# Cumulative response of ecosystem carbon and nitrogen stocks to chronic $CO_2$ exposure in a subtropical oak woodland

**Bruce A. Hungate[1], Paul Dijkstra[1], Zhuoting Wu[1,2], Benjamin D. Duval[1,3], Frank P. Day[4], Dale W. Johnson[5], J. Patrick Megonigal[6], Alisha L. P. Brown[4] and Jay L. Garland[7]**

[1]Department of Biological Sciences and The Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ 86011, USA; [2]US Geological Survey, Flagstaff, AZ 86001, USA; [3]US Dairy Forage Research Center, USDA-ARS, Madison, WI 53706, USA; [4]Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529, USA; [5]Department of Natural Resources and Environmental Science, University of Nevada, Reno, NV 89557, USA; [6]Smithsonian Environmental Research Center, Edgewater, MD 21037, USA; [7]Environmental Protection Agency, Microbiological and Chemical Exposure Assessment Research Division, Cincinnati, OH 45268, USA

Author for correspondence:
*Bruce A. Hungate*
*Tel: +1 928 523 0925*
*Email: bruce.hungate@nau.edu*

Received: *24 January 2013*
Accepted: *10 April 2013*

*New Phytologist* (2013) **200:** 753–766
**doi**: 10.1111/nph.12333

**Key words:** carbon cycling, elevated $CO_2$, global change, long-term experiment, nitrogen cycling, scrub oak, soil carbon, subtropical woodland.

## Summary

- Rising atmospheric carbon dioxide ($CO_2$) could alter the carbon (C) and nitrogen (N) content of ecosystems, yet the magnitude of these effects are not well known. We examined C and N budgets of a subtropical woodland after 11 yr of exposure to elevated $CO_2$.
- We used open-top chambers to manipulate $CO_2$ during regrowth after fire, and measured C, N and tracer $^{15}N$ in ecosystem components throughout the experiment.
- Elevated $CO_2$ increased plant C and tended to increase plant N but did not significantly increase whole-system C or N. Elevated $CO_2$ increased soil microbial activity and labile soil C, but more slowly cycling soil C pools tended to decline. Recovery of a long-term $^{15}N$ tracer indicated that $CO_2$ exposure increased N losses and altered N distribution, with no effect on N inputs.
- Increased plant C accrual was accompanied by higher soil microbial activity and increased C losses from soil, yielding no statistically detectable effect of elevated $CO_2$ on net ecosystem C uptake. These findings challenge the treatment of terrestrial ecosystems responses to elevated $CO_2$ in current biogeochemical models, where the effect of elevated $CO_2$ on ecosystem C balance is described as enhanced photosynthesis and plant growth with decomposition as a first-order response.

## Introduction

Many experiments have examined the responses of plant production and ecosystem carbon (C) balance to rising atmospheric $CO_2$ (Reich *et al.*, 2006a; Norby & Zak, 2011). Results from these feature prominently in assessments of potential feedbacks between the biosphere and the changing atmosphere (Dolman *et al.*, 2010). Compared to responses of photosynthesis and plant growth to elevated $CO_2$, the response of soil C is less well understood, because changes in soil C content are difficult to detect (Smith, 2004). Increased C in soil in response to elevated $CO_2$ is sometimes found (Jastrow *et al.*, 2005; Iversen *et al.*, 2008, 2012), although more frequently there is no effect, whether because of low statistical power or the absence of an important effect is unclear (Hungate *et al.*, 2009). Ecosystem-scale inventories assessing C balance responses to elevated $CO_2$ also often show no effect (Hungate *et al.*, 1997b; Gielen *et al.*, 2005; Gill *et al.*, 2006; Niklaus & Falloon, 2006; Adair *et al.*, 2009), although in aggregate some analyses suggest an effect is apparent (Luo *et al.*, 2006). Thus, global models projecting future C dynamics of the biosphere have strong support for the effects of $CO_2$ on plant growth (Denman *et al.*, 2007), but less empirical support for assumed effects on total ecosystem C storage. Our first goal in this work was to construct a complete C inventory for a subtropical oak woodland after 11 yr of exposure to elevated $CO_2$, to test whether the $CO_2$ treatment altered total system C accumulation, and determine how any changes in C accumulation were distributed among plant and soil pools.

Total ecosystem C content is a function of plant growth and accumulation of plant biomass and detritus and also of C losses through microbial decomposition. Microbial decomposition is typically assumed to be a first-order process (Parton *et al.*, 1987), responding predictably and constantly to changes in substrate supply, and thus is not expected to respond to elevated $CO_2$ independently of changes in substrate accumulation (Denman *et al.*, 2007). Challenging this idea, inputs of C to soil can stimulate mineralization of native soil organic matter (Lohnis, 1926; Broadbent & Norman, 1947; Broadbent & Bartholomew, 1949; Van Veen *et al.*, 1991), and increased atmospheric $CO_2$ has been shown to promote microbial activity (Dieleman *et al.*, 2010) and even soil C loss (Hoosbeek, 2004; Trueman & Gonzalez-Meler, 2005; Carney *et al.*, 2007; Hagedorn *et al.*, 2008; Paterson *et al.*, 2008; Taneva & Gonzalez-Meler, 2008; Langley *et al.*, 2009; Trueman *et al.*, 2009; Drake *et al.*, 2011; Reid *et al.*, 2012).

Thus, soil processes influence potential C accumulation in response to increasing atmospheric $CO_2$, yet how and to what extent are not well understood. Our second goal in this work was to examine changes in soil microbial activity during the 11 yr of $CO_2$ enrichment, and to test whether patterns of $CO_2$ effects on soil microbial activity might help explain any effects (or lack of effects) of elevated $CO_2$ on soil C stocks.

Carbon cycling in ecosystems is linked to cycles of other elements (Finzi *et al.*, 2011), such as nitrogen (N). Simulations of land carbon uptake using models with coupled N and C dynamics usually differ, and in many cases differ strongly, from those ignoring N (e.g. compare Cramer *et al.*, 2001 and Thornton *et al.*, 2007), because N limits plant growth and C storage (LeBauer & Treseder, 2008), and because N cycling is sensitive to environmental change (Galloway *et al.*, 2008). With N cycling included, simulations project smaller increases in terrestrial C storage in response to rising $CO_2$, because N availability limits plant growth and its response to elevated $CO_2$ (Thornton *et al.*, 2007; McMurtrie *et al.*, 2008; Sokolov *et al.*, 2008; Jain *et al.*, 2009; Wang & Houlton, 2009; Friedlingstein & Prentice, 2010; Zaehle *et al.*, 2010).

While model simulations bear out the importance of including N, these models do not necessarily demonstrate a consistent pattern of effect. Results differ in magnitude, direction and mechanism, suggesting that additional data and analyses are needed to evaluate conditions under which C–N coupling is important. For example, some simulations project only a modest limitation of terrestrial C uptake with coupled C–N interactions in the long term (at equilibrium), but strong effects of C–N interactions on the dynamics of C cycling and storage after disturbance (Gerber *et al.*, 2010). Although the models generally agree that including N limitation of plant production reduces the terrestrial C sink, the magnitude of this effect is highly variable (Arneth *et al.*, 2010). Experiments also indicate that C–N interactions are critical modulators of the long-term $CO_2$ fertilization response, but different experiments provide support for different mechanisms underlying that modulation. In some cases, C–N interactions appear to constrain strongly the $CO_2$ response (Reich *et al.*, 2006a,b; Norby *et al.*, 2010; Garten *et al.*, 2011), but in others, plants appear able to access the extra N needed to support the growth response (Johnson *et al.*, 2006; Drake *et al.*, 2011). Effects of $CO_2$ concentration on microbial N transformations that influence the plant–soil distribution of N are extremely variable, with negative, positive and neutral effects observed for the same processes (Díaz *et al.*, 1993; Zak *et al.*, 1993; Morgan *et al.*, 1994; Zanetti *et al.*, 1996; Hungate *et al.*, 1997a,c; Johnson *et al.*, 1997; Hofmockel & Schlesinger, 2007; van Groenigen *et al.*, 2011, 2012). Furthermore, other concomitant global environmental changes will modulate N constraints on C balance responses to elevated $CO_2$, including changes that alter N cycling directly, such as warming, altered precipitation and atmospheric N deposition, as well as indirect effects, such as changes in plant species composition. There is considerable debate as to the magnitude of the impact of such effects on ecosystem C sequestration, however (Jenkinson *et al.*, 1999; Nadelhoffer *et al.*, 1999; Arneth *et al.*, 2010). Thus, both model simulations and data can be invoked to support N cycling constraining, increasing, or having little effect on the terrestrial C sink. Our third goal in this research was to compare C and N inventories in response to 11 yr of $CO_2$ exposure in a subtropical woodland, in order to test how rising $CO_2$ affects these elements in concert.

One of the challenges in investigating C–N interactions in ecosystem experiments is that the timescale of measurements of N cycling rates is typically far shorter than the timescale of N cycling processes that influence ecosystem responses. Elevated $CO_2$ can alter multiple processes within the soil N cycle simultaneously, with strong temporal dynamics, and with opposing impacts on plant N availability, making it very difficult to extrapolate short-term measurements to long-term effects. Following an isotope tracer over multiple years can help overcome this challenge. $^{15}N$ tracers reflect short-term effects on N cycling processes and integrate these into long-term effects on $^{15}N$ distribution among plant and soil components within the system. Because the $^{15}N$ is added in labile form, losses of added $^{15}N$ will be relatively larger than losses of total ecosystem N, so can be detected with greater sensitivity. Our fourth goal in this research was to use a long-term $^{15}N$ tracer to characterize changes in N distribution and N losses in response to elevated $CO_2$.

Here, we report a whole system inventory of the C and N content of a scrub-oak ecosystem after 11 yr of experimental $CO_2$ exposure. We also show how soil microbial activity responded to chronic $CO_2$ exposure. We also report recovery and distribution of a $^{15}N$ tracer applied early in the experiment, in order to assess how elevated $CO_2$ alters the system-level distribution of labile N over the timescale of a decade.

## Materials and Methods

The scrub-oak experiment occurred at the Merritt Island National Wildlife refuge on the east coast of Florida, USA (28°38′N, 80°42′W). After controlled burning, 16 open-top chambers were established over the regrowing vegetation, each covering 9.42 $m^2$ ground area, with 8 chambers receiving ambient air and 8 receiving ambient air + 350 ppm V $CO_2$ (referred to as the 'elevated $CO_2$' treatment). A large blower circulated air through each chamber at a rate of 24–30 $m^3$ $min^{-1}$, replacing the chamber air volume 1.3–1.6 times $min^{-1}$ (Dijkstra *et al.*, 2002). The chambers increased air temperature and vapor pressure deficit while decreasing light (Dore *et al.*, 2003), microenvironmental effects that did not significantly alter growth or species composition (Seiler *et al.*, 2009). The experiment began in May 1996 and was maintained until June 2007.

