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Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of CO_2-enrichment

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Abstract

Increased partitioning of carbon (C) to fine roots under elevated [CO_2], especially deep in the soil profile, could alter soil C and nitrogen (N) cycling in forests. After more than 11 years of free-air CO_2 enrichment in a *Liquidambar styraciflua* L. (sweetgum) plantation in Oak Ridge, TN, USA, greater inputs of fine roots resulted in the incorporation of new C (i.e., C with a depleted $\delta^{13}C$) into root-derived particulate organic matter (POM) pools to 90-cm depth. Even though production in the sweetgum stand was limited by soil N availability, soil C and N contents were greater throughout the soil profile under elevated [CO_2] at the conclusion of the experiment. Greater C inputs from fine-root detritus under elevated [CO_2] did not result in increased net N immobilization or C mineralization rates in long-term laboratory incubations, possibly because microbial biomass was lower in the CO_2-enriched plots. Furthermore, the $\delta^{13}CO_2$ of the C mineralized from the incubated soil closely tracked the $\delta^{13}C$ of the labile POM pool in the elevated [CO_2] treatment, especially in shallower soil, and did not indicate significant priming of the decomposition of pre-experiment soil organic matter (SOM). Although potential C mineralization rates were positively and linearly related to total SOM C content in the top 30 cm of soil, this relationship did not hold in deeper soil. Taken together with an increased mean residence time of C in deeper soil pools, these findings indicate that C inputs from relatively deep roots under elevated [CO_2] may increase the potential for long-term soil C storage. However, C in deeper soil is likely to take many years to accrue to a significant fraction of total soil C given relatively smaller root inputs at depth. Expanded representation of biogeochemical cycling throughout the soil profile may improve model projections of future forest responses to rising atmospheric [CO_2].

Keywords: ^{13}C, carbon mineralization, elevated [CO_2], fine roots, *Liquidambar styraciflua*, mineral-associated organic matter, net nitrogen mineralization, particulate organic matter, soil carbon, soil depth

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Introduction

The ecosystem processes that control carbon (C) uptake and storage in CO_2-enriched forested ecosystems are important components of land surface models used to project atmospheric and climatic change (Bonan, 2008). A common response of trees growing in elevated CO_2 is increased production of fine roots (Luo *et al*., 2006). In particular, increased root production in relatively deep soil under elevated [CO_2] has been observed in a number of CO_2-enrichment experiments in forested ecosystems (reviewed in Iversen, 2010). The access to additional mineral nitrogen (N) provided by deeper roots (Iversen *et al*., 2011) could help to sustain a CO_2-fertilization effect (Iversen, 2010). An increase in root-derived C input to the soil could also alter soil biogeochemical cycling by increasing the mineralization of previously stored soil organic matter, which could further increase N availability (Phillips *et al*., 2010), but also increase C loss from the soil (Fontaine *et al*., 2007). Alternatively, if root inputs to deeper soil are resistant to mineralization, storage of C and associated N in the soil could increase and progressively limit the N available for plant uptake (Luo *et al*., 2004). The implications of deeper rooting distributions for future forest responses to rising atmospheric CO_2 remain uncertain because deeper soil processes are poorly represented in ecosystem and land surface models (Iversen, 2010) in

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part because relatively few data exist for model parameterization and testing (Rumpel & Kogel-Knabner, 2011).

Here, we explored whether greater C and N inputs from increased fine-root production and mortality in a CO_2-enriched forest plantation affect C and N cycling throughout the soil profile. We tested these concepts at the Oak Ridge National Laboratory (ORNL) Free-Air CO_2 Enrichment (FACE) experiment located in a *Liquidambar styraciflua* L. (sweetgum) plantation in eastern Tennessee, USA. Net primary productivity increased in response to CO_2 enrichment during this nearly 12-year experiment, and the majority of the additional C fixed under elevated [CO_2] was allocated belowground to the production of fine roots (Norby *et al*., 2004). Furthermore, the largest increases in root biomass production and mortality under elevated [CO_2] were at relatively deep soil depths (i.e., below 30 cm, Iversen *et al*., 2008) where biogeochemical cycling could be expected to differ from that in surface soils (Rumpel & Kogel-Knabner, 2011). Sweetgum production at the ORNL FACE site was limited by soil N availability (Iversen & Norby, 2008), and the CO_2-fertilization effect declined over time (Norby *et al*., 2010), which was attributed to declining soil N availability (Garten *et al*., 2011). The conclusion and final harvest of the ORNL FACE experiment during the 12th year of treatment provided a unique opportunity to examine the effects of long-term CO_2-enrichment on C and N cycling and storage throughout the soil profile.

We tested three hypotheses: (1) FACE-derived C (i.e., new C with a depleted $\delta^{13}C$ fixed into plant biomass during the FACE experiment) from fine-root inputs would be incorporated into SOM under elevated [CO_2], including in deeper soil where large increases in root production and mortality were observed. Increased root C inputs under elevated [CO_2] would result in greater soil C content in the CO_2-enriched treatment at the end of the experiment. We also hypothesized that greater C inputs under elevated [CO_2] would lead to increased microbial activity, resulting in: (2) increased N immobilization rates (i.e., decreased net N mineralization rates) and, (3) increased C mineralization rates. We show here that FACE-derived C was incorporated into root-derived soil pools to 90-cm soil depth. Although C and N content were greater throughout the soil profile under elevated [CO_2] at the conclusion of the experiment, potential net N immobilization and C mineralization rates did not differ between ambient and elevated [CO_2]. The greater residence time of C in deeper soil indicates that inputs from deep roots under elevated [CO_2] may increase the potential for long-term storage of C and N in forested ecosystems.

Materials and methods

Experimental design

The ORNL FACE experiment was conducted in a *Liquidambar styraciflua* L. plantation on soil characterized as a moderately well-drained Aquic Hapludult with a silty clay loam texture (Norby *et al*., 2001b). The experimental manipulation comprised five 25-m diameter treatment rings, of which four were encircled by a FACE apparatus (Norby *et al*., 2001b). Beginning in 1998, two of the rings received elevated [CO_2] at a targeted level of 565 ppm during the daytime throughout the growing season (April to November, Riggs *et al*., 2010). The CO_2 used to fumigate the elevated [CO_2] treatment was depleted in ^{13}C, with an average $\delta^{13}C$ of $-51‰$ (Norby *et al*., 2001b). The other three rings were maintained at ambient [CO_2] (~396 ppm, Riggs *et al*., 2010). The experiment was concluded in 2009 during the 12th year of fumigation, at which time plant and soil material was harvested.

Soil pit excavation

Two soil pits (80-cm wide × 80-cm long × 90-cm deep) were excavated in each treatment ring (n = 10 pits) in late June, 2009. Sweetgum trees were within approximately 60 cm of at least two sides of each pit. After removing the litter layer, pits were excavated with hand tools in depth increments of: 0–5, 5–15, 15–30, 30–45, 45–60, and 60–90 cm. Soil from each depth increment was immediately sieved through 10-mm mesh to remove roots and homogenize soil. A large subsample (>2 kg) was refrigerated at 4 °C for 6–16 days until use in the soil incubation experiment described below.

Initial soil characteristics

Subsamples of the 10-mm sieved soil fraction were taken to determine gravimetric water content and water-holding capacity (see Table S1 in Supporting Information). The oven-dry mass of fine roots remaining in the 10-mm sieved soil fraction was determined after re-sieving a 1 kg (oven-dry weight equivalent) subsample of fresh soil through a 2-mm mesh and removing any remaining roots by hand.

The C and N content of two SOM pools was quantified by wet-sieving a 20 g subsample of air-dried, sieved soil (2-mm fraction) through a 53 μm sieve. Each subsample was first dispersed by shaking for 15 h at low speed on a reciprocating shaker in 100 mL of 5 g/L sodium hexametaphosphate (Cambardella & Elliott, 1993). As in Cambardella & Elliott (1993), we refer to the larger size fraction (53–2000 μm), which included sand and sand-sized organic matter, as particulate organic matter (POM), and the smaller size fraction (<53 μm), which included silt, clay, and silt- and clay-sized organic matter, as mineral-associated organic matter. We use these common terms for brevity and do not intend to imply a specific stabilization mechanism (i.e., von Lützow *et al*., 2006).

The $^{13}C/^{12}C$ ratio of fine roots, POM, MOM, and whole soil (2-mm fraction), was determined using continuous-flow isotope ratio mass spectrometry (Integra CN, SerCon Ltd.,

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Crewe, UK). Results from the isotope analysis are expressed in delta notation ($\delta^{13}C$, ‰): $\delta^{13}C = [R_{SAMPLE} - R_{STANDARD}] \times 1000$, where R is the $^{13}C/^{12}C$ ratio. The working standard for isotope analysis (glucose, $\delta^{13}C = -10.2‰$) was calibrated against National Institute of Standards and Technology (Gaithersburg, MD, USA) reference material (NIST 8542, sucrose). Soil C and N concentrations were determined in the same analysis as $\delta^{13}C$, and fine-root C and N concentrations were determined on an elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA). Soil and plant samples were dried for at least 24 h at 70 °C prior to analysis to reduce variability associated with moisture. Concentrations were multiplied by the total mass of each pool to determine root and soil C and N content. The fraction of C derived from tree input under CO_2-enrichment (f) was calculated for each depth increment as: $f = \left(\frac{\delta_e - \delta_a}{\delta_r - \delta_a}\right)$, where δ_e is the $\delta^{13}C$ of soil excavated from the elevated [CO_2] treatment, δ_a is the $\delta^{13}C$ of soil excavated from the ambient [CO_2] treatment, and δ_r is the $\delta^{13}C$ of fine roots harvested from the elevated [CO_2] treatment (Table 1; Fig. 1). For a given depth increment, δ_e and δ_r were determined for each soil pit, whereas δ_a was an average of the ambient [CO_2] treatment (n = 3 treatment plots). We assumed that the majority of soil C inputs were from fine roots because it has been shown previously that there is little C transfer between the leaf litter layer and mineral soil in forests (Garten, 2009). Bioturbation by earthworms at ORNL FACE may have incorporated leaf litter into mineral soil, but only in shallow soil horizons (Y. Sánchez de León, pers. comm.). Leaf litter $\delta^{13}C$ in elevated [CO_2] (−39.8 to −41.1‰, Garten *et al*., 2011) did not differ substantially from fine-root $\delta^{13}C$ at shallow soil depths (−39.6 to −40.3‰, Table 1), so leaf litter input to surface soils, via bioturbation or other processes, would not affect our calculations or conclusions.

