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Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest

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Abstract. Atmospheric CO_2 concentrations have risen 40% since the start of the industrial revolution. Beginning in 1996, the Duke Free-Air CO_2 Enrichment experiment has exposed plots in a loblolly pine forest to an additional 200 μL/L CO_2 compared to trees growing in ambient CO_2. This paper presents new belowground data and a synthesis of results through 2008, including root biomass and nutrient concentrations, soil respiration rates, soil pore-space CO_2 concentrations, and soil-solution chemistry to 2 m depth. On average in elevated CO_2, fine-root biomass in the top 15 cm of soil increased by 24%, or 59 g/m^2 (26 g/m^2 C). Coarse-root biomass sampled in 2008 was twice as great in elevated CO_2 and suggests a storage of ~20 g C·m^{-2}·yr^{-1}. Root C and N concentrations were unchanged, suggesting greater belowground plant demand for N in high CO_2. Soil respiration was significantly higher by 23% on average as assessed by instantaneous infrared gas analysis and 24-h integrated estimates. N fertilization decreased soil respiration and fine-root biomass by ~10–20% in both ambient and elevated CO_2. In recent years, increases in root biomass and soil respiration grew stronger, averaging ~30% at high CO_2. Peak changes for root biomass, soil respiration, and other variables typically occurred in midsummer and diminished in winter. Soil CO_2 concentrations between 15 and 100 cm depths increased 36–60% in elevated CO_2. Differences from 30 cm depth and below were still increasing after 10 years' exposure to elevated CO_2, with soil CO_2 concentrations >10 000 μL/L higher at 70- and 100-cm depths, potentially influencing soil acidity and rates of weathering. Soil solution Ca^{2+} and total base cation concentrations were 140% and 176% greater, respectively, in elevated CO_2 at 200 cm depth. Similar increases were observed for soil-solution conductivity and alkalinity at 200 cm in elevated CO_2. Overall, the effect of elevated CO_2 belowground shows no sign of diminishing after more than a decade of CO_2 enrichment.

Key words: elevated CO_2; loblolly pine forest; root biomass; root carbon and nitrogen; soil pore space CO_2; soil respiration.

Introduction

Because of the previous history of deforestation, temperate forests are important sinks of atmospheric carbon globally, and are expected to continue as sinks as the concentration of atmospheric CO_2 rises this century (e.g., Houghton et al. 1999, Jackson and Schlesinger 2004, Raupach et al. 2007). In general, trees growing in elevated CO_2 photosynthesize more rapidly and have at least modestly increased growth compared to trees growing at ambient CO_2 (DeLucia et al. 1999, Norby et al. 1999, Gielen et al. 2005, Hyvönen et al. 2007). Because of this CO_2 fertilization effect and other factors, model scenarios from the Intergovernmental Panel on Climate Change suggest that terrestrial ecosystems may sequester >20% of anthropogenic CO_2 emissions this century (IPCC 2001, 2007). Field experiments and other analyses suggest that such rates of sequestration may be overly optimistic, particularly for ecosystems that are not fertilized or actively managed (Sionit et al. 1981, Jackson et al. 1994, Rastetter et al. 1997, Gill et al. 2002, Luo et al. 2004, Jasoni et al. 2005, Körner 2006).

The fraction of net primary production (NPP) allocated belowground in temperate forests is large but varies widely, ranging from ~20% to 65% (e.g., Waring et al. 1998). Of the carbon that is allocated belowground in forests, one-half to three-quarters is typically respired back to the atmosphere (e.g., Raich and Nadelhoffer 1986, Högberg et al. 2002, Palmroth et al. 2005). Previous results from elevated CO_2 experiments have shown that belowground net primary production and fine-root biomass both tend to increase with CO_2 enrichment (e.g., Fitter et al. 1995, Hungate et al. 1997, Matamala and Schlesinger 2000, King et al. 2001, Norby et al. 2004, Finzi et al. 2007). Rates of soil respiration also typically increase with increased atmospheric CO_2, coupling faster outputs with the faster inputs observed through increased photosynthesis (e.g., Canadell et al. 1995, Luo et al. 1996, Bernhardt et al.

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2006, Gill et al. 2006, Hoosbeek et al. 2007, Wan et al. 2007).

Through changes in productivity, respiration, and other variables, elevated CO_2 can also influence weathering of carbonates, silicates, and other soil minerals by altering the concentration of CO_2 in soil pore spaces (e.g., Sposito 1989, Andrews and Schlesinger 2001, Pendall et al. 2001, Karberg et al. 2005). Soil CO_2 is produced primarily by respiration from belowground plant tissue and from heterotrophic organisms in the soil. Some of this CO_2 dissolves in water and forms carbonic acid (H_2CO_3), which reacts with soils via cation exchange and mineral dissolution. For instance, calcium carbonate ($CaCO_3$) reacts with protons in acidic soil solutions to produce Ca^{2+} and bicarbonate ions (HCO_3^-). An increase in the concentration of CO_2 in soil pore spaces could therefore increase rates of weathering. In fact, a number of elevated CO_2 experiments have shown a significant increase in CO_2 concentrations in soil pores and some evidence for increased rates of weathering (e.g., Andrews and Schlesinger 2001, Oh and Richter 2004, Karberg et al. 2005; but see Oh et al. 2007).

How long ecosystems can continue to respond to elevated CO_2 is a question that influences assumptions about future gains in productivity and the ability of forests to continue as carbon sinks in the coming century (e.g., Hungate et al. 2003, Heath et al. 2005, Finzi et al. 2007). To date, the longest running field manipulation of atmospheric CO_2 in a forest ecosystem is the Duke Free-Air CO_2 Enrichment (FACE) experiment. Beginning with a prototype in 1994 and the fully replicated experiment in 1996, the Duke FACE experiment has exposed a loblolly pine forest to an additional 200 μL/L CO_2 (elevated CO_2 treatment) compared to trees growing at ambient CO_2. This paper presents new belowground data and a synthesis of results for the first twelve years of the experiment, including results for root biomass and nutrient concentrations, rates of soil respiration, soil pore space CO_2 concentrations, and soil solution chemistry. Using these and other data, we examine the sustained response of the ecosystem to elevated CO_2, including which, if any, variables show evidence for a decrease in their response to atmospheric CO_2 during that time frame.

Methods

Site description

The experiment consists of four 30 m diameter control plots and four 30 m diameter plots fumigated with CO_2 to maintain the atmospheric CO_2 concentration at 200 μL/L above ambient (i.e., ~570 μL/L). Three of the control plots are fumigated with ambient air only (~370 μL/L), and a fourth provides a no-ring control. Manipulation of atmospheric CO_2 began in 1994 for one plot (the "prototype") and in August of 1996 for the replicated experiment. CO_2 enrichment was nearly continuous, with the exception of time periods when the air temperature was below 5°C or when sustained wind speeds exceeded 5 m/s. Additional details on the site and on the experimental system are described in Hendrey et al. (1999), Schlesinger and Lichter (2001), and Finzi et al. (2006).

Root biomass and soil respiration measurements

Root biomass was measured bimonthly in soil cores taken from the organic litter layer (O horizon) and the top 15 cm of mineral soil (A horizon) using a 5 cm diameter corer (AMS Incorporated, American Falls, Idaho, USA). Through April 2005, four random subsamples, one from each quadrat of each ring, were taken from each plot in the replicated experiment; from July 2005 through December 2007, six random subsamples were taken from the same six plots plus the prototype ring established in 1994 and the no-ring control (48 samples for eight plots in total). Samples from 15–30 cm depth were also taken annually using the same replication scheme. Live roots were separated by hand from the O- and A-horizon samples and thoroughly cleaned in deionized water. (Roots that had lost structural integrity, broke easily, or had dark brown-black pith were considered dead and were not used in the analyses.) Roots were separated into two size classes, fine roots (diameter < 2 mm) and coarse roots (diameter ≥ 2 mm), and dried to constant mass at 55°C. Coarse-root biomass was sampled additionally on 12 and 13 May 2008 at a much larger scale. Sixteen pits were dug, one from each CO_2 and fertilizer treatment combination, 0.64 m long by 0.64 m wide and 0.32 m deep. All live roots >2 mm diameter were separated from the soil, washed in deionized water, and separated into five diameter classes: 2–5 mm, 5–10 mm, 10–20 mm, 20–40 mm, and >40 mm. The roots were dried to constant mass at 55°C, then weighed.

