

Effects of elevated atmospheric CO₂ on fine root production and activity in an intact temperate forest ecosystem

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Abstract

We investigated the effects of elevated atmospheric CO₂ concentrations (ambient + 200 ppm) on fine root production and soil carbon dynamics in a loblolly pine (*Pinus taeda*) forest subject to free-air CO₂ enrichment (FACE) near Durham, NC (USA). Live fine root mass (LFR) showed less seasonal variation than dead fine root mass (DFR), which was correlated with seasonal changes in soil moisture and soil temperature. LFR mass increased significantly (by 86%) in the elevated CO₂ treatment, with an increment of 37 g(dry weight) m⁻² above the control plots after two years of CO₂ fumigation. There was no long-term increment in DFR associated with elevated CO₂, but significant seasonal accumulations of DFR mass occurred during the summer of the second year of fumigation. Overall, root net primary production (RNPP) was not significantly different, but annual carbon inputs were 21.7 gC m⁻² y⁻¹ (68%) higher in the elevated CO₂ treatment compared to controls. Specific root respiration was not altered by the CO₂ treatment during most of the year; however, it was significantly higher by 21% and 13% in September 1997 and May 1998, respectively, in elevated CO₂. We did not find statistically significant differences in the C/N ratio of the root tissue, root decomposition or phosphatase activity in soil and roots associated with the treatment. Our data show that the early response of a loblolly pine forest ecosystem subject to CO₂ enrichment is an increase in its fine root population and a trend towards higher total RNPP after two years of CO₂ fumigation.

Keywords: elevated CO₂, forest ecosystem, *Pinus taeda*, root production, root phosphatase activity, root respiration

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Introduction

A substantial body of literature suggests that the increasing concentration of atmospheric CO₂ has enhanced the net primary production (NPP) of land plants. Higher terrestrial NPP could lead to carbon accumulation in plant tissues and/or soils and explain the fate of CO₂ that is released to the atmosphere by human activities that is not accounted for by oceanic and atmosphere sinks (Schimel 1995). The role of the world's forests as a sink for atmospheric CO₂ is a subject of active debate. Some models imply a strong sink in eastern North America (Fan *et al.* 1998) and a corresponding

source in the Southern Hemisphere (Denning *et al.* 1999), which contrast with forest inventories that indicate weaker sinks in North America (Houghton *et al.* 1999) and in Neotropical forests of South America (Phillips *et al.* 1998). Accurate estimates of primary production, carbon accumulation, and turnover in terrestrial ecosystems exposed to experimental CO₂ enrichment are critical to evaluate the origin of the sink for carbon in forests.

Stimulation of photosynthesis by high CO₂ does not always result in an increase in aboveground biomass (Drake *et al.* 1997), but often results in greater carbon allocation to roots (Rogers *et al.* 1994). For example, yellow-poplar saplings grown in open-top chambers at elevated CO₂ consistently showed greater photosynthesis without a corresponding increase in aboveground plant

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mass during three growing seasons (Norby *et al.* 1992). Rather, these trees diverted carbon from leaf production to fine root production with a consequent increase in the C flux through the root system. This is also the case for many studies of tree seedlings growing at elevated CO₂, where higher root production increases carbon losses from the root system (Griffin *et al.* 1997; Janssens *et al.* 1998; Cheng 1999). There is no general relationship between the amount of fine root biomass per unit of foliage, because water supply (Schluze *et al.* 1996), nutrient availability (Axelsson 1981; Gower *et al.* 1992) and competition between plants (Casper & Jackson 1997) affect root production and turnover in most ecosystems. Trees growing in elevated CO₂ may invest more carbon in roots to satisfy their greater demands for nutrients and water.

Elevated CO₂ can increase the total input of organic material to the soil by: (i) increasing litter production (Zak *et al.* 1996; Finzi *et al.* 2000) (ii) increasing root production (Berntson & Bazzaz 1996; Fitter *et al.* 1996; Godbold *et al.* 1997), and (iii) increasing root turnover rates and exudation (Berntson & Bazzaz 1997; Fitter *et al.* 1997; Thomas *et al.* 1999). Increased carbon allocation to roots may lead to changes in soil carbon pools (Norby *et al.* 1987; Zak *et al.* 1993; Rygielwicz *et al.* 1997; Janssens *et al.* 1998) and in the rates of carbon transfer between the different soil pools (Van Veen *et al.* 1991; Van de Geijn & Van Veen 1993; Canadell *et al.* 1996). However, the integrated effects of elevated CO₂ concentration on C cycling in the soil are unclear since allocation is just one of the many variables that determines carbon cycling (Canadell *et al.* 1996).

A more rapid return of C to the atmosphere may occur if elevated CO₂ enhances root and soil respiration rates to a greater extent than root production, therefore minimizing the potential for soils to act as a sink for atmospheric carbon dioxide (Fitter *et al.* 1996; Hungate *et al.* 1997). Some studies have shown that elevated CO₂ reduces specific root respiration by decreasing the N content of the roots (Crookshanks *et al.* 1998) and/or by directly inhibiting respiratory enzymes (González-Meler *et al.* 1996; González-Meler & Siedow 1999). Griffin *et al.* (1997) have shown that specific soil CO₂ efflux, normalized to root biomass, increased only when there was adequate N; however, Vose *et al.* (1997) found no effect of N treatment on total soil CO₂ efflux.

Estimates of fine root production and turnover are fraught by methodological problems (Publicover & Vogt 1993). The production and turnover of roots occur simultaneously, and roots undergo multiple cycles of growth, death, and replacement during the year (Santantonio & Grace 1987). Despite the disparity between available estimation methods (Vogt *et al.* 1998), there is a general agreement that mortality and decom-

position should be included in estimates of fine root production. Sequential soil coring has been widely used to estimate root primary production in many forest ecosystems (Persson 1979, 1983; Gholz *et al.* 1986; Nadelhoffer & Raich 1992) including those dominated by *Pinus taeda* (Harris *et al.* 1977). This method has shown the importance of root turnover in the cycling of C, N and P in forest ecosystems (Aerts *et al.* 1992). Because of the problems associated with all techniques for measuring root production, there is an increasing need to compare the results of different methodologies in the same ecosystem (Vogt *et al.* 1998). In this regard, our study by sequential soil coring will establish the basis for comparison with the results of other methodologies now being conducted at the same site.

