

Soil carbon sequestration in a pine forest after 9 years of atmospheric CO₂ enrichment

JOHN LICHTER*, SHARON A. BILLINGS†, SUSAN E. ZIEGLER‡, DEEYA GAINDH*, REBECCA RYALSS§, ADRIEN C. FINZI¶, ROBERT B. JACKSON||, ELIZABETH A. STEMMLER** and WILLIAM H. SCHLESINGER††

*Environmental Studies Program, Biology Department, Bowdoin College, Brunswick, ME 04011, USA, †Department of Ecology and Evolutionary Biology, Kansas Biological Survey, University of Kansas, Lawrence, KS 66047, USA, ‡Department of Earth Sciences, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada A1B 3X5, §Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA, ¶Department of Biology, Boston University, Boston, MA 02215, USA, ||Department of Biology, Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA, **Chemistry Department, Bowdoin College, Brunswick, ME 04011, USA, ††Cary Institute of Ecosystem Studies, Millbrook, NY 12545, USA

Abstract

The impact of anthropogenic CO₂ emissions on climate change may be mitigated in part by C sequestration in terrestrial ecosystems as rising atmospheric CO₂ concentrations stimulate primary productivity and ecosystem C storage. Carbon will be sequestered in forest soils if organic matter inputs to soil profiles increase without a matching increase in decomposition or leaching losses from the soil profile, or if the rate of decomposition decreases because of increased production of resistant humic substances or greater physical protection of organic matter in soil aggregates. To examine the response of a forest ecosystem to elevated atmospheric CO₂ concentrations, the Duke Forest Free-Air CO₂ Enrichment (FACE) experiment in North Carolina, USA, has maintained atmospheric CO₂ concentrations 200 µL L⁻¹ above ambient in an aggrading loblolly pine (*Pinus taeda*) plantation over a 9-year period (1996–2005). During the first 6 years of the experiment, forest-floor C and N pools increased linearly under both elevated and ambient CO₂ conditions, with significantly greater accumulations under the elevated CO₂ treatment. Between the sixth and ninth year, forest-floor organic matter accumulation stabilized and C and N pools appeared to reach their respective steady states. An additional C sink of ~30 g C m⁻² yr⁻¹ was sequestered in the forest floor of the elevated CO₂ treatment plots relative to the control plots maintained at ambient CO₂ owing to increased litterfall and root turnover during the first 9 years of the study. Because we did not detect any significant elevated CO₂ effects on the rate of decomposition or on the chemical composition of forest-floor organic matter, this additional C sink was likely related to enhanced litterfall C inputs. We also failed to detect any statistically significant treatment effects on the C and N pools of surface and deep mineral soil horizons. However, a significant widening of the C:N ratio of soil organic matter (SOM) in the upper mineral soil under both elevated and ambient CO₂ suggests that N is being transferred from soil to plants in this aggrading forest. A significant treatment × time interaction indicates that N is being transferred at a higher rate under elevated CO₂ ($P = 0.037$), suggesting that enhanced rates of SOM decomposition are increasing mineralization and uptake to provide the extra N required to support the observed increase in primary productivity under elevated CO₂.

Keywords: acid hydrolysis, carbon sequestration, ¹³C stable isotope, drought effects, FACE experiment, forest floor, ice-storm effects, loblolly pine, particle-size fractions, progressive nitrogen limitation, soil carbon sink, soil organic matter

Received 13 March 2008 and accepted 12 May 2008

Correspondence: John Lichter, tel. +1 207 725 3653, fax +1 207 725 3405, e-mail: jlichter@bowdoin.edu

Introduction

Forest soils may function as dynamic sinks for anthropogenic CO₂ if enhanced primary productivity associated with rising atmospheric CO₂ concentrations increases organic matter inputs to soils without corresponding increases in decomposition (Lichter *et al.*, 2005). Soil C sinks may also develop if organic matter inputs are sequestered in chemically recalcitrant compounds (Davies *et al.*, 2001; Hoosbeek *et al.*, 2007) or are physically protected within soil aggregates (Christensen, 1996; Jastrow *et al.*, 1996, 2005). Experimental studies have shown enhanced C sinks in the soil organic matter (SOM) of a prairie ecosystem as rates of microaggregate formation increased under elevated CO₂ (Jastrow *et al.*, 2000, 2005; Williams *et al.*, 2000, 2004). However, other studies have failed to detect significant changes in soil C in response to elevated CO₂ (Hungate *et al.*, 1996, 1997; Hagedorn *et al.*, 2003; Lichter *et al.*, 2005), or have demonstrated soil C loss when microbial respiration and decomposition accelerated with changes in microbial community composition or activity (Heath *et al.*, 2005; Carney *et al.*, 2007; Billings & Ziegler, 2008). These different results suggest that the source–sink dynamics of soils may depend on inherently variable local factors that make generalization difficult. Nevertheless, a fundamental understanding of the capacity of soils for long-term sequestration of atmospheric C is necessary for predicting ecosystem response to rising atmospheric CO₂ concentrations and the potential feedbacks to the global C cycle (Luo *et al.*, 2003, 2006).

In a recent review of atmospheric CO₂ experiments across a range of ecosystem types, Van Groenigen *et al.* (2006) concluded that significant C accumulation in soils required rates of N supply well above typical inputs derived from atmospheric deposition, and that biological N fixation was an unlikely source of additional N in most ecosystems because of other nutrient constraints. This result is consistent with studies of ecosystem recovery following decades of agricultural SOM depletion, showing that soil C accumulation is controlled by the availability of N from atmospheric deposition and symbiotic N fixation (Knops & Tilman, 2000). Sequestration of C in soils is, thus, likely to be constrained by the availability of exogenous sources of N (Hungate *et al.*, 2003).

Several researchers have postulated that, in general, the productivity response to elevated CO₂ should also be constrained by the availability of N or other limiting soil nutrients (Diaz *et al.*, 1993; Melillo *et al.*, 1993; Rastetter *et al.*, 1997). Because N is an intrinsic component of organic matter, C sequestration in long-lived plant tissues and soils will also sequester N, possibly

making it unavailable for plant uptake and growth. Progressive N limitation (PNL) may, therefore, accompany C sequestration in plants and soils stimulated by CO₂ fertilization, gradually attenuating the CO₂ response (Luo *et al.*, 2004; Finzi *et al.*, 2006; Johnson, 2006). Mechanisms that might alleviate or delay PNL include increased nutrient-use efficiency of plants as indicated by higher C:N ratios of plant tissue (Vitousek, 1982; Calfapietra *et al.*, 2007), mining of N from SOM pools of low C:N ratio and transfer to plants (Rastetter *et al.*, 1997; Luo *et al.*, 2004), and generally increased rates of microbial activity in soils that can result in increased availability of N to plants. There is evidence that each of these mechanisms may alleviate PNL in ecosystems under elevated CO₂, at least temporarily. Widening ecosystem C:N ratios have been observed under elevated CO₂ in woodlands and forests (e.g. Finzi *et al.*, 2006; Hungate *et al.*, 2006; Norby & Iversen, 2006). Mining of soil N from low C:N pools has been observed in a scrub oak woodland (Hungate *et al.*, 2006) and in a grassland ecosystem (Gill *et al.*, 2006), and isotopic evidence suggests increases in rates of microbial activity influencing plant N supplies in a desert ecosystem exposed to elevated CO₂ (Billings *et al.*, 2002, 2004).

The Duke Forest Free-Air CO₂ Enrichment (FACE) experiment was the first of several ecosystem-scale experiments designed to explore the effects of CO₂ fertilization on plant productivity, water relations, and biogeochemical cycling. The experiment was constructed in an aggrading loblolly pine plantation. After 6 years of CO₂ fumigation, significant increases in tree growth (DeLucia *et al.*, 2005), primary productivity (Finzi *et al.*, 2002; Norby *et al.*, 2005), forest-floor C sequestration (Lichter *et al.*, 2005), root growth and turnover (Matamala & Schlesinger, 2000; Matamala *et al.*, 2003; Pritchard *et al.*, 2008), and soil respiration (Bernhardt *et al.*, 2006; Taneva *et al.*, 2006) were observed in response to the elevated atmospheric CO₂ treatment. Even after 9 years of experimental CO₂ fertilization, attenuation of the CO₂-induced productivity enhancement had not been observed (Finzi *et al.*, 2006). Finzi *et al.* (2007) conducted a N mass-balance study of several FACE experiments and concluded that increased N demand accompanying CO₂ fertilization at the Duke Forest and elsewhere was met through greater uptake from soils. Consistent with this result, Billings & Ziegler (2005, 2008) reported increased activity of microorganisms adept at mineralizing recalcitrant SOM pools under N limitation. Here, we present time series measurements covering the first 9 years of experimental CO₂ fertilization (1996–2005) to document changes in forest-floor C and N content, mineral-soil C and N content, aboveground litterfall, C turnover, and

in the chemistry of forest floor and SOM. Our results extend earlier studies quantifying soil C and N content and turnover by 3 years at the Duke Forest FACE experiment to better understand the capacity of forests to sustain enhanced productivity and C sequestration under elevated CO₂ (i.e. Schlesinger & Lichter, 2001; Lichter *et al.*, 2005).