Soil respiration rates were measured monthly using two independent techniques, a portable infrared gas analyzer (IRGA) used for instantaneous measurements of diurnal peak respiration rates, and a soda-lime method used to obtain an integrated daily value. The portable IRGA system consisted of the PP Systems Soil Respiration System, with an EGM-4 Environmental Gas Monitor attached to an SRC-1 Soil Respiration Chamber (PP Systems, Hertfordshire, UK). Measurements were taken monthly from July 1996 through December 2007 from 12 PVC couplings (10 cm in diameter) installed to 3 cm depth in the mineral soil in each of the fully replicated FACE rings. On each date, the CO_2 flux from each collar was measured for a 60-s period between 1100 and 1700 hours, centered on the time of the daily maximum rate (1400 hours). During each efflux measurement, soil temperature was measured adjacent to each coupling from a type-K thermocouple installed at 3 cm depth in the mineral soil.

The soda-lime method was used to measure soil CO_2 efflux integrated over 24-h periods. Four PVC rings (30.5 cm in diameter and 15 cm in height) were

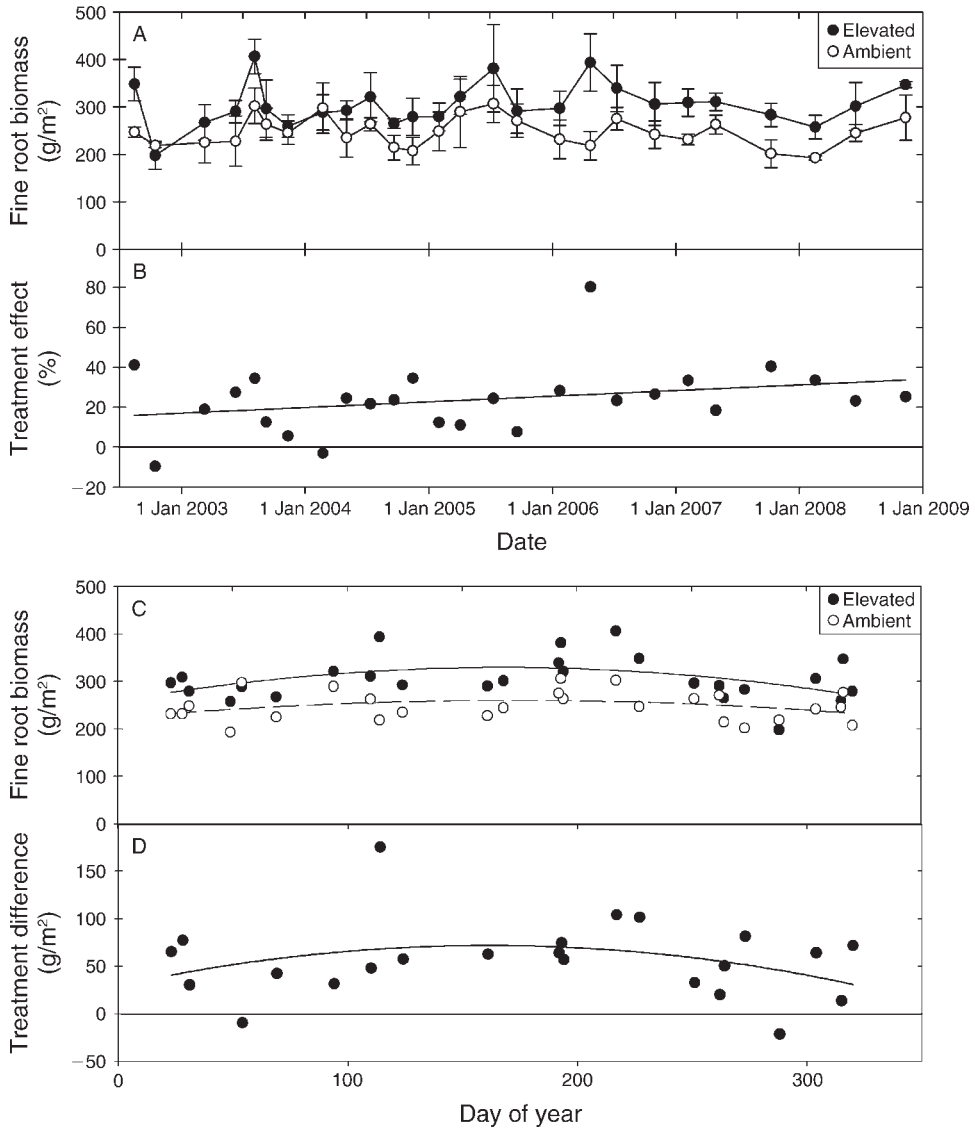

FIG. 1. Biomass of fine roots (diameter < 2 mm) from the O horizon plus the 0–15 cm depth increment of the A horizon (A) by date and (C) by day of year; (B) CO_2 treatment effect ([elevated − ambient]/ambient) by date with linear regression line ($P < 0.001$) and (D) treatment difference by day of year. All values are means ± SE; $n = 3$ or 4 per treatment. For panel (C), the solid line is the polynomial regression for the elevated treatment, and the dashed line is the polynomial regression for the ambient treatment.

permanently inserted to 3 cm depth in the mineral soil in each of the original six FACE plots. In October 2005 two additional rings were installed in each of the six original FACE plots, and six were installed in each of the prototype and no-ring control plots. Measurements were taken approximately monthly from January 1997 through December 2007. For each sample, a glass jar was filled with ~45 g of 4–8 mesh soda lime (particle size 2–5 mm), dried overnight at 105°C, sealed, and weighed. In the field, each jar was quickly opened and placed inside a PVC ring, which was immediately closed with a Plexiglas lid covered with reflective foil. An O-ring with Apiezon grease (M&I Materials Limited, Manchester, UK) prevented outside air from infiltrating the chamber. After 24 hours in the field, the jars were removed from the chambers, quickly capped, dried overnight at 105°C, and reweighed. The net mass was multiplied by 1.69 to correct for water lost during the CO_2 adsorption (Grogan 1998).

Soil pore space CO_2 and $\delta^{13}C$ analysis

The concentration of CO_2 in the soil atmosphere was measured monthly in the field using a portable EGM-1 IRGA (PP Systems, Hertfordshire, UK) connected to permanently installed PVC gas wells with Kynar tubing (Elf Atochem North America, Incorporated, Philadelphia, Pennsylvania, USA) extending just above the soil surface (Richter et al. 1995, Andrews and Schlesinger

2001). Each plot contained four gas wells at 15, 30, and 70 cm depths, and two gas wells at 100 and 200 cm depths each (a total of 16). Gas samples were drawn directly from the well through a magnesium perchlorate ($Mg(ClO_4)_2$) water trap into the IRGA, and the maximum CO_2 values were recorded. Simultaneous soil temperature measurements were made by plugging Type K thermocouple probes (permanently installed at the depths of each gas well) into a hand-held thermocouple thermometer. Soil CO_2 concentrations at 100 and 200 cm depths occasionally exceeded the maximum capability of the IRGA at over 65 535 μL/L (1996–2003) and over 100 000 μL/L (2003–present), as noted in the results.

The FACE experiment is located in the Blackwood Division of the Duke Forest (Orange County, North Carolina, USA). The site has a mean annual precipitation of 1115 mm and winter and summer mean temperatures of 3.8°C (January, 1998–2005 mean) and 24.6°C (July, 1998–2005 mean), respectively. In 1983 it was planted with 3-year-old half-sibling seedlings of loblolly pine (*Pinus taeda*) at 2.4-m spacing. In 1996, when the replicated experiment began, the pine trees were ~14 m tall and accounted for 98% of stand basal area. Since planting, a diverse mixture of understory hardwood species has colonized the site, including red maple (*Acer rubrum*) and sweet gum (*Liquidambar styraciflua*). Soils are fine, mixed, active, thermic Ultic Hapludalfs from the Enon series. They are slightly acidic (0.1 mol/L $CaCl_2$, pH = 5.75), with well-developed horizons and mixed clay mineralogy.