The objectives of this study were to measure the seasonal growth, turnover and respiration of fine roots in a loblolly pine forest located in the Blackwood Division of the Duke Forest, Orange County, North Carolina, USA, where FACE technology is used to increase the ambient CO₂ concentration by 200 ppm. In this study the forest primary production has increased by 25% during two years of CO₂ enrichment (DeLucia *et al.* 1999) with a 50–60% increase in the photosynthetic rate (Ellsworth 1999). We tested the hypothesis that forest trees growing in elevated CO₂ will increase their C allocation to roots, in order to meet increasing nutrient demands. In addition, we address whether the effects of elevated CO₂ on root production and turnover could lead to greater soil C sequestration at high CO₂.

Materials and methods

The Free-Air CO₂ Enrichment (FACE) facility in the Duke Forest, which is located in the central Piedmont region of North Carolina (35°97'N 79°09'W), is a 16-y-old loblolly pine (*Pinus taeda*) forest, planted in 1983 after the harvest of similar vegetation. The density of pine trees is 1733 stems ha⁻¹, which represents more than 98% of the total basal area. Various deciduous species have invaded the site and a few have reached the canopy. The most significant invaders are *Liquidambar styraciflua* (620 stems ha⁻¹), *Ulmus alata* (226 stems ha⁻¹) and *Acer rubrum* (207 stems ha⁻¹). Soils are Ultic Alfisols of the Enon Series and are low in available nitrogen and phosphorus. Soils are seasonally poorly drained, with a bulk density of 1.07 g cm⁻³ at 0–20 cm depth and pH of 4.7–5.0. The site is remarkably homogeneous with respect to soil properties, and fine roots are found mostly in the upper 20 cm of the soil profile (Fig. 1).

The FACE experiment consists of six experimental plots, each 30 m × 30 m size, which are surrounded by an array of vertical vent pipes that extend to the top of the forest canopy to deliver CO₂ to treatment plots. Three

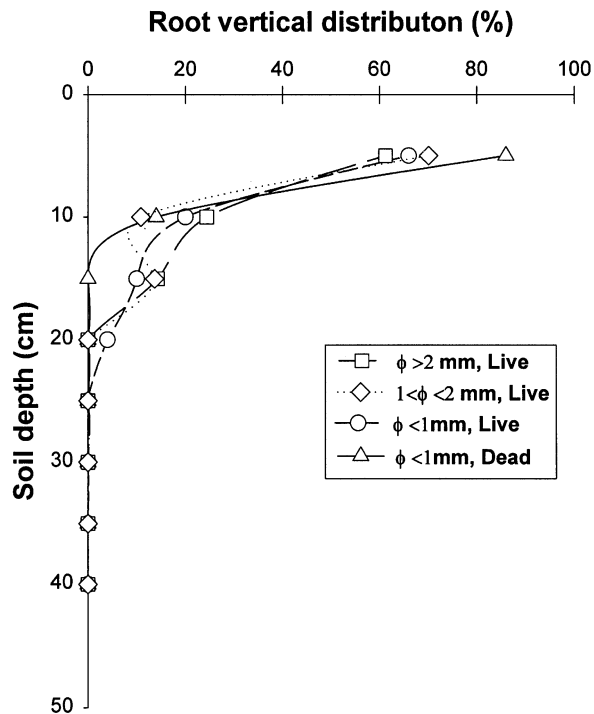


Fig. 1 Vertical distribution of fine roots collected during May 1997 in a loblolly pine plantation near the FACE site. Soil was sampled using a metal coring device of 5-cm diameter and to a depth of 50 cm. Soil cores were separated into 5-cm intervals starting from the surface soil. Roots from these intervals were sorted by diameter size into three groups: (i) $\phi \leq 1$ mm; (ii) $1 < \phi < 2$ mm; and (iii) $\phi \geq 2$ mm. Each group was sorted into live and dead roots. The x -axis shows the percentage of mass of each root group found within each depth interval with respect to the total mass of that particular group.

plots, designated as 'controls', are fully instrumented, but they receive ambient air only. Three experimental plots receive a continuous supplement of CO₂ to maintain an atmospheric concentration 200 $\mu\text{L L}^{-1}$ above ambient. Fumigation began on 27 August 1996, and the elevated CO₂ plots were fumigated for 81% and 79% of 1997 and 1998, respectively. The fumigation of the plots stops when temperature drops below 5 °C and when the wind speed is higher than 5 m s^{-1} for more than 5 min. Most of the nontreatment periods were at night due to low temperatures from mid December through mid March. Details of the FACE enrichment system can be found in Hendrey *et al.* (1999).

Seasonal measurement of fine roots

Below-ground biomass was collected in both FACE treatment and control plots at bimonthly intervals between June 1997 and November 1998. On each date five soil cores (5 cm diameter, 20 cm deep) were

randomly removed from each plot with a soil corer with slide-hammer. Soil collections were carried out over a three-day period; one pair of plots (one ambient and one elevated CO₂) was sampled each day, so that root activity could be measured immediately. Samples were stored on ice and immediately transported to the laboratory, where they were stored at 4 °C until processed. Roots were separated carefully from soil by hand, and sorted into three different diameter groups: (i) $\phi \leq 1$ mm, (ii) $1 < \phi < 2$ mm, and (iii) $\phi \geq 2$ mm. Roots from different tree species were not separated. Live and dead roots were distinguished based on the colour of the vascular tissue, strength and flexibility. Roots were considered alive if they showed some tensile strength and had white or yellow vascular tissue. Sorted roots were rinsed with water, dried at 65 °C for four days, and weighed. Mean root biomass per plot was calculated for live and dead root diameter groups at each sampling time. At each sampling time, roots from the same plot were pooled and the total carbon and nitrogen content of the live fine root tissues was determined by combustion of a ground subsample in an elemental analyser (Carlo Erba NA 1500).

