INTERACTIVE EFFECTS OF ATMOSPHERIC CO_2 AND SOIL-N AVAILABILITY ON FINE ROOTS OF *POPULUS TREMULOIDES*

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Abstract. The objective of this experiment was to understand how atmospheric carbon dioxide (CO_2) and soil-nitrogen (N) availability influence *Populus tremuloides* fine-root growth and morphology. Soil-N availability may limit the growth response of forests to elevated CO_2 and interact with atmospheric CO_2 to alter litter quality and ecosystem carbon (C) and N cycling. We established a $CO_2 \times N$ factorial field experiment and grew six genotypes of *P. tremuloides* for 2.5 growing seasons in 20 large open-top chamber/root-box experimental units at the University of Michigan Biological Station in northern lower Michigan (USA). In this paper we describe an integrated examination of how atmospheric CO_2 and soil-N availability influence fine-root morphology, growth, mortality, and biomass. We also studied the relationship between root biomass and total soil respiration.

Over 80% of the absorbing root length of *P. tremuloides* was accounted for by roots <0.4 mm in diameter, and specific root length (100–250 m/g) was much greater than reports for other temperate and boreal deciduous trees. Elevated atmospheric CO_2 increased the diameter and length of individual roots. In contrast, soil N had no effect on root morphology. Fine-root length production and mortality, measured with minirhizotrons, was controlled by the interaction between atmospheric CO_2 and soil N. Rates of root production and mortality were significantly greater at elevated CO_2 when trees grew in high-N soil, but there were no CO_2 effects at low soil N. Fine-root biomass increased 137–194% in high-N compared to low-N soil, and elevated atmospheric CO_2 increased fine-root biomass (52%) in high soil N, but differences in low soil N were not significant. Across all treatments, dynamic estimates of net fine-root production were highly correlated with fine-root biomass (soil cores; $r = 0.975$). Mean rates of soil respiration were more than double in high-N compared to low-N soil, and elevated atmospheric CO_2, when compared to ambient atmospheric CO_2, increased mean rates of soil respiration 19% in 1995 and 25% in 1996. Across all treatments, total root biomass was linearly related to mean rates of soil respiration ($r^2 = 0.96$).

Our results indicate that atmospheric CO_2 and soil-N availability strongly interact to influence *P. tremuloides* fine-root morphology, growth, and C turnover. Aspen-dominated ecosystems of the future are likely to have greater productivity fueled by greater nutrient uptake due to greater root length production. Further, it appears that elevated atmospheric CO_2 will result in greater C inputs to soil through greater rates of fine-root production and turnover, especially in high-fertility soils. Increased C inputs to soil result in greater rates of soil respiration. At this time, it is not clear what effects increased rates of root turnover will have on C storage in the soil.

Key words: atmospheric CO_2 and soil-N availability, interaction; carbon and nitrogen; fine-root turnover and biomass; global climate change; minirhizotrons; morphology, roots; nitrogen; nutrient cycling; Populus tremuloides; root growth and morphology; soil respiration.

INTRODUCTION

Understanding the direct and indirect ecological effects of increasing atmospheric carbon dioxide (CO_2)

is one of the most important ecological issues facing humankind. With a partial pressure of ~35 Pa and a relatively uniform distribution around the planet, CO_2 is a trace gas in the atmosphere that currently limits the rate of photosynthesis in most plant species. Using meta-analysis to summarize 500 experiments, Curtis and Wang (1998) reported that doubling atmospheric CO_2 increased average rates of photosynthesis 54%,

and increasing primary productivity could have a profound influence on such ecological processes as nutrient cycling, natural selection, competition, and succession.

Leaf nitrogen (N) is directly related to photosynthetic rate (Field and Mooney 1986, Evans 1989), and N limits primary production in many temperate ecosystems (Vitousek and Howarth 1991). Most of the N in leaves is acquired from the soil, and trees depend upon fine absorbing roots and mycorrhizae for the uptake of N and other growth-limiting nutrients. Trees growing in an atmosphere increasingly rich in CO$_2$ have the potential to fix more carbon (C) in photosynthesis if they can acquire enough N. As pointed out by BassiriRad et al. (1997), plants can potentially meet the increased physiological demand for soil N by either producing a more extensive root/mycorrhizal system and/or increasing the physiological capacity of roots/myhorrhize to acquire soil N. Potential alterations in the deployment of fine roots are, therefore, a critical direct response to elevated CO$_2$ (Berntson and Woodward 1992). Most of the existing literature indicates that root growth is enhanced under elevated CO$_2$ (Rogers and Runion 1994, Curtis and Wang 1998). However, there are very few studies longer than 360 d in duration (<10 worldwide, Curtis and Wang 1998). One of the objectives of this study was to understand the direct effects of elevated atmospheric CO$_2$ and soil-N availability on *Populus tremuloides* fine-root growth and morphology. Changes in mycorrhizal colonization and the physiological capacity of roots to assimilate N were evaluated in companion studies (J. Lussenhop, *unpublished data,* Rothstein et al. 2000). Our hypothesis was that elevated atmospheric CO$_2$ would increase fine-root growth and mortality regardless of soil-N availability, and that this response would be sustained over the course of the experiment.

Respiration is a critical component of net ecosystem carbon exchange, and understanding the relationships among net canopy assimilation, root respiration, and microbial respiration is a prerequisite for understanding C budgets estimated by eddy correlation (Russell and Voroney 1998). There are only a few measurements of soil respiration from long-term elevated-CO$_2$ experiments conducted in the field (Luo et al. 1996, Vose et al. 1997, Ball and Drake 1998, Edwards and Norby 1999). These studies all suggest that elevated CO$_2$ will increase soil respiration, but concurrent field studies of root growth and soil respiration remain mostly unexplored. It is important to understand the direct relationship between root growth and soil respiration because more than half of the C assimilated in many ecosystems is allocated to the production and maintenance of fine roots (Vogt et al. 1986, Hendrick and Pregitzer 1993), making soil respiration the major flux of C back to the atmosphere in many ecosystems (Hungate et al. 1997, Russell and Voroney 1998). Our sec-

ond objective was to quantify the relationship between root biomass and soil respiration across atmospheric-CO$_2$–soil-N treatments.

There are also important indirect ecosystem feedback mechanisms that could limit the growth response of terrestrial ecosystems to increasing atmospheric CO$_2$. This aspect of understanding ecosystem response to elevated atmospheric CO$_2$ is controversial and remains unresolved (but see Norby and Cotrufo [1998]). The controversy revolves around the widely observed influence of elevated CO$_2$ on litter chemistry. A common observation from elevated-CO$_2$ experiments is an increase in leaf C/N ratio (Bezemer and Jones 1998), in part driven by a decline in leaf N at elevated CO$_2$ (Bezemer and Jones 1998, Curtis and Wang 1998). This observation has fostered the hypothesis that greater inputs of recalcitrant litter will decrease soil-N availability and, in time, dampen the growth response of terrestrial ecosystems to elevated atmospheric CO$_2$ (Shimmel 1990, Diaz et al. 1993).

The alternative N-cycling hypothesis centers on the idea that elevated CO$_2$ will increase inputs of easily metabolized C compounds through increased root/mycorrhizal turnover (root exudation, and death and decay of fine roots/mycorrhizal hyphae; Zak et al. 1993). In reality, both of these arguments are gross simplifications of a complex set of organic-matter transformations that are mediated by soil microbes and soil invertebrates (Klironomos et al. 1996). It is clear, however, that processing of plant detritus by the soil biota will regulate N availability and C storage in the soil as the concentration of CO$_2$ increases in the earth's atmosphere. A dominant fraction of the C assimilated in net primary production passes rapidly through the plant biomass into the soil in all terrestrial ecosystems, mostly as leaf and root/mycorrhizal litter. The time steps involved are relatively short—new leaves and roots have life spans that range from days to a few years. In elevated-CO$_2$ experiments, Arnone and Körner (1995) estimated that for each mole of C sequestered in aboveground living-plant biomass in tightly controlled artificial ecosystems, 5 mol of C were sequestered in the soil (roots/mycorrhizae, microbes, and soil organic matter). Understanding the mechanisms that control soil C and N cycling under elevated CO$_2$ remains one of the most uncertain and important pieces of the global-change puzzle (Vitousek 1994). Fine roots play an important part in this unresolved issue.