Beginning in March 2005, a nitrogen fertilization treatment was added to the experiment. Each plot was subdivided into two halves and a narrow trench was dug to a depth of 70 cm. An impermeable partition was installed in the trench, which was backfilled with soil removed during the excavation. Beginning in 2005, half of each plot was fertilized each year with pelletized ammonium nitrate at a rate of 11.2 g $N \cdot m^{-2} \cdot yr^{-1}$. This amount was selected to follow the protocol of an international forest nutrition network in which fertilization is designed to remove all N limitation, allowing comparisons across multiple experiments; N deposition at the site is ~1.4 g $N \cdot m^{-2} \cdot yr^{-1}$. The experimental design including the N addition was a split-plot in a randomized complete block design with three replicates; CO_2 treatment was the whole-plot factor and N treatment was the subplot factor.

For stable carbon isotope analysis, samples were taken from the soil gas wells into pre-evacuated 75-cm^3 Whitey stainless steel gas cylinders (Whitey Company, Highland Heights, Ohio, USA) as described in Andrews and Schlesinger (2001). Determination of $\delta^{13}C$ was performed by stable isotope ratio mass spectrometry at several locations (1996–2001: Duke University Phytotron, Series 2, VG ISOGAS, Middlewich, Cheshire, England, UK; 2002–2003: Duke Environmental Stable Isotope Laboratory, Finnigan-MAT Delta Plus XL continuous flow isotope ratio mass spectrometer with Finnigan-MAT GasBench, ThermoFinnigan, Bremen, Germany; 2004–present: University of Illinois-Chicago Stable Isotope Facility, Finnigan-MAT Delta Plus XL continuous flow isotope ratio mass spectrometer with Finnigan-MAT GasBench, ThermoFinnigan, Bremen, Germany). Natural gas is the source of the 200 μL/L CO_2 used for fumigation at high CO_2. The $\delta^{13}C$ signature of this comparatively depleted air is -43.1 ± 0.6‰ (mean ± SE) compared with a PeeDee belemnite standard (Bernhardt et al. 2006). In consequence, the ^{13}C of atmospheric CO_2 in the elevated CO_2 plots is ~12‰ more negative: −20‰ compared with −8‰ for ambient air. New photosynthates in ambient CO_2 have a ^{13}C signature of approximately −28‰. Loblolly pine needles and fine roots (<1 mm diameter) grown in elevated CO_2 have $\delta^{13}C = -39.3$ (±1.4‰ and ±0.5‰ for needles and roots, respectively [Ellsworth 1999, Matamala and Schlesinger 2000, Bernhardt et al. 2006]).

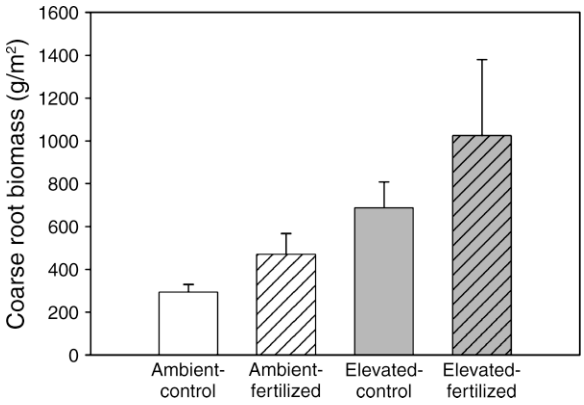

FIG. 2. Biomass of coarse roots (g/m^2, diameter > 2 mm) from 0.41-m^2 soil pits dug to 32 cm depth in March 2008 (mean + SE) for the four CO_2–nitrogen fertilization treatment combinations: ambient CO_2 unfertilized (ambient-control), ambient CO_2 fertilized (ambient-fertilized), elevated CO_2 unfertilized (elevated-control), and elevated CO_2 fertilized (elevated-fertilized). The CO_2 treatment is significant ($P = 0.03$), while the nitrogen treatment is not ($P = 0.21$).

Soil solution chemistry

Soil solutions were collected from the O horizon using tension-free lysimeters and from three depths in the mineral soil in each experimental ring. Samples from the mineral soil were collected under vacuum (60 kPa) from tension lysimeters installed at 15, 70 and 200 cm depth constructed from Rhizon samplers (15 and 70 cm depths; Rhizosphere Research Products, Wageningen, The Netherlands) and 5-cm length Prenart Teflon tubing (200 cm depth: Prenart Equipment ApS, Frederiksberg, Denmark). All soil solution samples were collected into 4-L acid-washed amber glass bottles. The lysimeters were installed during 1996, with initial sampling in July 1996. Samples were collected roughly biweekly through January 2002 and then triweekly from April 2004 through June 2005.

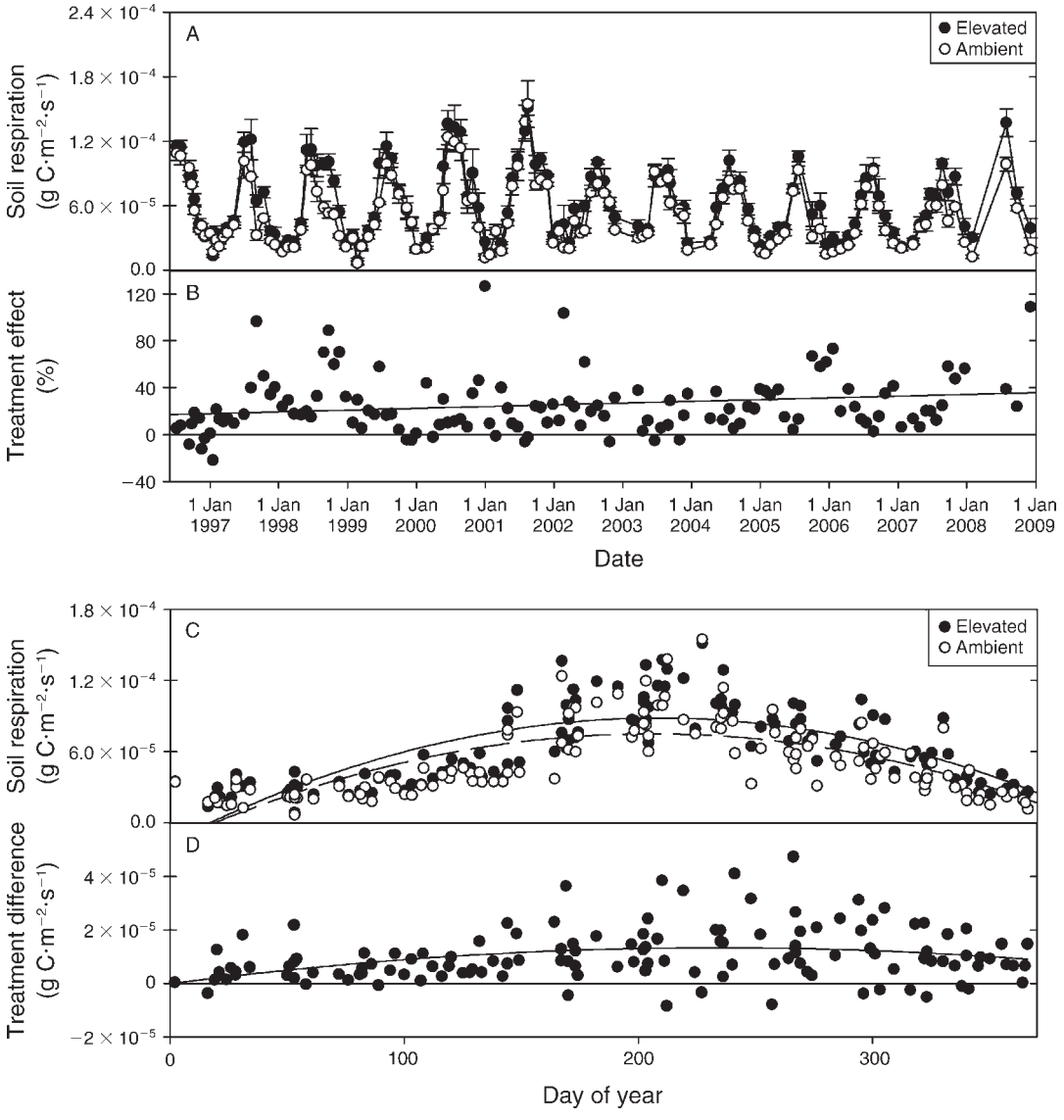

FIG. 3. Soil C efflux, measured using an infrared gas analyzer, (A) by date and (C) by day of year; (B) CO_2 treatment effect ([elevated − ambient]/ambient) by date with linear regression line ($P = 0.01$) and (D) treatment difference (elevated − ambient) by day of year. Values plotted show mean ± SE. In panel (C), the solid line is the polynomial regression for the elevated treatment, and the dashed line is the polynomial regression for the ambient treatment.

