Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine

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Summary

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• Availability of growth limiting resources may alter root dynamics in forest eco-systems, possibly affecting the land–atmosphere exchange of carbon. This was evaluated for a commercially important southern timber species by installing a factorial experiment of fertilization and irrigation treatments in an 8-yr-old loblolly pine (*Pinus taeda*) plantation.
• After 3 yr of growth, production and turnover of fine, coarse and mycorrhizal root length was observed using minirhizotrons, and compared with stem growth and foliage development.
• Fertilization increased net production of fine roots and mycorrhizal roots, but did not affect coarse roots. Fine roots had average lifespans of 166 d, coarse roots 294 d and mycorrhizal roots 507 d. Foliage growth rate peaked in late spring and declined over the remainder of the growing season, whereas fine roots experienced multiple growth flushes in the spring, summer and fall.
• We conclude that increased nutrient availability might increase carbon input to soils through enhanced fine root turnover. However, this will depend on the extent to which mycorrhizal root formation is affected, as these mycorrhizal roots have much longer average lifespans than fine and coarse roots.

Key words: seasonal phenology, fine root turnover, minirhizotron, nutrient availability, water availability, loblolly pine, root dynamics.

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Introduction

The flux of C through the production and turnover of fine roots in forest ecosystems has long been recognized as a major component of terrestrial C and nutrient cycles (Cox *et al.*, 1978; Grier *et al.*, 1981; Vogt, 1991; Ruess *et al.*, 1996), and may have important implications for future climate through effects on C storage in soils. Rising anthropogenic N deposition already exceeds that available naturally (Galloway *et al.*, 1995; Vitousek *et al.*, 1997), and global precipitation is expected to increase in response to CO_2-induced climate change (Kattenberg *et al.*, 1996). Both factors could alter patterns of fine root production and turnover in forest ecosystems. Despite this, uncertainty remains in our estimates of fine root production and turnover because of the inherent difficulty of measurement. In a recent review, Nadelhoffer

(2000) pointed out that we do not even know the direction of the trend in fine root production as a proportion of total below- and above-ground production with increased N availability.

Many methods have been developed to estimate fine root turnover, but they may arrive at strikingly different results (Kurz & Kimmins, 1987; Publicover & Vogt, 1993; Vogt *et al.*, 1998). Traditional biomass-based methods (max–min, decision matrix and compartment flow) are plagued by statistical problems and the inability to account for simultaneous root production and mortality (Nadelhoffer, 2000). However, they do provide reasonable estimates of standing crop. Conversely, methods such as minirhizotrons or root observation windows are excellent for monitoring the demography of populations of fine roots, but estimation of biomass and absolute fluxes of C in bulk soil can be problematic. In addition,

there is no standard methodology for differentiating between dynamic and static root fractions in forest ecosystems. Many investigators conveniently classify all roots ≤ 2 mm diameter as 'fine roots', but using minirhizotrons we have observed roots of 2 mm diameter persisting for several years in semi-mature stands of loblolly pine (J. S. King, unpublished). Recent evidence demonstrates that nutrient contents and rates of respiration are dramatically higher for hardwood roots less than 0.5 mm diameter compared with larger roots (Pregitzer *et al.*, 1997; Pregitzer *et al.*, 1998). In addition, fine root longevity has been shown to be inversely related to diameter in apple (Wells & Eissenstat, 2001). These findings suggest that much of what has been termed 'fine roots' in the literature is probably a mix of static and dynamic root fractions, further reducing confidence in our estimates of fine root production and turnover.

The seasonal association of the growth of the two major resource-acquiring tissues, foliage and fine roots, is also poorly understood. Curiously, since the watershed paper of Lyr & Hoffman (1967), very few studies have examined concurrent above- and below-ground growth in intact forest stands. The evidence that does exist suggests that production and turnover of fine roots are synchronized with canopy development in the spring and leaf senescence in the fall (McClaugherty *et al.*, 1982; Hendrick & Pregitzer, 1992, 1993, 1996; Halter, 1998). Pregitzer *et al.* (2000) recently hypothesized that the stimulation of root growth in the spring is keyed to the same environmental cues as foliage growth (e.g. cumulative heat sum) and therefore above- and below-ground growth should be synchronized. As foliage and fine roots may be competitive sinks for C, it is important to determine if forest trees temporally partition the growth of these modular tissues. For deciduous species, if the spring flush of root growth occurs concurrent to that of foliage, the fixed amount of stored C must be split between the two growth sinks. Evergreen species, especially those living in mild climates, may be able to fuel early spring root growth with currently assimilated C, as photosynthesis proceeds when temperature rises above zero (Strain *et al.*, 1976).

To assess the effects of resource availability on above- and below-ground production and phenology, we monitored the production and senescence of foliage and fine roots, and stem volume in a loblolly pine stand that had been subject to fertilization and irrigation treatments for 3 yr. Loblolly pine is the most commercially valuable pine in the USA. It constitutes more than 95% of all trees planted on industrial forest land in the south-eastern USA (Baker & Langdon, 1990). Loblolly pine is an early successional species with high rates of growth, and it is known to be tolerant of low nutrient levels but is highly responsive to changes in resource availability (Griffin *et al.*, 1995). Using biomass-based methods, previous work at this site reported substantial declines in fine root production in response to fertilization (Albaugh *et al.*, 1998). Therefore, we expected to see reduced fine root production in fertilized

plots. We hypothesized that smaller diameter roots would be more dynamic than larger roots, and reasoned that competition for limited stored reserves would cause the growth of fine roots to occur at times during the growing season when foliage and stem growth were minimal.