Initial NH_4^+ and NO_3^- concentrations were determined by extracting a ~12 g (oven-dry weight equivalent) subsample of field-moist, sieved soil (10-mm fraction) from each pit and depth increment with 100 mL of 2 M KCl. Samples were shaken at low speed on a reciprocating shaker for 1 h, and soil was allowed to settle overnight at 4 °C. The extractant was filtered from the soil using Whatman #1 filter paper that had been preleached with distilled water; an aliquot was frozen at −20 °C until analysis for total [NH_4^+] (Phenate method, 12-107-06-1-A) and [NO_3^-] (Nitrate method, 12-107-04-1-B) on a Lachat QuikChem 8500 autoanalyzer (Lachat Instruments, Loveland, CO, USA).

Microbial biomass was determined on paired subsamples using the chloroform fumigation extraction method (Horwath & Paul, 1994). One ~12 g subsample (oven-dry weight equivalent) of field-moist, sieved soil (10-mm fraction) from each pit and depth increment was extracted with 50 mL of 0.5 M K_2SO_4 in a manner similar to the 2 M KCl extractions. A paired sample was fumigated with chloroform and incubated in the dark for 5 days; after incubation, the fumigated samples were extracted with 50 mL of 0.5 M K_2SO_4. All samples were frozen at −20 °C until analysis for total N (method 12-107-04-3-B) on the Lachat paired with an in-line digestor (QuickChem In-line Sample Preparation Module, QC8000 Series). The difference in total N (mg N/kg soil) between fumigated and unfumigated samples was divided by an extraction efficiency of 0.54 (Joergensen & Mueller, 1996) to convert total N to microbial biomass N. Due to sample loss during analysis, microbial biomass data from ring 3 (ambient [CO_2], no FACE apparatus) were excluded from statistical analyses.

Soil incubations

Approximately 12 g (oven-dry weight equivalent) of field-moist, sieved soil (10-mm fraction) from each pit and depth increment was placed into 20-mL glass scintillation vials. The soil moisture in each vial was adjusted to 60% of water-holding capacity. Three replicate scintillation vials from each pit and depth increment were placed together in a 0.95 L Mason jar, along with a container holding ~10 mL of distilled water to maintain humidity (Hart *et al*., 1994). Mason jars (5 rings × 2 pits per ring × 6 depth increments = 60 jars) were tightly capped and incubated in the dark at 20 ± 2 °C for 243 days. Five Mason jars incubated with water (but no soil scintillation vials) served as blanks to correct for CO_2 in the ambient atmosphere.

Potential net N mineralization

One scintillation vial from each jar was sacrificed on days 1, 29, and 243 to measure potential net N mineralization and net nitrification. Samples were extracted with 100 mL of 2 M KCl, and analyzed for [NH_4^+] and [NO_3^-]. Net N mineralization rates were calculated as the difference in NH_4-N between final and initial samples, and net nitrification rates were calculated in a similar manner. Net changes in N were standardized per unit of oven-dry soil.

Potential C mineralization

The headspace air in each jar was sampled via an air-tight septum after 1, 3, 7, 16, 29, 61, 120, and 243 days. At each sampling date, a well-mixed 5-mL subsample was injected into an EGM-4 CO_2 gas analyzer (PP Systems, Amesbury, MA, USA). Potential C mineralization rates were determined as the difference in headspace [CO_2] between sampling dates, which was corrected for [CO_2] in ambient atmosphere and standardized by the dry-soil equivalent contained in each jar. Our measurements of potential C mineralization represent heterotrophic mineralization of SOM under constant laboratory conditions (~20 °C) at an ideal soil moisture content (60% of water-holding capacity). Hence, these values do not reflect *in situ* rates, which would also include respiration by microbes associated with an active rhizosphere. However, our approach allowed for a unique comparison among treatments and depth intervals that was not possible in the field.

The ^{13}C signature of the headspace CO_2 was determined on samples in one pit per ring (excluding ring 3) after 1, 7, 29, 61, 120, and 243 days. A well-mixed, 12-mL headspace subsample was injected into evacuated vacutainers (Labco exetainers, Labco Limited, Buckinghamshire, UK), and the samples were

Table 1 Fine-root and soil carbon (C) and nitrogen (N) pools throughout the soil profile under ambient and elevated [CO_2]

		Fine roots				Whole soil					
Treatment	Soil depth (cm)	Standing crop (g C/m^3 soil)*	^{13}C (‰)†	N content (g N/m^3 soil)*	C/N ratio	C content (kg C/m^3 soil)	^{13}C (‰)†	Fraction FACE-derived C‡	New C (kg C/m^3 soil)§	N content (kg N/m^3 soil)	C/N ratio
Ambient [CO_2]	0–5	480 ± 30	−28.8 ± 1.1	13.9 ± 0.9	35.2 ± 1.1	30.8 ± 1.0	−27.1 ± 0.2	–	–	2.29 ± 0.04	13.4 ± 0.2
	5–15	212 ± 30	−29.3 ± 0.1	5.0 ± 0.8	44.7 ± 3.4	18.3 ± 0.5	−25.4 ± 0.3	–	–	1.60 ± 0.06	11.4 ± 0.2
	15–30	83 ± 7	−29.2 ± 0.1	1.4 ± 0.1	58.4 ± 5.9	11.3 ± 0.4	−23.6 ± 0.1	–	–	1.15 ± 0.04	9.9 ± 0.1
	30–45	31 ± 4	−29.2 ± 0.1	0.4 ± 0.1	71.8 ± 3.0	5.7 ± 0.7	−21.9 ± 0.1	–	–	0.75 ± 0.06	7.5 ± 0.4
	45–60	12 ± 2	−29.0 ± 0.3	0.2 ± 0.1	77.6 ± 0.8	3.4 ± 0.5	−22.1 ± 0.6	–	–	0.54 ± 0.05	6.2 ± 0.3
	60–90	17 ± 9	−28.9 ± 1.0	0.1 ± 0.1	134.8 ± 6.8	2.8 ± 0.4	−22.0 ± 0.5	–	–	0.47 ± 0.04	5.8 ± 0.6
	0–90¶	77 ± 3	−28.9 ± 0.1	1.7 ± 0.1	45.9 ± 2.1	8.1 ± 0.4	−23.1 ± 0.2	–	–	0.87 ± 0.04	9.3 ± 0.2
Elevated [CO_2]	0–5	646 ± 14 (35)	−40.3 ± 0.8 (−40)	16.6 ± 0.4 (20)	39.2 ± 0.3	36.8 ± 3.7 (19)	−33.9 ± 2.2 (−25)	0.52 ± 0.07	19.7 ± 4.5	2.63 ± 0.08 (15)	13.9 ± 0.9
	5–15	381 ± 151 (80)	−39.6 ± 0.1 (−36)	7.5 ± 2.9 (51)	52.4 ± 2.5 (17)	20.1 ± 0.3 (10)	−28.9 ± 1.8 (−13)	0.25 ± 0.05	5.1 ± 1.1	1.77 ± 0.06 (11)	11.3 ± 0.5
	15–30	159 ± 46 (92)	−39.3 ± 1.2 (−34)	2.7 ± 0.9 (87)	59.7 ± 1.6	14.4 ± 0.3 (27)	−25.4 ± 0.9	0.13 ± 0.04	1.8 ± 0.6	1.35 ± 0.09 (17)	10.7 ± 0.5
	30–45	44 ± 4 (40)	−37.4 ± 0.8 (−28)	0.6 ± 0.1 (41)	69.1 ± 7.8	7.0 ± 2.5 (23)	−22.9 ± 0.4	0.10 ± 0.01	0.7 ± 0.2	0.83 ± 0.24 (11)	8.3 ± 0.6
	45–60	26 ± 15 (111)	−36.0 ± 0.8 (−25)	0.3 ± 0.2 (104)	79.7 ± 4.1	4.2 ± 1.9 (25)	−23.5 ± 0.1	0.12 ± 0.01	0.5 ± 0.2	0.59 ± 0.17 (10)	6.8 ± 1.2
	60–90	13 ± 1 (−22)	−35.3 ± 0.4 (−23)	0.1 ± 0.1 (22)	94.3 ± 4.1 (−30)	3.2 ± 0.8 (15)	−23.3 ± 0.3	0.03 ± 0.01	0.1 ± 0.1	0.51 ± 0.08 (8)	6.1 ± 0.6
	0–90¶	121 ± 23	−36.9 ± 0.8	2.4 ± 0.5	50.5 ± 1.0	9.6 ± 0.8	−24.5 ± 0.1	–	2.2 ± 0.4	0.98 ± 0.11	9.9 ± 0.3
ANOVA	CO_2 treatment	**$F_{1,18}$ = 5.7, P = 0.03**	**$F_{1,12}$ = 176.8, P < 0.0001**	**$F_{1,18}$ = 8.2, P = 0.01**	$F_{1,18}$ = 0.1, P = 0.74	**$F_{1,18}$ = 10.3, P = 0.005**	**$F_{1,12}$ = 25.7, P = 0.0003**	–	–	**$F_{1,18}$ = 9.1, P = 0.008**	$F_{1,18}$ = 2.4, P = 0.14
	Soil depth	**$F_{5,18}$ = 53.1, P < 0.0001**	**$F_{5,12}$ = 4.6, P = 0.01**	**$F_{5,18}$ = 173.3, P < 0.0001**	**$F_{5,18}$ = 76.6, P < 0.0001**	**$F_{5,18}$ = 195.9, P < 0.0001**	**$F_{5,12}$ = 26.1, P < 0.0001**	**$F_{5,6}$ = 21.5, P = 0.0009**	**$F_{5,6}$ = 39.1, P = 0.0002**	**$F_{5,18}$ = 161.8, P < 0.0001**	**$F_{5,18}$ = 68.7, P < 0.0001**
	Treatment × Depth	$F_{5,18}$ = 0.2, P = 0.94	**$F_{5,12}$ = 4.5, P = 0.02**	$F_{5,18}$ = 0.7, P = 0.65	**$F_{5,18}$ = 4.3, P = 0.009**	$F_{5,18}$ = 1.5, P = 0.26	**$F_{5,12}$ = 3.1, P = 0.05**	–	–	$F_{5,18}$ = 0.9, P = 0.50	$F_{5,18}$ = 0.2, P = 0.95

