

Fine root dynamics in a loblolly pine forest are influenced by free-air-CO₂-enrichment: a six-year-minirhizotron study

SETH G. PRITCHARD*, ALLAN E. STRAND*, M. LUKE McCORMACK*, MICHEAL A. DAVIS†, ADRIEN C. FINZI‡, ROBERT B. JACKSON§, ROSER MATAMALA¶, HUGO H. ROGERS|| and RAM OREN§

*Department of Biology, College of Charleston, Charleston, SC 29401, USA, †Department of Biology, University of Southern Mississippi, Hattiesburg, MS 39406-5018, USA, ‡Biology Department, Boston University, Boston, MA 02215, USA, §Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA, ¶Argonne National Laboratory, Argonne, IL 60439, USA, ||USDA-ARS National Soil Dynamics Laboratory, 411 S. Donahue Drive, Auburn, AL 36832, USA

Abstract

Efforts to characterize carbon (C) cycling among atmosphere, forest canopy, and soil C pools are hindered by poorly quantified fine root dynamics. We characterized the influence of free-air-CO₂-enrichment (ambient +200 ppm) on fine roots for a period of 6 years (Autumn 1998 through Autumn 2004) in an 18-year-old loblolly pine (*Pinus taeda*) plantation near Durham, NC, USA using minirhizotrons. Root production and mortality were synchronous processes that peaked most years during spring and early summer. Seasonality of fine root production and mortality was not influenced by atmospheric CO₂ availability. Averaged over all 6 years of the study, CO₂ enrichment increased average fine root standing crop (+23%), annual root length production (+25%), and annual root length mortality (+36%). Larger increase in mortality compared with production with CO₂ enrichment is explained by shorter average fine root lifespans in elevated plots (500 days) compared with controls (574 days). The effects of CO₂-enrichment on fine root proliferation tended to shift from shallow (0–15 cm) to deeper soil depths (15–30) with increasing duration of the study. Diameters of fine roots were initially increased by CO₂-enrichment but this effect diminished over time. Averaged over 6 years, annual fine root NPP was estimated to be 163 g dw m⁻² yr⁻¹ in CO₂-enriched plots and 130 g dw m⁻² yr⁻¹ in control plots ($P = 0.13$) corresponding to an average annual additional input of fine root biomass to soil of 33 g m⁻² yr⁻¹ in CO₂-enriched plots. A lack of consistent CO₂ × year effects suggest that the positive effects of CO₂ enrichment on fine root growth persisted 6 years following minirhizotron tube installation (8 years following initiation of the CO₂ fumigation). Although CO₂-enrichment contributed to extra flow of C into soil in this experiment, the magnitude of the effect was small suggesting only modest potential for fine root processes to directly contribute to soil C storage in south-eastern pine forests.

Keywords: elevated carbon dioxide, fine roots, minirhizotrons, root turnover

Received 6 March 2007 and accepted 15 August 2007

Introduction

The influence that rising atmospheric [CO₂] is having on forests, including the exchange of energy and materials among soil, aboveground biomass, and the atmo-

sphere, is currently of great interest (Lal, 2005). Accelerating rates of anthropogenic CO₂ emissions into the atmosphere, and accompanying forcing effects on global climate, carbon (C) and nitrogen (N) cycles, and ecosystem function, portend even greater urgency for coming years.

Large-scale field experiments on intact forests are currently underway to test the hypothesis that forests

Correspondence: Seth G. Pritchard, tel. +1 843 953 5995, fax +1 843 953 5453, e-mail: pritchards@cofc.edu

will sequester C in a CO₂-enriched world, and that this will slow the rate of increase of atmospheric CO₂ (DeLucia *et al.*, 2005). In 1996, the free-air CO₂-enrichment (FACE) forest atmosphere carbon transfer and storage (FACTS-1) research facility was established in the Duke forest in central North Carolina, USA (Hendrey *et al.*, 1999). The primary goal of this experiment is to quantify the effect of elevated atmospheric CO₂ (ambient +200 ppm) on canopy C assimilation, the transfer of C among ecosystem C pools, and the potential for C storage in above and belowground C reservoirs.

So far, the stimulation of net primary productivity (NPP) by CO₂-enrichment at Duke FACE has persisted after more than 8 years amid speculation that nutrient limitations will eventually constrain a positive CO₂ response (Oren *et al.*, 2001; Körner 2003; Luo *et al.*, 2004a, b; Finzi *et al.*, 2006; Johnson, 2006). Over the period 1996 through 2002 NPP was 18–24% higher in CO₂-enriched plots compared with controls resulting in an additional soil C sink of 52 g m⁻² yr⁻¹ (Lichter *et al.*, 2005; Finzi *et al.*, 2006). The accumulation of additional C in the CO₂-enriched plots was mostly confined to the organic horizon of the soil profile, a response driven by enhanced litter production under elevated CO₂. The potential contribution of fine root production and mortality to soil C sequestration, on the other hand, is not well understood (Matamala & Schlesinger, 2000; Pritchard *et al.*, 2001a, b; Lichter *et al.*, 2005).

The stimulatory effect of elevated atmospheric [CO₂] on fine roots of individual trees growing in pots is well established. In many instances, root growth has been shown to exhibit a larger proportional increase compared with shoot growth in plants subjected to elevated atmospheric CO₂ concentrations (Rogers *et al.*, 1994, 1996; Pritchard & Rogers, 2000). Tingey *et al.* (2000) reviewed available literature on conifer seedlings grown in greenhouses and field chambers and found that CO₂-enrichment stimulated fine root growth from –3% to 145% (median increase of 54%). Fine root density increased 135% in *Pinus sylvestris* grown with CO₂ enrichment (Janssens *et al.*, 1998) and increases of 95–240% in fine root production were reported for *Quercus petraea*, *P. sylvestris*, and *Fraxinus excelsior* (Crookshanks *et al.*, 1998). Effects of CO₂ enrichment on fine roots within intact ecosystems dominated by older, more mature plants, on the other hand, have rarely been characterized. In a study on a 15-year-old FACE grown sweetgum forest, annual production of fine roots more than doubled, an effect that explained a large proportion of the 22% increase in NPP observed in CO₂-enriched plots (550 ppm) (Norby *et al.*, 2004). Similarly, allocation of biomass to belowground structures increased in FACE grown poplar saplings resulting in a

35–84% increase in fine root standing biomass (Lukac *et al.*, 2003).

Large stimulatory effect of elevated atmospheric CO₂ on fine root production and turnover has important implications for forest C and N cycling. It is often assumed that small diameter (fine) roots are quantitatively most important for mediating C flow into soils because of their short lifespans and rapid decomposition rates (Guo *et al.*, 2004); in some forests, the rate of passage of C and nutrients through fine root pools (i.e. nonwoody roots <1 mm diameter) is several orders of magnitude higher than through aboveground tissues (Ruess *et al.*, 2003). In fact, the proportion of photosynthate allocated to support fine root construction could account for 30–50% of total global terrestrial primary productivity (Caldwell, 1977; Jackson *et al.*, 1997; Ruess *et al.*, 2003).

