INFLUENCE OF ROOT BRANCHING ORDER ON FINE ROOT SUBSTRATE QUALITY AND DECOMPOSITION IN A *Pinus palustris* ECOSYSTEM

By

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To my parents and my grandparents, for fostering my education

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Fine root decomposition represents a large flux of carbon and nutrients in forest ecosystems. Yet we have poor understanding of how fine root decomposition and its controls vary among roots of different structure and function within the fine root network. This study investigated variation in morphological and chemical indices of decomposer substrate quality among fine roots of different orders and species, including *Aristida stricta*, *Pinus palustris*, and *Quercus laevis*. A buried litterbag study was also conducted, to determine if differences in initial substrate quality among orders would result in different rates of mass loss and nutrient (nitrogen, phosphorus, and calcium) release over one year. Trends in substrate quality indices generally supported the hypothesis that the smallest, most distal roots comprise a higher quality substrate for decomposers and could decay faster than larger, more proximal roots. After twelve months of decomposition, we were not able to separate orders precisely, but composited them into groupings (orders 1–3, "first order" versus orders 4–5, "fourth order"), based on extant branch patterns and root length and diameter. Surprisingly, no differences between orders were observed, either in decomposition rate, as modeled by a single exponential decay function, or in net nutrient release. Both orders had decay rate constants of about 0.3 yr^{-1} and lost from 19–27% of their original mass and nutrient content. Concentrations of nitrogen, phosphorus, and calcium

were nearly constant throughout decomposition, resulting in nutrient dynamics that closely followed mass loss patterns. Mass loss and nutrient dynamics were poorly correlated with initial substrate quality indices. These unexpected patterns are likely due to large errors in estimation of mass loss, but recalcitrant forms of mycorrhizal nitrogen, phosphorus, or calcium present in distal first order roots may partially explain the lack of correlation between apparent substrate quality and root decomposition rates. Future studies must be able to estimate mass loss among orders with greater precision and should attempt to account for the influence that tissues of mycorrhizal fungi may have on fine root decomposition.

CHAPTER 1
LITERATURE REVIEW

Studies of carbon (C) cycling are fundamental to terrestrial ecosystems because they allow us to monitor the flow of energy, in the form of C compounds, into, within, and out of ecosystems. Yet while C cycling has been well studied aboveground, belowground C fluxes and their controls are not as well understood (Norby and Jackson 2000). Because belowground C fluxes compose a substantial portion of the total C budget in terrestrial ecosystems, they represent a critical missing link in our understanding of C cycling (Zak and Pregitzer 1998; Norby and Jackson 2000).

Decomposition of fine roots is a fundamental process of C and nutrient cycling in forested ecosystems. Although coarse roots may comprise the bulk of the belowground standing biomass in woody species (Carins et al. 1997), fine roots account for a disproportionate amount of C flux due to higher rates of production and mortality (Grier et al. 1981).Grier et al. (1981) report that fine roots made up only 2–12% of total biomass in 180- and 23-year old *Abies amabilis* forests, but their production and turnover accounted for 69–55% of the net primary productivity, respectively. Likewise, Steinaker and Wilson found that fine roots accounted for 80–90% of all litter produced in a young (<40 yr old), natural *Populus tremuloides* forest. Despite their low standing biomass, fine root decomposition represents a substantial C flux.

Decomposition rates of plant litter, including root detritus, are controlled primarily by three factors: climactic or environmental conditions, the composition of the decomposer community, and the substrate quality of the plant litter (Aerts 1997). While climate seems to be the most important control on aboveground decomposition (Aerts 1997), decomposition of roots may be controlled first and foremost by the substrate quality of root litter (Silver and Miya 2001). This difference in controls of above- and belowground decomposition may result from the

ability of the soil to buffer belowground decomposition from the extremes of moisture and temperature found on the soil surface (Silver and Miya 2001).

Because of the importance of substrate quality in controlling rates of root decomposition, researchers have often tried to correlate decay rates with various physical or chemical indices of litter quality. For example, high concentrations of nitrogen (N, Berg 1984; Gijsman et al. 1997; John et al. 2002), phosphorus (P, Berg 1984; Conn and Day 1997; Gijsman et al. 1997; Scheffer & Aerts 2000; Silver and Miya 2001), calcium (Ca, Bloomfield et al. 1993; Silver and Miya 2001), and C "extractives" (generally the more soluble portion of C compounds in plant material, McClaugherty et al 1984; Berg et al. 1987), are frequently positively correlated with decay rates of plant litter. In contrast, cellulose (Chen et al. 2002; John et al. 2002) and lignin (Berg 1984; Arunachalam et al. 1996; Silver & Miya 2001; Chen et al. 2002; John et al. 2002) concentrations and the C:N (Cotrufo and Ineson 1995; Arunachalam et al. 1996; Gijsman et al. 1997; King et al. 1997; Silver and Miya 2001), C:P (Gijsman et al. 1997; King et al. 1997; Scheffer and Aerts 2000), lignin:N (Arunachalam et al. 1996; Gijsman et al. 1997; Silver and Miya 2001; Chen et al. 2002) and lignin:P (Silver and Miya 2001) ratios are negatively correlated with decomposition rate. We have also included the ratio of root surface area to volume in this study, since root litter with a high surface area to volume ratio exposes a greater proportion of its tissues to microbial attack, which may speed decomposition.

Our ability to accurately assess belowground C fluxes due to fine root decomposition rests upon improved comprehension of variation in morphology and physiology among fine roots (Norby and Jackson 2000; Wells et al. 2002; Tierney and Fahey 2002; Guo et al. 2004). Previous studies of fine root decomposition have viewed fine roots as homogenous cohorts, defining fine roots as being less than 5, 2, or 1 mm in diameter. It was assumed that variation among roots of

these sizes was either inconsequential, or that such variation was normally distributed and could be averaged over fine roots of different sizes within the chosen diameter range. It is now increasingly recognized, however, that many parameters of root form and function, such as diameter, specific root length (SRL), longevity, and N concentration vary within the fine root guild (Pregitzer et al. 1997; Wells and Eissenstat 2001; Pregitzer et al. 2002; Wells et al. 2002; Guo et al. 2004). Furthermore, some fine root parameters, such as SRL and N concentration, are skewed toward the smallest roots (Guo et al. 2004), and averaging measurements over all roots may result in an inaccurate understanding of fine root dynamics. Consequently, this variation among fine roots has probable implications for C fluxes of ecosystems (Pregitzer et al. 1998).

To capture some of this variability, fine roots have been subdivided into diameter classes within the 2 mm range, yet subdividing fine roots into several diameter classes has shortcomings as well. Selection of diameter limits is arbitrary or at least subjective, and diameter classes do not fully capture functional differences that correlate with root branching patterns (Pregizter et al. 1997; Pregizter et al. 1998; Fitter 2002; Pregizter et al. 2002; Pregitzer 2002). Such differences in structure and function control patterns of C allocation to fine roots (Fitter 2002) and ultimately the fate of C as it leaves the plant (Guo et al. 2004), via mortality and then decomposition.

Root order classifications offer a method for demarcating functional variation among fine roots that is based on the hierarchical branching architecture of the root system (Pregizter et al. 1997; Fitter 2002; Pregitzer 2002). Implicit in the concept of root order is the idea that roots within a root network are linked: If a root dies, all the daughter roots that branch from it will also die. This phenomenon is not completely captured using diameter classes, the smallest of which commonly in use is 0–0.5 mm. There may be as many as three to five interconnected root orders among all roots less than 0.5 mm in diameter (Pregitzer et al. 1997).

Although few data are available, several researchers have found variation among root orders in morphology and tissue chemistry traits used as indices of substrate quality (Pregitzer et al. 1997; Pregitzer et al. 2002; Guo et al. 2004), which, in turn, could lead to variation in fine root decomposition rates. Concentrations of N have been shown to decrease across order, from distal to proximal (Pregitzer et al. 1997; Pregitzer et al. 2002; Guo et al. 2004), while C:N ratios (Pregitzer et al. 1997; Pregitzer et al. 2002) and cellulose concentrations (Guo et al. 2004) increased concurrently. This implies that the smallest, most distal roots may decompose faster than more proximal root orders. Extractives concentration and lignin concentration have not been shown to vary substantially across orders (Guo et al. 2004), perhaps indicating that they will contribute little to variation in decay rates, at least within a species.

Unfortunately, many substrate quality indices have not yet been measured among different root orders. Root Ca concentration was the single best predictor of decay rates in a global review of root decomposition, correlating positively with decay rates (Silver and Miya 2001), but patterns of Ca concentration across orders are unknown. High P concentrations and low C:P ratios have sometimes been correlated with increased plant litter decay rates (Heal and French 1974; Schlesinger and Hasey 1981), yet P concentrations among different root orders have never been published. High lignin:N ratios, which are thought to inhibit decomposition (Arunachalam et al. 1996; Gijsman et al. 1997; Silver and Miya 2001; Chen et al. 2002), have also gone unreported in the fine root literature. Furthermore, the effects of these litter quality indices may vary with order, further influencing the decomposition patterns among roots of different order.

Another factor that limits our understanding of litter quality among root orders is the unresolved issue of nutrient retranslocation (Gordon and Jackson 2000). At present, no conclusive evidence of nutrient retranslocation in roots has been found

(Gordon and Jackson 2000), and most root decomposition studies rest on the assumption that retranslocation is insubstantial. Yet, if roots do retranslocate one or more nutrients before they die, this could substantially alter the tissue chemistry and substrate quality of root litter, which may modify fine root decay rates. Furthermore, the amount of retranslocation of some nutrients could be variable across root orders. Conversely, if no retranslocation occurs in roots, as is commonly assumed, mortality of the smallest, most distal roots could represent a substantial N cost to the plant, since these roots have higher N concentrations (Pregitzer et al. 1997; Pregitzer et al. 2002; Guo et al. 2004) and shorter lifespans than more proximal roots (Wells et al. 2002). The difficulty of reliably collecting fresh, undecomposed root litter will likely make the issue of root retranslocation intractable for some time to come.