Materials and methods

Site description and experimental design

The Duke Forest FACE experiment was constructed in a 15-year-old stand of loblolly pine (*Pinus taeda*) in Chapel Hill, NC, USA. Before planting 3-year-old seedlings in 1983, the 32 ha site was logged of 40- to 60-year-old mixed stands of loblolly pine and Virginia pine (*Pinus virginiana*), and was drum-chopped and burned. Since then, sweet gum (*Liquidambar styraciflua*), red maple (*Acer rubrum*), red bud (*Cercis canadensis*), and dogwood (*Cornus florida*) have established from seed and re-sprouted from stumps throughout the site; however, loblolly pine comprises 98% of the aboveground biomass. Soils are clay loams of the Enon series described as Ultic alfisols derived from mafic bedrock (US Department of Agriculture, 1977). They are relatively homogeneous, acidic, and have well-developed profiles of mixed clay mineralogy. The topsoil is underlain by 5 m of saprolite, below which lies highly fractured granodiorite or diorite bedrock (Andrews & Schlesinger, 2001). The elevation ranges up to 15 m across the 32 ha site with local topographic relief generally <1°. The static water table lies at 6 m depth, but the site drains poorly and surface soils are often saturated during periods of high precipitation. The mean annual temperature is 15.5 °C and the mean annual precipitation is 1140 mm.

Beginning on August 27, 1996, the elevated atmospheric CO₂ treatment was maintained continuously 24 h day⁻¹ at 200 µL L⁻¹ above ambient in three 30 m diameter circular treatment plots (Hendrey *et al.*, 1999). Three identical control plots were maintained at ambient atmospheric CO₂ levels. Starting in December 2002, the elevated CO₂ treatment was reduced to daylight hours only. The fumigation CO₂ derives from natural gas and consequently has a strongly depleted δ¹³C signature (i.e. -43.0 ± 0.6), where

$$\delta^{13}\text{C} = \left[\frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{reference}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{reference}}} \right] \times 1000.$$

We traced the incorporation of new C into SOM by change in the δ¹³C signature to estimate the turnover rates of C in bulk mineral soils and SOM pools.

Sample collection and laboratory analysis

Samples of the forest-floor and upper mineral soil (0–15 cm depth) were collected in August 1996, October 1999, August 2002, and August 2005. The October 1999 sampling was delayed by Hurricane Frank. During each sample collection, forest-floor samples were separated from the mineral soil by hand, and mineral soil samples were retrieved in a 4.76 cm diameter soil core driven with a slide hammer at stratified random positions within each of the six FACE plots. All samples were dried at 50 °C for 5 days before weighing. For the August 2005 sampling, the forest-floor samples were subsequently divided into Oi and Oa subhorizons before chemical analyses. The mineral soils were dried and sieved with a 2 mm brass screen to remove stones and coarse roots, and the volume and mass of the stones were determined for each sample to estimate field bulk density (g cm⁻³). Coarse roots were removed manually from the forest-floor samples, which were ground with a ball mill to a fine powder for chemical analyses. The sieved mineral soil samples were subsampled, and the free light fraction (plant residues) was removed via density fractionation. To do so, a well-mixed 5 g subsample of mineral soil was suspended in 35 mL of 1.85 g cm⁻³ polytungstate solution and centrifuged for 60 min (Christensen, 1992; Cambardella & Elliott, 1993; Lichter *et al.*, 2005). The free light fraction floating on the solution surface was removed by aspiration, rinsed, and dried at 105 °C before chemical analysis.

The forest-floor and bulk mineral soil samples were analyzed for %C, %N, ¹³C, and ¹⁵N by the Stable Isotope Laboratory at the University of California, Davis. Stable isotope ratios of C and N were measured by continuous flow isotope ratio mass spectrometry (IRMS) with a 20–20 mass spectrometer (PDZEuropa, Northwich, UK) after sample combustion to CO₂ and N₂ at 1000 °C in an on-line elemental analyzer (PDZEuropa ANCA-GSL). The gases were separated on a Carbosieve G column (Supelco, Bellefonte, PA, USA) before introduction to the IRMS. The working standards used were a mixture of ammonium sulfate and sucrose, which were periodically calibrated against international isotope standards (i.e. Pee Dee Belamnite, PDB).

We characterized organic matter from the forest floor and the free light fraction of the upper mineral soil with tetramethylammonium hydroxide thermochemolysis gas chromatography (Frazier *et al.*, 2003). Chemical signatures for lignin-derived compounds, carbohydrate-derived compounds, and membrane fatty acids were quantified from the forest-floor Oa and Oi subhorizons, as well as the free light fraction of upper mineral soil. Compounds characteristic of the three compound classes were quantified using measurements of

peak area relative an internal standard (i.e. eicosane; Sigma-Aldrich, Milwaukee, WI, USA).

In late October and early November of 2005, a second set of mineral soil samples was collected for particle-size separation and chemical fractionation of SOM. These samples were collected from the six plots described above and from two additional plots: a 'prototype' plot that has been exposed to elevated CO₂ since 1994, and its counterpart control. A 5 cm diameter soil core was driven from the surface of the mineral soil to a depth of 30 cm in four random locations within each FACE plot. The samples from each plot were composited, dried, and sieved with a 2 mm screen. The fine roots remaining in the sieved soils were removed by hand.

For particle-size fractionation, 25 g subsamples were separated into water-stable aggregates by mixing with 75 mL of deionized water and agitating vigorously. The slurry mixtures were then wet-sieved through a series of sieves (2000, 500, and 53 µm) into a receiving pan for 30 min on a shaker (New Brunswick Scientific, Edison, NJ, USA). Each fraction was washed and decanted onto the sieve(s) 10 times to ensure complete separation of the three size fractions. Sieve size was chosen based on Schlesinger & Lichter (2001), who assessed bulk soil and SOM size fraction δ¹³C values at this site to trace new C inputs into SOM pools after 3 years of exposure to elevated CO₂. We compared δ¹³C values and new C incorporation in SOM pools among samples collected at 3 years (Schlesinger & Lichter, 2001) and 9 years (this study).

For the chemical fractionation, a second set of 10 g subsamples from the late 2005 sampling was subjected to acid hydrolysis (Leavitt *et al.*, 1996). Plant residues were removed by immersing subsamples in a NaCl (1.2 g cm⁻³) solution, mixing, and picking out the floating material. The remaining soil was washed free of salt, dried, and then examined under a 20 × microscope. Visible plant parts were removed via forceps. Removal of recognizable plant material was critical to limit the influence of the relatively ¹³C-depleted organic matter of new plant tissue on the δ¹³C signatures (Leavitt *et al.*, 1996). Subsamples of the plant-free soils weighing 3 g were subjected to 18 h of acid hydrolysis with boiling 6 N HCl (NF/FCC grade, Fisher Scientific, Pittsburgh, PA, USA). After cooling, the acid-soil mixture was filtered and the residual soil, designated as the nonhydrolyzable fraction, was washed and prepared for chemical analysis. The supernatant was heated to evaporate the liquid. The remaining material, designated as the hydrolyzable fraction, was washed and prepared for chemical analysis. Soil fractions from both physical and chemical fractionation methods along with bulk soil subsamples were dried at 70 °C for at least

48 h. We weighed all SOM fractions to calculate the percentage of total soil mass in each, and pulverized the bulk soil subsamples and soil fractions to a fine powder. The SOM fractions and bulk soils were analyzed by the stable isotope laboratory at the University of Arkansas for %C and δ¹³C with a Finnigan Delta⁺ mass spectrometer (Finnigan MAT, Bremen, Germany) coupled to a Carlo Erba elemental analyzer (NA1500 CHN Combustion Analyzer, Carlo Erba Strumentazione, Milan, Italy) via a Finnigan Conflo II Interface (Bremen, Germany). Samples were analyzed using plant leaf (NIST 1547 and NIST 1570A) and low organic soil reference standards in conjunction with working standards of acetanilide and spinach leaves. The δ¹³C values were measured relative to high purity, reference gas standards expressed relative to international standard PDB.