Solution samples were filtered in the laboratory through 0.45-μm filters and stored below 4°C until analyzed. Alkalinity was measured by titration with 0.01 mol/L HCl to an end point of pH 5.0, using a Brinkmann titrator fitted with a combination electrode. Conductivity was measured with a YSI Model 32 conductivity meter (Yellow Springs Instruments Incorporated, Yellow Springs, Ohio, USA). Concentrations of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), and potassium (K^{+}) were determined by flame atomic absorption spectroscopy (Model 3100, Perkin-Elmer, Norwalk, Connecticut, USA). Concentrations of anions (sulfate, nitrate, chloride) were determined by ion chromatography (ICS-2000, Dionex Corporation, Sunnyvale, California, USA). Dissolved silica was measured colorimetrically by autoanalysis using an ammonium molybdate method. Dissolved organic carbon was measured by persulfate digestion and infrared detection using a Shimadzu TOC-5000A (Shimadzu Corporation, Kyoto, Japan). We calculated the sum of base cations (millimoles of charge per liter) from the sum of individual cation molar concentrations ($2[Ca^{2+}] + 2[Mg^{2+}] + [Na^{+}] + [K^{+}]$), assuming that elemental concentrations measured by atomic absorption spectroscopy were equivalent to dissolved cation concentrations.

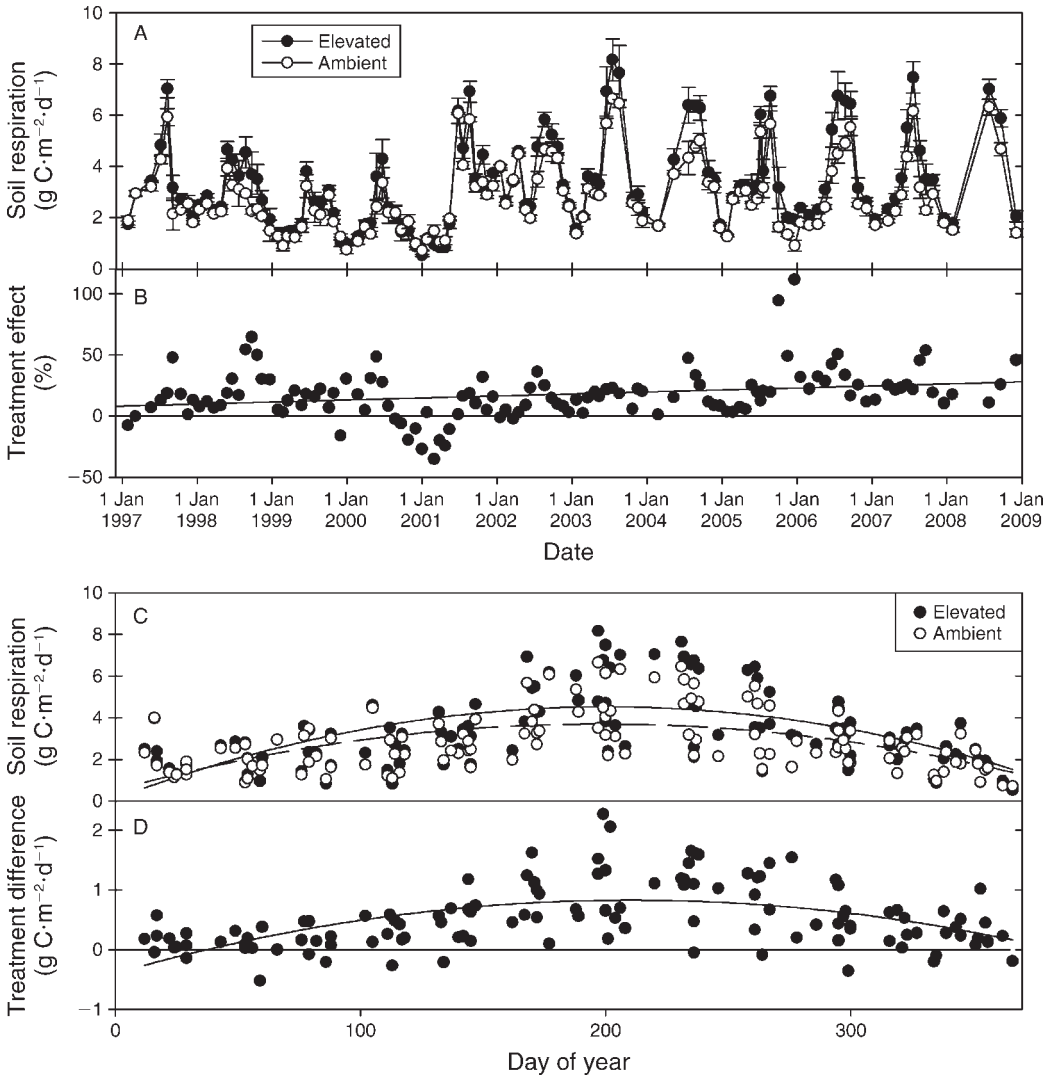

FIG. 4. Soil C efflux, measured using the soda-lime technique, (A) by date and (C) by day of year; (B) CO_2 treatment effect ([elevated − ambient]/ambient) by date with linear regression line ($P < 0.01$) and (D) treatment difference (elevated − ambient) by day of year. Values plotted show mean ± SE. In panel (C), the solid line is the polynomial regression for the elevated treatment, and the dashed line is the polynomial regression for the ambient treatment.

Statistics

Because the FACE experiment is based on continued measurements through time on the same experimental plots, we tested the main effect of CO_2 using a repeated-measures analysis of variance (Proc mixed, SAS version 9.1 [SAS 2008]), with rings blocked in pairs based on pretreatment soil parameters. Within-ring data was averaged for each sampling date during statistical determinations ($n = 3$–4 rings per treatment). The N fertilization treatment that began in the spring of 2005 was a split-plot factor nested within CO_2 rings (half of each ring receiving ammonium nitrate fertilizer and the other half remaining unfertilized). Regression analysis was used to test the slope of the CO_2 effect through time as another way to examine the $CO_2 \times$ time interaction.

RESULTS

The amount of fine roots in elevated CO_2 averaged 24% higher than in ambient CO_2 for the past half-decade of the experiment ($P < 0.05$; Fig. 1). Across all dates, the mass of <2 mm diameter roots in the organic horizon and top 15 cm of mineral soil were 248 g and 307 g for ambient and elevated CO_2, respectively (a difference of 26 g C/m^2 compared with 2100 g organic C/m^2 in the top 15 cm). The observed differences varied by the time of year, with the greatest increases in root biomass in elevated CO_2 occurring in mid to late summer (Fig. 1). Fine-root C and N concentrations in the unfertilized treatment averaged 43.7% and 1.02%, and were not significantly different for roots in the ambient and elevated CO_2 treatments (data not shown; $P > 0.30$ for

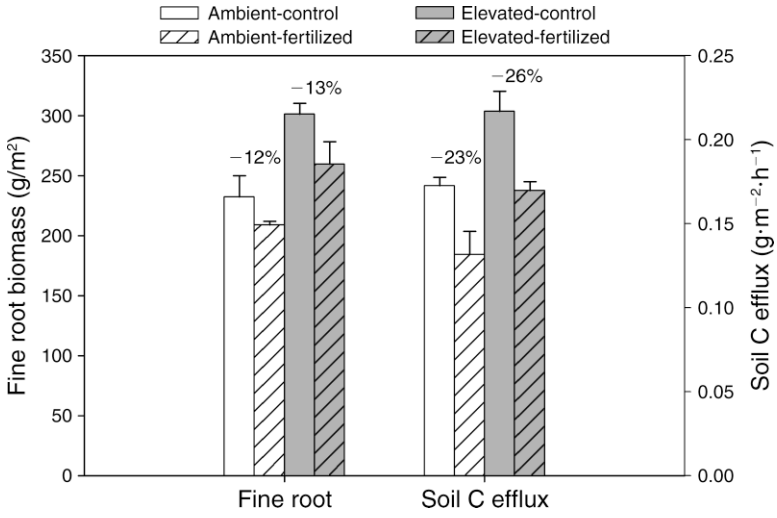

FIG. 5. Average soil CO_2 efflux and fine-root biomass for the years 2007 and 2008 (mean + SE).

each). This lack of a change in root C and N concentrations, combined with the observed increases in root biomass at elevated CO_2, suggests a higher belowground N demand for plants growing at elevated CO_2. In the fertilized treatment, fine-root C and N concentrations averaged 44.2% and 1.27%, and were not significantly different between CO_2 treatments. Fine-root C did not significantly differ between nitrogen treatments ($P > 0.80$), but fine-root N was significantly higher in the fertilized treatment ($P < 0.001$). Using the regularly sampled biomass cores, mean coarse-root biomass in the top 15 cm of soil was 17% greater on average in elevated compared with ambient CO_2, but the variation was much higher than for fine-root biomass and was not statistically significant ($P = 0.25$). However, when the large soil pits were dug in March of 2008, coarse-root biomass in elevated CO_2 was more than double that in ambient CO_2 (Fig. 2, $P < 0.05$).

