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SOIL CARBON SEQUESTRATION AND TURNOVER IN A PINE FOREST AFTER SIX YEARS OF ATMOSPHERIC CO_2 ENRICHMENT

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Abstract. During the first six years of atmospheric CO_2 enrichment at the Duke Forest free-air CO_2 enrichment (FACE) experiment, an additional sink of 52 ± 16 g $C \cdot m^{-2} \cdot yr^{-1}$ accumulated in the forest floor (O-horizon) of the elevated CO_2 treatment relative to the ambient CO_2 control in an aggrading loblolly pine (*Pinus taeda* L.) forest near Chapel Hill, North Carolina, USA. The experiment maintained an atmospheric CO_2 concentration 200 μL/L above ambient levels in replicated ($n = 3$) FACE rings throughout the six-year period. This CO_2-induced C sink was associated with greater inputs of organic matter in litterfall and fine-root turnover. There was no evidence that microbial decomposition was altered by the elevated CO_2 treatment. Consistent with ecosystem recovery following decades of intensive agriculture, the C and N content of the mineral soil increased under both the elevated CO_2 treatment and the ambient CO_2 control during the six-year period. This increase is attributed to accumulation of plant residues derived from fine roots with relatively high turnover rates rather than accumulation of refractory or physically protected soil organic matter (SOM). The elevated CO_2 treatment produced no detectable effect on the C and N content of the bulk mineral soils or of any particulate organic matter size fraction. Because the fumigation gas was strongly depleted in ^{13}C, the incorporation of new C could be traced within the ecosystem. Significant decreases in $\delta^{13}C$ of soil organic carbon (SOC) under the elevated CO_2 treatment were used to estimate the mean residence times of intra-aggregate particulate organic matter and mineral-associated organic matter as well as the annual C inputs required to produce the observed changes in $\delta^{13}C$. Our results indicate that forest soils such as these will not significantly mitigate anthropogenic C inputs to the atmosphere. The organic matter pools receiving large annual C inputs have short mean residence times, while those with slow turnover rates receive small annual inputs.

Key words: *^{13}C stable isotope; CO_2-induced NPP enhancement; elevated CO_2; forest free-air CO_2 enrichment (FACE) experiment; intra-aggregate particulate organic matter (iPOM); loblolly pine; mineral-associated organic matter;* Pinus taeda; *soil organic matter (SOM); soil N.*

INTRODUCTION

Regrowth of temperate forests constitutes a large C sink that may be supplemented by enhanced tree growth and increased production of refractory soil organic matter associated with rising atmospheric CO_2 concentration (Houghton et al. 1999, Ciais et al. 2000, Schimel et al. 2000, Houghton 2003). To date, atmospheric CO_2 enrichment experiments have demonstrated significant increases in net primary productivity (NPP) and C storage in forest vegetation (Curtis and Wang 1998, DeLucia et al. 1999, Körner 2000, Hamilton et al. 2002, Norby et al. 2002), but the evidence for significant C sequestration in soils is less conclusive (Hungate et al. 1996, Van Kessel et al. 2000, Schlesinger and Lichter 2001, Van Groenigen et al. 2002). Whether forest soils will constitute a significant, long-term C sink depends on the sustainability of the observed CO_2-induced NPP enhancements, which in turn depends largely on whether microbial decomposition and turnover of limiting nutrients such as N keep up with plant demand (Zak et al. 2000, McMurtie et al. 2001, Finzi et al. 2002, Luo et al. 2004).

Soil N turnover may be affected in different ways by the increased availability of C substrate depending on the amount and chemistry of the additional substrate (Zak et al. 2000, 2003). If the additional C inputs are in the form of simple carbohydrates and organic acids, microbial growth and biosynthesis will be stimulated, increasing microbial N demand and immobilization in microbial biomass. In this case, plant demand for N would not be met over the long term and the CO_2-induced NPP enhancement would eventually attenuate. However, if the increased C inputs are largely recalcitrant compounds containing lignin and tannins, microbial metabolism will be reduced; lessening N immobilization and thereby sustaining plant demand. To

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date, there has been little evidence that elevated CO_2 significantly affects decomposition and N availability to plants in forest ecosystems (Zak et al. 2000, 2003, Finzi and Schlesinger 2003). Although increases in heterotrophic soil respiration are generally observed (Insam et al. 1999, Andrews and Schlesinger 2001, Phillips et al. 2002), detailed studies quantifying microbial N content, gross mineralization, and immobilization of N have not detected significant elevated CO_2 effects on microbial biomass and N turnover (Zak et al. 2000, 2003, Finzi and Schlesinger 2003). Thus, it appears from these experimental studies that CO_2-induced NPP enhancements observed in temperate forests may be sustained over the long term.

Assuming the long-term sustainability of enhanced NPP and C inputs to soils under rising atmospheric CO_2 with no change in decomposition, a dynamic C sink will develop in soils simply because C inputs increase while C losses remain constant. This sink will wholly depend on the continued supply of additional C inputs unless organic matter is physically protected from decomposers within soil aggregates (Post et al. 2004), or is chemically protected in refractory compounds such as humic substances (Norby et al. 2001). Because subtle changes in soil N cycling and in the formation of soil aggregates and production of humic substances may elicit important ecosystem effects over time, an accurate understanding of the role of soils in ecosystem C sequestration can only be determined with whole-ecosystem elevated-CO_2 experiments conducted over long periods.

The free-air CO_2 enrichment (FACE) experiment at the Duke Forest was designed to investigate the response of an intact forest ecosystem (i.e., an aggrading loblolly pine plantation) to the atmospheric CO_2 concentration expected in the year 2050 (i.e., ~560 μL/L). During the first six years of experimental CO_2 fumigation (1996–2002), a large CO_2-induced enhancement of NPP (12%, with a range of 8–20%) was observed in increased woody increment (DeLucia et al. 1999, Hamilton et al. 2002), increased litter production (Finzi et al. 2001), increased root production (Matamala and Schlesinger 2000, Matamala et al. 2003), and higher concentrations of dissolved organic carbon (DOC) in throughfall precipitation (Lichter et al. 2000). At the end of three years, Schlesinger and Lichter (2001) detected a significant additional C sink (~183 g C/m^2) in the forest floor (i.e., soil organic horizon) of the elevated CO_2 treatment of this aggrading forest. However, significant C sequestration in the mineral soil was not detected under the elevated CO_2 treatment. Here we document the effects of six years of experimental CO_2 fumigation on soil C sequestration and turnover at the Duke Forest FACE experiment. Our objective was to quantify soil C pools, inputs, turnover, and long-term storage to better understand the capacity of forest soils for C sequestration.