The possibility of sequestering a meaningful proportion of anthropogenic CO₂ emissions in soil is promising because of the size of the soil C pool. Globally, soils store 3.3 times more C than the atmosphere, and 4.0 times more C than the aboveground terrestrial biomass pool (Lal, 1999; Fitter, 2005). Although the rate of accretion of soil organic matter is generally slow, even in aggrading forests (Lichter *et al.*, 2005), fine root turnover may account for >65% of C transferred into mineral soils (Richter *et al.*, 1999). Therefore, understanding the effects of CO₂ enrichment on fine root dynamics at the ecosystem level is critical for understanding forest NPP and the potential of forest soils to sequester atmospheric C over long time periods (Luo, 2003; Matamala *et al.*, 2003; Norby *et al.*, 2004).

In addition to directly influencing flow of C into soil, stimulation of fine roots by CO₂-enrichment may enhance C storage in the canopy and litter layer via effects on nutrient acquisition. The progressive nitrogen limitation (PNL) hypothesis predicts that long-term stimulation of forest productivity by rising atmospheric CO₂ concentrations will be limited by ecosystem N availability (Oren *et al.*, 2001; Hungate *et al.*, 2003; Körner, 2003; Luo *et al.*, 2004a, b; Finzi *et al.*, 2006). But stimulation of fine roots by elevated CO₂ could increase root exploitation of deep soil N reserves (Jobbágy & Jackson, 2001), or transient N-rich microsites (Hodge, 2006), thereby postponing or alleviating nutrient limitations and prolonging a positive response to rising atmospheric CO₂ concentrations (Schäfer *et al.*, 2003; Luo *et al.*, 2004a, b; Johnson, 2006; van Groenigen *et al.*, 2006). The observation that CO₂ enrichment leads to greater accumulation of N in forest biomass even under N-limited conditions lends support to this idea (Johnson, 2006). In the Duke forest FACE experiment, Finzi *et al.* (2006) recently reported that CO₂-enriched plots

have accumulated additional N (relative to ambient plots) that cannot be accounted for by N-deposition and heterotrophic N fixation and suggested that more efficient uptake of N by fine roots and mycorrhizae may explain the origin of the extra N (see also Zak *et al.*, 2000). Although its generality in forests is unknown, other CO₂-enrichment experiments on trees have indicated a stimulation of root exploration into deeper soils suggesting root exploration of larger soil volumes (Pritchard *et al.*, 2001b; Norby *et al.*, 2004; Johnson *et al.*, 2006).

We attempted to address the knowledge gap regarding spatial and temporal patterns of fine root growth in the Duke FACE experiment by employing minirhizotrons to quantify fine root length standing crop, production, and mortality for 6 years (1998–2004). Currently, minirhizotrons represent the most direct and, arguably, the most accurate method for characterizing fine root dynamics, especially when production and mortality are synchronous processes (Johnson *et al.*, 2001; Higgins *et al.*, 2002; Hendricks *et al.*, 2005; Majdi *et al.*, 2005).

Methods

Study site description

In 1996, the FACTS-1 FACE experiment was established in a 15-year-old loblolly pine plantation in the Duke Forest in Orange County, NC, USA. The forest was established from 3-year-old loblolly pine seedlings planted in 1983 at a 2.4 m × 2.4 m spacing. Since then, several species of deciduous trees have become established including red maple (*Acer rubrum*), winged elm (*Ulmus alata*), sweetgum (*Liquidambar styraciflua*), tulip poplar (*Liriodendron tulipifera*), and redbud (*Cercis canadensis*). Density of loblolly pine trees, however, represents more than 98% of the total basal area in this forest. The soil series is Enon loam (fine, mixed, thermic Ultic Hapludalfs).

This experiment has been described many times (e.g. Matamala & Schlesinger, 2000) and will not be described in detail here. Briefly, the FACTS-1 experiment is a randomized block design with three replications of each of two CO₂ concentrations maintained with FACE technology (Hendrey *et al.*, 1999). CO₂-enriched plots are maintained at approximately 200 ppm above ambient atmospheric CO₂ levels (average concentration of enriched plots was 567 ppm from 1996 through 2004; Taneva *et al.*, 2006). Each of the six experimental plots is 30 m in diameter. Beginning August, 1996, fumigation was constant except when temperature dropped below 5 °C or the wind speed was higher than 5 m s⁻¹ for more

than 5 min. In 2003 fumigation was limited to daylight hours only.

Minirhizotron analysis of root dynamics

The methods of fine root analyses for this experiment have been described previously (Pritchard *et al.*, 2001a). Briefly, a total of 72 minirhizotrons (12 per plot) were installed into each of the six FACE rings June 23–26, 1998. Minirhizotrons are clear plastic tubes (OD = 56 mm) that allow repeated, noninvasive measurement of root growth. These clear tubes were installed at an angle of 45° from vertical to a vertical depth of ~30 cm. FACE rings were divided equally into four sectors; three tubes were installed at random with respect to vegetation into each of the four sectors. The portion of the minirhizotron tube extending above the ground was covered with a closed cell polyethylene sleeve, and the end was sealed with a rubber cap to exclude light and minimize heat exchange between the air and the tube. A PVC cap was then installed over the end to protect the rubber cap from UV damage, and to further protect and insulate the tube. In order to prevent minirhizotron tubes from moving, aluminum brackets were clamped to tubes and anchored into the ground with 40 cm stainless-steel rods. No data were collected for the first 5 months after tube installation to allow time for roots to colonize the tube surface, and to allow soil adjacent to minirhizotrons to equilibrate with bulk soil.

From 22 October 1998 through October 2004, a BTC-100x microvideo camera (Bartz Technologies, Santa Barbara, CA, USA) was used to record images of roots growing along the soil/minirhizotron tube interface. Roots were imaged approximately monthly during the 6-year-period (61 sessions). The camera was equipped with an indexing handle that allowed precise and consistent camera placement (Johnson & Meyer, 1998) enabling us to track the fate of individual roots through time. Video images of roots were replayed in the laboratory and 16 frames for each tube (total of 67 access tubes remained functional for the duration of the study) were digitized (a total of >65 000 images). Images were named using the format favored by ICP-Lab (Bartz Technologies) in order to facilitate animation of a given frame through time. Animations were played for each of the 16 locations for each minirhizotron using ICP-Lab to ensure that files were named correctly and that excessive image shift did not occur.

Data were extracted from digital images using Roo-Tracker software (Dave Tremmel, Duke University, Durham, NC, USA). At each date, root diameters, total length of live roots, new root length production, and

root length mortality were recorded. Roots were considered dead when they either disappeared from view, or upon their structural disintegration. In some cases, nonfunctional roots may have been classified as alive as long as they remained present and intact, and thus errors would be overestimations of fine root longevity, standing crop and mortality rate.