As noted above, fine-root turnover is an important source of substrate for microbial and invertebrate metabolism simply because fine roots and mycorrhizae account for a large percentage of net primary production (Fogel and Hunt 1979, Vogt et al. 1986, Hendrick and Pregitzer 1993, Hungate et al. 1997). Fine roots are modular plant organs, and they have life expectancies that are often shorter than leaves (Hendrick and Pregitzer 1992, Pregitzer et al. 1993). Dynamic esti-

mates of fine-root growth and mortality for trees growing under elevated CO_2 have only been quantified in a handful of studies (Pregitzer et al. 1995, Berntson and Bazzaz 1996, Tingey et al. 1996, Kubiske et al. 1998). In deciduous trees, most root length can be accounted for by short-lived, absorbing roots that have diameters <0.5 mm (Hendrick and Pregitzer 1993, Pregitzer et al. 1997), and these roots, their exudates, and their associated mycorrhizal hyphae represent the majority of belowground C input to the soil. We know almost nothing about the litter quality of these ephemeral organs and how root litter quality will respond to different atmospheric CO_2 and soil N treatments. Our final objective was to quantify the C/N ratio of fine roots. Our hypothesis was that trees growing in soil with greater available N would have roots with significantly lower C/N ratios, and that elevated CO_2 would not significantly influence fine root C/N ratio. Although C/N ratio is a very crude estimate of litter quality, understanding C/N ratio is a first step toward understanding how elevated CO_2 and soil N influence belowground litter quality. To summarize our introduction, the overall goal of this study was an integrated examination of how atmospheric CO_2 and soil-N availability influence the fine-root systems of *Populus tremuloides,* the dominant timber species in the Lake States and the most widely distributed tree in North America.

METHODS

Experimental design

The experiment was conducted at the University of Michigan Biological Station (UMBS; 45°35′ N, 84°42′ W), near Pellston, Michigan, USA. The immediate area was open and level (<1% slope), and the soil was a uniform, well-sorted Rubicon sand (Entic Haplorthod). In the summer of 1993 we removed the O, A, and upper B soil horizons (15 cm) over the entire area with a backhoe.

The experiment was a two-factor, randomized complete-block design. One fixed effect, soil N, consisted of two soil treatments. Soil with high N availability consisted of 100% A horizon collected from a nearby Kalkaska sand (Typic Haplorthod). Soil with low N availability was prepared by mixing 20% A-horizon Kalkaska sand and 80% C-horizon Rubicon sand. Both of these soils are very common in northern Lower Michigan. We have successfully used these soil preparations to alter N availability in other experiments (Pregitzer et al. 1995, Kubiske et al. 1997). Differences in organic matter between the two soil treatments in this experiment (low N = 3.6 g C/kg; high N = 12.5 g C/kg) resulted in net N-mineralization rates of 62 ng $N \cdot g^{-1} \cdot d^{-1}$ in the low-N soil and 318 ng $N \cdot g^{-1} \cdot d^{-1}$ in the high-N soil. Extractable PO_4^{3-}, assayed using the Bray P1 method (Kuo 1996), was 13.7 mg/kg in the low-N soil and 10.4 mg/kg in the high-N soil (see Curtis et al. [2000] for additional soil characterization). After

the two soil treatments were prepared, 10 open-bottom root boxes (3.3 m^2 × 0.45 m deep) lined with 1.3-cm-thick styrofoam insulation and plastic film were filled with one of the two soil treatments (2 soil treatments × 10 boxes = 20 soil boxes total). Before the boxes were filled with soil, eight minirhizotron tubes were installed in each (see below). The boxes were filled with soil in the fall of 1993 and allowed to settle to a uniform bulk density over winter.

Open-top chambers (Heagle et al. 1989) were constructed and placed over the 20 root boxes in early spring 1994. These were used to control the second fixed effect, atmospheric CO_2. Ambient daytime (0701–1900) atmospheric CO_2 (35.7 ± 0.06 Pa over the 2.5 growing seasons) was maintained in one half of the chambers and the daytime atmosphere was elevated to twice ambient CO_2 in the other 10 chambers (70.7 ± 0.06 Pa). Further details about the open-top chambers and their operation and performance can be found in Curtis et al. (2000). The experiment was balanced (4 main treatments × 5 replications = 20 chamber/root-box experimental units).

On 7 June 1994 we planted two softwood cuttings of six different local genotypes of *Populus tremuloides* Michx. into each chamber. Before planting, the cuttings were graded for uniformity; individuals averaged 17.3 (±0.45) cm in height with 12.3 (±0.3) leaves. The parent aspen clones grow on the Pellston plain which is a part of UMBS. In the field, three genotypes exhibit early leaf senescence and three exhibit late senescence (Barnes 1959). Harvest of the entire experiment began by block on 8 July 1996 following ~2.5 growing seasons. The largest trees were 3.3 m tall and had basal diameters of 10 cm at the time of harvest. Additional information about tree propagation, growth, physiology, and destructive harvest can be found in Curtis et al. (2000) and Zak et al. (2000b).

Root morphology

Root samples were collected during August and September of 1996 as the experiment was harvested to study fine-root morphology, and relationships between fine-root morphology and root carbon and nitrogen concentration. A portion of one horizontal woody lateral root, ~20 cm long and 1.0 mm in diameter, was randomly collected from each chamber in blocks 3, 4, and 5 of the experiment. Each of the root segments was very carefully excavated from the soil, making every effort to extract the roots without disrupting the small lateral branches. Following excavation each segment had several attached lateral branches. Four individual segments from the intact root systems were detached and preserved in a 20% methanol solution and later scanned and digitized in order to provide a representative picture of the variation in fine-root morphology. The remainder of the root systems were immediately frozen and later thawed for analysis. Once the root

system was unfrozen, it was kept at 1°C in deionized water to prevent desiccation.

Each of the 15 branching root systems was dissected by order using methods described by Pregitzer et al. (1997, 1998). The smallest lateral roots were considered first-order roots following the approach of Fitter (1982, 1987). Individual root diameters and lengths were measured with a microscope (15×) fitted with an ocular micrometer. The diameters and lengths of 4877 individual roots, 1003–1507 per treatment, were measured. This amounted to 8.14 m of total fine-root length. Individual roots were composited by order within each of the 15 chambers, weighed to enable calculation of specific root length (in meters per gram), and then analyzed for C and N concentration with a Carlo Erba NA 1500 Series II elemental analyzer (CE Elantech, Lakewood, New Jersey, USA).

To illustrate the distribution of fine-root length by root diameter across all treatments, we prepared a relative cumulative root-length distribution function for all the dissected roots. The 4877 individual roots that were hand-dissected were sorted into 0.05-mm-diameter classes ranging from 0.05 through 3.0 mm, and the cumulative length of roots in class was calculated. Relative cumulative length (percentage of total length) was then plotted by root-diameter class.

Root-morphology treatment effects and interaction terms were analyzed for significant differences using a three-factor (soil N, atmospheric CO$_2$, root order) balanced, randomized block design ANOVA ($n = 3$). All variates were tested for normality using Bartlett's test for equality of variances. All variates met the assumption of normality following the transformation of root length (square root), C and N concentration (arcsine), and C/N ratio (arcsine). Separate ANOVAs were run for root length, root diameter, specific root length, C and N concentration, and C/N ratio using the GLM procedures in SAS 6.12 (SAS Institute 1990). A posteriori pairwise comparisons of interaction means within and among root orders were tested for significance using Tukey's hsd test in the GLM procedure of SAS.

