. menziesii* in February with an average soil temperature of 2°C, similar to the conditions in our plots. Because early season growth often relies heavily on starch reserves while later-season growth is supported by current photosynthate (Larcher 2003), in our study it is likely that the higher rainfall in 2000 allowed for greater starch accumulation that year, which in turn promoted high root production early in 2001. It was unfortunate that 2001 was the only year we were able to take images in March. There was no snow on the ground by March of that year and we were able to get the equipment through the plots. In other years, there was too much precipitation or the camera system was being repaired.

We found only a few isolated instances of significant autumn root growth in our species, but none compared to the 20%-60% of maximum peaks reported by Engler (1903), which included some of the same species we used in our study. In the study of Engler there was no summer drought condition in any year which could explain the peaks in autumn. However, Engler was working with saplings (Table 4.1), and the physiological responses of young trees may differ from that of older trees (Lambers *et al*. 1998). Young trees are more likely to have multiple flushes of growth in a year compared to older trees because older trees have higher demands on current photosynthate and tend to maintain a root-to-shoot balance (Reich *et al.* 1980). Lyr and Hoffman (1967) recorded autumn peaks in some species at depths of more than 1 m. In our study, our observations were restricted to a depth of 45 cm, which may be why we did not record more fall peaks of root growth during our study.

The timing of the root growth peaks was not the same each year, but conifers and the broadleaved species demonstrated similar patterns in timing of peak summer production from year to year (Figures 4.3 and 4.4). Reich *et al.* (1980) reported synchrony of root flushes in *Quercus alba* and *Q. marilandica* seedlings; however, we found no previous studies showing consistency of yearly peaks across so many species.

The relative strength of the shoot and root systems as sinks for carbon results in a trade-off in the production of above- and belowground biomass. In this study, we observed that while fine root production could begin early in the spring, it was extremely low at the time of leaf production. After the leaves were fully expanded, root production increased to a peak in late summer and then dropped-off in the fall (Figures 4.3 and 4.4). This alternation of shoot and root growth has been reported repeatedly in studies with seedlings (e.g. *Q. alba*, *Q. marilandica*: Reich *et al*. 1980, *Hevea brasiliensis*: Thaler and Pages 1996) as well as in mature trees (e.g. *Q. alba*: Reich et al. 1980)

Our patterns of annual root production concur with Tierney *et al*.'s (2003) observation that in northern forests which rarely experience dry summers, fine root production tracks temperatures with usually one peak in mid-summer. Although our study site can have dry years, it does not generally experience summer water limitation, and root production basically tracked temperatures each year.

Tierney *et al*. (2003) suggested that the one peak vs. two peak patterns of growth are related to the geographical location of the study site. They linked the presence or absence of summer drought conditions to location. We suggest this may not always be true. But for a few species in 2001, we observed one peak of production over three years in Poland, while studies in geographically similar locations like Germany and Switzerland (Resa 1877, Buesgen 1901, Engler 1903, Puhe 2003) reported two peaks with the second in the fall (Table 4.1).

There are four possible explanations for different patterns of production recorded in similar locations: age of study species, maximum depth of observation, weather conditions the previous year and weather conditions the current year. First, young trees are more likely to have multiple flushes of growth (Reich *et al.* 1980) and second, fall peaks of production were observed at greater depths (Lyr and Hoffman 1967). Third, high starch accumulation the previous-year is used to support high root production early in the spring (Larcher 2003). Fourth, if weather conditions lead to insufficient water availability during the middle of the growing season, there can be a second peak of growth in the fall when soil moisture is again sufficient and the temperatures are still warm enough regardless of typical conditions at the study site. Soil moisture can be insufficient when there is low summer precipitation or when there are increased temperatures leading to increased transpiration which depletes the water faster than it is replenished (Diller 1935).

Variable weather patterns can thus lead to variable patterns of root growth from year to year, as observed in our data. This suggests phenology results based on only 1-year-worth of

data, although common in the literature (Table 4.1), could be considered inadequate for observing true patterns of root production.

Our results suggest that generalizations cannot be made with regard to root production at the species level. *Picea abies* is a good example of this. Engler (1903) and Puhe (2003) both report an early and a fall peak for *P. abies*. This study reports only one late summer peak for the same species. Neither Engler's nor our site experienced summer drought, yet we observed different patterns in root production for the same species at similar latitudes (Switzerland and Poland). It is probable that the differences in root growth peaks are not due to precipitation but to differences in soil characteristics. Soil characteristics and temperature interact with precipitation to influence soil moisture availability (Lowry 1962, Weber and Nkedirim 1998). Engler's site was a nursery on a mountainside at 650 m. Our site at 150 m elevation had a very sandy and fairly nutrient-poor soil.