Whether or not roots retranslocate nutrients before dying, the available data suggest that the assumption that fine root decomposition rates can be studied without regard to a root's position within the root network (i.e. order) may be invalid (Pregitzer et al. 1997). The systematic differences in morphological and tissue chemistry variables among root orders could result in parallel variation in fine root litter quality and decay rates (Pregitzer et al. 1997). At present, this hypothesis remains tested, and there are currently no data on patterns across order for several potential litter quality indices, including Ca and P concentrations and lignin:N, C:P, and lignin:P ratios. Given the important role of morphology and tissue chemistry variables as drivers of fine root decomposition and to better understand the belowground C fluxes from decomposition and their controls, this study will address the following objectives: 1) quantify variation in morphological and chemical litter quality indices among fine root orders; 2) quantify and compare rates of decomposition among different orders of fine roots; and 3) examine the effects of litter quality indices on the decomposition of fine roots of different orders. We

hypothesize that: 1) across species, morphology and tissue chemistry indices of substrate quality will vary predictably such that distal roots will have lower values of length, diameter, cellulose concentration, C:N ratio, C:P ratio, lignin:N ratio, and lignin:P ratio and higher values of surface area to volume ratio and higher concentrations of N, P, and extractives than more proximal root orders, while calcium and lignin concentrations will remain constant across orders; 2) decay rates will vary within the fine root guild, decreasing across order as one moves from distal to more proximal roots; 3) differences in decay rates among orders will be correlated with initial fine root tissue chemistry

CHAPTER 2
DIFFERENCES IN FINE ROOT MORPHOLOGY AND TISSUE CHEMISTRY AMONG ROOT ORDERS

Introduction

Fine roots (<2 mm in diameter) exert an important influence on carbon (C) and nutrient fluxes in many forested ecosystems (Jackson et al. 1997). While fine roots may account for as little as 2–12% of total living biomass they are a large sink for photosynthate allocation, accounting for as much as 55–69% of net primary productivity (Grier et al. 1981). Fine roots are also a large source of detrital inputs to the soil, comprising up to 90% of annual litter inputs, above- and belowground, in a young (<40 yr old), natural *Populus tremuloides* stands (Steinaker and Wilson 2005). In spite of the important role of fine roots in C and nutrient budgets of forest ecosystems, most studies of decomposition have concentrated on decay of aboveground litter, resulting in only a rudimentary understanding of belowground decomposition (Silver and Miya 2001; Chen et al. 2002).

Despite the lack of information on belowground decomposition, it is generally agreed that both above- and belowground decomposition are controlled by the same three factors: environment (e.g. climate, soil moisture, and fertility), litter chemistry, and the soil community (Aerts 1997). Of these factors, the environment in which decomposition takes place is thought to be the most important control on decay of aboveground litter; litter chemistry is of secondary importance, followed by the decomposer community (Aerts 1997). The importance of litter chemistry as a regulator of decomposition rates has led to the development of litter quality indices based on one or more chemical constituents of the litter in order to model decay rates of aboveground litter. Commonly cited indices of aboveground litter quality include concentrations of nitrogen (N, Pandey and Singh 1982; Berg 1984; McClaugherty and Berg 1987; White et al. 1988; Taylor et al. 1989), phosphorus (P, Schlesinger and Hasey 1981; Staaf and Berg 1982;

Taylor et al. 1989), calcium (Ca, Van Cleve 1974; Bloomfield et al. 1993), C "extractives" (generally the readily soluble fraction of plant material, Allison and Vitousek 2004), acid-soluble cellulose (Muller et al. 1988; Aber et al. 1990), and acid-insoluble lignin (Fogel and Cromack 1977; Meentemeyer 1978; Schlesinger & Hasey 1981; Melillo et al. 1982; Pandey and Singh 1982), as well as ratios of C:N (Jensen 1929; White et al. 1988; Taylor et al. 1989; Hendricks et al. 2002), C:P (Xuloc-Tolosa et al. 2002; Hirobe et al. 2004; Xu et al. 2004; Xu and Hirata 2005; Rejmánková and Houdková 2006), lignin:N (Melillo et al. 1982; Blair 1988; White et al. 1988; Taylor et al. 1989; Hendricks et al. 2002), lignin:P (Aerts 1997), and surface area to mass (Gillon et al. 1993; Maloney and Lambert 1995).

In contrast, for decomposition belowground, litter chemistry seems to be the most important regulator of decay, while environmental factors play a secondary role, perhaps due to the ability of the soil to buffer belowground decomposition from the extremes of temperature and moisture found at the soil surface (King et al. 1997; Silver and Miya 2001). Given the primary role of litter chemistry in controlling rates of belowground decomposition, researchers have attempted to model decay of belowground litter using many of the same litter quality indices utilized in aboveground decomposition studies. Belowground litter quality indices have included concentrations of N (Berg 1984; Conn and Day 1997; Gijsman et al. 1997; John et al. 2002), P (Berg 1984; Conn and Day 1997; Gijsman et al. 1997; Scheffer & Aerts 2000), Ca (Bloomfield et al. 1993; Silver and Miya 2001), C extractives (McClaugherty et al. 1984; Berg et al. 1987), acid-soluble cellulose (Scheffer and Aerts 2000; Chen et al. 2002; John et al. 2002), and acid-insoluble lignin (Arunachalam et al. 1996; Silver & Miya 2001; Chen et al. 2002; John et al. 2002), as well as ratios of C:N (Cotrufo and Ineson 1995; Arunachalam et al. 1996; Gijsman et al. 1997; King et al. 1997; Silver and Miya 2001), C:P (Gijsman et al. 1997) and lignin:N

(McClaugherty et al. 1984; Arunachalam et al. 1996; Gijsman et al. 1997; Chen et al. 2002). The surface area to volume ratio (SA:V ratio) is also included in the current study as a potential indicator of fine root decomposition rates because roots that have a high surface area relative to their volume will expose a greater proportion of their tissues to degradation by microbial enzymes.

Although past studies of fine root decomposition have measured many different litter quality indices, they have generally assumed that variation in litter quality (and thus in decay rates) among roots <2 mm in diameter is either negligible or that it is normally distributed and can simply be averaged over all fine roots without regard to their size or position within the root system. Consequently, differences in litter chemistry or decomposition rates within the fine root guild have seldom been reported. However, there is a growing body of evidence suggesting that there are significant physiological differences (nutrient concentrations, maintenance respiration rates, turnover rates, etc.) that follow root branching structure as quantified by fine root order (Pregitzer 2002).

This variation among fine roots is likely to have important implications for rates of biogeochemical cycling in forest ecosystems (Pregitzer 2002), including rates of decomposition. At the ecosystem scale, root decomposition is controlled firstly by rates of root turnover, which determine the flow of root detritus to decomposer organisms, and secondly, by the rate at which decomposers mineralize dead root material. Both of these factors likely depend on root order. Fine root lifespans have been shown to increase as one moves from distal to proximal within the fine root branching system (Pregitzer 2002), implying that rates of turnover vary within the fine root system, with small, distal roots turning over more rapidly and potentially supplying a disproportionate amount of detrital inputs to the soil (Wells and Eissenstat 2001). Moreover, fine

root tissue chemistry has also been shown to vary with branch order, potentially impacting decay rates at the individual root level. Recent research has shown a systematic decrease in N concentration and increase in C:N ratio as one moves from distal to more proximal root orders (Pregitzer et al. 1997; 2002; Guo et al. 2004). Since decomposition rates often correlate positively with N concentration and negatively with C:N ratio, it seems reasonable to believe that fine root decay rates will also vary by order, tracking the trends in N concentration or C:N ratio.

Unfortunately, our knowledge of the variation in litter chemistry across different root orders is rather limited. Only some of the potential litter quality parameters, such as N concentration, C fraction concentrations, and the C:N ratio, have ever been measured across fine root orders, and these only for a handful of species and life forms, primarily trees. More work is needed to determine fine root tissue chemistry trends across orders and how these patterns vary among a wider variety of taxa and life forms.

Given the importance of litter chemistry as a control on fine root decomposition rates and the need for improved understanding of the variation in litter chemistry among fine roots, our objective was to describe the heterogeneity in morphology and tissue chemistry within the fine root guild across three species, representing various taxa, life forms, and mycorrhizal types. We compared root length, diameter, surface area to volume ratio (SA:V ratio), concentrations of N, P, Ca, and serial C fractions and the ratios of C:N, lignin:N, C:P, and lignin:P of different fine root orders. We hypothesized that across species, as one moves from distal root tips to the more proximal root orders, 1) there will be significant differences in morphological and tissue chemistry traits that have the potential to influence fine root decomposition rates, such that 2) length, diameter, cellulose concentration, C:N ratio, C:P ratio, lignin:N ratio, and lignin:P ratio

will increase; 3) SA:V ratio, and concentrations of N, P, and extractive C, will decrease; and 4) concentrations of calcium and lignin will remain fairly constant across orders.

Materials and Methods

Study Site and Species

Roots were collected from a common site at the Joseph W. Jones Ecological Research Center at Ichauway in southwest Georgia, USA (31˚13′N, 84˚29′ W), which is in the Lower Coastal Plain and Flatwoods Province described by McNab and Avers (1994). The collection site was on an upland sand ridge with an overstory dominated by *Pinus palustris* P. Mill. (longleaf pine) and scrub oaks, predominantly *Quercus laevis* Walt. (turkey oak) and *Q. margaretta* Ashe ex Small (runner oak) (Goebel et al. 2001; Wilson et al. 2002). *Aristida stricta* Michx. (wiregrass) dominates the understory (Mitchell et al. 1999; Goebel et al. 2001). The soils have been classified as Typic Quartzipsamments and are characterized by coarse sands over 2.5 m deep containing little organic matter and having weak soil horizons and little aboveground litter due to frequent fire (Goebel et al. 2001). Representing a variety of taxa, growth habits, and mycorrhizal types, three of the dominant species of the longleaf pine-wiregrass ecosystem were chosen for study: *P. palustris*, an ectomycorrhizal conifer; *Q. laevis*, an ectomycorrhizal deciduous broadleaf tree; and *A. stricta*, an arbuscular mycorrhizal C4 grass.

Root Excavation

During October 2005, three replicate root samples of each species were collected from arbitrarily selected plants growing in a common site. Roots were carefully excavated by hand using spades and shovels. For all species, root networks were followed toward the base of the plant until an estimated five to seven orders were obtained, and then severed from the rest of the root system. Following collection, all roots were then briefly rinsed of soil in tap water, placed in zipper seal bags and stored on ice for transportation back to the lab within 4 hr.

To ensure that the roots collected were of the desired species, the following two precautions were taken: (1) until species could be distinguished by traits such as form, size, and color, roots were followed toward the base of the plant so that we could be sure the excavated roots belonged to the species of interest, and (2) roots of a given species were collected from areas where that species was concentrated and where species with similar roots were mostly excluded. Since roots of most grass species at the site looked very similar, *A. stricta* roots were clipped directly from individual plants that were excavated to a radius and depth of approximately 30–40 cm, leaving their root systems attached. This method allowed for collection of the most proximal root orders at the base of the plants, which would have been impractical to collect for the trees.