Fine-root biomass

Live fine-root biomass was estimated using soil cores (45 cm long by 10 cm in diameter) collected in June 1996, 2 wk before the beginning of the final harvest. Twelve soil cores were extracted from each of the 20 chambers by dividing the chambers into quarters and randomly removing three cores from each quarter. Cores were immediately transported on ice to Michigan Technological University where they were separated from soil using a hydropneumatic root elutriator (Gillison Variety Fabrication, Benzonia, Michigan, USA). Recovery of roots from a washed soil sample is >99% (Smucker et al. 1982). We have found that careful hand sorting of soil cores only recovers ~60% of the fine-

root biomass we can recover using the root elutriator (A. J. Burton, *unpublished data*).

After washing, all roots >1.0 mm in diameter were removed from the samples. Live roots 0.5–1.0 mm in diameter were sorted by hand, oven-dried, and weighed; dead roots were discarded. The biomass of live roots <0.5 mm in diameter was estimated using a modified Newman's line-transect method (Newman 1966, Hendrick and Pregitzer 1993). All dry masses were corrected for ash content, which was always <5% of total dry mass. Root N content for the 0.5–1.0 mm diameter size class was determined by combusting samples from the biomass cores (Carlo Erba NA 1500 Series II elemental analyzer), and multiplying N concentration by dry mass. Root N content for the roots <0.5 mm diameter was determined by using the weighed treatment mean N concentration of roots from the first four orders dissected by hand and multiplying this concentration by the biomass in each chamber. The weighed mean N concentration was determined for each treatment by weighting the mean N concentration of the four root orders dissected by hand by the proportional length of roots in each of the four orders.

A mixed model (randomized complete block design) was used to statistically analyze the fine-root biomass and N-content data after testing to be sure all variates were normally distributed. If significant interactions were found, then a posteriori pairwise comparisons were investigated using Tukey's hsd test in the GLM procedure of SAS 6.12 (SAS Institute 1990).

Fine-root dynamics

Eight 2-m-long clear plastic minirhizotron tubes (Ann Arbor Plastics, Ann Arbor, Michigan, USA) were installed in each of the 20 root boxes before the treatment soil was placed into the boxes at the beginning of the experiment. Six tubes were installed horizontally, four at 10-cm soil depth, and two at 20-cm soil depth; two additional tubes were installed at a 30° angle to sample the soil to a vertical depth of 45 cm. Tubes were installed on all sides of the root boxes. The goal was to completely sample the treated soil space, with more intensive sampling near the surface where the majority of the fine roots are concentrated (Hendrick and Pregitzer 1993). One-hundred-sixty individual 9 × 13 mm ''root windows'' were sampled along each of the 160 minirhizotron tubes on each sample date using a BTC-2 Minirhizotron Video Camera System (Bartz Technology, Santa Barbara, California, USA). Each root window was roboticly etched into the plastic and numbered sequentially in a line along the long axis of the tube. The individual tubes were securely anchored to assure that images of the same roots were collected each time we used the video camera system.

There were no roots at the beginning of the experiment. Plants were allowed to grow until 1 August 1994 (57 d) when the first minirhizotron images were col-

lected. Three sets of images were collected in 1994 (1–7 August, 29 August–14 September, and 29 September–14 October) and six sets in 1995 (31 May–2 June, 21–23 June, 17–21 July, 7–11 August, 23–28 August, and 26–29 October). In total, the growth and mortality of 16 646 individual roots was monitored over the 1994–1995 growing seasons following methods previously described (Hendrick and Pregitzer 1992, Pregitzer et al. 1993, 1995). Additional images were collected in 1996, but a lack of funding prevented their analysis.

Cumulative root production was calculated as a running total of new root length produced during each time interval. Cumulative root mortality was calculated as the running total of root loss during each time interval. Net root production was calculated as cumulative root production minus cumulative root mortality for each time interval. Sample interval midpoints were used in statistical analyses. Treatment main effects and interactions were assessed using repeated-measures ANOVA (Meredith and Stehman 1991) in which orthogonal, quadratic polynomials were fitted to the data as calculated for unequally spaced sample dates (Robson 1959). The polynomial coefficients were then used as primary data to test for treatment effects using standard ANOVA (Steel and Torrie 1980). Data were log-transformed to meet the assumptions of ANOVA when necessary; ANOVA assumptions were evaluated by inspection of residuals and normal probability plots. Additional statistical details are described by Pregitzer et al. (1995). To investigate the relationship between results obtained using the minirhizotrons and soil cores, we correlated (Pearson's product-moment correlation coefficient) treatment mean net production (minirhizotrons) with treatment mean fine-root biomass <0.5 mm in diameter (soil cores).

Soil respiration

Soil respiration in each of the 20 chambers was measured using the static-chamber technique (sensu Goodroad and Keeney 1984, Steudler et al. 1989, Raich et al. 1990). In 1993 we permanently located a 400-cm² aluminum sampling base for gas sampling in each open-top chamber. Each sampling base extended 3 cm below the soil surface and was constructed with a U-shaped gutter into which the static chamber was placed for in situ gas sampling. Sample frames were located in each open-top chamber one year prior to our measurements of soil respiration so that roots were able to fully colonize the soil below each chamber. During the 1995 and 1996 field seasons we placed static chambers on sampling frames in a block and collected aliquots of headspace gas at 5-min intervals over a 20-min collection period. All field sampling occurred on a block basis. Gas samples (5 mL) were stored in evacuated 3-mL serum vials and transported to the laboratory for analysis. Carbon dioxide concentration in headspace gas was determined by gas chromatography,

and we estimated soil-respiration flux as the increase in CO_2 concentration over the 20-min sampling period. Five sets of soil-respiration measurements were taken from each chamber following this protocol in 1995 (26 June, 17 July, 9 and 28 August, and 1 October) and again in 1996 (4 and 25 May, 8 and 21 June, and 4 July). We are aware that static chambers underestimate soil respiration (Russell and Voroney 1998), but the relative treatment differences should not be greatly influenced by the method we employed.

Separate mixed models (randomized complete block design) were used to analyze soil respiration from 1995 and 1996. The two years were analyzed separately because data were collected during different parts of the growing season in 1995 (26 June–1 October) vs. 1996 (4 May–4 July), and because the variance structure of the data from the two years differed. The MIXED procedure was implemented in SAS (SAS Institute staff 1990). The random effect was the block variable and the fixed effects were atmospheric CO_2 and soil-N availability. Tukey's hsd test in the GLM procedure of SAS (SAS Institute 1990) was used to conduct a posteriori pairwise comparisons of 1996 interaction means. All assumptions of normality and equal variances were met.

Simple linear regression was used to understand the relationship between root biomass and soil respiration. Separate regressions were developed for fine-root biomass (root biomass <0.5 mm diameter + root biomass 0.5–1.0 mm diameter), coarse root biomass (root biomass >1.0 mm diameter, data from Zak et al. 2000b) and total root biomass (fine + coarse root biomass). The mean flux of CO_2 from the surface of the soil was calculated for the five measurements in 1996 (4 May–4 July) because this period of soil respiration most closely corresponded with the date of harvest. The mean (1996) rates of soil CO_2 flux from each chamber were used in all three regression analyses.

RESULTS

Fine-root morphology and carbon and nitrogen concentrations

Long, thin main-root axes with short lateral branches characterize the fine roots of *Populus tremuloides* (Fig. 1). Almost all of the total root length is accounted for by roots that are <0.5 mm in diameter, with a median root diameter of ~0.2 mm (Fig. 2). The CO_2 and soil-N treatments significantly altered fine-root morphology (Table 1). Roots became longer and thicker as order increased, and elevated CO_2 resulted in individual root segments that were both longer and thicker than roots produced under ambient CO_2 (Table 2). The soil-N treatments had no effect on root length and diameter. Across all treatments, first-order roots were only ~1.5 mm in length and 0.15 mm in diameter (Table 2).