		Particulate organic matter					Mineral-associated organic matter				
Treatment	Soil depth (cm)	C content (kg C/m^3 soil)	Fraction FACE-derived C‡	New C (kg C/m^3 soil)§	N content (kg N/m^3 soil)	C/N ratio	C content (kg C/m^3 soil)	Fraction FACE-derived C‡	New C (kg C/m^3 soil)§	N content (kg N/m^3 soil)	C/N ratio
Ambient [CO_2]	0–5	11.0 ± 1.1	–	–	0.51 ± 0.04	21.6 ± 0.4	18.5 ± 0.9	–	–	1.60 ± 0.07	11.6 ± 0.2
	5–15	3.8 ± 0.1	–	–	0.19 ± 0.01	20.1 ± 0.2	12.7 ± 0.6	–	–	1.29 ± 0.04	9.8 ± 0.2
	15–30	1.6 ± 0.1	–	–	0.08 ± 0.01	21.7 ± 1.1	10.0 ± 0.4	–	–	1.01 ± 0.03	9.8 ± 0.2
	30–45	0.5 ± 0.1	–	–	0.03 ± 0.01	19.1 ± 2.1	5.8 ± 0.7	–	–	0.67 ± 0.05	8.6 ± 0.6
	45–60	0.4 ± 0.1	–	–	0.03 ± 0.01	15.4 ± 1.7	4.0 ± 0.5	–	–	0.49 ± 0.05	8.0 ± 0.6
	60–90	0.4 ± 0.1	–	–	0.03 ± 0.01	15.0 ± 2.0	3.0 ± 0.4	–	–	0.43 ± 0.04	7.0 ± 0.9
	0–90¶	1.6 ± 0.1	–	–	0.08 ± 0.01	19.9 ± 0.7	6.7 ± 0.5	–	–	0.74 ± 0.04	9.1 ± 0.4
Elevated [CO_2]	0–5	13.6 ± 3.7 (23)	0.71 ± 0.02	9.8 ± 2.8	0.67 ± 0.22	20.9 ± 1.0 (−3)	20.1 ± 1.7	0.39 ± 0.02	7.8 ± 0.3	1.79 ± 0.14 (12)	11.3 ± 0.1
	5–15	4.3 ± 1.1 (13)	0.53 ± 0.06	2.4 ± 0.9	0.19 ± 0.05	22.6 ± 0.6 (13)	14.2 ± 1.1	0.17 ± 0.01	2.5 ± 0.1	1.45 ± 0.09 (12)	9.8 ± 0.2

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Table 1 (continued)

Treatment	Soil depth (cm)	Particulate organic matter: C content (kg C/m^3 soil)	Fraction FACE-derived C‡	New C (kg C/m^3 soil)§	N content (kg N/m^3 soil)	C/N ratio	Mineral-associated organic matter: C content (kg C/m^3 soil)	Fraction FACE-derived C‡	New C (kg C/m^3 soil)§	N content (kg N/m^3 soil)	C/N ratio
	15–30	2.2 ± 0.1 (34)	0.35 ± 0.01	0.7 ± 0.1	0.09 ± 0.01	25.4 ± 2.9 (18)	12.0 ± 1.5	0.06 ± 0.02	0.7 ± 0.3	1.18 ± 0.07 (16)	10.2 ± 0.8
	30–45	0.8 ± 0.1 (42)	0.29 ± 0.02	0.2 ± 0.1	0.03 ± 0.01	25.1 ± 3.0 (31)	6.9 ± 2.9	−0.01 ± 0.05	0.2 ± 0.2	0.77 ± 0.23 (15)	8.5 ± 1.2
	45–60	0.6 ± 0.1 (45)	0.42 ± 0.08	0.2 ± 0.1	0.03 ± 0.01	18.9 ± 1.1 (23)	4.2 ± 1.8	−0.15 ± 0.10	0.1 ± 0.1	0.54 ± 0.17 (9)	7.5 ± 0.9
	60–90	0.5 ± 0.1 (16)	0.19 ± 0.01	0.1 ± 0.1	0.03 ± 0.01	14.8 ± 0.8 (−2)	3.1 ± 0.9	−0.15 ± 0.08	0.1 ± 0.1	0.46 ± 0.08 (7)	6.6 ± 0.9
	0–90¶	2.0 ± 0.3	–	1.0 ± 0.3	0.09 ± 0.02	21.2 ± 1.0	7.6 ± 1.6	–	0.9 ± 0.1	0.83 ± 0.12	9.1 ± 0.5
ANOVA	CO_2 treatment	**$F_{1,18}$ = 6.5, P = 0.02**	–	–	$F_{1,18}$ = 1.2, P = 0.29	**$F_{1,18}$ = 6.8, P = 0.02**	$F_{1,18}$ = 3.0, P = 0.10	–	–	**$F_{1,18}$ = 5.2, P = 0.04**	$F_{1,18}$ = 0.2, P = 0.66
	Soil depth	**$F_{5,18}$ = 212.9, P < 0.0001**	**$F_{5,6}$ = 20.3, P = 0.001**	**$F_{5,6}$ = 30.1, P = 0.0003**	**$F_{5,18}$ = 56.8, P < 0.0001**	**$F_{5,18}$ = 8.1, P = 0.0004**	**$F_{5,18}$ = 66.5, P < 0.0001**	**$F_{5,6}$ = 12.9, P = 0.004**	**$F_{5,6}$ = 59.1, P < 0.0001**	**$F_{5,18}$ = 66.9, P < 0.0001**	**$F_{5,18}$ = 14.3, P < 0.0001**
	Treatment × Depth	$F_{5,18}$ = 0.2, P = 0.96	–	–	$F_{5,18}$ = 0.6, P = 0.68	$F_{5,18}$ = 1.2, P = 0.35	$F_{5,18}$ = 0.3, P = 0.93	–	–	$F_{5,18}$ = 0.3, P = 0.92	$F_{5,18}$ = 0.1, P = 0.98

Data are treatment means ± standard error of the mean for each soil depth increment (n = 2 elevated rings, n = 3 ambient rings). Standard errors less than the last significant digit were rounded to 0.01, 0.1, or 1. Parentheses indicate a percent increase under elevated [CO_2] where there was a significant difference between CO_2 treatments.

Bold values indicate significant P values.

*Fine roots in the 10-mm soil fraction (i.e., roots that were included in the laboratory incubations; average diameter range was 0.3–0.5 mm). Treatment averages for root C and N were used to replace missing data at deeper soil depths where root biomass was low; there was enough sample for $\delta^{13}C$ analysis in all cases. Note that root standing crop C and N pools were an order of magnitude less than the soil pools.

†Data were averaged over two pits within each treatment ring with the exception of $\delta^{13}C$ values which correspond to the soil pits used in measurements of $\delta^{13}CO_2$ (i.e., the four treatment rings having a FACE apparatus; $\delta^{13}C$ values for POM and MOM are in Fig. 1). However, there was no statistical difference in $\delta^{13}C$ between the two pits within each ring ($F_{1,48}$ = 0.1, P = 0.76 for fine roots and $F_{1,48}$ = 0.2, P = 0.63 for whole-soil).

‡Fraction FACE-derived C was calculated using both soil pits in each ring receiving elevated [CO_2], where ring was the statistical replicate (n = 2). The standard error of the fraction of C derived under elevated [CO_2] was calculated as in Phillips & Gregg (2001).

§New C (i.e., C with a depleted $\delta^{13}C$) was calculated for each depth increment as the product of the C content of each soil pool and the fraction of FACE-derived C. New C was assumed to equal 0 where the fraction FACE-derived C was <0.

¶Integration of data across soil depths (i.e., summation or weighted average).