Root Processing

After returning the excavated roots to the lab, roots were stored at 4°C for up to two weeks, then transferred to a freezer (-20°C) until they could be freeze-dried within one to three weeks. After freeze-drying, roots of each species were refreshed in deionized water (1°C) to increase pliability. They were then teased apart into individual root networks, and soil and other foreign material were removed in deionized water (1°C). Then the roots were dissected into the first five root orders using methods similar to those of Pregitzer et al. (2002) and Guo et al. (2004). As the method of excavation of *A. stricta* roots allowed us to collect the most proximal orders, order classification for this species followed the "centrifugal", or ontogenetic topological system described by Berntson (1997), in which the largest roots at the plant base are considered first order roots. In contrast, orders were assigned to *P. palustris* and *Q. laevis* roots using a centripetal approach (Berntson 1997). Only the centripetal method of designating root order was appropriate for the tree species, since the whole root system was not excavated. Despite the pains taken throughout root sample collection, there were inevitably roots with incomplete branching

due to breakage during excavation. This makes assigning orders to roots difficult when using a centripetal ordering system. Similar to Guo et al. (2004), in cases where we could not be certain of root order, the order was estimated using a combination of the extant branching and a combination of the root's length and diameter.

In the case of ectomycorrhizal species (*P. palustris* and *Q. laevis*), roots that showed signs of ectomycorrhizal colonization (bifurcation, fungal mantle, etc.) were treated as first order roots. Throughout dissection, roots were kept moist in deionized water (1°C). During dissection, a subsample of approximately 15–80 roots of each order per sample collected was measured for diameter at the midpoint and length, using a 40x stereomicroscope with an ocular micrometer (± 0.025mm), and each root measured was treated as a replicate sample during data analysis. Broken roots were not used for morphology assessments because the entire root length was not available. If roots were too long to be measured for length with the stereomicroscope, a ruler was used to measure length to the nearest 0.5 mm. Length (l) and diameter (d) data were used to calculate the SA:V ratio using equation 2-1. For the chemical analyses, some root orders were

$$\text{SA:V} = l \cdot d \cdot \pi \,/\, l \cdot \pi \,(0.5 \cdot d)^2 \qquad (2\text{-}1)$$

composited in order to obtain enough sample mass to perform all of the chemical analyses. The orders composited were first and second order of *P. palustris* and *Q. laevis* and orders 4–7 of *A. stricta*. We found significant, but barely discernable differences in diameter of the two most distal orders of *P. palustris*, the three most distal orders of *Q. laevis*, and the four most distal orders of *A. stricta* (Table 2-2), lending some support to the decision of which orders to composite for tissue chemistry analyses.

Root Chemical Analyses

After dissection, roots were ground and homogenized using a SPEX 8000-D mixer mill (SPEX, Edison, NJ). Ground root material of each species and order were subjected to a variety

of tissue chemistry analyses. Total C and N were measured with a Thermo FlashEA 1112 NC analyzer (Thermo Fisher Scientific, Inc. Waltham, MA, USA). P and Ca were analyzed by a common digestion in sulfuric acid (Parkinson and Allen 1975). P concentration was then determined using a Lachat QuikChem Series 8000 flow injection analyzer (Lachat Instruments Inc., Milwaukee, WI, USA), while Ca concentration was measured on a Perkin-Elmer Model 5100 PC atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). Root serial C fraction concentrations (C "extractives", acid soluble cellulose, and acid insoluble lignin), were analyzed using a modification of Goering and Van Soest's (1970) forage fiber technique. Ash content was determined by combustion in a Fisher Scientific Isotemp model 126 muffle furnace (Thermo Fisher Scientific, Inc. Waltham, MA, USA) for 6 hr at 500°C. All tissue chemistry concentrations have been expressed on an ash-free, dry mass basis (Bledsoe et al. 1999).

Data Analysis

The effect of order on fine root morphology and tissue chemistry was evaluated for each species by a one-way ANOVA, using the GLM procedure of SAS (SAS 9.1 2002-2003, Cary, NC, USA). All data were rank transformed to satisfy the assumptions of normality and homogeneity of variance, and Fisher's protected LSD was used to determine which morphology and tissue chemistry parameters were significantly different among orders, within each species.

Results

Fine Root Morphology

Fine root order had a significant effect on root length, diameter, and SA:V ratio for each species (Table 2-1), as evidenced by a trend in increasing root length and diameter and decreasing SA:V ratio from distal to proximal (Table2-2). The only exceptions to this pattern were found in *A. stricta* where second order was generally longer than first order and where sixth order was significantly smaller in diameter (but not length) than seventh order. Although this

difference in diameter was very slight, it resulted in a significant difference in SA:V ratio between sixth and seventh orders (Table 2-2). The cortex on seventh order roots generally appeared to be intact, whereas in the more proximal orders of *A. stricta*, the cortex appeared to have degenerated (Henry and Deacon 1981), possibly explaining the larger than expected diameter of seventh order roots of that species.

Fine Root Tissue Chemistry

Fine root tissue chemistry parameters often varied significantly with order (Table 2-1), but patterns across order were often not as clear as those of fine root morphology were (Tables 2-3). Although order did have a significant effect on C concentrations in *A. stricta* and *Q. laevis*, C concentrations did not vary systematically across orders for any of the species. Concentrations of N and P generally decreased across orders, from distal to proximal for the tree species. Patterns of increasing C:N and C:P ratios seemed to be driven primarily by increasing N and P concentrations, as the differences in C concentrations were relatively small (coefficients of variation ≤3.6% across all orders for each species, data not shown). Trends in N and P concentrations were less apparent for *A. stricta*. N concentration was significantly higher in roots greater than or equal to fourth order, but there were no significant differences among orders one through three of *A. stricta*. Similarly, there were no clear trends across order in P concentration or C:N and C:P ratios for this species. Among the C fractions, extractives did not vary consistently across orders, while cellulose increased in the trees from proximal to distal, but did not vary significantly in *A. stricta*. Patterns of lignin across orders revealed different trends, depending on the species, but tended to decrease from distal to proximal within root branching networks. Although differences among species were not statistically tested, the extractives concentration was substantially higher and the lignin concentration substantially lower in *A. stricta* compared to the trees.

Discussion

Fine Root Morphology and Tissue Chemistry Patterns

As we hypothesized, there were significant systematic differences in morphology among root orders (Table 2-1), and length and diameter increased across root orders from distal to proximal, while the SA:V ratio decreased (Table 2-2). Variation in fine root morphology was far greater between root orders than within a root order, as reflected in the small standard errors of the mean (Table 2-2), especially for root diameter, where the coefficients of variation within an order were less than 10% for each species, while the coefficient of variation across orders ranged from 54.6% for *P. palustris* to 94.1% for *A. stricta*.

Tissue chemistry also varied significantly across order (Table 2-1), and many of the variables measured varied systematically across orders (Table 2-3). For the tree species, N and P concentrations tended to decrease from distal to proximal, and the C:N and C:P ratios tracked the differences in N and P, as C concentration was relatively constant across orders (Table 2-3). In contrast, patterns of N and P concentration in *A. stricta*, the lone grass species, did not exhibit a systematic correlation with order. Instead, the smallest, most distal orders (≥ fourth order) had a 60% higher N concentration than the three more proximal orders.

Among the C fractions, cellulose concentrations increased from distal to proximal. However, while we expected extractives to decrease from distal to proximal, extractives concentrations did not exhibit systematic variation across orders. Moreover, lignin concentrations were not constant across orders as predicted, but tended to decrease across orders from distal to proximal (Table 2-3). This was surprising, since it might be expected that larger, more distal roots would have higher concentrations of structural materials like lignin. However, other studies have reported a decrease in lignin from distal to proximal among root orders (Guo et al. 2004) or diameter classes (Hendricks et al. 2000; Dornbush et al. 2002).The acid-insoluble

lignin fraction is not chemically pure and may contain suberin, cutin, condensed tannin-protein complexes, and other highly recalcitrant compounds, in addition to true, structural lignin. First order roots could have high concentrations of tannins, associated with defense against herbivory or pathogens, while being relatively low in true lignin.

While we predicted an increase in lignin:N and lignin:P ratios would increase from distal to proximal, lignin:N ratio only met this expectation in the case of *Q. laevis,* showing no strong trends for the other species. Lignin:P ratios, in contrast did exhibit a clear trend to increase systematically from distal to proximal, although in *A. stricta*, lignin:P ratios first increased from roots greater than or equal to fourth order to third order roots, and then decreased from third to first order.

Although this study presents clear evidence of variation in tissue chemistry among different fine root orders, the patterns of that variation across orders likely have been obscured somewhat by compositing of the smallest, most distal root orders. This is particularly the case for *A. stricta*, where four orders were composited. While this undoubtedly reduced the precision with which we were able to report tissue chemistry variables, we felt this was a reasonable compromise for several reasons. First, by speeding the root dissection by order, it allowed us to process enough sample mass to describe trends across root orders for more tissue chemistry variables than have been reported in past studies of root order, including P and C concentrations and C:P and lignin:P ratios, which heretofore had not been measured among root orders. Second, the smallest, most distal three to four orders in *A. stricta* and the smallest, most distal two to three orders of the trees were all similar in diameter within a species, while there was a substantial jump in diameter to the next most proximal root order. These distal root orders may function as "modular units" such that whole networks of two to four root orders die as one unit

(Pregitzer et al. 2002). It is possible that the larger, more proximal roots are permanent or semi-permanent: The C cost of losing such large diameter roots alone would likely be great, but additionally, all more distal subsidiary roots would also be lost. Finally, the precision with which we measured tissue chemistry is equal to or exceeds that of most fine root studies. For example, all of the composited root orders in *A. stricta* had mean diameters under 0.2 mm, smaller than the smallest diameter classes commonly used to report root nutrient concentrations (typically <0.5 mm or larger, Gordon and Jackson 2000). This was also true for the two most distal orders of oak and pine that were lumped together, which all had mean diameters of <0.1 mm and ≤0.3 mm, respectively.

Despite the fact that some root orders were composited in this study, the patterns of fine root morphology and tissue chemistry described here are similar to those found by other researchers. Guo et al. (2004) reported systematic patterns of length, diameter, and N and cellulose concentrations across fine root orders in *P. palustris*. They also found that extractives and lignin concentrations did not change much across orders, decreasing slightly from distal to proximal. In this study, extractives varied across orders, but not in a systematic fashion, while lignin generally decreased from distal to proximal across orders. However, the extractives concentrations for *P. palustris* in this study were generally lower and the lignin concentrations higher than in Guo et al. (2004), though cellulose concentrations were similar. These differences are perhaps owing to a combination of methodology and site differences. Nevertheless, overall trends are not dissimilar.

