CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest

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Summary

• Greater fine-root production under elevated [CO_2] may increase the input of carbon (C) and nitrogen (N) to the soil profile because fine root populations turn over quickly in forested ecosystems.

• Here, the effect of elevated [CO_2] was assessed on root biomass and N inputs at several soil depths by combining a long-term minirhizotron dataset with continuous, root-specific measurements of root mass and [N]. The experiment was conducted in a CO_2-enriched sweetgum (*Liquidambar styraciflua*) plantation.

• CO_2 enrichment had no effect on root tissue density or [N] within a given diameter class. Root biomass production and standing crop were doubled under elevated [CO_2]. Though fine-root turnover declined under elevated [CO_2], fine-root mortality was also nearly doubled under CO_2 enrichment. Over 9 yr, root mortality resulted in 681 g m^{-2} of extra C and 9 g m^{-2} of extra N input to the soil system under elevated [CO_2]. At least half of these inputs were below 30 cm soil depth.

• Increased C and N input to the soil under CO_2 enrichment, especially below 30 cm depth, might alter soil C storage and N mineralization. Future research should focus on quantifying root decomposition dynamics and C and N mineralization deeper in the soil.

Key words: fine roots, root biomass, root diameter, root mortality, root N content, root turnover, soil C storage.

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Introduction

'Fine' roots (i.e. roots < 2 mm in diameter that are the most active in water and nutrient uptake, Pregitzer, 2002, 2003) comprise up to one-third of annual net primary production in terrestrial ecosystems (Jackson *et al.*, 1997). Further, the production of fine roots is expected to increase under elevated atmospheric [CO_2], especially in nitrogen (N)-limited forests where increased below-ground carbon (C) allocation may facilitate N acquisition (Zak *et al.*, 1993; Eissenstat *et al.*, 2000; Norby & Jackson, 2000; BassiriRad *et al.*, 2001). Fine root populations turn over quickly, often within 1–9 yr in forested ecosystems (Gill & Jackson, 2000; Norby & Jackson, 2000; Matamala *et al.*, 2003), and root detritus has a greater probability of being retained in the soil organic matter pool than surface litter because it is in intimate contact with the soil profile (Gale & Cambardella, 2000; Gale *et al.*, 2000). The stabilization of root-derived C in long-term soil pools (i.e. pools ranging from centuries to millennia in age, Schlesinger, 1997) may mitigate some portion of future atmospheric and climatic change. Forested ecosystems may be an especially important sink for rising [CO_2] given that forest soils currently store nearly half of below-ground terrestrial C (Dixon *et al.*, 1994).

The annual dynamics of C and N input from root population growth and mortality (i.e. turnover) are important parameters in models projecting biosphere responses to atmospheric and climatic change in N-limited ecosystems (Aber *et al.*, 1997; Kirschbaum *et al.*, 2003; Franklin, 2007). Decaying fine roots of woody plants represent an important flux of labile C and

N to the soil (Aerts *et al.*, 1992), because they turn over quickly (Eissenstat *et al.*, 2000; Norby & Jackson, 2000) and have relatively high concentrations of N and carbohydrates (Guo *et al.*, 2004). However, it is currently uncertain whether the input of labile root C and N to the soil profile will stimulate microbial degradation of organic matter and increase soil N availability (i.e. the 'priming effect'; cf. Kuzyakov *et al.*, 2000; Xiao *et al.*, 2007), allowing sustained forest responses to rising atmospheric [CO_2] (Zak *et al.*, 2000; Phillips, 2007). Alternatively, greater input of labile C could increase microbial immobilization of available N and constrain forest production (de Graaff *et al.*, 2007).

Whether increased fine-root production will lead to changes in soil N availability and C storage depends largely on the turnover rate of the fine-root population (Norby & Jackson, 2000), the chemical characteristics of root litter (Sollins *et al.*, 1996; Zak *et al.*, 2000; Silver & Miya, 2001), and the depth at which litter is input into the soil profile (Hunt, 1977; Fontaine *et al.*, 2007). Rising atmospheric [CO_2] is projected to decrease root turnover (as reviewed in Eissenstat *et al.*, 2000) by altering a suite of fine-root characteristics that are linked to greater root longevity. For example, CO_2 enrichment has been shown to increase root diameter (cf. Pritchard & Strand, 2008b), decrease root N concentration (Cotrufo & Ineson, 1995), increase mycorrhizal infection (Pritchard *et al.*, 2008a) and increase rooting depth (Norby *et al.*, 2004; Pritchard *et al.*, 2008b). Changes in root characteristics or rooting depth may increase root longevity (cf. Wells & Eissenstat, 2001; Baddeley & Watson, 2005; Joslin *et al.*, 2006; Withington *et al.*, 2006) by increasing nutrient gain per unit C cost (i.e. construction or maintenance respiration; cf. Eissenstat *et al.*, 2000).

Fine-root turnover is hard to measure because of the 'hidden' nature of below-ground processes (Norby & Jackson, 2000; Matamala *et al.*, 2003; Pritchard & Strand, 2008; Strand *et al.*, 2008), and the fact that roots grow in a branched pattern, ranging from smaller-diameter younger roots, to larger-diameter older roots (Pregitzer, 2002, 2003). Root production and mortality have been estimated with destructive soil sampling methods ranging from root in-growth cores to sequential biomass cores (Vogt *et al.*, 1998; Majdi *et al.*, 2005), and whole-ecosystem C and N budgets have been used to constrain estimates of below-ground inputs (Vogt *et al.*, 1998). Digitized images from minirhizotron cameras are now generally accepted to be a more accurate measure of root length production in ecosystems where production and mortality occur simultaneously (Johnson *et al.*, 2001; Tierney & Fahey, 2001), although long-term minirhizotron datasets are needed to adequately track the turnover rate of fine-root populations (Strand *et al.*, 2008). Quantitative relationships are necessary to derive root biomass and N content from minirhizotron measurements of root length and diameter (Vogt *et al.*, 1998; Tingey *et al.*, 2000; Johnson *et al.*, 2001), but this is complicated by the fact that root mass per unit length and root N concentration are both highly related to root diameter (Pregitzer *et al.*, 2002).