Specific root area and specific root length were measured in June 1997 and July 1998. In both cases, live fine roots ($\phi \leq 1$ mm, LFR) that were picked from the soil cores were spread on a transparent film and photocopied. The photocopy was then scanned and digitized using an image-analysis system. The number of black pixels (b) was linearly correlated to the area (A) and length (L) of the wires used to calibrate the software ($A = 0.0085 + 0.0013b$, $r^2 = 0.998$ and $L = 0.1194 + 0.042b$, $r^2 = 0.996$). The regressions were then applied to calculate the area and length of the roots.

Root net primary production (RNPP) was calculated for the fine roots, ≤ 1 mm diameter, for a one-year interval, starting in November 1997 and ending in November 1998, in each experimental plot and then averaged by CO₂ treatment ($n = 3$). To estimate fine root production, we used a modification of the Santantonio & Grace (1987) compartment flow method because there was no seasonal pattern in the growth of fine roots:

$$\text{RNPP} = I + T,$$

where I is the fine root increment calculated from the regression of LFR biomass vs. time determined by sequential soil coring for each experimental plot over the period of November 1997 to November 1998; and T is the root turnover rate calculated as the sum of mortality plus decomposition for each experimental plot for the same period. Mortality is the net change in the pool of dead roots ($\phi \leq 1$ mm diameter, DFR) over the time interval. Decomposition is the sum of the estimated DFR

that decomposed during each sampling interval from November 1997 to November 1998.

To calculate annual decomposition, two root decomposition studies were carried out, one during the winter of 1997–98 and another during the summer of 1998. For the winter decomposition experiment, LFR from the sequential harvest collection of June 1997 were composited by plot, and 5–6 oven-dried subsamples of approximately 0.16 g were packed into 5×10 cm 0.2-mm mesh litterbags. Litterbags were placed in the plot from which the roots were collected. Litterbags were buried in the top 10 cm of the soil profile by inserting them in the ground in September 1997; they were collected after six months in the field (March 1998). Samples were rinsed with water and oven-dried for three days at 65 °C. Dry mass and ash content were measured. For the summer decomposition experiment, LFR from the sequential collection of March 1998 were placed in the field in May 1998 as previously and collected in November 1998. We estimated monthly decomposition rates by estimating monthly decay coefficients from monthly averages of soil temperature at 15-cm depth. Winter and summer decay coefficients were used to estimate the contribution of decomposed roots to total root NPP following Santantonio & Grace (1987) (Table 3).

Root activity

Samples of approximately 0.1 g of fresh mass of LFR were used for measurements of root respiration. Root respiration measurements were performed at room temperature within 6 h after each soil core was collected. Preliminary experiments showed that rates of root respiration were stable for at least 8 h after a soil core was collected. Fine roots were rinsed with water and kept in 10 mM TES buffer mixed in 0.2 mM CaCl_2 (pH 7.0) for 15 min to avoid wounding respiration effects. Root respiration rates were measured using a Clark-type oxygen electrode (Rank Brothers, Cambridge, UK), as oxygen consumption in an air-saturated buffer contained in the closed cuvette. Root samples were then oven-dried at 65 °C until constant mass was obtained. Root respiration rates were expressed in $\text{nmolO}_2 \text{g}^{-1} \text{s}^{-1}$, which were transformed to $\mu\text{gC g}^{-1} \text{s}^{-1}$ using 1.25 as the respiratory quotient (Penning de Vries *et al.* 1974; Poorter *et al.* 1991). Annual root respiration was calculated as the sum of the specific root respiration multiplied by the averaged root biomass per unit area in control and fumigated plots starting November 1997 and ending November 1998.

During most soil collections, approximately 0.1 g of fresh mass of LFR and 1 g of fresh mass soil were used to measure root and soil phosphatase activity. Both measurements were performed on the day the soil was collected. Phosphatase activity was measured by the

hydrolysis of p-nitrophenyl phosphatase (p-NPP) to p-nitrophenol (p-NP) after an incubation of roots and soil for 1 h at 37 °C (Alef *et al.* 1995). The root samples were oven-dried at 65 °C. A subsample of fresh soil was oven-dried at 70 °C for 3 days to obtain the soil fresh/dry mass ratio. Enzyme activity was expressed as the $\mu\text{mol p-NP g}^{-1} \text{min}^{-1}$ root or soil dry mass.

Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (SAS) program. Annual fine root increment per plot was estimated from the rate of biomass increment (slope of the regression) multiplied by time (12 months). We used a Student's *t*-test to determine statistical differences among the slopes of the regressions obtained in ambient and elevated CO_2 plots. Because the root densities for random sequential cores within each ring cannot be assumed to be independent, we used a Student's *t*-test on paired samples to evaluate whether the biomass changes between two dates were significantly different from zero for the calculation of fine root mortality. Residuals were tested for normality to verify the assumption of the *t*-test. RNPP was calculated as the sum of annual increment and turnover per plot, and a *t*-test was used to test for the significant differences among CO_2 treatments. We used a multivariate analysis of repeated measures to analyse the effect of CO_2 enrichment over time on dead fine root biomass, root respiration and root and soil phosphatase activity. We used a one-way anova to test for the significant differences among elevated and ambient CO_2 treatments on root respiration and root and soil phosphatase activity on individual dates. Also, simple linear regressions were used to test for relationships among soil moisture, soil temperature, root biomass, root respiration and root and soil phosphatase activity.

Results

Fine root dynamics

Roots larger than 1 mm diameter contributed 67% of the total root dry mass in the soil (Fig. 2a,b). Roots, $\phi \geq 2$ mm and $1 < \phi < 2$ mm, were not distributed uniformly in the soil, which resulted in a substantial variability in estimates of root biomass [the coefficient of variations (CV) were 41% and 30%, respectively]. Large roots, $\phi \geq 2$ mm, showed no biomass trend over time, whereas medium roots, $1 < \phi < 2$ mm, decreased significantly under both elevated and ambient CO_2 levels during the course of the experiment (Fig. 2a,b).