Like the fine-root data, values for soil respiration were also significantly higher in elevated CO_2 as assessed by both instantaneous infrared gas analysis and 24-h integrated estimates (Figs. 3 and 4). The average increase in soil respiration across all dates was 23%, with the soda-lime technique showing a slightly higher CO_2 effect than infrared gas analysis. Peak increases in soil respiration with CO_2 showed an even stronger seasonal pattern than increases in fine-root biomass did; differences were consistently higher in midsummer to early fall, with the CO_2 response in soil respiration diminishing or disappearing in winter (Figs. 3 and 4). Across the first 12 years of the experiment, the soil respiration data also show a slowly increasing trend in the treatment effect with CO_2 (Figs. 3 and 4) ($P < 0.01$ using the soda-lime technique, $P = 0.01$ using the gas-exchange measurements). Averaged across all dates, soil respiration was higher in sectors with higher average fine-root biomass (linear regression $R^2 = 0.47$, $P < 0.0001$ for soda lime, $R^2 = 0.26$, $P = 0.01$ for gas exchange technique).

In the period 2007–2008, N fertilization, which began in the spring of 2005, significantly decreased soil respiration in both elevated and ambient CO_2 plots (Fig. 5; $P < 0.05$). The observed differences with N fertilization were 23% ($P = 0.03$) and 26% ($P = 0.004$) in ambient and elevated CO_2, respectively, using gas-exchange measurements, and 7% ($P = 0.26$) and 17% ($P = 0.006$), respectively, using the 24-h soda-lime technique (Fig. 5). Fine-root biomass in fertilized subplots decreased 12% ($P = 0.05$) and 13% ($P = 0.01$) in ambient and elevated CO_2, respectively.

Concentrations of CO_2 in the soil pore spaces at 15, 30, 70, and 100 cm depths were 36–60% higher in elevated CO_2 than in ambient CO_2 on average (Figs. 6–8), with potentially large effects on soil weathering. Relative and absolute differences were greatest at intermediate depths (Figs. 6–8). At 70 cm depth, soil CO_2 was >10 000 μL/L and 60% higher than at the same depth in ambient CO_2. Values at 30 cm and 100 cm depth were 44% and 39% higher, respectively, but absolute differences showed different dynamics for the two depths. Treatment differences of >10 000 μL/L at 100 cm depth showed no seasonal effect with CO_2, just as at 70 cm; absolute differences of a few thousand μL/L at 30-cm depth peaked strongly in mid- to late summer (Fig. 8), similar to the data for soil respiration (Figs. 3 and 4). Increases at 2 m depth were smaller, only 16% higher for elevated CO_2 rings after the 10th year. Treatment effects at 30, 100, and 200 cm depths were still increasing slowly but steadily after the first 10 years of the experiment (Fig. 8). Differences in pore space $^{13}CO_2$ values stabilized over progressively longer time periods from shallow to deep layers (Fig. 9). The carbon isotope signatures stabilized within a couple of years of the start of CO_2 fumigation at 15 cm depth. In

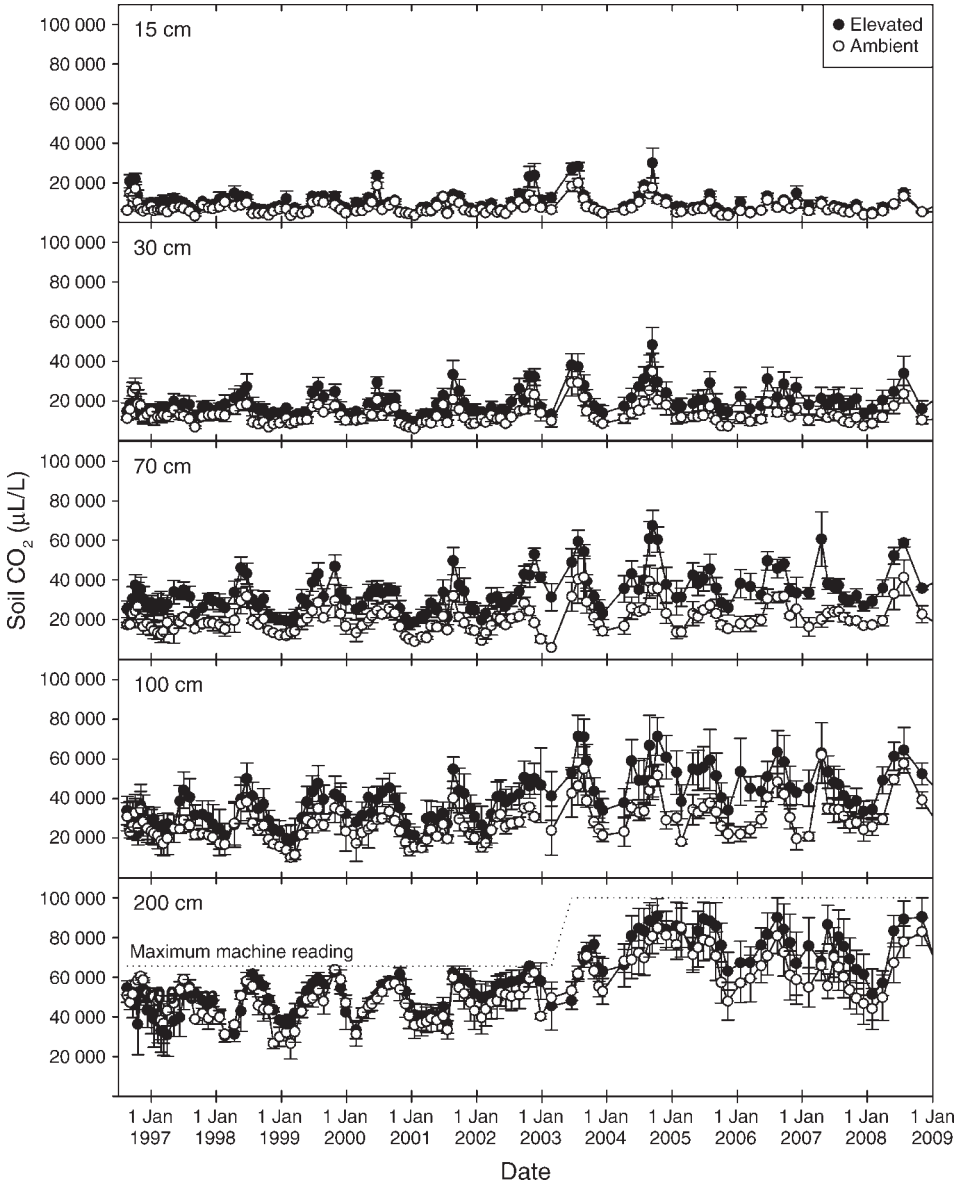

FIG. 6. Soil CO_2 concentration (mean ± SE) by date for five depths.

contrast, $\delta^{13}C$ values at the deepest sampling depth of 200 cm appear to have been relatively stable only since the 2003 growing season (Fig. 9). Even that ~7-year stabilization period seems surprisingly rapid given the presence of long-term pools of soil organic matter created from photosynthesis before the high-CO_2 fumigation began.