In addition to Guo et al. (2004), Pregitzer et al. (1997; 2002) demonstrated systematic trends across orders in root length, diameter, N concentration, and C:N ratio, while C concentration remained constant across orders. These patterns were found in a variety of species

and ecosystems around North America, making it unlikely that the patterns of morphology and tissue chemistry presented in this work are exceptional or site-dependent. Additionally, trends in N concentration and C:N ratio were more pronounced in the trees than in the herbaceous species (Pregitzer et al. 1997), similar to *A. stricta* in this study, although in the latter case, this could be due in part to compositing the four most distal root orders.

We are not aware of any other papers to date that report trends across fine root orders for P or Ca concentrations. Extant studies utilizing diameter classes approximate some of the same trends as are described here. Gordon and Jackson (2000) present a review of trends in nutrient concentrations along a diameter gradient. Although there was a lot of variability, the overall pattern for mean P concentration declined from about 1.2 g/kg for roots about 0.25 mm in diameter to about 0.5 g/kg for roots 5 mm in diameter. Over the same diameter range, Ca concentrations declined from an average of approximately 7 g/kg to about 3 g/kg. In both cases, the decline appears to have occurred most rapidly in the smallest, most distal roots, leveling off in roots larger than about 1 mm in diameter.

Ecological Significance

Our results indicate that both fine root morphology and tissue chemistry varied, often systematically, among root orders from the most distal roots to the most proximal orders. Due to the relationships between root morphology and tissue chemistry, litter quality, and decomposition rates, fine root orders are likely to decompose at different rates (Pregitzer et al. 1997), at scales both of ecosystems and of individual roots. At the ecosystem scale, root mortality rates are likely to be skewed towards the more distal orders, affecting which roots die and enter the detrital pool. Pregitzer et al. (1997) observed that if one of the more proximal roots dies, all connected roots that are more distal than itself will also die, compounding the C cost associated with its death. Because of the high C cost associated with mortality of larger, more

proximal fine roots, they suggested that rates of mortality (and hence detrital input) vary within the fine root guild, with smaller, more distal roots more likely to die and become detritus. This has been borne out by the work of Wells and Eissenstat (2001), who found a negative correlation between fine root diameter and survivorship. Yet, despite the evidence that the smallest fine roots are also the most likely to enter the detrital pool, past decomposition studies, using primarily the buried bag technique, have focused predominantly on larger fine roots (0.5–2.0 mm), which are easier to sample but may be semi-permanent components of the root system (Pregitzer et al. 1997).

Nutrient concentrations in roots were generally low (Gordon and Jackson 2000). This may be partly due to our treatment of the roots during root processing. After collection, roots were frozen, lysing the cells. They were subsequently refreshed by placing them in deionized water, potentially leaching nutrients and extractives from ruptured cells. These methods may have underestimated nutrient concentrations. Yet if this is the case, it is important to note that the true differences in nutrient concentrations among orders would have been even greater than those reported here, as nutrients were likely more easily leached from smaller roots.

Low nutrient concentrations may also be a reflection of a lack of available soil nutrients in *P. palustris-A. stricta* forests. In fact, N mineralization rates in these ecosystems are among the lowest rates found in North American forests (Wilson et al. 1999). Root decomposition likely plays an important role in recycling nutrients within these systems, particularly N. In the aboveground litter of these ecosystems, largely from *P. palustris* and *A. stricta*, N can be mobilized for over a year (Hendricks et al. 2002). Although some N release occurs after that time, much of the N in aboveground litter is thermally mineralized by fire and gets returned to the atmosphere by prescribed fire (return interval 1–3 years), while other nutrients may be

retained in the ash. Belowground, N is not removed from the system by fire and seems to be released with little or no prior immobilization (Parton et al. 2007; Chapter 3). The relative contribution of root and shoot detritus to the N budget in *P. palustris-A. stricta* ecosystems may deserve further attention.

Conclusions

The results of this work clearly support the conclusion of previous researchers that roots below an arbitrary diameter limit cannot be treated as a homogenous cohort (Pregitzer 2002)., Within the fine root guild, there are significant differences in the morphological and chemical variables that have been shown to influence decomposition rates. The smallest, most distal root orders in this study generally had higher concentrations of N, and P, higher SA:V ratios, lower cellulose concentrations and lower C:N, C:P, lignin:N, and lignin:P ratios than the larger more proximal roots (Table 2-2 and Table 2-3). This implies that the smallest, most distal root orders likely provide a better quality substrate for decomposer organisms and may decay at faster rates relative to the comparatively large fine roots used in most buried bag studies. Of course, this hypothesis is dependent upon the assumption that nutrient retranslocation does not occur before the death of individual fine roots, but most root decomposition studies assume implicitly that little retranslocation occurs in roots. While this assumption may have to be accepted until more evidence is available, decomposition studies of fine roots can no longer overlook variation in litter quality within the fine root guild. The hypothesis that the variation in fine root morphology and tissue chemistry will lead to dissimilar patterns of decomposition among root orders requires future testing. Additionally, while we chose our study species to include different mycorrhizal types (arbuscular and ectomycorrhizal), life forms (a grass and two trees), and broad taxonomic groups (angiosperms and a gymnosperm) we made no direct comparisons among species, since a different topological system was used for the tree species than for *A. stricta*, and since the extent

to which orders were composited also would have made direct tests of species effects difficult. Future studies should attempt to account for variability in root morphology and tissue chemistry in explaining patterns of fine root decomposition. Nevertheless, this study has documented clear and often systematic differences in fine root morphology and tissue chemistry across fine root orders and among several species. This variability creates differences in the substrate quality of root litter of different orders, and should result in faster decay rates for relatively more distal root orders.

Table 2-1. Results of one-way ANOVAs analyzing the effect of root order on factors reported to influence the substrate quality of decomposing plant materials: All data were rank transformed prior to analysis.

	Species					
	Aristida stricta		*Pinus palustris*		*Quercus laevis*	
Variable	df	*P* value	df	*P* value	df	*P* value
Length	6	<0.0001	4	<0.0001	4	<0.0001
Diameter	6	<0.0001	4	<0.0001	4	<0.0001
SA:V ratio	6	<0.0001	4	<0.0001	4	<0.0001
Carbon	3	0.0047	3	0.0674	3	0.0011
Nitrogen	3	0.0398	3	0.0151	3	<0.0001
C:N ratio	3	0.0524	3	0.0151	3	<0.0001
Extractives	3	0.7075	3	0.0027	3	0.0398
Cellulose	3	0.2921	3	0.0002	3	0.0005
Lignin	3	0.0011	3	0.0002	3	0.0133
Calcium	3	0.0151	3	0.4050	3	0.0267
Phosphorus	3	0.0345	3	0.0015	3	<0.0001
C:P ratio	3	0.0267	3	0.0043	3	0.0002
Lignin:N ratio	3	0.2373	3	0.0621	3	0.0005
Lignin:P ratio	3	0.0002	3	0.0345	3	0.0018

Table 2-2. Fine root morphology among several species and root orders: Values represent means followed by one standard error and the range. Different superscript letters indicate significant differences ($P<0.05$) among orders within a given species, as determined by one-way ANOVAs on rank transformed data.

			Length (mm)			Diameter (mm)			SA:V ratio (mm^{-1})		
Species	Order	*n*	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Aristida stricta	1	48	0.6 [a]	0.1	0.2 – 2.0	0.108 [a]	0.004	0.050 – 0.175	40.4 [a]	2.0	22.9 – 80.0
	2	48	2.6 [b]	0.2	0.5 – 7.5	0.087 [b]	0.005	0.025 – 0.150	56.6 [b]	5.0	26.7 – 160.0
	3	49	8.5 [a]	1.0	0.8 – 29.0	0.103 [a,b]	0.004	0.050 – 0.175	42.5 [a]	2.0	22.9 – 80.0
	4	48	29.5 [c]	1.9	9.3 – 67.5	0.164 [c]	0.005	0.075 – 0.250	25.6 [c]	0.9	16.0 – 53.3
	5	105	75.1 [d]	4.7	12.2 – 278.0	0.288 [d]	0.006	0.175 – 0.450	14.5 [d]	0.3	8.9 – 22.9
	6	105	143.1 [e]	8.2	31.0 – 396.5	0.572 [e]	0.010	0.350 – 0.800	7.2 [e]	0.1	5.0 – 11.4
	7	106	86.0 [f]	6.1	15.0 – 287.5	0.932 [f]	0.016	0.625 – 1.550	4.4 [f]	0.1	2.6 – 6.4
Pinus palustris	1	265	1.1 [a]	0.0	0.2 – 3.6	0.254 [a]	0.003	0.125 – 0.400	16.5 [a]	0.2	10.0 – 32.0
	2	302	3.2 [b]	0.1	0.5 – 14.9	0.308 [b]	0.003	0.200 – 0.450	13.4 [b]	0.1	7.3 – 20.0
	3	147	30.1 [c]	1.5	4.7 – 101.0	0.390 [c]	0.005	0.250 – 0.575	10.5 [c]	0.1	7.0 – 16.0
	4	124	80.6 [d]	3.7	16.0 – 218.0	0.609 [d]	0.010	0.375 – 0.950	6.8 [d]	0.1	4.2 – 10.7
	5	94	246.8 [e]	11.9	41.0 – 625.0	1.009 [e]	0.017	0.700 – 1.475	4.1 [e]	0.1	2.7 – 5.7
Quercus laevis	1	44	0.7 [a]	0.1	0.2 – 1.6	0.085 [a]	0.003	0.050 – 0.125	50.0 [a]	2.0	32.0 – 80.0
	2	47	3.2 [b]	0.2	1.2 – 6.5	0.103 [b]	0.003	0.075 – 0.150	40.4 [b]	1.2	26.7 – 53.3
	3	93	15.8 [c]	1.2	1.8 – 77.0	0.134 [c]	0.003	0.075 – 0.200	30.9 [c]	0.6	20.0 – 53.3
	4	90	26.9 [d]	2.0	1.4 – 95.0	0.233 [d]	0.003	0.125 – 0.325	17.6 [d]	0.3	12.3 – 32.0
	5	90	79.0 [e]	4.6	10.4 – 251.0	0.374 [e]	0.004	0.300 – 0.450	10.8 [e]	0.1	8.9 – 13.3

Notes: All orders are presented beginning with the most distal root tips at the top, and each succeeding order is proximal to the previous. Orders in *A. stricta* followed a centrifugal or ontogenetic pattern and are presented in the tables with order numbers reversed to facilitate comparison to *P. palustris* and *Q. laevis*, which followed a centripetal ordering system. Abbreviations: SA:V ratio, surface area to volume ratio; SE, standard error of the mean

Table 2-3. Fine root tissue chemistry among several species and root orders: Values represent means of three replicate samples with one standard error in parentheses. Different superscript letters indicate significant differences ($P<0.05$) among orders, within a given species, as determined by one-way ANOVAs on rank transformed data.