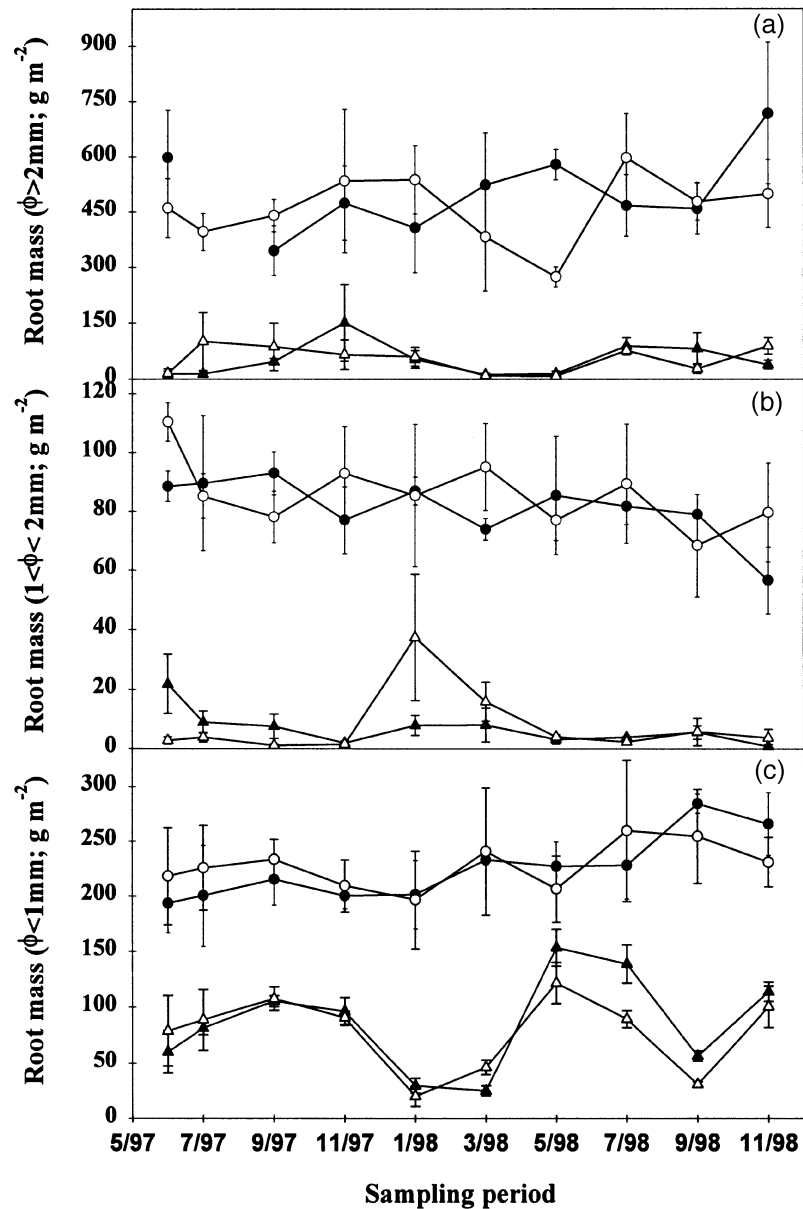


Fig. 2 Fine root biomass of live roots (circles) and dead roots (triangles) collected in a 16-y-old loblolly pine plantation located in the Duke forest under ambient (clear symbols) and elevated CO₂ (ambient + 200 ppm CO₂) (dark symbols). Roots were collected by-monthly from June 1997 to November 1998 to a depth of 20 cm. Roots were sorted by diameter size into three groups: (a) $\phi \geq 2$ mm (b) $1 < \phi < 2$ mm and (c) $\phi \leq 1$ mm, and each group was sorted into live and dead roots.

Fine roots, $\phi \leq 1$ mm, live and dead, represented 33% of the total root mass. Spatial variation in the biomass of roots $\phi \leq 1$ mm diameter was much smaller than for larger roots; the CV was typically 17% (Fig. 2c). LFR mass showed little seasonal variation despite the large range of soil temperature and moisture that occurred during the study (Fig. 3). LFR mass did not vary consistently over time in the ambient CO₂ treatment, in which the average LFR mass was 233 ± 7 g m⁻² (Fig. 2c). The increment in LFR was 42 and 80 gDW m⁻² y⁻¹ in the ambient and elevated CO₂ treatments, respectively (Table 1). This accounted for a significant increase of 37.8 g m⁻² (86%) in the treatment plots vs. the controls during two years of CO₂ fumigation ($P = 0.017$; Table 1). DFR showed a seasonal variation that was marginally negatively corre-

lated to soil moisture ($P = 0.056$) and positively correlated to soil temperature ($P = 0.070$). DFR exhibited minimum values during the winter and early spring, and biomass peaked during late spring and summer. There was no change in the DFR dynamics due to elevated CO₂ over time ($P = 0.28$), but elevated CO₂ resulted in an accumulation of DFR mass in May, July and September 1998, that was statistically greater than the DFR mass in control plots in July ($P = 0.03$) (Fig. 2c). Annual mortality, as the sum of mortality and decomposition, was not different between treatments (Table 1).