A yearly root turnover index was calculated as annual root production divided by average annual fine root standing crop. Calculating turnover index as annual mortality divided by average annual root standing crop produced similar results since mortality and production were nearly equivalent during each of the 6 years of study. Production and mortality (in $\text{g dw m}^{-2} \text{yr}^{-1}$) were estimated by determining the proportion of average annual root length standing crop that was produced during a given year (with minirhizotrons) and then multiplying this value by the average annual standing crop ($\text{g dw m}^{-2} \text{yr}^{-1}$) for similar-sized roots ($\leq 2.0 \text{ mm}$) quantified with soil cores by R Matamala (1999; unpublished data) and R. Jackson (2003 and 2004; unpublished data) (Majdi & Andersson, 2005). Because no coring was done during 2000, 2001, and 2002, root production and mortality for these years were estimated by regressing biomass production and mortality for 1999, 2003, and 2004 with corresponding annual root length production/mortality measurements for those years. After the relationship between biomass production/mortality and length production/mortality was determined, the resulting equation was used to estimate biomass production/mortality from root length production for years in which no soil core data were available. We were unable to convert root length measurements to biomass from specific root length (SRL) values, a method advocated by other investigators (e.g., Tingey *et al.*, 2000) because SRL data were available for 1998 only and minirhizotron analyses indicated that effects of CO_2 -enrichment on diameters of fine roots (and therefore SRL) were not consistent throughout the study (see Fig. 5b).

Environmental data

Details of soil temperature and volumetric soil moisture measurements are described elsewhere (Palmroth *et al.*, 2005). Briefly, volumetric soil moisture content was measured with four frequency domain reflectometry probes installed in the top 30 cm of mineral soil in each of the six plots. Soil moisture data, therefore, represent the mean of 12 probes for both elevated and ambient treatments. Soil temperature was measured with a single sensor in each plot situated at a 10–15 cm soil depth. Both soil moisture and temperature data were collected twice per minute and half-hour averages were

stored. Averaged soil moisture and temperature values corresponding to minirhizotron sampling intervals were then calculated for comparison with monthly fine root production and mortality values.

Statistical analyses

The experimental design was a split block design with three replications (three elevated plots and three ambient plots). A block consisted of two plots to which ambient and elevated CO_2 treatments were assigned. Each ring contained 12 subsample minirhizotron access tubes. Minirhizotron frames were grouped into two depth classes, 0–15 (frames 1–8) and 15–30 (frames 9–16) cm. Statistical analyses were done using PROC MIXED in SAS [SAS Institute (1999) SAS/Stat User's Guide, Version 8 SAS Institute Inc., Cary, NC]. Repeated measures ANOVA was used to test for effects of CO_2 (two levels), depth (two levels) and year (6 years), and their interactions on yearly fine root production, mortality, NPP, standing crop, and root diameter. In all cases, plot means were used in statistical analyses.

In order to characterize and compare root longevity, we used the Kaplan–Meier test for survival analyses and significance of treatment effects were determined from a Cox proportional hazards model. Model fitting was performed with the 'survival' package implemented in R (R Development Core, 2004).

Because of variability inherent to minirhizotron studies, statistical trends were designated when $0.05 < P < 0.15$ and significant differences when $P < 0.05$.

Results

Root length standing crop

There was a trend ($P = 0.15$) suggesting an increase in fine root length standing crop in FACE plots compared with controls (Table 1; Fig. 1a and b). Averaged over all 6 years of the study, CO_2 -enriched plots maintained 23% more fine root length than control plots. We observed a sharp increase in root length density in the year following the onset of data collection (i.e. 1999) in the 0–15 cm soil horizon. Subsequently, standing crop (length) in shallow soil diminished until May 2003 when it then began to increase again. Standing crop of roots in the 15–30 depth also increased following initiation of image collection until May 2000 and then began to decline. Shifts in root length density from shallow to deeper soils over the course of the study are reflected by a significant horizon \times year interaction ($P = 0.0001$; Fig. 1c). Although we found no significant $\text{CO}_2 \times$ horizon effect, the modest (and insig-

Table 1 Summary of repeated measures analyses of variance for effects of year, CO₂ (elevated vs. ambient), and soil horizon (0–15 vs. 15–30 cm) on fine root (length) cumulative yearly production, cumulative yearly mortality, average yearly standing crop, and annual turnover index

Source	Production	Mortality	Standing crop	Diameters
CO ₂	0.02	0.04	0.15	0.53
Horizon	0.0001	0.0001	0.001	0.42
Year	0.0001	0.0001	0.0001	0.0001
CO ₂ × year	0.16	0.10	0.73	0.0001
Horizon × year	0.0013	0.0001	0.0001	0.24
CO ₂ × horizon	0.88	0.31	0.93	0.66
CO ₂ × horizon × year	0.97	0.28	0.86	0.60

	Yearly length production			Yearly length mortality		
	eCO ₂	aCO ₂	% change	eCO ₂	aCO ₂	% change
1999	22.1	14.5	+52	21.0	12.8	+64
2000	8.9	7.7	+16	13.7	11.1	+24
2001	6.3	4.8	+30	6.4	5.9	+08
2002	4.2	3.3	+26	5.4	3.7	+45
2003	7.2	5.7	+27	3.6	4.1	-12
2004	5.4	5.7	-04	4.6	2.7	+70

Below are means of yearly length production and yearly length mortality for ambient and CO₂-enriched plots. *P*-values in bold signify statistically significant results.

nificant) stimulation of fine root length standing crop by CO₂ enrichment appeared to shift from shallow to deeper soil with increasing duration of the experiment (Fig. 1c).

Fine root production and mortality

Fine root production and mortality appeared to follow seasonal patterns (Fig. 2a and b). In most years, largest peaks in production occurred during late spring or early summer as soil temperatures increased and soils began to dry out (Fig. 2c and d). Seasonal patterns of mortality were similar to those observed for production suggesting synchronous production and mortality. We were unable to detect a significant relationship between monthly production or mortality and average soil temperature or soil moisture over those same sampling intervals because of significant month to month and year to year variability (data not shown). Yearly production of fine root length was stimulated 25% in CO₂-enriched plots compared with controls (Table 1; *P* = 0.02). No significant CO₂ × year interaction was observed for root length production; CO₂ enrichment increased yearly length production by 52%, 16%, 30%, 26%, and 27% in 1999, 2000, 2001, 2002, and 2003, respectively (Fig. 3). In 2004, root length production was virtually identical in elevated and ambient plots.

Yearly cumulative root length mortality was 36% greater in CO₂-enriched compared with control plots averaged over the entire 6-year-study (*P* = 0.04; Table 1; Fig. 2b and Fig. 3). The effect of CO₂ enrichment on cumulative yearly mortality varied considerably from year to year (Table 1; Fig. 3). Production per year and mortality per year were linearly related illustrating the synchronous nature of production and mortality and also that many fine roots produced in a given year also died that same year (data not shown).