A third set of soil samples was collected for comparison with deep soil samples obtained during the construction of the FACE apparatus. In 1996, excavation for the FACE towers offered an opportunity to sample the entire soil profile. Samples collected at 0–7.5, 7.5–15, 15–35, and 60–90 cm depths were sieved (2 mm), dried, and archived. In February 2005, four soil cores (4.76 cm diameter) were collected from random positions beneath the boardwalks within each of the six FACE plots. Each core was divided into sections of 0–25, 25–50, 50–75, 75–100, 100–125, and 125–150 cm depth. Samples were sieved, dried, and prepared for chemical analyses as were the upper mineral soil samples (0–15 cm depth) described above.

Forest-floor dynamics

The mean turnover rate (k) of soil horizons under nonsteady-state conditions can be estimated from

$$C_t = C_0 e^{-kt} + \frac{I}{k} (1 - e^{-kt}), \quad (1)$$

where C_t is the C content at time t , C_0 is the initial carbon content, I is the mean annual C input from litterfall and root turnover, and k is the decomposition constant (Davidson & Hirsch, 2001; Schlesinger & Lichter, 2001). We measured C_t , C_0 , and I directly for the forest floor, and solved for k by iteration for each of the six FACE plots. The value of k derived in this manner represents the proportion of organic matter that decomposes each year for the entire forest floor (e.g. Lichter *et al.*, 2005). It is not directly comparable with the value of k derived from litterbag studies because it summarizes the average annual decomposition rate for the entire forest floor including relatively new litter and older, partially decomposed organic matter. The mean residence time (MRT) is $1/k$, and the predicted steady-state C content (C^*) is I/k . Total decomposition of

insoluble organic C occurring in each FACE plot over the 9-year time period was calculated as:

$$\sum \text{Decomposition} = C_0 + \sum I - C_t, \quad (2)$$

A comparison of the total decomposition of insoluble organic C between elevated CO₂ and control plots indicates whether microbial decomposition was altered by the elevated CO₂ treatment.

To estimate carbon inputs to the forest floor, we summed monthly litterfall measurements over the 9-year time period and multiplied litter dry weight by mean %C. Because we do not have reliable estimates of fine-root turnover in the forest floor that excluded the upper mineral soil, we relied solely on measurements of aboveground litter production to estimate carbon inputs to the forest floor. Using mini-rhizotrons, Pritchard *et al.* (2008) estimated fine-root turnover of $\sim 78 \text{ g C m}^{-2} \text{ yr}^{-1}$ under elevated and $62 \text{ g C m}^{-2} \text{ yr}^{-1}$ under ambient CO₂ in the upper 30 cm of soil including the forest floor for a 6-year period 1998–2004. Of these amounts, $< 10 \text{ g C m}^{-2} \text{ yr}^{-1}$ was likely to accumulate in the forest floor based on fine-root profile information, whereas aboveground litterfall averaged across the treatment plots was two orders of magnitude greater (i.e. $382 \pm 22.9 \text{ g C m}^{-2} \text{ yr}^{-1}$).

We used information about change in the $\delta^{13}\text{C}$ ratio of the forest floor and estimates of the $\delta^{13}\text{C}$ ratio of litterfall and new roots to validate our estimates of C turnover in the forest floor. After estimating k for each of the elevated rings, we weighted Eqn (1) with estimates of the $\delta^{13}\text{C}$ of the original C pool, the $\delta^{13}\text{C}$ of the C pool after 9 years, and the average $\delta^{13}\text{C}$ of C inputs during the 9-year period [i.e. $C_t(\delta^{13}\text{C}_t) = C_0 e^{-kt}(\delta^{13}\text{C}_0) + (I/k)(1 - e^{-kt})(\delta^{13}\text{C}_{\text{new}})$] to estimate the $\delta^{13}\text{C}$ ratio of the forest floor for the elevated rings at the end of 9 years. These estimates were then compared with our measurements of the mean forest-floor $\delta^{13}\text{C}$ for the elevated CO₂ rings as a check on the estimates of forest-floor C turnover under the elevated CO₂ treatment.

Mineral SOM dynamics

For the bulk mineral soil, the $\delta^{13}\text{C}$ value of the fumigation gas allows an indirect estimation of the steady-state C turnover and annual C inputs for the elevated CO₂ rings. The fumigation gas rapidly becomes incorporated into plant biomass and begins to alter the $\delta^{13}\text{C}$ value of C inputs to the soil as a result of root turnover (Matamala *et al.*, 2003). Unlike the forest floor, we do not have direct estimates of the annual C inputs, I , for the bulk mineral soil, which with estimates of C pools would allow us to derive an estimate of k . However, we can indirectly estimate k from the change in $\delta^{13}\text{C}$ value of SOM in the elevated CO₂ rings, and then

multiply this estimate by the C pool size to estimate the inputs required to produce the observed change in $\delta^{13}\text{C}$. In doing so, we assume that the C pools are in steady state in terms of k and I . See Lichter *et al.* (2005) for the methods of inferring C turnover based on change in the $\delta^{13}\text{C}$ values of SOM.

Statistical analyses

The mean of samples collected within each FACE plot was considered the experimental unit for all statistical analyses testing for an elevated CO₂ treatment effect. The sample size is therefore only three or four per treatment for these analyses, which provides limited statistical power. However, the development of time series over several years should help detect significant trends in soil properties. We used both linear and non-linear mixed-effects models to describe change in soil properties over time and to test for statistically significant treatment effects. Mixed-effects models are useful for analyzing longitudinal, repeated measures, and multilevel data (Pinheiro & Bates, 2000). They incorporate both fixed and random effects and model the covariance structure related to repeated measurements of the experimental unit over time. For our analyses, the experimental treatment and year (i.e. change over time) were fixed effects, and variation in measurements of the six experimental rings over time was considered a random effect. For the forest-floor properties, we used a nonlinear asymptotic model to compare the asymptote, initial conditions, and rate of increase between elevated and control rings. For the mineral soil properties, we used a linear model in which the fixed effects were crossed as a factorial (i.e. time \times treatment). The P -value for the time \times treatment interaction term indicated the influence of the elevated CO₂ treatment on the response variable during the period of the experiment. Statistical analyses were performed with S-PLUS v. 8.0 (Insightful Corporation, Seattle, WA, USA) (Insightful Corporation, 1988, 2007). A self-starting asymptotic model command (i.e. SSasympt) in S-PLUS or R was used to fit the forest-floor time series for each experimental FACE ring. The mean parameter estimates of elevated and ambient CO₂ rings were subsequently compared with a t -test.