Materials and Methods

Study site

This study was conducted at The South-east Tree Research and Education Site (SETRES), which is located in the Georgia–Carolina Sandhills in Scotland County, NC, USA (35° N, 79° W) on an infertile, excessively well-drained, sandy, siliceous, thermic Psammentic Hapludult soil (Wakula series). Available water at field capacity is 18–20 cm (approx. 10% volumetric water content) in the upper 2 m of the profile. Annual precipitation averages 1210 mm, but extended droughts periodically occur during the growing season. Annual temperature averages 17°C (Murthy *et al.*, 1996).

In 1992, 16 50 × 50 m treatment plots with 30 × 30 m measurement plots were established in a plantation of 8-yr-old loblolly pine. Plant material consisted of a mix of 10 one-half sib families of loblolly Piedmont selections that were hand-planted on a 2.4 × 3.0 m spacing in March, 1985. Final tree stocking density was 1260 stems per hectare. Treatments were applied in a randomized complete-block design with two levels of fertilization and two levels of irrigation, replicated four times. Nutrient treatments have been maintained since March 1992, and irrigation has been applied since April 1993. See Albaugh *et al.* (1998) for complete site and treatment descriptions.

The fertilization treatments were: ambient soil conditions (control); and optimum nutrition. Optimum nutrition was defined as maintaining dormant season foliar nitrogen concentration at 1.3% dry mass from a branch terminal in the upper third of the crown in dominant or codominant trees (Adams & Allen, 1985; Allen, 1987). Other nutrients were applied in balance with N to maintain nutrient : N ratios of 0.1 for phosphorus, 0.35 for potassium, 0.12 for calcium and 0.06 for magnesium. Foliar boron concentrations were maintained above 12 p.p.m. Foliar nutrient status was monitored monthly and fertilizer applied as necessary to maintain target values. Irrigation treatments were: natural precipitation (control); and precipitation plus supplemental irrigation. Irrigation events were targeted to maintain soil moisture between field capacity and 40% of field capacity in the surface 50 cm of the profile, as determined by time domain reflectometry (TDR) (Topp & Davis, 1985). Measurements were taken with a 50-cm TDR probe from 10 random locations within each plot at biweekly intervals from March to November. The irrigation system consisted of Rain Bird irrigation nozzles (Rain Bird Corporation, Azuga, CA, USA) mounted on 35 cm high risers positioned on a 10 × 10 m grid throughout the

treatment plots. During the growing season our soil water targets were achieved in 1994 and 1995; however, two separate drought events in 1993 caused available soil water content in the irrigated plots to drop to 23% and 5% for a total of 8 wk (Albaugh *et al.*, 1998).

Plant measurements

Before establishment of treatments, one tree per plot that was near the mean plot height and diameter was selected for nondestructive growth measurements. During the period 5 April 1994 to 12 April 1995, height was measured monthly and diameter biweekly on these trees. Foliage growth was assessed by determination of a foliage growth index. Fascicle length by flush was measured as the average fascicle length from three fascicles measured in the center of each flush. The fascicles were measured from their connection to the shoot to the tip of the longest needle in the fascicle. Fascicles were not measured until they could be handled without damage to the tree (between 1 and 2 cm long). The measurements were completed on the terminal of a branch in the upper third of the crown. Measurements were made biweekly from March to November and monthly during the rest of the year. Loblolly pine exhibits an indeterminate flushing pattern. Early in the measurement period only one flush of growth was present and fascicle length was measured on this single flush. Later in the growing season, when multiple flushes were present, a fascicle length for each flush was recorded. The foliage growth index was calculated as the sum of fascicle length for all flushes present on the tree when measurements were completed. The index represents the pattern of foliage development throughout the year given the multiple-flushing growth pattern.