Our fairly consistent results across eleven tree species for a particular year underscore the benefit of a multi-species study for looking at fine root production. Most studies we have read have not included more than one species or have not distinguished among species in a mixed forest (Table 4.1). Exceptions to this were Resa (1877), Wieler (1894), Engler (1903) and Lyr and Hoffman (1967), but no recent studies. These types of long-term, multi-species studies are essential to improving our understanding of forest ecosystems and belowground carbon dynamics.

Acknowledgements

The authors would like to thank Bartosz Bułaj and Jakub Olesiński for their invaluable help organizing activities in Poland and the countless hours they worked collecting minirhizotron images. We appreciate the diligent efforts of Mariellen Withers for processing the tens of thousands of images that generated these data. We thank the Forestry Personnel with the Siemianice Experimental Forest for collecting the temperature and precipitation data.

J. Withington was supported by an NSF Root Biology Training Grant (NSF DBI 9602255), a William J. Fulbright Foundation Fellowship to Poland and The Women's Welsh Clubs of America. This research was supported by an Eastern European International Supplement to an NSF grant (IBN 9596050). NSF also contributed to the work through the following grants: DEB 01298944 (PSU), DEB 0090069 (UMN) and DEB 0128958 (UMN).

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Chapter 5

Synthesis

Biologists interested in carbon balance and carbon sequestration in ecosystems, especially the potential changes in these processes associated with global warming, generally assume that production and turnover of belowground biomass roughly equals production and turnover aboveground. Our data, based on experiments using minirhizotrons to monitor root growth and mortality, suggest that this assumption is flawed; belowground biomass turnover was not correlated with aboveground biomass turnover in our temperate tree species.

Chapter 2 described a study on two different plastics used for minirhizotron tubes and their potential effects on the results of a root lifespan study. It revealed a problem with assuming that materials, like plastics, are not reactive with the soil and will not affect rhizosphere processes. Plastics, such as cellulose acetate butyrate, can have reactions with the soil solution and influence root growth and lifespan in some species. This means that root studies employing butyrate may be subject to problems with their data. We suggested that all studies using minirhizotron systems report the tube material used and that researchers should be aware of the potential influence of the plastic tubes on their data.

Because different species may have different allometric relationships, eleven species with a wide range in leaf lifespan (0.5 yr to 8 yr) were examined for root dynamics. The eleven species had a similar range in root lifespan (0.5 yr to 3 yr). Root lifespan was calculated in three ways: as median lifespan, as an average lifespan based on the ratio of standing crop to annual root production and as the average amount of time for cumulative root mortality to equal cumulative root production. None were correlated with leaf lifespan.

Only root nitrogen concentration was correlated with median fine root lifespan. Across the eleven tree species, roots with higher nitrogen concentrations did not live as long as roots with lower nitrogen concentrations, potentially linked to increased herbivory. Fungal biomass

associated with the roots likely contributed to these nitrogen measurements; however, both fungal and plant material with high levels of nitrogen attract herbivores, decreasing root lifespan. Also, nitrogen concentrations have been positively correlated with respiration rates (Willis and Yemm 1955) and organisms with high respiration rates tend to have shorter lifespans (e.g. homeothermic animals: Lindstedt and Calder 1981, leaves: Reich *et al*. 1997).

Although other studies have found relationships between specific root length (SRL), tissue density and diameter and root lifespan across species (Anderson *et al*. 2003, Craine *et al*. 2002, Kern *et al*. 2004, Ryser 1996, Wells and Eissenstat 2001), this study did not. Our values of SRL were only measured once, and SRL can fluxuate with field conditions (Eissenstat 1991, Ryser 1998), especially with soil moisture availability (Bell and Sultan 1999). If we had collected more tissue for more estimates over the year, we might have had a better estimate of SRL to put into the regression analysis. Root diameter was not related to fine root lifespan across the eleven species. It is possible that the one average value used for the correlation matrix was insufficient to represent the variability of root diameter of a species (Ryser 1998). When looking at roots within a species, the Cox proportional hazard analysis allowed us to maintain the pairing of each individual root's diameter with its lifespan; diameter had a significant influence on root lifespan for only four of the eleven species.