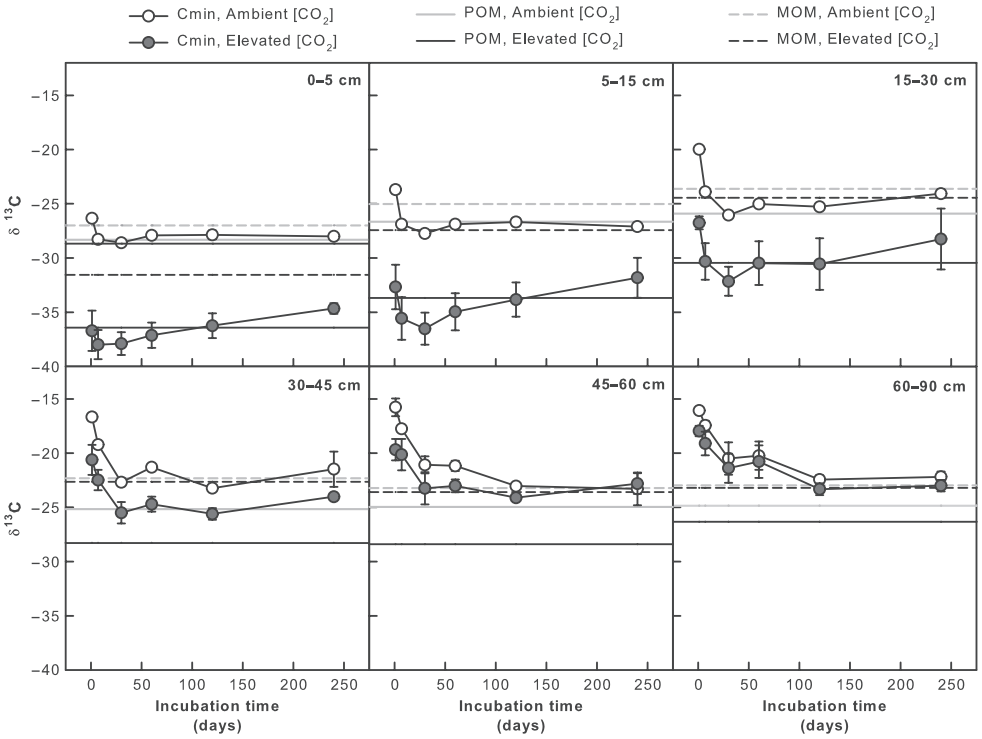

Fig. 1 The $\delta^{13}C$ of CO_2 mineralized (C_{min}) from soil during a 243-day laboratory incubation and the initial $\delta^{13}C$ of two soil fractions. The $\delta^{13}CO_2$ data (represented by circles) are the mean of each treatment ±1 SEM (error bars are often too small to see). Lines are the average $\delta^{13}C$ of two soil pools within each treatment, where POM is the soil fraction 53–2000 μm in size and MOM is the soil fraction <53 μm. For comparison with $\delta^{13}CO_2$ data, which were taken only from samples corresponding to one pit in the plots encircled by FACE apparatus, we present only the $\delta^{13}C$ of the soil fractions corresponding to the same pit. However, averaged across soil depths, there was no significant difference in POM or MOM $\delta^{13}C$ between the two soil pits in each treatment ring (POM ^{13}C, $F_{1,48} = 0.03$, $P = 0.87$ and MOM ^{13}C, $F_{1,48} = 1.8$, $P = 0.19$). The relative enrichment of ^{13}C in the POM and MOM pools at deeper soil depths in both the ambient and elevated [CO_2] treatment, reflected in the $\delta^{13}C$ of respired CO_2, was likely due to a combination of factors, including a decline in atmospheric $\delta^{13}CO_2$ since 1780 (~1.5‰, Francey *et al.*, 1999), a relatively larger contribution in deeper soil of microbially derived C products enriched in ^{13}C (Bostrom *et al.*, 2007), and a history of agriculture that likely included maize at the ORNL FACE site prior to 1943.

analyzed for $^{13}CO_2$ at the University of California, Davis Stable Isotope Facility (Davis, CA, USA).

Jars were uncapped after each set of headspace measurements and flushed for 1 min (flow rate = 5.1 L/min) to equilibrate the headspace with the ambient atmosphere. Jars were then re-capped and returned to the dark. Beginning on day 7, distilled water was added as needed to replace water lost from evaporation and microbial respiration (determined as a change in soil mass between measurements) to maintain soil moisture at 60% of water-holding capacity.

Statistics

Data were analyzed using the mixed-model procedure in SAS (SAS version 9.1, Cary, NC, USA). Before analysis, soil bulk density (Table S1) was used to express mass-based soil pools and fluxes per unit soil volume to standardize comparisons among depth increments. Data from each depth interval were averaged over two pits within each treatment ring, and ring was treated as the statistical replicate ($n = 2$ elevated [CO_2] rings and $n = 3$ ambient [CO_2] rings). Treatment and soil depth were treated as fixed effects. We specified the 'Kenward-Roger' option in the model statement to estimate denominator degrees of freedom for small sample sizes. For repeated measurements (i.e., potential C mineralization and $\delta^{13}CO_2$), incubation length was treated as a repeated measure, and the autoregressive (1) covariance structure was specified. Non-normal variables were log-transformed prior to analysis. Post-hoc treatment comparisons were determined with a *t*-test using the LSMEANS procedure in SAS. Differences between treatments and among soil depths were considered significant at $P < 0.10$ (Iversen *et al.*, 2011).

Results

Root and soil C and N content

The elevated (CO_2) treatment increased the amount of C in the fine-root standing crop, whole-soil, and POM pools, and increased the amount of N in the fine-root,

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whole-soil, and MOM pools. Root and soil C and N content declined with soil depth, but there were no interactions between CO_2 treatment and soil depth (Table 1). The largest relative increases in C content under elevated [CO_2] were in the fine-root and POM pools. The effect of elevated [CO_2] on the C/N ratio of the fine-root pool depended on the soil depth (treatment × soil depth interaction). Relative to ambient [CO_2], fine-root C/N increased under elevated [CO_2] at 5–15 cm ($P = 0.08$), but decreased under elevated [CO_2] at 60–90 cm ($P = 0.0008$). In contrast, elevated [CO_2] generally increased the C/N ratio of the POM fraction (with the exception of very shallow and very deep soil), and had no effect on the C/N of whole soil or MOM (Table 1). The C/N ratio increased with soil depth in the fine-root pool, but decreased with depth in the soil fractions.

Root and soil isotopic signatures

The $\delta^{13}C$ of fine roots, whole-soil, POM, and MOM depended on CO_2 treatment and soil depth; the largest depletion in root and soil ^{13}C under elevated [CO_2] generally occurred at shallower soil depths (Table 1 for fine roots and whole-soil, Fig. 1 for POM and MOM). Treatment × depth interactions were $F_{5,12} = 3.6$, $P = 0.03$ for POM, and $F_{5,12} = 2.6$, $P = 0.09$ for MOM. CO_2-enrichment caused fine-root and POM pools to be significantly more depleted in ^{13}C throughout the soil profile (fine roots, $P < 0.02$ to 90-cm depth and POM, $P < 0.05$ to 60-cm depth), indicating that a substantial fraction of these pools consisted of FACE-derived C (Table 1). In contrast, whole-soil and MOM were more depleted in ^{13}C under elevated [CO_2] in only the shallowest soil depths (0–15 cm, $P < 0.02$ and $P < 0.05$, respectively). The fraction of FACE-derived C in MOM at depths deeper than 45 cm was difficult to interpret, perhaps because of differences among plots that existed prior to the experimental manipulation. Within each CO_2 treatment, whole soil, POM, and MOM generally became more enriched in ^{13}C in deeper soil. The $\delta^{13}C$ of fine roots in the elevated [CO_2] treatment also increased in deeper soil. The amount of FACE-derived C (i.e., new C with a depleted $\delta^{13}C$) declined with depth in all soil pools (Table 1).

Initial soil and microbial N pools

Initial extractable soil [NH_4^+] was ~24% less under elevated [CO_2] than under ambient [CO_2], and [NH_4^+] declined with soil depth (Fig. S1). Initial extractable [NO_3^-] depended on the CO_2 treatment and soil depth (Fig. S1). Integrated across all soil depths, microbial biomass N was initially ~29% less under elevated [CO_2] than under ambient [CO_2], and it declined with soil depth (Table 2).

Potential net N mineralization

Potential net N mineralization (i.e., the net amount of organic N mineralized into NH_4-N per day) in long-term laboratory incubations changed over time and depended on the soil depth (Table 2). After 1 day, NH_4^+ was strongly immobilized, likely because of the large amount of C initially available for microbial biosynthesis at the start of the incubation. However, there was no difference in potential net N mineralization rate between ambient and elevated [CO_2], and the rate of NH_4^+ immobilization declined with soil depth. After 29 days, potential net N mineralization was greater under elevated [CO_2] at 0–5 cm depth ($P = 0.01$) and less under elevated [CO_2] at 5–15 cm depth ($P = 0.08$). After 243 days of incubation, NH_4^+ immobilization was less under elevated [CO_2] 0–5 cm depth ($P = 0.0005$). At the end of the incubation, the amount of extractable NH_4-N in the soil did not differ between ambient and elevated [CO_2] (Fig. S1).

Potential net nitrification (i.e., the net amount of NH_4-N mineralized into NO_3-N per day) in long-term laboratory incubations changed over time and depended on the soil depth (Table 2). After 1 day, NO_3-N was weakly immobilized throughout the soil profile, but there was no difference in potential net nitrification between ambient and elevated [CO_2] or with soil depth. The potential net nitrification rate over 29 days was less under elevated [CO_2] compared with ambient [CO_2]. Over 243 days, NO_3^- was strongly mineralized, but there was no difference in potential net nitrification rate between ambient and elevated [CO_2]. Potential net nitrification declined with soil depth in both treatments after 29 and 243 days. At the end of the incubation, there was no difference in extractable NO_3-N between ambient and elevated [CO_2] (Fig. S1). Integrated throughout the soil profile, extractable NO_3-N was nearly 100% of total inorganic N at the last sampling date, whereas NO_3-N averaged only 26% of initial extractable inorganic N.

