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Fine root dynamics in a developing *Populus deltoides* plantation

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Summary A closely spaced (1 × 1 m) cottonwood (*Populus deltoides* Bartr.) plantation was established to evaluate the effects of nutrient availability on fine root dynamics. Slow-release fertilizer (17:6:12 N,P,K plus micronutrients) was applied to 225-m^2 plots at 0, 50, 100 and 200 kg N ha^{-1}, and plots were monitored for two growing seasons. Fine root production, mortality, live root standing crop and life span were analyzed based on monthly minirhizotron observations. Fine root biomass was measured in soil cores. Fine root dynamics were controlled more by temporal, depth and root diameter factors than by fertilization. Cumulative fine root production and mortality showed strong seasonal patterns; production was greatest in the middle of the growing season and mortality was greatest after the growing season. Small diameter roots at shallow soil depths cycled more rapidly than larger or deeper roots. The strongest treatment effects were found in the most rapidly cycling roots. The standing crop of live roots increased with fertilizer treatment according to both minirhizotron and soil coring methods. However, production and mortality had unique treatment response patterns. Although cumulative mortality decreased in response to increased fertilization, cumulative production was intermediate at 0 kg N ha^{-1}, lowest with 50 kg N ha^{-1}, and highest with 200 kg N ha^{-1}. Aboveground growth responded positively to fertilization up to an application rate of 50 kg N ha^{-1}, but no further increases in growth were observed despite a threefold increase in application rate. Median fine root life span varied from 307 to over 700 days and increased with depth, diameter and nutrient availability.

Keywords: cottonwood, fine root production, nitrogen fertilizer, root longevity, short rotation woody crops, stand development.

Introduction

Fine root biomass is a relatively small, yet highly active C pool in forest ecosystems. The fraction of live fine root biomass to total tree biomass can range from less than 1% in mature forests to over 15% in young forests (Vogt 1991). Although this pool is proportionally small, carbon rapidly cycles through it. Fine root production varies from 10 to 60% of total net primary production (Vogt et al. 1986, Nadelhoffer and Raich 1992); fine root life spans vary from less than 20 to over 200 days (Eissenstat and Yanai 1997), indicating replacement of the feeder root system occurs once or more per year. This high activity implies that the fine root pool has an important role in the C and N cycles.

The wide range in fine root production and turnover is attributed to different measurement techniques and sensitivity to several external and internal factors (Eissenstat et al. 2000, Gill and Jackson 2000). Seasonality appears to dominate these factors and variation in fine root production is correlated with key phenological events (e.g., bud burst and bud set) during the growing season (Atkinson 1983, Hendrick and Pregitzer 1996, Thomas et al. 1996, Burton et al. 2000, Johnson et al. 2000, Joslin et al. 2001). However, several other environmental controlling factors have been identified including nutrient availability (Hendricks et al. 1993), drought (Santantonio and Hermann 1985, Joslin et al. 2001), temperature (Teskey and Hinckley 1981), atmospheric carbon dioxide (CO_2) concentration (Pregitzer et al. 1995, Tingey et al. 2000), pathogens (Kosola et al. 1995), symbionts (Eissenstat et al. 2000) and invertebrate herbivory (Wells et al. 2002). There are also internal factors controlling production and turnover such as root diameter (Coleman et al. 2000, Wells and Eissenstat 2001), depth in soil profile (Hendrick and Pregitzer 1996, Coleman et al. 2000) and genotypic variation (Eissenstat 1991). The effects of stand age are not well understood. Conifer seedlings have low fine root turnover (Hallgren et al. 1991); however, as trees mature, a majority of fine roots turn over every year (Eissenstat and Yanai 1997). In stand-level comparisons, conifer root production increases with age, relative to live-root biomass (Vogt et al. 1982). Yet it is unclear how production and mortality change through the stages of stand development from initiation to maturity. Understanding responses to external environmental factors will require information on internal

controls to make informed comparisons among studies.

The magnitude of the different controlling factors can be quite variable; however, the direction of the response is typically well defined. For instance, root production and turnover increase with temperature, atmospheric CO_2 concentration and shoot growth activity; however, they decrease with increasing drought, diameter and depth (Bloomfield and Vogt 1996, Eissenstat and Yanai 1997). The direction of response has not been defined for nitrogen (N) availability. Nitrogen availability is of major interest for several reasons including the limitations N imposes on growth in many forest types (Binkley 1986), increases in forest fertilization programs (Allen et al. 1990, Chappell et al. 1992), environmental concerns over anthropogenic N inputs (Vitousek et al. 1997) and the potential for tree root systems to mitigate nitrate-contaminated ground and surface water as riparian or wastewater filters (Myers et al. 1996, Schultz et al. 2000, Aronsson and Perttu 2001, Isebrands and Karnosky 2001).

The response of fine root production to N has been studied using destructive sequential coring, nondestructive observational, and indirect N budget approaches with equivocal results (Hendricks et al. 1993). In general, with increasing nutrient availability, sequential coring results show decreased fine root production and nondestructive methods show increased fine root production (Nadelhoffer 2000), but there are important exceptions (Hendricks et al. 1993, Eissenstat and Yanai 1997).