In February and March 1994, six acrylic minirhizotron tubes measuring 5.5 cm diameter were installed to a depth of 55 cm adjacent to each of the 16 measurement trees at a 45° angle pointing towards the tree, for a total of 96 tubes. However, excessive fouling of minirhizotron tubes by soil fungi reduced replication to three in the current study (i.e. a total of 72 tubes). The tubes were installed in line with the tree row, with two of the tubes entering the soil 30 cm from the measurement tree (one on either side of the tree), two at 60 cm and the remaining two at 90 cm. Beginning 5 April 1994, minirhizotrons were sampled approximately every 30 d for the following year. Sampling consisted of photographing a transect of 40 contiguous frames (1.17 cm^2) along the inner top surface of each minirhizotron using a Bartz BTC-2 camera (Bartz Technology, Santa Barbara, CA, USA) affixed to an indexed ratcheting handle. The handle allowed for precise camera placement at each sample date, ensuring observation of the same roots over time. Images were recorded on standard VHS format videotapes and were manually digitized using ROOTRACKER software (Duke University Phytotron, Durham, NC, USA). Roots were classified as fine (nonmycorrhizal roots < 1 mm diameter) or coarse (nonmycorrhizal roots 1–2 mm diameter), and given a condition code, 'live' or 'dead'. Dead roots were those that disappeared between sample dates, or that were visibly deteriorating (black, sloughing material). Mycorrhizal roots were defined as those displaying a dichotomous branching pattern or on which the fungal mantle was visible. The condition ('live' or 'dead') was also noted for mycorrhizal roots. Cumulative root length production, mortality and net root production (the integral of the difference between root production and mortality) for each category of root were determined over the course of the study following the method of Berntson & Bazzaz (1996). Estimates of average annual root turnover for each plot were calculated as the ratio of average annual length production and mortality to average annual live root standing crop (Burton *et al.*, 2000). Average root life span in days was estimated by dividing rates of turnover into 365. In order to directly compare rates of length extension of needles and fine roots, the relative growth rate formula (Hunt, 1990) was modified for application to the foliage and nonmycorrhizal fine root length data as follows: RLER = $(\ln L2 - \ln L1)/(t_2 - t_1)$, where RLER stands for 'relative length extension rate', L is length of needles or fine roots, t is time, and subscripts refer to successive measurement dates.

Statistics

The study site at SETRES is set up as a randomized complete block design of replicated irrigation and fertilization treatments. Within this statistical framework, root length production and mortality data were analysed using repeated measures analysis of variance to account for serial correlation of growth measurements collected from the same trees over time (Moser *et al.*, 1990; Meredith & Stehman, 1991). Root length measurements were pooled over all minirhizotrons in a plot, thereby treating the tree as the experimental unit. Orthogonal quadratic polynomials were fit to all data (stem volume, foliage index, etc.) as calculated for unequal sampling intervals (Robson, 1959). Coefficients of the polynomials were then used as primary data to test for significance of treatment effects using standard ANOVA (Steel & Torrie, 1980). In this approach, analysis of the mean (M) term of the polynomials is equivalent to the usual whole-unit comparison of the treatment main effect, while the linear term (L) is a test of a time × treatment interaction, such that the slope of the response is not the same for all treatments. Finally, the quadratic term (Q) tests for complex quadratic time interactions (different curvature or rates of change of response functions). Polynomial coefficients were log-transformed to normalize variances across treatments, meeting the assumptions of ANOVA, that was confirmed by inspection of residuals and normal probability plots. Root turnover data were analysed using split-plot ANOVA, with irrigation and fertilization comprising main effects and root type (fine, coarse and mycorrhizal) being treated as the split-plot effect. Treatment effects were considered significant if $P \leq 0.05$.
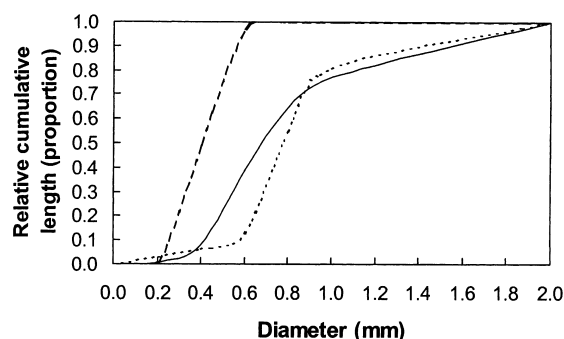
Fig. 1 Cumulative length distribution by diameter for dead (dotted line), live (unbroken line) and mycorrhizal (dashed line) roots observed in minirhizotrons on 6 October 1994 in an 11-yr-old loblolly pine plantation after 3 yr of irrigation and fertilization treatments on a coastal plain experimental site (SETRES), Scotland County, NC, USA.

Results

The length distributions of the roots viewed in the minirhizotrons differed by root class (Fig. 1). At the end of the growing season (6 October 1994), 96% of the mycorrhizal root length occurred in roots with diameters between 0.2 and 0.6 mm. Live nonmycorrhizal roots showed a much broader diameter distribution, with 77% of the root length occurring in roots with diameters between 0.4 mm and 1.0 mm. The remaining root length occurred in roots with diameters between 1.0 and 2.0 mm. Finally, the dead roots that remained visible by this date had a diameter distribution similar to that of live roots. Eighty-one per cent of the dead root length occurred in roots with diameters between 0.2 mm and 1.0 mm, with the remaining percentage in roots with diameters between 1.0 and 2.0 mm.