Methods

Site description and experimental design

The Duke Forest free-air CO_2 enrichment (FACE) experiment was constructed in a loblolly pine (*Pinus taeda* L.) forest derived from seedlings planted in a 2.4 × 2.4 m spacing in 1983 (see Plate 1). The 32-ha site was logged of 40- to 60-year-old mixed stands of loblolly, shortleaf (*Pinus echinata* Mill.), and Virginia pine (*Pinus virginiana* Mill.), and was drum chopped and burned prior to tree planting (J. Edeburn, *personal communication*). In 1996, at the start of the experiment, the pine seedlings had grown to 14 m in height and composed 98% of the aboveground biomass. Deciduous tree species including sweet gum (*Liquidambar styraciflua* L.), red maple (*Acer rubrum* L.), red bud (*Cercis Canadensis* L.), and dogwood (*Cornus florida* L.) had sprouted from stumps and established from seed dispersed from the surrounding vegetation.

The soils are clay loams, classified as low-fertility Ultic Alfisol of the Enon series, which are typical of many upland areas in the southeastern USA (U.S. Department of Agriculture 1977). They are relatively homogeneous, derive from mafic bedrock, and exhibit acidic, well-developed profiles of mixed clay mineralogy. Boreholes show up to 1 m of topsoil underlain by 5 m of saprolite, below which lies a highly fractured granodiorite or diorite bedrock (Andrews and Schlesinger 2001). Variation in elevation ranges up to 15 m across the 32-ha site, but topographic relief is generally less than 1°. The static water table lies at a 6 m depth, but the site drains poorly and surface soils often become saturated in the spring. The mean annual temperature is 15.5°C and the mean annual precipitation, 1140 mm.

The FACE experiment consists of six circular rings, 30 m in diameter. Three of the six rings are fumigated with CO_2 to maintain an atmospheric concentration 200 μL/L above ambient (Hendrey et al. 1999). The three remaining control rings are identical to the elevated CO_2 treatment rings except that they are fumigated with ambient air. The experiment was begun on 27 August 1996 and the elevated CO_2 treatment was maintained continuously over the following six years except for brief periods during Hurricane Fran in 1996 and Hurricane Floyd in 1999.

The CO_2 used for fumigation is derived from natural gas and consequently is strongly depleted in ^{13}C relative to ^{12}C (i.e., $\delta^{13}C = -43.0 \pm 0.6$), where

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

Raising the atmospheric CO_2 concentration by 200 μL/L with this CO_2 gas reduces the $\delta^{13}C$ of the atmosphere in elevated rings from −8 to −20‰. We tracked the incorporation of this isotopic signature into soil organic matter (SOM) and indirectly estimated the

PLATE 1. Duke Forest FACE experiment. Photo credit: J. Lichter.

turnover rates of bulk soils and SOM fractions based on the change in $\delta^{13}C$.

Sample collection and laboratory analyses

Pretreatment samples were collected of the mineral soil during the construction of the experiment from the locations of sixteen excavations around the periphery of the experimental rings where the support towers for the FACE apparatus would later be installed. These samples were collected at 0–7.5, 7.5–15, 15–30, 30–60, and 60–90 cm depths, and were sieved (<2 mm), dried, and archived for future use. Subsamples collected from the 0–7.5 cm and 7.5–15 cm depths were mixed to estimate %C, %N, and $\delta^{13}C$ in the upper 15 cm of bulk mineral soils and SOM fractions for comparison with samples collected in later years.

In early October of 1999, after three growing seasons of experimental CO_2 fumigation, and again in late August of 2002, after six years of experimental CO_2 fumigation, 12 soil cores 4.76 cm in diameter were collected from stratified, random positions within each FACE ring. The forest floor was first separated from the mineral soil by hand, and the mineral soil was collected in two sections: 0–15 cm depth and 15–30 cm depth. Deeper soil horizons were not sampled because 99% of root biomass is contained within the top 30 cm (Matamala and Schlesinger 2000) and because a gravelly, difficult to penetrate hardpan occurs at 30–35 cm depth. All samples were dried for five days at 50°C and weighed. The mineral soil samples were sieved (<2 mm) to remove stones and coarse roots. The volume and mass of stones were determined for each mineral soil sample and subtracted from the sample mass and volume to estimate field bulk density (g/cm^3). Coarse roots were removed by hand from the forest floor samples, which were ground in their entirety to a fine powder for chemical analysis. The sieved mineral soil samples were subsampled for chemical analyses and SOM fractionation. From six of the 12 upper mineral soil samples collected at 0–15 cm depth in 1996, 1999, and 2002, a well-mixed 5-g subsample was separated into water-stable, intra-aggregate particulate organic matter (iPOM) and mineral-associated organic matter (Christensen 1992, Cambardella and Elliott 1993). To isolate these SOM fractions, the free light fraction (plant residues) was first removed by density fractionation. The 5-g subsample of mineral soil was suspended in 35 mL of 1.85 g/cm^3 polytungstate solution, and centrifuged for 60 minutes. The free light fraction floating on the solution surface was subsequently aspirated off into an

aluminum weighing dish. The polytungstate solution was then filtered (Whatman #50, Whatman International Ltd., Maidstone, UK), and the particles rinsed with deionized water into the weighing dish containing the aspirated particles. The free light fraction was dried at 105°C and prepared for isotopic analysis. The heavy fraction obtained from centrifugation was vacuum filtered (Whatman #50) and dispersed with a 0.5 mol/L sodium hexametaphosphate solution on a reciprocal shaker for 18 hours. The dispersed solution was rinsed over 250 and 53-μm brass screens (see Cambardella and Elliott 1993, Six et al. 1998, Gill et al. 1999). The <53-μm fraction was captured in a pan. The 250–2000-μm fraction is considered coarse iPOM, the 53–250-μm fraction is fine iPOM, and the <53-μm fraction is the mineral-associated organic matter (OM). Each fraction was dried, weighed, and prepared for chemical analysis.

All bulk soil samples and SOM fractions collected in 1996 and 1999 were analyzed for %C, %N, and ^{13}C using a SIRA Series II isotope ratio mass spectrometer (Micromass, Manchester, UK), operated in automatic trapping mode after Dumas combustion of samples in an elemental analyzer (NA1500 Series 1, Carlo Erba Instrumentazione, Milan, Italy). The reference CO_2, obtained from Oztech (Dallas, Texas, USA), was calibrated against a standard, and data were expressed as $\delta^{13}C$ after correction for the isotope contribution of the O_2 used in sample combustion. Samples collected in 2002 were processed for %C, %N, and $\delta^{13}C$ with a Carlo Erba NA1500 elemental analyzer interfaced with a ThermoFinnigan Delta Plus XL continuous flow isotope ratio mass spectrometer via a ThermoFinnigan Conflo III interface (Finnigan MAT, Bremen, Germany). The raw ^{13}C measurements were normalized to internal standards calibrated to international reference materials. Samples collected in 1996 and 1999 were rerun with the second instrument to test for consistency between the two instruments. The two instruments provided similar %C measurements ($\bar{x}_A = 1.48$, $\bar{x}_B = 1.43$; $n = 23$, $t = 1.96$, $P = 0.066$). To develop a time series for the C content of physically protected and refractory SOM in the upper 15 cm of mineral soil, we summed the C contents of the various iPOM and mineral-associated organic matter fractions and showed change in this SOM pool over the six-year period along with change in the free light fraction.