Greater soil acidity from CO_2-derived carbonic acid may strongly influence soil weathering. The deepest soil depth sampled (200 cm) was where we observed the largest changes in soil solution chemistry with elevated CO_2 (Table 1). Soil solution Ca^{2+} and total base cation concentrations were 140% and 176% greater, respectively, in elevated compared with ambient CO_2 (Table 1; $P < 0.01$ and $P < 0.05$, respectively). Similar increases with CO_2 were observed for electrical conductivity and alkalinity (Table 1). Greater base cation concentrations most likely arose from increased weathering and/or release from cation exchange sites attributable to the higher pore space CO_2 concentrations observed in the experiment (Figs. 6–8) and subsequent additional acidity. At shallower depths, differences in soil solution chemistry were smaller and, in most cases, not significantly different (Table 1). Treatment by time interaction terms were significant for several solutes (Table 1). Most significant interactions at the deeper depths have positive coefficients, indicating increasing differences in elevated relative to ambient plots; where significant interactions occurred in the O horizon, coefficients were generally negative.

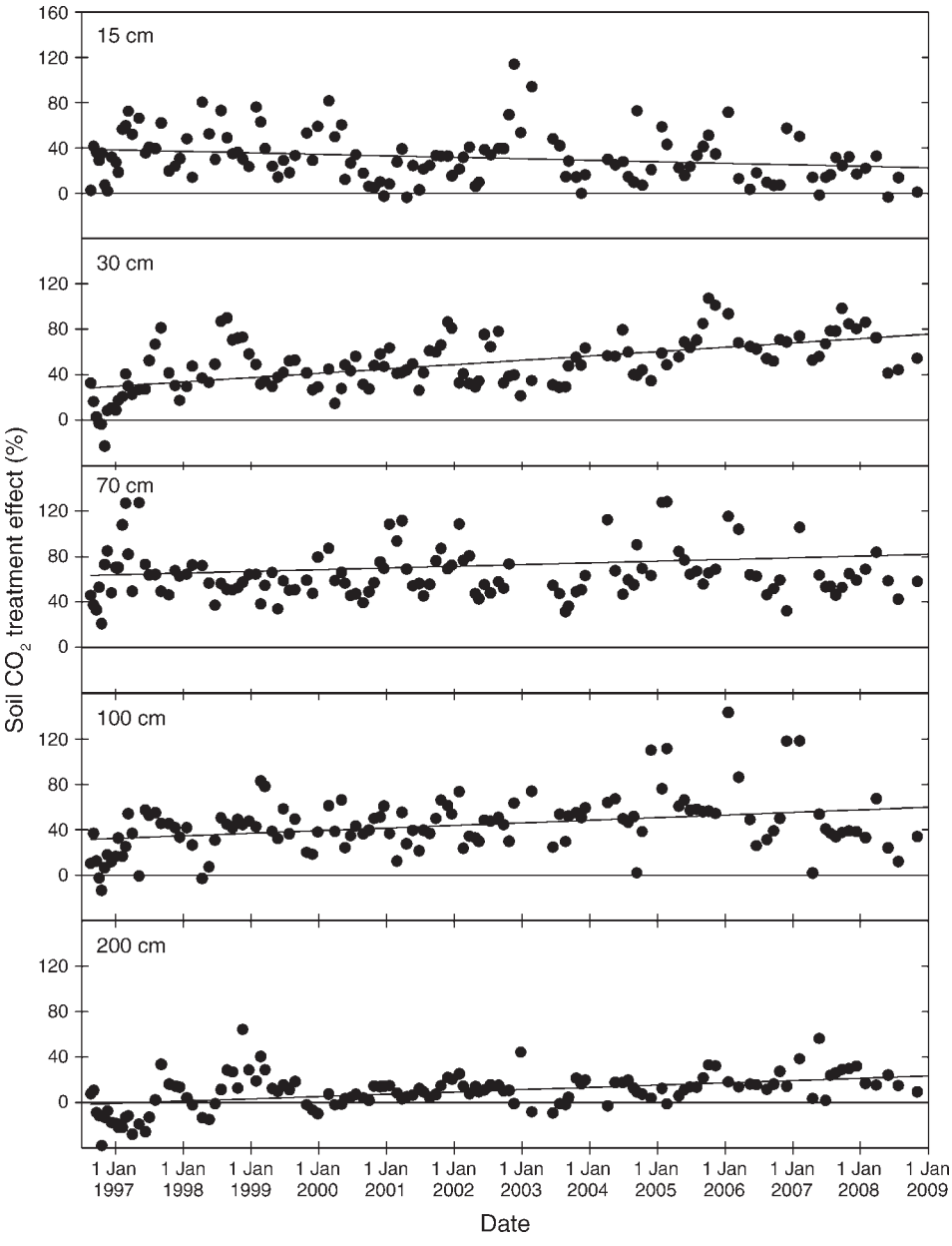

FIG. 7. The relative treatment effect of soil CO_2 concentration (μL/L) ([elevated − ambient]/ambient) by date for five depths ($P < 0.001$ for linear regression lines for depths 30 and 200 cm; $P < 0.05$ for 100 cm; $P > 0.60$ for 15 cm and 70 cm).

DISCUSSION

After more than a decade of CO_2 enrichment, the responses of belowground variables to CO_2 show no sign of slowing, and in fact continue to grow through time, suggesting that the system is not yet fully in equilibrium with its high-CO_2 environment. Concentrations of CO_2 in soil air are still increasing in all layers, except at 15 and 70 cm (Fig. 7). The treatment effects for root biomass and soil respiration are also still increasing in both absolute and relative terms (elevated vs. ambient CO_2). These and other continued increases belowground contrast with changes in many aboveground variables at Duke FACE, including photosynthesis and leaf area and biomass, that maintain a positive CO_2 response but where the relative response is not continuing to widen. They also contrast with the results of some other CO_2 experiments where "root closure," defined as a dynamic equilibrium between production and mortality, occurred within a few years of CO_2 enrichment (e.g., the scrub oak system of Day et al. [2006]).

We found substantial increases in root biomass and respiration throughout the course of the 12-year experiment, consistent with results of other experiments. All four forest FACE experiments and other experi-