Cumulative fine (nonmycorrhizal) root length production (Fig. 2a) greatly exceeded mortality (Fig. 2b) from April 1994 to October 1994, when a large mortality event occurred causing a turnover of virtually all of the fine roots produced up to that time (Fig. 2c). This was immediately followed by a second period in which length production exceeded mortality, from November 1994 until March 1995, causing an increase in net production. A second mortality event then occurred, causing another large turnover of fine roots in the spring of 1995. This pattern was consistent for all treatments, and fertilization was significant in the M and L terms (Table 1), indicating that
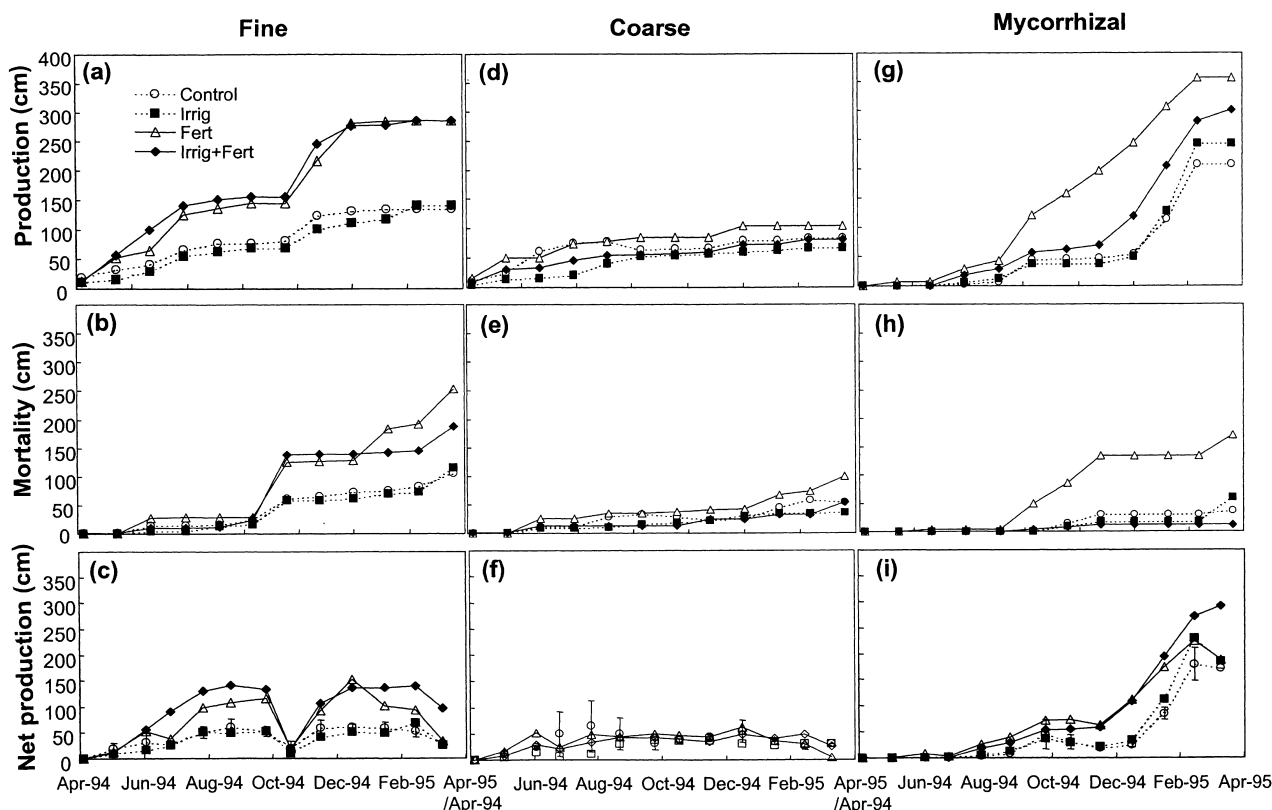
Fig. 2 Cumulative length production, cumulative length mortality, and net production, respectively, of fine roots (a, b, c), coarse roots (d, e, f), and mycorrhizal roots (g, h, i) observed in minirhizotrons in an 11-yr-old-loblolly pine plantation after 3 years of irrigation and fertilization treatments on a coastal plain experimental site (SETRES), Scotland County, NC, USA. Open circles, control; filled squares, irrigated; open triangles, fertilized; filled diamonds, irrigated and fertilized.

Table 1 P-values from the repeated measures analysis of variance on stem volume index (D_2H), foliage development index and cumulative production, cumulative mortality and net production of fine roots (< 1 mm diameter), coarse roots (1–2 mm diameter) and ectomycorrhizal roots in an 11-yr-old loblolly pine plantation after 3 yr of fertilization and irrigation in Scotland County, NC, USA

Parameter	Source	M	L	Q
Stem volume (D^2H)	Irrigation	ns	ns	0.046
	Fertilization	ns	0.008	0.013
	Irrigation ×fertilization	ns	ns	ns
Foliage development index	Irrigation	ns	ns	ns
	Fertilization	0.009	0.017	ns
	Irrigation ×fertilization	ns	ns	ns
Cumulative fine root production	Irrigation	ns	ns	ns
	Fertilization	0.023	0.013	ns
	Irrigation ×fertilization	ns	ns	ns
Cumulative fine root mortality	Irrigation	ns	ns	ns
	Fertilization	0.035	0.027	ns
	Irrigation ×fertilization	ns	ns	ns
Net fine root production	Irrigation	ns	ns	ns
	Fertilization	0.006	0.007	ns
	Irrigation ×fertilization	ns	ns	ns
Cumulative coarse root production	Irrigation	ns	ns	ns
	Fertilization	ns	ns	ns
	Irrigation ×fertilization	ns	ns	ns
Cumulative coarse root mortality	Irrigation	ns	ns	ns
	Fertilization	ns	ns	ns
	Irrigation ×fertilization	ns	ns	ns
Net coarse root production	Irrigation	ns	ns	ns
	Fertilization	ns	ns	ns
	Irrigation ×fertilization	ns	ns	ns
Cumulative mycorrhizal root production	Irrigation	ns	ns	ns
	Fertilization	0.028	0.024	ns
	Irrigation ×fertilization	ns	ns	ns
Cumulative mycorrhizal root mortality	Irrigation	ns	ns	ns
	Fertilization	ns	ns	ns
	Irrigation ×Fertilization	ns	ns	ns
Net mycorrhizal root production	Irrigation	ns	ns	ns
	Fertilization	0.011	0.019	ns
	Irrigation ×fertilization	ns	ns	ns