Species	Order	Nitrogen (g/kg)	Phosphorus (g/kg)	Calcium (g/kg)	C:N ratio	C:P ratio
Aristida stricta	1	7.2 (0.5) [a]	1.2 (0.1) [a]	0.7 (0.1) [a]	71.3 (4.3) [a]	427.8 (17.5) [a]
	5	4.7 (0.6) [b]	0.8 (0.0) [b]	0.4 (0.0) [a,b]	111.5 (12.7) [a]	604.9 (32.6) [b]
	6	4.1 (0.4) [b]	1.1 (0.1) [a,b]	0.3 (0.0) [b]	123.1 (10.8) [a]	440.3 (36.1) [a]
	7	4.7 (0.7) [b]	1.3 (0.1) [a]	0.3 (0.1) [b]	109.0 (16.9) [a]	373.6 (31.6) [a]
Pinus palustris	1	10.0 (1.0) [a]	2.4 (0.1) [a]	0.7 (0.1) [a]	50.8 (5.1) [a]	204.4 (11.1) [a]
	3	8.0 (0.9) [a,b]	2.0 (0.2) [a]	0.9 (0.1) [a]	66.5 (7.6) [a,b]	258.0 (27.7) [a,b]
	4	6.6 (0.8) [b,c]	1.6 (0.1) [b]	0.7 (0.0) [a]	80.7 (10.9) [b,c]	331.1 (16.8) [b,c]
	5	5.1 (0.6) [c]	1.4 (0.1) [b]	0.7 (0.0) [a]	102.9 (11.8) [c]	375.6 (26.7) [c]
Quercus laevis	1	14.1 (0.1) [a]	1.0 (0.1) [a]	2.1 (0.2) [a]	37.1 (0.5) [a]	530.0 (54.9) [a]
	3	9.2 (0.1) [b]	0.7 (0.0) [b]	1.6 (0.1) [b]	61.4 (0.7) [b]	844.5 (34.6) [b]
	4	7.8 (0.1) [c]	0.6 (0.0) [c]	1.4 (0.1) [b]	72.1 (1.1) [c]	995.0 (25.7) [c]
	5	7.0 (0.2) [d]	0.5 (0.0) [d]	1.4 (0.3) [b]	80.2 (2.2) [d]	1105.6 (53.2) [c]

Notes: All orders are presented beginning with the most distal root tips at the top, and each succeeding order is proximal to the previous. Orders in *A. stricta* followed a centrifugal or ontogenetic pattern and are presented in the tables with order numbers reversed to facilitate comparison to *P. palustris* and *Q. laevis*, which followed a centripetal ordering system. The most distal four orders in *A. stricta* and the most distal two orders in *P. palustris* and *Q. laevis* were composited for tissue chemistry analyses.

Table 2-3. Continued

Species	Order	Extractives (g/kg)	Cellulose (g/kg)	Lignin (g/kg)	Lignin:N ratio	Lignin:P ratio
Aristida stricta	1	536.6 (5.7) [a]	268.8 (4.7) [a]	194.6 (5.0) [a]	27.3 (1.6) [a]	164.1 (10.1) [a]
	5	526.7 (14.0) [a]	306.6 (17.2) [a]	166.7 (3.2) [a]	37.0 (4.5) [a]	200.5 (13.7) [a]
	6	546.9 (11.8) [a]	307.1 (8.9) [a]	145.9 (5.6) [b]	36.2 (2.0) [a]	129.6 (5.9) [b]
	7	554.0 (27.4) [a]	308.9 (23.1) [a]	140.8 (7.4) [b]	30.6 (2.9) [a]	105.9 (5.8) [c]
Pinus palustris	1	299.1 (10.2) [a]	229.7 (3.3) [a]	471.2 (9.2) [a]	48.3 (5.6) [a]	193.7 (11.9) [a]
	3	232.1 (6.0) [b]	279.0 (9.4) [b]	488.9 (3.6) [a]	62.8 (7.4) [a]	243.1 (24.0) [a,b]
	4	243.4 (5.3) [b]	317.6 (4.4) [c]	439.0 (8.3) [b]	68.4 (7.9) [a]	282.3 (17.1) [b]
	5	282.7 (7.3) [a]	325.1 (1.6) [c]	392.2 (7.3) [c]	78.2 (7.9) [a]	286.7 (23.6) [b]
Quercus laevis	1	284.0 (20.7) [a]	210.9 (13.2) [a]	505.1 (8.7) [a]	35.8 (0.6) [a]	511.5 (52.1) [a]
	3	216.0 (1.8) [b]	221.9 (8.9) [a]	562.1 (8.0) [b]	61.1 (0.1) [b]	840.1 (24.7) [b]
	4	216.9 (9.3) [b]	246.8 (4.9) [b]	537.5 (10.0) [b,c]	68.7 (2.0) [c]	948.5 (41.8) [b,c]
	5	226.1 (7.1) [a,b]	261.2 (3.1) [c]	513.2 (9.0) [a,c]	73.8 (2.0) [c]	1019.7 (63.6) [c]

Notes: All orders are presented beginning with the most distal root tips at the top, and each succeeding order is proximal to the previous. Orders in *A. stricta* followed a centrifugal or ontogenetic pattern and are presented in the tables with order numbers reversed to facilitate comparison to *P. palustris* and *Q. laevis*, which followed a centripetal ordering system. The most distal four orders in *A. stricta* and the most distal two orders in *P. palustris* and *Q. laevis* were composited for tissue chemistry analyses.

CHAPTER 3
FINE ROOT DECOMPOSITION RATES AND NUTRIENT DYNAMICS AMONG DIFFERENT ROOT ORDERS OF *PINUS PALUSTRIS*

Introduction

Although fine roots may account for as much as 80% of annual detritus inputs in some forest ecosystems (Steinaker and Wilson 2005), the lack of work defining fine root decomposition rates, how those rates vary among functional classes of fine roots, and the mechanisms controlling patterns of fine root decay represent a persistent gap in our understanding of carbon (C) and nutrient fluxes through ecosystems (Chen et al. 2002; Pregitzer 2002). Most work on the patterns and controls of decomposition has assessed aboveground litter with comparatively few studies describing patterns belowground (Silver and Miya 2001; Chen et al. 2002), even though rates of decay and controls on decomposition may be fundamentally different for belowground decomposition (Seastedt 1988; Bloomfield et al. 1993; Hendricks et al. 2000; Silver and Miya 2001; Langley and Hungate 2003).

Studies of both above- and belowground decomposition have revealed the importance of physical and chemical characteristics of plant litter in regulating how quickly the decomposer community mineralizes C and nutrients in the litter (Aerts 1997; Silver and Miya 2001). Consequently, researchers have tried to explain observed rates of mass loss and nutrient dynamics using the indices of microbial substrate quality based on the chemical properties of the litter. These indices include concentrations of nitrogen (N, Pandey and Singh 1982; Berg 1984; McClaugherty and Berg 1987; White et al. 1988; Taylor et al. 1989), phosphorus (P, Schlesinger and Hasey 1981; Staaf and Berg 1982; Berg 1984; Taylor et al. 1989), calcium (Ca, Van Cleve 1974; Bloomfield et al. 1993; Silver and Miya 2001), C “extractives” (the readily soluble fraction of organic compounds in plant material, McClaugherty et al. 1984; Berg et al. 1987; Allison and Vitousek 2004), acid-soluble cellulose (Muller et al. 1988; Aber et al. 1990;

Scheffer and Aerts 2000; Chen et al. 2002; John et al. 2002), and acid-insoluble lignin (Fogel and Cromack 1977; Meentemeyer 1978; Schlesinger & Hasey 1981; Melillo et al. 1982; Pandey and Singh 1982), as well as interactions among these variables, such as the C:N (Jensen 1929; White et al. 1988; Taylor et al. 1989; Cotrufo and Ineson 1995; Arunachalam et al. 1996), C:P (Xuloc-Tolosa et al. 2002; Hirobe et al. 2004; Xu et al. 2004; Xu and Hirata 2005; Rejmánková and Houdková 2006), and lignin:N (Melillo et al. 1982; McCLaugherty et al. 1984; Blair 1988; White et al. 1988; Taylor et al. 1989) ratios.

Although litter quality indices offer some predictive power in estimating decomposition rates, the extent to which litter quality and decay rates vary within the fine root guild is not well understood (Pregitzer 2002). Moreover, heterogeneity of structure and function among fine roots is likely to lead to variation in litter quality, and thus to variation in decomposition rates among fine roots (Pregitzer et al. 1997). This variability in structure and function among fine roots is intimately linked to root branching structure. For example, N concentrations and C:N ratios have been reported to vary systematically with root order (Pregitzer et al. 1997; 2002; Guo et al. 2004). It seems plausible that fine root decomposition rates could also vary by order, tracking the changes in N and C:N ratio across orders (Pregitzer et al. 1997).

In a companion paper, we reported a number of morphological and tissue chemistry parameters with the potential to influence decomposition rates of fine roots, describing patterns of variation across several root orders and three species. We suggested that the systematic differences across orders in fine root morphology and tissue chemistry could lead to attendant variation in decomposition rates across orders. The objectives of this study were: 1) to investigate variation in decomposition rates among fine root orders of *Pinus palustris* Mill. (longleaf pine), one of the species studied in the companion paper; 2) to model the decay rates

for each order using exponential decay models; 3) to correlate our decay rate constants with initial morphology and tissue chemistry parameters; and 4) to explore the variation in patterns of nutrient mineralization and immobilization among root orders and their relationship with initial litter quality. We hypothesized that: 1) decay rates will exhibit variation within the fine root guild, decreasing across order as one moves from distal to more proximal roots; 2) differences in decay rates among orders will be correlated with fine root tissue chemistry; 3) net nutrient release will be higher, or net accumulation lower in more distal root orders, due to higher initial nutrient concentrations.

Materials and Methods

Study Site and Experimental Design

This study took place at the Joseph W. Jones Ecological Research Center at Ichauway in southwest Georgia, USA (31°13′N, 84°29′ W) in the Lower Coastal Plain and Flatwoods Province described by McNab and Avers (1994). The climate is characterized by long, hot summers and short, cool winters (Lynch et al. 1986). Mean annual temperature is 20°C, ranging from 21–34°C in the summer and from 5–17°C in winter (Goebel et al. 2001). Mean annual precipitation is 131 cm and is distributed evenly throughout the year (Goebel et al. 2001).