In June–July 2007, all aboveground material was harvested from the chambers (see Seiler *et al.*, 2009), and roots and soils were collected using multiple cores in each chamber (see Day *et al.*, 2013). For aboveground biomass, all shoots were cut at the base of the stem, weighed immediately, and subsampled for the determination of water content and elemental analysis of leaves and stems. Ten surface cores (0–10 cm) and five deep cores were collected from each plot at 10 cm increments; all cores were 7 cm diameter. Core depth varied among plots from 2 to 3 m due to differences in the depth to the water table and the spodic ($B_h$)

horizon. For purposes of the element inventory conducted here, depth increments were combined into 0–10, 10–30, 30–60 and 60–100 cm. Samples were hand-picked to remove large roots, and subsamples separated into coarse particulate organic matter, roots and mineral soil. Belowground biomass was also sampled indirectly using ground-penetrating radar (Stover *et al.*, 2007, Day *et al.*, 2013). Material on the forest floor was gathered from 1/8th of each plot by hand, collecting until no visibly identifiable plant fragments remained. Material was dried, sifted to remove adhering sand, and weighed.

We used a combination of density and biological fractionations to estimate soil carbon (C) pools of varying turnover rates. We used incubations to estimate labile and active soil C pools (and, by difference residual C), using the technique of Nadelhoffer (1990). We measured $CO_2$ production from laboratory incubations, combining short-term incubations of soils immediately after collection (McKinley *et al.*, 2009) with 541-d incubations conducted in the lab at Northern Arizona University. We used density fractionations as described previously (Hungate *et al.*, 2006; Carney *et al.*, 2007), separating light ($< 1.5\ \mathrm{g\ cm^{-3}}$), medium ($1.5–1.8\ \mathrm{g\ cm^{-3}}$), heavy ($1.8–2.2\ \mathrm{g\ cm^{-3}}$) and residual ($> 2.2\ \mathrm{g\ cm^{-3}}$) organic matter fractions. Total soil C, N, $^{15}$N and $^{13}$C were also measured on bulk samples collected from the cores. Our fractionation analysis focused on soils from the 0–60 cm depths. For bulk soil analyses where we measure total C, N and $^{15}$N, we present the data to 1 m to correspond with the depth of the root biomass inventory.

We measured microbial biomass using the chloroform-fumigation extraction method (Vance *et al.*, 1987) in mineral soil (0–15 cm) sampled in July 1997; June, July, September and December 1998; September 1999; and May 2004. Soil subsamples (20–25 g at field moisture content) were extracted in 75 ml 0.5 M $K_2SO_4$ before and after 24-h fumigation with ethanol-free chloroform. The $K_2SO_4$ extracts were dehydrated in a forced-air drying oven at 60°C, the salts ground in a mortar and pestle, and the resulting powder analyzed for C, N, $\delta^{15}$N and $\delta^{13}$C on a CE 2100 elemental analyzer coupled to a Thermo DeltaPLUS-XL isotope-ratio mass spectrometer (http://www.isotope.nau.edu). Microbial biomass was calculated as the difference in mass (of C, N, $^{13}$C or $^{15}$N) between fumigated and nonfumigated samples, divided by 0.54 to correct for extraction efficiency (Vance *et al.*, 1987). For samples collected after the $^{15}$N tracer application (June 1998), we also measured the $^{15}$N content of mineral soil (0–15 cm depth). After milling, soil N and $^{15}$N contents were determined as described above.

The $CO_2$ added to the elevated-$CO_2$ treated plots was depleted in $^{13}$C. We used a two-member mixing model to determine mineral soil C derived from new photosynthate (Leavitt *et al.*, 1994; Hungate *et al.*, 1996). Stem tissue produced in the elevated $CO_2$ treatment ($\delta^{13}C_{S,E}$) provided an integrative measure of the $\delta^{13}$C value of new photosynthate (average across five sampling dates, -42.6 ± 0.3 ‰). However, because mineral soil ($\delta^{13}C_{M,A}$) and stem $\delta^{13}$C ($\delta^{13}C_{S,A}$) differed in the ambient $C_a$ treatment, we calculated the $\delta^{13}$C signature of new carbon ($\delta\ ^{13}C_{new}$) as:

$$\delta^{13}C_{new} = \delta^{13}C_{S,E} - (\delta^{13}C_{S,A} - \delta^{13}C_{M,A}). \qquad \text{Eqn 1}$$

The $\delta\ ^{13}$C of the mineral soil in the ambient $CO_2$ treatment was used as the end member for organic matter fixed before the experiment began. Carbon, N, $^{15}$N, and $^{13}$C were determined for all plant and soil components using coupled Dumas combustion isotope-ratio mass spectrometry (Carlo-Erba elemental analyzer and Finnigan Delta-V mass spectrometer) at the Colorado Plateau Stable Isotope Laboratory (www.isotope.nau.edu).

For testing soil microbial activity, we collected soil and litter samples in May through July of 2004, after 8 yr of $CO_2$ treatment. Soil sampling, preparation of microbial inocula, carbon and nutrient amendments, and incubation conditions are described in Brown *et al.* (2009). Carbon substrates included glucose and hot-water extracts of roots and leaf litter collected from the ambient and elevated $CO_2$ treatments. Microbial inocula from litter, rhizosphere and bulk soil communities were also prepared from the two $CO_2$ treatments. We used the BD-oxy system (BD Oxygen Biosensor System, BD Biosciences, Bedford, MA, USA (Garland *et al.*, 2003; Väisänen *et al.*, 2005; Zabaloy *et al.*, 2008) to evaluate microbial respiration. The system uses a fluorophore that fluoresces as $O_2$ is consumed during the 48 h incubation. Normalized relative fluorescence was calculated as relative fluorescence after 48 h normalized by dividing by relative fluorescence after 1 h. The response to substrate addition was calculated as:

$$\text{Relative response} = (R_r - R_c)/R_c \times 100\% \qquad \text{Eqn 2}$$

($R_c$, normalized relative fluorescence in the absence of resource addition; $R_r$, normalized relative fluorescence with the added resource. Brown *et al.* (2009) present data from the ambient $CO_2$ treatment; here, we expand on this past analysis to evaluate responses of microbial respiration to elevated $CO_2$. We used ANOVA to test for effects of habitat (rhizosphere, litter or bulk soil), inoculum source (ambient or elevated $CO_2$), substrate source (ambient or elevated $CO_2$), substrate type (litter or root), N, and P. We used a separate ANOVA to test compare responses to the addition of glucose vs natural substrates extracted from roots and litter. Where appropriate, ANOVAs were designed as split-plots, to account for the nonindependence of inocula collected from individual experimental plots subject to multiple combinations of resource treatments in the BD-Oxy assay.

We used resampling to infer the effects and estimate the magnitude of the elevated $CO_2$ treatment on ecosystem C and N pools and recovery of tracer $^{15}$N. We estimated 5% and 95% confidence limits for the difference in means between elevated and ambient $CO_2$ treatments, using 1000 samples with replacement ($n = 8$ for each treatment).

## Results

Elevated $CO_2$ increased plant biomass, including the mass of C ($\mathrm{g\ C\ m^{-2}}$) in leaves, stems and coarse roots, and the total mass of

C in plants (Table 1). The mass of C in fine roots was not significantly affected by the elevated $CO_2$ treatment at the final harvest (Table 1), although fine roots did exhibit significant increases at other times during the experiment (Day *et al.*, 2013). On average, plant C accumulation by the end of the experiment was 71.5 g C $m^{-2}$ $yr^{-1}$ higher in elevated compared to ambient $CO_2$, roughly equally distributed aboveground (37.5 g $m^{-2}$ $yr^{-1}$) and belowground (33.5 g $m^{-2}$ $yr^{-1}$). The C content of the litter layer, coarse particulate organic matter, total mineral soil C, and the light and medium density fractions did not significantly respond to the $CO_2$ treatment, whereas the heavy density soil C pool significantly declined. Elevated $CO_2$ had no effect on soil C in the spodic horizon, with no significant effect on total mineral soil C, or on the light, medium and heavy density fractions (Table 2); thus, C in the deep soil was also insensitive to the $CO_2$ treatment. In general, increased mass of plant C caused by elevated $CO_2$ did not translate to increased C storage in other ecosystem reservoirs (Table 1).

Elevated $CO_2$ increased the N content of plants aboveground (Table 3), but the N contents of coarse and fine roots did not respond to elevated $CO_2$, yielding no effect on total plant N. The N content of most soil fractions was not significantly altered by elevated $CO_2$, except the medium density fraction at 30–60 cm, which increased, and the light fraction at 10–30 cm, which declined. Increased C in plant pools with only small changes in N means higher C to N ratios. Higher C to N ratios under elevated $CO_2$ were observed for leaves, coarse roots and the sum of all plant parts; elevated $CO_2$ also increased the C to N ratio of the litter layer (Table 4). Elevated $CO_2$ did not increase the C to N ratio of any soil pool; the only soil pool to respond – the heavy density fraction – actually declined in C to N ratio. Changes in plant and soil C to N ratios were compensatory, such that elevated $CO_2$ had no effect on the C to N ratio of the plant–soil system to 1 m depth.

Elevated $CO_2$ increased recovery of tracer $^{15}N$ in aboveground plant tissues, but reduced recovery in coarse roots, in the soil light fraction at 10–30 cm depth, and in the soil residual fraction at 0–60 cm (Table 5). Together, these changes resulted in a significant decline in whole-system $^{15}N$ recovery under elevated $CO_2$. Elevated $CO_2$ reduced the $\delta^{15}N$ of plant tissue (weighted average of all plant parts), a dilution of the added $^{15}N$ tracer with unlabeled $^{15}N$. This pattern indicates that elevated $CO_2$ increased plant access to N, either through new N inputs or redistribution from existing ecosystem N reservoirs. But, because total plant N did not respond to elevated $CO_2$, the increase in inputs of new N to plants were matched by N losses from plants, such that $CO_2$ enhanced N turnover through the plant system. In contrast to plant $\delta^{15}N$, the $\delta^{15}N$ of soils did