M, L and Q refer to mean, linear, and quadratic terms of orthogonal polynomials fitted to the original data in the repeated measures analysis of variance. Values marked 'ns' are statistically nonsignificant (e.g. $P > 0.05$).

rates of fine root production and mortality were responsive to nutrient availability. Averaged over the year, fine root standing crop was 91% greater in fertilized and irrigated and fertilized plots compared with control plots.

Length production and mortality of coarse roots was much less dynamic than that of fine roots (Fig. 2d,e). Both increased slowly over the course of the experiment, and were not significantly influenced by the treatments (Table 1). Net length production of coarse roots (Fig. 2f) was much less than that of fine roots, and varied little over the course of the experiment. Maximum coarse root standing crop was reached by October 1994 and remained fairly constant over the measurement year. It appears to have declined in April 1995 in fertilized plots, but since this occurred only in this treatment and none of the treatments were statistically significant, we feel this is probably a spurious measurement.

Cumulative production of mycorrhizal root length increased almost monotonically over the course of the measurement year (Fig. 2g), and was significantly increased by fertilization (Table 1). Mycorrhizal root length mortality occurred at very low rates for the duration of the study (Fig. 2h), but was increased by fertilization, although this response was not statistically significant. Net mycorrhizal root production peaked at approximately 300 cm length along the minirhizotrons (Fig. 2i), and was ultimately much greater than that of either fine (approx. 150 cm) or coarse roots (approx. 75 cm). Net mycorrhizal root production was significantly affected (M and L terms) by fertilization, averaging 268% higher in fertilized compared with unfertilized plots.

Average rates of root turnover determined from the length production, mortality and standing crop data ranged from 2.02 to 2.49 yr^{-1} for nonmycorrhizal fine roots, 1.04 to 1.55 yr^{-1} for coarse roots, and 0.32 to 0.98 yr^{-1} for mycorrhizal roots (Table 2). Analysis of variance revealed that the irrigation and fertilization treatments did not have a statistically significant effect on root turnover. Root type (fine, coarse or mycorrhizal), however, was significant in affecting turnover rate ($P = 0.036$), and therefore average root life span. Fine

Root type	Treatment	Turnover (yr^{-1})	Lifespan (d)
Fine roots	Control	2.02 (0.41)§	181
	Irrigation	2.17 (0.30)	168
	Fertilization	2.49 (0.30)	147
	Irrigation and fertilization	2.08 (0.23)	175
Coarse roots	Control	1.28 (0.09)	285
	Irrigation	1.09 (0.14)	335
	Fertilization	1.55 (0.45)	235
	Irrigation and fertilization	1.04 (0.36)	351
Mycorrhizal roots	Control	0.94 (0.07)	388
	Irrigation	0.98 (0.16)	372
	Fertilization	0.63 (0.12)	579
	Irrigation and fertilization	0.32 (0.00)	1140

Table 2 Measured rates of turnover and calculated root lifespan of fine roots (< 1 mm diameter), coarse roots (1–2 mm diameter) and mycorrhizal roots in an 11-yr-old loblolly pine plantation after 3 yr of fertilization and irrigation in Scotland County, NC, USA. Root type had a significant effect on turnover ($P = 0.036$); effects of irrigation and fertilization were not significant

§Turnover values are means ($n = 3$) with standard errors in parentheses. Note that $n = 2$ for mycorrhizal roots in both the irrigation and the irrigation and fertilization treatments.
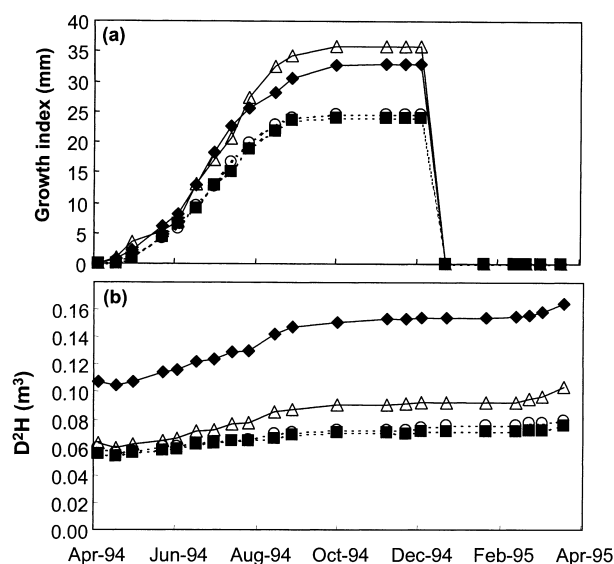
Fig. 3 Pattern of foliage growth (a) and stem volume index (b) in an 11-yr-old loblolly pine plantation after 3 yr of irrigation and fertilization treatments on a coastal plain experimental site (SETRES), Scotland County, NC, USA. Open circles, control; filled squares, irrigated; open triangles, fertilized; filled diamonds, irrigated and fertilized. The foliage growth index was calculated from fascicle length of all flushes present.

roots had an average turnover rate (with standard error in parenthesis) of 2.19 (0.15) yr^{-1}, which equals an average root life span of 166 d. Coarse roots had an average turnover rate of 1.24 (0.14) yr^{-1}, or an average life span of 294 d. Mycorrhizal roots had an average turnover rate of 0.72 (0.09) yr^{-1}, or an average life span of 507 d.