Two sites at the J.W. Jones Ecological Research Center were utilized during the study. At the first, roots were collected for litterbags. At the second, located approximately 6 km northwest of the collection site, the litterbags were buried. Both sites were located on xeric sand ridges, as described by Goebel et al. (2001). The soils (Goebel et al. 2001) are classified as Typic Quartzipsamments and are characterized by coarse sands over 2.5 m deep containing little organic matter and having weak soil horizons and little aboveground litter due to frequent fire (return interval 2 yr since 2000, 1–5 yr for several decades before). The vegetation of the collection site is dominated by open-canopied woodlands of *Pinus palustris* Mill. (longleaf pine)

and scrub oaks, predominantly *Quercus laevis* Walt. (turkey oak) (Mitchell et al. 1999; Goebel et al. 2001). The understory is dominated by *Aristida stricta* Michx. (wiregrass), but also includes many species of native legumes (Mitchell et al. 1999; Goebel et al. 2001). Similarly, the litterbag incubation site supports *P. palustris–A. stricta* woodlands, although due to greater continuity of fine fuels, the oaks are largely relegated to the understory by frequent prescribed fires.

An experiment to measure first year fine root decomposition mass loss and nutrient dynamics among root orders was established using a completely randomized design, replicated 3 times, during the summer of 2005. Root litterbags were incubated in the soil at three different plots within the litterbag site. Seven different litterbag harvests were used to monitor mass loss and nutrient (N, P, and Ca) dynamics over one year: zero days (excavated the day of burial), two weeks, one month, two months, three months, six months, and twelve months.

Litterbag Preparation and Placement

Detailed methods for collecting fine roots of *P. palustris* have been described elsewhere (Chapter 2). Briefly, during summer 2005, roots were collected by hand, rinsed, and placed in zipper seal bags. The bags were then placed on ice in a cooler, and transported to the lab within four hr. Thereafter, roots were stored at 4°C for less than two months.

Litterbags were cut from polyester cloth with a 50 μm mesh (Harmon et al. 1999; Chen et al. 2002) and measured 15 cm x 20 cm. This mesh size was small enough to exclude root ingrowth and loss of decomposed sample roots, while permitting access by soil bacteria and fungal hyphae. The zipper seal bags of roots were randomly assigned to 14 litterbags per replicate, with the stipulation that each litterbag have an approximately equal total initial root mass (14.6 ± 0.227 g, mean fresh weight ± standard error). The fresh weight of roots in each litterbag was recorded. Root networks were not dissected into root orders before being placed into litterbags. By leaving root networks intact we hoped to avoid artifacts associated with

altering the distribution of root size classes and disrupting the rhizosphere community (Dornbush et al. 2002), as well as destroying the connectivity among roots of sequential orders.

At the litterbag site, we utilized existing 50 m x 50 m plots established for an unrelated study, which began in 2000. We did not collect roots at this site to avoid disturbing ongoing research. Within three of the established plots, seven randomly selected locations for litterbag burial were marked with pin flags, representing the seven scheduled litterbag harvests. Litterbags were excluded from an inner 20 m x 20 m plot used for a concurrent study. Litterbags were attached to each flag using monofilament line. In order to ensure good soil contact, while minimizing soil disturbance during burial, a shovel was used to make a 45° angle slit in the soil, about 15 cm deep. The litterbags were carefully inserted into this slit, and the overlying soil was tamped down (Ostertag and Hobbie 1999).

After the designated incubation time, litterbags were carefully excavated by inserting a trowel into the soil directly below the litterbag and lifting the soil up to loosen it until the litterbag could be removed. Excavated litterbags were transported back to the lab on ice within 30 min. At the lab, they were immediately placed in a drying oven (70°C) for storage until the roots could be dissected.

Determination of Mass Loss

After oven-drying, litterbags that had been disturbed by animals were discarded. If neither of the two litterbags from a given plot and litterbag retrieval date had been disturbed, one of these was randomly selected for further analysis. The dried roots from these litterbags were subdivided into two groups: first–third orders (hereafter first order) versus fourth–fifth orders (hereafter fourth order), using fine forceps. We originally had planned to separate all five orders from each other in dissecting the roots from the excavated litterbags; however, after twelve months of decomposition, the roots had deteriorated to such an extent that accurate order

assignment was no longer possible. Thus, the root order groups were used, estimating the difference between third and fourth order from a combination of the extant branching and the mean length and diameter of third and fourth order roots, as reported previously (Chapter 2). We felt confident in dividing the roots at this point because differences in length and diameter among the first three orders in this species were not great, while there was a more substantial difference between orders 3 and 4 (Guo et al. 2004; Chapter 2), allowing visual estimation of order based on length and diameter, combined with extant branching (Guo et al. 2004). Root order classification followed Pregitzer et al. (2002), in which distal root tips are considered first order roots. Roots displaying structural modifications due to ectomycorrhizal colonization (bifurcation) were treated as first order roots.

After dissection, root orders were oven-dried at 70°C to constant mass and weighed. The roots were then ground and homogenized using a SPEX 8000-D mixer mill (SPEX, Edison, NJ), and subsampled for ash determination for 6 hr at 500°C. Mass loss was then calculated by subtracting the mass of each order after decomposition from its initial mass. Since roots were not separated into order groups before dissection, initial mass of each order in each litterbag was determined using equation 3-1, where I_{ijk} is the initial ash-free dry mass of order *k* in the litterbag

$$I_{ijk} = W_{ij\cdot}\cdot(D_{\cdot 1\cdot} / W_{\cdot 1\cdot})\cdot(P_{\cdot 1k} / D_{\cdot 1\cdot}) - A_{ijk} \qquad (3\text{-}1)$$

for the *j*th harvest date of the *i*th replicate, W is the total initial fresh weight, D is the total initial dry mass, P is the initial dry mass by order, and A is the mass of ash by order in a given litterbag. Since the estimated initial weight by order of the first set of litterbags harvested did not match the actual weight, the mass of each order of each litterbag within a replicate was adjusted by adding a constant, such that the initial percent mass remaining would equal 100%.

To correlate decay rates with initial tissue chemistry variables and to monitor nutrient dynamics over the course of decomposition, the dried and ground root tissues were analyzed for concentrations of C, N, P, and Ca. Total C and N were measured with a Thermo FlashEA 1112 NC analyzer (Thermo Fisher Scientific, Inc. Waltham, MA, USA). Concentrations of P and Ca were analyzed by digestion in sulfuric acid (Parkinson and Allen 1975), followed by determination of P concentration using a Lachat QuikChem Series 8000 flow injection analyzer (Lachat Instruments Inc., Milwaukee, WI, USA) and determination of Ca using a Perkin-Elmer Model 5100 PC atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). All masses and nutrient concentrations have been expressed on an ash-free, dry mass basis (Bledsoe et al. 1999).

Statistical Analyses

Using nonlinear regression (PROC NLIN, SAS 9.1 2002-2003, Cary, NC, USA), mass loss for each order was fit to a single exponential decay model (equation 3-2, Olson 1963; Wieder and Lang 1982) where X_0 is the initial mass in grams at time 0, X_t is the mass remaining in grams at

$$X_t/X_0 = e^{kt}, \quad (3\text{-}2)$$

time t (yr), and k is the decay rate constant (in units of yr^{-1}). Double exponential decay models were also tested; however, for first order roots, there were strong correlations among double exponential decay model parameters, indicating that a model with fewer parameters may be appropriate, and for fourth order roots, the model reduced to a single exponential decay model. Therefore, all subsequent analyses involved the single exponential decay model. Percent mass remaining at each litterbag harvest date was averaged across replicates before models were fit. Each single exponential decay model was solved for the rate constant, k, and 95% confidence intervals were used to test for differences in decay constants between orders. Differences among orders in final mass loss at the end of one year were assessed using t tests.

Net nutrient (N, P, and Ca) release or accumulation was estimated, and differences among orders were evaluated using *t* tests. The effect of initial tissue chemistry on decay rates and nutrient dynamics was investigated using the data from the first harvest of litterbags (0 days) to determine initial concentrations of N, P, Ca, and C:N and C:P ratios. To determine concentrations of extractives, cellulose, and lignin, we averaged the data from the previous study (Chapter 2) for each of these C fractions across orders (orders one through three vs. orders four through five), weighting the averages using the biomass data for each order of *P. palustris* reported in Guo et al. (2004). All statistical analyses were performed using SAS 9.1 (2002-2003, Cary, NC, USA)

Results

Mass Loss Patterns and Initial Substrate Quality

Mass loss occurred at a moderate rate for both orders (Figure 3-1): Decay constants (*k*) for first and fourth order roots were 0.326 yr^{-1} and 0.301 yr^{-1}, respectively. Order did not have a significant effect on the decay constants (Table 3-2), and the final percent mass loss after one year was virtually the same for both orders: 72.6 ± 0.32% (mean ± standard error) for first order versus 73.3 ± 0.06% for fourth order (P=0.9850).

Although first and fourth order roots did not differ significantly in first-year mass loss or decay rates, significant differences in initial tissue chemistry between the two orders were observed (Table 3-1). Concentrations of N, P, and Ca, for example, were 58%, 46%, and 73% higher in first order roots than in fourth order roots. First order roots also had lower estimated cellulose concentrations and lower C:N, C:P, lignin:N, and lignin:P ratios, but had higher estimated lignin concentrations than fourth order roots.

Nutrient Dynamics

Concentrations of all three nutrients were nearly constant over the course of decomposition (data not shown), resulting in patterns of release that closely reflected patterns of mass loss (Figure 3-2). Periods of nutrient accumulation and release alternated during the first three months of decomposition, and both first and fourth order roots showed similar relative amounts of N, P, and Ca throughout the later stages of first year decomposition (Figure 3-2). After one year, both orders showed a net release of approximately 20–25% of all three nutrients. Fourth order roots released slightly more N, P, and Ca than first order roots, but differences in the final percent remaining of each nutrient were not significant (P=0.7970, P=0.7920, and P=0.8793, respectively). Initial concentrations of nutrients seemed poorly correlated with trends in accumulation and release of these nutrients. Although initial concentrations of N, P, and Ca were 58%, 46%, and 73% greater, respectively, in first order roots, the relative rates of nutrient release did not differ between the two orders.

Discussion

Mass Loss Patterns and Initial Substrate Quality

First-year mass loss for both orders was moderate, with both orders losing only about 27% of their original mass. The decay constants determined in this study compare well with k values for roots from the coniferous forests of the Pacific Northwest (range, 0.172–0.386 yr^{-1}, Chen et al. 2002), and with *P. strobus* roots and foliage decomposing in Wisconsin (k=0.300 and 0.360 yr^{-1}, respectively, Aber et al. 1990). Percent mass remaining after one year was also similar to that of *P. palustris* needle litter decomposing at a nearby site (Hendricks et al. 2002).