**Table 1** Inventory of carbon after 11 yr exposure to increased atmospheric $CO_2$ in a subtropical oak woodland[a]

| Carbon (g C $m^{-2}$) | Ambient | Elevated | Effect | 5% & 95% CLs |
|---|---|---|---|---|
| Aboveground | 624.5 ± 54.6 | 1043.0 ± 77.5 | 418.5 | (274.8 to 556.9) |
| Oak leaves | 212.2 ± 22.3 | 318.4 ± 29.6 | 106.2 | (47.6 to 157) |
| Oak stems | 347.1 ± 34.2 | 621.6 ± 60.8 | 274.5 | (164.8 to 374.2) |
| Other species | 38.3 ± 10.7 | 63.1 ± 10.2 | 24.7 | (1.7 to 47.4) |
| Standing dead | 26.9 ± 8.4 | 39.8 ± 13.8 | 13.0 | (−9.7 to 39.5) |
| Litter layer | 332 ± 41.2 | 368.1 ± 42.4 | 36.1 | (−57.9 to 127.7) |
| Roots | 2886.7 ± 90.2 | 3261.3 ± 174.6 | 374.6 | (73.6 to 674.5) |
| Fine roots | 909.4 ± 62.8 | 803.9 ± 43.3 | −105.5 | (−226.8 to 9.7) |
| Coarse roots | 1977.3 ± 102.8 | 2457.4 ± 177.7 | 480.1 | (168.9 to 790.0) |
| Plant | 3511.2 ± 102.0 | 4304.3 ± 221.3 | 793.1 | (437.4 to 1172.7) |
| CPOM (0–100 cm) | 1406.5 ± 386.4 | 1168.5 ± 272.1 | −238.0 | (−957 to 354.4) |
| Soil (0–100 cm) | 5513.1 ± 411.5 | 5025.6 ± 647.4 | −487.5 | (−1456.5 to 636.8) |
| Light, 0–60 cm | 2534.7 ± 260.2 | 2394.4 ± 333.3 | −140.4 | (−746.8 to 473.2) |
| 0–10 cm | 1530.9 ± 284.8 | 1415.8 ± 316.8 | −115.0 | (−760.1 to 565.5) |
| 10–30 cm | 480.2 ± 94.5 | 331.2 ± 36.5 | −149.1 | (−297.9 to 6.7) |
| 30–60 cm | 523.7 ± 149.9 | 647.3 ± 169.2 | 123.7 | (−214.9 to 474.5) |
| Medium, 0–60 cm | 1306.3 ± 302 | 1208.4 ± 177.3 | −97.9 | (−633.8 to 380.3) |
| 0–10 cm | 660.3 ± 115.3 | 560.7 ± 108.2 | −99.6 | (−346.4 to 158.9) |
| 10–30 cm | 370.9 ± 109.2 | 341.5 ± 55.2 | −29.4 | (−222.4 to 147.1) |
| 30–60 cm | 275 ± 157.8 | 306.2 ± 88.6 | 31.1 | (−267.6 to 289.2) |
| Heavy, 0–60 cm | 706.3 ± 120.5 | 396 ± 92.1 | −310.4 | (−553.2 to −86.0) |
| 0–10 cm | 110.9 ± 27 | 81.2 ± 19.9 | −29.7 | (−81.7 to 22.0) |
| 10–30 cm | 148 ± 23.7 | 83.5 ± 30.6 | −64.6 | (−122.9 to 2.0) |
| 30–60 cm | 447.4 ± 107.6 | 231.3 ± 87.2 | −216.1 | (−402.7 to −3.3) |
| Residual, 0–60 cm | 965.8 ± 1026.9 | 1026.9 ± 330.9 | 61.1 | (−782.3 to 925.2) |
| Soil (60–100 cm) | 1547.0 ± 129.3 | 1877.6 ± 359.8 | 330.7 | (−274.4 to 925.4) |
| Total ecosystem | 12309.8 ± 582.1 | 12744.1 ± 444.3 | 434.4 | (−723 to 1529.9) |

[a]Values are means ± SE of the mean for the Ambient and Elevated $CO_2$ treatments, the Effect of the $CO_2$ treatment (E–A), and the bootstrapped 5% and 95% CLs (confidence limits) for the treatment effect. CPOM, coarse particulate organic matter. Soil fractions are density fractions, including light (< 1.5 g $cm^{-3}$), medium (1.5–1.8 g $cm^{-3}$), heavy (1.8–2.2 g $cm^{-3}$) and residual (calculated as total soil total minus the sum of measured density fractions).

**Table 2** Soil carbon (C) in the spodic horizon of the subtropical oak woodland

| | Ambient | Elevated | | Ambient | Elevated | |
|---|---|---|---|---|---|---|
| | %C | | *P*-value | $\delta^{13}C$ | | *P*-value |
| Total C | 0.77 ± 0.10 | 0.60 ± 0.15 | 0.383 | −25.6 ± 0.1 | −25.1 ± 0.3 | 0.157 |
| Light | 18.3 ± 2.8 | 11.9 ± 1.2 | 0.151 | −25.3 ± 0.1 | −25.3 ± 0.1 | 0.943 |
| Medium | 14.2 ± 3.8 | 9.4 ± 2.0 | 0.288 | −25.6 ± 0.2 | −25.3 ± 0.1 | 0.178 |
| Heavy | 13.2 ± 2.3 | 12.2 ± 1.0 | 0.710 | −25.6 ± 0.1 | −25.2 ± 0.2 | 0.116 |

**Table 3** Inventory of ecosystem nitrogen ($g\ N\ m^{-2}$) after 11 yr exposure to increased atmospheric $CO_2$ in a subtropical oak woodland[a]

| | Ambient | Elevated | Effect | 5% & 95% CLs |
|---|---|---|---|---|
| Aboveground | 8.4 ± 0.8 | 13.1 ± 0.9 | 4.7 | (3.0 to 6.3) |
| Oak leaves | 4.7 ± 0.6 | 6.6 ± 0.6 | 1.9 | (0.5 to 3) |
| Oak stems | 3.0 ± 0.3 | 5.1 ± 0.5 | 2.2 | (1.2 to 3.1) |
| Other species | 0.5 ± 0.1 | 1.1 ± 0.2 | 0.5 | (0.2 to 0.9) |
| Standing dead | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.1 | (−0.1 to 0.3) |
| Litter layer | 5.7 ± 0.7 | 6.0 ± 0.9 | 0.3 | (−1.4 to 2.2) |
| Roots | 29.3 ± 1.8 | 27.8 ± 2.5 | −1.4 | (−6.4 to 3.1) |
| Fine roots | 8.3 ± 0.8 | 7.3 ± 0.9 | −1.0 | (−2.8 to 0.9) |
| Coarse roots | 21.0 ± 1.2 | 20.5 ± 2.5 | −0.4 | (−4.6 to 4.2) |
| Plant | 37.7 ± 1.8 | 41.0 ± 2.9 | 3.3 | (−1.8 to 8.3) |
| CPOM (0–100 cm) | 20.7 ± 5.7 | 15.2 ± 3.5 | −5.4 | (−15 to 3.0) |
| Soil (0–100 cm) | 159.5 ± 15.0 | 145.4 ± 17.5 | −14.2 | (−44.2 to 15.9) |
| Light, 0–60 cm | 55.9 ± 7.0 | 54.9 ± 8.9 | −1.0 | (−19 to 16.6) |
| 0–10 cm | 37.2 ± 7.6 | 37.6 ± 8.9 | 0.4 | (−17.9 to 18.5) |
| 10–30 cm | 9.6 ± 1.8 | 6.5 ± 0.7 | −3.1 | (−5.8 to −0.1) |
| 30–60 cm | 9.1 ± 2.8 | 10.8 ± 2.1 | 1.7 | (−4.2 to 6.4) |
| Medium, 0–60 cm | 30.7 ± 5.5 | 30.9 ± 4.1 | 0.2 | (−10.9 to 11) |
| 0–10 cm | 18.8 ± 3.4 | 15.4 ± 3.0 | −3.4 | (−10.4 to 3.5) |
| 10–30 cm | 7.9 ± 2.0 | 7.0 ± 1.0 | −0.9 | (−4.3 to 2.3) |
| 30–60 cm | 4.0 ± 1.5 | 8.5 ± 2.6 | 4.6 | (0.4 to 9.3) |
| Heavy, 0–60 cm | 17.3 ± 2.1 | 15.6 ± 4.9 | −1.7 | (−9.2 to 6.8) |
| 0–10 cm | 3.4 ± 0.8 | 2.4 ± 0.6 | −1.0 | (−2.7 to 0.5) |
| 10–30 cm | 4.1 ± 0.8 | 2.5 ± 1.1 | −1.6 | (−3.3 to 0.5) |
| 30–60 cm | 9.8 ± 1.5 | 10.7 ± 4.4 | 0.9 | (−6 to 8.9) |
| Residual, 0–60 cm | 55.6 ± 44.0 | 12.3 ± 13.5 | −43.3 | (−40 to 17.3) |
| Soil (60–100 cm) | 51.3 ± 3.2 | 55.3 ± 8.8 | 4.1 | (−10.8 to 18.2) |
| Total ecosystem | 274.8 ± 10.9 | 262.9 ± 13.9 | −12.0 | (−38.1 to 18.9) |

[a]Values are means ± SE of the mean for the Ambient and Elevated $CO_2$ treatments, the Effect of the $CO_2$ treatment (E–A), and the bootstrapped 5% and 95% CLs (confidence limits) for the treatment effect. CPOM, coarse particulate organic matter. Soil fractions are density fractions, including light (<1.5 g $cm^{-3}$), medium (1.5–1.8 g $cm^{-3}$), heavy (1.8–2.2 g $cm^{-3}$) and residual (calculated as total soil total minus the sum of measured density fractions).

not change with elevated $CO_2$, nor was whole-system $\delta^{15}N$ affected (Table 6).

While elevated $CO_2$ did not alter total ecosystem C, and effects on soil C were either nil or negative, several results indicate that elevated $CO_2$ increased soil microbial activity. Elevated $CO_2$ increased C mineralization in laboratory incubations, particularly for the first 24 h after collection in the field (Fig. 1, and see McKinley *et al.*, 2009), indicating a larger and more rapidly cycling labile soil C pool. Elevated $CO_2$ also increased the proportion of soil organic matter that occurred in the soil microbial biomass: averaged across seven sample dates from 1997 to 2004, more soil C, N and $^{15}N$ was contained in the soil microbial biomass in the elevated $CO_2$ treatment ($P = 0.012$ for C, $P = 0.096$ for N, and $P = 0.049$ for $^{15}N$; Fig. 2). When common inocula were presented with the labile substrates produced by leaves and roots, substrates produced in the elevated $CO_2$ treatment were respired more completely than substrates from the same sources in the ambient $CO_2$ treatment (Fig. 3a), indicating that the substrates produced in the high-$CO_2$ environment were more susceptible to microbial decay. For the litter and rhizosphere microbial communities, microbial inocula from the elevated $CO_2$ treatment consumed more $O_2$ than inocula collected from the ambient $CO_2$ treatment when presented with a common C substrate (Fig. 3b). Glucose induced a greater response in bulk soil inoculum from the ambient treatment (Fig. 3b), which may reflect $CO_2$-depletion of available soil C susceptible to priming (Brown *et al.*, 2009).

The incorporation of the depleted $\delta^{13}C$ signature into organic matter pools revealed rates and patterns of flow of 'new' C into the system, where new C is that fixed since $CO_2$ fumigation