Foliage production, as estimated by the foliage growth index, commenced in early April 1994, and continued through late August to early September, after which time very little additional foliage growth occurred (Fig. 3a). Fertilization was significant in the M and L terms (Table 1) due to greater

rates of foliage production at the higher level of nutrient availability. This resulted in a 41% increase in the foliage index by the time leaf area production ceased. Stem volume, as estimated by D^{2}H, displayed a steady increase over the course of the study, with the period of greatest growth occurring from April to early September 1994 (Fig. 3b). Fertilization was significant in the M, L and Q terms, and irrigation in the Q term (Table 1), indicating that stem volume growth rate was sensitive to both water and nutrient availability, with nutrient availability having the greatest effect. Stem volume increased an average 19% in fertilized plots compared with control plots, while the increase in irrigated and fertilized plots averaged 99% compared with control plots (Fig. 3b).

Calculation of the relative length extension rate (RLER) of foliage demonstrates that foliage growth occurred in a single episode beginning in early April 1994, that peaked in late May and then slowly declined for the remainder of the growing season, ending in October (Fig. 4a). By contrast, there were three periods of growth for fine roots (Fig. 4b). The first occurred from April to July 1994, the second was from October to November 1994 and the third occurred from February to April 1995. Each period of net root growth was followed by a period of net root mortality, as indicated by reductions in fine root RLER. There was some variation in the magnitude of foliage and root RLER in response to the treatments but, overall, the seasonal pattern of growth was remarkably constant. Further, relative length extension occurred at similar rates for both foliage and fine roots, reaching maximum rates between 2 and 2.5 mm cm^{-1} wk^{-1}.

Discussion

This study provides clear evidence that net production of the dynamic fraction of fine roots in loblolly pine increases in response to higher availability of N. Further, growth of above- and below-ground ephemeral tissues appears to be synchronized over the course of the growing season. Leaves
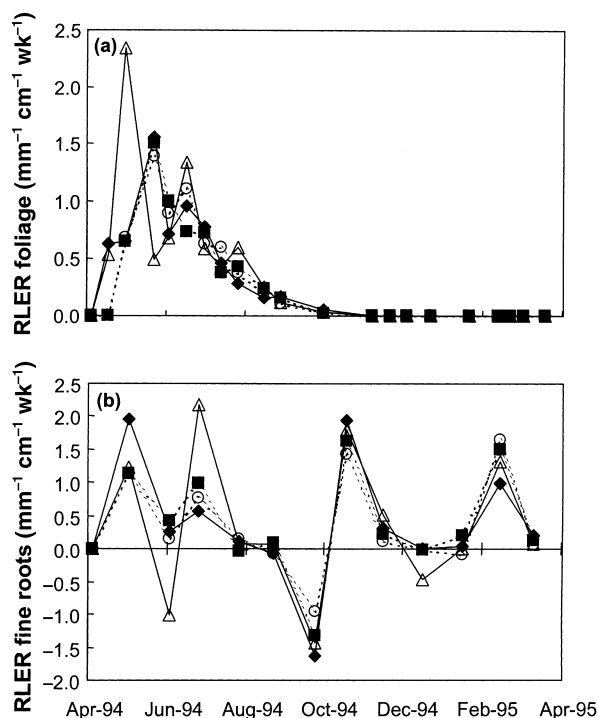
Fig. 4 Relative length extension rate (RLER, growth of new length per unit of existing length per week: RLER = $(\ln L2 - \ln L1)/(t_2 - t_1)$, where RLER stands for 'relative length extension rate', L is length of needles or fine roots, t is time, and subscripts refer to successive measurement dates) of foliage (a) and fine roots (b) in a semi-mature loblolly pine plantation after 3 yr of irrigation and fertilization treatments on a coastal plain experimental site (SETRES), Scotland County, NC, USA. Open circles, control; filled squares, irrigated; open triangles, fertilized; filled diamonds, irrigated and fertilized.

and fine roots of loblolly pine began growing at the same time in the spring, and fine roots experienced high mortality in fall, the time of year when second year needles were being shed.