Mass loss failed to conform to expected patterns in two ways. First, in contrast to our hypothesis, neither the decay rate constant nor the final mass loss after one year of decomposition differed between first and fourth order roots (Table 3-2). Second, the standard

errors around the mean percent mass remaining for each harvest date were unusually high (Figure 3-1). These deviations from expected decay patterns may be due, in part, to insufficient replication, leading to poor central tendency among litterbags of a given harvest date. A larger problem, however, was the variability in the conversion factors used to estimate the initial ash-free dry mass by order for each litterbag. Although the coefficients of variation were only 12.6% for the wet to dry conversion factor and 15.8–18.1% for the total weight to weight by order conversion factors, we were unable to predict accurately and consistently the ash-free dry weight by order for the same litterbags on which the conversion factors were based. Estimates of initial mass for the first harvest litterbags ranged from 71% lower to 83% higher (mean, 3.4 ± 10.6% higher) than the actual mass (data not shown). Although we attempted to correct for this error by adjusting the estimated initial mass of each replicate set of litterbags to equal 100% mass remaining at time=0, this did not eliminate unusual patterns of mass loss. Moreover, it is unknown how well the adjustment of initial mass data from subsequent litterbags matched the true initial mass.

While we tried to avoid artifacts associated with dissecting the roots before placing them in litterbags, such as altering the distribution of root size classes, disrupting the rhizosphere community (Dornbush et al. 2002), and breaking the continuity between root orders, this method resulted in a substantial loss of both accuracy and precision. Not only could we not accurately estimate the initial mass of each order in the litterbags, but precision in determining decomposition rates of different orders was also lost. Had we dissected the roots before decomposition, it would have been possible to estimate decay rates for all five individual root orders. Instead, after twelve months of decomposition, the roots were so fragmented and

degraded that orders had to be composited into only two broad order groupings (orders one through three vs. four through five).

Having only two distinct classes of roots to work with also prevented us from performing precise statistical correlations between mass loss among orders and potential indices of litter quality. Nevertheless, the fact that litter chemistry differed among root orders but decay rate did not suggests that *P. palustris* fine root decay rates may be driven by environmental or microbial factors, rather than by substrate quality. Alternatively, while higher nutrient concentrations and lower C:nutrient and lignin:nutrient ratios in first order roots suggested that they would be the faster decomposing of the two orders, higher concentrations of recalcitrant materials, such as lignin, may have offset this somewhat. The acid-insoluble lignin fraction of plant material typically contains not only structural lignin, but also suberin, tannin-protein complexes, and other highly reduced compounds (Hendricks et al. 2000). First order roots could have high concentrations of condensed tannin-protein complexes, which may be a defensive response to herbivory or soil pathogens. Similarly, an unknown proportion of the N in first order roots may be present as mycorrhizal chitin, which is not only high in N, but also recalcitrant (Langley and Hungate 2003). First order root tips of *P. palustris* are encircled by an ectomycorrhizal mantle of chitinous fungal tissue, which is not only resistant to decay, but also may block microbial enzymes from degrading the less durable tissues surrounded by the mantle (Langley and Hungate 2003). Thus, chitin N is expected to slow decomposition, potentially decoupling N concentration and C:N ratio from decomposition rates (Langley and Hungate 2003).

Nutrient Dynamics

As with mass loss, nutrient dynamics followed unexpected patterns and exhibited large amounts of variance. We predicted that first order roots would have higher net release or lower net accumulation of nutrients because of their higher initial nutrient concentrations, yet this did

not seem to be the case. Little or no positive correlation could be found between initial nutrient concentration and net release, though we were not able to test this statistically, given that the roots were only dissected into two groups.

Errors in estimation of initial mass in each litterbag affected patterns of nutrient release to an unknown extent. However, the proportion of each nutrient remaining after each litterbag incubation period depends not only on the proportion of mass remaining, but also upon the nutrient concentration at that time. Therefore, it is likely that nutrient dynamics results are slightly less affected by the errors in initial mass estimation than are pattern of mass loss.

One possible explanation for these patterns of nutrient dynamics is the potential presence of recalcitrant forms of nutrients in first order roots. First order roots had higher concentrations of lignin than fourth order roots. Some of the N in first order roots may have been bound in condensed tannin-protein complexes, which are included in the acid-insoluble lignin fraction. Bound in such complexes, N would be less easily released than expected, based on the higher N concentration in first order roots. Additionally, first order roots likely had higher concentrations of recalcitrant, ectomycorrhizal chitin, resulting in slower than predicted release of N in first order roots. Some mycorrhizal fungi are also known to accumulate stores of Ca-polyphosphates, which, though they are actually part of the fungal tissue, may contribute significant amounts of Ca and P to analyses of these nutrients in mycorrhizal roots (Kulaev 1975; Peterson and Howarth 1991; McFall et al. 1992; Solaiman and Saito 2001). Mycorrhizal fungi contribute only slightly to fine root biomass (<2%), yet a third or more of fine root P may be of fungal, rather than plant origin (McFall et al. 1992; Solaiman and Saito 2001). Much of the P and Ca of first order *P. palustris* roots may be of fungal origin. If the fungal forms of these nutrients are recalcitrant, this

could explain why rates of P and Ca release from first order roots were lower than expected, relative to fourth order.

Patterns of nutrient dynamics during decomposition may differ between above and belowground detritus. In this study, nutrient dynamics were marked by at most very short periods of nutrient accumulation. This contrasts sharply with patterns of N, P, and Ca dynamics reported by Hendricks et al. (2002), at a nearby site, where *P. palustris* needle litter showed no release of N, P, or Ca for over one year. Parton et al. (2007) described similar patterns of N dynamics over ten years of decomposition at sites in seven biomes. In roots, N release occurred immediately despite C:N ratios greater than 50, but in leaf litter, N was only released when C:N ratios decreased below 40. Microbial decomposers belowground may have greater access to moisture, organic matter, and extraneous nutrient sources, resulting in more rapid release of nutrients from fine roots (Parton et al. 2007). These patterns of N dynamics are likely accentuated at our study site, where regular prescribed fires (one to three year return interval) remove organic matter and organic forms of N from the soil surface, while other nutrients are retained in the ash. Belowground decomposition likely plays an important role in the recycling of nutrients in *P. palustris*-*A. stricta* ecosystems, and the relative contribution of aboveground and belowground decomposition in nutrient cycling at ecosystem scales deserves more attention.

Conclusions

The use of litterbags in decomposition studies creates an artificial environment for decomposition (Harmon et al. 1999). Nonetheless, the simplicity of this method makes it an attractive technique, as long as its limitations are understood. A mesh size of 50 μm was used in this study to prevent the loss of small and fragmented roots, as the smallest roots of *P. palustris* are only about 0.26 mm in diameter, far smaller than mesh sizes used in past studies (commonly 1 mm, Silver and Miya 2001). However, soil fauna involved in decomposition were certainly

prevented from reaching the roots within litterbags, and onset of fungal colonization may have been delayed (Harmon et al. 1999; Chen et al. 2002). Therefore, the results of this study may underestimate true rates of decay and nutrient release. Nonetheless, litterbag studies are useful in placing lower bounds on estimates of root decomposition rates, and because this method is widely used, results can be compared easily with many previous studies of root decomposition.

Unfortunately, the errors associated with estimating the initial mass of each order in the litterbags had an adverse effect on the accuracy and precision of observed patterns of decomposition in this study. Both the rates of decomposition among orders and the finding that order had no effect on decomposition rate warrant further testing. Subsequent studies of decomposition among root orders will need to overcome problems in estimating the initial mass of different orders. They will also need to strike a balance between precision, in terms of subdividing roots into individual orders, and accuracy, in terms of decomposing intact root networks rather than separated orders. It may be that the precision gained from separating roots orders into their own litterbags before burial outweighs the potential for artifacts associated with this technique. This method could at least place lower bounds on the rates of decay among root orders.

Table 3-1. Initial tissue chemistry for fine roots of *Pinus palustris*: Values represent means with one standard error in parentheses, and *P* values were generated by *t* tests for root order differences in the means of each tissue chemistry variable. Orders one through three (first order) and four through five (fourth order) were composited for analysis.

	First order	Fourth order	*P*
Nitrogen (g/kg)	9.3 (0.8)	5.9 (0.4)	0.0160
Phosphorus (g/kg)	3.0 (0.0)	2.0 (0.0)	0.0001
Calcium (g/kg)	2.8 (0.1)	1.6 (0.1)	0.0005
Extractives (g/kg)	272.2 (4.9)	268.9 (6.5)	0.7036
Cellulose (g/kg)	249.5 (5.1)	322.5 (2.6)	0.0002
Lignin (g/kg)	478.3 (6.4)	408.6 (7.3)	0.0020
C:N	59.7 (4.5)	90.9 (5.8)	0.0129
C:P	184.5 (2.7)	259.4 (5.8)	0.0003
Lignin:N	52.0 (3.3)	70.1 (4.0)	0.0252
Lignin:P	160.9 (4.4)	200.2 (6.2)	0.0066

Notes: Concentrations of extractives, cellulose, and lignin were estimated using a weighted average of the data presented in chapter two.

Table 3-2. Results of single exponential decay models for each order: The decay rate constant, *k* ± a 95% confidence interval is shown, followed by the *P* value for the exponential decay model. Orders one through three (first order) and four through five (fourth order) were composited for analysis.

	k (yr^{-1})	95% CI	*P*
First order	0.326	0.030 – 0.622	<0.001
Fourth order	0.301	0.190 – 0.413	<0.001

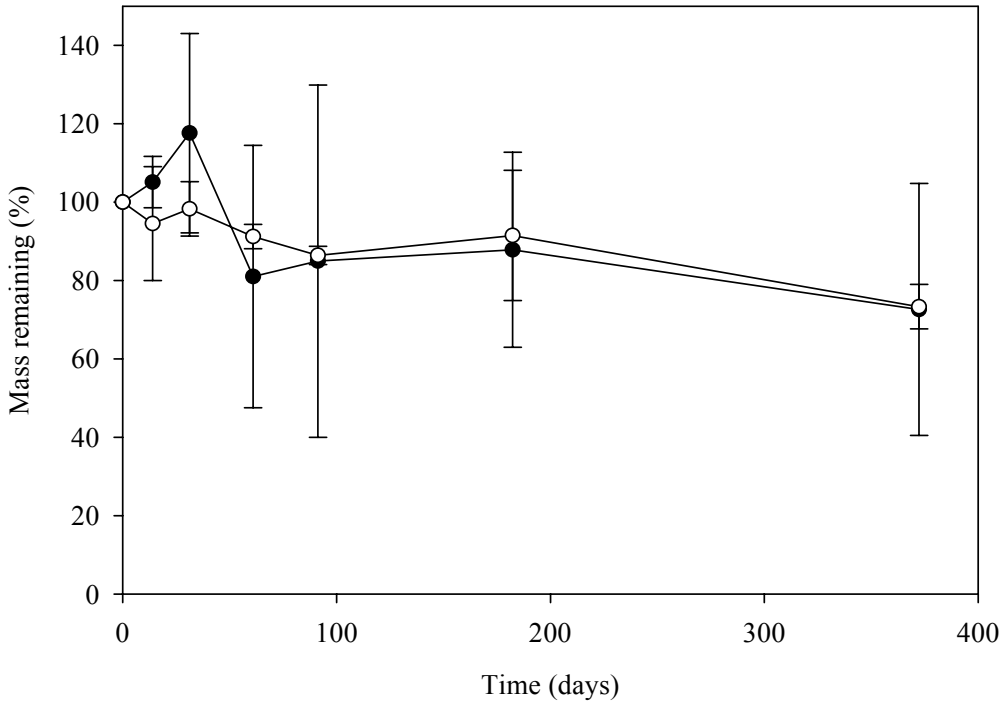

Figure 3-1. Mass loss from *Pinus palustris* roots decomposing over one year: Values are means ± one standard error, and one through three (first order) and four through five (fourth order) were composited for analysis. Closed circles represent first order roots, and open circles signify fourth order roots.