**Table 4** Carbon to nitrogen ratios (g : g) in ecosystem components after 11 yr of experimental exposure of a subtropical woodland to increased atmospheric $CO_2$[a]

| | Ambient | Elevated | Effect | CI |
|---|---|---|---|---|
| Aboveground | 74.7 ± 2.2 | 79.3 ± 2.1 | 4.6 | (0.3 to 9.1) |
| Oak leaves | 45.8 ± 1.0 | 48.3 ± 1.0 | 2.5 | (0.2 to 4.9) |
| Oak stems | 120.6 ± 7.9 | 121.2 ± 7.2 | 0.6 | (−13 to 14.5) |
| Other species | 71.9 ± 6.6 | 60.1 ± 7.4 | −11.8 | (−23.7 to −1.3) |
| Standing dead | 108.5 ± 3.8 | 113.4 ± 4.8 | 4.9 | (−4.5 to 13.8) |
| Litter layer | 58.6 ± 1.5 | 64.2 ± 1.9 | 5.6 | (0.7 to 10.6) |
| Roots | 101.3 ± 7.4 | 122.0 ± 7.5 | 20.7 | (2.3 to 39.7) |
| Fine roots | 114.0 ± 9.6 | 116.3 ± 9.8 | 2.2 | (−16.8 to 23.6) |
| Coarse roots | 97.0 ± 8.1 | 128.9 ± 8.1 | 32.0 | (7.1 to 59.3) |
| Plants | 94.6 ± 5.3 | 106.9 ± 5.6 | 12.4 | (0.8 to 23.4) |
| CPOM (0–100 cm) | 71.1 ± 9.1 | 76.2 ± 8.7 | 5.1 | (−10.6 to 17.9) |
| Soil (0–100 cm) | 36.8 ± 2.2 | 37.8 ± 2.0 | 0.9 | (−3 to 4.6) |
| Light, 0–60 cm | 46.8 ± 3.9 | 45.0 ± 1.4 | −1.8 | (−9.1 to 5.1) |
| 0–10 cm | 43.7 ± 6.9 | 38.8 ± 0.8 | −4.9 | (−17.9 to 3.7) |
| 10–30 cm | 50.4 ± 2.5 | 51.9 ± 2.3 | 1.5 | (−4.5 to 7.4) |
| 30–60 cm | 64.5 ± 9.7 | 60.8 ± 9.5 | −3.7 | (−21.8 to 11.2) |
| Medium, 0–60 cm | 41.2 ± 3.0 | 39.5 ± 3.0 | −1.7 | (−8.2 to 4.1) |
| 0–10 cm | 35.4 ± 0.6 | 37.2 ± 0.7 | 1.8 | (−0.3 to 4.4) |
| 10–30 cm | 45.6 ± 2.9 | 48.2 ± 2.9 | 2.7 | (−3.4 to 8.8) |
| 30–60 cm | 53.2 ± 8.3 | 44.9 ± 8.6 | −8.3 | (−26.8 to 7.7) |
| Heavy, 0–60 cm | 40.2 ± 4.2 | 29.4 ± 5.1 | −10.7 | (−18.5 to −3.5) |
| 0–10 cm | 32.8 ± 1.4 | 33.7 ± 1.4 | 0.9 | (−1.6 to 3.6) |
| 10–30 cm | 41.2 ± 5.1 | 38.4 ± 5.1 | −2.9 | (−10.8 to 5.3) |
| 30–60 cm | 45.5 ± 10.0 | 27.7 ± 10.6 | −17.8 | (−36.6 to −3.4) |
| Residual, 0–60 cm | 30.1 ± 1.4 | 33.2 ± 1.5 | 3.1 | (−0.4 to 6.8) |
| Soil (60–100 cm) | 31.6 ± 9.3 | 26.0 ± 9.4 | −5.5 | (−24.1 to 10.4) |
| Total ecosystem | 45.0 ± 2.1 | 48.9 ± 1.9 | 3.9 | (−0.1 to 8.1) |

[a]Values are means ± SE of the mean for the Ambient and Elevated $CO_2$ treatments, the Effect of the $CO_2$ treatment (E–A), and the bootstrapped 5% and 95% CLs (confidence limits) for the treatment effect. CPOM, coarse particulate organic matter. Soil fractions are density fractions, including light (<1.5 g $cm^{-3}$), medium (1.5–1.8 g $cm^{-3}$), heavy (1.8–2.2 g $cm^{-3}$) and residual (calculated as total soil total minus the sum of measured density fractions).

began in May 1996. By 2007, coarse roots contained 740 g C $m^{-2}$ of new C, 31% of the total C contained in coarse roots (Fig. 4), yielding a mean C residence time in coarse roots of 35.5 ± 4.2 yr. The total difference in coarse root biomass between E and A was 480 g C $m^{-2}$. This could have been caused entirely by a stimulation of new root C (probably the most parsimonious interpretation), but it is possible that treatments differed in patterns of use of 'old', stored C – an idea which should not be immediately dismissed, given that these plants use old C to build new roots (Langley *et al*., 2002). In the surface soil mineral fraction, the percent new C increased linearly (Fig. 5), with an overall mean residence time of C of 33.6 ± 2.1 yr. In the spodic horizon, there was no evidence of new C accumulation in the total mineral soil or in the light, medium, or heavy density fractions (Table 2). Overall, elevated $CO_2$ did not significantly alter the total C content of the system (Table 1), because increased C in plant reservoirs were compensated by reduced C from the soil (Fig. 6).

## Discussion

In this subtropical oak woodland, 11 yr of exposure to elevated $CO_2$ increased plant C by 22%, with a smaller (and not significant) effect on plant N of 9%, well within the range of responses typically observed in plants growing under a wide variety of experimental conditions (Norby *et al*., 2005; de Graaff *et al*., 2006; Luo *et al*., 2006). Absolute responses in the mass of C above- and belowground were similar, consistent with elevated $CO_2$ having little impact on the partitioning of biomass above- and belowground (Tingey *et al*., 2000), in contrast to the expectation that root growth would increase disproportionately (Stulen & den Hertog 1993). In our experiment, the relative response aboveground was actually larger than that belowground, because most of the biomass in this system is belowground. The mean residence time of C in coarse roots (revealed by incorporation of the $\delta^{13}C$ tracer) was sufficiently long that, at the final harvest, only about one third of the C in coarse roots represented new growth over the course of this experiment. By contrast, all of the standing aboveground biomass at the final harvest had accumulated after fire. Thus, repeated cycles of fire disturbance and recovery might yield a larger cumulative response of new C in coarse roots.

The increased C content of plants suggests the potential for elevated $CO_2$ to enhance ecosystem C uptake. Yet, increased C contained in plants was not reflected in the C content of soil, neither in the top meter nor in the deeper spodic horizon. Possibly, the experiment lacked sufficient power to detect soil C accumulation (Smith, 2004). Alternatively, other mechanisms may have operated to prevent soil C accumulation in this ecosystem. We can place boundary conditions on the power problem: integrated

**Table 5** Inventory of tracer $^{15}N$ (mg excess $^{15}N\ m^{-2}$) after 11 yr exposure to increased atmospheric $CO_2$ and 9 yr of integration of the added $^{15}N$ tracer in a subtropical oak woodland[a]

| | Ambient | Elevated | Effect | 5% & 95% CLs |
|---|---|---|---|---|
| Aboveground | 2.8 ± 0.4 | 3.7 ± 0.4 | 0.9 | (0.1 to 1.8) |
| Oak leaves | 1.6 ± 0.2 | 1.8 ± 0.3 | 0.2 | (−0.3 to 0.7) |
| Oak stems | 1 ± 0.2 | 1.6 ± 0.2 | 0.6 | (0.2 to 0.9) |
| Other species | 0.1 ± 0 | 0.2 ± 0 | 0.1 | (0 to 0.2) |
| Standing dead | 0.1 ± 0 | 0.1 ± 0 | 0.0 | (0 to 0.1) |
| Litter layer | 2 ± 0.3 | 2 ± 0.3 | 0.0 | (−0.8 to 0.8) |
| Roots | 7.7 ± 1.2 | 4.5 ± 0.6 | −3.3 | (−5.2 to −1.4) |
| Fine roots | 2.1 ± 0.4 | 1.4 ± 0.2 | −0.7 | (−1.5 to 0) |
| Coarse roots | 5.6 ± 1.1 | 3.1 ± 0.6 | −2.6 | (−4.5 to −0.7) |
| Plant | 10.5 ± 1.0 | 8.2 ± 0.8 | −2.4 | (−4.2 to −0.4) |
| CPOM (0–100 cm) | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.1 | (−0.2 to 0.3) |
| Soil (0–100 cm) | 83.7 ± 16.4 | 59.2 ± 11.4 | −24.5 | (−53 to 3.7) |
| Light, 0–60 cm | 28.5 ± 4 | 29.2 ± 4.8 | 0.7 | (−8.9 to 10) |
| 0–10 cm | 21.6 ± 4.4 | 24 ± 5 | 2.4 | (−8 to 12.8) |
| 10–30 cm | 4 ± 0.7 | 2.2 ± 0.2 | −1.8 | (−2.9 to −0.7) |
| 30–60 cm | 2.9 ± 0.9 | 3.1 ± 0.7 | 0.1 | (−1.8 to 1.8) |
| Medium, 0–60 cm | 15.2 ± 2.9 | 14.9 ± 2.4 | −0.3 | (−5.6 to 5.6) |
| 0–10 cm | 11.2 ± 2.1 | 10.1 ± 2.2 | −1.2 | (−6 to 3.7) |
| 10–30 cm | 2.8 ± 0.7 | 2.5 ± 0.4 | −0.3 | (−1.5 to 0.8) |
| 30–60 cm | 1.1 ± 0.4 | 2.3 ± 0.8 | 1.2 | (−0.1 to 2.6) |
| Heavy, 0–60 cm | 5.1 ± 0.8 | 4.3 ± 1.1 | −0.8 | (−2.7 to 1.3) |
| 0–10 cm | 1.9 ± 0.5 | 1.5 ± 0.4 | −0.3 | (−1.3 to 0.7) |
| 10–30 cm | 1.2 ± 0.2 | 0.8 ± 0.4 | −0.4 | (−1 to 0.3) |
| 30–60 cm | 2.1 ± 0.3 | 2 ± 0.8 | −0.1 | (−1.5 to 1.3) |
| Residual, 0–60 cm | 34.8 ± 10.8 | 14.2 ± 9.5 | −20.7 | (−51.7 to −0.8) |
| Soil (60–100 cm) | 5.8 ± 1.1 | 6.4 ± 1.7 | 0.6 | (−2.5 to 4) |
| Total ecosystem | 102.6 ± 15.7 | 76.4 ± 9.0 | −26.2 | (−55.1 to −0.8) |

[a]Values are means ± SE of the mean for the Ambient and Elevated $CO_2$ treatments, the Effect of the $CO_2$ treatment (E–A), and the bootstrapped 5% and 95% CLs (confidence limits) for the treatment effect. CPOM, coarse particulate organic matter. Soil fractions are density fractions, including light (<1.5 g $cm^{-3}$), medium (1.5–1.8 g $cm^{-3}$), heavy (1.8–2.2 g $cm^{-3}$) and residual (calculated as total soil total minus the sum of measured density fractions).

**Table 6** $\delta^{15}N$ signatures (mean ± SEM) of plant, soil and whole system N at the final harvest in July 2007

| | Ambient $CO_2$ | Elevated $CO_2$ | *P*-value* |
|---|---|---|---|
| Plant | 76.3 ± 5.4 | 55.0 ± 4.2 | 0.008 |
| Soil | 103.7 ± 13.9 | 85.4 ± 11.6 | 0.422 |
| System | 100.4 ± 11.6 | 80.8 ± 10.0 | 0.317 |

**P*-values are for one-way ANOVAs testing the effect of elevated $CO_2$.

over the top meter of soil, the mean effect of $CO_2$ on total soil C was a decline of $-44.3\ g\ C\ m^{-2}\ yr^{-1}$, with the 90% confidence interval spanning a range of $CO_2$ effects from more rapid losses of soil C ($-132.4\ g\ C\ m^{-2}\ yr^{-1}$) to gain ($+57.9\ g\ C\ m^{-2}\ yr^{-1}$). This range exhibits the power limitations typical when assessing responses of total soil C to elevated $CO_2$ (Hungate *et al.*, 2009). Isolating components of the total soil C reservoir can help overcome the problem of limited power (e.g. Iversen *et al.*, 2012). In our case, we found that by year 6 of the experiment, elevated $CO_2$ had reduced the C contained in the light density (Carney *et al.*, 2007) and in the acid-hydrolysable (Langley *et al.*, 2009) fractions of soil C. These findings are consistent with the response we observed at the final harvest reported here where elevated $CO_2$ reduced the heavy density fraction of soil C (Table 1) and decreased soluble C susceptible to glucose-induced priming (Fig. 3). The pattern of declining soil C in soil fractions is difficult to reconcile with the concept of soil C accumulation as a first-order response to enhanced plant growth.