Over the course of the experiment, total production and mortality were nearly balanced in both ambient plots (production and mortality were 6.95 and 6.73 m root length m⁻² of minirhizotron viewing area per year, respectively) and elevated plots (production and mortality were 9.01 and 9.13 m root length m⁻² of minirhizotron viewing area per year). Exposure to CO₂-enriched air did not influence root turnover index (cumulative yearly root length production/average yearly standing crop) (*P* = 0.58; Table 2). Turnover index in CO₂-enriched plots was 0.61 compared with 0.58 in ambient plots. Moreover, expressing relative turnover as cumulative yearly root mortality/average yearly standing crop also yielded statistically insignificant results (data not shown); relative production turnover index and mortality turnover index were related in a positive linear fashion (data not shown). Although we found no significant effect on

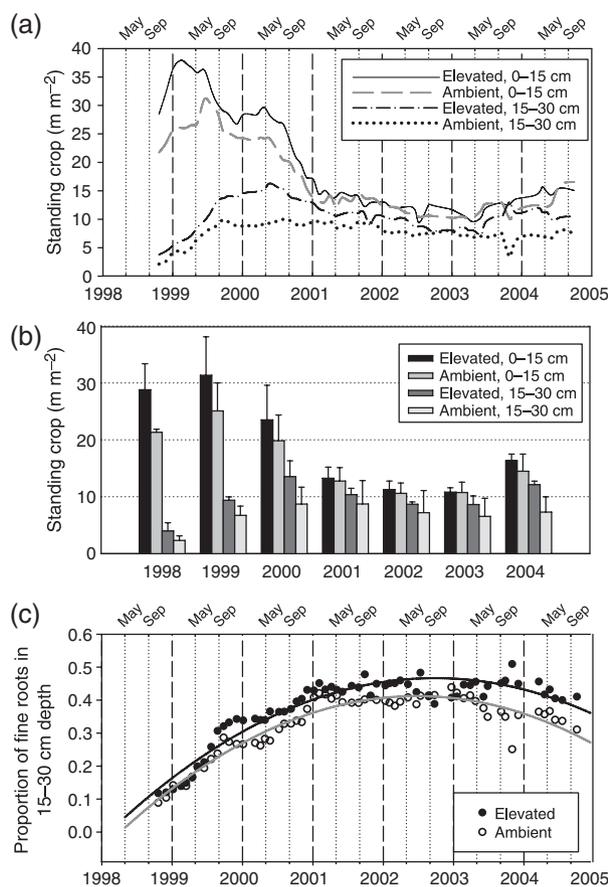


Fig. 1 (a) Approximately monthly fine root length standing crop from November 1998 through October 2004 quantified with minirhizotrons for 0–15 and 15–30 cm soil depths for ambient and elevated (ambient +200 ppm) experimental plots, (b) average yearly fine root length standing crop for 0–15 and 15–30 cm depths, and (c) proportion of total root length (0–30 cm) present at the 15–30 cm soil depth.

annual root turnover using this method, survivorship analyses of individual roots showed that fine roots in CO₂-enriched plots had shorter mean lifespans compared with fine roots in control plots (500 compared with 574 days; S. G. Pritchard *et al.*, unpublished result).

Fine root diameter

Average diameter of fine roots viewed with minirhizotrons in this study was 0.55 mm (0.52 mm in ambient and 0.56 mm in elevated plots) and most roots were <0.75 mm in diameter (Fig. 4). Diameter of fine roots varied neither with depth nor year. No significant main effect of CO₂ treatment was found ($P = 0.53$; Table 1). A significant CO₂ × year interaction, however, was observed, ($P = 0.0001$; Fig. 5a and b). Diameters of roots visible in minirhizotron images were 16% larger during

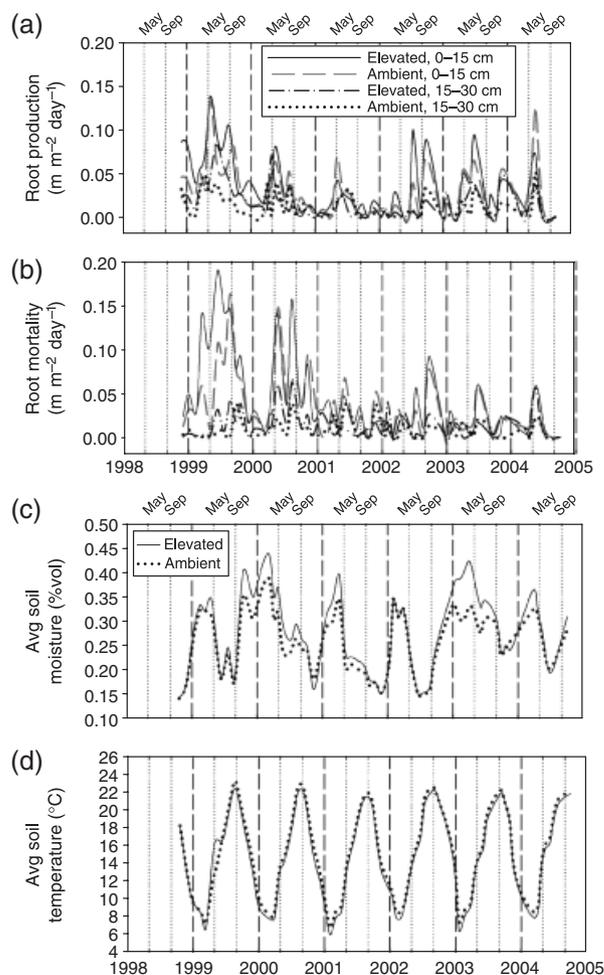


Fig. 2 Root production (a) and mortality (b) during 61 sampling intervals from November 1998 through October 2004 for 0–15 and 15–30 cm soil depths for CO₂-enriched (ambient +200 ppm) and ambient forest plots. Average soil moisture (c) and average soil temperature (d) for ambient and CO₂-enriched plots. Volumetric soil moisture content data represent means of 12 frequency domain reflectometry probes installed in the top 30 cm of mineral soil for elevated and control plots. Soil temperature was measured with a single sensor in each plot situated at a 10–15 cm soil depth.

the first year of the study but the stimulatory effect of the CO₂ treatment disappeared over time ($P = 0.0001$; $R^2 = 0.90$; Fig. 5b).

Estimations of fine root NPP

Fine root NPP (biomass production) averaged over the 6-year-study was 130 g dw m⁻² yr⁻¹ in control plots compared with 163 g dw m⁻² yr⁻¹ in CO₂-enriched plots (not significant; Table 2). Fine root mortality per year averaged 126 and 152 g dw m⁻² yr⁻¹ in ambient and elevated plots, respectively ($P = 0.30$). Fine root produc-

tion and mortality varied significantly from year to year ($P = 0.0001$; Fig. 6) but no $\text{CO}_2 \times \text{year}$ interaction was observed. In general, fine root NPP was positively related to the amount of rainfall over the period of active stem growth and was negatively related to average ambient air temperatures over this same period

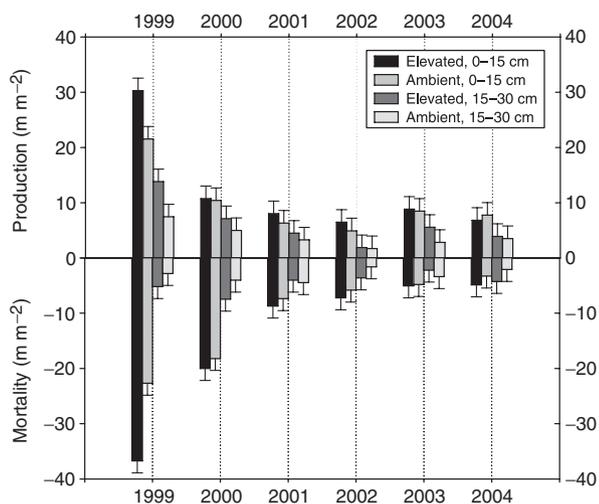


Fig. 3 Cumulative yearly fine root length production for 0–15 and 15–30 cm soil depths for 1999 through 2004. The last sampling period for 2004 was in October so production and mortality for November and December are not included in those means.