Results

Forest-floor C dynamics

During the first 6 years of the experiment, forest-floor mass, C content, and N content increased linearly under both elevated and ambient CO₂, and a significant treatment \times time interaction indicated that organic matter

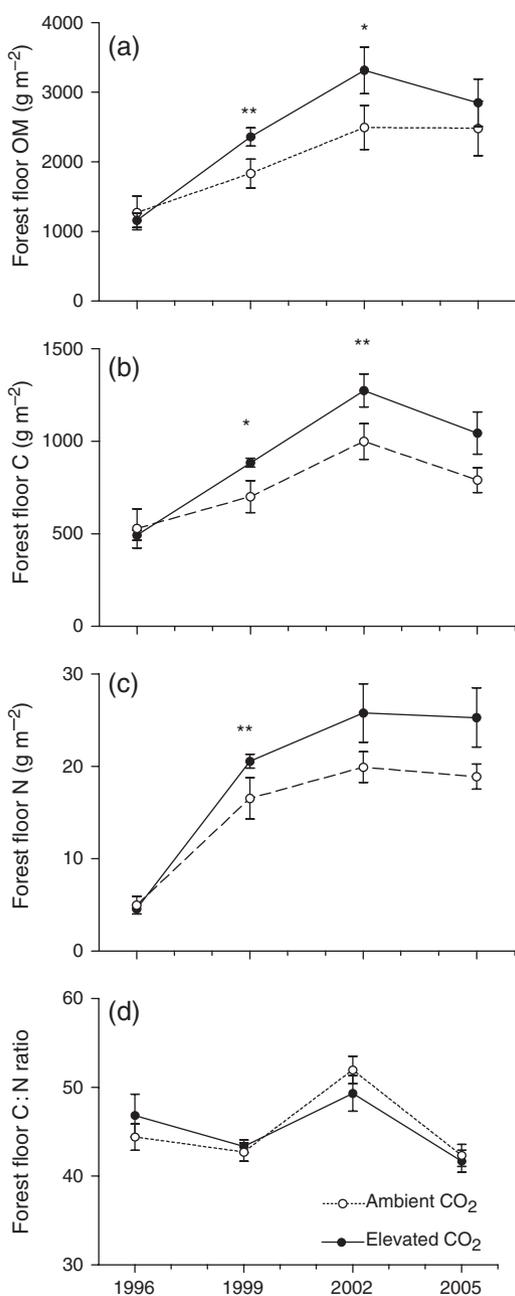


Fig. 1 Time series illustrating changes in forest-floor mass (a), C content (b), N content (c), and C:N ratio (d) between 1996 and 2005.

accumulated more rapidly under the elevated CO₂ treatment (Fig. 1a–c). Between years 6 and 9, these forest-floor properties leveled off, each appearing to reach its respective steady state. Nonlinear mixed-effects analyses showed that treatment effects influencing the estimated steady-state asymptotes and rates of increase were not statistically significant (Table 1). The C:N ratio of the forest floor under both elevated and ambient CO₂ concentrations fluctuated without a

discernable treatment effect (Fig. 1d). Consistent with a first-order exponential model describing incorporation of new C into the forest floor, the $\delta^{13}\text{C}$ signature of the forest floor of elevated CO₂ rings decreased by a smaller amount during each successive 3-year sampling period (Fig. 2).

Annual C inputs to the forest floor increased significantly during the 9-year period in both elevated and ambient CO₂ rings; however, the time \times treatment interaction illustrating the effect of the elevated CO₂ treatment was only statistically significant through the first 6 years of the experiment (Fig. 3a; Lichter *et al.*, 2005). Cumulative C inputs summed over the entire time series were greater under elevated CO₂ with an average annual increase of litterfall of $48 (\pm 14) \text{ g C m}^{-2}$ (Fig. 3b). Estimates of total decomposition, turnover rate (k), MRT, and steady-state C content derived from Eqns (1) and (2) along with estimates of the C content and inputs to the forest floor are given for each of the six FACE plots (Table 2a). In contrast to the results after 6 years of experimental CO₂ fumigation (Lichter *et al.*, 2005), there were no statistically significant treatment effects for total decomposition, k , MRT, or the steady-state C content of the forest floor after 9 years of CO₂ fumigation (Table 2b). Using change in the $\delta^{13}\text{C}$ signature of the forest floor under elevated CO₂ (Fig. 2) along with Eqn (1), we checked our estimate of k for the elevated CO₂ plots and found a good agreement with the mean value of k estimated for the elevated CO₂ plots from measurements of C inputs and losses (i.e. 0.41 vs. 0.39 ± 0.03 , Table 2).

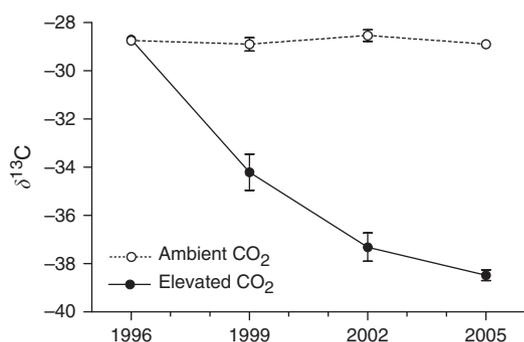
The chemical composition of the Oi and Oa portions of the forest floor and organic matter in the upper mineral soil was assessed using tetramethylammonium hydroxide thermochemical GC–MS (Frazier *et al.*, 2003). The analysis yielded chemical signatures for lignin and fatty acid-derived compounds; however, no treatment differences were detected among the three soil horizons (data not shown).

SOM dynamics

Time series measurements of C and N concentrations and contents in the upper bulk mineral soil (0–15 cm depth) failed to detect any statistically significant changes over the 9-year period (Table 3). However, significant changes in the C:N ratio in both elevated and ambient CO₂ rings were observed. The time \times treatment interaction was also statistically significant, indicating that the C:N ratio of SOM in the upper 15 cm of mineral soil increased at a greater rate under the elevated CO₂ treatment than under the ambient CO₂ control (Fig. 4). Change in the $\delta^{13}\text{C}$ signature of the upper mineral soil under the elevated CO₂ treatment

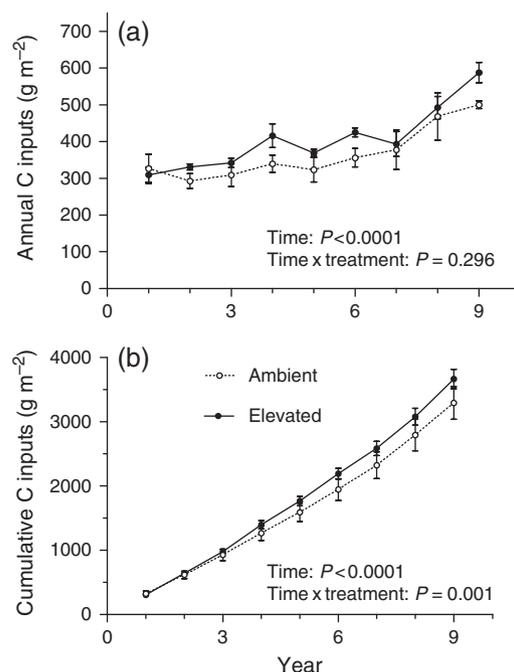
Table 1 Parameter values of a nonlinear mixed-effects model for forest floor properties

	Ambient CO ₂	Elevated CO ₂	<i>t</i> -Statistic	<i>P</i> -value
Forest floor mass (g dry OM m ⁻²)				
Asymptote	3120 ± 843	3214 ± 458	0.098	0.928
Initial amount	1239 ± 236	1138 ± 81	0.403	0.719
Rate of increase	0.327 ± 0.176	0.773 ± 0.397	1.028	0.386
Forest floor carbon (g C m ⁻²)				
Asymptote	918 ± 82	1189 ± 114	1.93	0.133
Initial amount	519 ± 106	484 ± 22	0.32	0.778
Rate of increase	0.339 ± 0.057	0.748 ± 0.418	0.971	0.431
Forest floor nitrogen (g N m ⁻²)				
Asymptote	20.2 ± 1.3	26.5 ± 3.8	1.55	0.237
Initial amount	5.0 ± 1.0	4.5 ± 0.4	0.42	0.708
Rate of increase	0.339 ± 0.057	0.748 ± 0.418	0.40	0.719

**Fig. 2** Change in $\delta^{13}\text{C}$ signature of the forest floor under elevated and ambient CO₂ concentration.

was also significant. Our estimate of turnover (*k*) of SOM in the upper bulk mineral soil (0–15 cm depth) was 0.039, generating an MRT of 25.7 years. The annual C input required to maintain steady-state C levels in the upper 15 cm of mineral soil was 89.5 g C m⁻² yr⁻¹.

The particle-size fractionations of SOM from the second set of mineral soil samples collected from 0 to 30 cm depths in 2005 generated estimates of the C inputs necessary to produce the observed changes in $\delta^{13}\text{C}$ values (Fig. 5a). The large and mid-sized particle-size fractions exhibited $\delta^{13}\text{C}$ values similar to the values for bulk soil ($-30.5 \pm 0.7\text{‰}$ averaged for both size fractions), whereas the smallest size fraction exhibited relatively enriched $\delta^{13}\text{C}$ values ($-27.2 \pm 0.5\text{‰}$). The C inputs required to generate the $\delta^{13}\text{C}$ signatures that we observed in the bulk soils from the upper 30 cm depth, $109.1 \pm 13.8 \text{ g C m}^{-2} \text{ yr}^{-1}$, were consistent with those derived from the upper 15 cm depth. Approximately 20% of C inputs enter the smallest and most stable size fraction. Acid hydrolysis fractionation of SOM also indicated that ~20% of C inputs enter the most stable fraction (Fig. 5b).