Ephemeral, perennial and mycorrhizal roots

We hypothesized that growth patterns of smaller diameter roots would be more dynamic than larger roots. In support of our hypothesis, we found that fine roots, less than 1.0 mm diameter, were highly dynamic, while coarse roots, 1.0–2.0 mm diameter, appeared to be part of the perennial root system. The distribution of cumulative root length by diameter reveals that almost 80% of the fine root length occurred in roots less than 1.0 mm in diameter. Similarly, Hendrick & Pregitzer (1993) reported that in sugar maple-dominated forests in Michigan approximately 80% of the cumulative root length observed in minirhizotrons was composed of roots less than 0.6 mm diameter. Pregitzer *et al.* (1997) hand-dissected lateral roots of *Acer saccharum* and *Fraxinus americana* and also found that over 80% of the length occurred in roots less than 1.0 mm diameter. This suggests that our fine root length distributions are not an

artifact of the minirhizotrons. Allocation of the majority of root length to the very smallest diameter roots in a variety of forest taxa suggests the size class below which root properties and function shift from those of perennial to ephemeral roots is much smaller than the commonly used 2.0 mm diameter size class.

The seasonal demography of roots observed in this study reinforces this point. By calculating root life span from measured rates of turnover, we found that roots less than 1.0 mm in diameter were highly dynamic, with average life spans of 166 d. By contrast, roots that were 1.0–2.0 mm diameter were estimated to live, on average, 294 d. These results are consistent with Wells & Eissenstat (2001) who reported that apple roots with diameters less than 0.3 mm had median life spans of under 2 months, while those greater than 0.5 mm diameter were likely to over-winter and function for more than one growing season. Although our analysis did not consider differences in demography between roots that differed in diameter by tenths of a millimeter, our data show that the majority of small diameter root length, both live and dead, occurred in roots with diameters between 0.5 mm and 1.0 mm. Further, similarity between the diameter distributions of live and dead roots suggests that the ephemeral fraction in loblolly pine is indeed mainly composed of roots with diameters less than 1.0 mm, and these account for the majority of root turnover on an annual basis.

The exception to the above analysis was roots that formed mycorrhizal symbioses. Almost all of the mycorrhizal root length occurred in roots with diameters between 0.2 mm and 0.6 mm. This may be because infection occurs in living primary cortical cells of physiologically 'young' growing root tips (Marks & Foster, 1973; Anderson, 1992), which apparently remain small in diameter over the life of the association. Interestingly, the steadily increasing net production (very low rates of cumulative length mortality) measured over the course of this study indicates that mycorrhizal infection greatly increased the longevity of the infected roots, with estimated average lifespans of 507 d. This finding is consistent with studies (Garret, 1960) reporting that mycorrhizae remain functional longer and are more resistant to certain root pathogens than nonmycorrhizal roots of similar diameter. Mycorrhizae are obviously important modifiers of root turnover and should receive greater attention when estimating cycling of C and nutrients in forest ecosystems (Fogel, 1980).

Consistent with Wells & Eissenstat (2001), our results indicate that the term 'fine roots' can be a misleading simplification of the relationship between root function and morphology, obscuring our understanding of below-ground dynamics. Roots of very similar diameters can have dramatically different demography depending on their physiological state. Thus, the challenge for future research is to determine not only the size of the 'fine root' pool, but also the proportion comprising ephemeral, perennial and mycorrhizal roots along with estimated turnover rates for each fraction. Because roots

of extremely small diameter have very high specific root length (Pregitzer *et al.*, 1997, 1998) they comprise only a small fraction of total root biomass at any given time, even though they contain the majority of the length. Therefore, sampling techniques that aggregate all roots below a given diameter have limited capacity to detect changes in the pool size of the most dynamic roots, and therefore confound responses of perennial, ephemeral, and mycorrhizal roots.

Response of fine roots to N availability

Soil nutrient availability differentially affected the seasonal dynamics of fine, coarse and mycorrhizal roots, but irrigation had little effect. Irrigation probably had little effect because soil moisture content differed little between irrigated and nonirrigated plots during this study because of adequate precipitation (King *et al.*, 1997). Contrary to our hypothesis, cumulative (and net) fine root production was greatly stimulated (+108%) by increased nutrient availability. This result conflicts with that of Albaugh *et al.* (1998) for the same site: they reported significantly decreased cumulative fine root production due to fertilization in 1994 and 1995. We attribute the discrepancy to the different methods used to sample fine roots and determine production. The former study used sequential coring to estimate standing crop of all roots ≤ 2.0 mm diameter and the decision matrix approach to calculate production (Fairley & Alexander, 1985). We used minirhizotrons to observe directly the cumulative production and mortality of root length for fine (< 1 mm diameter), coarse (1–2 mm diameter) and mycorrhizal roots. We believe what was termed 'fine roots' in the two studies actually refers to different populations of roots that were differentially sampled by the respective methodologies.

Our data suggest that the dynamic responses of the ephemeral 'fine' roots went totally undetected by the sequential core sampling in the study of Albaugh *et al.* (1998), probably because of their very small diameter and relatively low biomass. In the current study, net production and average rate of turnover of the perennial 'coarse' roots (1–2 mm diameter) were little affected by nutrient availability. Small differences in the standing crop of this root fraction detected by sequential coring, combined with the assumptions used in the decision matrix resulted in an estimated decrease in cumulative production in the analysis of Albaugh *et al.* (1998). Interestingly, we found that cumulative and net production of mycorrhizal roots also increased significantly with fertilization. This could be due to greater allocation of labile C below ground from increased leaf area and higher photosynthetic rates under fertilization (Murthy *et al.*, 1996; Albaugh *et al.*, 1998). The relatively long lifespan of this root fraction could actually reduce turnover under increased nutrient availability, decreasing the amount of C deposited into the soil on an annual basis.