We quantified C and N input from fine-root mortality under ambient (i.e. 'current') and elevated [CO_2] at several soil depths in a deciduous sweetgum forest in eastern North America by combining allometric relationships derived from samples of individual roots with a long-term minirhizotron dataset. Fine-root production and mortality length estimates from minirhizotron images taken in ORNL FACE from 1998 to 2003 have been published previously (Norby *et al.*, 2004). Our research expanded these data by developing continuous relationships that enabled us to estimate root biomass and N content from root length and diameter. We used these relationships to calculate annual and cumulative root biomass and N input as a function of soil depth over the entire span of the experiment to date (a period of 9 yr ranging from 1998 to 2006). Our main questions were: do C and N inputs from root mortality increase in response to long-term CO_2 enrichment; and, if so, what are the implications for soil C storage and N cycling?

Materials and Methods

Site description

We conducted our research at the Oak Ridge National Laboratory (ORNL), free-air CO_2 enrichment (FACE) experiment in a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee, USA. ORNL FACE has been described in detail elsewhere (Norby *et al.*, 2001, 2002, 2004), but briefly, the experiment consists of five 25-m-diameter rings, of which four have a FACE apparatus installed. Two rings blow air enriched with CO_2 to achieve a concentration in the canopy of *c.* 560 ppm, while two rings blow a current [CO_2] of *c.* 380 ppm; the fifth ring serves as a current [CO_2] treatment without a FACE apparatus. CO_2 enrichment was initiated in 1998 when the sweetgum trees were 10 yr old and 12 m tall. The soil at the FACE site is described as an Aquic Hapludult, and consists of alluvium washed from upland soils (Norby *et al.*, 2001, 2002).

Minirhizotron images

Cellulose acetate butyrate minirhizotron tubes (Bartz Technology, Santa Barbara, CA, USA) of 5.1 cm inner diameter were installed in each FACE ring in July 1997 at a 60° angle from vertical to a depth of 60 cm (as described in Norby *et al.*, 2004). Measurements were made using five tubes per ring. Minirhizotron images were collected in each of 91 frames per tube (12.4 mm wide × 18 mm long) with a BTC-2 minirhizotron camera with a Smucker handle (Bartz Technology). Images were collected every 2 wk during the growing season (April–October), and monthly during the winter (December–March, Table 1). Before 2004, we did not film during winter months because cold temperatures resulted in shrinkage of the minirhizotron tubes, but relatively milder

Table 1 Minirhizotron sampling scheme at ORNL FACE

Year	First sampling date	Last sampling date	Number of sessions	Date of peak standing crop
1998	19 Feb 1998	30 Oct 1998	17	27 Jul
1999	18 Mar 1999	26 Oct 1999	15	21 Jun
2000	7 Mar 2000	30 Oct 2000	18	26 Jun
2001	14 Mar 2001	9 Oct 2001	16	27 Aug
2002	15 Mar 2002	22 Oct 2002	17	16 Jul
2003	11 Mar 2003	8 Dec 2003	23	30 Sep
2004	17 Mar 2004	30 Mar 2005	22	14 Oct
2005	15 Apr 2005	15 Mar 2006	21	7 Sep
2006	29 Mar 2006	7 Mar 2007	22	10 Nov

winters since 2004 allowed us to film throughout the winter. For the purposes of this experiment,we considered a 'year' to be the period between leaf-out in the spring (April) and leaf-out the next spring. Images were captured with the Targa + video board (True Vision, Indianapolis, USA, 1998–2002) or I-CAP system (Bartz Technology, 2003–2006), and digitized to obtain root diameter and length using ROOTS (Michigan State University, East Lansing, MI, USA, 1998–2002) or RooTracker (Duke University, Durham, NC, USA, 2003–2006) software. The same analyst has processed all of the minirhizotron images captured since the inception of the experiment.

Root biomass and N content

To determine allometric relationships between root diameter, biomass and N content, we sampled 30-cm-deep by 5-cm-diameter cores from the current and elevated CO_2 treatments on 7 June, 8 July, and 27 September 2005 (15–30 cm only), and on 30 May, 24 July, and 14 September 2006 (in 2005, five subsamples per ring, and in 2006, six subsamples per ring). Individual cores were divided into depths of 0–15 cm and 15–30 cm in the field, and refrigerated at 4°C until processed.

Roots were separated from the soil using a hydropneumatic elutriator with a 530 μm filter (Gillison Variety Fabrications, Benzonia, MI, USA), and large pieces of organic matter were removed by hand with forceps. After washing, moist roots were refrigerated at 4°C up to 1 month until they were scanned on a flatbed scanner (400 dpi, Epson Expression 1680, Epson, Long Beach, CA, USA). The high-resolution images obtained from the scanner were digitized using the *WinRhizo* root-scanning software program (Regent Instruments, Inc., Québec, Canada) to determine the total length, volume and surface area of root in diameter classes ranging from 0 to 4 mm in 0.1 mm increments. After the entire population of roots in each core was scanned, three small subsamples, ranging from two to 20 individual roots (10–200 mg total mass depending on root size), were removed from each 0–15 cm core (five to six cores per ring), and from a composite of the 15–30 cm cores because they contained fewer roots (one composite per ring). Subsamples were selected by hand to encompass the range of diameter classes contained in the core or composite sample (i.e. approx. within 0–0.4 mm, 0.4–0.8 mm and 0.8–4 mm size classes). The small subsamples were rescanned using the same *WinRhizo* parameters as described earlier and oven-dried at 70°C for at least 48 h to determine root mass per unit length (RML, mg cm^{-1}). After oven-drying, subsamples were ground on a Wig-L-Bug dental grinder (Crescent Dental Manufacturing Co., Chicago, IL, USA), and total N content was determined on an elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA).