We also found that the presence of a suberized hypodermal layer (=exodermis) in the roots of the two *Acer* species could explain their relatively long lifespans. Brundrett and Kendrick (1987) also found the presence of an exodermis linked to longer-lived roots of woodland plants. In species with high production, we expected to see high mortality rates indicating high turnover. We did not. The exodermis can help these roots survive for long periods, protecting them from desiccation, pathogens and herbivory. We found exodermis in nonmycorrhizal roots of many of the other species, which we feel could explain the longer lifespans we observed in white versus pigmented roots.

Ecosystem carbon models can greatly benefit from accurate belowground measurements leading to accurate predictions of annual fine root production. To understand soil nutrient dynamics, it is necessary to know how much seasonal variation there is in production and if these patterns are consistent from year to year. Within these two areas, there was particular interest in examining similarities between conifers and broadleaved species.

For all three years and for all eleven species, soil moisture was not limiting in the summer months, and root production patterns seemed to follow average monthly temperatures. Root production peaked in the summer (Figure 4.1). In 2000 and 2002, the peaks were in late

summer; in 2001, the main peak was two months earlier. The peaks for all eleven species occurred at very similar times for all three years; they shifted synchronously.

Although the eleven species had peaks in production at similar times each year, total annual production per plot from year to year was quite variable. Total annual production per plot varied greatly from year to year for each species (Table 4.1). The five broadleaved species exhibited similar year to year patterns with 2001 having the highest production for all and 2002 being the lowest. The conifers did not have a uniform pattern across years.

This study demonstrates the importance of using data from multiple years to calibrate ecosystem models. Even just two years may not be sufficient to make statements about patterns when, as was shown in Chapter 4, production can vary greatly from year to year. Although our data from 2003 was minimal, it was a major drought year in Europe. All species had very little root production after February of that year. We suspect when the rain returned in the fall, there was the possibility for another pulse of root growth. We agree with Tierney *et al*. (2003) that patterns of peaks (one or two) in annual root production are due to the weather patterns at the study site but suggest that different geographical locations are unnecessary to observe these differences.

Currently our boreal forests are mostly conifers in the *Pinaceae* family, like tamarack (*Larix laricina*), black spruce (*Picea mariana*) and white spruce (*P. glauca*). With global warming, the composition of the boreal forests could change rapidly. Consider, for example, if the spruce were replaced with Norway maple (*Acer platanoides*), also a boreal species but one which can tolerate much warmer temperatures. If researchers modeling carbon cycling in boreal forests assume that the maples will have higher belowground biomass turnover because of warmer soil temperatures and higher aboveground turnover, the results from *A. platanoides* and *P. abies* in our study suggest this assumption would be incorrect and could lead to very inaccurate cycling models. *Acer platanoides'* annual root production was 4-times larger than *P. abies*, and its median root lifespan was twice as long (Tables 3.2 and 3.3). For this hypothetical boreal forest that would mean an 8-fold increase in carbon storage belowground, even though the aboveground biomass would be cycling 8-times as fast. Without accurate measurements on root production and lifespan, the models on carbon balance can be off by a significant amount.

Future directions

Anatomy

One of the basic tenets in biology is that form follows function, yet few ecologists study the basic form of their study species while trying to understand its function in an ecosystem. Considering what is known about root physiology, information on anatomy and development can be used to propose anatomical traits that influence root lifespan (Eissenstat and Achor 1999). These traits include factors that increase root density such as the size and number of cortical cells, those that increase physical resistance to stress or herbivory such as thicker cell walls and an exodermis and those which affect root uptake efficiency such as an exodermis and the number of cortical cell layers. It was interesting to find an exodermis present in so many species, and it is possible that an exodermis may be present in the roots of many more species. In particular, a survey of fine root anatomy could help us determine if the presence of an exodermis is fairly common in long-lived roots. I would also like to examine the relationship between root anatomy and root lifespan in a more detailed study. Larger concentrations of lignin in root vascular tissue make it more energetically expensive to construct than ground tissue (Kozlowski 1992); so, does a root system with a greater ratio of stele to total diameter have a longer lifespan than a root system with a smaller ratio due to the construction cost of vascular tissue? Some studies have shown that mycorrhizal infections can increase root longevity (e.g. *Pinus taeda*: King *et al.* 2002) and others have shown the opposite (*Populus gererosa inter americana*: Hooker *et al*. 1995). I would like to examine this relationship in more depth specifically asking if the proportion of cortical layers infected with mycorrhizal hyphae correlates positively with root lifespan, indicating a protective nature of the mycorrhizal association.