In addition to differing methodologies, variation in N treatment regimes makes comparisons among studies difficult (Gower et al. 1996, Gill and Jackson 2000). Generally, past studies considering N effects on fine root production and turnover compared only two N regimes that were experimentally controlled by N amendments, use of different soil types, or comparison of sites of different quality, i.e., N mineralization rates. Among these different studies, a wide range of nutrient availability differences has been imposed or considered; however, few studies have incorporated multiple nutrient availability regimes to fully understand the response function.

In this study, we sought to resolve some of the uncertainty in fine root production responses to N by controlling some sources of variation. We selected a uniform study site low in available N, established a uniformly spaced, clonal plantation, maintained an optimal water regime through irrigation, and provided four N regimes in balance with other macronutrients and micronutrients. Observations were made for two growing seasons to account for developmental effects and to provide insight into the differences between seedling and ecosystem studies.

Materials and methods

Study site

The study site was a 0.35 ha plantation located in the Hugo Sauer Nursery at the North Central Research Station in Rhinelander, WI (Oneida County, 89°25′ W, 45°38′ N). The soil in the study area is a Croswell loamy sand (Entic Haplorthod) (Oneida County Soil Survey, 1993, USDA Soil Conservation Service). Soil properties are shown in Table 1. The climate is continental, with cold winters and mild summers. Temperatures average 19.4 °C in July and –12.1 °C in January. The frost-free growing season usually lasts 80–100 days, and precipitation averages about 80 cm year^{-1} (Oneida County Soil Survey, 1993, USDA Soil Conservation Service).

During the 1997 growing season, green stem cuttings of top-performing *Populus deltoides* Bartr. clone D-105 (Riemenschneider and Isebrands 1996) were rooted, grown in a greenhouse and moved to a shade house where they were overwintered. The container-grown rooted cuttings were planted at the study site on May 1–2, 1998, in a randomized complete block design consisting of four blocks with four fertilizer treatments. Slow-release fertilizer (17:6:12 N,P,K plus micronutrients, 3- to 4-month release, N derived from ammonium nitrate) was applied at 0, 50, 100 or 200 kg N ha^{-1} (hereafter referred to as 0N, 50N, 100N and 200N, respectively) on May 5–7, 1998 and April 13, 1999. Each 225-m^2 treatment plot contained 196 trees (14 × 14) at 1 × 1 m spacing. Measurement plots contained 64 trees and were surrounded by three border rows. Alleyways (3 m wide) divided the plots for irrigation lines and tractor equipment. Irrigation supplemented precipitation so that the plantation received at least 2.5 cm of water each week from May through August of each year.

Strict weed control was imposed throughout the experiment to ensure all roots observed were from the target species. In summer 1997, the site was sprayed with 4.7 l ha^{-1} glyphosate (N-(phosphonomethyl) glycine). Tilling was performed in spring and fall before planting. The preemergent herbicide linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) was applied at 1.1 kg ha^{-1} before planting in 1998. The preemergent herbicide imazaquin/pendimethlin (*N*-(1-ethylpropyl)-3) was applied at 4.7 l ha^{-1} before leaf emergence in 1999.

After planting, glyphosate was regularly applied directly to weeds within plots. Plot borders were tilled mechanically. Interactions among trees in adjacent plots were minimized by severing lateral roots by drawing a coulter disk along plot borders to a depth of 45 cm in midsummer and early spring.

Minirhizotron techniques

Root dynamics were monitored to a vertical depth of 36 cm below the soil surface with extruded, acrylic minirhizotron tubes (5 cm internal diameter and 90 cm length placed at an angle of

Table 1. Study site soil properties. In each plot, three soil cores were collected and composited by depth. Means ± standard errors (n = 4) are shown.

Soil depth (cm)	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	C (g kg^{-1})	N (g kg^{-1})	pH
0–30	818 ± 14	143 ± 39	39 ± 9	240 ± 4	14 ± 0.2	5.4 ± 0.1
30–60	865 ± 20	104 ± 26	31 ± 8	50 ± 2	0.3 ± 0.1	5.6 ± 0.2

45° to the surface). Five tubes per plot were observed monthly. Roots (< 2 mm in diameter) growing along the upper surface of the tube were imaged with a high resolution (26 μm pixel^{-1}) micro-video camera (Bartz Technology, Santa Barbara, CA). About 40 consecutive images (1.48 cm^2 per image) were digitized per tube, covering the entire 36 cm depth. Therefore, each tube had a total observed surface area of 59.2 cm^2. Rootracker (Duke University, Durham, NC) image analysis software was used to quantify root length, width and condition. Three root condition categories were used: (1) new, (2) previously observed and (3) missing. Because of the subjectivity in determining the condition of previously observed roots, only roots that disappeared were considered dead.

Destructive techniques

Live root biomass was determined in soil cores. Eight random soil cores (5 cm diameter, 30 cm deep) from each plot were taken in October 1998. Roots were separated from soil with a Gillison root washer (Gillison's Variety Fabrication, Benzonia, MI) (Smucker et al. 1982, Pallant et al. 1993). Roots were divided into two diameter size classes (< 1 mm and ≤ 1 mm), dried at 70 °C, and weighed.