The second explanation for not finding soil C accumulation in response to elevated $CO_2$ is that it does not occur, because increased C input to soil is compensated by increased C loss. Elevated $CO_2$ could enhance export of C through leaching of dissolved organic matter. But, if elevated $CO_2$ increased leaching of C in this experiment, this response had no influence on the C content or $\delta^{13}C$ composition of the spodic horizon; the absence of any effect on $\delta^{13}C$ is especially unlikely if leaching was an important pathway for C loss. These findings indicate that elevated $CO_2$ did not substantially alter leaching losses of C from the system.

In contrast to the absence of any apparent effect on leaching, there was compelling evidence that elevated $CO_2$ increased the rate of C cycling through the soil: elevated $CO_2$ significantly increased the size and rate of C flow through the labile soil C pool (Fig. 1), it enhanced the proportion of soil C (and N, and $^{15}N$) that were cycling through the soil microbial biomass (Fig. 2), and it increased the decomposability of labile plant substrates and promoted a physiologically more responsive microbial community (Fig. 3). Elevated $CO_2$ also increased fungal biomass,

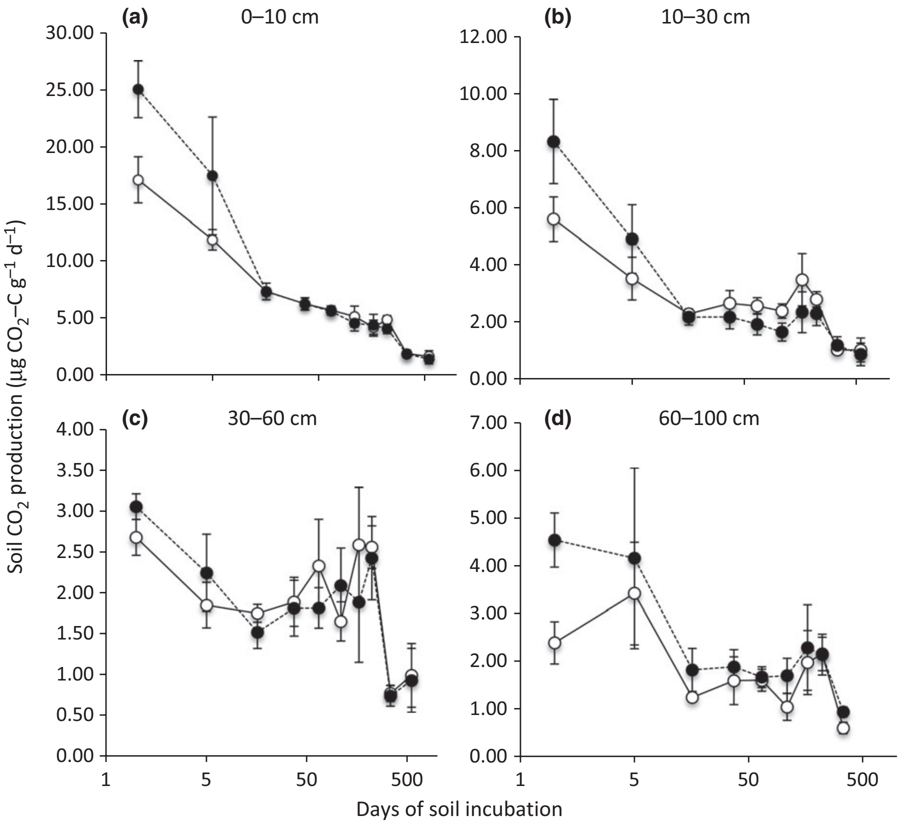


**Fig. 1** $CO_2$ production during soil incubations for four soil depths (a, 0–10 cm; b, 10–30 cm; c, 30–60 cm; d, 6–100 cm) in a subtropical oak woodland exposed to 11 yr of increased $CO_2$. Ambient $CO_2$, open circles; elevated $CO_2$, closed circles. Bars show ± 2 SEM.

as measured by ergosterol (Klamer *et al.*, 2002), by direct measurements of mycorrhizal fungal biomass (Langley *et al.*, 2003), and by the ratio of fungi to bacteria in the soil microbial biomass, as indicated by the analysis of phospholipid fatty acid profiles (Carney *et al.*, 2007). These results indicate that higher microbial activity was associated with a shift in the composition of the microbial community.

Increased soil microbial activity may also explain why the effect of elevated $CO_2$ on the C : N of plant tissues and the litter layer was not apparent, and indeed in some cases may even have been reversed, in soil organic matter. Specifically, elevated $CO_2$ increased the C : N ratio of individual plant tissues (Table 3) as commonly observed (Cotrufo *et al.*, 1998; Norby *et al.*, 2001), of the entire plant biomass, above- and belowground, and of the litter layer. Yet, this shift was not observed in soil organic matter after 11 yr of continuous inputs of plant material to the soil organic matter pool. There are two possibilities for this discrepancy: (1) either the inputs of plant material were too low compared to background soil organic matter to drive a change in soil organic C : N; or (2) by increasing soil microbial activity and the processing of C in the soil system, elevated $CO_2$ caused a compensatory response, tending to reduce soil C : N. Our finding that elevated $CO_2$ reduced the total mass of soil N in the medium density fraction, but increased it in the heavy fraction, is consistent with this second explanation. The medium fraction has a higher C : N ratio than the heavy fraction, and the medium fraction is thought to cycle into the heavy fraction as the soil organic matter is processed by microbial activity and interactions with minerals (Camberdella & Elliott 1992). Thus, the pattern we observe may indicate increased processing and turnover of soil N, promoting transfer to pools with lower C : N ratios, and a tendency for $CO_2$ to decrease soil C : N.

Some previous measurements at this site indicated that elevated $CO_2$ reduced or had no effect on microbial activity during the first 18 months of the experiment, with reduced gross N mineralization (Hungate *et al.*, 1999) and either reduced or no impact on microbial biomass N (measured as ninhydrin-reactive N) and microbial activity (measured as fluorescein diacetate hydrolysis) in the rhizosphere (Schortemeyer *et al.*, 2000), although the mechanism(s) for these changes were not apparent. These early responses were apparently transient, and did not indicate the decadal-scale response of soil microorganisms to elevated $CO_2$. The measurements reported here of microbial biomass, the size of the labile soil C pool, and the distribution and retention of $^{15}N$ cycling through the system are more representative of the entire duration of the experiment (e.g. Fig. 2). Results from this experiment are consistent with the general finding that elevated $CO_2$ stimulates soil microbial activity (de Graaff *et al.*, 2006; Dieleman *et al.*, 2012), and the turnover of soil organic matter (Marhan *et al.*, 2010; Phillips *et al.*, 2012; Dawes *et al.*, 2013).

Elevated $CO_2$ can stimulate microbial activity by increasing soil water content, especially in grasslands (Hungate *et al.*, 1997a; Morgan *et al.*, 2004), and this response can counterbalance the increased C inputs from enhanced plant growth at elevated $CO_2$, causing no change in soil C accumulation (Marhan *et al.*, 2010). In the scrub-oak experiment reported here, elevated $CO_2$ slightly

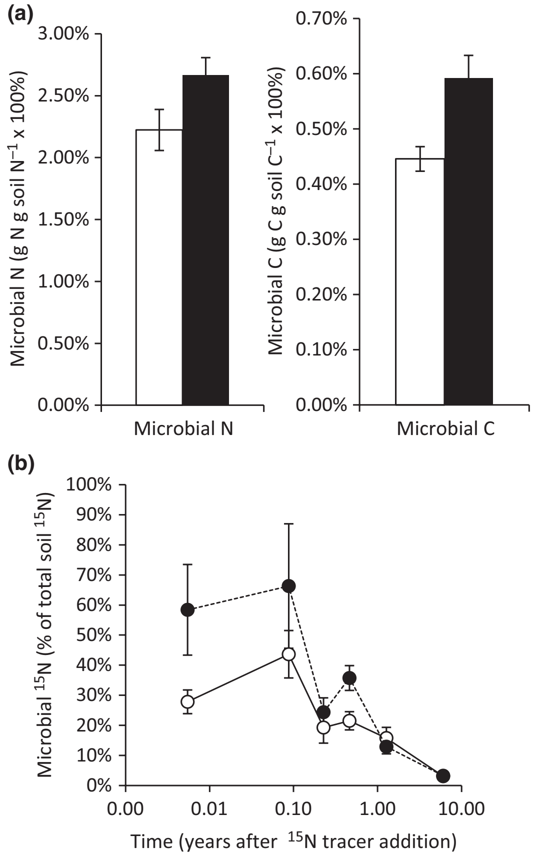


**Fig. 2** (a) Soil microbial biomass nitrogen (N) and carbon (C) from the ambient (open bars) and elevated (closed bars) $CO_2$ treated plots. Microbial C and N (as a proportion of total soil C and N) are shown as means across seven sample dates spanning 1997 to 2004, years 2–9 of $CO_2$ exposure. (b) Tracer $^{15}N$ in the microbial biomass (as a proportion of tracer $^{15}N$ in total soil) over time after label addition (log scale). Bars show ± 2 SEM.

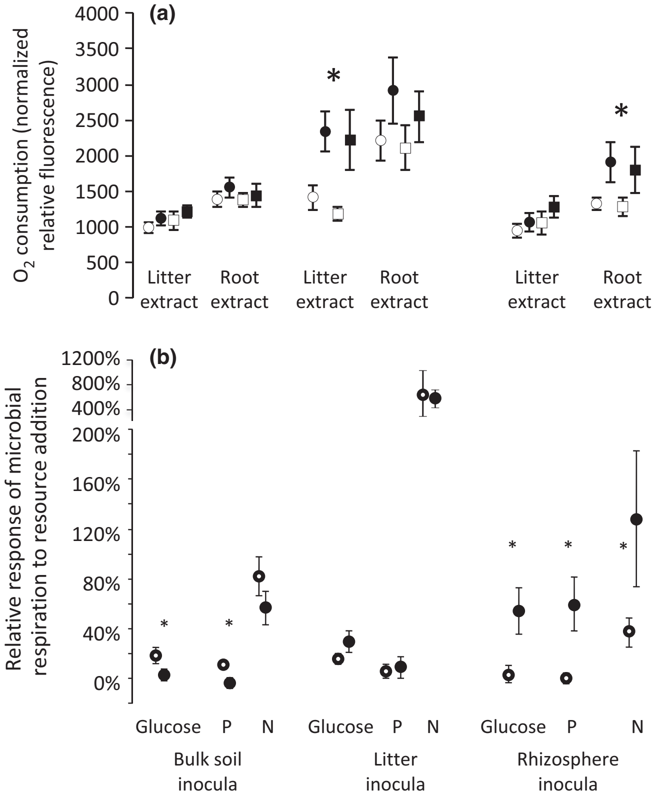


**Fig. 3** (a) Total respiration ($O_2$ consumption, expressed as normalized relative fluorescence) of microbial inocula from three soil habitats (bulk soil, litter, rhizosphere) on extracts of litter and roots. Circles, inocula from ambient $CO_2$; squares, from elevated $CO_2$. Open symbols, substrates produced in the ambient $CO_2$ treatment; closed symbols, substrates produced in the elevated $CO_2$ treatment. Significant differences between substrates produced under ambient and elevated $CO_2$ conditions (two-way ANOVAs, effect of substrate origin); *, $P < 0.050$. (b) The relative responses of microbial respiration to single resource additions (glucose, N, or P) for microbial inocula from the bulk soil, litter and rhizosphere communities in the ambient (open circles) and elevated (closed circles) $CO_2$ treatments. *Significant differences in resource limitation for individual comparisons ($t$-tests) of inocula from the ambient and elevated $CO_2$ treatments. For full statistical results, see Supporting Information Tables S1 and S2. Bars show ± 2 SEM.

increased surface soil water content during the first several years (Hungate *et al.*, 2002), but this effect disappeared with leaf area development (Li *et al.*, 2007), and elevated $CO_2$ had no effect on soil temperature (Hymus *et al.*, 2003). Thus, the changes in microbial activity and organic matter turnover that we observed are unlikely to have been driven by differences in temperature, although increased soil moisture may have played a role early on.