Fine roots lost 10% and 22% of their initial mass during six months of field decomposition in the winter and in the summer, respectively (Table 2). Fine roots collected in June 1997 and used in the winter decomposition study

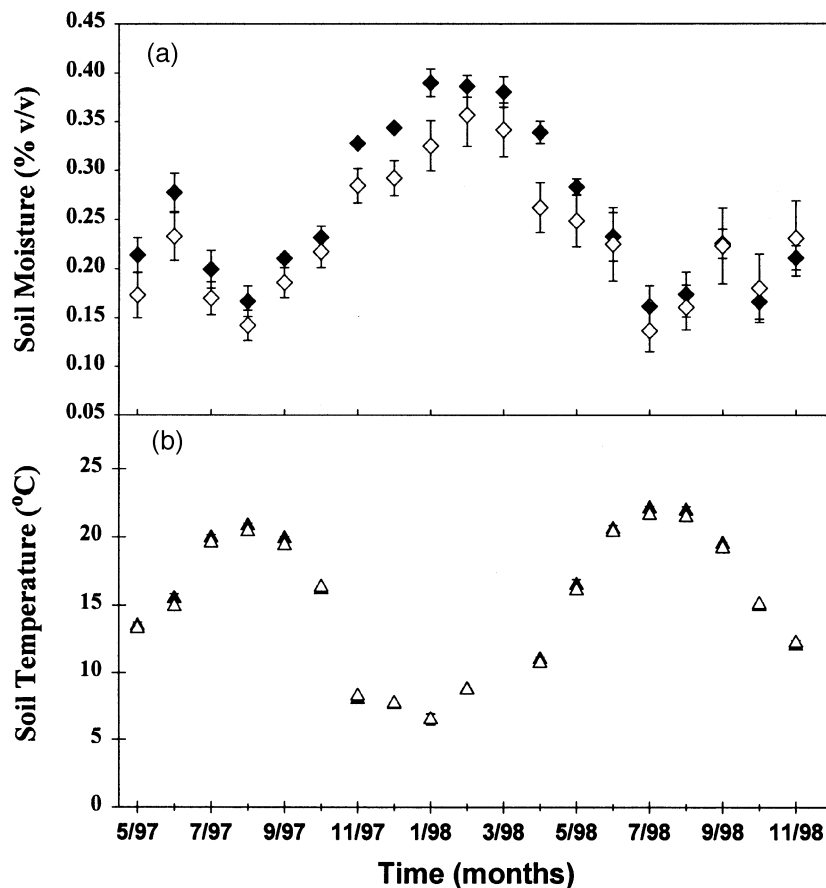


Fig. 3 (a) Soil moisture and (b) soil temperature. Soil moisture was measured using a TDR Campbell Sci Model CS615, with four sensors in each plot of the FACE site. Values are mean and SE of the percentage volumetric soil moisture content integrated within the top 30 cm of the soil profile and averaged by CO₂ treatment (courtesy of K. Schäfer and R. Oren <http://www.env.duke.edu>). Soil temperature was measured at 15 cm of the soil profile using a permanently installed thermocouple wires in all plots values are means and standard errors averaged by CO₂ treatment (data from Andrews 1999).

	Ambient [gDW m ⁻² y ⁻¹]	Elevated [gDW m ⁻² y ⁻¹]	Ambient (g C m ⁻² y ⁻¹)	Elevated (g C m ⁻² y ⁻¹)
C inputs				
Root increment	42.8 ± 7.5	80.0 ± 5.7*	17.6 ± 3.1	32.2 ± 2.7*
Root turnover:				
Mortality	10.2 ± 15.1	17.9 ± 20.9	3.8 ± 5.5	6.6 ± 8.4
Decomposition	26.8 ± 2.0	36.3 ± 0.9*	9.8 ± 0.9	14.1 ± 1.0*
Total Root NPP	79.8 ± 21.8	134.2 ± 26.9	31.2 ± 8.1	52.9 ± 10.9
C losses				
Root respiration			349.4 ± 33.4	401.0 ± 59.1

Root turnover estimates are revised over preliminary data given by DeLucia *et al.* (1999) and Allen *et al.* (2000).

* significant differences at $P < 0.05$

had lower initial C/N ratios than roots collected in March 1998 and used in the summer study (Table 2). The decay coefficient for the winter decomposition study was much smaller than the coefficient for the summer months despite the higher C/N ratio of root tissues decomposing during the summer (Table 2). Elevated CO₂ did not affect

C/N ratio of the fine roots or their decomposition during either period (Table 2). Because root decomposition was not affected by CO₂ treatment, we used a single set of monthly decay coefficients to estimate annual decomposition for the ambient and elevated CO₂ plots following the Santantonio & Grace (1987) model (Table 3).

Table 2 Results from winter (September 1997 to March 1998) and summer (May 1998 through November 1998) root decomposition experiments. LFR, of less than 1 mm in diameter, derived from trees grown in control and elevated CO₂ treatments. Decomposition of LFR was carried out in the same treatment site from which the roots were originally harvested. Values are means ± SE of 3 replicates

	Winter		Summer	
	Ambient	Elevated	Ambient	Elevated
Initial root C/N	47.69 ± 2.07	48.19 ± 2.81	55.63 ± 5.54	59.21 ± 7.84
Mass remaining (%)	92.52 ± 4.26	88.16 ± 2.25	76.86 ± 2.46	79.87 ± 5.16
Decay coefficient (months)	0.024 ± 0.000	0.024 ± 0.004	0.049 ± 0.008	0.048 ± 0.008

Table 3 Estimated monthly decay coefficients that were used to calculate monthly decomposition. Monthly decay coefficients (K_j) were estimated by using Santantonio & Grace (1987) equations: $K_j = K_{ref} \exp\{[(\ln k_1 - \ln k_2)/(T_1 - T_2)] * (T_j - T_{ref})\}$, where k_1 is the summer decay coefficient (Table 1), T_1 is the mid summer temperature (19.8 °C), k_2 is the winter decay coefficient (Table 1), T_2 is the mid winter temperature (11.1 °C) and summer decay and temperature were used as References.

Month	K_j
November 97	0.019
January 98	0.017
March 98	0.020
May 98	0.036
July 98	0.057
September 98	0.047
November 98	0.026

Annual decomposition was significantly greater [9.5 gDW m⁻² or 35.4%] in the elevated CO₂ treatments compared to the ambient plots ($P = 0.014$, Table 1).