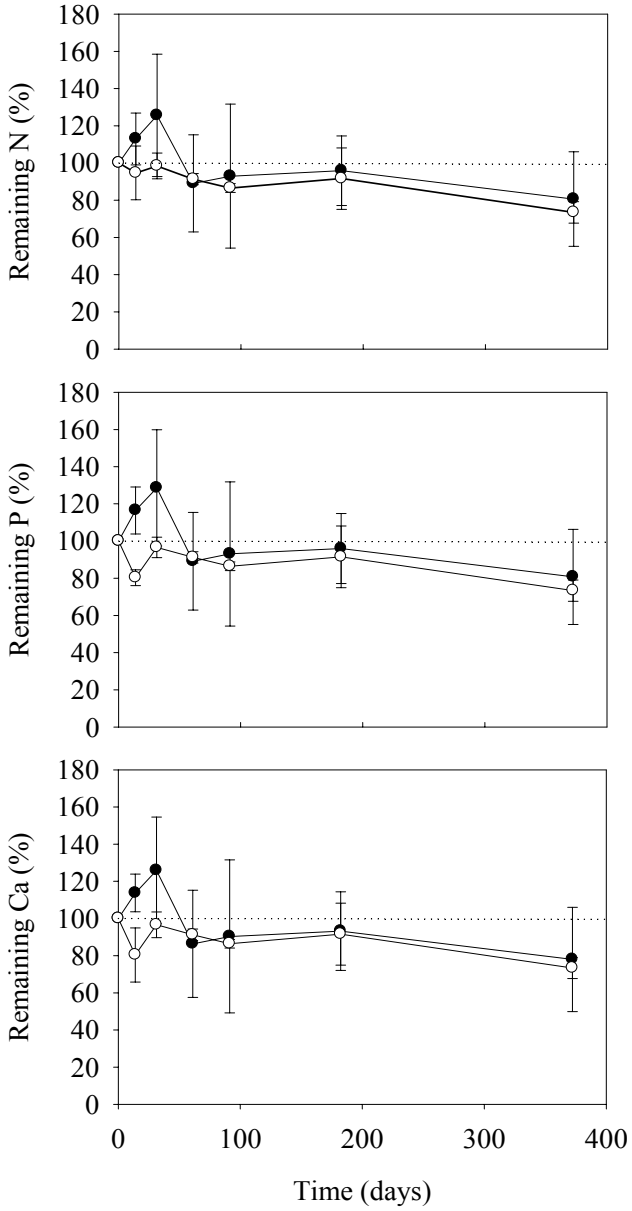

Figure 3-2. Patterns of nutrient accumulation and release in fine roots of *Pinus palustris* decomposing over one year: Values are means ± one standard error, and one through three (first order) and four through five (fourth order) were composited for analysis. Closed circles represent first order roots, and open circles signify fourth order roots.

CHAPTER 4
SUMMARY AND CONCLUSIONS

Fine root detritus represents a considerable flux of carbon (C) and nutrients in forest ecosystems, yet patterns and controls of fine root decomposition and how they vary within the fine root guild are poorly understood (Chen et al. 2002; Pregitzer 2002). While past studies of root decomposition have revealed the importance of fine root substrate quality in explaining decay rates, they have not accounted for variation among fine roots of different form and function (Pregitzer 2002).

To investigate this area more thoroughly, detailed analyses of root morphology and tissue chemistry believed to influence fine root decomposition rates were carried out among three species, *Aristida stricta*, *Pinus palustris*, and *Quercus laevis*, to determine how these factors varied among root orders among different species. Fine root length, diameter, and surface area to volume ratio (SA:V ratio) were included in the morphology analyses, and tissue chemistry measures included concentrations of nitrogen (N), phosphorus (P), calcium (Ca), labile C extractives, cellulose, and lignin, as well as C:N, C:P, lignin:N, and lignin:P ratios. We hypothesized that fine root morphology and tissue chemistry parameters would differ among root orders in ways likely to lead to variation in decay rates of different orders. We also predicted that these parameters would vary systematically across orders, with the smallest, most distal root orders likely to comprise a higher quality substrate for the microbial decomposer community and decompose more rapidly than larger, more proximal fine roots.

We observed distinct differences in morphological and tissue chemistry traits expected to influence decomposition rates. The SA:V ratio in each species increased systematically from the most distal roots to the most proximal. For *P. palustris* and *Q. laevis*, N and P concentrations generally decreased across orders from distal to proximal, while cellulose concentrations, C:N

ratio, C:P ratio, lignin:N ratio, and lignin:P ratio increased. These trends were somewhat less evident for *A. stricta*, perhaps due to a combination of species differences and compositing more of the smallest orders for *A. stricta* than we did for the trees. Regardless of species, variation in substrate quality among root orders was apparent, and our results support the hypothesis that rates of mass loss and nutrient release from decomposing roots will vary among different orders of fine roots, tracking the variation in fine root morphology and tissue chemistry.

Accordingly, a buried litterbag study was conducted to examine trends in fine root decomposition and nutrient dynamics among different root orders of *P. palustris* only. Rather than separating orders into different litterbags, we left root networks intact during decomposition, hoping to avoid artifacts caused by altering the distribution of root orders and eliminating connectivity among orders and associated rhizosphere communities. However, after twelve months of decomposition, the roots were degraded to the point that we were only able to separate the *P. palustris* roots into two groupings, orders one through three ("first" order) and orders four through five ("fourth" order).

Surprisingly, after modeling fine root decay using a single exponential decay model (Olson 1963; Wieder and Lang 1982), no differences in decomposition rate or the percent mass remaining among root orders were observed, despite significant and substantial differences in tissue chemistry. Distal first order roots had a decay constant (k) of 0.326 yr^{-1}, with 72.6% (mean ± standard error) of original mass remaining after one year, while fourth order roots had a k of 0.301 yr^{-1} and 73.3 % of original mass remaining.

Fine root nutrient dynamics also did not follow the expected pattern that distal, first order roots, with their higher nutrient concentrations would release more (or accumulate less) of their original nutrient content than proximal, fourth order roots. No significant differences in final

nutrient retention were observed among orders, despite the fact that first order roots had initial concentrations of N, P, and Ca that were 58%, 46%, and 73% higher, respectively, than those of fourth order roots. After one year, both orders retained about 75–80% of their original N, P, and Ca.

There are several possible explanations for these unexpected patterns of mass loss and nutrient dynamics. The method used to estimate the initial mass by order for each litterbag was found to produce errors of up to 83% in estimation of initial mass for litterbags that were buried less than 24 hours, for which the initial and final mass should be equal). These errors in initial mass estimation, combined with insufficient replication led to high standard errors among replicate litterbags on most sample dates. The influence of ectomycorrhizal fungi associated with roots of *P. palustris* may also help explain our unexpected results. The presence of recalcitrant chitin N (Langley and Hungate 2003) and possibly P and Ca could account for the lack of correlation between decomposition rates and initial tissue chemistry.

This work represents the first attempt to investigate root decomposition among orders within the fine root guild. The variation in morphology and tissue chemistry we reported clearly supports the assertion by Pregitzer (2002) that fine roots cannot be treated as a homogenous cohort. Yet that understanding of fine roots may conflict with the results of the litterbag study of decomposition rates, which did not differ by order. Due primarily to large errors in estimation of initial mass, more research is needed to confirm the findings presented here, and future studies must find a way to determine mass loss more accurately. Greater precision among root orders is needed as well. We separated roots of *P. palustris* into only two groups, based on order, yet there may be differences among orders within these groups that our methods were unable to detect. Placing different root orders into separate litterbags may be one potential method, although roots

certainly do not decay this way in nature. Furthermore, the effects of mycorrhizae on decomposition are mostly unknown. Is mycorrhizal chitin present in distal roots to the degree that mass loss and nutrient dynamics will be substantially influenced? Are recalcitrant forms of mycorrhizal P and Ca present, and if so, what is the extent to which they might influence decomposition among root orders? If a better understanding of this important flux of C and nutrients is desirable, then further research into controls of fine root decomposition among orders is strongly needed.

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BIOGRAPHICAL SKETCH

Noah A. Jansen was born and raised in Grand Rapids, Michigan. As a child, he spent many days collecting bugs, reptiles, and amphibians in a vacant lot next door and exploring the woods near his grandparents' cottage. These experiences, along with elementary school field trips to local nature centers and annual summertime camping trips, helped to develop a fascination with the natural world from an early age. Noah also acquired an interest in science as early as kindergarten, when his career ambition was to become a scientist (or possibly a firefighter, if the whole science thing didn't work out). These interests later blossomed into a college major. Noah received a bachelor's degree in environmental science (biology emphasis) from Calvin College (Grand Rapids, Michigan) in 2002. As an undergraduate research assistant during the summer of 2001, Noah assisted Dr. David Warners in assessing local populations of two species threatened in the state of Michigan. A manuscript resulting from this work, Computer mapping of *Silphium laciniatum* and *Stipa spartea* as a tool for conservation, was accepted for publication in *The Michigan Botanist*.

A desire to learn about other parts of the country led Noah out of his long-time home of Michigan and down to the University of Florida School of Forest Resources and Conservation, where a cooperative assistantship was offered him in conjunction with the Joseph W. Jones Ecological Research Center at Ichauway (Newton, Georgia). After graduation, Noah will continue work at the Jones Ecological Research Center as the lead technician of the Forest Ecology and Silviculture lab, working with Dr. Steve Jack.