Potential C mineralization

Potential C mineralization rates over the 243-day laboratory incubations were generally higher in surface soils from the elevated [CO_2] treatment, especially early in the incubation, but did not differ significantly between ambient and elevated [CO_2] ($F_{1,20} = 3.0$, $P = 0.10$, Table S2). A separate experiment indicated that length of soil refrigeration after pit excavation had no effects on the relative differences in potential C

Table 2 Soil microbial N biomass and net N fluxes throughout the soil profile under ambient and elevated [CO_2]

Treatment	Soil depth (cm)	Microbial biomass N (g N/m^3 soil)	Net N mineralization rate (mg NH_4-N m^{-3} soil day^{-1})			Net nitrification rate (mg NO_3-N m^{-3} soil day^{-1})		
			1 day	29 days	243 days	1 day	29 days	243 days
Ambient [CO_2]	0–5	154 ± 11	−957 ± 72	1 ± 49	−7 ± 2	−24 ± 144	562 ± 213	1027 ± 76
	5–15	71 ± 5	−475 ± 91	120 ± 28	−4 ± 1	−216 ± 173	111 ± 42	516 ± 36
	15–30	37 ± 9	−238 ± 53	38 ± 4	−2 ± 1	−21 ± 21	23 ± 5	180 ± 3
	30–45	4 ± 1	−114 ± 15	5 ± 3	−1 ± 1	−113 ± 125	−3 ± 2	28 ± 9
	45–60	1 ± 1	−127 ± 28	8 ± 2	0 ± 1	−57 ± 94	0 ± 1	12 ± 10
	60–90	2 ± 2	−93 ± 35	30 ± 18	0 ± 1	49 ± 33	2 ± 2	15 ± 7
	0–90¶	24 ± 1	−217 ± 12	32 ± 11	−1 ± 1	−41 ± 42	48 ± 17	156 ± 6
Elevated [CO_2]	0–5	134 ± 9 (−13)	−683 ± 333	102 ± 53 (7232)	1 ± 6 (120)	49 ± 177	133 ± 30 (−76)	1295 ± 176
	5–15	58 ± 10 (−18)	−379 ± 24	52 ± 24 (−57)	−1 ± 1	−116 ± 22	5 ± 16 (−95)	508 ± 108
	15–30	12 ± 9 (−67)	−353 ± 24	11 ± 8	−1 ± 1	−74 ± 74	−1 ± 10 (−106)	154 ± 16
	30–45	5 ± 2 (24)	−158 ± 54	2 ± 3	−1 ± 1	−100 ± 139	−9 ± 1 (−219)	16 ± 3
	45–60	1 ± 1 (−20)	−80 ± 47	7 ± 2	0 ± 1	−25 ± 80	−6 ± 2 (−1467)	8 ± 9
	60–90	0 ± 1 (−100)	−54 ± 28	8 ± 1	0 ± 1	−366 ± 189	−16 ± 8 (−790)	−2 ± 1
	0–90¶	17 ± 2	−197 ± 43	17 ± 1	0 ± 1	−165 ± 31	0 ± 8	157 ± 18
ANOVA	CO_2 treatment	**$F_{1,12}$ = 6.7, P = 0.02**	$F_{1,18}$ = 0.9, P = 0.36	$F_{1,18}$ = 0.1, P = 0.82	**$F_{1,18}$ = 5.3, P = 0.03**	$F_{1,18}$ = 0.3, P = 0.59	**$F_{1,18}$ = 5.0, P = 0.04**	$F_{1,18}$ = 0.5, P = 0.48
	Soil depth	**$F_{5,12}$ = 152.1, P < 0.0001**	**$F_{5,18}$ = 15.1, P < 0.0001**	**$F_{5,18}$ = 2.9, P = 0.04**	$F_{5,18}$ = 1.3, P = 0.31	$F_{5,18}$ = 0.7, P = 0.64	**$F_{5,18}$ = 7.0, P = 0.0008**	**$F_{5,18}$ = 203.2, P < 0.0001**
	Treatment × Depth	$F_{5,12}$ = 1.4, P = 0.29	$F_{5,18}$ = 0.9, P = 0.52	**$F_{5,18}$ = 2.4, P = 0.08**	**$F_{5,18}$ = 2.7, P = 0.05**	$F_{5,18}$ = 1.3, P = 0.30	$F_{5,18}$ = 2.1, P = 0.12	$F_{5,18}$ = 1.9, P = 0.14

Data are treatment means ± standard error of the mean for each soil depth increment (n = 3 ambient rings, n = 2 elevated rings), where data were averaged over two pits within each treatment ring. The exception was microbial biomass N, which was only measured in two of the three ambient rings (i.e., the rings with FACE apparatus) due to the loss of samples during total N analysis. Net N mineralization and nitrification rates were determined after 1, 29, or 243 days of incubation in the laboratory (note that the units are an order of magnitude less than microbial biomass N). Notes are as in Table 1.

Bold values indicate significant P values.

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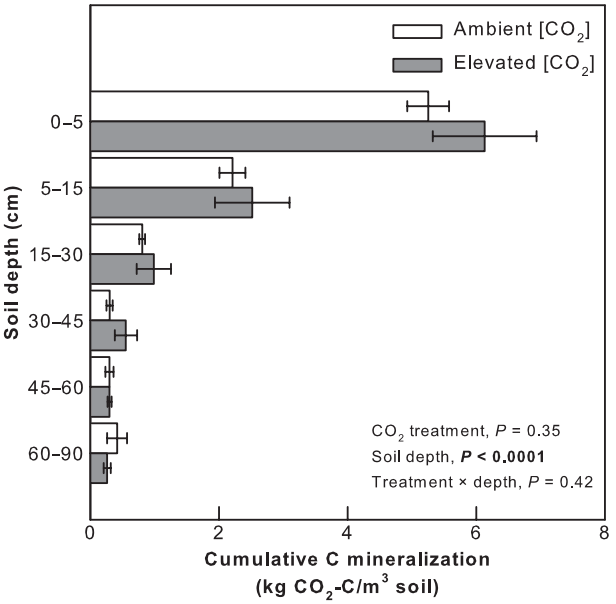

Fig. 2 Mean cumulative potential C mineralization ± 1 SEM of soil sampled from the ambient and elevated [CO_2] treatments and incubated for 243 days in the laboratory.

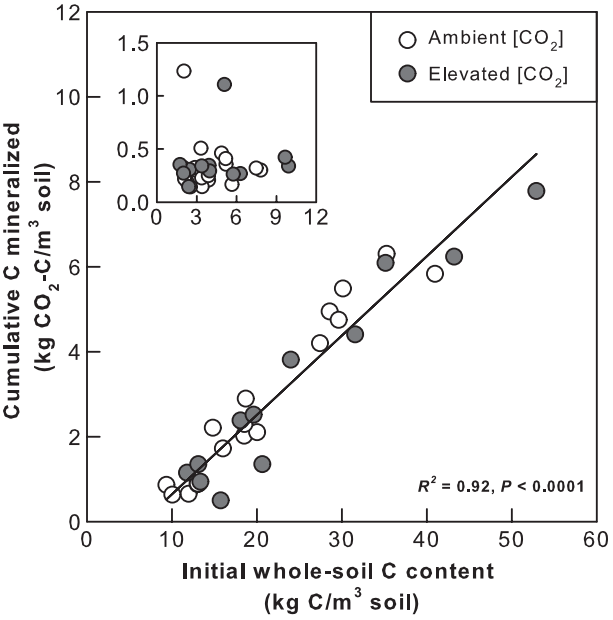

Fig. 3 Regression between cumulative potential C mineralization over a 243-day laboratory incubation and initial whole-soil (10-mm fraction) C content. Data are from each soil pit in the CO_2 treatments (n = 2 pits per treatment ring, 10 pits total for each soil depth). The linear relationship corresponds to soil depths from 0 to 30 cm (CO_2 mineralized = 0.187 × Soil C – 1.223); inset corresponds to soils sampled deeper than 30 cm where no significant relationship was present.

mineralization rates between the ambient and elevated [CO_2] treatments (Table S3). Furthermore, pre-incubation in the refrigerator may have helped to avoid the inclusion of large flushes of C and N associated with the sieving process in measurements over the first few weeks of the incubation (Sparling *et al.*, 1985).

After 243 days, there was no difference in cumulative C mineralized between the ambient and elevated [CO_2] treatments (P = 0.35, Fig. 2). Initial C content of whole-soil (10-mm fraction, including fine roots) explained 92% of the cumulative amount of C mineralized over the course of the 243-day incubation (Fig. 3). However, the relationship between initial soil C content and cumulative C mineralization was significant only at shallower soil depths (i.e., 0–30 cm); there was no relationship between soil C content and C mineralization at deeper soil depths (Fig. 3, insert).

Isotopic signature of C mineralized

The $\delta^{13}C$ of C mineralized from the laboratory incubations depended on soil depth (treatment × depth and day × depth interactions, $F_{5,12.7}$ = 5.7, P = 0.006 and $F_{25,57.4}$ = 5.9, P < 0.0001, respectively, Fig. 1). CO_2 efflux from the elevated [CO_2] treatment was depleted in ^{13}C from 0 to 45 cm soil depth, indicating the microbial mineralization of FACE-derived C inputs. At 0–5 and 5–15 cm depth increments, the CO_2 evolved from the incubations was more depleted in ^{13}C under elevated [CO_2] (~ −37‰ in elevated [CO_2] compared with ~ −28‰ in ambient [CO_2] at 0–5 cm, $F_{1,2.0}$ = 52.6, P = 0.02 and ~ −35‰ compared with ~ −26‰ at 5–15 cm, $F_{1,2.0}$ = 16.77, P = 0.06), although the $\delta^{13}CO_2$ signal changed over time in both treatments ($F_{5,9.9}$ = 9.0, P = 0.002 and $F_{5,10.0}$ = 37.8, P < 0.0001, respectively), and the absolute difference in ^{13}C enrichment declined over the course of the incubation (treatment × time interaction, $F_{5,9.9}$ = 2.7, P = 0.09 and $F_{5,10.0}$ = 5.7, P = 0.01, respectively). At the 15–30 and 30–45 cm depth increments, the CO_2 evolved from the incubations was also more depleted in ^{13}C under elevated [CO_2] (~ −30‰ in elevated [CO_2] compared with ~ −24‰ in ambient [CO_2] at 15–30 cm, $F_{1,2.1}$ = 10.0, P = 0.08 and ~ −24‰ compared with ~ −21‰ at 30–45 cm, $F_{1,1.7}$ = 17.0, P = 0.07). The $\delta^{13}CO_2$ also changed over time at the 15–30 and 30–45 cm depth increments in both treatments ($F_{5,9.9}$ = 19.6, P < 0.0001 and $F_{5,8.3}$ = 13.4, P = 0.0009, respectively) as the decomposition of different SOM pools progressed. However, there was no interaction between treatment and time on $^{13}CO_2$ enrichment at the 15–30 and 30–45 cm soil depths ($F_{5,9.9}$ = 0.4, P = 0.81 and $F_{5,8.3}$ = 0.3, P = 0.88, respectively).