Root net primary production, calculated as the sum of root increment, mortality and decomposition, was 80 and 134 gDW m⁻² y⁻¹ in the ambient and elevated CO₂ treatment, respectively (Table 1). Thus root NPP was 54 gDW m⁻² (68%) greater in the elevated CO₂ treatments compared to the ambient plots. This increase was not statistically significant ($P = 0.191$; Table 1). The average C concentration of LFR and DFR was 40.7 ± 0.5 and 37.6 ± 1.1, respectively, and no differences due to the CO₂ treatment were found. Annual carbon inputs were calculated from RNPP by multiplying the LFR mass increment by the LFR C concentration and by multiplying DFR by their tissue C concentration for each experimental plot. Annual carbon inputs were 21.7 gC m⁻² y⁻¹ higher, but not statistically significant, in the elevated CO₂ treatment ($P = 0.185$, Table 1).

Specific root length and surface area of the LFR were similar during the period studied, and the root area index was slightly higher in July 1998 under both the elevated CO₂ treatment and controls (Table 4). There were no statistically significant effects of elevated CO₂

on specific root length, root surface area, or root area index.

Root respiration

Specific root respiration showed a seasonal trend that was marginally correlated to soil temperature ($P = 0.078$) and soil moisture ($P = 0.084$), with lower respiration rates during the winter and higher respiration rates during summer (Fig. 4). Elevated CO₂ did not alter the specific root respiration over time ($P = 0.78$), but the respiration rate of roots from fumigated plots was marginally higher by 21% and 13% in September 1997 ($P = 0.060$) and May 1998 ($P = 0.067$), respectively. Respiration was 41% greater in the elevated compared to ambient CO₂ treatment in November 1998, but this increase was not statistically significant ($P = 0.18$) (Fig. 4). Annual carbon losses due to fine root respiration were estimated to be 51.6 g m⁻² y⁻¹ higher in the elevated CO₂ treatment compared to the control plots; however, this effect was also not statistically significant (Table 1).

Root and soil phosphatase activity

Root phosphatase activity was correlated with soil moisture ($P = 0.024$) and soil temperature ($P = 0.047$), but soil phosphatase activity was not. There was no effect of CO₂ treatment on the activity of the root and soil phosphatases over time ($P = 0.51$ and $P = 0.41$, respectively). Root and soil phosphatase activity were marginally significantly reduced by 33% and 32%, respectively, in elevated CO₂ in July 1997 ($P = 0.064$ and $P = 0.072$, respectively, Fig. 5). Root phosphatase was again reduced by 34% in May 1998 in the elevated CO₂ treatment, but this difference was not statistically significant (Fig. 5).

Discussion

Our data indicate that plant growth at elevated concentrations of atmospheric CO₂ increased annual increment of live root biomass and the annual decomposition of dead roots (Table 1). There was a

	June 1997		July 1998	
	Ambient	Elevated	Ambient	Elevated
SRL (m g^{-1})	12.73 ± 1.22	12.5 ± 0.38	12.17 ± 0.95	11.14 ± 0.64
SRA ($\text{m}^2 \text{kg}^{-1}$)	18.51 ± 1.26	20.11 ± 1.20	18.87 ± 0.54	17.89 ± 1.02
RAI ($\text{m}^2 \text{m}^{-2}$)	3.72 ± 0.34	3.92 ± 0.72	5.044 ± 1.35	4.15 ± 0.77

Table 4 Specific root length (SRL), surface area (SRA) and root area index (RAI) of LFR, 1 mm diameter, derived from Loblolly pine trees grown in ambient and elevated CO_2 treatments in the FACE site. LFR were collected after 10 (June 1997) and 23 (July 1998) months of CO_2 enrichment to a depth of 20 cm. Values are means \pm SE of 3 replicates

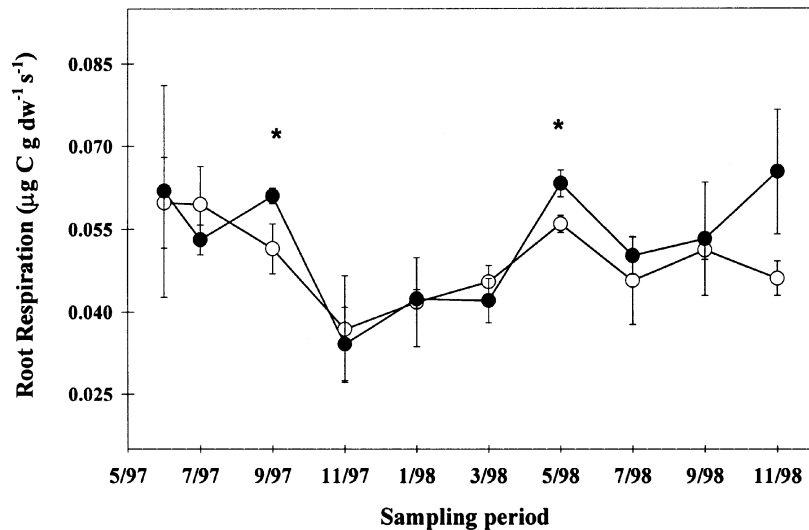


Fig. 4 LFR respiration ($\phi \leq 1 \text{ mm}$) was measured at each root collection from June 1997 to November 1998 in the ambient (clear symbols) and elevated CO_2 (ambient + 200 ppm CO_2) (dark symbols) treatments. Values are means and SE of 3 replicates. * significant differences at $P < 0.1$.

Table 5 Percentage effect of elevated vs. ambient CO_2 $\{[(E-A)/A] \times 100\}$ on several parameters measured in the FACE site over the period of 1997 and 1998.