(rainfall data and temperature data from Moore *et al.*, 2006) (Fig. 6b and c).

Discussion

Our data on fine root responses to FACE suggest a more modest effect of elevated CO_2 than has been reported previously for seedlings exposed to elevated atmospheric CO_2 in open top field chambers (OTCs) and also for larger trees exposed to FACE. Although effects on fine roots were modest, the effect was consistent over the entire duration of the 6-year-study. Furthermore, the current study also suggests that stimulatory effects of atmospheric CO_2 enrichment on fine roots shifted to deeper soil through time.

Fine root length standing crop

Atmospheric CO_2 enrichment increased standing crop of roots by 23% averaged over the period from October 1998 through October 2004. R. Jackson *et al.* (unpublished result) found that FACE resulted in a 19% increase in fine root biomass from 2002 through 2005 in this experiment using destructive soil coring. The increase in standing root crop observed here mirrors the 23% increase in ecosystem NPP reported for four forest FACE experiments implemented across a broad range of productivity (Norby *et al.*, 2005). It is also consistent

Table 2 Summary of repeated measures analyses of variance for effects of year and CO_2 on fine root NPP (production in $\text{g dw m}^{-2} \text{yr}^{-1}$), mortality ($\text{g dw m}^{-2} \text{yr}^{-1}$) and yearly turnover index (year^{-1})

Source	P value		
	Production	Mortality	Turnover index
CO_2	0.13	0.30	0.58
Year	0.0001	0.0001	0.0001
$\text{CO}_2 \times \text{year}$	0.89	0.83	0.94

	Mean		
	Production	Mortality	Turnover index
1999	249.3	239.8	0.98
2000	130.6	194.1	0.56
2001	86.9	96.1	0.47
2002	59.0	74.5	0.43
2003	233.2	144.0	0.72
2004	119.5	88.2	0.42

Fine root NPP and mortality values were derived from turnover indexes derived from minirhizotrons and fine root standing biomass determined from soil cores provided by R. Matamala (unpublished data from 1999) and R. Jackson (unpublished data from 2003 and 2004). Data for production and mortality per year for elevated and ambient treatments are presented in Fig. 4.

P-values indicate statistically significant results.

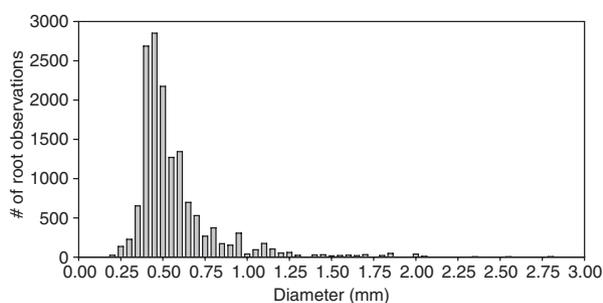


Fig. 4 Distribution of fine root diameter measurements over the 6-year observation period (1998–2004).

with the 18–24% increase in NPP reported for this study for the period 1997–2002 (Finzi *et al.*, 2006). The increase in fine root standing crop resulting from CO₂ enrichment was, however, substantially lower than has been reported previously for other forest species. For instance, Norby *et al.* (2004) found that FACE grown sweetgum (550 ppm vs. ambient CO₂) maintained more than twice as many roots during summer months. In a 5-year study of root demography in a shortgrass steppe ecosystem, root length standing crop was 41% greater in CO₂ enriched compared with control plots (Milchunas *et al.*, 2005). On the other hand, exposure of Douglas fir to CO₂ enrichment had no effect on fine root standing crop, production, or mortality in a 3-year-minirhizotron study conducted in field chambers (Johnson *et al.*, 2006). FACE tended to decrease root density in a Mojave Desert community (Phillips *et al.*, 2006). In other studies, atmospheric CO₂-enrichment increased fine root density 135% in small *P. sylvestris* trees (Janssens *et al.*, 1998) and increases in fine root production of 95–240% were observed in *Quercus petraea*, *P. sylvestris*, and *F. excelsior* (Crookshanks *et al.*, 1998). Elevated atmospheric CO₂ did not affect fine root production, mortality, or standing crop of *Pseudotsuga menziesii*, however (Johnson *et al.*, 2006). Exposure to elevated CO₂ tended to decrease fine root production in a scrub-oak 7 years after treatment initiation (Brown *et al.*, 2007). The magnitude of CO₂ effects on fine roots apparently varies with ecosystem type, exposure duration and/or with soil conditions.

We observed no clear seasonal pattern in fine root standing crop in spite of seasonality of fine root production. This is explained by the fact that production and mortality occurred simultaneously which dampened seasonal fluctuations in standing crop. FACE did not influence seasonality of production, mortality, or fine root standing crop in our study. Similarly, based on soil cores, Matamala & Schlesinger (2000) observed no seasonal pattern in live fine root standing crop in this forest from 1997 to 1999.

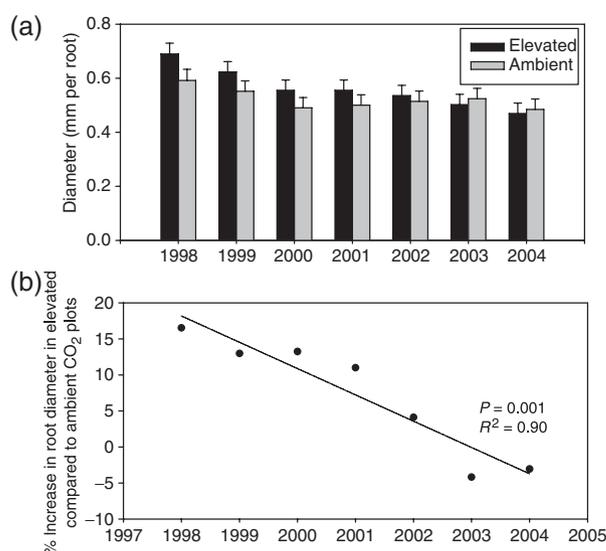


Fig. 5 Diameter of fine root in ambient and CO₂-enriched plots during years 1998 through 2004 (a); and (b) linear regression showing the decrease in the CO₂ effect on root diameter during the course of the study. *Significant difference at $P < 0.05$.