Estimation of root biomass and N content from length measurements

We used the relationships between root mass per unit length, root [N] and root diameter (see the Results section) to estimate root biomass from root length and diameter measurements derived from digitized root images from 1998 to 2006. Root production was calculated from the appearance and incremental growth of individual roots, while root mortality was calculated from the disappearance of individual roots (without subsequent reappearance) from the minirhizotron frames as in Johnson *et al.* (2001). The biomass of individual roots was scaled to a volume of soil using the depth-of-field approach; we assumed that the depth of field in each minirhizotron window was 2 mm (cf. Johnson *et al.*, 2001). Annual mortality, production, and root N input (i.e. the N content of roots lost to mortality) were calculated based on the maximum diameter observed for each individual root within a given year, and standing crop was calculated based upon the maximum diameter observed on or before the date of peak standing crop. Root biomass and N content were summed within individual tubes for each of four depth increments. We assumed that roots did not resorb N before senescence (Nambiar & Fife, 1991; Aerts *et al.*, 1992; Gordon & Jackson, 2000), and that the N content of roots when they died was predicted by the relationship derived from live roots. We estimated production, mortality and N input over the winter when filming was infrequent (see Table 1) by

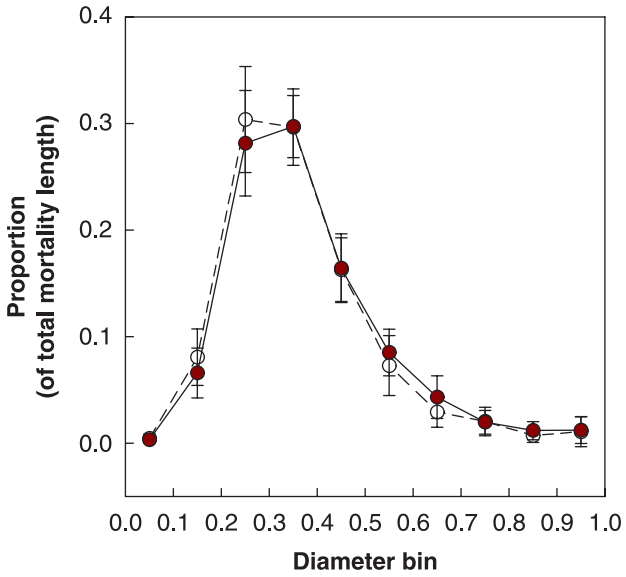

Fig. 1 Distribution of fine-root mortality length in diameter classes (i.e. bins) of 0.1 mm. The length of root in diameter classes > 1 mm was included in the analysis, but the proportion was minimal and was not included in the figure. Data are averaged over all years of the experiment (1998–2006); the error shown is ± 1 pooled standard error (SE). $n = 3$ in the current [CO_2] treatment (open circles) and $n = 2$ in the elevated [CO_2] treatment (closed circles).

subtracting the standing crop in the last session in a given year from that of the first session in the following year (using the associated diameters in the first and last filming sessions). Thus, our estimates of root production and mortality are net values, as roots probably formed and disappeared between filming intervals (Johnson *et al.*, 2001). We calculated root turnover as in Gill & Jackson (2000), where turnover (yr^{-1}) was equal to annual biomass mortality ($g\ m^{-2}\ yr^{-1}$) divided by the peak standing crop ($g\ m^{-2}$).

Statistics

We used the 'NLIN' procedure in SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA) to fit a power function to the relationships between root mass per length ($mg\ cm^{-1}$) and diameter, and root [N] and diameter for the subsamples taken from cores in 2005 and 2006. We used the SAS 'Mixed' procedure to test for differences in root biomass production, mortality, peak standing crop, N input and turnover under elevated [CO_2] from 1998 to 2006 using year as a repeated measure (ANOVA tables can be found in Supplementary material, Table S1). Ring within each treatment or treatment × depth combination was the subject of the repeated measure (cf. Littell, 1996, $n = 2$ elevated [CO_2] plots, $n = 3$ current [CO_2] plots); CO_2 treatment and soil depth were treated as fixed effects. We used the autoregressive (1) covariance structure in our model because it yielded the best goodness of fit (as determined by the Akaike's information criterion) when compared with other potential covariance structures (Littell, 1996; Littell *et al.*, 1998). Nonnormal data were log-transformed before analysis, and differences were considered significant at $P < 0.05$.

Results

All but 10 of the nearly 14 000 roots captured by minirhizotron filming between 1998 and 2006 were < 2 mm in diameter; 99% of the roots were < 1 mm in diameter. The distribution of root length lost to annual mortality among diameter classes did not significantly differ between the current and elevated [CO_2] treatments in any year ($P > 0.1$, Fig. 1). Across all years, the weighted average diameter of roots lost to mortality under elevated CO_2 tended to be only slightly larger on average (0.39 ± 0.01 mm under elevated CO_2, compared with 0.36 ± 0.01 mm under current CO_2; $P > 0.08$, Table 2). There were no interactions among diameter, treatment or depth ($P > 0.3$).

The relationship between root diameter and RML followed a positive power function ($r^2 = 0.96$, $P < 0.0001$, Fig. 2a). RML did not differ between the current and elevated [CO_2] treatments ($P > 0.05$), or with soil depth ($P > 0.05$; data shown are pooled across depths). The relationship between root diameter and [N] followed a negative power function ($r^2 = 0.65$, $P < 0.0001$, Fig. 2b), and also did not differ between the current and elevated [CO_2] treatments or by soil depth ($P > 0.05$). Root [C] was not related to root diameter (i.e. the slope was not significantly different from 0, $P > 0.1$), and root [C] averaged 46.6 ± 0.2% in the 0–15 cm soil depth, and 45.4 ± 0.1% in the 15–30 cm soil depth (data not shown, overall depth effect, $P < 0.005$).

Elevated [CO_2] more than doubled fine-root biomass production ($P < 0.003$), and biomass production changed over time in both treatments ($P < 0.001$, Table 2). Production did not differ with soil depth ($P > 0.2$); there were no interactions among treatment, depth or time ($P > 0.5$). Elevated [CO_2] approximately doubled peak standing crop across all years and soil depths ($P < 0.003$, Table 2). However, the depth dynamics of standing crop changed over time (soil depth × year interaction, $P < 0.05$). Combined across CO_2 treatments, peak standing crop was less below 45 cm soil depth than at either 0–15 or 15–30 cm in 1998 and 2000 ($P < 0.05$); in 2001 there was a large increase in standing crop, especially below 30 cm soil depth (Table 2). Thereafter, there was no difference in standing crop among depths ($P > 0.4$).