Aboveground measurements

Basal area growth and leaf nutrient content were used to monitor the aboveground response to nutrient amendments. Diameter at the root collar was measured with calipers in October 1998 and October 1999 in the measurement plots. Basal area was calculated based on root collar diameter and a stocking of 10,000 trees ha^{-1} minus any mortality, which was less than 0.2%. Leaf N content was determined from leaf samples collected from the four center trees in each plot in July 1998 and 1999. We collected leaf samples from the upper canopy in 1998 and the upper and lower canopy in 1999. Leaves collected in each plot were composited, dried at 70 °C, and analyzed for total N (Carlo Erba mass analyzer).

Data analysis

Temporal and treatment effects on fine root production, mortality and standing crop were evaluated by repeated measures analysis (Moser et al. 1990, Potvin et al. 1990, SAS, Cary, NC). Repeated measures analysis was appropriate because observations of the same minirhizotron tube location were repeated over time. A separate analysis was performed for each year. The form of fertilizer response functions was evaluated by polynomial contrasts (Snedecor and Cochran 1980). With four fertilizer treatments, it was possible to consider three (n – 1) contrasts including linear, quadratic and lack-of-fit. The ORPOL function in SAS IML generated coefficients for orthogonal polynomials at unequal spacing. The contrasts were applied to both multivariate and univariate analyses. Univariate treatment means were also separated with Tukey's Studentized Range Test.

Leaf N concentration, fine root biomass and basal area were analyzed by one-way randomized complete block analysis of variance. Treatment means for leaf N, basal area and biomass were separated with Tukey's Studentized Range Test and polynomial contrasts.

Individual root life spans determined with minirhizotrons were analyzed with survival distribution functions (Kalbfleisch and Prentice 1980, Lee 1992). Root survival time or life span was defined as days between initial appearance and disappearance. The survival distribution function defines the proportion of roots surviving at a given life span. Roots living past the last observation were considered right censored, i.e., survival time is at least as long as the time to final observation. Product-limit analysis in the SAS Lifetest Procedure was used to estimate survival distribution functions, and the effects of covarying factors were used to stratify the data (SAS). The log rank and Wilcoxon tests were used to determine treatment differences between survival curves. The Wilcoxon tests for early survival differences, and the log rank tests for late survival differences. Pair-wise mean comparisons were made among strata with Scheffe's multiple-comparison procedure (code provided by P.T. Savarese, SAS). Factors controlling fine root survival were introduced in a stepwise manner to determine ranking. Controlling factors that were tested included fertilizer treatment, year of appearance, season of appearance, initial diameter and depth.

Results

Fine root dynamics

Fine root dynamics were influenced by season, stand development stage and nutrient amendment, with seasonal patterns having the greatest effects on fine root production, mortality and live-root standing crop (Figure 1). Fine root production rate was highest in late August of the establishment year and in early July of the second growing season. Cumulative production reached a plateau after September in both years as soil temperature declined. Fine root mortality occurred at a steady rate during the growing season, but increased late in the growing season, and peak mortality occurred in November of both years. Soil temperature hovered near 0 °C during winter, corresponding with little change in cumulative root production or mortality from December to April. As a result, fine root production was correlated with soil temperature (1999: r^2 = 0.827, $P < 0.005$; 1998: $r^2 = 0.687$, $P < 0.005$).

Live-root standing crop patterns were similar to cumulative production because mortality was relatively low until late in the season and was only a fraction of cumulative production. As mortality increased in October, production rate nearly ceased. Consequently, live-root standing crop peaked in August and decreased for the remainder of the season (Figure 1). This pattern occurred in both growing seasons, but the decline in standing crop was greater in the second growing season because of greater mortality that year.

Stand development affected the rate of fine root production, mortality and standing crop. Cumulative production during the first growing season was two thirds that of the second season. Cumulative mortality represented about 11% of production in 1998 and 31% in 1999. The greatest mean 1998 live-root standing crop was 4.87 ± 1.18 mm cm^{-2} in October.