**Fig. 3** Time series showing annual (a) and cumulative (b) C inputs to forest floor under both elevated and ambient atmospheric CO₂.

We did not detect significant elevated CO₂ effects on the C and N concentrations and contents of the deep mineral soil horizons (Table 4). There was a significant change in the $\delta^{13}\text{C}$ signature of SOM in the upper 25 cm of mineral soil under the elevated CO₂ treatment similar to the change observed in the upper 15 cm (Table 3), as well as a marginally significant change in $\delta^{13}\text{C}$ signature between 125 and 150 cm depth (i.e. $P = 0.077$), suggesting root turnover at these depths. Total organic C content summed across the upper 150 cm of mineral soil in ambient and elevated plots was 6996.3 ± 284.5

Table 2 Estimates of C pools, inputs, turnover, and long-term steady-state C storage for the forest floor given for (a) each experimental FACE plot and (b) treatment effects over a 9-year period

(a) Plot estimates								
Plot no. – treatment	C ₀	∑ I	C ₀ + ∑ I	C ₉	Total decomposition	k	MRT (year)	C*
1 – Ambient	328	2789	3117	694	2423	0.443	2.26	700
2 – Elevated	454	3403	3857	833	3024	0.450	2.22	840
3 – Elevated	547	3928	4475	1225	3250	0.347	2.88	1256
4 – Elevated	478	3673	4151	1075	3076	0.372	2.69	1097
5 – Ambient	578	3606	4184	917	3267	0.434	2.30	924
6 – Ambient	681	3487	4168	760	3408	0.509	1.96	760

(b) Treatment effects				
	Total decomposition	k	MRT	C*
Ambient CO ₂ control	3032 ± 307	0.46 ± 0.02	2.18 ± 0.11	795 ± 67
Elevated CO ₂ treatment	3116 ± 68	0.39 ± 0.03	2.60 ± 0.20	1064 ± 121
df	1, 4	1, 4	1, 4	1, 4
t	0.26	1.85	1.89	1.95
P	0.80	0.14	0.13	0.12

Note: All results given as g C m^{-2} except for the unitless variable k and MRT. Treatment effects were tested with the t -test. Error values are \pm SE for each mean.

and $7813.4 \pm 732.4 \text{ g C m}^{-2}$, respectively. Total soil N (0–150 cm depth) in ambient and elevated plots was 397.6 ± 15.2 and $462.1 \pm 27.8 \text{ g C m}^{-2}$, respectively.

Discussion

Forest-floor dynamics

The incremental accumulation of organic matter in the forest floor under elevated atmospheric CO₂ represents a dynamic C sink of $\sim 271 \text{ g C m}^{-2}$ over 9 years or $\sim 30 \text{ g C m}^{-2} \text{ yr}^{-1}$. This sink is dependent on enhanced C inputs through aboveground litterfall. We observed no treatment effects on the chemical composition of forest-floor and upper mineral SOM or in the rate of decomposition of the forest floor. Although forest-floor organic matter and C and N contents appear to have reached their respective steady states after 6 years, variability in organic matter inputs to the forest floor over time and between the elevated and ambient CO₂ treatment plots leaves the question of steady states uncertain.

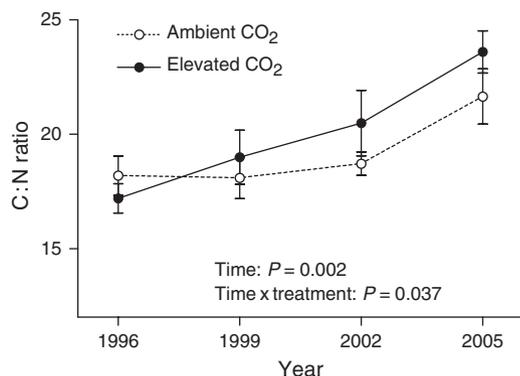
Interannual variability in the treatment effects on litterfall and forest-floor C content is likely to be related to extreme weather events. In December of 2002, a severe ice storm resulted in a substantial reduction in living biomass and an increase in detrital inputs to the forest floor (McCarthy *et al.*, 2006). The time series of annual C inputs to the forest floor, of which aboveground litterfall comprises >95%, showed a significant

treatment effect during the first 6 years of the experiment (Lichter *et al.*, 2005), followed by 2 years without a significant treatment difference, and then a return to a significant (i.e. $\sim 17\%$) net annual increment in the 9th year similar in magnitude to the treatment effect before the storm (Fig. 3). The influence of CO₂ fertilization on litterfall in the aftermath of the ice storm was likely overwhelmed by inputs of broken branches and leaves that were randomly distributed in relation to the elevated CO₂ treatment. The return of the treatment effect on litterfall in the third year following the ice storm suggests a continuous influence of elevated CO₂ on litterfall that was masked by storm damage during the seventh and eighth years.

In addition to the effects of the ice storm, a substantial drought (1999–2002; Weaver, 2005) may also have influenced the accumulation of organic matter in the forest floor, as well as SOM pools in the upper mineral soil. An increase in the C:N ratio of forest-floor organic matter between 1999 and 2002 under both elevated and ambient CO₂ suggests that overall rates of decomposition decreased during that 3-year period. To explore temporal variation in forest-floor decomposition rates, we calculated a decomposition coefficient (k) for each of the six treatment plots for each 3-year time interval (i.e. $n = 18$) using Eqn (1). The overall decomposition coefficients for each of the three time intervals (i.e. 1996–1999, 1999–2002, 2002–2005) averaged over the six rings were 0.321 ± 0.041 , 0.278 ± 0.013 , and 0.420 ± 0.018 , respectively. Relatively low rates of decomposition during the

Table 3 Time series of bulk mineral soil properties for the upper 15 cm depth

Property	Year	Ambient	Elevated	df	<i>t</i>	<i>P</i>
Field bulk density	1996	–	–			
	1999	1.17 (0.04)	1.12 (0.02)			
	2002	1.13 (0.04)	1.06 (0.02)	10	1.23	0.245
	2005	1.09 (0.06)	1.12 (0.03)			
%C	1996	1.36 (0.06)	1.53 (0.07)			
	1999	1.31 (0.07)	1.59 (0.07)			
	2002	1.85 (0.25)	1.94 (0.05)	16	–0.03	0.337
	2005	1.66 (0.21)	1.56 (0.13)			
%N	1996	0.08 (0.007)	0.09 (0.001)			
	1999	0.07 (0.007)	0.09 (0.01)			
	2002	0.10 (0.010)	0.10 (0.004)	16	–1.34	0.198
	2005	0.08 (0.011)	0.07 (0.007)			
C:N	1996	18.19 (0.86)	17.19 (0.64)			
	1999	18.09 (0.91)	18.99 (1.18)			
	2002	18.71 (0.51)	20.49 (1.43)	16	2.27	0.037
	2005	21.65 (1.21)	23.60 (0.92)			
Total C (g m ⁻²)	1996	1977 (18)	2142 (94)			
	1999	1901 (52)	2208 (136)			
	2002	2407 (141)	2734 (96)	16	–0.61	0.553
	2005	2164 (152)	2096 (175)			
Total N (g m ⁻²)	1996	109 (6.4)	125 (3.2)			
	1999	106 (8.5)	119 (13.6)			
	2002	133 (12.3)	145 (7.0)	16	–1.00	0.333
	2005	102 (11.0)	93 (10.3)			
δ ¹³ C	1996	–26.00 (0.14)	126.09 (0.40)			
	1999	–26.24 (0.18)	–28.05 (0.20)			
	2002	–26.64 (0.18)	–29.75 (0.08)	16	–5.75	<0.0001
	2005	–27.40 (0.04)	–30.86 (0.32)			

**Fig. 4** Change in the C:N ratio of the upper bulk mineral soil (0–15 cm depth) over 9 years of experimental CO₂ fertilization.

first and second time intervals were followed by a large increase in decomposition during the final 3-year interval. Temporal variation in soil moisture and decomposition rate potentially explain the wide fluctuations in C:N ratio of the forest floor; and the increase in C:N ratios between 1999 and 2002 is consistent with a slowing of decomposition during those years. Con-

sely, a decrease in C:N ratios after 2002 is consistent with a return to normal rainfall.