Elevated [CO_2] nearly doubled fine-root mortality ($P < 0.005$, Fig. 3). In contrast to production, the depth dynamics of mortality changed over time (soil depth × year interaction, $P < 0.05$); combined across CO_2 treatments, root mortality was much lower below 45 cm soil depth than at

Table 2 Weighted average root diameter, and annual biomass production and peak standing crop estimates

		Weighted average diameter (mm)		Production (g m^{-2} yr^{-1})		Peak standing crop (g m^{-2})	
Year	Soil depth	Current [CO_2]	Elevated [CO_2]	Current [CO_2]	Elevated [CO_2]	Current [CO_2]	Elevated [CO_2]
1998	0–15 cm	0.37 ± 0.00	0.35 ± 0.01	87 ± 29	57 ± 21	34 ± 14	43 ± 30
	15–30 cm	0.37 ± 0.03	0.46 ± 0.09	46 ± 17	66 ± 2	38 ± 11	32 ± 9
	30–45 cm	0.40 ± 0.07	0.38 ± 0.07	15 ± 7	26 ± 10	9 ± 4	23 ± 13
	45–60 cm	0.36 ± 0.03	0.44 ± 0.05	9 ± 6	23 ± 13	4 ± 2	10 ± 5
	Total profile	0.38 ± 0.01	0.40 ± 0.01	157 ± 52	171 ± 4	85 ± 28	107 ± 4
1999	0–15 cm	0.41 ± 0.02	0.43 ± 0.00	129 ± 18	118 ± 1	66 ± 14	54 ± 25
	15–30 cm	0.41 ± 0.06	0.43 ± 0.07	79 ± 58	98 ± 45	64 ± 42	53 ± 18
	30–45 cm	0.34 ± 0.02	0.36 ± 0.04	11 ± 3	34 ± 17	5 ± 1	23 ± 8
	45–60 cm	0.43 ± 0.03	0.46 ± 0.04	21 ± 11	36 ± 20	21 ± 9	30 ± 16
	Total profile	0.42 ± 0.03	0.43 ± 0.02	239 ± 79	286 ± 9	157 ± 61	160 ± 22
2000	0–15 cm	0.44 ± 0.01	0.42 ± 0.06	86 ± 11	177 ± 35	28 ± 3	46 ± 5
	15–30 cm	0.44 ± 0.04	0.49 ± 0.01	68 ± 33	110 ± 65	35 ± 15	52 ± 27
	30–45 cm	0.44 ± 0.05	0.45 ± 0.03	69 ± 55	144 ± 59	6 ± 3	49 ± 7
	45–60 cm	0.44 ± 0.04	0.41 ± 0.05	47 ± 46	105 ± 45	1 ± 1	17 ± 9
	Total profile	0.45 ± 0.02	0.45 ± 0.02	270 ± 104	536 ± 165	69 ± 15	164 ± 36
2001	0–15 cm	0.40 ± 0.03	0.36 ± 0.00	105 ± 36	118 ± 36	59 ± 25	125 ± 14
	15–30 cm	0.39 ± 0.05	0.42 ± 0.06	118 ± 65	188 ± 109	94 ± 40	199 ± 130
	30–45 cm	0.41 ± 0.06	0.42 ± 0.00	85 ± 57	166 ± 81	81 ± 59	145 ± 70
	45–60 cm	0.49 ± 0.09	0.50 ± 0.07	105 ± 90	381 ± 206	76 ± 68	300 ± 155
	Total profile	0.43 ± 0.05	0.43 ± 0.04	414 ± 206	853 ± 530	311 ± 161	768 ± 418
2002	0–15 cm	0.35 ± 0.03	0.39 ± 0.02	52 ± 17	129 ± 15	45 ± 28	91 ± 17
	15–30 cm	0.42 ± 0.01	0.41 ± 0.08	79 ± 7	123 ± 48	57 ± 22	108 ± 62
	30–45 cm	0.38 ± 0.03	0.41 ± 0.02	70 ± 34	221 ± 31	48 ± 36	152 ± 58
	45–60 cm	0.38 ± 0.04	0.47 ± 0.01	45 ± 27	329 ± 116	24 ± 16	251 ± 113
	Total profile	0.39 ± 0.02	0.42 ± 0.03	245 ± 61	802 ± 258	175 ± 75	603 ± 265
2003	0–15 cm	0.27 ± 0.01	0.28 ± 0.01	61 ± 24	78 ± 14	39 ± 17	73 ± 7
	15–30 cm	0.31 ± 0.01	0.36 ± 0.04	29 ± 11	72 ± 17	40 ± 7	64 ± 15
	30–45 cm	0.33 ± 0.05	0.33 ± 0.02	34 ± 23	74 ± 7	30 ± 16	133 ± 1
	45–60 cm	0.36 ± 0.03	0.35 ± 0.03	26 ± 5	92 ± 42	30 ± 16	138 ± 31
	Total profile	0.31 ± 0.02	0.32 ± 0.03	149 ± 54	316 ± 40	139 ± 46	407 ± 46
2004	0–15 cm	0.42 ± 0.06	0.30 ± 0.05	70 ± 23	89 ± 40	87 ± 19	94 ± 26
	15–30 cm	0.28 ± 0.02	0.32 ± 0.01	40 ± 16	73 ± 11	43 ± 14	86 ± 5
	30–45 cm	0.33 ± 0.03	0.35 ± 0.01	70 ± 53	83 ± 23	81 ± 62	160 ± 28
	45–60 cm	0.28 ± 0.04	0.38 ± 0.01	29 ± 20	127 ± 22	45 ± 31	185 ± 37
	Total profile	0.36 ± 0.01	0.33 ± 0.02	209 ± 79	372 ± 118	255 ± 87	525 ± 106
2005	0–15 cm	0.28 ± 0.03	0.35 ± 0.08	19 ± 5	75 ± 19	15 ± 5	70 ± 19
	15–30 cm	0.30 ± 0.03	0.43 ± 0.11	29 ± 18	105 ± 41	31 ± 16	107 ± 41
	30–45 cm	0.32 ± 0.02	0.29 ± 0.02	26 ± 20	85 ± 38	35 ± 23	93 ± 8
	45–60 cm	0.29 ± 0.02	0.34 ± 0.07	22 ± 16	77 ± 31	25 ± 19	88 ± 20
	Total profile	0.30 ± 0.01	0.35 ± 0.07	96 ± 56	341 ± 158	107 ± 61	358 ± 108
2006	0–15 cm	0.28 ± 0.06	0.34 ± 0.01	28 ± 8	87 ± 6	19 ± 4	58 ± 13
	15–30 cm	0.29 ± 0.07	0.40 ± 0.11	32 ± 17	67 ± 15	27 ± 13	44 ± 2
	30–45 cm	0.25 ± 0.01	0.29 ± 0.05	18 ± 16	67 ± 2	12 ± 9	111 ± 40
	45–60 cm	0.26 ± 0.08	0.43 ± 0.10	23 ± 18	32 ± 1	24 ± 20	34 ± 2
	Total profile	0.30 ± 0.03	0.37 ± 0.06	102 ± 49	254 ± 29	82 ± 44	247 ± 59