Forest floor dynamics

In non-steady-state conditions, the mean turnover rate (k) of soil horizons or SOM pools can be estimated from

$$C_T = C_0 e^{-kT} + \frac{I}{k}(1 - e^{-kT}) \qquad (1)$$

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 (Olson 1963, Davidson and Hirsch 2001, Schlesinger and Lichter 2001). For the forest floor, we measured C_T, C_0, and I directly, and solved for k by iteration for each of the six FACE rings. The value of k derived from in this manner represents the proportion of organic matter that decomposed each year for the forest floor considered as a unit. It is not directly comparable to the value of k derived from litter-bag studies because it quantifies the mean annual decomposition rate for the entire thickness of forest floor including relatively new litter and older, partially decomposed organic matter, rather than new litter alone. Because there is no evidence to the contrary (Finzi et al. 2001), we assumed that the decomposability of new organic matter derived after the experiment began was similar to that of older native soil organic matter and used a single C pool model to determine the mean decomposition rate for the forest floor of each FACE ring. The mean residence time (MRT) is simply $1/k$, and the predicted state-state C content (C^*) is I/k. Total decomposition of insoluble organic C occurring in each FACE ring over the six-year time period was calculated as

$$\sum \text{decomposition} = C_0 + \sum I - C_T. \qquad (2)$$

A comparison of total decomposition between elevated CO_2 and control rings determined whether or not the gross rate of microbial decomposition was altered by the elevated CO_2 treatment.

To estimate C inputs to the forest floor, we summed monthly litterfall measurements over the six-year time period and added estimates of fine-root turnover. Matamala and Schlesinger (2000) estimated fine-root turnover for 1997 and 1998 from sequential sampling, whereas Pritchard et al. (2001) estimated fine-root turnover for 1999 from mini-rhizotrons. The two methods reported consistent rates of fine-root turnover although both studies failed to show statistically significant treatment effects because of limited statistical power. Unfortunately, we do not have direct estimates of root turnover for the subsequent three years. However, Matamala et al. (2003) show a linear trend of decreasing $\delta^{13}C$ of fine roots between 1997 and 2001 as the fumigation CO_2 was assimilated by plants, which indicates that no major change in root turnover occurred during this time period. We therefore assume that the estimated rates of fine-root turnover for 1997 through 1999 were maintained between 1999 and 2002. Because fine-root turnover makes up only three to four percent of C inputs to the forest floor, any error in our estimates of forest-floor turnover related to changes in fine-root turnover after the initial three years of the experiment is unlikely to be large.

We used information about change in $\delta^{13}C$ of the forest floor and estimates of $\delta^{13}C$ in litterfall and new roots to test our estimates of C turnover in the forest floor under the elevated CO_2 treatment. After estimating k for each of the elevated rings, we weighted Eq. 1 with estimates of $\delta^{13}C$ of the original C pool, $\delta^{13}C$

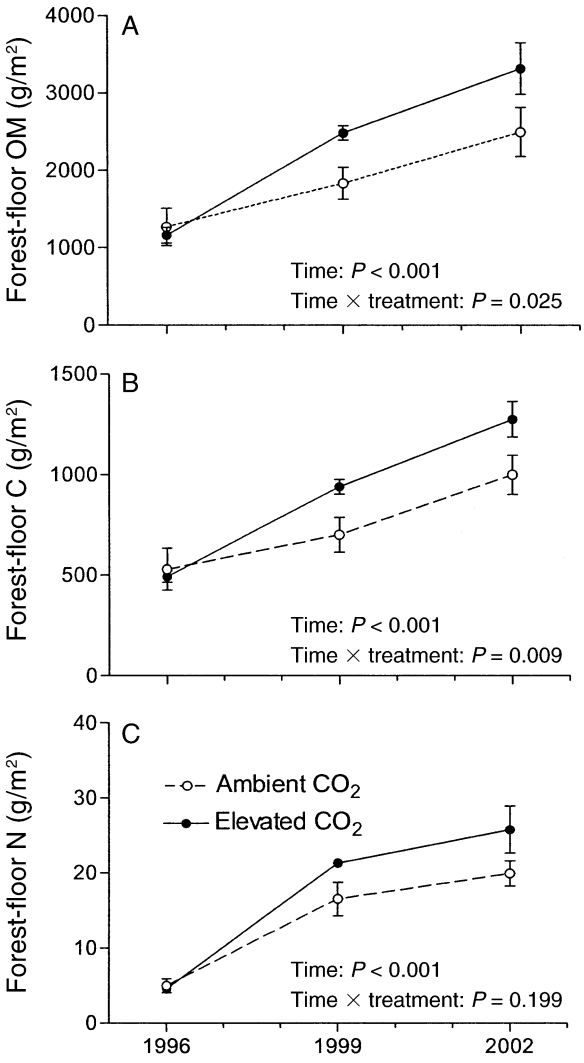

FIG. 1. Accumulation of soil organic matter in the forest floor under the elevated CO_2 treatment (solid circles) and the ambient CO_2 control conditions (open circles) during the first six years of the Duke Forest free-air CO_2 enrichment (FACE) experiment: (A) forest-floor organic mass (OM), (B) forest-floor carbon content, and (C) forest-floor N content. Error bars represent ±SE. *P* values are from a mixed-effects analysis for the effects of time summed over treatment and for the effects of the elevated CO_2 treatment (i.e., the time × treatment interaction).

of the C pool after six years, and the mean $\delta^{13}C$ of C inputs during the six-year period [i.e., C_t ($\delta^{13}C_T$) = C_0e^{-kT} ($\delta^{13}C_0$) + $1/k(1 - e^{-kT})(\delta^{13}C_{new})$] to estimate of the $\delta^{13}C$ of the forest floor for the elevated rings at the end of six years. These estimates were then compared with our measurements of the mean forest floor $\delta^{13}C$ for the elevated CO_2 rings as a check on our estimates of forest-floor C turnover under the elevated CO_2 treatment.