SOM dynamics

The increase in the C:N ratio of the upper mineral soil under both elevated and ambient CO₂ is indicative of the removal of soil N during forest aggradation (Rastetter *et al.*, 1997; Hooker & Compton, 2003; Johnson, 2006). The more rapid increase in C:N ratio under the elevated CO₂ treatment than under the ambient CO₂ control suggests that the enhanced N demand associated with the elevated CO₂ treatment was met by transfer of nitrogen from narrow C:N pools in the soil (Rastetter *et al.*, 1997; Luo *et al.*, 2004; Gill *et al.*, 2006). Such a transfer of N was not detected in the bulk SOM pools in part because the absolute quantity of N immobilized in tree biomass is a small fraction of the total quantity of N in the soil (Finzi *et al.*, 2006). In addition, the estimates of soil N content are based on the chemical analysis of soil subsamples, the results of which are multiplied by estimates of field bulk density for each soil sample. Therefore, the estimates of total N content

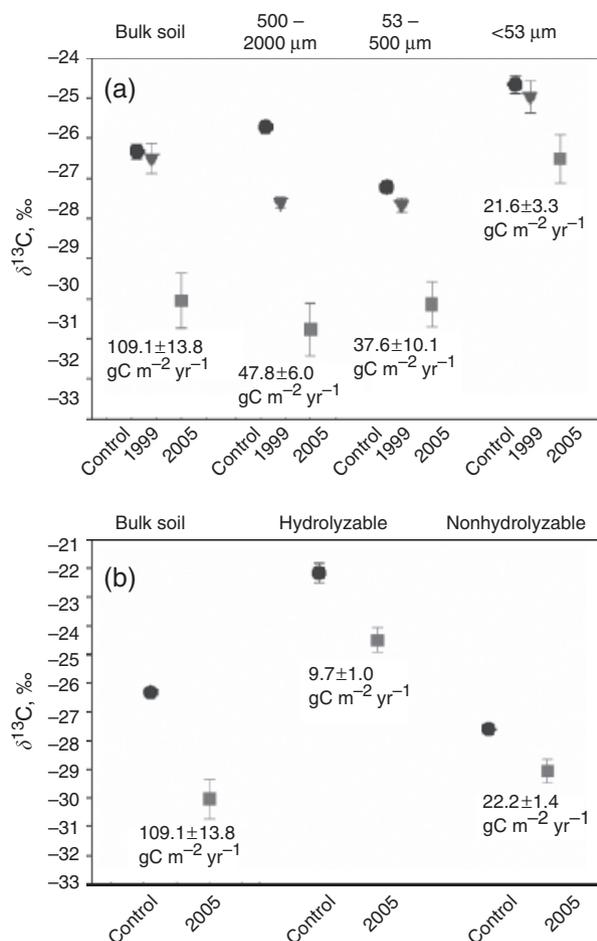


Fig. 5 (a) $\delta^{13}\text{C}$ signatures of bulk mineral soil and particle-size fractions in control plots (black circles) and after 3 years (gray triangles) and 9 or 11 years (light-gray squares) of exposure to elevated CO_2 at the Duke FACE experiment (0–30 cm). (b) Bulk soil and acid hydrolysis fractions in control plots (black circles) and elevated CO_2 plots (gray squares). Data collected at 3 years of elevated CO_2 exposure in (a) are redrawn from Schlesinger & Lichter (2001). Rates below symbols are net rates of soil organic carbon (SOC) accretion across the study period, and reflect the exposure of the three elevated CO_2 plots to 9 years of CO_2 fumigation and one plot (the ‘prototype’ plot; Oren *et al.*, 2001) to 11 years of elevated CO_2 . Rates in (b) for the two acid hydrolysis fractions do not add to the bulk soil rate because of the likely loss of CO_2 during hydrolysis, and the removal of all visible plant parts before hydrolysis; see the text for details of method.

are unavoidably less well constrained than are estimates of C:N ratios, which are derived directly from laboratory measurements of C and N concentrations.

Our results describing soil C pools under elevated atmospheric CO_2 are consistent with numerous studies, indicating a limited capacity of soils for CO_2 -stimulated carbon sequestration (Hungate *et al.*, 1997; Gill *et al.*, 2002; Hagedorn *et al.*, 2003). In contrast, Jastrow *et al.* (2005) found a substantial increase in soil C in the upper

15 cm of mineral soil under elevated CO_2 associated with a large increase in fine-root turnover under elevated CO_2 . Importantly, much of this new C became incorporated into microaggregates, especially in the upper 5 cm. Microaggregates increase the duration of SOM by physically limiting access of soil microorganisms (Christensen, 1996). Jastrow *et al.* (2005) concluded that studies measuring SOM from the upper 10–20 cm depths may mask potential changes in the uppermost soil by including deeper soil less likely to have been influenced by the elevated CO_2 treatment. This may be true with previous studies at the Duke Forest FACE experiment, where soil samples were collected from the upper 15 cm depth for SOM fractionation studies (i.e. Schlesinger & Lichter, 2001; Lichter *et al.*, 2005).

At Oak Ridge, Jastrow *et al.* (2005) estimated a net increment of $44 \pm 9 \text{ gC m}^{-2} \text{yr}^{-1}$ of additional soil C storage in the upper 15 cm of mineral soil under elevated CO_2 . Such an increase in C inputs to the mineral soil is unlikely at the Duke Forest FACE experiment, where only $\sim 89 \text{ gC m}^{-2}$ of annual inputs are required to produce the observed change in $\delta^{13}\text{C}$ signature of the upper 15 cm of mineral soil under the elevated CO_2 treatment, and only about 25%, or $18 \text{ gC m}^{-2} \text{yr}^{-1}$ of additional carbon would enter the upper 15 cm of mineral soil under the elevated CO_2 treatment. After 9 years, a total accumulation of 162 gC m^{-2} would not be readily detected given the inherent spatial heterogeneity of soil properties and the statistical limitations of the experiment (Hungate *et al.*, 1996; Lichter *et al.*, 2005).

Consistent with Jastrow *et al.* (2005), we found soil fractionation important for revealing important features of mineral soil C formation with forest development, even when aggregating mineral soil from the surface to 30 cm depth. Smaller particle-size fractions were enriched in ^{13}C relative to larger fractions, consistent with other studies describing ^{13}C enrichment of the relatively old SOM typically associated with silt and clay (Ehleringer *et al.*, 2000). Chemical fractionation data present additional challenges for interpretation; in spite of the recalcitrant nature of the nonhydrolyzable fraction and its presumably older C (Leavitt *et al.*, 1996), this fraction exhibited depleted $\delta^{13}\text{C}$ values relative to the hydrolyzable fraction ($P < 0.05$; $-29.1 \pm 0.04\%$ vs. $-24.5 \pm 0.4\%$), suggesting substantial annual inputs into this SOM pool. However, these data may reflect losses of ^{13}C -depleted C during acid hydrolysis via decarboxylation that generated relatively enriched $\delta^{13}\text{C}$ values of hydrolyzable organic material (Billings, 2006). Because C lost during acid hydrolysis is likely lost preferentially from the relatively hydrolyzable fraction, such losses do not affect our estimates of C inputs entering the nonhydrolyzable fraction. Here, we focus on the nonhydrolyzable and smallest particle-size fractions,

Table 4 Mineral soil properties from 0 to 150 cm depth after 9 years of CO₂ fertilization