Data are means ± 1 SE of the mean (n = 3 in the current [CO_2] treatment, and n = 2 in the elevated [CO_2] treatment) at four soil depths, and also for the entire soil profile (0–60 cm). The weighted average diameter is of the population of roots lost annually to mortality. The length of root of a given diameter was used to weight diameter estimates proportionally. Thus, relatively rare roots with a large diameter did not bias the estimated diameter of the root population. Peak standing crop was determined to be when root biomass was greatest in at least three of the five rings.

shallower depths (i.e. the 0–15 and 15–30 cm depth increments) from 1998 to 2000 ($P < 0.05$); in 2001 there was a large increase in root mortality input, especially at 45–60 cm soil depth. Thereafter, there was no difference in mortality among depths ($P > 0.1$). Elevated [CO_2] doubled the N input from fine-root mortality ($P < 0.01$), and the amount of N input from fine-root mortality changed over time in both treatments ($P < 0.0001$, Fig. 4). There were no differences in

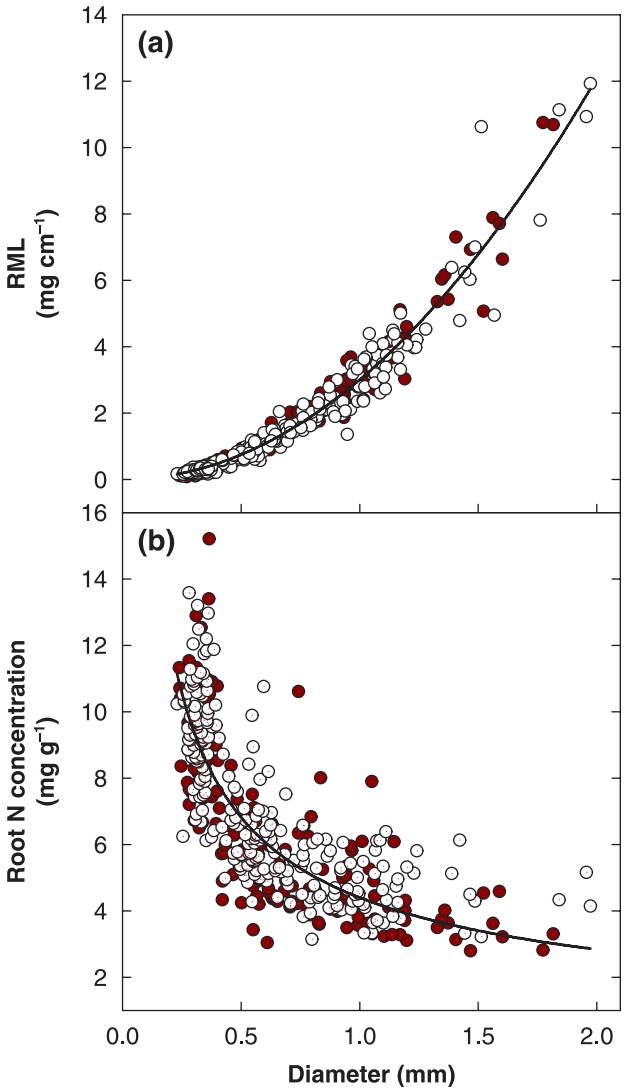

Fig. 2 The relationship between root diameter and root mass per unit length (RML) (a) or root nitrogen (N) concentration (b). (a) The relationship between root diameter and RML follows a positive power function, RML = 3.00 × diameter$^{2.01}$, that does not differ between the current and elevated [CO_2] treatments or with soil depth. Open circles (n = 292), current [CO_2] treatment; closed circles (n = 195), elevated [CO_2] treatment. Data are pooled across collection dates in 2005 and 2006 and across depths. (b) The relationship between root diameter and [N] follows a negative power function that does not differ between treatments or with soil depth. The relationship is [N] = 4.40 × diameter$^{-0.63}$, where n = 243 in the current [CO_2] treatment (open circles) and n = 190 in the elevated [CO_2] treatment (closed circles). Data are pooled across collection dates in 2005 and 2006 and across depths. Some data points are missing from the initial subsample collections because sample weights were too small for combustion to determine root [N].