Root Nitrogen

The regression correlation coefficient between leaf nitrogen concentration and leaf lifespan was -0.74 in my study. The regression correlation coefficient between root nitrogen:carbon ratio and root lifespan was -0.70 (Figure 3.6). Other values of N/C ratio and fine root lifespan obtained from the literature fit the regression relationship with our data (Figure **5.1**).

Reich (1993) reported *r*= -0.86 for the correlation between leaf N concentration and leaf lifespan for >40 species, and Reich *et al*. (1992) reported *r*= -0.72 for their LEAVES data set. With root N/C and longevity data on more species, we may find, like with the leaf data, the relationship between root nitrogen concentration and root longevity is fundamental across species, although the relationship may be different for herbaceous and woody plants. Craine *et al*. (2002) reported *r*= -0.54 for the relationship between N/C ratio and fine root lifespan for 33 grassland species, mostly grasses and forbs. Measuring root nitrogen concentration would be much easier than waiting three years to estimate median root longevity. If this is a fundamental relationship, it could be a useful tool for researchers needing to estimate root longevity.

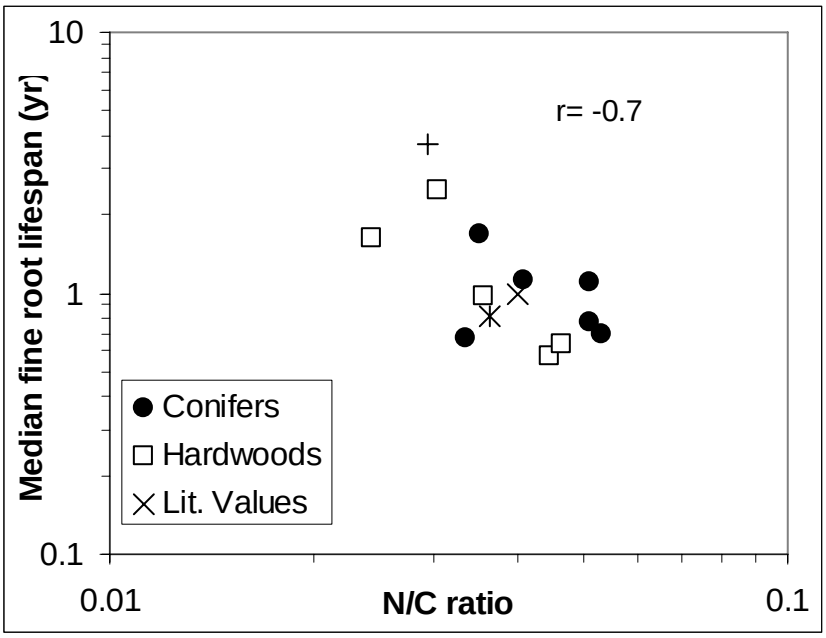

Figure **5.1**: Relationship between fine root N/C ratio and median fine root lifespan presented on a log-log scale. The (+) represents *Quercus alba* (Joslin and Henderson (1997). The (X) represents *Acer saccharum* from sites in Pennsylvania (Wells 1999) and the (∗) represents *A. saccharum* from Michigan (Hendrick and Pregitzer 1993, Pregitzer *et al*. 2002).

Fine Root Lifespan

The *Pinaceae* is an important gymnosperm plant family in temperate and boreal ecosystems. The six *Pinaceae* species in my study had a wide range of leaf lifespans and allowed

us to look for significant relationships within a family. But we still had only 11 species total to look for a relationship between root and leaf lifespan. Most of the leaf studies which have shown relationships between leaf lifespan and leaf traits have included hundreds of species from a wide range of habitats (e.g. Reich *et al*. 1992). As in leaves, would a relationship between fine root lifespan and root traits be stronger with data on species from a wider range of habitats? Many tropical broadleaved trees from many plant families are evergreen; many shrubs in Mediterranean climates are evergreen. Would these types of evergreens have a positive relationship between fine root and leaf lifespan? Would the inclusion of root lifespan data on broadleaved, deciduous and evergreen trees from tropical and neotropical habitats to our current data set still show no relationship between root and leaf lifespan or would the expansion of the data set reveal a positive relationship between the two?