At soil depths deeper than 45 cm (45–60 and 60–90 cm depth increments), CO_2-enrichment had no effect

on the ^{13}C signature of CO_2 evolved from the incubations ($F_{1,2.1} = 4.3$, $P = 0.17$ and $F_{1,3.1} = 1.8$, $P = 0.27$, respectively). However, the $\delta^{13}C$ of mineralized CO_2 declined over time ($F_{5,8.9} = 9.4$, $P = 0.002$ and $F_{5,10} = 15.3$, $P = 0.0002$, respectively); there was no interaction between treatment and incubation time on $^{13}CO_2$ enrichment at the deepest soil depths ($F_{5,8.9} = 1.1$, $P = 0.45$ and $F_{5,10} = 0.21$, $P = 0.95$ for the 45–60 and 60–90 cm depth increments, respectively).

Discussion

Hypothesis 1: Increased C content throughout the soil profile under elevated [CO_2]

As we hypothesized, substantial FACE-derived C inputs (i.e., new C with a depleted $\delta^{13}C$) were incorporated into SOM pools throughout the soil profile at ORNL FACE after more than 11 years of CO_2-enrichment. The largest fraction of FACE-derived C, ranging from 71% in the upper soil to 19% in deeper soil (Table 1), was found in the relatively labile POM pool, which is root-derived (Garten & Brice, 2009). This novel quantification of the effect of elevated [CO_2] on SOM formation in deeper soil supports minirhizotron observations that indicated increased fine-root C inputs under elevated [CO_2], especially at relatively deep soil depths, over the first 9 years of the experiment (Iversen *et al*., 2008), and indicates that inputs of fine-root detritus were an important source for SOM formation at this site.

A smaller fraction of C in the MOM pool was FACE-derived. MOM consists mainly of the byproducts generated by POM decomposition and is dominated by microbially derived compounds (Grandy & Neff, 2008). The fact that FACE-derived C inputs were observed in the MOM pool only at relatively shallow soil depths (Table 1) indicates that microbial processing of POM occurred more quickly in shallow soil.

Soil C content was ~19% greater throughout the soil profile after more than 11 years of CO_2-enrichment (Table 1). This finding suggests greater C accrual in elevated [CO_2] compared with ambient [CO_2] during the experiment, consistent with the conclusion of a meta-analysis that indicated increased ecosystem C storage under elevated [CO_2] (Luo *et al*., 2006). Differences in C content between ambient and elevated [CO_2] were greatest in shallow soil (Table 1), likely because C inputs were greatest at these depths (Iversen *et al*., 2008), and consistent with the previous report of Jastrow *et al*. (2005) indicating greater C accrual in the 0–5 cm depth increment in the CO_2-enriched plots at ORNL FACE during the first 6 years of the experiment. Calculating C accrual throughout the entire soil profile, however, is problematic in that it depends on the assumption of equivalent C content in ambient and elevated [CO_2] at the beginning of the experiment. Johnson *et al*. (2004) reported no significant difference in pretreatment C content to a depth of 90 cm, although there was a tendency toward greater C content in elevated [CO_2] which could account for some of the difference we measured 13 years later. However, the pretreatment samples were taken from the periphery of the FACE plots and were processed differently from the samples we report here; hence, they are not directly comparable. A full analysis of C accrual during the experiment based on a consistent sampling protocol is forthcoming.

Observed increases in soil C under elevated (CO_2) at ORNL FACE stand in contrast to patterns observed across a number of other long-term CO_2-enrichment experiments in forested ecosystems. Despite increased C inputs under elevated [CO_2], soil C content did not differ between CO_2 treatments after 12 years of CO_2-enrichment at the Duke FACE site in a loblolly pine (*Pinus taeda*) plantation in North Carolina, USA (Drake *et al*., 2011). There were also no difference in soil C content between ambient and elevated [CO_2] after 6 years of CO_2-enrichment at POP-EuroFACE in *Populus* spp. plantations near Viterbo, Italy (Hoosbeek & Scarascia-Mugnozza, 2009), and after 5 years of CO_2-enrichment at the BangorFACE experiment in a mixed-deciduous species plantation in Wales, UK (Hoosbeek *et al*., 2011). Furthermore, soil C content declined under elevated [CO_2] at the Rhinelander FACE site in mixed-aspen plantations in northern Wisconsin, USA (Talhelm *et al*., 2009), and at the scrub oak open-top chamber CO_2-enrichment experiment in Florida, USA (Langley *et al*., 2009).

Priming of the decomposition of recalcitrant SOM by the inputs of new, relatively labile, C (Kuzyakov, 2010) was pinpointed as the cause of soil C loss (or a lack of additional soil C accrual) in response to greater C inputs under elevated [CO_2] across a number of CO_2-enrichment experiments in forests (Hoosbeek & Scarascia-Mugnozza, 2009; Langley *et al*., 2009; Drake *et al*., 2011; Hoosbeek *et al*., 2011). Greater soil C content under elevated [CO_2] at the conclusion of the ORNL FACE experiment does not preclude the occurrence of priming (i.e., Kuzyakov, 2010); rather, it indicates that C inputs from increased forest production under elevated [CO_2] were greater than C losses via priming or other processes. However, we did not observe significant priming in our laboratory incubations. The $\delta^{13}CO_2$ of the C mineralized from laboratory soil incubations closely tracked the $\delta^{13}C$ of the relatively labile POM pools in each treatment, especially at shallow soil depths where the largest absolute increases in soil C

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content under elevated [CO_2] were observed (Fig. 1). Priming of recalcitrant SOM (which would have had a pretreatment $\delta^{13}C$) would have resulted in an enrichment of the ^{13}C signal of CO_2 in the elevated [CO_2] treatment (i.e., the $\delta^{13}CO_2$ in ambient and elevated [CO_2] would have been more similar), and would also have increased C mineralization rates. However, these were laboratory incubations that necessarily excluded the influence of living roots. Increases in root-derived exudates under elevated [CO_2] stimulated microbial activity and increased SOM mineralization at Duke FACE (Phillips *et al*., 2010) and root exudates may have fueled the priming of recalcitrant SOM *in situ* at ORNL FACE.

The causes of differences in C accrual under elevated [CO_2] among the different CO_2-enrichment experiments in forested ecosystems remain uncertain. As we show here, roots are an important source for SOM formation, and many sites exhibited smaller relative increases in root inputs to the soil (Day *et al*., 2006; Pregitzer *et al*., 2008; Pritchard *et al*., 2008) compared with large stimulations in root production and mortality under elevated [CO_2] at ORNL FACE (Norby *et al*., 2004; Iversen *et al*., 2008). Furthermore, small changes in large and variable soil C pools are notoriously difficult to quantify, even over decadal scales (Luo *et al*., 2011), and C accrual can be site- and species-specific. Sandy soil at the Florida site may have limited capacity for C accrual (Langley *et al*., 2009), and there were differences across sites in the physical protection and stabilization of new C in SOM (as reviewed in Hoosbeek & Scarascia-Mugnozza, 2009). Also, soil C accrual at Rhinelander FACE differed beneath different tree species (Talhelm *et al*., 2009; although there was no species-specific effect on soil C content at BangorFACE, Hoosbeek *et al*., 2011), and soil C dynamics in the CO_2-enriched plots at POP-EuroFACE changed after coppicing (Hoosbeek & Scarascia-Mugnozza, 2009). A recent synthesis concludes that rather than providing a definitive answer on the amount of C stored in CO_2-enriched forests, FACE experiments are most useful for providing process-level information to inform models (Norby & Zak, 2011). We focus the remainder of our discussion on the effects of CO_2-enrichment on C and N mineralization throughout the soil profile.

Hypothesis 2: Increased microbial N immobilization (i.e., decreased net N mineralization) under elevated [CO_2]

Soil N availability exerts strong controls over the production response of forests to CO_2-enrichment (Luo *et al*., 2004), and may limit the accrual of C in plant biomass and soil pools over time (Hungate *et al*., 2003). Microbial communities, which mediate soil C and N dynamics (Hart *et al*., 1994), are expected to respond to greater C inputs under elevated [CO_2] (He *et al*., 2010). To assess the effect of elevated [CO_2] on soil N availability, we measured potential net N mineralization, which in root-free laboratory incubations is the difference between two processes, gross N mineralization and gross N immobilization by microbes. Positive net N mineralization indicated that over the course of the incubation, gross N mineralization was greater than gross N immobilization, whereas the opposite was true for negative net N mineralization. We also estimated the size of the microbial community by quantifying microbial biomass N. Contrary to what we hypothesized, we did not observe a significant stimulation in microbial biomass N after more than 11 years of CO_2-enrichment. Microbial biomass N was ~29% less under elevated [CO_2] when summed over the soil profile (Table 2). Although a previous study at the ORNL FACE site showed no significant differences in bacterial community composition after 10 years of CO_2 enrichment in the top 15 cm of the soil (Austin *et al*., 2009), a decline in microbial biomass N under elevated [CO_2] could be related to a change in microbial function (e.g., He *et al*., 2010), or altered fungal to bacteria ratios. Fungi tend to have wider C/N ratios than bacteria, and therefore less N demand (Hu *et al*., 2001). Ziegler & Billings (2011) observed an increase in fungal biomass under elevated [CO_2] at the Duke FACE experiment, and similar increases in fungal abundance in response to elevated [CO_2] have been seen in other ecosystems (reviewed in Carney *et al*., 2007).