Elevated $CO_2$ can also increase microbial activity by enhancing the supply of C substrates to soil microorganisms, a response consistent with past reports that, in this experiment, elevated $CO_2$ stimulated the 'priming effect' (Carney *et al.*, 2007; Langley *et al.*, 2009), the phenomenon where there occurs 'extra decomposition of native soil organic matter in a soil receiving an organic amendment' (Bingeman *et al.*, 1953). In the experiment described here, the $O_2$ consumption assay indicates that C derived from the litter and roots is more labile in the elevated $CO_2$ treatment (Fig. 3), leading to a larger quantity of labile organic matter (Fig. 1). The higher rates of microbial activity observed are consistent with the notion that these new inputs of labile C to soil increased mineralization of native soil organic matter (Van Veen *et al.*, 1991; Carney *et al.*, 2007). This phenomenon has been observed for some time (Lohnis, 1926; Broadbent & Norman, 1947; Broadbent, 1948) and evidence for it has grown: isotope tracer experiments in soil incubations show that substrate additions can more than treble the decomposition rate of native soil organic matter in the short term (Cheng & Johnson, 1998; Cheng *et al.*, 2000). Substrate additions can influence the oxidation of old soil C reservoirs, for example, in deep soil (Fontaine *et al.*, 2007), and can shape the response of soil C to elevated $CO_2$ (Hoosbeek, 2004; Trueman & Gonzalez-Meler, 2005; Carney *et al.*, 2007; Hagedorn *et al.*, 2008; Paterson *et al.*, 2008; Taneva & Gonzalez-Meler, 2008; Langley *et al.*, 2009; Trueman *et al.*, 2009; Drake *et al.*, 2011; Reid *et al.*, 2012). Increased oxidation of old soil organic matter is likely a transient response to a change in the rate of labile C inputs. In the experiment described here, the reduction in soil C observed by year 6 (Carney *et al.*, 2007) was

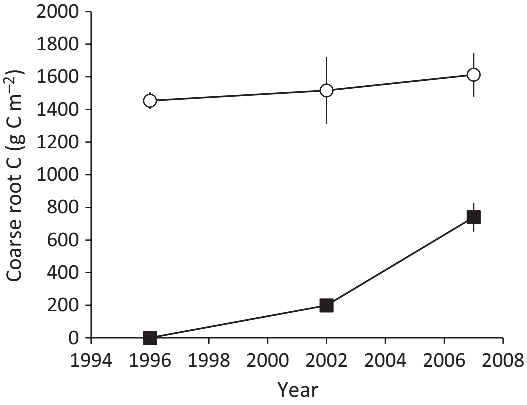


**Fig. 4** Coarse root carbon (C) over time in the scrub-oak experiment, showing 'old' (open circles) and 'new' (closed squares) carbon for the elevated $CO_2$ plots, where new is defined as carrying a $^{13}C$ isotopic signature of the $CO_2$ added to the elevated $CO_2$ plots. Modeling % old C as exponential decay over time yielded a decomposition constant of 0.0325 $yr^{-1}$, considerably lower than decomposition assessed by litterbags (0.22 $yr^{-1}$ for ambient, 0.29 $yr^{-1}$ for elevated). Bars show ± 2 SEM.

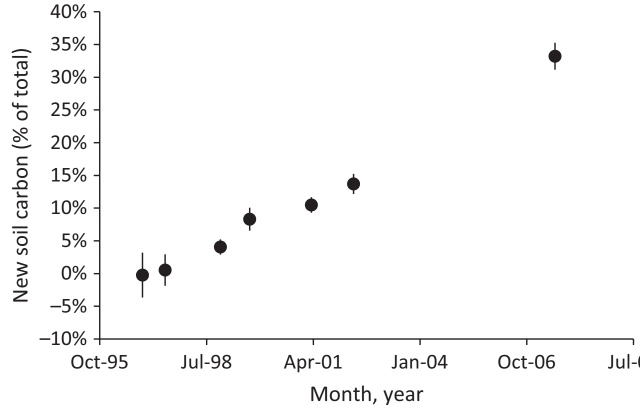


**Fig. 5** New carbon in surface mineral soils over time. Bars show ± 2 SEM.

comparable to that found after 11 yr, suggesting that the substrates susceptible to priming-induced loss had mostly been degraded during the first 6 yr.

The implications of this response are not limited to C: increased C input to soil, enhancing microbial activity and turnover, can also increasing nutrient availability to plants (Zak *et al.*, 1993). Observations elsewhere that elevated $CO_2$ increases microbial activity in concert with greater plant N acquisition from soil are also consistent with this interpretation (Drake *et al.*, 2011), although without direct evidence of increased soil organic matter turnover, increased root exploration is a simpler explanation. Results presented here call into question the notion that feedbacks stimulating soil microbial turnover and N availability necessarily lead to plant N accumulation and increased plant growth. On the one hand, we did find that elevated $CO_2$ stimulated plant N uptake and $^{15}N$ dilution in plant tissues, likely driven by increased turnover of soil organic matter mediated by microorganisms (Figs 1, 3; Johnson *et al.*, 1998, 2001; Finzi *et al.*, 2007). On the other hand, increased microbial activity

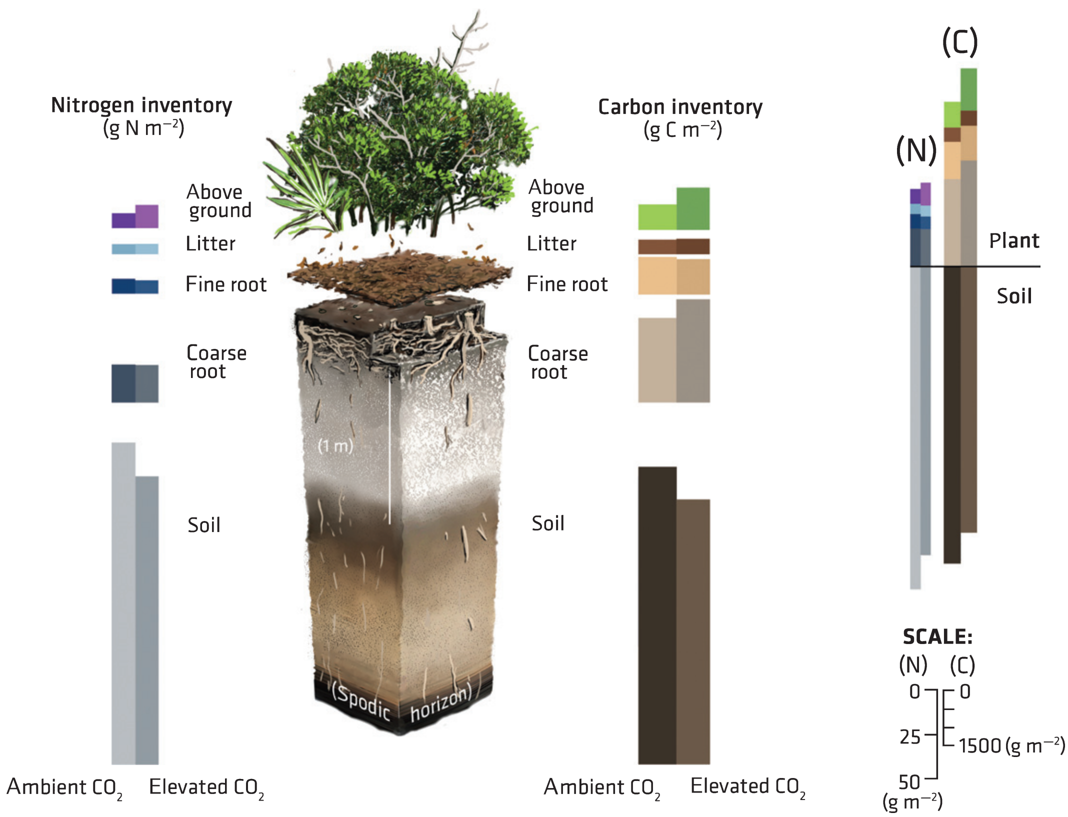


**Fig. 6** Summary of ecosystem carbon (C) and nitrogen (N) inventories in a subtropical woodland after 11 yr of exposure to elevated $CO_2$.

likely promoted N losses, accounting for our finding that elevated $CO_2$ reduced recovery of added tracer $^{15}N$ (Table 4).

In this experiment, spanning more than a decade in a naturally occurring ecosystem, photosynthesis and aboveground plant growth exhibited strong responses to chronic exposure to elevated atmospheric $CO_2$ (Dijkstra *et al.*, 2002; Seiler *et al.*, 2009), leading to the increased aboveground C content reported here, as well as increased C in coarse roots (Day *et al.*, 2013; Fig. 6). The elevated $CO_2$ treatment did not affect C in fine roots at the final harvest, although fine roots responded sporadically in this experiment, with particularly strong responses following the initial fire disturbance and after a hurricane in year 8 (Day *et al.*, 2013). Elevated $CO_2$ did not increase soil C, and in fact tended to decrease it, likely a consequence of increased microbial activity. Elevated $CO_2$ also increased plant N uptake, possibly driven by higher microbial activity and increased soil N availability, but these responses were also associated with reduced recovery of a long-term $^{15}N$ tracer, likely indicating enhanced ecosystem N losses. Thus, $CO_2$ altered the C and N cycles in this ecosystem, but not in ways that promoted large or even detectable increments in total ecosystem C mass. The effect of elevated $CO_2$ on soil C turnover via the 'priming effect' was large enough to modulate net carbon balance. This finding is not unique, and treatment of this phenomenon in models of soil C cycling is likely warranted (Heimann & Reichstein, 2008; Chapin *et al.*, 2009). While the importance of priming is becoming evident, the challenge to include the phenomenon in models is not trivial: priming is still poorly quantified and the mechanisms remain inscrutable. Meeting this challenge could improve substantially our understanding of terrestrial C cycling, replacing, or at least modifying, the stabilizing first-order kinetics of decomposition used in virtually all current models of the soil C cycle (Luo & Weng, 2011). The response of soil C to labile substrate inputs suggests a previously unrecognized sensitivity of what was thought to be a long-term, stable C sink in the biosphere.

## Acknowledgements

This research was supported by the US Department of Energy (DE-FG-02-95ER61993, and subcontract 95-59, MPOOO02), and by the National Science Foundation (DEB 9873715, 0092642, and 0445324). The National Aeronautics and Space Administration at the Kennedy Space Center, the US Fish and Wildlife Service at Merritt Island National Wildlife Refuge provided generous support throughout the $CO_2$ project. Thanks to Bert Drake for visionary leadership and opportunity. Victoria Albarracin, Mike Roberts, Mary Hummerick, Jan Bauer and Lanfang Levine assisted in the laboratory.