Specific root length (SRL) decreased with root order and was unaffected by the CO_2 and soil-N treatments

Low N, Ambient CO$_2$

Low N, Elevated CO$_2$

High N, Ambient CO$_2$

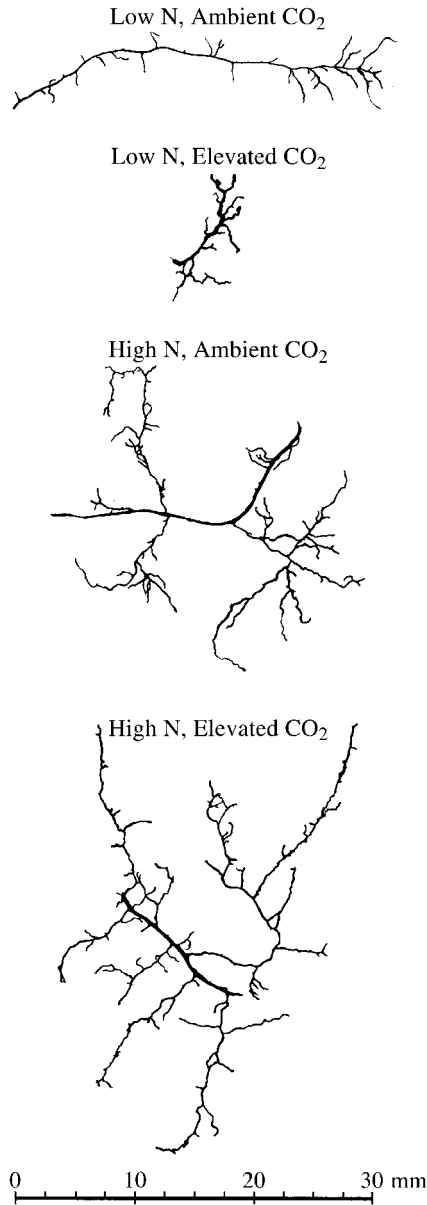High N, Elevated CO$_2$

0   10   20   30 mm

FIG. 1. Representative portions of the fine-root system of *Populus tremuloides* from each of the four experimental treatments. Treatments combined ambient atmosphere CO$_2$ or elevated (twice-ambient) CO$_2$ with low or high soil-N availability.

(Table 1). Root C concentration was also not influenced by any of the treatments (Table 1). However, root N concentration decreased in the higher order roots and was significantly lower at elevated CO$_2$ (Tables 1 and 2). Interestingly, the soil-N treatments had only a marginally significant influence on fine-root N concentration (Table 1). The C/N ratio increased as root order increased and within a given root order was highest in the elevated CO$_2$–low soil-N treatment (Table 2).

Fine-root length production and mortality

Fine-root length production and mortality both exhibited a very clear treatment response. The mean ($P < 0.001$), linear ($P < 0.001$) and quadratic ($P < 0.01$) effects were all highly significant terms in both the production (Fig. 3A) and mortality (Fig. 3B) functions. Atmospheric CO$_2$ ($P < 0.05$), soil-N availability ($P < 0.001$) and the interaction between atmospheric CO$_2$ and soil-N availability ($P < 0.05$) were significant mean effects. Soil-N availability also exhibited significant linear ($P < 0.001$) and quadratic effects ($P < 0.001$), and the quadratic interaction term between atmospheric CO$_2$ and soil-N availability was significant ($P < 0.05$). The significant mean effect interaction terms are obvious in Fig. 3A and B. Atmospheric CO$_2$ had no influence on fine-root length production and mortaility at low N availability, but rates of root length extension and mortality were greater at elevated CO$_2$ when trees were grown in high-N soil. The linear and quadratic effects are also obvious in Fig. 3A and B. Rates of root length extension and mortaility were much greater at high soil N, especially when the trees were grown at elevated atmospheric CO$_2$.

Net root production is the difference between root length growth and root length mortality (production minus mortality), and net production controls the shape of the fine-root growth-response curves. Again, the mean ($P < 0.001$), linear ($P < 0.01$) and quadratic ($P < 0.01$) terms in the net root-production functions were all statistically significant. Soil N ($P < 0.001$) was the only significant mean effect. Net production was statistically greater at high soil N compared with low soil N (Fig. 3C). The soil-N linear ($P < 0.001$) and quadratic $P < 0.001$) terms were also significant. This means that rates of net root production were greater at high soil N compared with low N, but that CO$_2$ did not significantly influence net production (Fig. 3C). It is
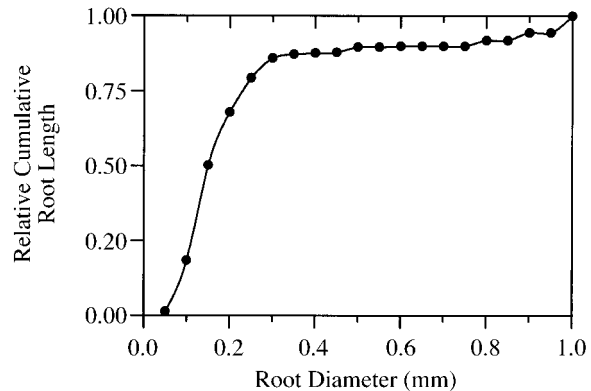
FIG. 2. The proportion of total cumulative root length, by root diameter class. The diameters and lengths of 4877 individual fine roots were measured across all treatments for a total fine-root length of 8.14 m.

TABLE 1. Results of analysis of variance of atmospheric CO_2 and soil-N availability on *Populus tremuloides* fine-root architecture and chemistry.

Source of variation	df	Root length (mm)		Root diameter (mm)		Specific root length (m/g)		Root carbon (g/kg)	
		MS	P	MS	P	MS	P	MS	P
CO_2	1	0.00235	0.0239	0.01386	0.0148	2 988.6	0.4341	0.0005	0.5160
Soil N	1	0.00021	0.4827	0.00012	0.8150	5 740.6	0.2806	0.0439	0.0771
Root order	3	0.04654	<0.0001	0.04544	<0.0001	42 101.0	0.0002	0.0144	0.3660
$CO_2 \times$ Soil N	1	0.00001	0.8660	0.00510	0.1271	1 112.0	0.6322	0.0033	0.6200
$CO_2 \times$ Root order	3	0.00043	0.3939	0.00325	0.2176	4 745.4	0.4073	0.0035	0.8489
Soil N $\times$ Root order	3	0.00006	0.9344	0.00031	0.9281	5 195.3	0.3674	0.0064	0.6952
$CO_2 \times$ Soil N $\times$ Root order	3	0.00031	0.5323	0.00026	0.9441	1 413.4	0.8271	0.0044	0.7994

obvious from Fig. 3 that net production was greatest at elevated CO_2–high soil N.

Together, the production and mortality functions in Fig. 3 demonstrate that the fine roots of young *P. tremuloides* trees grow and die much faster when soil-N availability is high. Atmospheric CO_2 increased rates of root growth and mortality, but only in the high-N soil treatment.

Fine-root biomass and nitrogen content

Both atmospheric CO_2 and soil N significantly influenced fine-root biomass. Roots <0.5 mm in diameter accounted for the majority of fine-root biomass (Table 3). Fine-root biomass was significantly greater at elevated CO_2 in the high-N soil, but there were no significant differences due to the CO_2 treatment at low soil N (Table 3). These results explain the significant interaction among the atmospheric-CO_2 and soil-N treatments (Table 3). Soil N increased the biomass of the smallest diameter roots 194%, while atmospheric CO_2 increased biomass 52% (main effects, Table 3). The pattern for fine roots 0.5–1.0 mm in diameter was basically the same, except the atmospheric-CO_2 and soil-N interaction term was not significant (Table 3).

The minirhizotron data and biomass estimates from soil cores are two independent methods of studying fine roots. It is interesting to note that the statistical treatment responses from these two methods are identical. Both methods document a highly significant response to elevated CO_2 at high soil N and no response to atmospheric CO_2 at low soil N. To document the correspondence of results from two independent methods we correlated final cumulative net root length production measured with the minirhizotrons in each chamber to the biomass of roots <0.5 mm in diameter in each chamber. The Pearson product-moment correlation coefficient between these two measures of fine-root response was 0.975 ($P = 0.02$). The fine-root biomass estimates in Table 3 represent a ''snapshot'' of the treatment response 2.5 growing seasons after the treatments were initiated, while the minirhizotron data depict the dynamic response measured over two growing seasons (Fig. 3). The correspondence in results among the two independent methods of measuring fine-root response is noteworthy.