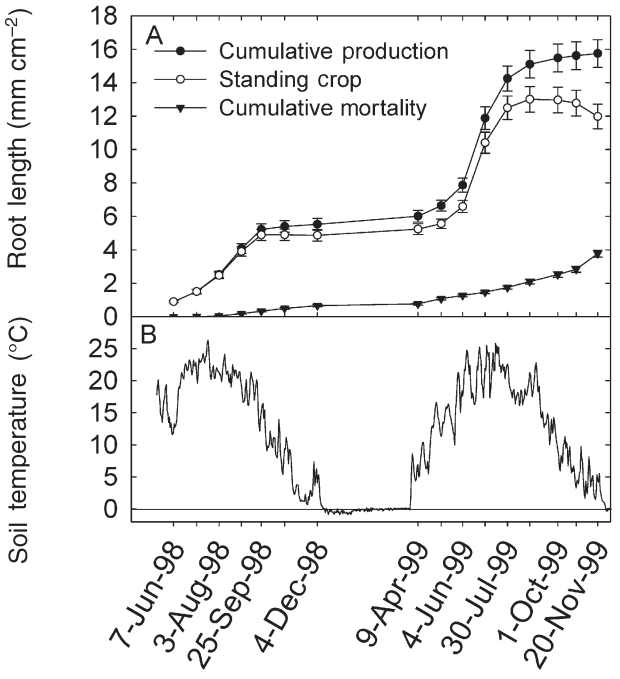

Figure 1. (A) Cottonwood plantation fine root (0–2 mm diameter) cumulative production, cumulative mortality and resulting live-root standing crop in 1998 and 1999. Each value represents the mean fine root length density (mm cm^{-2}) of all treatment plots. Minirhizotron observations were collected 16 times beginning on June 7, 1998 and ending on November 20, 1999. Vertical bars indicate the standard error of the mean for each observation date. (B) Mean daily soil temperature at 15-cm soil depth. Means were calculated from hourly readings in one plot from each of the four treatments.

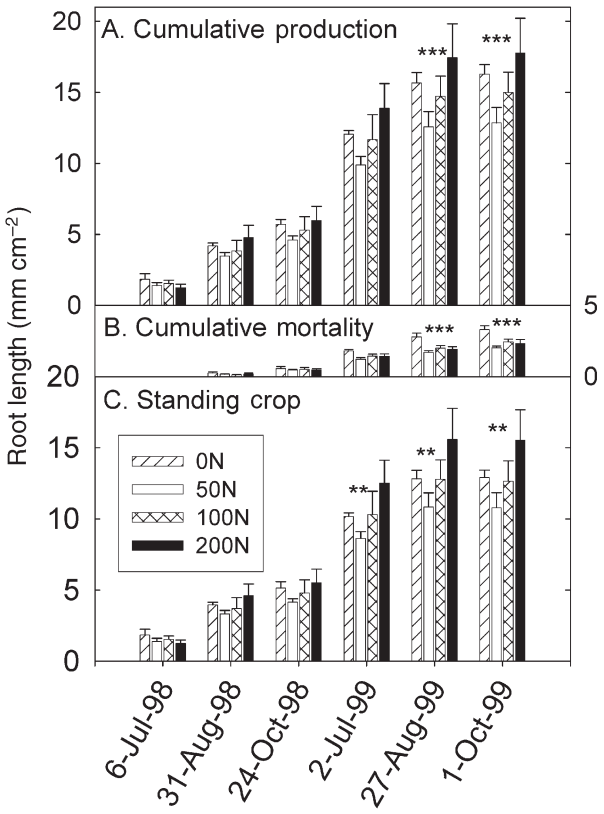

Figure 2. Fertilizer nitrogen (N) effects on fine root length production (A), mortality (B) and standing crop (C) on six selected dates in 1998 and 1999. Each date represents four replications per treatment. Treatments were slow-release fertilizer applied at 0, 50, 100 or 200 kg N ha^{-1} (0N, 50N, 100N and 200N, respectively). Vertical bars are the standard error of the mean. Value of F-test significance: * = linear contrast, $0.05 < P < 0.1$; ** = linear contrast, $0.01 < P < 0.05$; and *** = quadratic contrast, $0.05 < P < 0.1$.

In 1999, the standing crop increased 1.6-fold, reaching 13.18 ± 2.55 mm cm^{-2} by late August.

Nutrient amendments had important influences on fine-root dynamics that became more pronounced during the study. In 1998, cumulative production and standing crop increased linearly with nutrient additions (Figure 2). There was a decrease in the 1999 production and mortality from the 0N to intermediate fertilizer treatments (50N and 100N), and then cumulative production increased to its highest value with 200N fertilizer treatment, exceeding that of the control. Although cumulative mortality also increased as fertilizer addition increased from the 50N treatment to the 200N treatment, it did not exceed that of the control treatment (Figure 2). The relatively high mortality in the control treatment and relatively low mortality in the 200N treatment resulted in standing crop increasing linearly with fertilizer addition in 1999 (Figure 2, linear multivariate effect $P = 0.05$, quadratic multivariate effect not significant), despite the nonlinear response of production and mortality. A similar linear response of fine root standing crop to nutrient addition was found when fine root biomass was sampled from cores collected in October 1998 (Table 2; linear effect $P = 0.02$).

We tested roots less than 0.6 mm in diameter in the upper 20 cm soil layers and found results similar to the entire root population. In 1998, these actively cycled roots increased linearly with fertilizer treatment in both root production and standing crop (linear contrast $P < 0.06$). In 1999, root production (fertilizer effect $P = 0.03$, linear contrast $P = 0.01$) and standing crop (linear contrast $P = 0.05$) responses were similar to those in 1998 in addition to a quadratic response in mortality ($P = 0.06$) to fertilizer treatment.

We used both direct and indirect approaches to test for treatment effects on root morphology. The direct approach tested the effects of N fertilizer on morphological traits such as root length, diameter and volume. No treatment or temporal effects were observed for morphological traits. To confirm the lack of morphological effects indirectly, we counted the number of roots. Root count results were similar to those found for cumulative root length. In 1998, production, mortality and standing crop increased linearly with fertilizer treatment ($P < 0.05$). In 1999, production and mortality were lowest at intermediate fertilizer rates (quadratic contrast $P < 0.06$) and standing crop increased linearly with fertilizer treatment ($P < 0.03$).