Fine root production

Understanding patterns in fine root production increases our knowledge of plant growth strategies and ecosystem carbon cycling. It is a simple characteristic that is not so simple to predict. More basic research is needed to better understand root growth patterns and how they relate to plant growth strategies. If we continued to measure root production in the plots in Siemianice, would the one peak pattern continue to be observed each year? With measurements of soil moisture and temperature, would we observe the peaks following soil temperature closely as long as soil moisture was adequate? Lyr and Hoffman (1967) saw a second peak at deeper depths in the soil than our current tubes can measure. I would like to add two 100-150 cm tubes to each plot to follow roots at 60-100 cm depths to observe root production at deeper depths. It is quite likely that we would also see a small peak in the fall at closer to 1 m depth.

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Appendix A

Equations

Data analyses and data transformation steps are generally written out in manuscripts, especially when the focus of the investigation does not focus on the equations. However, some of

these steps can be hard to follow. What follows are some of the equations used in the thesis chapters.

Equation 1 (Chapters 2, 3, 4: Methods)

Calculating root birth and death dates

Root birth date= ½*[imaging date on which root 1st seen – date of previous imaging session]

Root death date= ½*[imaging date on which root 1st gone – date of previous imaging session]

= ½*[imaging date root 1st wrinkled – date of previous imaging session]

Equation 2 (Chapters 2, 3, 4: Methods)

Calculating root depth in soil from minirhizotron windows

Root depth in soil=

Equation 3 (Chapter 2: Methods)

Calculating root mass from root counts in minirhizotron windows

Root length= (root count) * (slope of regression line of root number to root length)

Root mass= (root length) * (specific root length of species)$^{-1}$

Equation 4 (Chapters 2, 3, 4: Methods)

Cox proportional hazard model

Ratio of risk for individual i to risk of individual j= $\exp \{\beta_1(x_{i1}-x_{j1})+ \ldots+\beta_k(x_{ik}-x_{jk})\}$

Equation 5 (Chapter 2: Discussion)

Fraction of fine tissue production contributed by fine roots

Fine root production fraction =

Equation 6 (Chapter 3: Methods)

Average root lifespan from cumulative root production and cumulative mortality

Say cumulative root production = 100 roots on day 50

and cumulative root mortality = 100 roots on day 375

then number of days for mortality to equal production = 325.

We made this calculation 5 times for each species and then calculated the harmonic mean to control for very large or very small estimates.

Average lifespan (P-M)= $\{[\text{Sum (estimates}^{-1})] * 1/5\}^{-1}$

Equation 7 (Chapter 3: Methods)

Average root lifespan from standing crop and annual root length production against tubes

Root length= (root count) * (slope of regression line of root number to root length)

Average root lifespan (SC/Prod)= ½ * [max standing crop near tubes (2000, 2001) * avg. annual root length production $(2000, 2001)^{-1}$]

Equation 8 (Chapter 4: Methods)

Average root production based on plot percent of annual production

Plot average= 100*(number of roots born) / (total number of roots born that year)

Average production= (Sum of plot averages) / (number of plots)

VITA

Jennifer Marie Withington

Jennifer was born in Cleveland, OH in 1972. She was a member of the 1990 Ohio State Champion Academic Decathalon Team at Willoughby South High School and graduated in 1990. Jennifer graduated with a B.S. from Heidelberg College of Tiffin, OH in 1994 with a double major in Environmental Biology and Mathematics. She graduated with a M.S. in Biology from the University of Iowa in 1997; her thesis was on "The Effects of Habitat Fragmentation on the Mycorrhizal Associations of *Phlox pilosa*" in the lab of Dr. Stephen Hendrix. Jennifer chose to continue her study of root ecology at Penn State. She was accepted as a part of the second round of students in an NSF Training Grant in Root Biology.

Jennifer enjoys singing in choirs, both church and school. She prefers singing mezzo soprano but will sing alto when needed. One of her most cherished choir experiences was in Poland. In 2001, during her residence on a Fulbright Fellowship to collect data for her dissertation, Jennifer got to sing with the *a capella* choir of the Medical University of Poznan. She sang in Polish, Italian and Latin- mostly hymns. The best pieces, though, were a set from *Carmina Burana*- what a performance rush!

Jennifer met her husband, RP Withington III, at Penn State at the Graduate Student Association volleyball games in summer 1998. They dated during the school years and emailed lots during the three summers while she was in Poland doing fieldwork. They were married in October 2001, six weeks after she returned from her last field season in Poland. R. Preston Withington IV was born in August 2003. He is a delightful bundle of energy and joy and the reason it took Momma a bit longer to finish her work than anticipated.