	%C	%N	C:N	Total C (g m ⁻²)	Total N (g N m ⁻²)	δ ¹³ C (‰)
0–25 cm depth						
Ambient	0.973 (0.064)	0.038 (0.003)	26.25 (3.090)	3917.80 (257.69)	153.43 (11.27)	–25.72 (0.29)
Elevated	1.420 (0.178)	0.040 (0.006)	27.13 (0.006)	4323.73 (719.26)	160.71 (25.60)	–28.56 (0.95)
<i>P</i> value	0.614	0.805	0.795	0.623	0.808	0.045
25–50 cm depth						
Ambient	0.328 (0.012)	0.022 (0.002)	15.28 (1.30)	1102.96 (19.35)	74.01 (5.66)	–23.34 (0.25)
Elevated	0.360 (0.014)	0.026 (0.020)	14.48 (0.95)	1188.44 (55.90)	83.58 (8.14)	–23.31 (0.33)
<i>P</i> value	0.164	0.406	0.645	0.222	0.389	0.685
50–75 cm depth						
Ambient	0.233 (0.027)	0.019 (0.002)	12.35 (0.66)	875.67 (124.72)	68.92 (4.27)	–22.99 (0.49)
Elevated	0.242 (0.025)	0.121 (0.001)	11.41 (0.83)	875.26 (125.85)	76.91 (4.75)	–23.23 (0.52)
<i>P</i> value	0.819	0.431	0.408	0.819	0.433	0.756
75–100 cm depth						
Ambient	0.141 (0.003)	0.012 (0.001)	11.29 (0.23)	514.73 (11.41)	44.95 (1.35)	–23.09 (0.40)
Elevated	0.167 (0.023)	0.016 (0.001)	10.65 (0.51)	587.60 (74.45)	55.04 (4.80)	–23.60 (0.41)
<i>P</i> value	0.317	0.101	0.314	0.388	0.113	0.427
100–125 cm depth						
Ambient	0.107 (0.014)	0.009 (0.001)	11.00 (1.07)	391.72 (50.89)	32.91 (4.34)	–24.32 (0.71)
Elevated	0.122 (0.009)	0.012 (0.001)	9.77 (0.18)	457.46 (33.20)	46.77 (2.40)	–24.00 (0.25)
<i>P</i> value	0.414	0.190	0.321	0.340	0.012	0.439
125–150 cm depth						
Ambient	0.056 (0.011)	0.006 (0.001)	9.54 (0.20)	211.63 (40.09)	22.22 (4.34)	–23.29 (0.71)
Elevated	0.097 (0.007)	0.010 (0.001)	9.23 (0.46)	365.46 (27.38)	38.72 (2.41)	–25.83 (0.80)
<i>P</i> value	0.034	0.029	0.574	0.034	0.029	0.077

both typically thought to be composed of slow-turnover soil organic carbon (SOC) (Tiessen & Stewart, 1983; Leavitt *et al.*, 1996; Billings, 2006).

Schlesinger & Lichter (2001) used the same particle-size fractions at this site to reveal only slow accumulation of mineral soil C in this forest after 3 years of exposure to ¹³C-depleted CO₂, congruent with another study of C dynamics in an aggrading loblolly pine forest (Richter *et al.*, 1999). Their work indicated little accumulation of new C in the smallest particle-size fractions. After 9 years of ¹³C tracer incorporation, δ¹³C values of our smallest particle-size and nonhydrolyzable fractions suggest that a significant proportion of new C in the mineral soil (~20%) has accrued in well-protected, stable pools over the last 9 years. Although these pools can contain a high proportion of relatively old C that often appears to accumulate over hundreds of years (Tiessen & Stewart, 1983; Leavitt *et al.*, 1996; Trumbore, 2000), the δ¹³C signatures of these fractions in the current study, however, suggest that this C can form over relatively short time frames during the development of a young pine forest.

Conclusions

Previously, after 6 years of experimental CO₂ fumigation, we estimated a long-term steady-state sink of

454 ± 146 g C m⁻² (4.52 ± 1.46 mg C ha⁻¹) in the forest floor under the elevated CO₂ treatment based on estimates of C inputs and turnover (Lichter *et al.*, 2005). After 9 years, as described in the current study, our estimate decreased to 271 g C m⁻² (i.e. ~30 g C m⁻² yr⁻¹). Although a net increment of this magnitude represents a substantial increase in forest-floor C storage under elevated CO₂ (i.e. 29%), a long-term C sink of this magnitude would not greatly lessen the effects of the anthropogenic CO₂ emissions expected over the coming decades (Lichter *et al.*, 2005). We observed no statistically significant treatment effects in the C and N contents of mineral soil down to 1.5 m depth. Although there is some uncertainty in our estimates of soil C pools, the results of this long-term CO₂ fertilization study indicate that mineral soils in coniferous forests do not play a major role in atmospheric CO₂ sequestration. However, of the mineral soil C formed during the past 9 years in this forest, approximately 20% has been allocated to stable pools that will likely remain protected from microbial activity and associated release as CO₂. Changes in the C:N ratio of the upper mineral soil over the 9-year period does suggest that N is being removed to support the increase in living and detrital biomass under elevated CO₂ treatment. Finzi *et al.* (2006) estimate that an additional 26 g N m⁻² is required to satisfy the increased N demand under elevated CO₂

in this forest. Although the treatment effect on upper mineral soil N content was not statistically significant, the difference in the amount of N mobilized under elevated and ambient CO₂ between 2002 and 2005 is in this range (Table 4), suggesting that enhanced rates of SOM decomposition enable this forest to mine the additional N needed to support the observed CO₂-growth enhancement.

Acknowledgements

We thank Tom Millican, Glenn Piercey, Jeff Phippen, Alexis Reed, Vaughn Salisbury, and Guihong Zhang for field assistance, Paulette Messier and Lucas Amundson for conducting the GC-MS work, and Kevin Harrison for completing the original soil sampling in 1996. This research was supported by the National Science Foundation (DEB-0235576) and the Office of Science (BER), US Department of Energy, Grant no. DE-FG02-95ER62083, and the US Department of Energy's National Institute of Climate Change Research (NICCR; DE-FC02-03ER63613).

References

- Andrews JA, Schlesinger WH (2001) Soil CO₂ dynamics, acidification, and chemical weathering in a temperate forest with experimental CO₂ enrichment. *Global Biogeochemical Cycles*, **15**, 149–162.
- Bernhardt ES, Barber JJ, Phippen JS, Taneva L, Andrews JA, Schlesinger WH (2006) Long-term effects of free air CO₂ enrichment (FACE) on soil respiration. *Biogeochemistry*, **77**, 91–116.
- Billings S, Schaeffer SM, Evans RD (2004) Response of soil microbial activity and N mineralization to elevated CO₂ in Mojave Desert soils. *Global Biogeochemical Cycles*, **18**, GB1011, doi: 10.1029/2003GB002137.
- Billings S, Ziegler S (2005) Linking microbial activity and soil organic matter transformations in forest soils under elevated CO₂. *Global Change Biology*, **11**, 203–212.
- Billings S, Ziegler S (2008) Altered patterns of soil carbon substrate usage and heterotrophic respiration in a pine forest with elevated CO₂ and N fertilization. *Global Change Biology*, **14**, 1025–1036.
- Billings SA (2006) Soil organic matter dynamics and land use change at a grassland/forest ecotone. *Soil Biology & Biochemistry*, **38**, 2934–2943.
- Billings SA, Schaeffer SM, Zitzer S, Charlet T, Smith SD, Evans RD (2002) Alterations of nitrogen dynamics under elevated carbon dioxide in an intact Mohave Desert ecosystem: evidence from nitrogen-15 natural abundance. *Oecologia*, **131**, 463–467.
- Calfapietra C, De Angelis P, Gielen B *et al.* (2007) Increased nitrogen-use efficiency of a short rotation poplar plantation in elevated CO₂ concentration. *Tree Physiology*, **27**, 1153–1163.
- Cambardella CA, Elliott ET (1993) Methods for physical separation and characterization of soil organic matter fractions. *Geoderma*, **56**, 449–457.
- Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO₂ leads to loss of soil carbon. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 4990–4995.
- Christensen B (1996) Carbon in primary and secondary organo-mineral complexes. In: *Structure and Organic Matter Storage in Agricultural Soils* (eds Carter MR, Stewart BA), pp. 97–165. CRC Press, Boca Raton, FL.
- Christensen BT (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. *Advances in Soil Science*, **20**, 1–90.
- Davidson EA, Hirsch IA (2001) Fertile forest experiments. *Nature*, **411**, 431–433.
- Davies G, Ghabbour EA, Steelink C (2001) Humic acids: marvelous products of soil chemistry. *Journal of Chemical Education*, **78**, 1609–1614.
- DeLucia EH, Moore DJ, Norby RJ (2005) Contrasting responses of forest ecosystems to rising atmospheric CO₂: implications for the global C cycle. *Global Biogeochemical Cycles*, **19**, 1–9, GB3006.
- Diaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature*, **363**, 616–617.
- Ehleringer JR, Buchmann N, Flanagan LB (2000) Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications*, **10**, 412–422.
- Finzi AC, DeLucia EH, Hamilton JG, Richter DD, Schlesinger WH (2002) The nitrogen budget of a pine forest under free-air CO₂ enrichment. *Oecologia*, **132**, 567–578.
- Finzi AC, Moore DJP, DeLucia EH *et al.* (2006) Progressive nitrogen limitation of ecosystem processes under elevated CO₂ in a warm-temperate forest. *Ecology*, **87**, 15–25.
- Finzi AC, Norby RJ, Calfapietra C *et al.* (2007) Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO₂. *Proceedings of the National Academy of Sciences*, **104**, 14014–14019.
- Frazier SW, Nowack KO, Goins KM, Cannon FS, Kaplan LA, Hatcher PG (2003) Characterization of organic matter from natural waters using tetramethyl ammonium hydroxide thermochemolysis GC-MS. *Journal of Analytical and Applied Pyrolysis*, **70**, 99–128.
- Gill RA, Anderson LJ, Polley HW, Johnson HB, Jackson RB (2006) Potential nitrogen constraints on soil carbon sequestration under low and elevated atmospheric CO₂. *Ecology*, **87**, 41–52.
- Gill RA, Polley HW, Johnson HB, Anderson LJ, Maherali H, Jackson RB (2002) Nonlinear grassland responses to past and future atmospheric CO₂. *Nature*, **417**, 279–282.
- Hagedorn F, Spinnler D, Bundt M, Blaser P, Siegwolf R (2003) The input and fate of new C in two forest soils under elevated CO₂. *Global Change Biology*, **9**, 862–872.
- Heath J, Ayres E, Possell M, Bardgett RD, Black HIJ, Grant H, Ineson P, Kerstiens G (2005) Rising atmospheric CO₂ reduces sequestration of root-derived soil carbon. *Science*, **309**, 1711–1713.
- Hendrey GR, Ellsworth DS, Lewin KE, Nagy J (1999) A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂. *Global Change Biology*, **5**, 293–309.
- Hooker TB, Compton JE (2003) Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural abandonment. *Ecology*, **13**, 299–313.
- Hoosbeek MR, Vos JM, Meinders MJB, Velthorst EJ, Scarascia-Mugnozza GE (2007) Free atmospheric CO₂ enrichment