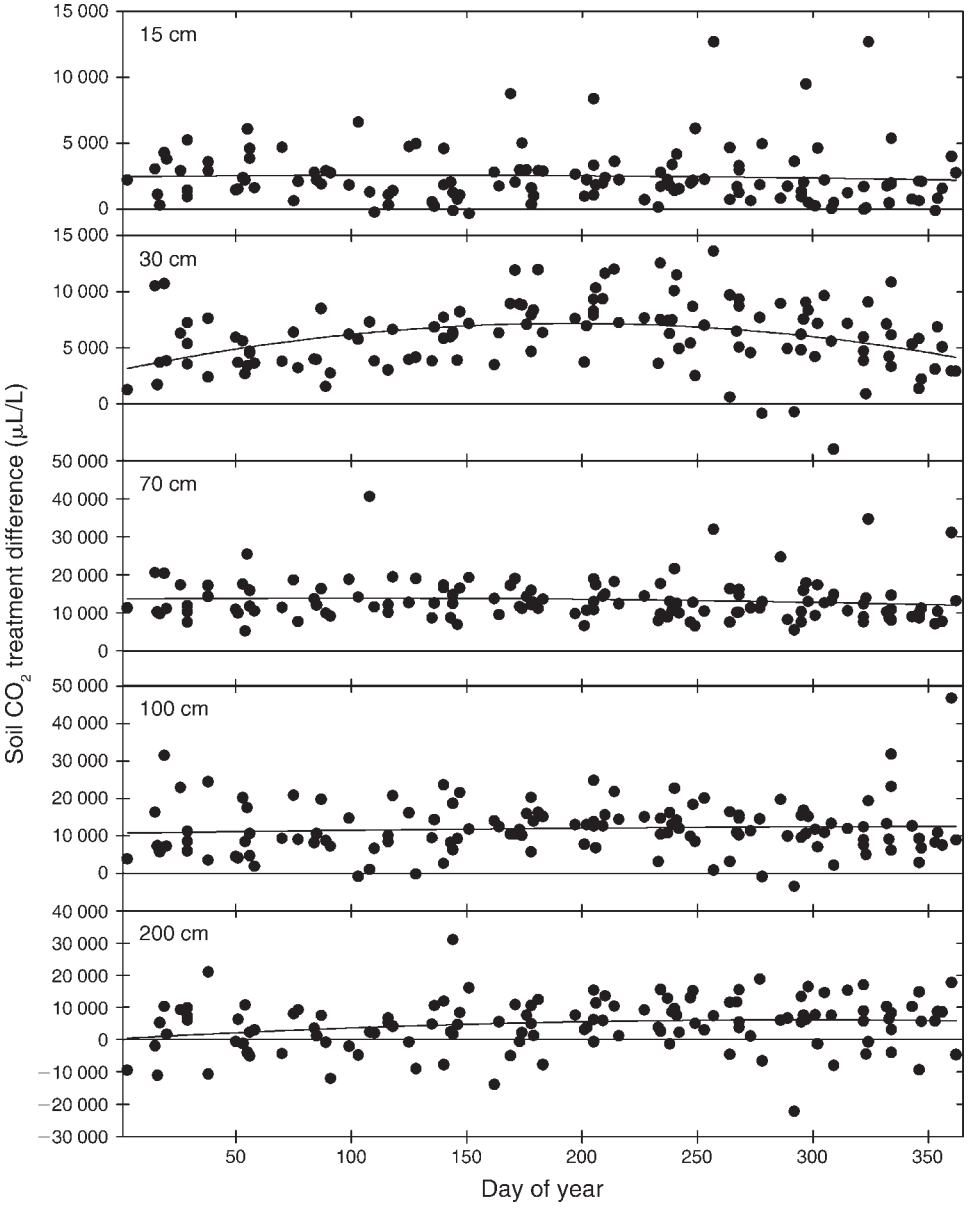

FIG. 8. Soil CO_2 concentration treatment difference (elevated − ambient) by day of year for five depths.

ments have typically shown increased belowground biomass and respiration (e.g., Körner and Arnone 1992, Lewis and Strain 1996, Hungate et al. 1997, Matamala and Schlesinger 2000, Gill et al. 2002, Norby et al. 2004, King et al. 2005, Hoosbeek et al. 2007, Pritchard et al. 2008). In general, root responses to elevated CO_2 are often greater than aboveground responses in the same systems. The balance of these changes, though, do not guarantee large carbon storage belowground. For instance, soil respiration has increased considerably in our ecosystem (Figs. 3 and 4) and in fine roots (Drake et al. 2008).

Manipulative experiments are often limited in the ability of investigators to sample belowground variables extensively. In our case, sampling once a year for deeper root biomass (15–30 cm) did not reveal a significant CO_2 response ($P = 0.75$), with mean values of fine-root biomass at that depth averaging 43.7 and 41.6 g/m^2 in ambient and elevated CO_2, respectively (although see the fairly strong CO_2 effect characterized by Pritchard et al. [2008] at that depth using minirhizotrons). Similarly, more than a decade of soil coring for root biomass revealed a trend toward increased coarse-root biomass (17% on average), but the response was not statistically significant ($P = 0.25$). We were able to detect a significant doubling of coarse-root biomass only with the opportunity to dig much larger soil pits in the spring of 2008 (Fig. 2). Other experiments may be missing

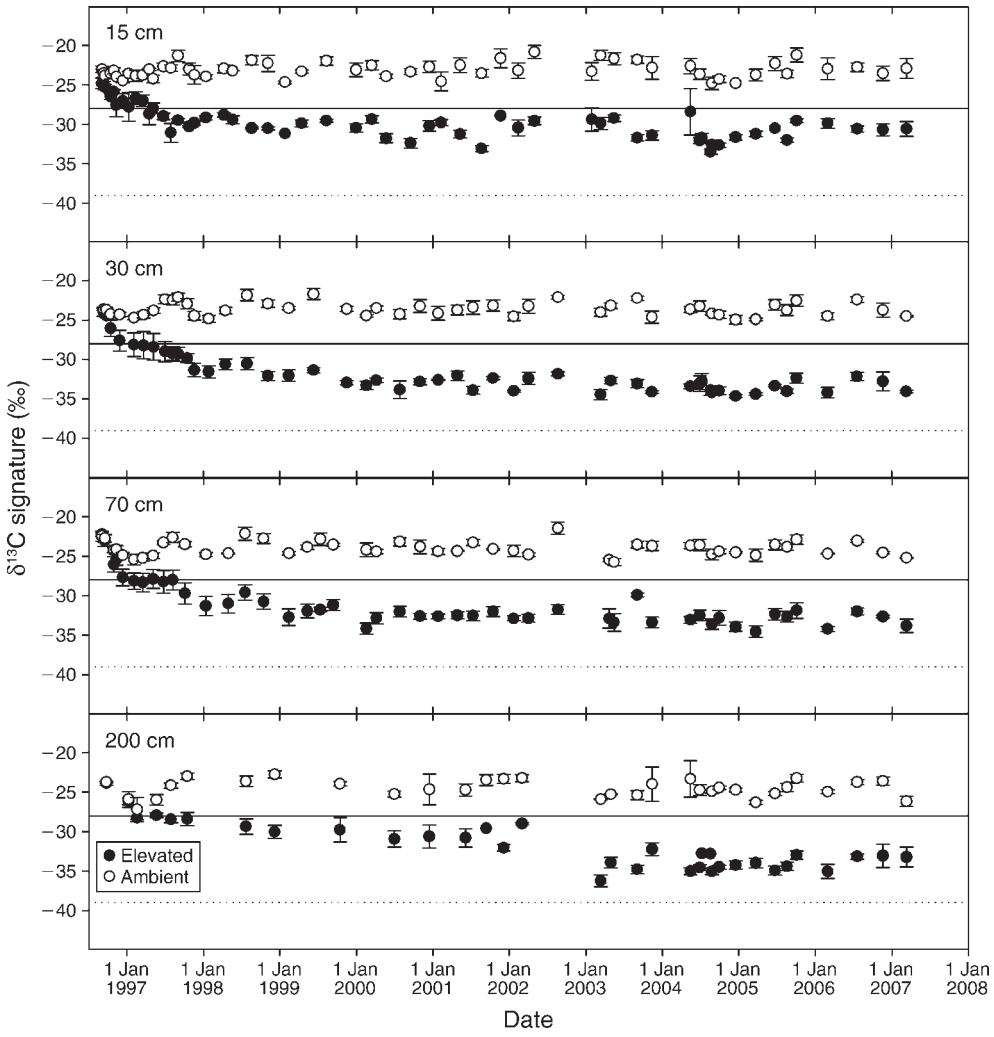

FIG. 9. Values (mean ± SE) of $\delta^{13}C$ for soil CO_2 at four depths belowground. The $\delta^{13}C$ signature of atmospheric CO_2 in the elevated plots is ~12‰ more negative (−20‰ compared with −8‰ for ambient air), reflecting the use of natural gas-derived CO_2 for the fumigation (see *Methods*). New photosynthates in ambient CO_2 have a ^{13}C signature of approximately −28‰ (solid line). Loblolly pine needles and fine roots (<1 mm diameter) grown in elevated CO_2 have $\delta^{13}C = -39.3 \pm 1.4$‰ and ±0.5‰ (SE) for needles and roots, respectively (dotted line).

TABLE 1. Soil solutes from the sampling period 1996–2005 (means ± standard errors, $n = 3$).

Soil parameters	O horizon		15 cm	
	Ambient	Elevated	Ambient	Elevated
Conductivity (μS/cm)	47.1 ± 2.7	52.6 ± 1.6†	46.9 ± 8.7	46.9 ± 5.8
Alkalinity ($mmol_c$/L)	0.116 ± 0.017	0.139 ± 0.008	0.133 ± 0.021	0.117 ± 0.011
Calcium (mmol/L)	0.160 ± 0.015	0.171 ± 0.005	0.134 ± 0.032	0.115 ± 0.024
Magnesium (mmol/L)	0.064 ± 0.001	0.076 ± 0.005†	0.049 ± 0.011	0.056 ± 0.007
Sodium (mmol/L)	0.033 ± 0.002	0.037 ± 0.004	0.055 ± 0.009	0.080 ± 0.025
Potassium (mmol/L)	0.056 ± 0.003	0.079 ± 0.013†	0.007 ± 0.001	0.006 ± 0.002
Total base cations ($mmol_c$/L)	0.538 ± 0.030	0.608 ± 0.019†	0.429 ± 0.086	0.427 ± 0.052
Chloride (mmol/L)	0.066 ± 0.004	0.068 ± 0.001†	0.078 ± 0.016	0.078 ± 0.005
Sulfate (mmol/L)	0.046 ± 0.002	0.047 ± 0.001†	0.092 ± 0.016	0.108 ± 0.022
Nitrate (mmol/L)	0.021 ± 0.008	0.019 ± 0.004	0.019 ± 0.018	0.001 ± 0.001
Ammonium (mmol/L)	0.023 ± 0.006	0.026 ± 0.007	0.002 ± 0.0002	0.001 ± 0.0002†
DOC (mmol/L)	3.55 ± 0.13	3.90 ± 0.23	0.380 ± 0.060	0.222 ± 0.076*
Dissolved Si (mmol/L)	0.082 ± 0.014	0.071 ± 0.002	0.146 ± 0.018	0.162 ± 0.009

Notes: Total base cations = $2[Ca^{2+}] + 2[Mg^{2+}] + [K^+] + [Na^+]$. Dissolved Si data are for 1996–2001 only.
* $P < 0.05$; ** $P < 0.01$. Asterisks indicate significant treatment differences for a given depth.
† Significant treatment-by-time interactions ($P < 0.05$).

similar changes because of the limitations on destructive sampling such experiments may employ.