Fine root survival

Fine root survival was affected by N treatment, tree age, sea-

Table 2. Fine root biomass and leaf N concentration. Treatments were slow-release fertilizer applied at 0, 50, 100 or 200 kg N ha^{-1} (0N, 50N, 100N and 200N, respectively). Fine root biomass (< 1 mm diameter) soil cores were sampled in October 1998. Midsummer leaf N concentrations were taken from one canopy position in 1998 and two canopy positions in 1999. Within a column, means ± standard error ($n = 4$) followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey's HSD).

Treatment	Fine root biomass ($g\ m^{-2}$)	Leaf N concentration ($mg\ g^{-1}$)		
		1998	1999	
		Upper canopy	Lower canopy	Upper canopy
0N	31.5 ± 3.7 b	37.2 ± 1.2 a	19.8 ± 1.1 b	22.2 ± 1.4 b
50N	34.7 ± 1.6 ab	36.7 ± 0.2 a	23.2 ± 0.3 ab	32.8 ± 0.3 a
100N	38.0 ± 1.3 ab	36.3 ± 1.3 a	26.5 ± 0.9 ab	33.4 ± 1.2 a
200N	38.9 ± 0.6 a	38.0 ± 0.7 a	26.9 ± 0.9 a	32.1 ± 0.7 a

son of root initiation, root diameter and soil depth (χ^2 $P <$ 0.0001, Figure 3). When these factors were introduced in a stepwise manner, they ranked depth > diameter > age > N treatment > season. Therefore, the fourfold range of N availability created by our treatments had less effect on fine root longevity than did root diameter, soil profile location and tree age. Nonetheless, fertilizer N had a significant influence on survival distribution functions (Figure 3D) and a positive influence on median fine root life span (Table 3). As with cumulative mortality data (Figure 2B), the shape of the life span treatment response was nonlinear. A linear regression fitted to median life span as a function of fertilizer N explained 77% of the variation, whereas a quadratic regression explained 97% of the variation, demonstrating the nonlinear nature of the response. But unlike cumulative mortality, where there was no minimum at the intermediate treatments, no such optimum occurred with life span. Instead, median life span rose rapidly from 0N to 50N and then leveled off at high fertilizer N treatments. A similar treatment response pattern was observed when small diameter roots (< 0.6 mm) at shallow depths (< 20 cm) were considered separately; survival increased with nutrient availability (Figure 3E) in a nonlinear manner. However, median life span for these active roots was 30 and 27% shorter when grown with 0N and 50N, respectively, than for

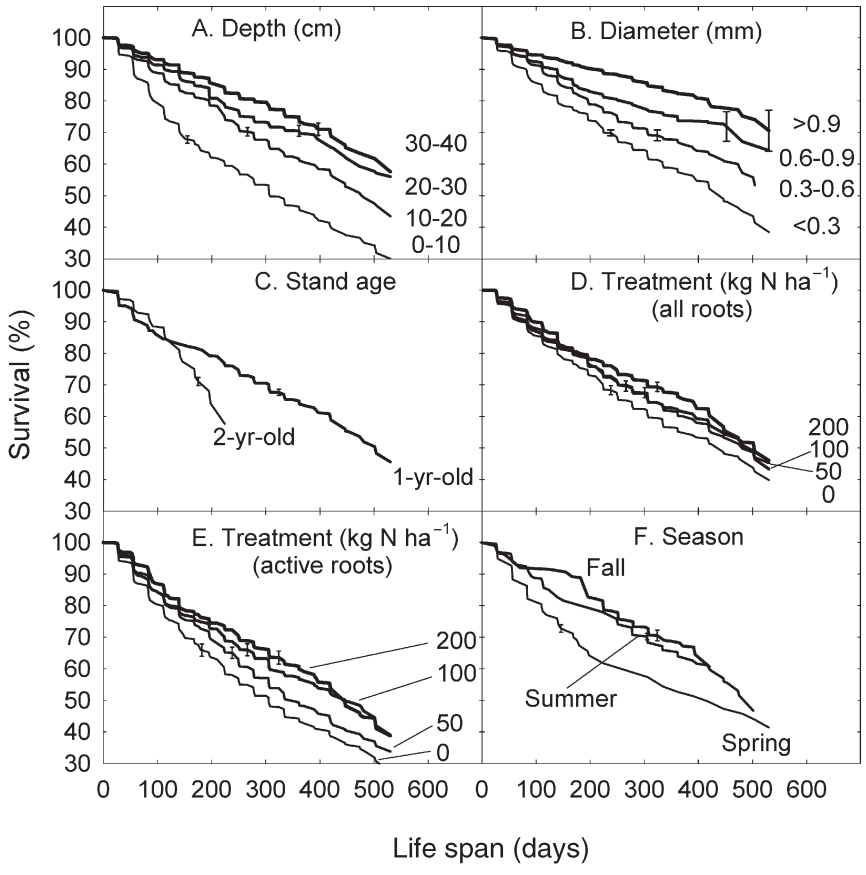

Figure 3. Survival distribution functions and the effects of soil depth (A), initial root diameter (B), stand age (C), fertilizer nitrogen (N) for all roots (D) or for the most active roots, i.e., small diameter (< 0.6 mm) and shallow depth (< 20 cm) (E), and season of root initiation (F). Each graph reflects the percent of roots that reached a given life span. For clarity, error bars for the 95% confidence interval are only placed near 70% survival.