## References

**Adair EC, Reich PB, Hobbie SE, Knops JMH. 2009.** Interactive effects of time, $CO_2$, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. *Ecosystems* 12: 1037–1052.

**Arneth A, Harrison SP, Zaehle S, Tsigaridis K, Menon S, Bartlein PJ, Feichter J, Korhola A, Kulmala M, O'Donnell D *et al.* 2010.** Terrestrial biogeochemical feedbacks in the climate system. *Nature Geoscience* 3: 525–532.

**Bingeman CW, Varner JE, Martin WP. 1953.** The effect of the addition of organic materials on the decomposition of an organic soil. *Soil Science Society of America Proceedings* 17: 34–38.

**Broadbent FE. 1948.** Nitrogen release and carbon loss from soil organic matter during decomposition of added plant residues. *Soil Science Society of America Journal* 12: 246–249.

**Broadbent FE, Bartholomew WV. 1949.** The effect of quantity of plant material added to soil on its rate of decomposition. *Soil Science Society of America Journal* 13: 271–274.

**Broadbent FE, Norman AG. 1947.** Some factors affecting the availability of the organic nitrogen in soil long dash a preliminary report. *Soil Science Society of America Journal* 11: 264–267.

**Brown AL, Garland JL, Day FP. 2009.** Physiological profiling of soil microbial communities in a Florida scrub-oak ecosystem: spatial distribution and nutrient limitations. *Microbial Ecology* 57: 14–24.

**Cambardella CA, Elliott ET. 1992.** Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal* 56: 777–783.

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

**Chapin FS III, McFarland J, McGuire AD, Euskirchen ES, Ruess RW, Kielland K. 2009.** The changing global carbon cycle: linking plant-soil carbon dynamics to global consequences. *Journal of Ecology* 97: 840–850.

**Cheng WX, Johnson DW. 1998.** Elevated $CO_2$, rhizosphere processes, and soil organic matter decomposition. *Plant and Soil* 202: 167–174.

**Cheng WX, Sims DA, Luo YQ, Coleman JS, Johnson DW. 2000.** Photosynthesis, respiration, and net primary production of sunflower stands in ambient and elevated atmospheric $CO_2$ concentrations: an invariant NPP:GPP ratio? *Global Change Biology* 6: 931–941.

**Cotrufo MF, Ineson P, Scott A. 1998.** Elevated $CO_2$ reduces the nitrogen concentration of plant tissues. *Global Change Biology* 1: 43–54.

**Cramer W, Bondeau A, Woodward FI, Prentice IC, Betts RA, Brovkin V, Cox PM, Fisher V, Foley JA, Friend AD *et al.* 2001.** Global response of terrestrial ecosystem structure and function to $CO_2$ and climate change: results from six dynamic global vegetation models. *Global Change Biology* 7: 357–373.

**Dawes MA, Hagedorn F, Handa IT, Streit K, Ekblad A, Rixen C, Korner C, Hattenschwiler S. 2013.** An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study. *Oecologia* 171: 623–637.

**Day FP, Schroeder RE, Stover DB, Brown ALP, Butnor JR, Dilustro J, Hungate BA, Dijkstra P, Duval BD, Seiler TJ *et al.* 2013.** The effects of 11 yr of $CO_2$ enrichment on roots in a Florida scrub-oak ecosystem. *New Phytologist* 199: 74–88.

**Denman KL, Brasseur G, Chidthaisong A, Ciais P, Cox PM, Dickinson RE, Hauglustaine D, Heinze C, Holland E, Jacob D *et al.* 2007.** Couplings between changes in the climate system and biogeochemistry. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, UK: Cambridge University Press, 501–568.

**Diaz S, Grime JP, Harris J, McPherson E. 1993.** Evidence of a feedback mechanism limiting plant-response to elevated carbon-dioxide. *Nature* 364: 616–617.

**Dieleman WI, Luyssaert S, Rey A, de Angelis P, Barton CV, Broadmeadow MS, Broadmeadow SB, Chigwerewe KS, Crookshanks M, Dufrene E *et al.* 2010.** Soil [N] modulates soil C cycling in $CO_2$-fumigated tree stands: a meta-analysis. *Plant, Cell & Environment* 33: 2001–2011.

**Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes JS *et al.* 2012.** Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of $CO_2$ and temperature. *Global Change Biology* 18: 2681–2693.

**Dijkstra P, Hymus G, Colavito D, Vieglais D, Cundari C, Johnson D, Hungate BA, Hinkle CR, Drake BG. 2002.** Elevated atmospheric $CO_2$ stimulates shoot growth in a Florida scrub-oak ecosystem. *Global Change Biology* 8: 90–103.

**Dolman AJ, van der Werf GR, van der Molen MK, Ganssen G, Erisman JW, Strengers B. 2010.** A carbon cycle science update since IPCC AR-4. *Ambio* **39**: 402–412.

**Dore S, Hymus GJ, Johnson DP, Hinkle CR, Valentini R, Drake BG. 2003.** Cross validation of open-top chamber and eddy covariance measurements of ecosystem $CO_2$ exchange in a Florida scrub-oak ecosystem. *Global Change Biology* **9**: 84–95.

**Drake JE, Gallet-Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB, Johnsen KS, Lichter J, McCarthy HR, McCormack ML *et al.* 2011.** Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated $CO_2$. *Ecology Letters* **14**: 349–357.

**Finzi AC, Cole JJ, Doney SC, Holland EA, Jackson RB. 2011.** Research frontiers in the analysis of coupled biogeochemical cycles. *Frontiers in Ecology and the Environment* **9**: 74–80.

**Finzi AC, Norby RJ, Calfapietra C, Gallet-Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME *et al.* 2007.** Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated $CO_2$. *Proceedings of the National Academy of Sciences, USA* **104**: 14 014–14 019.

**Fontaine S, Barot S, Barre P, Bdioui N, Mary B, Rumpel C. 2007.** Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **450**: 277–280.

**Friedlingstein P, Prentice IC. 2010.** Carbon–climate feedbacks: a review of model and observation based estimates. *Current Opinion in Environmental Sustainability* **2**: 251–257.

**Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008.** Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* **320**: 889–892.

**Garland JLR, Roberts MS, Levine LH, Mills AL. 2003.** Community-level physiological profiling performed with an oxygen-sensitive fluorophore in a microtiter plate. *Applied & Environmental Microbiology* **69**: 2994–2998.

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

**Gerber S, Hedin LO, Oppenheimer M, Pacala SW, Shevliakova E. 2010.** Nitrogen cycling and feedbacks in a global dynamic land model. *Global Biogeochemical Cycles* **24**, GB1001.

**Gielen B, Calfapietra C, Lukac M, Wittig VE, De Angelis P, Janssens IA, Moscatelli MC, Grego S, Cotrufo MF, Godbold DL *et al.* 2005.** Net carbon storage in a poplar plantation (POPFACE) after three years of free-air $CO_2$ enrichment. *Tree Physiology* **25**: 1399–1408.

**Gill RA, Anderson LJ, Polley HW, Johnson HB, Jackson RB. 2006.** Potential nitrogen constraints on soil carbon sequestration under low and elevated atmospheric $CO_2$. *Ecology* **87**: 41–52.

**de Graaff M-A, van Groenigen K-J, Six J, Hungate BA, van Kessel C. 2006.** Interactions between plant growth and soil nutrient cycling under elevated $CO_2$: a meta-analysis. *Global Change Biology* **12**: 2077–2091.

**van Groenigen KJ, van Kessel C, Hungate BA. 2012.** Increased greenhouse-gas intensity of rice production under future atmospheric conditions. *Nature Climate Change* **3**: 288–291.

**van Groenigen KJ, Osenberg CW, Hungate BA. 2011.** Increased soil emissions of potent greenhouse gases under increased atmospheric $CO_2$. *Nature* **475**: 214–216.

**Hagedorn F, van Hees PAW, Handa IT, Hättenschwiler S. 2008.** Elevated atmospheric $CO_2$ fuels leaching of old dissolved organic matter at the alpine treeline. *Global Biogeochemical Cycles* **22**: GB2004.

**Heimann M, Reichstein M. 2008.** Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**: 289–292.

**Hofmockel KS, Schlesinger WH. 2007.** Carbon dioxide effects on heterotrophic dinitrogen fixation in a temperate pine forest. *Soil Science Society of America Journal* **71**: 140–144.

**Hoosbeek MR. 2004.** More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): cause of increased priming effect? *Global Biogeochemical Cycles* **18**: GB1040.

**Hungate BA, Chapin FS, Zhong H, Holland EA, Field CB. 1997a.** Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia* **109**: 149–153.

**Hungate BA, Dijkstra P, Johnson DW, Hinkle CR, Drake BG. 1999.** Elevated $CO_2$ increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Global Change Biology* **5**: 781–789.

**Hungate BA, van Groenigen K-J, Six J, Jastrow JD, Luo Y, de Graaff M-A, van Kessel C, Osenberg CW. 2009.** Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. *Global Change Biology* **15**: 2020–2034.

**Hungate BA, Holland EA, Jackson RB, Chapin FS, Mooney HA, Field CB. 1997b.** The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**: 576–579.

**Hungate BA, Jackson RB, Field CB, Chapin FS. 1996.** Detecting changes in soil carbon in $CO_2$ enrichment experiments. *Plant and Soil* **187**: 135–145.

**Hungate BA, Johnson DW, Dijkstra P, Hymus G, Stiling P, Megonigal JP, Pagel AL, Moan JL, Day F, Li JH *et al.* 2006.** Nitrogen cycling during seven years of atmospheric $CO_2$ enrichment in a scrub oak woodland. *Ecology* **87**: 26–40.

**Hungate BA, Lund CP, Pearson HL, Chapin FS. 1997c.** Elevated $CO_2$ and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. *Biogeochemistry* **37**: 89–109.

**Hungate BA, Reichstein M, Dijkstra P, Johnson D, Hymus G, Tenhunen JD, Hinkle CR, Drake BG. 2002.** Evapotranspiration and soil water content in a scrub-oak woodland under carbon dioxide enrichment. *Global Change Biology* **8**: 289–298.

**Hymus GJ, Johnson DP, Dore S, Anderson HP, Hinkle CR, Drake BG. 2003.** Effects of elevated atmospheric $CO_2$ on net ecosystem $CO_2$ exchange of a scrub-oak ecosystem. *Global Change Biology* **9**: 1802–1812.

**Iversen CM, Keller JK, Garten CT Jr, Norby RJ. 2012.** Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of $CO_2$-enrichment. *Global Change Biology* **18**: 1684–1697.

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

**Jain A, Yang X, Kheshgi H, McGuire AD, Post W, Kicklighter D. 2009.** Nitrogen attenuation of terrestrial carbon cycle response to global environmental factors. *Global Biogeochemical Cycles* **23**: GB4028.

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

**Jenkinson DW, Goulding K, Powlson DS. 1999.** Nitrogen deposition and carbon sequestration. *Nature* **400**: 629.

**Johnson DW, Ball JT, Walker RF. 1997.** Effects of $CO_2$ and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. *Plant and Soil* **190**: 29–40.

**Johnson DW, Hoylman AM, Ball JT, Walker RF. 2006.** Ponderosa pine responses to elevated $CO_2$ and nitrogen fertilization. *Biogeochemistry* **77**: 157–175.