Parameter	% CO_2 effect		Reference
	1997	1998	
Photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	50–60*	160 ^a	Ellsworth (1999); D.S. Ellsworth, submitted
[N] Green leaves (mg g^{-1}) ^b	-4	-18	Finzi <i>et al.</i> (2000)
Litterfall ($\text{g m}^{-2} \text{y}^{-1}$)	1	21*	DeLucia <i>et al.</i> (1999)
NPP ($\text{g m}^{-2} \text{y}^{-1}$)	16	25*	DeLucia <i>et al.</i> (1999)
Soil C flux ($\text{gC m}^{-2} \text{y}^{-1}$)	11	27*	Andrews (1999)

^a for summer measures; ^bcalculated from Fig. 2; * significant differences.

trend toward higher RNPP, although not significant, in this loblolly pine forest during two years of CO_2 enrichment. LFR mass increased in the elevated CO_2 treatment, and the increment was 37 g m^{-2} (86%) greater than in the control plots after two years of CO_2 fumigation. This is the first time such an effect has been shown for adult trees growing in the field at high CO_2 , and this result is consistent with previous findings of increased fine root density for tree

seedlings growing at high CO_2 in open-top chambers (OTC) or greenhouses.

During the past decade, studies in open-top chambers have shown that tree seedlings exposed to elevated CO_2 grow faster. Their ability to sustain greater growth rates depended on their ability to take up nutrients and, in general, root density, root mass, and/or root length were also enhanced in elevated CO_2 (Norby 1994; Rogers *et al.* 1994). Fewer studies have examined root turnover,

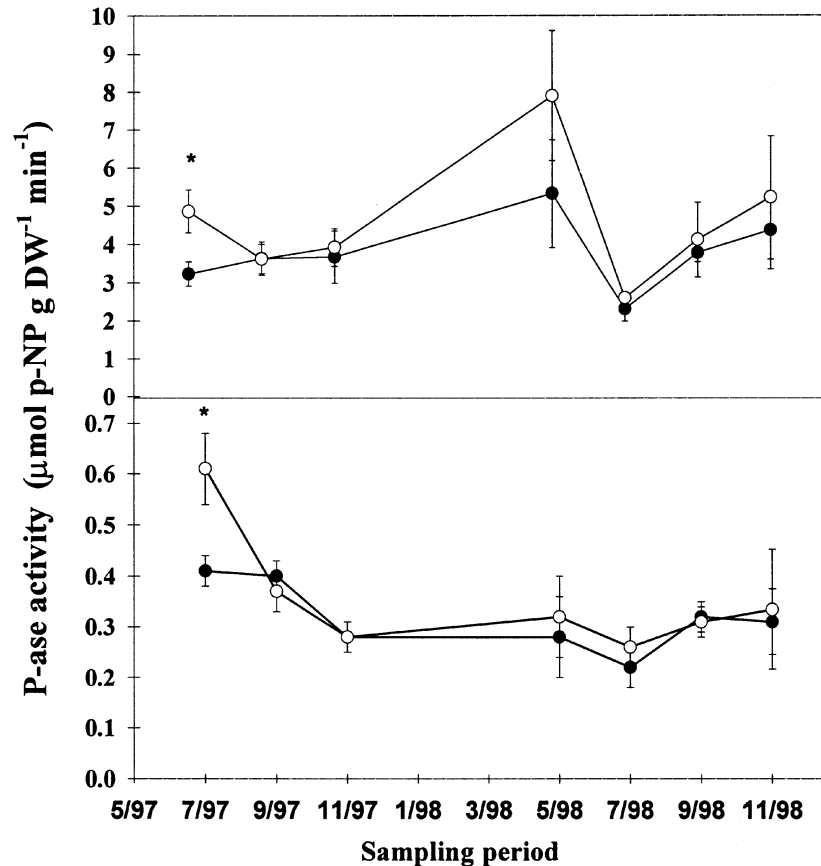


Fig. 5 LFR ($\phi \leq 1$ mm) and soil phosphatase activity was measured at several root collections between June 1997 and November 1998 in the ambient (clear symbols) and elevated CO₂ (ambient + 200 ppm CO₂) (dark symbols) treatments. Values are means and SE of 3 replicates. * significant differences at $P < 0.1$.

respiration or exudation rates and those that did show no consistent changes among tree species exposed to high CO₂ (Berntson & Bazzaz 1997; Crookshanks *et al.* 1998; Janssens *et al.* 1998; Thomas *et al.* 1999; Johnson *et al.* 2000). Our expectations that forest ecosystems will accumulate CO₂ from the atmosphere and help alleviate the expected global warming of the planet come from these studies. However, tree seedlings differ from adult trees (total mass, RGR, LAI, root structure, volume of soil explored), so it is uncertain whether mature forests will respond to elevated CO₂. One of the first responses of this 16-y-old loblolly pine forest exposed to Free-Air CO₂ Enrichment is to increase fine root production which contributes to the overall increase in net primary productivity observed in the elevated CO₂ treatment (DeLucia *et al.* 1999). Increased root production in elevated CO₂ accompanied the stimulation of photosynthesis (Ellsworth 1999) (Table 5). Higher RNPP in this forest allows greater soil exploration and nutrient and water uptake to meet the resource demands of the trees.

DFR root mass began to accumulate at the beginning of the second season of fumigation (Fig. 2c) as a result of greater root production. This accumulation ceased during late summer and disappeared by November

1998. An increase in the annual decomposition, due to greater DFR mass rather than changes in specific decomposition rates, accompanied this disappearance. Despite this, there was no clear effect of elevated CO₂ on root turnover, which showed a nonsignificant 46% increase in the elevated compared to the ambient CO₂ treatment. Increased turnover of fine roots has been shown in some grasslands (Fitter *et al.* 1997), where the half-life of root cohorts in peat soil was significantly shorter under elevated compared to ambient CO₂. Similarly, elevated CO₂ increased root turnover in seedlings of *Liriodendron tulipifera* (Norby *et al.* 1992), *Pinus radiata* (Thomas *et al.* 1999), *Populus euramericana* (Pregitzer *et al.* 1995) and *Betula papyrifera* (Berntson & Bazzaz 1996). It seems likely that a change in root turnover is largely a function of the increase in root production (Berntson & Bazzaz 1996) and of a greater root activity in plants grown at elevated CO₂.