Table 3. Root longevity (life span), turnover rates and mean separations influenced by treatment, morphology, depth and temporal factors. Median life span was not reached with many factors, so it was predicted based on 70% survival. Mean separations involved pairwise comparisons among survival distribution functions (Figure 3) using Scheffe's multiple-comparison procedure. Means with similar letters are not significantly different ($\alpha = 0.05$)

Factor analyzed	Factor regimes	Median life span (days)[1]	Turnover (% year^{-1})[2]	Mean separation
Fertilizer N (kg N ha^{-1})	0N	435	84	a
	50N	480	76	b
	100N	491	74	b
	200N	503	73	c
Depth (cm)	0 to 10	307	119	a
	10 to 20	436	84	b
	20 to 30	**565**	65	c
	30 to 40	**620**	59	c
Diameter (mm)	< 0.3	438	83	a
	0.3 to 0.6	**511**	71	b
	0.6 to 0.9	**663**	55	c
	> 0.9	**728**	50	c
Age	1998	502	73	b
	1999	**390**	94	a
Season	Spring	412	89	a
	Summer	**510**	72	b
	Fall	**532**	69	b

[1] Median life span (days) for bold typeface values was estimated from 70% survival (x) as: $y = 0.9706x + 213.67$ ($r^2 = 0.884$, $P = 0.0015$) because 50% survival was not reached. The prediction function was developed from data in regular typeface.

[2] Turnover was estimated from the inverse of median life span.

the combined sample. Higher N treatment showed a similar but less extreme pattern with only 9 and 11% shorter median life span for 100N and 200N, respectively, when compared with the combined sample. Thus, thin shallow roots displayed more active turnover rates and were more responsive to fertilizer treatments than the combined root population.

Of the factors considered, fine root depth had the strongest influence on survival. Survival increased with greater depth (Figure 3A). Roots at soil depths between 30 and 40 cm had twice the life span of those in the top 10 cm of soil (Table 3). But rooting density was greater at the surface; 58% of the roots were observed in the top 20 cm of soil and only 20% were observed in the deepest soil layer examined.

Depth affected the fine root survival response to treatments. Root survival in the surface 20 cm showed highly significant treatment effects (χ^2 $P < 0.0001$), but treatment responses were much less significant below soil depths of 20 cm (Wilcoxon χ^2 $P = 0.10$; log rank χ^2 $P = 0.01$).

Fine root turnover rate estimated by inverting median lifespan (Table 3) also showed important depth effects. The equivalent of the entire standing crop in the surface 10 cm was replaced in less than 1 year, whereas only 59% of the fine roots between the soil depths of 30 and 40 cm turned over each year (Table 3). To understand how soil C input varies with depth, turnover was converted to C at various depths based on fine root biomass data (Table 2) and C concentration. Carbon turnover was 45.3 ± 4.0, 35.0 ± 1.3, 19.3 ± 2.0, and 6.7 ± 0.8 g C m^{-2} year^{-1} at 10, 20, 30 and 40 cm depth increments, respectively. These C turnover values demonstrate that annual soil C input from fine root turnover declined with depth, and that the 20 to 30 cm depth increment had less than half the input of the surface 10 cm.

Initial root diameter ranked second to depth in controlling fine root survival (Figure 3B). The median life span of roots less than 0.3 mm in diameter was 14 months and that of larger diameter roots was estimated to be nearly 2 years (Table 3). Therefore, the turnover rate was 66% greater for the smallest roots than for the largest roots. However, of all the roots observed, most were of small diameter; less than 4.5% had diameters greater than 0.6 mm. Diameter distribution with depth did not explain increased survival with depth. All depth categories contained a range of root diameter sizes; large roots made up 7% of the roots in the top 20 cm and 10% of the roots in the 30–40 cm depth category, and there was poor correlation between depth and diameter ($r^2 < 0.001$). Effect of fertilizer treatment on survival was also strongest in the smaller diameter classes. Root survival showed highly significant treatment effects (χ^2 $P < 0.0001$) in roots less than 0.6 mm, whereas no treatment differences were found for roots greater than 0.6 mm in diameter.

Survival was influenced by temporal factors including plantation age and the season of initiation. Roots initiated in 1998 had longer life spans than roots initiated in 1999 (Figure 3C). Greater survival of roots initiated during the first year naturally caused increased life span and decreased turnover (Table 3). During the first 113 days after initiation, roots from both years had similar survival patterns (Wilcoxon χ^2 $P = 0.0798$). During subsequent days, 1999 roots had shorter life spans than 1998 roots (log rank χ^2 $P < 0.0001$). Temporal effects were also evident for roots initiated during different seasons of the year (χ^2 $P < 0.0001$). Roots initiated during spring had lower survival than roots initiated during fall, and those initiated during summer were intermediate (Figure 3F). Survival differed among the spring, summer and fall seasons (χ^2 $P < 0.0067$).