Decreased microbial biomass, or a shift to a more fungal-dominated microbial community, may explain the difference in potential net N mineralization rates under ambient and elevated [CO_2]. In contrast to what we hypothesized, we observed dampened rates of potential net N mineralization and immobilization in the CO_2-enriched plots, although shallow and deep soils exhibited different patterns in some cases. When integrated throughout the rooting zone (0–90 cm), initial extractable NH_4-N was 24% less under elevated [CO_2] (Fig. S1), likely because of decreased net N mineralization rates under elevated [CO_2]. After 29 days of incubation, potential net N mineralization was 47% less throughout the rooting zone under elevated [CO_2] compared with ambient [CO_2], and net nitrification in the CO_2-enriched treatment was a small fraction of that in ambient [CO_2] during the same time period. After 243 days, NH_4^+ immobilization was 64% less under elevated [CO_2] compared with ambient [CO_2] (Table 2).

Declining soil N pools in other CO_2-enrichment experiments in forested ecosystems (i.e., Hoosbeek & Scarascia-Mugnozza, 2009; Langley *et al*., 2009) have been presented as evidence of priming of the decomposition

of SOM, resulting in increased net N mineralization and sustained increases in forest production under elevated [CO_2]. The converse may be true at ORNL FACE. Increasing N content in relatively recalcitrant or protected SOM pools over time could potentially be the cause of changes in stand N dynamics at ORNL FACE, including a decrease in microbial community biomass (Table 2) or a shift to a more fungal-dominated community, declining N availability (Garten *et al*., 2011), and progressive N limitation of forest production under elevated [CO_2] (Norby *et al*., 2010).

We did indeed observe increases in the N content of most root and soil pools under elevated [CO_2], especially in shallower soil (Table 1), although increased N content was somewhat surprising given limited soil N availability at this site (Iversen & Norby, 2008). The increase in soil N content concurrent with soil C has been associated with the protection of organic matter in soil aggregates (Jastrow *et al*., 2005). An exception was the root-derived POM pool, where we observed a 10–30% increase in C/N under elevated [CO_2] from 5 to 60 cm soil depth (Table 1), which allowed soil C accumulation in POM without additional N inputs (i.e., Hungate *et al*., 2003). The increase in POM C/N under elevated [CO_2] was somewhat larger than the increase observed for leaf litter C/N across a range of CO_2-enrichment experiments (~7%, Norby *et al*., 2001a), and could be related to diameter-dependent variation in fine-root C/N (i.e., root C/N ratio is strongly and positively related with root diameter, Iversen *et al*., 2008), or the mining of N to support increased plant N uptake (i.e., Norby *et al*., 2010). In both treatments, the C/N ratio of whole-soil generally declined with soil depth, approaching an average microbial biomass C/N (~8.5, Cleveland & Liptzin, 2007), indicating that SOM was highly processed in deeper soil.

In contrast to the C/N ratio of soil pools, the C/N ratio of fine roots increased with soil depth (Table 1), likely because deeper roots were able to acquire less N per unit of C investment (Eissenstat *et al*., 2000) due to relatively smaller gross N fluxes in deeper soil (Iversen *et al*., 2011). We did not observe an increase in root diameter with depth (*data not shown*) that would explain the increase in root C/N in deeper soil (i.e., Iversen *et al*., 2008). Also, changes in fine-root C/N under elevated [CO_2] were more variable than that of POM, and depended on the soil depth (i.e., increased root C/N in shallow soil, and decreased root C/N in deep soil). Ultimately, the N content of the fine-root pool was greater overall under elevated [CO_2] because of greater root biomass. Surprisingly, the ^{13}C signature of fine roots was less depleted under elevated [CO_2] in deeper soils relative to shallower soils. An increase in the ^{13}C of fine roots in deeper soil may be due to the presence of older C in deeper roots, either because the fine-root population at depth was relatively long-lived (Gaudinski *et al*., 2001), or because new roots at depth were produced in part with labile prefumigation C that was stored in belowground woody structures (e.g., Langley *et al*., 2002; Vargas *et al*., 2009). It is also possible that dead roots were more difficult to differentiate from living roots in deeper soil due to decreased root decomposition, and therefore decreased degradation and fragmentation of the root tissue. While interesting in the context of root turnover and plant C storage pools, differences in root $\delta^{13}C$ with soil depth do not affect the assumptions of the current study. The $\delta^{13}C$ of roots was used solely for calculations of FACE-derived C, and these calculations were done separately for each depth increment. If we had assumed that the $\delta^{13}C$ of roots throughout the soil profile in the CO_2-enriched plots was equal to that of shallow roots, we would have underestimated the amount of FACE-derived C at depth.

Hypothesis 3: Increased potential C mineralization rates under elevated [CO_2]

Contrary to what we hypothesized, there was no significant increase in potential C mineralization rates under elevated [CO_2] (Fig. 2), although there was a trend toward somewhat greater rates in shallower soil in the CO_2-enriched treatment in the first few weeks of the incubation (Table S2). An *in situ* experiment also found that the decomposition rate of 60-cm long birch dowels decaying in the soil over a period of 82 weeks was not greater in the CO_2-enriched plots compared with the ambient [CO_2] plots (Fig. S2). Ultimately, relatively small increases in C mineralization rates in the CO_2-enriched plots were not enough to compensate for additional C inputs, resulting in the accumulation of FACE-derived C throughout the soil profile under elevated [CO_2] after more than 11 years of CO_2-enrichment (Table 1).

The significantly depleted $\delta^{13}C$ of CO_2 mineralized in the laboratory incubations of soil sampled from the elevated [CO_2] treatment at 0–45 cm soil depth relative to soil sampled from the ambient [CO_2] at 0–45 cm soil depth indicates that FACE-derived C was utilized by microbes during the incubation (Fig. 1). However, the steady enrichment of the $\delta^{13}CO_2$ (i.e., the C mineralized) over time in laboratory incubations of soil sampled from the elevated [CO_2] treatment indicates a gradual shift toward the decomposition of older C sources as labile FACE-derived C was consumed. In contrast to shallower soil, relatively little FACE-derived C was mineralized in the laboratory incubations of soil sampled from deeper in the soil profile in the elevated

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[CO_2] treatment (Fig. 2). The lack of a FACE-derived C signal at depth is particularly surprising given the large fraction of new C in the relatively labile POM pool in deeper soil (Table 1). The highly enriched $\delta^{13}C$ of C mineralized in the laboratory incubations (i.e., $\delta^{13}CO_2$) relative to the $\delta^{13}C$ of SOM sampled from deeper soil indicates that microbial-derived compounds, which tend to be more enriched in ^{13}C (Bostrom *et al*., 2007), were the main source of C mineralized at depth. Taken together with our observations of increased root inputs to deeper soil, and depleted $\delta^{13}C$ in deeper POM pools, the fact that FACE-derived C was not mineralized from deeper soil indicates that soil C accrual was likely occurring at depth, albeit at slower rates than in surface soil.

The processing and mineralization of organic matter differs between shallow and deeper soil depths (Rumpel & Kogel-Knabner, 2011). Although soil C content (10-mm fraction) predicted 92% of cumulative C mineralization at the 0–30 cm depth increment (Fig. 3), cumulative C mineralization deeper than 30 cm was not related to soil C content (Fig. 3, insert). This finding indicates that microbial C mineralization at deeper soil depths is limited by something other than soil C, which could be a function of several interacting factors. Low microbial biomass combined with small and spatially segregated inputs of root-derived C could limit the rate at which microbial exoenzymes encountered organic substrates (Rumpel & Kogel-Knabner, 2011); poorer root litter quality deeper in the soil profile (i.e., increased root C/N ratios, Table 1) could limit root decomposition rates (Gholz *et al*., 2000); or increased soil clay content in deeper soil at ORNL FACE (M. Mayes, pers. comm., Mayes *et al*., in press) could limit the decomposability of relatively labile organic material (Rumpel & Kogel-Knabner, 2011). C mineralization rates in deeper soil ranged from one-hundredth to one-half of those in shallower soil (Fig. 2; Tables S2 and S4; Fig. S2).

Differences in the mechanisms of C cycling in deeper soil have implications for C storage under elevated [CO_2]. Root inputs to deep soil, which have been observed to be relatively greater under elevated [CO_2] at ORNL FACE (Iversen *et al*., 2008) and in nearly three-quarters of other CO_2-enrichment experiments in forests (Iversen, 2010), may not be decomposed as quickly as root inputs in shallower soil horizons. Although the amount of root inputs and the C accrued in deeper soil were small relative to shallower soil and more difficult to quantify, C inputs to deeper soil may be one potential mechanism whereby ecosystem C storage is increased under elevated [CO_2]. However, most land surface models simulate soil C dynamics as one homogenous layer that does not capture changes in the processing of C with depth in the soil (Iversen, 2010). Based on our calculations, which indicate decreased C turnover in deeper soil (Table S4), models that use a decomposition rate based on the shallow soil profile (e.g., Parton *et al*., 1988) would underestimate annual C accumulation in the POM pool by ~18 g m^{-2} $year^{-1}$ over the soil profile to 90 cm (steady-state decomposition rates calculated as in Lichter *et al*., 2005). This underestimation is approximately one-half of annual C accumulation in land actively managed for C sequestration (Post & Kwon, 2000). Therefore, a separate representation of soil C dynamics at deeper soil depths may increase the accuracy of model projections of the potential of future forests to store C and mitigate some portion of rising atmospheric [CO_2].