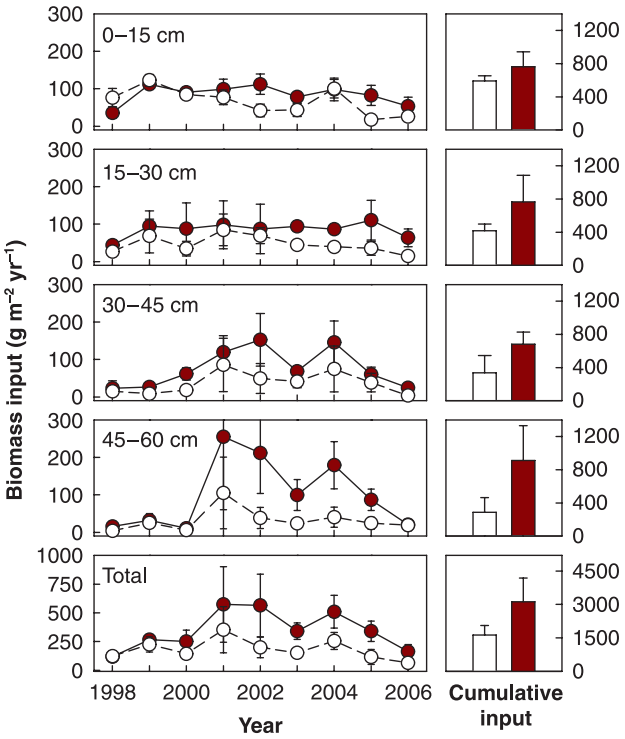

Fig. 3 Average annual root biomass input (± 1 SE) by soil depth (in 15 cm increments) and for the total soil profile (0–60 cm) as calculated from the relationship derived in Fig. 2(a). Within each year, n = 3 in the current [CO_2] treatment (open circles), and n = 2 in the elevated [CO_2] treatment (closed circles). Cumulative biomass inputs over the experiment to date (1998–2006) are shown as bars for each soil depth and for the total soil profile, where n = 3 in the current [CO_2] treatment (open bars), and n = 2 in the elevated [CO_2] treatment (closed bars).

N input with soil depth (P > 0.6), and no interactions among treatment, depth or time (P > 0.1). Over 9 yr, elevated [CO_2] nearly doubled the cumulative input of C (P < 0.03) and N (P < 0.02) from fine-root mortality (Figs 3, 4). There was no depth effect, or depth × treatment interactions on the cumulative inputs of C or N (P > 0.5).

Averaged across all soil depths, turnover (calculated from root mortality and peak standing crop) was lower under elevated [CO_2] (P < 0.05, Fig. 5). Turnover decreased over time in both treatments as the minirhizotron tubes were colonized (P < 0.0001), and stabilized in 2001 (i.e. turnover did not differ significantly among years after 2001, P > 0.1). We were unable to examine the effect of soil depth on turnover rate before 2001 because there were few roots below 30 cm (i.e. Table 2). However, we examined depth effects from 2001 onwards when root population turnover appeared stable. After 2001, the root population in both treatments turned over more slowly below 30 cm depth (on average 1.1 ± 0.09 yr^{-1} in the current and 0.8 ± 0.06 yr^{-1}in the elevated [CO_2] treatments) than closer to the soil surface (0–15 cm, 1.3 ± 0.09 yr^{-1} in the current and 1.1 ± 0.10 yr^{-1} in the elevated [CO_2] treatments, P < 0.05). There was no effect of year (P > 0.2), and there were no interactions among treatment, year or soil depth after 2001 (P > 0.4).

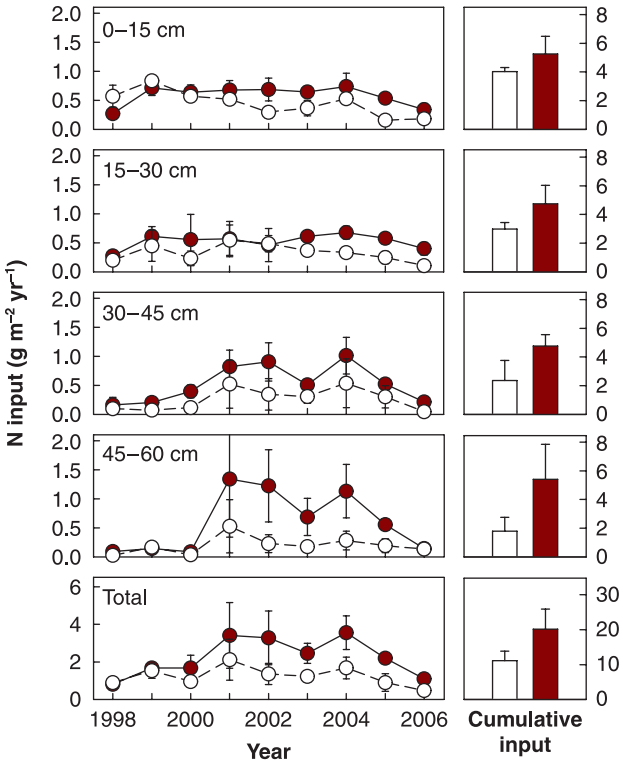

Fig. 4 Average annual nitrogen (N) input from root mortality (± 1 SE) by soil depth (in 15 cm increments) and for the total soil profile (0–60 cm) as calculated from the relationship derived in Fig. 2(b). Within each year, $n = 3$ in the current [CO_2] treatment (open circles), and $n = 2$ in the elevated [CO_2] treatment (closed circles). Cumulative N inputs over the experiment to date (1998–2006) are shown as bars for each soil depth and for the total soil profile, where $n = 3$ in the current [CO_2] treatment (open bars), and $n = 2$ in the elevated [CO_2] treatment (closed bars).

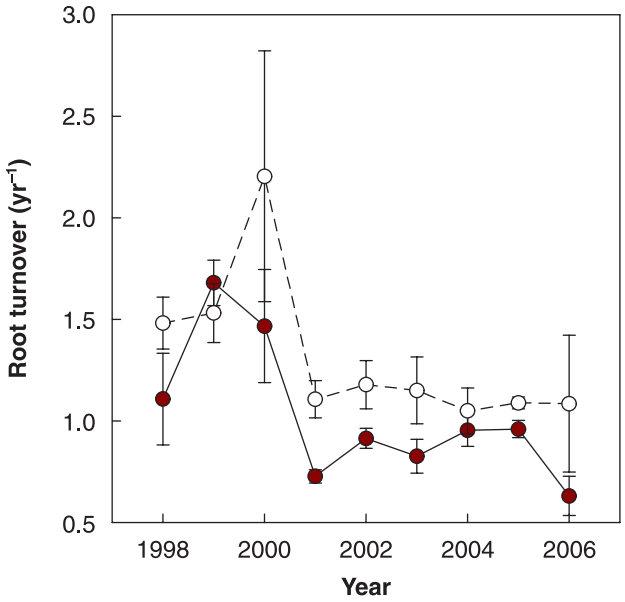

Fig. 5 Root turnover (± 1 SE) calculated as in Gill & Jackson (2000): turnover (yr^{-1}) = root mortality (g m^{-2} yr^{-1})/peak standing crop (g m^{-2}). Data are cumulative turnover rates across all soil depths. Within each year, $n = 3$ in the current [CO_2] treatment (open circles), and $n = 2$ in the elevated [CO_2] treatment (closed circles).