Finzi *et al.* (2006) recently reported for the Duke FACE study that CO₂-enriched plots are accruing ecosystem N at the rate of $12 \text{ g N m}^{-2} \text{ yr}^{-1}$. This rate far exceeds N-deposition ($\sim 1.4 \text{ g N m}^{-2} \text{ yr}^{-1}$) and heterotrophic N fixation ($\sim 1.4 \text{ g N m}^{-2} \text{ yr}^{-1}$) combined. Several investigators have suggested that N accrual that cannot be accounted for in existing N budgets might be explained by more thorough root exploration, particularly from sources deeper in soil as suggested here (Jobbágy & Jackson, 2001; Bernhardt *et al.*, 2006; Finzi *et al.*, 2006; Johnson, 2006). The apparent widening of the positive effect of CO₂ enrichment on root proliferation in deeper soil through time might also be contributing to recent widening of the CO₂ effect on soil air CO₂ concentration also observed at depths $> 15 \text{ cm}$ (Bernhardt *et al.*, 2006). The 23% increase in fine root standing crop resulting from CO₂-enrichment in this experiment is mirrored by a 24% increased in midday soil respiration reported for the same plots (Bernhardt *et al.*, 2006). Atmospheric CO₂ enrichment also stimulated production of mycorrhizae and rhizomorphs in deep soil (S. G. Pritchard *et al.*, unpublished result) which could further contribute to increased uptake of N from deeper soil. More thorough exploration of soil in space (deeper roots and more mycorrhizae) and time (intensification of foraging through faster turnover) may increase uptake of transient N 'hotspots' thereby facilitating sustained increase in NPP at the whole forest level and delaying PNL.

A consistent shift in root proliferation into deeper soils was also reported for FACE grown sweetgum

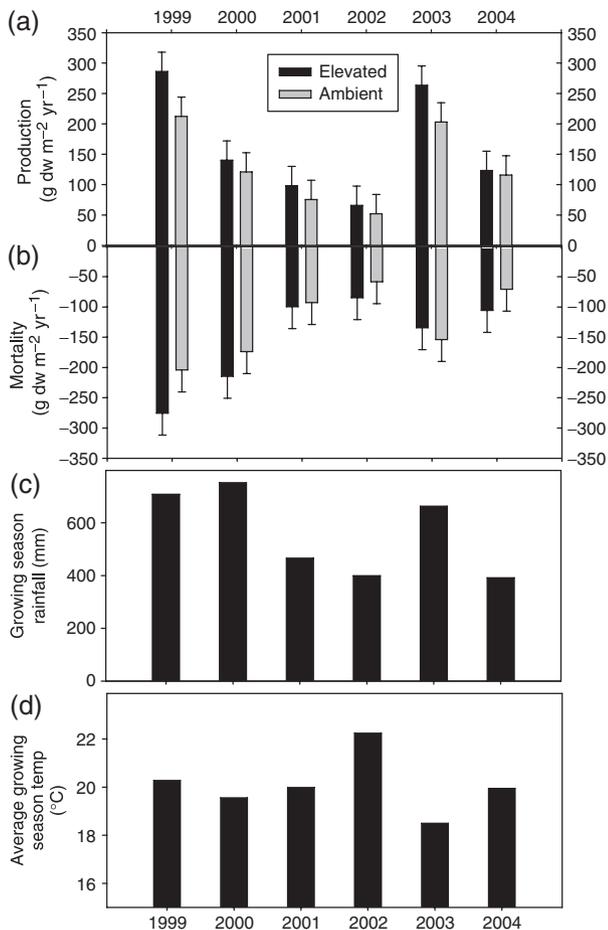


Fig. 6 Estimates of fine root NPP (yearly production in $\text{g dw m}^{-2} \text{yr}^{-1}$) (a) and yearly mortality ($\text{g dw m}^{-2} \text{yr}^{-1}$) (b) for 1999 through 2004 (a). Production and mortality were estimated by determining the proportion of average annual root length standing crop that was produced during a given year (with minirhizotrons) and then multiplying this value by the average annual standing crop ($\text{g dw m}^{-2} \text{yr}^{-1}$) quantified with soil cores by Matamala (1999) and Jackson (2003 and 2004). Because no coring was done during 2000, 2001, and 2002, root production and mortality for these years were estimated by regressing biomass production and mortality for 1999, 2003, and 2004 with root length production for those years. After the relationship between biomass production/mortality and length production/mortality was determined, the resulting equation was used to estimate biomass production/mortality from root length production for years in which not soil core data were available. Total rainfall during the period of active tree growth (c) and average ambient air temperatures (d) during this same period were reported previously by Moore *et al.* (2006).

(Norby *et al.*, 2004). Elevated atmospheric CO_2 also preferentially increased fine root proliferation in deep soils in experiments conducted on oak palmetto scrub (Day *et al.*, 1996), a model longleaf pine ecosystem (Pritchard *et al.*, 2001b), and *Pinus radiata* (Thomas

et al., 1999) grown in OTC experiments. Others, however, have reported no clear effect of CO_2 enrichment on vertical root distribution patterns in FACE grown *Populus* (Lucac *et al.*, 2003) and root stimulation is commonly confined to shallow soil in annual herbaceous plants (Pritchard & Rogers, 2000; Pritchard *et al.*, 2006) and grasslands (Arnone *et al.*, 2000; Milchunas *et al.*, 2005). Stimulation of fine roots deeper in soil, as is suggested by our data, could lead to greater potential for soil C storage than equivalent increases in root growth in shallow soils because mass loss rates of litter decrease with increasing soil depth (Gill & Burke, 2002). But spatial patterns of greater root growth in high CO_2 at the ecosystem level (where CO_2 may have contrasting effects on trees vs. herbaceous plants), in addition to the role of fine root proliferation in delaying PNL needs to be resolved in order to accurately predict the capacity of various ecosystems to sequester atmospheric C. Accordingly, we installed minirhizotrons access tubes deeper into the soil profile in spring of 2005 in this experiment in order to resolve this issue and to determine if CO_2 enrichment is preferentially stimulating root growth at greater depths than we are currently able to sample with existing minirhizotron access tubes (from 0 to ~ 30 cm soil depth).

Seasonality of production and mortality

Our data suggest that root production and mortality were highest during the first 2 years of the study (1998–2000), decreased through 2002 and then rose in 2004. This might be linked to a wound response (compensatory fine root proliferation) triggered by tube installation in early summer of 1998 followed by a die-back of these roots. A significant CO_2 by year interaction for average diameter of fine roots also seems to support this idea. Other studies reported that root distribution along minirhizotron tubes did not fully equilibrate for 2 years or more (Ruess *et al.*, 2003). Phillips *et al.* (2006) observed that fine root production in a 2-year minirhizotron study in the Mojave Desert FACE study was rapid during the first 6 months following the initiation of image collection and then decreased thereafter as was also reported for a shortgrass steppe (Milchunas *et al.*, 2005).