- (FACE) increased respiration and humification in the mineral soil of a poplar plantation. *Geoderma*, **138**, 204–212.
- Hungate BA, Dukes JS, Shaw MR, Luo Y, Field CB (2003) Nitrogen and climate change. *Science*, **302**, 1512–1513.
- Hungate BA, Holland EA, Jackson RB, Chapin FS, Mooney HA, Field CB (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388**, 576–579.
- Hungate BA, Jackson RB, Field CB, Chapin FS (1996) Detecting changes in soil carbon in CO₂ experiments. *Plant and Soil*, **187**, 135–145.
- Hungate BA, Johnson DW, Dijkstra P *et al.* (2006) Nitrogen cycling during seven years of atmospheric CO₂ enrichment in a scrub oak woodland. *Ecology*, **87**, 26–40.
- Insightful Corporation (1988, 2007) *S-plus 8.0: Guide to Statistics*, Vol. I. Insightful, Seattle, Washington, USA.
- Jastrow JD, Boutton TW, Miller RM (1996) Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. *Soil Science Society of America Journal*, **60**, 801–807.
- Jastrow JD, Miller M, Matamala R, Norby RJ, Boutton TW, Rice CW, Owensby CE (2005) Elevated atmospheric carbon dioxide increases soil carbon. *Global Change Biology*, **11**, 2057–2064.
- Jastrow JD, Miller RM, Owensby CE (2000) Long-term effects of elevated CO₂ on below-ground biomass and transformations to soil organic matter in grassland. *Plant and Soil*, **224**, 85–97.
- Johnson DW (2006) Progressive N limitation in forests: review and implications for long-term responses to elevated CO₂. *Ecology*, **87**, 64–75.
- Knops JMH, Tilman D (2000) Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. *Ecology*, **81**, 88–98.
- Leavitt S, Follett R, Paul E (1996) Estimation of slow- and fast-cycling soil organic carbon pools from 6N HCl hydrolysis. *Radiocarbon*, **38**, 231–240.
- Lichter J, Barron SH, Bevacqua CE, Finzi AC, Irving KF, Stemmler EA, Schlesinger WH (2005) Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO₂ enrichment. *Ecology*, **86**, 1835–1847.
- Luo Y, Hui D, Zhang D (2006) Elevated CO₂ stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology*, **87**, 53–63.
- 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, White LW, Canadell J *et al.* (2003) Sustainability of terrestrial carbon sequestration: a case study in the Duke Forest with an inversion approach. *Global Biogeochemical Cycles*, **17**, 1–19, 1021.
- Matamala R, Gonzalez-Meler MA, Jastrow JA, Norby RJ, Schlesinger WH (2003) Impacts of fine-root turnover on forest NPP and soil C sequestration potential. *Science*, **302**, 1385–1387.
- Matamala R, Schlesinger W (2000) Effects of elevated atmospheric CO₂ on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology*, **6**, 967–979.
- McCarthy HR, Oren R, Kim H-S, Johnsen KH, Maier C, Pritchard SG, Davis MA (2006) Interaction of ice storms and management practices on current carbon sequestration in forests with potential mitigation under future CO₂ atmosphere. *Journal of Geophysical Research*, **111**, D15103.
- Melillo JM, McGuire AD, Kicklighter DW, Moore B, Vorosmarty CJ, Schloss AL (1993) Global climate change and terrestrial net primary production. *Nature*, **363**, 234–240.
- Norby RJ, DeLucia EH, Gielen B *et al.* (2005) Forest response to elevated CO₂ is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences*, **102**, 18052–18056.
- Norby RJ, Iversen CM (2006) Nitrogen uptake, distribution, turnover, and efficiency of use in a CO₂-enriched sweetgum forest. *Ecology*, **87**, 5–14.
- Oren R, Ellsworth DS, Johnsen KH *et al.* (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature*, **411**, 469–472.
- Pinheiro JC, Bates DM (2000) *Mixed-Effects Models in S and S-plus*. Springer-Verlag, New York, New York, USA.
- Pritchard SG, Strand AE, McCormack ML *et al.* (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air CO₂ Enrichment (FACE): a six year mini-rhizotron study. *Global Change Biology*, **14**, 588–602.
- Rastetter EB, Ågren GI, Shaver GR (1997) Responses of N-limited ecosystems to increased CO₂: a balanced-nutrition, coupled-element-cycles model. *Ecological Applications*, **7**, 444–460.
- Richter DD, Markewitz D, Trumbore SE, Wells CG (1999) Rapid accumulation and turnover of soil carbon in a re-establishing forest. *Nature*, **400**, 56–58.
- Schlesinger W, Lichter J (2001) Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature*, **411**, 466–469.
- Taneva L, Phippen JS, Schlesinger WH, Gonzalez-Meler MA (2006) The turnover of carbon pools contributing to soil CO₂ and soil respiration in a temperate forest exposed to elevated CO₂ concentration. *Global Change Biology*, **12**, 983–994.
- Tiessen H, Stewart J (1983) Particle size fractions and their use in studies of soil organic matter 2. Cultivation effects on organic matter composition in size fractions. *Soil Science Society of America Journal*, **47**, 509–514.
- Trumbore S (2000) Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecological Applications*, **10**, 399–411.
- US Department of Agriculture (1977) *Soil Survey of Orange County*. Soil Conservation Service, North Carolina, USA.
- Van Groenigen K-J, Six J, Hungate BA, de Graaff MA, van Breemen N, van Kessel C (2006) Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 6571–6574.
- Vitousek PM (1982) Nutrient cycling and nutrient use efficiency. *American Naturalist*, **119**, 553–572.
- Weaver JC (2005) *The drought of 1998–2002 in North Carolina – precipitation and hydrologic conditions*. US Geological Survey Scientific Investigations Report 2005-5053, 88 pp.
- Williams MA, Rice CW, Omay A, Owensby C (2004) Carbon and nitrogen pools in a tallgrass prairie soil under elevated carbon dioxide. *Soil Science Society of America Journal*, **68**, 148–153.
- Williams MA, Rice CW, Owensby CE (2000) Carbon dynamics and microbial activity in tallgrass prairie exposed to elevated CO₂ for 8 years. *Plant and Soil*, **227**, 127–137.