The total amount of N (N content = N concentration $\times$ biomass) of the fine roots was primarily driven by changes in root biomass (Table 3). The treatment responses were significant and correspond more or less directly with the changes in fine-root biomass. The ex-

TABLE 2. Root morplology, root N concentration and carbon-to-nitrogen ratio (C/N) for the first four root orders of *Populus tremuloides*.

Root order	Root length (mm)			Root diameter (mm)			Specific root length (m/g)	Root nitrogen (g/kg)		
	Ambient CO_2	Elevated CO_2	Marginal means	Ambient CO_2	Elevated CO_2	Marginal means		Ambient CO_2	Elevated CO_2	Marginal means
1	1.47 (0.202)	1.41 (0.144)	1.44[a] (0.118)	0.14 (0.005)	0.16 (0.009)	0.15[a] (0.005)	244.5[a] (25.9)	21.21 (1.23)	18.09 (1.12)	19.65[a] (0.53)
2	3.85 (0.319)	4.77 (0.001)	4.31[b] (0.323)	0.18 (0.014)	0.18 (0.013)	0.18[a] (0.009)	167.1[b] (13.1)	18.52 (1.41)	15.12 (1.68)	16.82[a] (0.67)
3	9.55 (0.341)	11.28 (0.001)	10.31[c] (0.591)	0.22 (0.012)	0.27 (0.027)	0.25[b] (0.016)	124.3[b] (17.2)	13.69 (0.73)	10.72 (0.75)	12.29[b] (0.39)
4	10.06 (0.001)	14.1 (0.002)	11.68[d] (1.076)	0.25 (0.018)	0.33 (0.044)	0.29[b] (0.020)	107.3[b] (18.8)	12.59 (0.72)	11.69 (3.80)	12.22[b] (0.81)
Marginal means	6.20[a] (0.80)	7.30[b] (1.17)		0.20[a] (0.011)	0.23[b] (0.018)			16.50[a] (0.89)	14.50[b] (1.17)	

Notes: Tukey's hsd pairwise comparisons contrast significant main effects and interactions from the ANOVA summarized in Table 1. Data are means with 1 SE in parentheses. Within a row or column of marginal means for the same dependent variable, means with the same lowercase superscript letter are not significantly different at $P < 0.05$.

TABLE 1. Extended.

Root nitrogen (g/kg)		C/N ratio	
MS	P	MS	P
0.0020	0.0029	0.1145	<0.000
0.0068	0.0705	0.0072	0.0007
0.0296	<0.0001	0.1434	<0.000
0.0020	0.3139	0.0432	0.0064
0.0001	0.9810	0.0158	0.0397
0.0007	0.7973	0.7973	0.0832
0.0032	0.1961	0.0303	0.0024

ception to this generalization is due to the fact the elevated-CO_2 treatment stimulated biomass production, especially at high soil N, but decreased N concentration (Table 2). This resulted in N-content treatment responses that were not as pronounced as biomass treatment responses (Table 3).

Soil respiration

Mean rates of soil respiration in 1995 and 1996 were strongly affected by the two main treatments. Soil N increased mean rates of soil respiration by 123% in 1995 and 104% in 1996 (Table 4). This response is not surprising because the high-N soil had 3.5 times as much organic C as the low-N soil and because the soil N treatment had a highly significant impact on root growth (Table 3). Elevated CO_2 also had a pronounced impact on mean rates of soil respiration, increasing mean rates by 19% in 1995 and 25% in 1996 (Table 4). The interaction between the atmospheric CO_2 and soil N treatments was highly significant in 1996, with a response parallel to that of fine-root biomass. The mean rate of CO_2 flux from the high-N soil surface was significantly greater at elevated CO_2 compared to ambient CO_2, but there were no significant differences among the CO_2 treatments at low soil N (Table 4).

TABLE 2. Extended.

C/N ratio			
Ambient CO_2, low soil N	Elevated CO_2, low soil N	Ambient CO_2, high soil N	Elevated CO_2, high soil N
21.7[b]	23.9[b]	19.0[b]	21.7[b]
(0.6)	(2.5)	(1.4)	(1.2)
25.9[b]	31.5[ab]	21.3[b]	28.5[ab]
(0.5)	(6.3)	(2.0)	(2.4)
34.0[ab]	46.3[a]	31.2[ab]	36.4[ab]
(2.5)	(2.0)	(2.9)	(2.3)
33.9[ab]	...	38.2[ab]	37.7[ab]
(2.5)		(3.1)	(10.4)

Across all treatments, there were highly significant linear relationships among mean 1996 rates of soil respiration and root biomass (Fig. 4). By comparing the slopes of the regressions among fine-root biomass vs. coarse-root biomass with mean rates of soil respiration (Fig. 4A vs. B) it is clear that, per gram of C, fine roots contribute much more to total soil CO_2 flux than do coarse roots. The linear relationship between total root biomass and mean rates of soil respiration is remarkable (Fig. 4C).

DISCUSSION

Fine-root morphology

The availability of sufficient nutrients to sustain plant growth is a key factor in understanding the response of terrestrial ecosystems to rising atmospheric CO_2. Plants could facilitate increased nutrient acquisition by changing the morphology of their root systems. There are numerous ways this could happen, e.g. an increase in root hairs, number of lateral branches, or the length of individual lateral branches to increase absorptive root length, or an increase in specific root length (SRL) to facilitate greater exploration of the soil. Because rates of photosynthesis commonly increase at elevated CO_2 (Curtis and Wang 1998), the energy to support the cost of increased fine-root growth and maintenance is potentially available. Given the importance of root morphology to nutrient acquisition, especially absorbing root length (Nye and Tinker 1977), it is surprising how few studies of root morphology have been conducted.

We found that both the length and diameter of individual short lateral roots were significantly greater under elevated vs. ambient atmospheric CO_2, but SRL exhibited no statistical treatment response and decreased with root order. These results are similar to other studies. Bernston and Woodward (1992), Rogers et al. (1992), King et al. (1997) and Crookshanks et al. (1998) all report that elevated CO_2 increased root length. In the more anatomical study, Crookshanks et al. (1998) report that elevated CO_2 accelerated cortical-cell expansion rates and increased formation of lateral roots in *Arabidopsis thaliana.* Although there is very little literature documenting the influence of elevated CO_2 on root morphology, it is very consistent—all studies suggest that elevated CO_2 changes root morphology by increasing absorbing root length. It is especially important to understand how the deployment of absorbing root length is influenced by elevated CO_2, because this factor, along with nutrient uptake capacity per unit root length, is critical in regulating nutrient acquisition (Caldwell et al. 1992). Given the fact several studies report that elevated CO_2 does not increase rate of nutrient uptake per unit root length (BassiriRad et al. 1997, Rothstein et al. 2000), additional research focusing on root morphology and nutrient acquisition is needed.
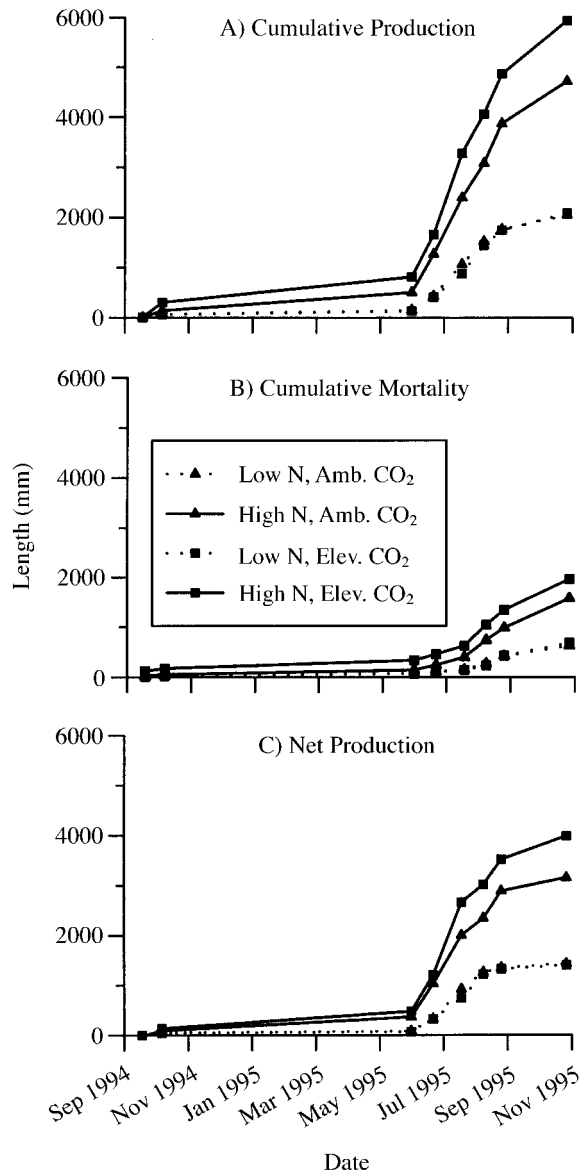
FIG. 3. *Populus tremuloides* cumulative fine-root production (A), cumulative fine-root mortality (B), and net fine-root production (C) for trees grown in ambient atmospheric CO_2 and twice-ambient (elevated) atmospheric CO_2, and at low soil-N availability and high soil-N availability.