Aboveground responses

Basal area responses to the fertilizer treatments increased from 1998 through 1999 (Figure 4). At the end of the first growing season, basal area was lowest in the control and highest in 100N, but the treatment differences were not significantly different ($P = 0.55$). In 1999, nutrient amendments increased basal area ($P = 0.0094$). Fertilizer amendments had a positive effect on leaf N concentration only in the second growing season (Table 2). The upper canopy had higher N concentrations than the lower canopy, and leaf N concentration on cuttings in the fertilizer treatments increased substantially compared with

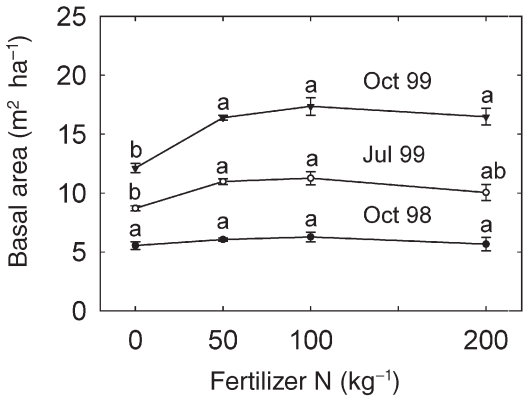

Figure 4. Basal area response to fertilizer nitrogen (N) on different dates in 1998 and 1999. Means ± standard errors (n = 4) having the same letter are not statistically different (α = 0.05, Tukey's HSD).

the controls. Leaf N was less responsive to N fertilizer in the lower canopy than in the upper canopy.

Discussion

Fine root responses to nutrient availability

We observed a nonlinear response of fine root production to soil N availability. As N increased initially from 0N to 50N, root production decreased, but at an N availability of 200N, root production matched control values (Figure 2). Had comparisons been made between any two of these N treatments, entirely different conclusions would have been reached. This finding sheds a new light on our understanding of fine root dynamics in response to N availability. It suggests that there may be two mechanisms for root responses to N. The first is the classic pattern of decreased root production associated with increased soil N availability and increased aboveground growth (Keyes and Grier 1981). We observed this pattern between 0N and 50N. This pattern can be explained by greater aboveground investment without adverse consequences for nutrient acquisition in the face of a decreased belowground investment. The second pattern is more difficult to explain. In response to high N regimes, aboveground growth is sustained (Figure 4), yet fine root production and standing crop increased substantially as N increased from 50N to 200N (Figure 2). We speculate that, during nutrient deficiency (0N), belowground C is plentiful and is used by root systems to acquire nutrients, whereas shoot growth is limited by low N availability. As N increases sufficiently to increase shoot growth (50N), C is diverted away from fine root production by shoot growth demands, with little consequence for overall plant nutrient acquisition. As shoot N continues to increase (100N and 200N), photosynthetic capacity may increase (Linder and Rook 1984) beyond the point that saturates shoot growth, with excess C available to the roots, allowing root production to rebound.

Past studies of the effects of nutrient availability on fine root production have generally considered only two fertilizer treatments. Within this literature, there is disagreement about both the direction and magnitude of the response, partly due to differences in the species and sites tested. In general, a decline in fine root productivity with increased nutrient availability in many reports (Keyes and Grier 1981, Kurz 1989, Vogt et al. 1990, Gower et al. 1992, Burton et al. 2000) contrasts with an equal number of reports showing little or no change to an over twofold increase in fine root production (Persson 1980*a*, Aber et al. 1985, Nadelhoffer et al. 1985, Fahey and Hughes 1994, Majdi and Persson 1995, Pregitzer et al. 1995, Majdi and Nylund 1996, Majdi and Kangas 1997, Kubiske et al. 1998, Pregitzer et al. 2000) in both coniferous and hardwood ecosystems. Our results appear to unify these contradictory results by showing that the response of fine root production to nutrient availability is nonlinear with a minimum at moderate fertility. This result emphasizes the need to control as many confounding factors as possible, and to study the response to a wide range of nutrient availabilities rather than to just two treatments with the assumption that the response is linear or unidirectional.

The magnitude of nutrient-availability treatment differences increased over the 2 years of our observations. A similar strengthening of the treatment response was observed when soils with contrasting mineralization rates were used (Persson 1980*b*, Pregitzer et al. 1995, Kubiske et al. 1998, Pregitzer et al. 2000). In each of these corroborating studies, as in our study, young trees were grown in noncompetitive conditions. Under these conditions of site exploration, fine root production exceeds mortality, demonstrating that standing crop is increasing, and therefore root turnover is not in steady state. In contrast, mature *Populus* stands with full site occupancy have fine root production rates that are equivalent to mortality rates (Coleman et al. 2000), thus mean annual standing crop remains relatively constant. We emphasize that root turnover in young trees must be considered in non-steady state until annual production and mortality are equivalent.