Root turnover accounts for an important part of the carbon flowing to the roots, and root turnover is thought to be one of the most important mechanisms of carbon transfer to the soil (Van Veen *et al.* 1991; Canadell *et al.* 1996). Hungate *et al.* (1997) showed that elevated CO₂ enhanced carbon partitioning to roots, but root exudation

and root respiration accounted for most of the increase, releasing large amounts of CO₂ to the atmosphere and leading to a small accumulation of carbon in the soil. We have quantified belowground carbon fluxes in order to address whether growth at elevated CO₂ would lead to greater soil C sequestration in this pine forest ecosystem. Our collaborators have measured a 248 gC m⁻² y⁻¹ increase in the CO₂ efflux from the soil surface in the elevated CO₂ treatment during 1998 (DeLucia *et al.* 1999; Andrews 1999). Our estimates of root respiration explain 52 gC m⁻² y⁻¹ or 20% of the extra soil CO₂ efflux in the elevated plots. The remainder is likely to be due to higher heterotrophic respiration associated with an increase in root turnover, increased aboveground litterfall (DeLucia *et al.* 1999; Finzi *et al.* 2000), increased root exudation in the elevated CO₂ treatment (Cheng 1999), and increased mycorrhizal colonization of roots (O'Neill *et al.* 1987; Runion *et al.* 1997). Greater carbon allocation to labile carbon pools in the soil appears to increase carbon cycling, rather than carbon storage in this ecosystem. For example, the increase in root production in the elevated CO₂ treatment, accounts for only 21.7 gC m⁻² y⁻¹, which is substantially less than the differences in carbon losses by respiration.

Our estimates of RNPP must be accepted with some caution for several reasons. First, dead root mass was potentially overestimated, as live roots may have been mistaken for dead roots because of a loss of tensile strength during extended droughts periods in the summer. Secondly, annual estimates of decomposition (Table 2) were comparatively low and may have affected our estimates of annual decomposition. For example King *et al.* (1997) reported decay coefficients of 0.009–0.0016 per day for loblolly pine roots of 0–5 mm in diameter measured over the period of two years in North Carolina. Overall, our annual root production estimates fall in the lower range reported for temperate and boreal forests. The reported range of annual fine root production for pine forests is substantial, from 69 to 1090 g m⁻² y⁻¹, with a mean of 331 (± 55) g m⁻² y⁻¹ (calculated from Nadelhoffer & Raich 1992), which is higher than our estimates of 80 (± 22) and 134 (± 27) g m⁻² y⁻¹ for ambient and elevated CO₂, respectively. Much of the differences observed in previous studies may be accounted for by methodological differences, the age of the forests, and the size classes of root studied (Gholz *et al.* 1986; Nadelhoffer & Raich 1992). Our RNPP estimates are lower than estimates of annual fine root production ($\phi \leq 1$ mm) obtained in a 9-y-old slash pine forest in north Florida (Gholz *et al.* 1986), where estimated root production was 264 g m⁻² y⁻¹ and root turnover rate was 259 g m⁻² y⁻¹. Our root turnover rates are also lower than estimates obtained in a 33-y-old loblolly pine plantation in South Carolina (63 gC m⁻² y⁻¹)

(Richter *et al.* 1999). Our estimates of RNPP show that the Duke forest has moderate belowground productivity and turnover. The mean residence time of fine roots is 3 years, calculated by dividing the mean standing biomass by the annual net production in the ambient treatment, most probably due to poor soil resources (Adams & Allen 1985; Schoettle & Fahey 1994).

Root surface area is particularly important for the acquisition of P and other macronutrients with low mobility in soils. In our study, the length and surface area of the roots were not changed by the CO₂ treatment; although increases in specific root respiration and fine root production in elevated CO₂ may have enhanced total nutrient uptake by the loblolly pine trees. During the first two years of CO₂ fumigation, we saw no change of the N concentration in the leaves (Finzi *et al.* 2000) or fine roots in the high CO₂ treatment.

We measured phosphatase activity of roots and soil as an index of changes in the nutrient demands of loblolly pine trees grown at high CO₂. Conroy *et al.* (1988) showed an increase in the growth of *Pinus radiata* seedlings growing at high CO₂ only when phosphorus nutrition was adequate. At our study site, soils are inherently phosphorus-poor, and mineralization takes place in the near-surface of the mineral soil, where plant roots, soil microorganisms and P-absorbing minerals compete strongly for PO₄³⁻ available in solution (Walbridge *et al.* 1991; Gallardo & Schlesinger 1994). Most P available for biogeochemical cycling is found in organic compounds (Schlesinger 1997), which must be hydrolysed to inorganic P by phosphatases, released from roots and microorganisms. In July 1997, we found a marginal reduction in the root phosphatase activity and lower phosphatase activity in the bulk soil in the elevated CO₂ treatment compared to the ambient plots. This response was not observed again for the remainder of our study. DeLucia *et al.* (1997) found decreased root phosphatase activity and increased root exudation of oxalic acid in the rhizosphere of ponderosa pine seedlings growing at high CO₂. Organic acids, such as oxalate, can increase the solubility of P and Al in soils and may influence nutrient availability (Fox & Comerford 1992). Oxalate mobilizes soluble P in the soil solution by chelation and precipitation of Fe and Ca (Graustein *et al.* 1977). DeLucia *et al.* (1997) suggest that exudation of organic acids is a mechanism that pines trees use to solubilize P and increase P uptake under elevated CO₂. It is also possible that mycorrhizae may have increased the rate of P uptake, although this was not investigated in this study.

Our data show that the early response of a loblolly pine forest ecosystem subject to CO₂ enrichment is an increase in its fine root population and a trend towards higher total RNPP after two years of CO₂ fumigation.

Our data also provide support for the hypothesis that elevated atmospheric CO₂ will lead to increased below-ground carbon allocation. The allocation of the extra carbon, particularly to increases in root production and respiration rates, suggests that the CO₂ enrichment would most probably increase the flux of C through the ecosystem without causing a large accumulation of C in the soil.

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