Almost nothing is known about species-specific differences in root morphology and how root morphology will respond at the species level to elevated CO_2. A comparison of the fine-root morphology of two deciduous trees that grow sympatrically in northern Michigan, *Populus tremuloides* and *Acer saccharum,* illustrates some of the differences that can occur among taxa. The great majority of absorbing roots of both species have very small diameters; median diameters range from 0.2 to 0.3 mm (Fig. 2; Pregitzer et al. 1997). Specific root lengths are variable among the two spe-

cies. The mean specific root length of first-third order roots of *P. tremuloides* ranges from 245 to 124 m/g (Table 2), while the mean SRL of first-third order roots of *A. saccharum* ranges from 131 to 80 m/g (Pregitzer et al. 1997). These changes mirror the different architecture of the two species. *P. tremuloides* has a more exploratory root system with long, thin axes and relatively fewer lateral branches (Fig. 1), while *A. saccharum* has a much more highly branched root system (Pregitzer et al. 1997, 1998). Based on differences in SRL, the C cost of constructing first-third order roots of *P. tremuloides* is 35–47% less than the cost of constructing first-third order roots of *A. saccharum.* It is important to realize that root-system morphology, although very poorly understood, may vary widely among species, just as leaf morphology and leaf function vary among species (Reich et al. 1992). *P. tremuloides* with its long, thin, relatively inexpensive fine roots (compared to those of *A. saccharum*) may exhibit a different demographic response to rising atmospheric CO_2 than a species with roots that have lower SRL. At this time, there is no reason to expect different taxa to exhibit identical morphological and demographic responses to elevated atmospheric CO_2.

Fine-root carbon and nitrogen concentrations

Contrary to our original hypothesis, the two soil-N treatments did not significantly alter fine-root N concentration (Table 1). High soil N greatly increased fine-root biomass (Table 3), total plant biomass (Zak et al. 2000b), and the total amount of N in trees (Zak et al. 2000b), but the concentration of N in fine roots was only marginally higher in this treatment. Elevated atmospheric CO_2, on the other hand, resulted in significantly lower fine-root N concentrations, averaging 15% less across the first four root orders (Table 2). Root N concentration decreased with root order (Table 2), a pattern we have observed in other deciduous trees (Pregitzer et al. 1997). Rates of fine-root respiration are correlated with fine-root N concentration (Ryan et al. 1996, Pregitzer et al. 1998), making the small-diameter roots at the distal end of the root system the most metabolically active.

Total fine root C concentrations were not influenced by the atmospheric-CO_2 or soil-N treatments (Table 1). The C/N ratios of fine roots were higher at elevated CO_2 compared to ambient CO_2 across both levels of soil-N availability, but most of these differences were not statistically significant (Table 2). In general, root C/N ratio increased with root order (Table 2), a pattern consistent with previous studies (Pregitzer et al. 1997).

There is very little information on the influence of elevated atmospheric CO_2 on tree root tissue quality. Both King et al. (1997), and Rothstein et al. (2000) found that elevated atmospheric CO_2 did not affect the concentration of total non-structural carbohydrates (TNC) in fine roots, although Rothstein et al. (2000)

TABLE 3. *Populus tremuloides* fine-root biomass and root nitrogen content.

Dependent variable and root category†	Interaction means (n = 5 chambers)				Main-effect means (n = 10 chambers)					
	Low soil N		High soil N		Soil N			CO$_2$		
	Ambient CO$_2$	Elevated CO$_2$	Ambient CO$_2$	Elevated CO$_2$	Low	High	Change (%)	Ambient	Elevated	Change (%)
Biomass (g/m^3)										
Roots < 0.5-mm diam. [***]	46.8[c] (2.2)	55.0[c] (7.0)	112.7[b] (7.6)	186.9[a] (9.3)	50.9 (5.1)	149.8 (5.1)	194***	79.7 (5.1)	120.9 (5.1)	52***
Roots 0.5–1.0 mm diam. [NS]	14.0 (1.4)	16.0 (2.2)	31.9 (0.6)	39.1 (2.4)	15.0 (1.3)	35.5 (1.3)	137***	22.9 (1.3)	27.6 (1.3)	20*
Root N (g/m^3)										
Roots < 0.5-mm diam. [*]	0.90[c] (0.04)	0.92[c] (0.12)	2.34[b] (0.16)	2.99[a] (0.15)	0.91 (0.09)	2.67 (0.09)	193***	1.62 (0.09)	1.95 (0.09)	20*
Roots 0.5–1.0 mm diam. [NS]	0.11 (0.01)	0.12 (0.02)	0.31 (0.02)	0.34 (0.03)	0.11 (0.01)	0.32 (0.01)	191***	0.21 (0.01)	0.23 (0.01)	10[NS]

Notes: N content = N concentration × biomass. Data are means with 1 SE in parentheses. Within a row, interaction means with the same lowercase superscript letter are not significantly different at P < 0.05 (Tukey's hsd pairwise comparisons). For interaction means, n = 5 chambers; for main-effect means, n = 10 chambers.

† Significance of N × CO$_2$ interaction is indicated in brackets.

* P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant.

did document a significant decline in TNC at high soil N. Gebauer et al. (1998) demonstrated that the concentration of total phenolics and condensed tannins was relatively high in the lateral roots of *Pinus taeda* and that total phenolics increased in lateral roots at elevated CO$_2$, consistent with the carbon-nutrient balance hypothesis (Bryant et al. 1983). In our study, root C/N ratios were relatively stable across all treatments. This finding combined with the few other studies on root litter quality suggests that root litter quality may not be as responsive to elevated CO$_2$ and soil-N availability as leaf litter quality. We emphasize that in deciduous trees the smallest roots, generally <0.5 mm in diameter, account for most of the fine-root litter inputs. These very fine roots have much lower C/N ratios than larger diameter roots; C/N ratios of very fine roots are similar to those of leaf litter (Table 2; Pregitzer et al. 1997). More studies on the effects of varying soil-N availability and elevated CO$_2$ are needed to determine the generality of these results.