Soil organic matter (SOM) dynamics

For the mineral SOM pools, the unique $\delta^{13}C$ signature of the fumigation gas provides an opportunity to indirectly estimate steady-state C turnover and annual C inputs for the elevated CO_2 rings. The fumigation gas rapidly becomes incorporated into plant biomass and begins to alter the $\delta^{13}C$ signature of C inputs to the soil within a few months (Matamala et al. 2003). Unlike the forest floor, we do not have direct estimates of the annual C inputs, *I*, with which to combine estimates of C pools to derive an estimate of *k*. However, for SOM pools that have reached steady state, we can indirectly estimate *k* from the change in $\delta^{13}C$ of SOM in the elevated CO_2 rings, and then multiply this estimate by the C pool size to estimate the C inputs required to produce the observed change in $\delta^{13}C$. To use change in $\delta^{13}C$ to estimate the steady-state *k* and *I* for various SOM pools, we used a first-order model:

$$1 - f = e^{-kT} \quad (3)$$

where *f* is the fraction of organic matter replaced by new C with depleted $\delta^{13}C$ (i.e., $f = [\delta^{13}C_T - \delta^{13}C_0]/[\delta^{13}C_{new} - \delta^{13}C_0]$; Balesdent et al. 1988) and solved for *k*. In a similar coniferous forest in South Carolina, Richter et al. (1999) showed that approximately 67% of annual soil C inputs to the upper 15 cm of mineral soil derived from fine-root turnover and 33% from downward transport of DOC. The $\delta^{13}C$ signature of new roots under the elevated CO_2 treatment is -39.2 ± 0.83 (Matamala et al. 2003). New aboveground plant litter produced under the elevated CO_2 treatment has $\delta^{13}C$ of -42.5 ± 0.64. Assuming new DOC produced under the elevated CO_2 treatment has a similar $\delta^{13}C$ ratio and weighting the $\delta^{13}C$ estimates of fine-root C inputs and DOC inputs by 0.67 and 0.33, respectively, we derived an estimate for the $\delta^{13}C$ of new C inputs of -40.3. Because we do not have direct estimates of the proportion of annual C inputs derived from root turnover vs. those derived from downward transport of DOC at the Duke Forest FACE experiment, we cannot quantify

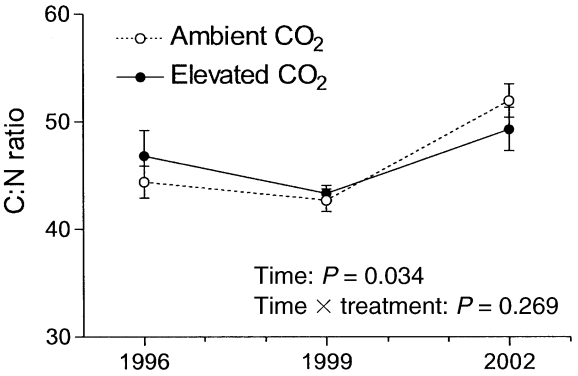

FIG. 2. Carbon-to-nitrogen ratio of the forest floor under the elevated CO_2 treatment (solid circles) and the ambient CO_2 control conditions (open circles) during the first six years of the Duke Forest free-air CO_2 enrichment (FACE) experiment. Error bars represent ±SE. *P* values are from a mixed-effects analysis for the effect of time summed over treatment and for the effect of the elevated CO_2 treatment (i.e., the time × treatment interaction).

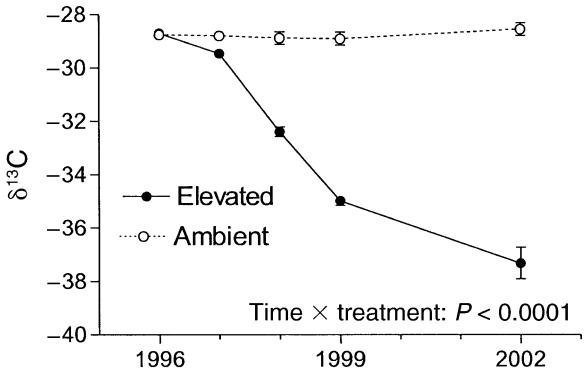

FIG. 3. Change in $\delta^{13}C$ of the forest floor under the elevated CO_2 treatment (solid circles) and the ambient CO_2 control conditions (open circles) during the first six years of the Duke Forest free-air CO_2 enrichment (FACE) experiment. Error bars represent ±SE. The *P* value from a mixed-effects analysis is given for the effects of the elevated CO_2 treatment (i.e., the time × treatment interaction).

the uncertainty of our estimates of SOM turnover based on change in $\delta^{13}C$.

Statistical analyses

For all statistical analyses testing for an elevated CO_2 treatment effect, the mean of samples collected within each FACE ring was considered the experimental unit. The sample sizes are thus three elevated CO_2 treatment plots and three ambient CO_2 control rings. While this experimental design provides limited statistical power, given the complexity and expense of an atmospheric CO_2 manipulation of this scale, it is a feasible experimental design. For those response variables for which we have data at a single time step, a one-way ANOVA was used to test for statistically significant treatment effects. However, for those variables for which we have measurements at three or more time steps, we used a linear mixed-effects model to describe change in soil properties over time and to test for statistically significant treatment effects. Mixed-effects models are commonly used to describe longitudinal, repeated-measures, and multilevel data (Pinheiro and Bates 2000). The model incorporates both fixed and random effects, and accounts for the covariance structure related to repeated measurements of the experimental unit over time. Fixed effects are those associated with levels of experimental factors and random effects are associated with individual experimental units. For our data, fixed effects were the elevated CO_2 treatment and time (i.e., year), and random effects were those associated with variation occurring in measurements of the six experimental plots over time. The fixed effects were crossed as a factorial (i.e., treatment × time). The model generates an intercept term describing the mean value of the response variable at the start of the experiment, a treatment term describing differences in the intercepts (i.e., initial conditions) of the treatment levels (i.e., elevated and ambient CO_2), a ''time'' term that describing change in the response variable over time averaged over the treatment levels, and a treatment × time interaction term identifying significant change over time attributable to the treatment. The *P* value for the treatment × time interaction term indicates the influence of the elevated CO_2 treatment on the response variable during the period of the experiment.

We used S-Plus, version 6.2 (Insightful 1988, 2003) for all statistical analyses with the exception of the pretreatment spatial analyses. To determine the best statistical model for the mixed-effects analyses, we tested models using different methods of describing the covariance structure and within-group correlation with the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) statistics, and chose the model with the lowest AIC and BIC values (Pinheiro and Bates 2000). A general positive-definite type of covariance structure generally provided the best fit to our data.