In the present study, however, the large initial stimulation of fine root growth followed by a steady decline over several years cannot be dismissed as artifactual because of the correlation between fine root production, standing crop, and environmental conditions (rainfall and temperature). For instance, the decrease in fine root production beginning in 2000 was apparently linked to a sharp drop in leaf area index in this forest which began in 2001 caused by a drought in 2001–2002

(McCarthy *et al.*, 2006a) and was exacerbated by an ice storm in 2002 (McCarthy *et al.*, 2006b). It is also interesting to note that drought at the end of 2000 reduced late season root production (but not mortality; Fig. 2) resulting in a lower standing crop of roots during 2001. Droughts during 2001 and 2002 kept the standing crop low. At the end of 2002 there was a resurgence in late season production corresponding to the end of the drought. However, by this time, leaf area index (and presumably total assimilate supply) was low and fine root standing crop did not begin to recover until 2004 when leaf area index also recovered.

We found that few new roots were produced during winter when soil temperatures were lowest and soil moisture was most plentiful. Root production was generally highest during late spring and early summer. The peak in fine root production in spring and early summer is presumably linked to soil warming coupled with expansion and rapid photosynthesis in foliage. More modest periods of root production were observed during autumn in several years which might be linked to abscission of second year needles (King *et al.*, 2002). A similar pattern of fine root production with peaks in spring and fall was also observed in an 8-year-old loblolly pine plantation by King *et al.* (2002). Peak periods of root production in a model longleaf pine community were observed in late winter or early spring and again in late summer (Pritchard *et al.*, 2001a, b). It has been suggested that in climates prone to summer droughts, root production tends to be highest in spring and then declines in summer (Hendrick & Pregitzer, 1996; Joslin *et al.*, 2001). In cooler climates where water availability does not limit tree growth during the growing season, however, root production tends to be higher in later summer (Tyron & Chapin, 1983; Burke & Raynal, 1994). Stronger seasonal patterns of fine root production in which almost all production occurs in spring are common for northern forests (e.g. Burke & Raynal, 1994) but temporal patterns of fine root production are evidently more plastic in pines which do not lose their foliage during the winter (Konôpka *et al.*, 2005). It is likely that periods of rapid root production coincide with periods of peak carbohydrate availability. Production during these periods, however, is likely contingent upon suitable temperatures and adequate soil moisture (Hendrick & Pregitzer, 1996; Joslin *et al.*, 2001; Fukuzawa *et al.*, 2007). Year to year variation in fine root NPP was largely controlled by the amount of rainfall during the period of active stem growth and by average ambient air temperatures (see also Moore *et al.*, 2006).

We found no difference in phenology of fine root growth or mortality in CO₂ enriched compared with control plots. In light of the discussion above, this may

suggest that the effects of CO₂ enrichment on soil temperature and moisture content were insufficient to appreciably alter root growth patterns. This contradicts findings of Thomas *et al.* (1999) who found that fine root growth of *P. radiata* was initiated earlier in the season but is consistent with other results at Duke FACE which showed no effect of elevated CO₂ on timing of basal area tree growth or timing of either peak leaf area index or peak litterfall (Moore *et al.*, 2006, H. McCarthy, personal communication). Timing of root growth was also unaffected in *Pinus ponderosa* grown with CO₂ enrichment (Tingey *et al.*, 1996).

Mortality was generally highest during spring and early summer but periods of high mortality also occurred in autumn. Fine root mortality is generally highest in autumn after leaves abscise, at least for deciduous forest species (Hendrick & Pregitzer, 1992; Kern *et al.*, 2004; Ponti *et al.*, 2004). Lowest rates of fine root mortality were generally observed during winter when soil temperatures were lowest and soil moisture was most plentiful. Similarly, Matamala & Schlesinger (2000) also observed that dead root biomass was negatively correlated with soil moisture and positively correlated with soil temperature.

It is important to note that production and mortality usually occurred simultaneously as reported previously for loblolly pine (King *et al.*, 2002). These observations indicate that traditional biomass-based methods, often used to estimate fine root production and mortality, are invalid, at least for this forest type, because they assume asynchronous production and mortality (Nadelhoffer, 2000; King *et al.*, 2002; Hendricks *et al.*, 2005). Synchronous fine root production and mortality has also been reported for several other tree species casting further doubt on methods for estimating fine root NPP based on sequential soil coring (Hendrick & Pregitzer, 1992; Dilustro *et al.*, 2002; Carter *et al.*, 2004; Ponti *et al.*, 2004; Hendricks *et al.*, 2005).

Fine root diameter

The increase in fine root diameter observed in CO₂-enriched plots in 1998 and 1999 did not persist. Stimulation of root diameters resulting from CO₂ enrichment decreased in a linear fashion with increasing duration of the experiment (from 0.7 mm in 1998 roots down to <0.5 mm in 2004 roots). A similar observation was made in a 4-year-minirhizotron study on *P. ponderosa* in which fine root diameter was highest at the beginning of the study and then decreased through time (Tingey *et al.*, 2000). Generally, diameter of a fine root is maximal shortly after it is produced and then gradually decreases through time as some cortical cells shrink or are sloughed off (personal observation). This could

partially explain the drop in average fine root diameters found here and in previous minirhizotron studies (Tingey *et al.*, 1995). In the current study, however, it is also possible that the decrease in root diameters was attributable to dry years late in the study (2001, 2002, and 2004). Diameters of individual fine roots were also increased in grass roots exposed to CO₂ enrichment, especially in shallow soil (Milchunas *et al.*, 2005). This 5-year study on grasses also found a decrease in average root diameter with increasing duration of the experiment. They attributed this decline to drought years toward the end of the study. The literature suggests that diameter of tree roots is commonly increased following growth in CO₂-enriched environments. For instance, short-term studies on *Pinus taeda* (Larigauderie *et al.*, 1994; King *et al.*, 1997) found that SRL decreased in CO₂-enriched seedlings. In *P. sylvestris*, exposure to elevated CO₂ reduced SRL after 3 months of exposure but that effect disappeared by the sixth month (Crookshanks *et al.*, 1998). Median diameter of *P. ponderosa* fine roots on the other hand was not affected by atmospheric CO₂ enrichment (Tingey *et al.*, 2000). Although CO₂ enrichment sometimes increases fine root diameters, that effect sometimes diminishes with duration of study, as we found in the current study, and apparently varies among species. In those cases where atmospheric CO₂ enrichment increases mean or median fine root diameters, a decrease in absorptive capacity might be expected since diameter is negatively related to uptake potential (Eissenstat *et al.*, 2000). Larger diameter fine roots may also have a greater dependency on mycorrhizae (Eissenstat, 1992). This, along with greater C allocation to individual roots might combine to explain the large stimulation of mycorrhizal colonization often reported for trees growing in CO₂-enriched atmospheres (Treseder, 2004).

The diameter of roots observed here is consistent with other minirhizotron root studies. For example, Norby *et al.* (2004) found that 95% of fine roots sampled in FACE grown sweetgum had diameters <1.0 mm and 80% had diameters <0.5 mm. Similarly, 80% of 8-year-old loblolly pine fine roots sample with minirhizotrons had diameters <1.0 mm (King *et al.*, 2002). In the Mojave Desert FACE minirhizotron experiment, >90% of fine roots had a diameter of <0.3 mm (Phillips *et al.*, 2006).