Fine-root length production and mortality

Soil-N availability had a highly significant influence on fine-root production and mortality. Both cumulative fine-root production and mortality more than doubled over the 2.5-growing-seasons experiment in the high soil-N treatment compared with the low-N treatment (Fig. 3). Pregitzer et al. (1995) and Kubiske et al. (1998) also found that high soil N greatly increased fine-root growth and mortality in *Populus* trees. There has been debate in the literature about the influence of soil-N availability on fine-root turnover. Some evidence suggests that increasing soil-N availability will increase root turnover, while other evidence supports the idea of decreased root turnover as soil N increases

(Hendricks et al. 1993). Our results (Fig. 3), Pregitzer et al. (1995), and Kubiske et al. (1998) demonstrate without doubt that high soil-N availability increases fine-root turnover in young, fast-growing *Populus* trees. In this experiment, measurements of fine-root turnover using minirhizotrons were highly correlated with fine-root biomass estimates (Table 3) and total soil-respiration measurements (Fig. 4). All three independent sets of measurements confirm that the absolute allocation of C to fine roots greatly increases in *Populus* at high soil N. We caution that these results should not be generalized to all tree species, and they may be influenced by tree ontogeny. Just as root morphology appears to vary among taxa, so too might patterns of C allocation and root demography in response to altered soil-N availability. For example, Tingey et al. (1996) found in *Pinus ponderosa* Dougl. Ex P. Laws. that elevated CO$_2$ increased fine-root area density and the occurrence of mycorrhizae, whereas N fertilization had no effect on fine-root area density.

Elevated atmospheric CO$_2$ increased root growth and mortality, but only at high soil N (Fig. 3). In a 1-yr experiment, Kubiske et al. (1998) also working with *Populus tremuloides,* found similar results. Tingey et al. (1996) found that elevated CO$_2$ increased fine-root area density in *Pinus ponderosa* regardless of soil-N availability. Pregitzer et al. (1995) reported that elevated CO$_2$ increased fine-root production and mortality at both high and low N availability. In a short-term experiment, Berntson and Bazzaz (1996) found that elevated CO$_2$ increased root production and mortality in *Betula papyrifera,* but not in *Acer rubrum*. Taken together, these few studies suggest that elevated atmospheric CO$_2$ will increase root turnover if soil N does not limit photosynthesis. However, this conclusion

TABLE 4. Mean rates of soil respiration (mg CO_2-C·m^{-2}·d^{-1}) in 1995 and 1996.

| | Interaction means (n = 5 chambers) | | | |
| | Low soil N | | High soil N | |
Year†	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
1995 [NS]	829.8	1087.9	2006.8	2274.0
	(32.4)	(142.2)	(109.5)	(163.8)
1996 [**]	664.8[c]	768.8[c]	1265.9[b]	1652.4[a]
	(45.5)	(50.2)	(39.2)	(63.4)

Notes: Data are means with 1 SE in parentheses. All units are milligrams CO_2-C per square meter per day. Within a row, interaction means with the same lowercase superscript letter are not significantly different at $P < 0.05$ (Tukey's hsd pairwise comparisons).

† Significance of N × CO_2 interaction is indicated in brackets.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant.

is highly speculative. Up to this point, dynamic estimates of root turnover at elevated CO_2 for plants growing in the field for >360 d have only come from the Placerville, California (USA) ponderosa pine experiment (Tingey et al. 1996) and our experiment. A more logical conclusion is that the evidence for generalizations about C allocation to roots and root demography is not sufficient at this time to make sweeping statements about belowground plant response to elevated CO_2. Given the diversity in life-forms, physiological adaptations, and inherent patterns of carbon allocation within and among taxa, we may see different patterns of root demography and C allocation to fine roots as atmospheric CO_2 increases. If soil N is nonlimiting, our studies demonstrate that the increase in C input to the soil from root turnover at elevated CO_2 can be substantial (Fig. 3, Pregitzer et al. 1995, Kubiske et al. 1998).

Fine-root biomass

Soil N had a dramatic influence on fine-root biomass. Across ambient and elevated atmospheric CO_2, mean biomass of roots <0.5 mm in diameter was 194% greater in high-N soil when compared to the low-N treatment (Table 3). The atmospheric-CO_2 and soil-N treatment interaction was also highly significant for this root size class, with a significant 65% increase due to elevated atmospheric CO_2 at high soil N, and a nonsignificant 17% increase at low soil N (Table 3). The pattern for fine roots 0.5–1.0 mm in diameter was similar, although the response was not as dramatic and the treatment interaction was not significant (Table 3). Several short-term studies of tree seedlings report a significant increase in fine-root biomass due to elevated CO_2 (Norby 1994). In many of these studies, fine-root biomass was very responsive to elevated CO_2, with growth increases exceeding those of other plant components (Norby 1994). However, as Norby (1994) articulates, fine-root mass is only a small portion of total-tree mass and, as trees develop, fine-root mass will become a relatively less important component of whole-tree C storage.

There are only a few experiments that have documented the belowground response to elevated CO_2 in

▲ Low N, Ambient CO_2 ■ Low N, Elevated CO_2
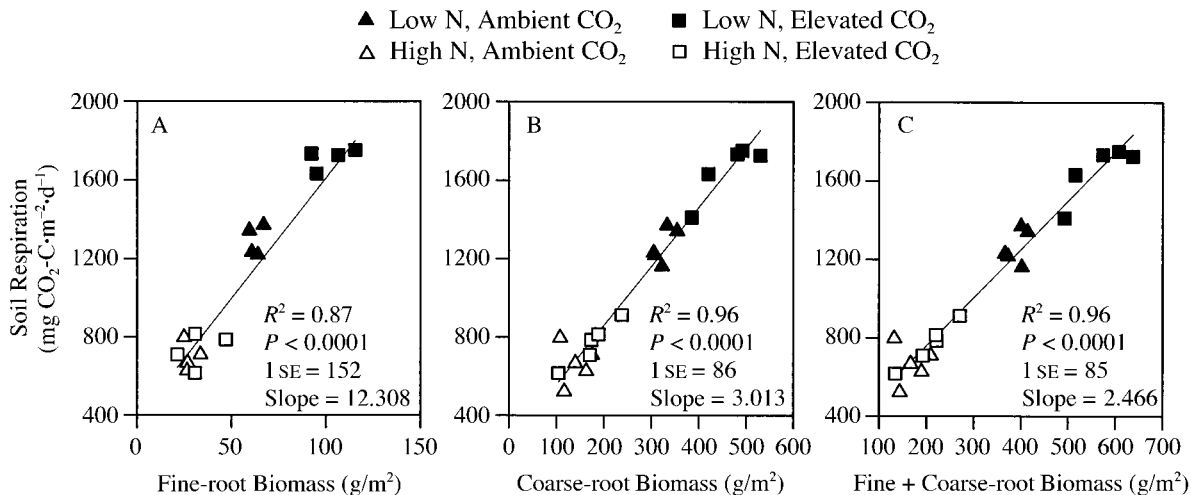△ High N, Ambient CO_2 □ High N, Elevated CO_2

FIG. 4. Linear relationships between fine, coarse, and total root biomass and soil respiration. Soil respiration values are 1996 mean fluxes from each chamber. Root biomass was measured during the final harvest (see *Methods: Fine-root biomass* for details). Fine roots are <1 mm in diameter; coarse roots are >1 mm in diameter.