RESULTS

Forest floor C dynamics

The dry mass of the forest floor increased linearly in both elevated CO_2 and ambient CO_2 rings over the

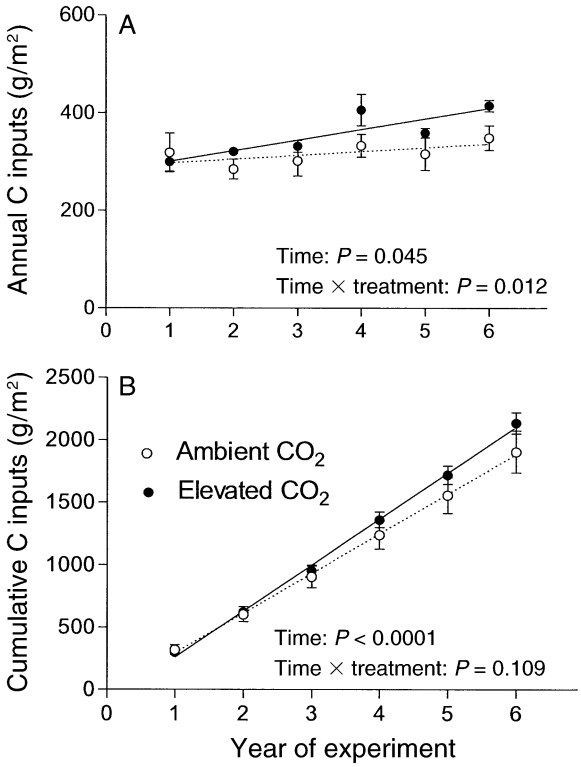

FIG. 4. Estimates of litterfall and fine-root carbon inputs to the forest floor under the elevated CO_2 treatment (solid circles) and ambient CO_2 control conditions (open circles) during the first six years of the Duke Forest free-air CO_2 enrichment (FACE) experiment for (A) annual inputs and (B) cumulative inputs. Error bars represent ±SE. *P* values from a mixed-effects analysis are given for the effect of time and the effect of the elevated CO_2 treatment (i.e., the time × treatment interaction).

TABLE 1. Estimates of C pools, inputs, turnover, and long-term steady-state C storage for the forest floor for (a) each experimental free-air CO_2 enrichment (FACE) ring and (b) treatment effects, and (c) one-way ANOVA results for the estimates.

a) By treatment ring

Ring	C_0	ΣI	$C_0 + \Sigma I$	C_6	Total decomposition	k	MRT (yr)	C^* (g C/m^2)
1) Ambient	328	1569	1897	803	1095	0.289	3.46	905
2) Elevated	454	2003	2457	1168	1289	0.241	4.15	1385
3) Elevated	547	2291	2837	1404	1433	0.224	4.46	1702
4) Elevated	478	2108	2586	1174	1412	0.259	3.86	1357
5) Ambient	578	2102	2680	1103	1578	0.288	3.47	1217
6) Ambient	681	2040	2720	928	1793	0.352	2.84	965

b) By treatment

Treatment	Total decomposition	k	MRT (yr)	C^*
Ambient CO_2 (control)	1488 ± 206	0.31 ± 0.02	3.26 ± 0.21	1029 ± 95
Elevated CO_2 (treatment)	1378 ± 45	0.24 ± 0.01	4.16 ± 0.17	1481 ± 111

c) One-way ANOVA results

Measurement	df	F	P
Total decomposition	1, 4	0.27	0.629
k	1, 4	8.49	0.043
Mean residence time	1, 4	10.98	0.029
C^*	1, 4	9.58	0.036

Notes: Table headings are C_0, initial carbon content; ΣI, cumulative carbon inputs; $C_0 + \Sigma I$, total carbon available for decomposition during the six-year period; C_6, total carbon content at year 6; total decomposition, the total amount of carbon that decomposed over the six-year period; k, the decomposition constant; MRT, mean residence time; and C^*, the steady-state carbon content. All results are given as g C/m^2 except for the unitless variable k and the mean residence time (MRT), given in years. In part b, the error values are ± SE for each mean. One-way ANOVA results are shown illustrating treatment effects after six years of experimental CO_2 fumigation.

six-year period (Fig. 1A). However, forest-floor mass increased at a faster rate (i.e., 155 ± 59 g·m^{-2}·yr^{-1}) under the elevated CO_2 treatment than under the control. This accelerated rate of organic matter accumulation under the elevated CO_2 treatment sequestered an additional 52 ± 16 g C·m^{-2}·yr^{-1} over the six-year period (Fig. 1B). However, an apparent increase in forest floor N content was not statistically significant (Fig. 1C). The C concentration of the forest floor did not change significantly; however, the N concentration decreased in both elevated and ambient CO_2 plots (df = 10, t = 2.46, P = 0.034). Consequently, the overall forest-floor C:N ratio increased from 42.5 to 50.6 (Fig. 2). In the elevated CO_2 FACE rings, the $\delta^{13}C$ of the forest floor declined exponentially (Fig. 3; Eq. 3) and indicated a lag period of 8–10 months before new C with its depleted $\delta^{13}C$ signature was incorporated into the forest floor. Such a lag period is consistent with the longevity of loblolly pine needles (Zhang and Allen 1996). That is, several months would be required before needles grown under the elevated CO_2 treatment would senesce and fall to the forest floor.

After the first year of the experiment, annual C inputs to the forest floor under the elevated CO_2 treatment exceeded those of the control by between 9 and 21% (Fig. 4). Cumulative C inputs to the forest floor under the elevated CO_2 treatment increased at a rate 50 ± 30 g C·m^{-2}·yr^{-1} greater than under the control; however, the effect was marginally significant (df = 28, t = 1.66, P = 0.109). This net increase in cumulative C inputs attributed to the elevated CO_2 treatment is very similar to the net increase of 52 ± 16 g C·m^{-2}·yr^{-1} that accumulated in the forest-floor C of the elevated CO_2 treatment (Fig. 1B).

The estimates of total decomposition, turnover rate (k), mean residence time (MRT), and steady-state C content (C^*) derived from the estimates of C pools and inputs for the forest floor are given for each of the six FACE rings (Table 1a). There was no distinguishable treatment effect on total decomposition over the six-year time period. However, there were statistically significant treatment effects for turnover rate k, MRT, and the predicted steady-state C content of the forest floor (Table 1b, c). The mean $\delta^{13}C$ of the forest floor in the elevated CO_2 plots predicted from the change in mean $\delta^{13}C$ of annual litter inputs was −38.43 ± 0.12. This prediction is greater than the observed forest-floor $\delta^{13}C$ of −37.31 ± 0.45; however, the difference was not statistically significant (t = 2.25, df = 2, P = 0.154).

SOM dynamics

We did not detect any statistically significant elevated CO_2 treatment effects in C and N concentrations or contents of the bulk mineral soils at either depth (Table 2). However, the C concentration of the bulk mineral soil at the 0–15 cm depth averaged over the elevated CO_2 and control rings increased significantly (df = 10, t = 2.53, P = 0.030), and produced a significant increase in C content of 72 ± 31 g C·m^{-2}·yr^{-1} (df = 10, t = 2.33, P = 0.042). The corresponding

TABLE 2. Bulk mineral soil properties for (a) 0–15 cm depth and (b) 15–30 cm depth under ambient vs. elevated CO_2.