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References

Austin EE, Castro HF, Sides KE, Schadt CW, Classen AT (2009) Assessment of 10 years of CO_2 fumigation on soil microbial communities and function in a sweetgum plantation. *Soil Biology and Biochemistry*, **41**, 514–520.

Bonan GB (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science*, **320**, 1444–1449.

Bostrom B, Comstedt D, Ekblad A (2007) Isotope fractionation and C-13 enrichment in soil profiles during the decomposition of soil organic matter. *Oecologia*, **153**, 89–98.

Cambardella CA, Elliott EA (1993) Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. *Soil Science Society of America Journal*, **57**, 1071–1076.

Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. *Proceedings of the National Academy of Sciences*, **104**, 4990–4995.

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, **85**, 235–252.

Day FP, Stover DB, Pagel AL *et al.* (2006) Rapid root closure after fire limits fine root responses to elevated atmospheric CO_2 in a scrub oak ecosystem in central Florida, USA. *Global Change Biology*, **12**, 1047–1053.

Drake JE, Gallet-Budynek A, Hofmockel KS *et al.* (2011) Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters*, **14**, 349–357.

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL (2000) Building roots in a changing environment: implications for root longevity. *New Phytologist*, **147**, 33–42.

Fontaine S, Barot S, Barre P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, **450**, 277–281.

Francey RJ, Allison CE, Etheridge DM, *et al.* (1999) A 1000-year high precision record of delta C-13 in atmospheric CO_2. *Tellus Series B*, **51**, 170–193.

Garten CT (2009) A disconnect between O horizon and mineral soil carbon – implications for soil C sequestration. *Acta Oecologica*, **35**, 218–226.

Garten CT, Brice DJ (2009) Belowground fate of N-15 injected into sweetgum trees (*Liquidambar styraciflua*) at the ORNL FACE experiment. *Rapid Communications in Mass Spectrometry*, **23**, 3094–3100.

Garten CT, Iversen CM, Norby RJ (2011) Litterfall ^{15}N abundance indicates declining soil nitrogen availability in a free air CO_2-enrichment experiment. *Ecology*, **92**, 133–139.

Gaudinski JB, Trumbore SE, Davidson EA, Cook AC, Markewitz D, Richter DD (2001) The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. *Oecologia*, **129**, 420–429.

Gholz HL, Wedin DA, Smitherman SM, Harmon ME, Parton WJ (2000) Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biology*, **6**, 751–765.

Grandy AS, Neff JC (2008) Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. *Science of the Total Environment*, **404**, 297–307.

Hart SC, Nason GE, Myrold DD, Perry DA (1994) Dynamics of gross nitrogen transformations in an old-growth forest – the carbon connection. *Ecology*, **75**, 880–891.

He Z, Xu M, Deng Y *et al*. (2010) Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. *Ecology Letters*, **13**, 564–575.

Hoosbeek MR, Scarascia-Mugnozza GE (2009) Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO_2 enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments. *Ecosystems*, **12**, 220–239.

Hoosbeek MR, Lukac M, Velthorst E, Smith AR, Godbold DL (2011) Free atmospheric CO_2 enrichment increased above ground biomass but did not affect symbiotic N_2-fixation and soil carbon dynamics in a mixed deciduous stand in Wales. *Biogeosciences*, **8**, 353–364.

Horwath WR, Paul EA (1994) Microbial biomass. In: *Methods of Soil Analysis: Part 2-Micrbiological and Biochemical Properties* (eds Weaver RW, Angle JS, Bottomley PS), pp. 753–771. Soil Science Society of America, Madison, WI, USA.

Hu S, Chapin FS III, Firestone MK, Field CB, Chiariello NR (2001) Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. *Nature*, **409**, 188–191.

Hungate BA, Dukes JS, Shaw MR, Luo YQ, Field CB (2003) Nitrogen and climate change. *Science*, **302**, 1512–1513.

Iversen CM (2010) Digging deeper: fine-root responses to rising atmospheric CO_2 concentration in forested ecosystems. *New Phytologist*, **186**, 346–357.

Iversen CM, Norby RJ (2008) Nitrogen limitation in a sweetgum plantation: implications for carbon allocation and storage. *Canadian Journal of Forest Research*, **38**, 1021–1032.

Iversen CM, Ledford J, Norby RJ (2008) CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. *New Phytologist*, **179**, 837–847.

Iversen CM, Hooker T, Norby RJ, Classen AT (2011) Net mineralization of N at deeper soil depths as a potential mechanism for sustained forest production under elevated [CO_2]. *Global Change Biology*, **17**, 1130–1139.

Jastrow JD, Miller RM, Matamala R, Norby RJ, Boutton TW, Rice CW, Owensby CE (2005) Elevated atmospheric CO_2 increases soil carbon. *Global Change Biology*, **11**, 2057–2064.

Joergensen RG, Mueller T (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the k(EN) value. *Soil Biology and Biochemistry*, **28**, 33–37.

Johnson DW, Cheng W, Joslin JD, Norby RJ, Edwards NT, Todd DE (2004) Effects of elevated CO_2 on nutrient cycling in a sweetgum plantation. *Biogeochemistry*, **69**, 379–403.

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. *Soil Biology & Biochemistry*, **42**, 1363–1371.

Langley JA, Drake BG, Hungate BA (2002) Extensive belowground carbon storage supports roots and mycorrhizae in regenerating scrub oaks. *Oecologia*, **131**, 542–548.

Langley JA, McKinley DC, Wolf AA, Hungate BA, Drake BG, Megonigal JP (2009) Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. *Soil Biology and Biochemistry*, **41**, 54–60.

Lichter J, Barron SH, Bevacqua CE *et al*. (2005) Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO_2 enrichment. *Ecology*, **86**, 1835–1847.

Luo Y, Su B, Currie WS *et al*. (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience*, **54**, 731–739.

Luo Y, Hui D, Zhang D (2006) Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology*, **87**, 53–63.

Luo Y, Melillo J, Niu S *et al*. (2011) Coordinated approaches to quantify long-term ecosystem dynamics in response to global change. *Global Change Biology*, **17**, 843–854.

von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. *European Journal of Soil Science*, **57**, 426–445.

Mayes MA, Heal K, Brandt CC, Philips JR, Jardine PM (in press) Relation between soil order and sorption of dissolved organic carbon in temperate subsoils. Soil Science Society of America Journal.

Norby RJ, Zak DR (2011) Ecological lessons from free-air CO_2 enrichment (FACE) experiments. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 181–203.

Norby RJ, Cotrufo MF, Ineson P, O'Neill EG, Canadell JG (2001a) Elevated CO_2, litter chemistry, and decomposition: a synthesis. *Oecologia*, **127**, 153–165.

Norby RJ, Todd DE, Fults J, Johnson DW (2001b) Allometric determination of tree growth in a CO_2-enriched sweetgum stand. *New Phytologist*, **150**, 477–487.

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. *Proceedings of the National Academy of Sciences*, **101**, 9689–9693.

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences*, **107**, 19368–19373.

Parton WJ, Stewart JWB, Cole CV (1988) Dynamics of C, N, P and S in grassland soils – a model. *Biogeochemistry*, **5**, 109–131.

Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**, 171–179.

Phillips RP, Finzi AC, Bernhardt ES (2010) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecology Letters*, **14**, 187–194.

Post WM, Kwon KC (2000) Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology*, **6**, 317–327.

Pregitzer KS, Burton AJ, King JS, Zak DR (2008) Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. *New Phytologist*, **180**, 153–161.

Pritchard SG, Strand AE, McCormack ML *et al*. (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air-CO_2-enrichment: a six-year-minirhizotron study. *Global Change Biology*, **14**, 588–602.

Riggs JS, Tharp ML, Norby RJ (2010) *ORNL FACE CO_2 Data*. Carbon Dioxide Information Analysis Center, Oak Ridge, TN, USA. Available at: http://cdiac.ornl.gov (accessed 20 October 2011).

Rumpel C, Kogel-Knabner I (2011) Deep soil organic matter – a key but poorly understood component of terrestrial C cycle. *Plant and Soil*, **338**, 143–158.

Sparling GP, West AW, Whale KN (1985) Interference from plant roots in the estimation of soil microbial ATP, C, N and P. *Soil Biology and Biochemistry*, **17**, 275–278.

Talhelm AF, Pregitzer KS, Zak DR (2009) Species-specific responses to atmospheric carbon dioxide and tropospheric ozone mediate changes in soil carbon. *Ecology Letters*, **12**, 1219–1228.

Vargas R, Trumbore SE, Allen MF (2009) Evidence of old carbon used to grow new fine roots in a tropical forest. *New Phytologist*, **182**, 710–718.

Ziegler SE, Billings SA (2011) Soil nitrogen status as a regulator of carbon substrate flows through microbial communities with elevated CO_2. *Journal of Geophysical Research*, **116**, G01011.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Extractable (in 2 M KCl) NH_4-N and NO_3-N throughout the soil profile under ambient and elevated [CO_2].
Figure S2. Decomposition of standard substrate as a function of depth at ORNL FACE using birch wood (Betula sp.) dowels.
Table S1. Initial soil characteristics.
Table S2. Potential C mineralization rates measured throughout the 243-day soil incubation.
Table S3. The effects of refrigeration length on extractable (in 2 M KCl) soil N as well as potential C mineralization rates.
Table S4. Steady-state decomposition rates of the particulate organic matter (POM) pool by soil depth increment.

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