TABLE 4. Extended.

| Main-effect means (n = 10 chambers) | | | | | |
| Soil N | | | CO$_2$ | | |
Low	High	Change (%)	Ambient	Elevated	Change (%)
958.8 (92.0)	2140.4 (92.0)	123***	1418.3 (92.0)	1681.0 (92.0)	19*
716.8 (42.9)	1459.1 (42.9)	104***	965.3 (42.9)	1210.6 (42.9)	25***

temperate deciduous trees grown in the field for more than 360 d. Norby et al. (1996) grew *Liriodendron tulipifera* L. and *Quercus alba* L. in open-top chambers for 3 and 4 yr, respectively. They found that elevated atmospheric CO$_2$ increased fine-root biomass significantly in both species, but that this tree component composed a relatively small percentage of total-tree biomass. Rey and Jarvis (1997) grew *Betula pendula* Roth. for 3.5 yr in open-top chambers and found that fine-root biomass increased more than any other tree component over the duration of the study. Our 2.5-growing-seasons field experiment also documents that *Populus tremuloides* very-fine-root biomass was more responsive to elevated atmospheric CO$_2$ than any other tree component (Zak et al. 2000). Although the data are few and still very short term from the perspective of forest development, all the published field experiments that have reported fine-root biomass support the idea that more fine-root C will be cycled through the soil as atmospheric CO$_2$ increases (Norby et al. 1992, 1996, Zak et al. 1993, 2000*a*, Johnson et al. 1994, Norby 1994, Pregitzer et al. 1995, Rey and Jarvis 1997). The exception to this generalization might be, as Fig. 3 and Johnson et al. (1998) suggest, situations where soil-N availability is limited. Our data (Fig. 3) clearly demonstrate another important point: inputs of C to soil from fine-root turnover are highly dynamic. Whether or not more C will be stored in the soil as atmospheric CO$_2$ increases root-C input is yet another unanswered question (Hungate et al. 1997).

Unresolved C-allocation issues

As the concentration of CO$_2$ increases in Earth's atmosphere, there are several aspects of C allocation to tree root systems that remain unresolved. Plant developmental rates may influence the way C is allocated to fine roots. In our experiment, trees were young and growing in their exponential phase in a soil matrix that, within a given treatment, was relatively uniform in terms of N availability. It is clear the *Populus tremuloides* trees we studied allocated absolutely more C to fine roots when soil-N availability was high and atmospheric CO$_2$ was elevated. Fine-root growth did not respond to elevated CO$_2$ when N was limiting given this set of experimental conditions. It remains to be seen if these patterns continue throughout the ontogeny of stand development, or if they will apply

to shade-tolerant trees that have slower inherent rates of growth. Because *Populus* is a fast-growing, shade-intolerant genus, we expect the patterns found in this study to carry forward in similar situations, such as *Populus* plantations grown for fiber production. It will be interesting to compare and contrast the results of early- and late-successional tree taxa, and young and older trees as these data become available. At this point in time, it is dangerous to make sweeping conclusions about how soil N and atmospheric CO$_2$ interact to influence C allocation to tree root systems since all the experimental data in hand come from young trees growing exponentially. It is also important to remember when modeling the response of forests to rising atmospheric CO$_2$ that the landscape is composed of stands of different age classes and varying composition. In reality, many of the forests around the world are young and they continue to be utilized by an ever-growing human population. To conclude that data from young trees is irrelevant is also a mistake, since many of the issues surrounding a better understanding of C storage in terrestrial ecosystems will involve young, managed forest ecosystems. We need a much better understanding of how trees respond throughout their typical period of growth and development to rising atmospheric CO$_2$.

The issue of C allocation to tree root systems is also confounded by methodological problems and semantics. In many instances, the proportional weight of different parts of the tree is used to quantify C allocation in response to changing soil-nutrient availability and increasing atmospheric CO$_2$. Typical measures in the literature are static root-to-shoot ratios and allometric analyses. Both of these approaches to understanding plant growth and development can be very useful, and both originated in agronomy where the issues of understanding the development of plants with an annual life cycle are much simpler. Understanding C allocation in trees is more complicated. Root/shoot ratios in trees, even young trees, can be misleading because the shoot (leaves, branches, bole) represents a mixture of C accumulated on different time steps (leaves once a year, bole C is accumulated over the life of the tree). In contrast, the fine-root system is very dynamic as Fig. 3 clearly demonstrates. Little is known about how increasing atmospheric CO$_2$ changes the allocation of C to fine roots over the lifespan of a tree, and little can

be inferred from root-to-shoot ratios. Since fine roots represent such a large part of the total C budget of trees, the issue of understanding how ontogeny and environment interact to control C allocation on a whole-tree basis will be a fruitful area for further research.

Soil respiration

Both atmospheric CO_2 and soil-N availability significantly increased mean rates of soil respiration in 1995 and 1996 (Table 4). Rates of soil respiration were linearly related to root biomass across all treatments, with fine roots contributing proportionally more to total soil CO_2 flux (Fig. 4). The fact that fine roots are more active metabolically should be expected, since they are the primary site for active nutrient uptake and assimilation. The smallest roots (<0.5 mm in diameter) located in the upper part of the soil profile have higher N concentrations and much higher rates of respiration than larger roots or roots located deeper in the soil profile (Pregitzer et al. 1998).

Literature estimates for the contribution of roots to total soil respiration range from 5 to 90%, with many values near or above 50% (Nakane et al. 1983, Ewel et al. 1987, Behera et al. 1990, Haynes and Gower 1995, Ryan et al. 1996). In well-drained soil it seems logical to expect root and total soil respiration to be closely correlated, and both should increase exponentially with temperature. All the field studies that have examined the influence of elevated CO_2 on total soil respiration report a significant increase at elevated CO_2 (Johnson et al. 1994, Luo et al. 1996, Griffin et al. 1997, Hungate et al. 1997, Vose et al. 1997, Ball and Drake 1998, Walker et al. 1998), except agricultural FACE (Free air carbon dioxide enrichment) experiments (Prior et al. 1997, Ineson et al. 1998). Our experiment is unusual in that fine-root dynamics (Fig. 3), root biomass, and soil respiration are reported together (Fig. 4). Mean rates of soil respiration across the four treatments follow the pattern one would expect if total soil respiration were strongly driven by root biomass (Fig. 4). We did not attempt to subdivide total soil respiration into its root and microbial components. Nevertheless, our data provide rather convincing evidence that, in our experiment, total soil respiration was strongly correlated to live-root biomass, probably the combination of root/mycorrhizal respiration and the rapid respiration of root exudates and decomposing fine roots and their associated mycorrhizae.

Summary and Implications

Populus tremuloides (trembling aspen) is a fast-growing, shade-intolerant species that has the most extensive geographic distribution of any tree in North America. It is important to wildlife, and it is a very important source of fiber in the Lake States and Canada, where aspen abundance and productivity are critical to the forest-products industry. Today, natural stands of trembling aspen are often managed on a short-rotation basis (25–60 yr), and even-aged stands are typically regenerated by clear-cutting. Several species and artificial hybrids in the genus *Populus* are also being planted on former agricultural fields throughout the United States in an effort to increase the supply, and decrease the cost, of fiber to the forest-products industry. The genus is also the target of intensive efforts to improve the genetic basis for pulp productivity, for example, by inserting herbicide- or insect-resistant genes into the genome or by decreasing lignin content (Stettler et al. 1996). Fast-growing plantations also have the potential to be used in atmospheric-C trading schemes. Obviously, the growth responses of trembling aspen to increasing atmospheric CO_2 have important ecological applications.

Our results demonstrate atmospheric CO_2 and soil-N availability strongly interact to influence *Populus tremuloides* fine-root morphology, growth, and C turnover. When sufficient soil N is available, aspen-dominated ecosystems of the future are likely to have greater productivity fueled by greater nutrient uptake due to greater root-length production. Further, it appears that elevated atmospheric CO_2 will result in greater C inputs to soil through greater rates of fine-root production and turnover, especially in high-fertility soils. Increased C inputs to soil result in greater rates of soil respiration. At this time, it is not clear what influence increased rates of root turnover will have on C storage in the soil, but since there is clearly more C moving into the soil at high N, it is entirely possible that some fraction of this C will find its way into stable soil organic matter.

In this experiment, limiting soil N did not statistically increase root growth and mortality. It is difficult to predict long-term trends in belowground production when soil N is limiting since ecosystem feedback on N availability is likely to work on longer time steps than the immediate growth responses we measured in this experiment. Still, it is clear that trembling aspen may not increase root growth when soil N is limiting and this could eventually result in leaf-level N deficiency. Low leaf N might eventually limit photosynthesis. We need longer term experiments to resolve these uncertainties. Our experiment also clearly demonstrates the critical role soil-N availability and root growth will play in determining ecosystem responses to increasing atmospheric CO_2.

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