Property	Year	0–15 cm depth				
		Ambient	Elevated	df	*t*	*P*
Field bulk density (g/cm^3)	1996	…	…			
	1999	1.17 (0.04)	1.12 (0.02)			
	2002	1.13 (0.04)	1.06 (0.02)	4	−3.16	0.034
%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)	10	−0.03	0.764
%N	1996	0.08 (0.007)	0.09 (0.001)			
	1999	0.07 (0.007)	0.09 (0.01)			
	2002	0.10 (0.01)	0.10 (0.004)	10	−0.74	0.473
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)	10	2.32	0.042
Total C (g/m^2)	1996	1977 (18)	2142 (94)			
	1999	1901 (52)	2208 (136)			
	2002	2407 (141)	2734 (96)	10	0.62	0.549
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)	10	−0.18	0.857
$\delta^{13}C$	1996	−26.00 (0.14)	−26.09 (0.40)			
	1999	−26.24 (0.18)	−28.05 (0.20)			
	2002	−26.64 (0.18)	−29.75 (0.08)	10	−6.21	0.0001

Notes: Values are given as the mean for each time step and treatment level with SE in parentheses. Results of a mixed-effects analysis time × treatment interaction indicate the influence of the elevated CO_2 treatment. Statistics (df, *t*, *P*) are for all three years pooled (time × treatment interaction).

increase in N content was marginally significant (df = 10, $t = 2.07$, $P = 0.065$). Apparent increases in C and N content at 15–30 cm depth for both elevated and ambient CO_2 plots were not statistically significant (Table 2). Consistent with the increase in C content of the mineral soil, the field bulk density decreased at both the 0–15 and 15–30 cm depths. There were also statistically significant increases in the C:N ratio for the elevated CO_2 treatment at the 0–15 cm depth (Table 2), and for both elevated CO_2 and control rings at the 15–30 cm depth (df = 10, $t = 4.42$, $P = 0.001$). Incorporation of new C into the mineral soil produced significant treatment differences in $\delta^{13}C$ of the bulk mineral soil at the 0–15 cm depth, but not at the 15–30 cm depth (Table 2).

The results of the density and physical fractionations of the upper mineral soil suggest that the increase in the C content of the bulk mineral soil at the 0–15 cm depth occurred entirely within the free light fraction (Fig. 5). There were no detectable increases in the C and N contents of any of the iPOM size fractions or of the mineral-associated organic matter fraction (Table 3). There was a statistically significant treatment effect on the N content of the free light fraction, but the corresponding result for C content was not significant. Changes in $\delta^{13}C$ were statistically significant for the free light fraction, fine iPOM, and mineral-associated organic matter fractions (Table 3).

Because the C content of the bulk mineral soil and of the free light fraction at the 0–15 cm depth increased during the first six years of the experiment, the assumption of steady-state conditions implicit in the method of deriving the MRT and annual C inputs from change in $\delta^{13}C$ was violated. However, because the C content of the iPOM and mineral-associated organic matter pools appear to be at steady state, the MRT and steady-state C inputs can be estimated for these SOM pools. The estimated MRT for each of the iPOM fractions as well as the mineral-associated organic matter was greater than 40 years with small annual inputs of C required to produce the observed changes (Table 4). Summing over the iPOM and mineral-associated fractions to estimate the turnover rate and annual C inputs to protected SOM pools, we derived a MRT of 44.8 years with annual C inputs of only 26 g $C \cdot m^{-2} \cdot yr^{-1}$.

DISCUSSION

Forest-floor dynamics

Over the first six years of the Duke Forest free-air CO_2 enrichment (FACE) experiment, organic C accumulated in the forest floor of the elevated CO_2 rings at a rate of 52 ± 16 g $C \cdot m^{-2} \cdot yr^{-1}$ greater than would be expected during reforestation as represented by C accumulation in the forest floor of the ambient CO_2 rings. This estimate supports the results of an ecosystem C assimilation model that produced a somewhat lower estimate of 36 g $C \cdot m^{-2} \cdot yr^{-1}$ for total forest-floor and soil C sequestration (i.e., Schaefer et al. 2003). This additional C sink resulted from increased C inputs of 50 ± 30 g $C \cdot m^{-2} \cdot yr^{-1}$ to the forest floor in response to CO_2 enhancement of primary production. That is, there

TABLE 2. Extended.

15–30 cm depth				
Ambient	Elevated	df	*t*	*P*
…	…			
1.45 (0.03) 1.40 (0.03)	1.49 (0.04) 1.42 (0.03)	4	−3.52	0.024
0.47 (0.02) 0.47 (0.04) 0.56 (0.02)	0.48 (0.04) 0.54 (0.06) 0.62 (0.04)	10	0.93	0.374
0.03 (0.001) 0.03 (0.003) 0.03 (0.001)	0.03 (0.003) 0.03 (0.004) 0.03 (0.002)	10	0.11	0.915
854 (28) 640 (52) 980 (61)	887 (49) 747 (46) 1091 (47)	10	0.43	0.678
65 (2.4) 64 (5.2) 67 (3.5)	70 (5.8) 74 (6.0) 71 (3.9)	10	−0.09	0.930
15.37 (0.31) 15.87 (0.31) 17.50 (0.39)	15.42 (0.61) 16.17 (0.52) 18.57 (0.41)	10	1.49	0.166
…	…			
−24.15 (0.42) −25.13 (0.36)	−24.96 (0.55) −27.11 (0.29)	4	−2.29	0.084

is no evidence that the overall rate of decomposition of the forest floor decreased under the elevated CO_2 treatment. Over the six-year period, total decomposition in the forest floor under elevated CO_2 was indistinguishable from that under the ambient CO_2 control (Table 3). The mean residence time (MRT) of the forest floor increased under the elevated CO_2 treatment relative to the control because the greater C inputs associated with the productivity enhancement could not be processed at the original rate without an increase in microbial biomass and activity. The longer MRT of the forest floor under elevated CO_2 explains the increase in the predicted steady-state C content. As such, the additional C sink in the forest floor of the elevated CO_2 treatment should be considered a dynamic C sink. That is, it is wholly dependent on the NPP enhancement and increased C inputs. Our estimate of forest-floor C accumulation in the control rings was comparable to an estimate from a similarly aged loblolly pine plantation at Oak Ridge, Tennessee, USA (i.e., 78 vs. 72 g $C \cdot m^{-2} \cdot yr^{-1}$; Johnson et al. 2003).

These results are consistent with measurements of N mineralization, N immobilization, and microbial N content that failed to detect an elevated CO_2 treatment effect at the Duke Forest and other FACE experiments (Finzi and Schlesinger 2003, Zak et al. 2003, Sinsabaugh et al. 2003). The lack of response by soil microbes indicates that either the native SOM pool is so large relative to annual C inputs that change in the latter has little effect on the soil microbial community (Zak et al. 2000), or that a high proportion of C inputs to the soil system consist of compounds that are resistant to microbial attack and therefore do not stimulate an increase in microbial biomass and biosynthesis (Zak et al. 2000, Finzi and Schlesinger 2003). At the Duke Forest, the native soil C pool in the mineral soil is approximately 29 times larger than the estimated annual C inputs and thus may overwhelm a treatment effect on microbial activity in the mineral soil (Hungate et al. 1996). In contrast, the native C pool of the forest floor is only 3.2 times the annual C inputs, so an increase in microbial biomass and activity would be expected if a significant proportion of the annual C inputs to the forest floor consisted of labile C compounds. Finzi and Schlesinger (2003) suggest that because approximately 50% of the C in aboveground litter is made up of refractory lignin compounds, poor substrate quality may explain the lack of a microbial response. Although Lichter et al. (2000) showed an increase in throughfall DOC inputs to soil associated with the elevated CO_2 treatment, the quantity of this presumably labile C substrate was negligible compared with the soluble C inputs of aboveground litter. Thus, the response of the microbial community appears to depend on the chemistry as well as the quantity of carbon substrate entering the soil system (Zak et al. 2000, 2003).