**Johnson DW, Norby RJ, Hungate BA. 2001.** Effects of elevated $CO_2$ on nutrient cycling in forests. In: Karnosky DF, Ceulemans R, Scarascia-Mugnozza GE, Innes JL, eds. *The impact of carbon dioxide and other greenhouse gases on forest ecosystems.* Report No. 3 of the IUFRO Task Force on Environmental Change. Wallingford, UK: CAB, 237–268.

**Johnson DW, Thomas RB, Griffin KL, Tissue DT, Ball JT, Strain BR, Walker RF. 1998.** Effects of carbon dioxide and nitrogen on growth and nitrogen uptake in ponderosa and loblolly pine. *Journal of Environmental Quality* **27**: 414–425.

**Klamer M, Roberts MS, Levine LH, Drake BG, Garland JL. 2002.** Influence of elevated $CO_2$ on the fungal community in a coastal scrub oak forest soil investigated with terminal-restriction fragment length polymorphism analysis. *Applied & Environmental Microbiology* **68**: 4370–4376.

**Langley JA, Dijkstra P, Drake BG, Hungate BA. 2003.** Ectomycorrhizal colonization, biomass, and production in a regenerating scrub oak forest in response to elevated $CO_2$. *Ecosystems* **6**: 424–430.

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

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

**Leavitt SW, Paul EA, Kimball BA, Hendrey GR, Mauney JR, Rauschkolb R, Rogers H, Lewin KF, Nagy J, Pinter PJ *et al.* 1994.** Carbon-isotope dynamics of free-air $CO_2$ enriched cotton and soils. *Agricultural and Forest Meteorology* **70**: 87–101.

**LeBauer DS, Treseder KK. 2008.** Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**: 371–379.

**Li J, Powell TL, Seiler TJ, Johnson DP, Anderson HP, Bracho R, Hungate BA, Hinkle CR, Drake BG. 2007.** Impacts of Hurricane Frances on Florida scrub-oak ecosystem processes: defoliation, net $CO_2$ exchange and interactions with elevated $CO_2$. *Global Change Biology* **13**: 1101–1113.

**Lohnis F. 1926.** Nitrogen availability of green manures. *Soil Science Society of America Journal* **22**: 171–177.

**Luo YQ, Hui DF, Zhang DQ. 2006.** Elevated $CO_2$ stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology* **87**: 53–63.

**Luo YQ, Weng ES. 2011.** Dynamic disequilibrium of the terrestrial carbon cycle under global change. *Trends in Ecology & Evolution* **26**: 96–104.

**Marhan S, Kandeler E, Rein S, Fangmeier A, Niklaus PA. 2010.** Indirect effects of soil moisture reverse soil C sequestration responses of a spring wheat agroecosystem to elevated $CO_2$. *Global Change Biology* **16**: 469–483.

**McKinley DC, Romero JC, Hungate BA, Drake BG, Megonigal JP. 2009.** Does deep soil N availability sustain long-term ecosystem responses to elevated $CO_2$? *Global Change Biology* **15**: 2035–2048.

**McMurtrie RE, Norby RJ, Medlyn BE, Dewar RC, Pepper DA, Reich PB, Barton CVM. 2008.** Why is plant-growth response to elevated $CO_2$ amplified when water is limiting, but reduced when nitrogen is limiting? A growth-optimisation hypothesis. *Functional Plant Biology* **35**: 521–534.

**Morgan JA, Knight WG, Dudley LM, Hunt HW. 1994.** Enhanced root-system C-sink activity, water relations and aspects of nutrient acquisition in mycotrophic *Bouteloua gracilis* subjected to $CO_2$ enrichment. *Plant and Soil* **165**: 139–146.

**Morgan JA, Pataki DE, Korner C, Clark H, Del Grosso SJ, Grunzweig JM, Knapp AK, Mosier AR, Newton PCD, Niklaus PA, Nippert JB, Nowak RS, Parton WJ, Polley HW, Shaw MR. 2004.** Water relations in grassland and desert ecosystems exposed to elevated atmospheric $CO_2$. *Oecologia* **140**: 11–25.

**Nadelhoffer KJ, Emmett BA, Gunderson P, Kjonnas OJ, Koopmans CJ, Schleppl P, Tietma A, Wright RF. 1999.** Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature* **398**: 145–148.

**Nadelhoffer KJ. 1990.** Microlysimeter for measuring nitrogen mineralization and microbial respiration in aerobic soil incubations. *Soil Science Society of America Journal* **54**: 411–415.

**Niklaus PA, Falloon P. 2006.** Estimating soil carbon sequestration under elevated $CO_2$ by combining carbon isotope labelling with soil carbon cycle modelling. *Global Change Biology* **12**: 1909–1921.

**Norby RJ, Cotrufo MF, Ineson P, O'Neill EG, Canadell JG. 2001.** Elevated $CO_2$, litter chemistry, and decomposition: a synthesis. *Oecologia* **127**: 153–167.

**Norby RJ, Delucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJ, Ceulemans R *et al.* 2005.** Forest response to elevated $CO_2$ is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences, USA* **102**: 18 052–18 056.

**Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE. 2010.** $CO_2$ enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences, USA* **107**: 19 368–19 373.

**Norby RJ, Zak DR. 2011.** Ecological lessons from Free-Air $CO_2$ Enrichment (FACE) experiments. *Annual Review of Ecology, Evolution, & Systematics* **42**: 181–203.

**Parton WJ, Schimel DS, Cole CV, Ojima DS. 1987.** Analysis of factors controlling soil organic levels of grasslands in the Great Plains. *Soil Science Society of America Journal* **51**: 1173–1179.

**Paterson E, Thornton B, Midwood AJ, Osborne SM, Sim A, Millard P. 2008.** Atmospheric $CO_2$ enrichment and nutrient additions to planted soil increase mineralisation of soil organic matter, but do not alter microbial utilisation of plant- and soil C-sources. *Soil Biology & Biochemistry* **40**: 2434–2440.

**Phillips RP, Meier IC, Bernhardt ES, Grandy AS, Wickings K, Finzi AC. 2012.** Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated $CO_2$. *Ecology Letters* **15**: 1042–1049.

**Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost J. 2006a.** Nitrogen limitation constrains sustainability of ecosystem response to $CO_2$. *Nature* **440**: 922–925.

**Reich PB, Hungate BA, Luo Y. 2006b.** Carbon–nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annual Review of Ecology, Evolution & Systematics* **37**: 611–636.

**Reid JP, Adair EC, Hobbie SE, Reich PB. 2012.** Biodiversity, nitrogen deposition, and $CO_2$ affect grassland soil carbon cycling but not storage. *Ecosystems* **15**: 580–590.

**Schortemeyer M, Dijkstra P, Johnson DW, Drake BG. 2000.** Effects of elevated atmospheric $CO_2$ concentration on C and N pools and rhizosphere processes in a Florida scrub oak community. *Global Change Biology* **6**: 383–391.

**Seiler TJ, Rasse DP, Li J, Dijkstra P, Anderson HP, Johnson DP, Powell TL, Hungate BA, Hinkle CR, Drake BG. 2009.** Disturbance, rainfall and contrasting species responses mediated aboveground biomass response over 11 years of $CO_2$ enrichment in a Florida Scrub-Oak ecosystem. *Global Change Biology* **15**: 356–367.

**Smith P. 2004.** How long before a change in soil organic carbon can be detected? *Global Change Biology* **10**: 1878–1883.

**Sokolov AP, Kicklighter DW, Melillo JM, Felzer BS, Schlosser CA, Cronin TW. 2008.** Consequences of considering carbon–nitrogen interactions on the feedbacks between climate and the terrestrial carbon cycle. *Journal of Climate* **21**: 3776–3796.

**Stover DB, Day FP, Butnor JR, Drake BG. 2007.** Effect of elevated $CO_2$ on coarse-root biomass in Florida scrub detected by ground-penetrating radar. *Ecology* **88**: 1328–1334.

**Stulen I, Den Hertog J. 1993.** Root-growth and functioning under atmospheric $CO_2$ enrichment. *Vegetatio* **104**: 99–115.

**Taneva L, Gonzalez-Meler MA. 2008.** Decomposition kinetics of soil carbon of different age from a forest exposed to 8 years of elevated atmospheric $CO_2$ concentration. *Soil Biology & Biochemistry* **40**: 2670–2677.

**Thornton PE, Lamarque J-F, Rosenbloom NA, Mahowald NM. 2007.** Influence of carbon-nitrogen cycle coupling on land model response to $CO_2$ fertilization and climate variability. *Global Biogeochemical Cycles* **21**: GB4018.

**Tingey DT, Phillips DL, Johnson MG. 2000.** Elevated $CO_2$ and conifer roots: effects on growh, life span and turnover. *New Phytologist* **147**: 87–103.

**Trueman RJ, Gonzalez-Meler MA. 2005.** Accelerated belowground C cycling in a managed agriforest ecosystem exposed to elevated carbon dioxide concentrations. *Global Change Biology* **11**: 1258–1271.

**Trueman RJ, Taneva L, Gonzalez-Meler MA, Oechel WC, BassiriRad H. 2009.** Carbon losses in soils previously exposed to elevated atmospheric $CO_2$ in a chaparral ecosystem: potential implications for a sustained biospheric C sink. *Journal of Geochemical Exploration* **102**: 142–148.

**Väisänen RKR, Robers MS, Garland JL, Frey SD, Dawson LA. 2005.** Physiological and molecular characterization of microbial communities associated with different water-stable aggregate size classes. *Soil Biology & Biochemistry* **37**: 2007–2016.

**Van Veen JA, Liljeroth E, Lekkerkerk LJA, Van De Geijn SC. 1991.** Carbon fluxes in plant-soil systems at elevated atmospheric carbon dioxide levels. *Ecological Applications* **1**: 175–181.

**Vance ED, Brookes PC, Jenkinson DS. 1987.** An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **19**: 703–707.

**Wang Y-P, Houlton BZ. 2009.** Nitrogen constraints on terrestrial carbon uptake: implications for the global carbon-climate feedback. *Geophysical Research Letters* **36**: L24403.

**Zabaloy MCL, Lehman RM, Frey SD, Garland JL. 2008.** Optimization of an oxygen-based approach for community-level physiological profiling of soils. *Soil Biology & Biochemistry* **40**: 2960–2969.

**Zaehle S, Friend AD, Friedlingstein P, Dentener F, Peylin P, Schulz M. 2010.** Carbon and nitrogen cycle dynamics in the O-CN land surface model: 2. Role of the nitrogen cycle in the historical terrestrial carbon balance. *Global Biogeochemical Cycles* **24**: doi:10.1029/2009GB003522.

**Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL. 1993.** Elevated atmospheric $CO_2$ and feedback between carbon and nitrogen cycles. *Plant and Soil* **151**: 105–117.

**Zanetti S, Hartwig UA, Luscher A, Hebeisen T, Frehner M, Fischer BU, Hendrey GR, Blum H, Nosberger J. 1996.** Stimulation of symbiotic N-2 fixation in *Trifolium repens* L under elevated atmospheric $pCO_2$ in a grassland ecosystem. *Plant Physiology* **112**: 575–583.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Tables S1 & S2** Results from ANOVAs testing responses of soil microbial respiration to $CO_2$ treatment, habitat, and substrate

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.