Treatment responses increased during the course of our experiment. In contrast, there are several reports of a relatively high initial response followed by a decreased response during the course of regularly applied nutrient amendments (Fahey and Hughes 1994, Haynes and Gower 1995). These latter studies enhanced nutrient availability by adding nutrient amendments to a mature forest in steady state. It is likely that the nutrient amendments forced a measurable short-term response and then a new steady state was reached, similar to the original. We emphasize that our results represent the response of establishing stands to nutrient availability, and they also demonstrate that many factors control the response of fine roots to nutrient availability. The fine root nutrient response was affected by the amount of nutrient applied, depth and diameter of roots observed, and the equilibrium between production and mortality. Understanding fine root production and turnover responses to treatment factors will require the control of such internal and external factors.

Root survival

Root survival distribution functions for the various nutrient treatments (Figure 3D) support the mortality data (Figure 2B). Root longevity was typically shorter in the 0N treatment than in the 200N fertilizer treatment (Table 3), especially in the smallest diameter roots at the surface (cf. Figures 3D and 3E). Therefore, our general results agree with studies showing increased survival with increasing nutrient availability (Pregitzer et al. 1993, Burton et al. 2000) and contrast with studies showing decreased survival (Mackie-Dawson et al. 1995, Pregitzer et al. 1995, Majdi and Kangas 1997, Kubiske et al. 1998, Johnson et al. 2000). Although cumulative mortality increased slightly with fertilization from 50N to 100N, fine root survival did not decrease at these intermediate N addition regimes. The small increase in cumulative mortality is explained by a larger pool of roots dying (Figure 2A) rather than by a higher death rate (Figure 3D).

The relatively small response of survival to nutrient availability compared with larger responses to depth and diameter (Figure 3) agrees with other reports. In general, root survival increases with depth in trees (Mackie-Dawson et al. 1995, Majdi and Kangas 1997, Burton et al. 2000, Coleman et al. 2000) and agronomic crops (Goins and Russelle 1996). This is consistent with soil environmental gradients in temperature, water content and CO_2 favoring greater longevity (Coleman et al. 2000). Root survival also increases with root diameter among a variety of species (Coleman et al. 2000, Wells and Eissenstat 2001). The shifts in both magnitude of treatment differences and in the relative rankings of the treatments for survival distribution functions with changes in depth and diameter demonstrate that the nutrient availability response is confounded by other environmental and plant factors. This illustrates that fine root survival response to nutrient availability is more complex than initially hypothesized, and that confounding factors should be considered when attempting to quantify fertility responses.

Our median root life spans, from 307 to over 700 days (Table 3), are high relative to those found for other tree species (Eissenstat and Yanai 1997), but see (Majdi and Kangas 1997, Burton et al. 2000, Coleman et al. 2000, Johnson et al. 2000, Lopez et al. 2001). Most minirhizotron studies collected observations at equal or even greater observation intervals than our monthly observations, so it is unlikely that greater longevity is an artifact of infrequent observations as reported by Johnson et al. (2001). It is more likely that the life spans that we observed were longer because they included the first year of stand establishment on a field site during which exploration of the rooting zone occurred. Life span during the first year was much greater than during the second year (Figure 3C). Although values for the second year were still high, they approach median root life spans reported elsewhere. As the stand fully occupies the site and reaches steady state, with similar fine root production and mortality rates, median root life span is expected to decline further. The long life spans we observed could also have been a result of physiological changes in the roots. In mature northern hardwoods in Michigan, roots initiated in the first year of the study lived longer than roots initiated in the second year of the study. First-year roots may have had more suberization of root cortical cells that extended their life spans (Hendrick and Pregitzer 1992). Our life span values are comparable with those reported by Eissenstat and Yanai (1997) when only the active roots are considered (Figure 3E), demonstrating the importance of describing the characteristics of the root population under consideration. In northern hardwood forests, roots initiated in late summer and fall had long life spans, extending over 1 year, (Hendrick and Pregitzer 1992, Tierney and Fahey 2001) comparable with our findings.

Cottonwood model system

The closely spaced stand of fast-growing cottonwood trees is an experimentally efficient model of a forest stand. Canopy closure occurred by the end of the first growing season, and roots had clearly explored the soil of each plot. However, the large difference between fine root production and mortality throughout the 2 years of observation demonstrates that the site was not at steady state (Figure 1).

The developmental shifts in fine root dynamics between the 2 years of observation have important implications for manipulative studies that consider seedlings and young trees as surrogates for ecosystem responses. In studies using nondestructive minirhizotron techniques, fine root production and turnover increase with N availability in young trees (Pregitzer et al. 1995, Kubiske et al. 1998), yet decreases in mature forest stands (Burton et al. 2000). Our results suggest that extrapolation from seedling studies to forest stands may not be valid, and developmental processes will require several growing seasons to be defined adequately.

We have demonstrated that many factors interact to affect the response of fine root dynamics to nutrient availability. Variation caused by factors such as depth, initial root diameter, individual root age and year or season of initiation can be easily evaluated with minirhizotron techniques. A range of nutrient availabilities is necessary to characterize the nonlinear nature of the fine root response to nutrient availability. Stage of stand development is another essential consideration because fine root responses change as stands age. Finally, responses should be considered over at least 2 years of observation to account for the possibility that nutrient-induced changes in root dynamics components are ephemeral.

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