Fertilization often decreases fine-root biomass and respiration in forest ecosystems (e.g., Gower and Vitousek 1989), as observed in our experiment. Soil respiration rates at Duke FACE decreased significantly by >20%, with fertilization in both ambient and elevated CO_2 plots in the third growing season after fertilization (Fig. 5). Fertilization also decreased fine-root biomass ~13% in the same plots (Fig. 5). Interestingly, the data for coarse-root biomass from the soil pits dug in 2008 show the opposite in both ambient and elevated treatments, a trend toward increased biomass. If N-induced decreases in fine-root biomass are insufficient to explain decreases in soil respiration, and if coarse-root biomass is, if anything, greater, then some other factor has to be contributing to the decreased soil CO_2 flux. Perhaps this factor is N-induced decreases in exudation, mycorrhizal colonization, or microbial respiration (e.g., Phillips and Fahey 2007). More intensive sampling, perhaps at the termination of the experiment, will be needed to determine the long-term effect of N fertilization on coarse and relatively deep root biomass at Duke FACE.

If progressive nitrogen limitation (PNL; e.g., McGuire et al. 1995, Luo et al. 2004) were occurring in this system, we would expect differences in productivity to diminish for trees in the elevated vs. ambient CO_2 plots (Oren et al. 2001). In fact there is little evidence from estimates of aboveground or total NPP in the replicated Duke experiment that PNL is occurring there or at other forest FACE experiments after more than a decade of manipulation (Finzi et al. [2007]; however, recent data at the Oak Ridge FACE experiment suggest that N limitation may be strengthening). To date the trees in these experiments have been able to increase either their nitrogen use efficiency, as for the European poplar FACE experiment (Calfapietra et al. 2007), or their uptake of soil nitrogen, as in the three United States forest FACE experiments (Finzi et al. 2007). At the Duke FACE site, CO_2-enriched plots are gaining N at the rate of ~12 g $N \cdot m^{-2} \cdot yr^{-1}$, well above estimated rates of N deposition (~1.4 g $N \cdot m^{-2} \cdot yr^{-1}$), heterotrophic N fixation (~1.4 g $N \cdot m^{-2} \cdot yr^{-1}$), and net N mineralization (0.6–3.2 g $N \cdot m^{-2} \cdot yr^{-1}$) (Finzi et al. 2006, 2007, Hofmockel and Schlesinger 2007, Hofmockel et al. 2007). Most likely this increased soil N uptake has come from greater root and mycorrhizal carbon allocation, increased exploitation of deeper soil layers, or greater organic matter decomposition, likely resulting from root-induced priming effects (e.g., Cheng and Kuzyakov 2005, Phillips 2007, Lichter et al. 2008, Pritchard et al. 2008). Results from N fertilization experiments in Duke Forest clearly show that N availability limits plant production at the site; nonetheless, there is no apparent interaction of fertilization and CO_2 belowground (Figs. 2 and 5) and no sign overall that the relative increase in NPP is declining in high CO_2.

Through its effects on carbonic acid and other chemical species, increased atmospheric CO_2 has the potential to increase weathering and the abundance of base cations in the soil (Berner 1992, Andrews and Schlesinger 2001, Karberg et al. 2005). Silicate minerals, for instance, react with carbonic acid in the soil to release bicarbonate ions HCO_3^-) and base cations such as Ca^{2+}. The acidifying effect of carbonic acid can also cause protons to displace base cations from soil exchange sites. Oh and Richter (2004) used column leaching experiments in the laboratory to show that the potential for soil acidification with elevated CO_2 is even higher than predicted by many models (though more recent work suggests that such estimates may be too high [Oh et al. 2007]). In the eight years since the study of Andrews and Schlesinger (2001), soil CO_2 concentrations have continued to increase steadily at 30, 100, and 200 cm at the Duke FACE site (Figs. 6 and 7). Cation concentrations and alkalinity in soil leachates more than doubled in elevated CO_2, as well, though primarily at

TABLE 1. Extended.

70 cm		200 cm	
Ambient	Elevated	Ambient	Elevated
40.3 ± 5.8	43.9 ± 11.0†	96.1 ± 18.9	199.8 ± 35.5**†
0.157 ± 0.034	0.204 ± 0.017	0.613 ± 0.118	1.652 ± 0.401*
0.073 ± 0.004	0.060 ± 0.012†	0.173 ± 0.025	0.416 ± 0.010**
0.047 ± 0.010	0.047 ± 0.006	0.131 ± 0.022	0.410 ± 0.115
0.132 ± 0.034	0.209 ± 0.099	0.268 ± 0.036	0.681 ± 0.269
0.004 ± 0.001	0.003 ± 0.001	0.007 ± 0.003	0.006 ± 0.002†
0.377 ± 0.054	0.424 ± 0.107	0.846 ± 0.089	2.333 ± 0.509*
0.097 ± 0.005	0.104 ± 0.021†	0.119 ± 0.019	0.222 ± 0.051
0.063 ± 0.029	0.065 ± 0.039†	0.110 ± 0.037	0.126 ± 0.049
0.001 ± 0.0003	0.001 ± 0.0001	0.001 ± 0.0004	0.010 ± 0.007
0.001 ± 0.0001	0.001 ± 0.0001†	0.001 ± 0.0002	0.001 ± 0.0003
0.134 ± 0.028	0.140 ± 0.025†	0.142 ± 0.028	0.288 ± 0.121†
0.228 ± 0.036	0.293 ± 0.016*	0.576 ± 0.040	0.735 ± 0.088

deeper depths (e.g., 200 cm; see Table 1). Such responses are likely to be important for nutrient availability and will apparently continue for some time with increased CO_2 concentrations.

Overall, the effects of elevated atmospheric CO_2 concentrations on root biomass and carbon cycling at Duke FACE are the greatest in summer, remain consistently higher in the elevated treatment, and show no sign of diminishing after more than a decade of research. These significant increases in root biomass and carbon cycling will not necessarily mean substantially more carbon storage at elevated than at ambient CO_2, however. For instance, average annual litterfall in elevated CO_2 has increased 48 g $C \cdot m^{-2} \cdot yr^{-1}$ (Lichter et al. 2008) and fine-root NPP is ~15 g $C \cdot m^{-2} \cdot yr^{-1}$ greater, but soil organic C has increased only a modest 30 g $C \cdot m^{-2} \cdot yr^{-1}$ (Lichter et al. 2008). Faster rates of soil respiration (see Figs. 3 and 4) and shorter fine-root life spans (500 vs. 574 days in elevated and ambient CO_2, respectively [Pritchard et al. 2008]) suggest that belowground carbon is cycling more quickly in elevated CO_2 as well (Drake et al. 2008). One surprising result that we found was the doubling in coarse-root biomass in the 2008 sampling with soil pits (Fig. 2). If this result holds more generally, an additional ~20 g $C \cdot m^{-2} \cdot yr^{-1}$ or more may be stored in coarse roots, and a possible shift in allometry for the pine trees in elevated CO_2 may be occurring. Clarifying this result will be critical in comprehensive soil sampling at the end of the experiment. Overall, though, the potential for carbon storage with increased CO_2 at the Duke FACE experiment appears to be modest at best. The long-term implications of these results are that terrestrial forests are unlikely to sequester a substantial portion of anthropogenic CO_2 emissions to the atmosphere.

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