Although the short interval between minirhizotron installation and beginning of the observations may have contributed to the observed responses caused by root wounding, we believe this effect, if it occurred, was small. The spatial arrangement of the minirhizotrons integrated a large volume of soil that initially had few roots (up to 90 cm from the measurement tree to a depth of 55 cm), and we observed very few severed roots during the installation process. In addition, the consistent increase of cumulative root length over the course of the study indicates that roots 'colonized' the minirhizotrons over time, rather than episodically responding to a wounding event. Although this may be an indication that our minirhizotrons had not yet equilibrated in the soil (Burton *et al.*, 2000), it should not have affected the relative responses to the treatments.

As recently discussed by Nadelhoffer (2000), there is no consensus as to the response of fine root production to increased N availability. There are reports in the literature of increased (Pregitzer *et al.*, 1995, 2000; Kubiske *et al.*, 1998; Burton *et al.*, 2000), decreased (Grier *et al.*, 1981; Vogt *et al.*, 1986; Gower *et al.*, 1992) and unchanged (Price & Hendrick, 1998) fine root production in response to increasing N availability. Some of the discrepancy is undoubtedly caused by differences in methodology, natural soil fertility and life-history traits of the dominant vegetation. In addition, there may not be a generalized response of fine root production to increasing N availability, but the currently available data are insufficient to test this hypothesis (Nadelhoffer, 2000). Consistent with other studies examining the effect of fertilization on root growth in loblolly pine (Sword *et al.*, 1996; Sword, 1998), we found that fine root production increased significantly with fertilization relative to control plots, but the timing of their shedding was unchanged. Therefore, increased turnover occurred with fertilization (i.e. more C was deposited in the soil) even though average root longevity appears to have remained unaltered. This response supports the hypothesis that fine root production responds to soil N availability similar to above-ground production and turnover (Hendricks *et al.*, 1993), which increased greatly with fertilization at our site (Albaugh *et al.*, 1998). Nutrient enrichment experiments have also documented greatly increased root proliferation in areas of soil with high nutrient availability (Jackson & Caldwell, 1991; Pregitzer *et al.*, 1993). Our results suggest that much of the uncertainty regarding fine root responses to nutrient availability may come from insufficient discrimination between physiologically distinct root fractions that differ in biomass and rates of turnover.

Seasonality of above- and below-ground production

We expected to see temporal asynchrony between the production of fine roots and above-ground growth because of competition for limited stored reserves of carbon. What we found is that the seasonal growth and mortality of ephemeral tissues was synchronized in loblolly pine, but the times of peak growth of foliage and fine roots were temporally offset.

By contrast, stem growth occurred at a slow, but almost continuous rate.

The strongly seasonal growth of fine roots is relatively well known for some northern forest ecosystems. Typically, fine root growth commences in early spring, before or coincident with foliage growth (Lyr & Hoffman, 1967; Hendrick & Pregitzer, 1993; Burke & Raynal, 1994; Fahey & Hughes, 1994), peaks in late spring or early summer and decreases rapidly in fall, coincident with decreasing soil temperature (McClaugherty *et al.*, 1982; Hendrick & Pregitzer, 1992, 1996; Burke & Raynal, 1994; Fahey & Hughes, 1994). These systems also exhibit strong seasonality in soil respiration which is highly correlated to changes in soil temperature, indicative of the annual cycle of root and microbial metabolism (King *et al.*, 2001). However, fine root growth in southern forests may have a much less distinct seasonal signal as a result of the mild climate and moderate soil temperatures, although shoot growth remains highly seasonal (Reed, 1939).

In the current study, fine root growth occurred throughout the year, but in three distinct phases corresponding to different leaf phenological periods. The first phase occurred from the end of March to August 1994, with initiation of root growth preceding that of foliage. The second phase of root growth occurred in October and November 1994, the time of year loblolly pine abscises second-year needles (Wahlenberg, 1960; Wells & Metz, 1963). Finally, the third phase of root growth occurred from early February to early April 1995, a period during which environmental conditions favor photosynthesis in loblolly pine, but shoot growth is minimal. An interesting, yet unexpected finding of the current study is that the relative rate of needle and fine root length extension was very similar, reaching a maximum of $2.0-2.5$ mm cm^{-1} wk^{-1}. This could be due to inherent limitations on rates of meristematic processes (cell division, differentiation, and expansion) and merits further consideration.

It is uncertain whether the seasonal pattern of fine root growth observed in this study is typical for our site because we only observed root growth for 1 yr. Studies have shown that fine root growth can vary from year to year at the same site (Burke & Raynal, 1994; Côté *et al.*, 1998), and it is possible that variation in climate could cause differences in year-to-year fine root growth at our site. Future studies of C cycling in terrestrial ecosystems would do well to distinguish the growth responses of the ephemeral and perennial root fractions, and to characterize the seasonal growth patterns of roots relative to resource availability, interannual variation in climate and above-ground plant responses. As a general hypothesis, we contend that the plasticity of seasonal fine root growth is probably greater in southern forests as a result of mild climate, compared with northern forests where the seasonal progression of soil temperature has much greater amplitude and growing seasons are much shorter.

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