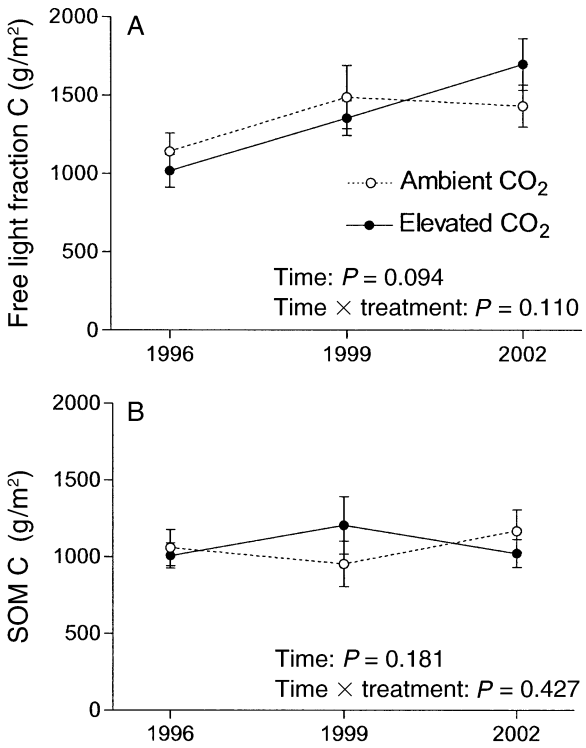

FIG. 5. Estimates of the total carbon content of (A) the free light fraction and (B) SOM at the 0–15 cm depth under the elevated CO_2 treatment (solid circles) and the ambient CO_2 control conditions (open circles) during the first six years of the Duke Forest free-air CO_2 enrichment (FACE) experiment. Error bars represent ±SE. *P* values derived from a mixed-effects analysis are given for the effect of time and the effect of the elevated CO_2 treatment (i.e., the time × treatment interaction).

TABLE 3. Properties of the free light fraction, intra-aggregate particulate organic matter (iPOM) size categories, and mineral-associated organic matter of the upper mineral soil (0–15 cm depth) in ambient and elevated free-air CO_2 enrichment (FACE) rings after six years of the experiment.

Treatment	%C	%N	C:N	Total C	Total N	$\delta^{13}C$
Light fraction						
Ambient CO_2	34.86 (0.72)	0.73 (0.03)	48.28 (1.46)	1431.4 (134.2)	30.1 (3.3)	−28.71 (0.57)
Elevated CO_2	35.10 (1.72)	0.75 (0.01)	47.69 (3.01)	1696.6 (164.0)	36.8 (2.5)	−34.45 (0.04)
df	10	10	10	10	10	10
t	−0.58	−0.31	−0.14	1.75	2.43	−9.05
P	0.574	0.760	0.889	0.110	0.035*	<0.0001*
Coarse iPOM (>250 μm)						
Ambient CO_2	1.04 (0.12)	0.05 (0.007)	19.30 (0.70)	473.0 (38.7)	24.8 (2.8)	−24.92 (0.60)
Elevated CO_2	1.44 (0.48)	0.05 (0.005)	22.45 (3.09)	562.3 (158.5)	22.6 (1.7)	−26.35 (0.38)
df	10	10	10	10	10	10
t	−0.83	−1.58	1.26	0.665	−0.05	−0.83
P	0.424	0.145	0.235	0.521	0.958	0.423
Fine iPOM (53–250 μm)						
Ambient CO_2	0.77 (0.04)	0.05 (0.004)	16.24 (0.70)	370.5 (42.7)	23.3 (3.1)	−25.96 (0.08)
Elevated CO_2	0.72 (0.05)	0.05 (0.004)	16.19 (0.42)	314.5 (52.1)	19.9 (3.5)	−27.63 (0.08)
df	10	10	10	10	10	10
t	−0.05	−0.20	1.45	−0.46	0.17	−2.59
P	0.956	0.843	0.176	0.652	0.504	0.027*
Mineral-associated organic matter (<53 μm)						
Ambient CO_2	0.63 (0.15)	0.05 (0.010)	12.54 (0.56)	326.3 (62.7)	28.9 (4.6)	−25.24 (0.29)
Elevated CO_2	0.66 (0.05)	0.05 (0.004)	12.73 (0.27)	326.9 (28.8)	26.0 (2.2)	−26.94 (0.09)
df	10	10	10	10	10	10
t	−0.45	−0.76	0.96	−0.47	−0.66	−2.89
P	0.661	0.463	0.361	0.646	0.523	0.016*

Notes: The table reports results of mixed-effects model analysis for the time × treatment interaction indicating the influence of the elevated CO_2 treatment on the total carbon content of the free light fraction and intra-aggregate particulate organic matter (iPOM) size categories. Results are given as means, with SE in parentheses.

Over the long term, N limitation may attenuate the CO_2-induced productivity enhancement at the Duke Forest FACE experiment. During the first six years of the experiment forest-floor C accumulated linearly, whereas forest-floor N began to level off toward a steady state during the latter three years. This discrepancy produced a significant widening of the C:N ratio of the forest floor between 1999 and 2002 in both elevated and ambient rings (Fig. 2). The widening of the C:N ratio of the forest floor is mirrored by increases in the C:N ratio of the mineral soil between 0 and 15 cm under the elevated CO_2 treatment and for both elevated and ambient CO_2 plots between 15 and 30 cm depth. These changes may reflect greater relative proportions of woody debris and refractory carbon compounds entering the soil system as the ecosystem matures (Gholz et al. 1985, Finzi and Schlesinger 2003). As such, acute N limitation of primary productivity may yet occur as the ecosystem matures (Richter and Markewitz 2001, Oren et al. 2001, Luo et al. 2004).

SOM dynamics

In contrast to the forest floor, we detected no statistically significant treatment effects on the C and N content of the bulk mineral soils or the iPOM and mineral-associated OM fractions. An elevated CO_2 effect was detected in the N content of the free light fraction although a corresponding change in the C content was not statistically significant (Table 3). Given that the estimated C inputs to protected SOC pools in mineral soil summed to only 26 g $C \cdot m^{-2} \cdot yr^{-1}$ and, assuming this amount represents a 12% increase over the annual C inputs to the control rings (similar to the aboveground NPP enhancement), only ~3 g $\cdot m^{-2} \cdot yr^{-1}$ of ad-

TABLE 4. Estimated turnover rates and carbon inputs required to produce the change in $\delta^{13}C$ for bulk mineral soils and SOM fractions at the 0–15 cm depth.