Discussion

Root biomass and N input

We quantified biomass and N input from fine-root mortality in a long-term CO_2 experiment in a sweetgum plantation by using continuous relationships to estimate the biomass and N content of individual roots from measurements of root length and diameter (Fig. 2a,b). Elevated [CO_2] nearly doubled the production of root biomass, and, contrary to what we expected, root production was not greatest near the soil surface (i.e. it was similar at all of the soil depths that we observed in both treatments, Table 2). In fact, it appeared that the largest increases in root production under elevated [CO_2] were deeper in the soil, though there was large variability associated with biomass estimates at depth (Table 2).

Overall, biomass and N input resulting from root mortality were twice as great in the elevated [CO_2] treatment relative to the current [CO_2] treatment (Figs 3, 4), even though the fine-root population turned over more slowly in the elevated relative to the current [CO_2] treatment (Fig. 5). Root mortality was greatest between November and March in both treatments (i.e. in the winter when filming was done at longer intervals; see Ledford *et al.*, 2007 for raw data). Ignoring overwinter dynamics in our calculations of root mortality would have underestimated annual biomass inputs via root mortality by up to 65%, even in years when we filmed during winter months (i.e. 2004–2006). These findings highlight the importance of quantifying root growth and mortality after leaf senescence, and also of decreasing the interval between minirhizotron filming dates when feasible (cf. Johnson *et al.*, 2001).

Root turnover

Root turnover is expected to decrease under elevated [CO_2] because of declining tissue [N] (Cotrufo & Ineson, 1995; Cotrufo *et al.*, 1998; Curtis & Wang, 1998; Long *et al.*, 2004), and associated declines in construction or maintenance respiration (Eissenstat *et al.*, 2000). However, elevated [CO_2] had no effect on sweetgum root [N] within a given diameter class (Fig. 2b). Instead, the decline in turnover rate we observed in response to CO_2 enrichment (Fig. 5) may be the result of increased root proliferation deeper in the soil profile. We found that in both treatments, the root population turned over more slowly below 30 cm than closer to the soil surface (i.e. 0–15 cm). Lower N availability and cooler temperatures

deeper in the soil profile may allow roots to live longer by reducing respiration costs (Eissenstat *et al.*, 2000; Burton *et al.*, 2000; Baddeley & Watson, 2005; Joslin *et al.*, 2006). Increased diameter has also been linked to greater root longevity in other ecosystems (Wells & Eissenstat, 2001), and the slight increase in root diameter that we observed under elevated [CO_2] (Table 2) may have also contributed to greater root longevity. Another mechanism for greater root longevity could be increased mycorrhizal infection (Eissenstat *et al.*, 2000). This has been observed in a CO_2-enriched loblolly pine plantation (Pritchard *et al.*, 2008a), but was beyond the scope of this investigation. Ultimately, a decline in root turnover under elevated [CO_2], or as roots grow deeper in the soil profile, may lead to delayed C and N input to the soil pool and result in increased storage of C and N in plant biomass.

Turnover estimated from production (data not shown) was initially greater, and more variable, than turnover estimated from root mortality (Fig. 5), indicating that the root population was not in equilibrium (cf. Burton *et al.*, 2000). However, the long-term nature of this dataset allowed us to observe the stabilization of root population turnover under current and elevated [CO_2] over time (Fig. 5; Tierney & Fahey, 2002; Guo *et al.*, 2008; Pritchard & Strand, 2008; Strand *et al.*, 2008). Contrary to the widespread assumption that soil disturbance and increased root proliferation associated with installation of minirhizotron tubes do not affect estimates of root production or mortality after 6–12 months (Joslin & Wolfe, 1999; Johnson *et al.*, 2001), we found that turnover calculated from total mortality and peak standing crop did not stabilize until year 4 of the experiment (Fig. 5). The initial variability in turnover rate was likely the result of soil disturbance and delayed colonization of the minirhizotron tubes; we have not identified any environmental signals (e.g. temperature or precipitation) that would explain the result. Our data indicate that short-term minirhizotron datasets should be viewed with caution (Strand *et al.*, 2008).

Root characteristics

Several authors have advocated the use of root order (i.e. the ontological order of a root's connection within a network of roots; Pregitzer, 2002; Pregitzer *et al.*, 2002; Guo *et al.*, 2008) over root diameter to describe root physiology and morphology. However, our results demonstrate that diameter is an excellent proxy for root mass and [N], especially given the difficulty of determining root order from minirhizotron images. Diameter explained 96% of the variation in root mass per length, and 65% of the variation in root [N] (Fig. 2a,b). These relationships did not differ from 0 to 30 cm soil depth, and are linear in a log–log space as predicted by allometric analysis (as reviewed in Enquist *et al.*, 2007; cf. Andersen *et al.*, in press). The relationship between root diameter and [N] may differ deeper in the soil profile, but we were unable to obtain enough biomass samples below 30 cm depth to develop robust relationships similar to those in Fig. 2. Continuous, root-specific relationships enabled us to avoid potentially confounding effects associated with differences between the diameter distribution of the minirhizotron data and the data used to quantify root biomass (Johnson *et al.*, 2001, Majdi *et al.*, 2005, Tingey *et al.*, 2005). For example, a root mass per length of 0.65 mg cm^{-1} was previously used to estimate the biomass of roots less than 0.5 mm in diameter in ORNL FACE (Norby *et al.*, 2004). This root mass per length corresponds to a root diameter of approx. 0.47 mm (Fig. 1), and overestimates the mass of a length of root in one of the most prominent diameter classes (0.2–0.3 mm, Fig. 1) by 150–450%.