Estimation of fine root NPP

The influence of environmental conditions on fine root NPP is rarely reported because of methodological difficulties (Zak *et al.*, 2000; Pritchard *et al.*, 2001a,b; Luo, 2003; Lal, 2005). Much of the confusion surrounding dynamic behavior of fine roots arises from the fact that

no two methods used to evaluate fine root demography yield the same results, even when sampling common experimental plots. This was effectively illustrated by Hendricks *et al.* (2005) when they quantified fine root production in longleaf pine-wiregrass forest ecosystems using minirhizotrons, soil cores, ingrowth cores, N-budget and C-budget techniques; they found that fine root production estimates ranged from 0 (with soil cores) to 4618 kg ha⁻¹ yr⁻¹ (with minirhizotrons). Based on their comparison of the leading methods for measuring fine root production and turnover, Hendricks *et al.* (2005) concluded that 'the minirhizotron technique yielded the most reliable root production estimates.'

Our estimations of fine root NPP were derived by multiplying the rate of fine root turnover (turnover yr⁻¹) by the average standing crop of fine roots for a given year obtained from destructive soil cores (from R. Matamala, 1999, unpublished core data and R. Jackson, 2003 and 2004, unpublished core data). We observed a consistent, albeit statistically insignificant, stimulation of fine root NPP in CO₂-enriched compared with control plots (163 and 130 g dw m⁻² yr⁻¹). Unfortunately, the current minirhizotron study represents the only effort to quantify fine root production and mortality in this study for the period 1999 through 2004. Matamala & Schlesinger (2000) estimated that fine root NPP, based on soil coring, during 1998 was 79.8 and 134.2 g m⁻² yr⁻¹ in ambient and FACE plots. This is below our 6-year average of 130 and 163 g m⁻² yr⁻¹ but is well within the range of interannual variability in fine root NPP reported here. Annual fine root productivity in this forest is on the lower end of the reported range of 69–1090 g m⁻² yr⁻¹ suggested for other pine forests (mean = 331 g m⁻² yr⁻¹; Nadelhoffer & Raich, 1992; Matamala & Schlesinger, 2000).

Implications for soil C storage

Consistent (but insignificant) effects of elevated CO₂ on root production and fast turnover times of fine roots in the soil, combine to suggest that fine root turnover may be contributing to C flow into soils of Southeastern pine forests. Averaged over the 6-year study, average annual fine root mortality was 36% greater in CO₂-enriched plots compared with controls. This increase in mortality (and thus C flow into soil) is partially explained by greater production (+25%) and shorter fine root lifespans (500 days in CO₂ enriched and 574 days in ambient plots). Past studies have reported inconsistent results regarding impacts of CO₂ enrichment on fine root longevity. For instance, in contrast to our results, Milchunas *et al.* (2005) reported relatively lower rates of mortality than production which they attributed to increased longevity of grass fine roots in CO₂ enriched

(750 days) compared with ambient plots (600 days). In other studies on pines, mean root lifespan was increased by CO₂-enrichment in *P. ponderosa* (102 vs. 74 days) (Johnson *et al.*, 2000) while the half life of fine roots of *P. radiata* ranged from 2.6 to 6.7 years in control plots compared with 0.9 to 1.3 years in CO₂-enriched plots (Thomas *et al.*, 1999). Currently, too few data exist to make any confident generalizations concerning the impact of elevated atmospheric CO₂ on fine root longevity (Arnone *et al.*, 2000; Tingey *et al.*, 2000). Although models have suggested that CO₂ enrichment is most likely to increase fine root longevity (Eissenstat *et al.*, 2000), that idea has not yet been borne out by empirical studies.

The rapid turnover of fine roots reported here does not agree with estimates of root C turnover of fine roots at Duke FACE of 4.2 years (Matamala *et al.*, 2003; but see Luo, 2003; Matamala *et al.*, 2004; Luo *et al.*, 2004a,b). Discrepancies between fine root longevity assessed with minirhizotrons vs. root C turnover assessed with isotopic methods have not been resolved although have been discussed elsewhere (e.g. Majdi *et al.*, 2005; Joslin *et al.*, 2006; Pritchard & Strand, 2008). Rapid turnover of a fraction of the fine roots reported here could explain the recent report that a substantial portion of soil respired C at the Duke FACE experiment is returned to the atmosphere within 1 year of being assimilated (Taneva *et al.*, 2006).

It is difficult to predict the extent to which the modest increases in fine root production observed here might contribute to C sequestration over very long time periods. The smallest, most ephemeral roots decompose rapidly because they have high N content, and a low lignin content relative to larger roots (Pregitzer *et al.*, 2002; Ruess *et al.*, 2003; Beedlow *et al.*, 2004; Guo *et al.*, 2004; Kalyn & Van Rees, 2006). It has also been suggested that production of vegetation is less important for formation of recalcitrant soil organic matter (i.e. humus), and therefore long-term C storage, than soil physical properties such as mineralogy (Trumbore, 1997; Beedlow *et al.*, 2004). Soil biotic conditions are also important because soil C transformations are controlled by soil microbes, and currently the long-term effects of atmospheric CO₂ enrichment on the soil food web remains largely unknown (Zak *et al.*, 2000). Longer running experiments will be required to determine if the lack of additional soil C sequestration observed in short-term CO₂-enrichment studies to date (Schlesinger & Lichter 2001; Heath *et al.*, 2005) will be reflected over longer time periods. In perhaps the longest running experiment on soil C accumulation in an aggrading forest, it was reported that 40 years after agricultural abandonment, a loblolly pine forest had accumulated 3780 g new C m⁻². But that new C resided mostly in

trees (80%) and the forest floor (20%) with <1.0% occurring in mineral soil (Richter *et al.*, 1999). Based on these findings, significant C sequestration, resulting from enhancements in fine root productivity, is unlikely.

In conclusion, the magnitude of stimulation of fine root and forest NPP by elevated CO₂ remained constant through the first 8 years of CO₂-enrichment. The effects of CO₂ enrichment on fine root production observed here, however, were modest compared with previous studies on large forest trees exposed to FACE (Norby *et al.*, 2004) and saplings/seedlings grown in OTCs. But even modest effects on finest root elements could have significant implications for long-term forest function and also for soil nutrient cycling because of their important functions. The finest, most distal populations of fine roots generally contain more N, respire faster, assimilate more soil resources, and represent better substrates for microbial decomposition compared to higher order, larger diameter roots (Pregitzer *et al.*, 1997, 1998, 2002). Furthermore, distal tips of fine roots are also the primary site for initiation of mycorrhizal partnerships which are critical for resource acquisition and could also influence whether or not forests can sustain higher productivity in a CO₂-enriched world.

Acknowledgements

I would like to thank Dave Moore, Evan DeLucia, and Heather McCarthy for sharing data and Steve Prior for help with minirhizotron installation. This work was supported by the Office of Science Biological and Environmental Research (BER), US Department of Energy, Grant numbers DE-FG02-95ER62083 and DE-FC02-06ER64156.

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