Soil	*f*	*k*	MRT (yr)	C inputs ($g \cdot m^{-2} \cdot yr^{-1}$)
Protected SOM (0–15 cm depth)	0.010	0.022	45.4	26
Coarse intra-aggregate particulate organic matter (iPOM)	0.093	0.021	46.8	12
Fine intra-aggregate particulate organic matter (iPOM)	0.116	0.025	40.4	8
Mineral-associated organic matter	0.113	0.022	45.5	7

Note: Key to variables: MRT, mean residence time; *f*, proportion of new carbon sequestered since the experiment began, out of the total carbon; and *k*, decomposition constant.

ditional C would have accumulated in protected SOM pools in the elevated CO_2 rings. That amount is far less than the uncertainty associated with the estimates of the C pool sizes. Consequently, it is not surprising that the analysis failed to detect a treatment effect.

These results reinforce numerous studies indicating that forest soils are unlikely to sequester significant additional quantities of atmospheric C associated with CO_2 fertilization because of the low rates of C input to refractory and protected SOM pools. For example, Schlesinger (1990) reviewed the rates of C accumulation in chronosequence studies and calculated that production of refractory humic substances in soils amounted to $<1\%$ of terrestrial primary production; an insignificant amount relative to global C sequestration. Using ^{14}C ages for soils collected from an aggrading southeastern forest similar to the Duke Forest FACE site that were archived over a 40-year period, Richter et al. (1999) showed that the mean rate of C accumulation was only 4 g $C \cdot m^{-2} \cdot yr^{-1}$. In a recent review of C sequestration potential of abandoned agricultural lands, Post and Kwon (2000) estimated the mean rate of C accumulation in soils of aggrading forests to be 34 g $C \cdot m^{-2} \cdot yr^{-1}$. Hagedorn et al. (2003) similarly found that the rate of C inputs to recalcitrant SOM fractions was too small to be consequential in forest soils. Hoosbeck et al. (2004) postulated a priming effect of labile C inputs that increased decomposition and thereby reduced the SOM content under elevated CO_2 in model ecosystems. Our results are consistent with these studies and provide a clear example of the limited potential of soils for sequestering anthropogenic C over the long term.

The rate of SOM accumulation following agricultural abandonment depends on the rate of C inputs and the rate of mineralization; each of which may be influenced by many variables. Post and Kwon (2000) report maximum rates of C accumulation in soils that range from 740 g $C \cdot m^{-2} \cdot yr^{-1}$ for tropical moist forest (Ramakrishnan and Toky 1981) to 3.4 g $C \cdot m^{-2} \cdot yr^{-1}$ for primary succession in tropical wet forest (Vitousek et al. 1983). For the upper 30 cm of mineral soil at the Duke Forest FACE experiment, we estimate that 89 ± 44 g $C \cdot m^{-2} \cdot yr^{-1}$ accumulated in both the elevated CO_2 and ambient CO_2 FACE rings in response to reforestation ($t = 2.01$, df $= 10$, $P = 0.072$). This rate of SOM accumulation falls at the high end of mean rates reported for southeastern U.S. forests. Schiffman and Johnson (1989) estimated a mean rate of 28.4 g $C \cdot m^{-2} \cdot yr^{-1}$ for the upper 33 cm of mineral soil over a 50-year time period, whereas Post and Kwon (2000) estimated a mean rate of 5.9 g $C \cdot m^{-2} \cdot yr^{-1}$ for the upper 68.5–91.4 cm of mineral soil over 110 years using data from Billings' (1938) classic account of old field succession in southeastern forests. Johnson et al. (2003) measured no change in SOC in the upper 40 cm of mineral soil 18 years after the establishment of a loblolly pine plantation at Oak Ridge, Tennessee, USA.

Given the Duke Forest site history and site preparation (i.e., drum-chopping and burning) prior to planting in 1982, it is likely that these soils are in an early stage of recovery and are currently accumulating C at a maximal rate as fine roots refill the soil volume. Our SOM fractionations suggest that most, if not all, of the C accumulation in the bulk mineral soils occurred in the free light fraction, not in the refractory or physically protected SOM pools. Being derived primarily from fine-root turnover, the free light fraction is short lived and thus will not constitute an important long-term C sink. Whereas studies of forest regrowth following agricultural abandonment over several decades have shown that N was transferred from the mineral soil to plant biomass and the forest floor (Richter et al. 2000, Richter and Markewitz 2001), our results suggest that N is accumulating in the upper 15 cm of mineral soil along with SOM.

Summary and implications

The results of six years of experimental CO_2 fumigation at the Duke Forest indicate a dynamic or transient C sink in the forest floor. However, the size of this sink is limited by the fast turnover time of organic C in the forest floor. Our steady-state prediction suggests that an additional 452 ± 146 g C/m^2 or 4.52 ± 1.46 Mg C/ha may be sequestered in these soils in response to rising atmospheric CO_2 concentration over the next fifty years. Given that the forested land in the eastern USA will never contain the amount of C in vegetation that was present prior to European settlement because of permanent land-use change, the additional amount of C that forest soils sequester in response to rising atmospheric CO_2 is the only potential long-term buffering of anthropogenic carbon emissions that U.S. forests can provide. The area of reforestation in the eastern USA has been estimated between 12×10^6 ha (Winjum et al. 1990) and 27×10^6 ha (Hart 1968). Taking the larger estimate and assuming that all area undergoing reforestation in the eastern USA responds to rising atmospheric CO_2 concentration as dramatically as the loblolly pine plantation in the North Carolina FACE experiment, 1.17×10^{14} g C could be expected to be sequestered in the forest floor of soils in eastern U.S. forests over the long term. This amount is only 7.4 percent of the fossil fuel CO_2 emitted by the USA during 2001 (Jackson and Schlesinger 2004). Worldwide the situation is probably similar. That is, C sequestration associated with reforestation will, at best, only account for that portion of historic atmospheric CO_2 increase related to the original land clearance and not diminish the larger portion of atmospheric CO_2 increase associated with fossil-fuel consumption. In terrestrial ecosystems, only the additional C sinks associated with CO_2-fertilization will reduce the impact of fossil-fuel consumption on atmospheric CO_2 concentration. Our results suggest that additional C sinks in soils are either dynamic in the sense that they depend

on increased rates of C input, which may attenuate over the long-term, or they are negligibly small because of low C inputs to physically protected and refractory soil C pools. Thus, we cannot depend on terrestrial ecosystems to significantly mitigate rising atmospheric CO_2 concentration and climate change.

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