Implications for long-term soil C and N storage

Elevated [CO_2] has increased annual N uptake in the ORNL FACE sweetgum stand by approx. 20%, but since 2000, nearly all the extra N taken up by the sweetgum trees under elevated [CO_2] was allocated to support fine-root growth (methods as described in Norby & Iversen, 2006; R. Norby, unpublished). Increased N uptake is necessary to support fine-root proliferation under elevated [CO_2] because small first-order roots have a high [N] (Pregitzer *et al.*, 2002, Fig. 2b), and we found no decline in individual root C : N under elevated [CO_2] (Fig. 2b).

Over 9 yr, approx. 681 g m^{-2} of the extra C, and 9 g m^{-2} of the extra N taken up by the sweetgum stand under elevated [CO_2] were returned to the soil via root mortality. Note that this calculation assumes that N is not resorbed from senescing roots (Nambiar & Fife, 1991; Aerts *et al.*, 1992; Gordon & Jackson, 2000). The average C : N of fine root detritus was approx. 75, which is somewhat greater than the global average C : N of living fine roots (approx. 40, Jackson *et al.*, 1997). Up to half of C and N input to the soil from root mortality was below 30 cm soil depth (Figs 3, 4), where soil properties such as oxygen availability, soil moisture, and temperature may stall the rate of microbial decomposition and the re-mineralization of N (Hunt, 1977; Baldock & Skjemstad, 2000). If all of the 'extra' N input from greater fine-root mortality under elevated [CO_2] were sequestered in soil organic matter, this would support approx. 135 g m^{-2} of extra soil C storage over all soil depths if C continues to be stored in soil organic matter pools with a C : N of approx. 15 (Jastrow *et al.*, 2005).

Jastrow *et al.* (2005) have previously shown C accrual in the top 5 cm of soil at ORNL FACE attributable to elevated [CO_2] to be approx. 44 g m^{-2} yr^{-1} (over the period 1997–2002). Our data on root C input over 9 yr cannot account for all of this accrual; we find only 8 g C m^{-2} yr^{-1} additional input from fine-root mortality under elevated [CO_2] in the top 15 cm of soil (39 g C m^{-2} yr^{-1} in elevated [CO_2] compared with 31 g C m^{-2} yr^{-1} in the current [CO_2] treatment.) We suspect this discrepancy may be explained in part by the minirhizotron system missing root growth in the top 5 cm of the soil (cf. Heeraman & Juma, 1993). Root biomass did not

decline in a linear or exponential fashion with soil depth in ORNL FACE as has been observed in other forested ecosystems (Jackson *et al.*, 1996; Matamala & Schlesinger, 2000, Table 2). Missed growth at the top of the soil profile could increase the additional root biomass input in the elevated [CO_2] treatment to approx. 20 g C m^{-2} yr^{-1} (*c.* 80 g m^{-2} yr^{-1} in elevated [CO_2] compared with *c.* 60 g m^{-2} yr^{-1} under current [CO_2]) given that up to 50% of root biomass in the top 15 cm at ORNL FACE is found from 0–5 cm depth (J. Jastrow, pers. comm.). Further, we have shown that overwinter mortality (between October and March) can contribute up to two-thirds of annual biomass input, and we may have missed C and N inputs before overwinter filming began in 2004. Lastly, leaf litter inputs at the top of the soil may contribute to soil C accrual; annual leaf litter inputs were approx. 225 g m^{-2} under elevated [CO_2] compared with 210 g m^{-2} under current [CO_2] (methods as in Norby *et al.*, 2002; R. Norby, unpublished).

Greater C and N sequestration in long-term soil pools under elevated [CO_2] is projected to decrease soil N availability and, ultimately, forest production (Luo *et al.*, 2004). However, limited soil N availability has not constrained forest production or stand N uptake in response to elevated [CO_2] thus far in any of the forested FACE experiments (Norby *et al.*, 2005; Finzi *et al.*, 2007; Iversen & Norby, 2008). Increased soil exploration by fine roots has facilitated greater N acquisition under elevated [CO_2] in forested ecosystems (Norby *et al.*, 2004; Norby & Iversen, 2006; Finzi *et al.*, 2007; Pritchard *et al.*, 2008b), and root proliferation could further stimulate soil N availability throughout the soil profile by supplying a 'fresh' source of organic matter and energy to the microbial community (cf. Fontaine *et al.*, 2007). However, it remains difficult to project future forest responses to global change because the relationship between forest N uptake and soil N availability is not well represented in ecosystem models (cf. Finzi *et al.*, 2007). Further, the soil organic matter dynamics that determine N mineralization in ecosystem models such as CENTURY are only simulated in the first 20 cm of the soil profile (Parton *et al.*, 1988; Ma & Shaffer, 2001). Continued data-model integration is an important goal in advancing our understanding of below-ground processes and their impact on ecosystem responses to global change (Jackson *et al.*, 2000; Classen & Langley, 2005).

Conclusion

Dynamic C and N cycling in soils are key components of ecosystem responses to atmospheric and climatic change and their feedbacks to the atmospheric CO_2 and global C cycle. Here, we have shown that in a forest growing in an elevated concentration of atmospheric CO_2, the flux of C and N into the soil nearly doubled owing to stimulated root production and mortality. Moreover, much of the C and N input occurred relatively deep in the soil profile where the dynamics of root decomposition and C and N mineralization are likely to be different from what is commonly observed and modeled in the upper profile. Contrary to expectations, root [N] did not decline under elevated [CO_2]; other mechanisms, including increased root diameter or rooting depth, may be responsible for the decline in root turnover we observed in response to CO_2 enrichment. Continued progress in understanding the interface between root detritus and soil C and N cycling, especially at depth in the soil, will improve our ability to predict ecosystem responses to atmospheric and climatic change.

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Supplementary Material

The following supplementary material is available for this article online:

Table S1 ANOVA table of repeated-measures analyses for main response variables

This material is available as part of the online article from:
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