

Cumulative response of ecosystem carbon and nitrogen stocks to chronic CO₂ exposure in a subtropical oak woodland

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Summary

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- Rising atmospheric carbon dioxide (CO₂) 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 CO₂.
- We used open-top chambers to manipulate CO₂ during regrowth after fire, and measured C, N and tracer ¹⁵N in ecosystem components throughout the experiment.
- Elevated CO₂ increased plant C and tended to increase plant N but did not significantly increase whole-system C or N. Elevated CO₂ increased soil microbial activity and labile soil C, but more slowly cycling soil C pools tended to decline. Recovery of a long-term ¹⁵N tracer indicated that CO₂ 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 CO₂ on net ecosystem C uptake. These findings challenge the treatment of terrestrial ecosystems responses to elevated CO₂ in current biogeochemical models, where the effect of elevated CO₂ 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 CO₂ (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 CO₂, 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂, to test whether the CO₂ 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 CO₂ 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 CO₂ 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 CO₂, 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 CO₂ enrichment, and to test whether patterns of CO₂ effects on soil microbial activity might help explain any effects (or lack of effects) of elevated CO₂ 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 CO₂, because N availability limits plant growth and its response to elevated CO₂ (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 (Arneeth *et al.*, 2010). Experiments also indicate that C–N interactions are critical modulators of the long-term CO₂ fertilization response, but different experiments provide support for different mechanisms underlying that modulation. In some cases, C–N interactions appear to constrain strongly the CO₂ 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 CO₂ 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 CO₂, 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; Arneeth *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 CO₂ exposure in a subtropical woodland, in order to test how rising CO₂ 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 CO₂ 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. ¹⁵N tracers reflect short-term effects on N cycling processes and integrate these into long-term effects on ¹⁵N distribution among plant and soil components within the system. Because the ¹⁵N is added in labile form, losses of added ¹⁵N 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 ¹⁵N tracer to characterize changes in N distribution and N losses in response to elevated CO₂.

Here, we report a whole system inventory of the C and N content of a scrub-oak ecosystem after 11 yr of experimental CO₂ exposure. We also show how soil microbial activity responded to chronic CO₂ exposure. We also report recovery and distribution of a ¹⁵N tracer applied early in the experiment, in order to assess how elevated CO₂ 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 m² ground area, with 8 chambers receiving ambient air and 8 receiving ambient air + 350 ppm V CO₂ (referred to as the 'elevated CO₂' treatment). A large blower circulated air through each chamber at a rate of 24–30 m³ 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), micro-environmental 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_n)

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 CO₂ 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 g cm⁻³), medium (1.5–1.8 g cm⁻³), heavy (1.8–2.2 g cm⁻³) and residual (> 2.2 g cm⁻³) organic matter fractions. Total soil C, N, ¹⁵N and ¹³C 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 ¹⁵N, 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 K₂SO₄ before and after 24-h fumigation with ethanol-free chloroform. The K₂SO₄ 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, δ¹⁵N and δ¹³C 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, ¹³C or ¹⁵N) between fumigated and nonfumigated samples, divided by 0.54 to correct for extraction efficiency (Vance *et al.*, 1987). For samples collected after the ¹⁵N tracer application (June 1998), we also measured the ¹⁵N content of mineral soil (0–15 cm depth). After milling, soil N and ¹⁵N contents were determined as described above.

The CO₂ added to the elevated-CO₂ treated plots was depleted in ¹³C. 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 CO₂ treatment (δ¹³C_{S,E}) provided an integrative measure of the δ¹³C value of new photosynthate (average across five sampling dates, -42.6 ± 0.3 ‰). However, because mineral soil (δ¹³C_{M,A}) and stem δ¹³C (δ¹³C_{S,A}) differed in the ambient C_a treatment, we calculated the δ¹³C signature of new carbon (δ¹³C_{new}) as:

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

The δ¹³C of the mineral soil in the ambient CO₂ treatment was used as the end member for organic matter fixed before the experiment began. Carbon, N, ¹⁵N, and ¹³C 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 CO₂ 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 CO₂ treatments. Microbial inocula from litter, rhizosphere and bulk soil communities were also prepared from the two CO₂ 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 O₂ 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:

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

(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 CO₂ treatment; here, we expand on this past analysis to evaluate responses of microbial respiration to elevated CO₂. We used ANOVA to test for effects of habitat (rhizosphere, litter or bulk soil), inoculum source (ambient or elevated CO₂), substrate source (ambient or elevated CO₂), 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 CO₂ treatment on ecosystem C and N pools and recovery of tracer ¹⁵N. We estimated 5% and 95% confidence limits for the difference in means between elevated and ambient CO₂ treatments, using 1000 samples with replacement (*n* = 8 for each treatment).

Results

Elevated CO₂ increased plant biomass, including the mass of C (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 CO₂ 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 CO₂, 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 CO₂ treatment, whereas the heavy density soil C pool significantly declined. Elevated CO₂ 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 CO₂ treatment. In general, increased mass of plant C caused by elevated CO₂ did not translate to increased C storage in other ecosystem reservoirs (Table 1).

Elevated CO₂ increased the N content of plants aboveground (Table 3), but the N contents of coarse and fine roots did not respond to elevated CO₂, yielding no effect on total plant N. The N content of most soil fractions was not significantly altered by elevated CO₂, 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 CO₂ were observed for leaves, coarse roots and the sum of all plant parts; elevated CO₂ also increased the C to N ratio of the litter layer (Table 4). Elevated CO₂ 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 CO₂ had no effect on the C to N ratio of the plant–soil system to 1 m depth.

Elevated CO₂ increased recovery of tracer ¹⁵N 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 ¹⁵N recovery under elevated CO₂. Elevated CO₂ reduced the δ¹⁵N of plant tissue (weighted average of all plant parts), a dilution of the added ¹⁵N tracer with unlabeled ¹⁵N. This pattern indicates that elevated CO₂ 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 CO₂, the increase in inputs of new N to plants were matched by N losses from plants, such that CO₂ enhanced N turnover through the plant system. In contrast to plant δ¹⁵N, the δ¹⁵N of soils did

Table 1 Inventory of carbon after 11 yr exposure to increased atmospheric CO₂ 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)

^aValues are means ± SE of the mean for the Ambient and Elevated CO₂ treatments, the Effect of the CO₂ 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	<i>P</i> -value	Ambient	Elevated	<i>P</i> -value
	%C			$\delta^{13}\text{C}$		
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)

^aValues 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 $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ 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 CO₂^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)

^aValues are means ± SE of the mean for the Ambient and Elevated CO₂ treatments, the Effect of the CO₂ treatment (E–A), and the bootstrapped 5% and 95% CIs (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 CO₂ 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 CO₂ 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 CO₂ 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 δ¹³C 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 CO₂ 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 $^{15}\text{N m}^{-2}$) 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)

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

	Ambient CO_2	Elevated CO_2	<i>P</i> -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 $-44.3 \text{ g C m}^{-2} \text{ yr}^{-1}$, with the 90% confidence interval spanning a range of CO_2 effects from more rapid losses of soil C ($-132.4 \text{ g C m}^{-2} \text{ yr}^{-1}$) to gain ($+57.9 \text{ g C m}^{-2} \text{ yr}^{-1}$). 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 $\delta^{13}\text{C}$ composition of the spodic horizon; the absence of any effect on $\delta^{13}\text{C}$ 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,

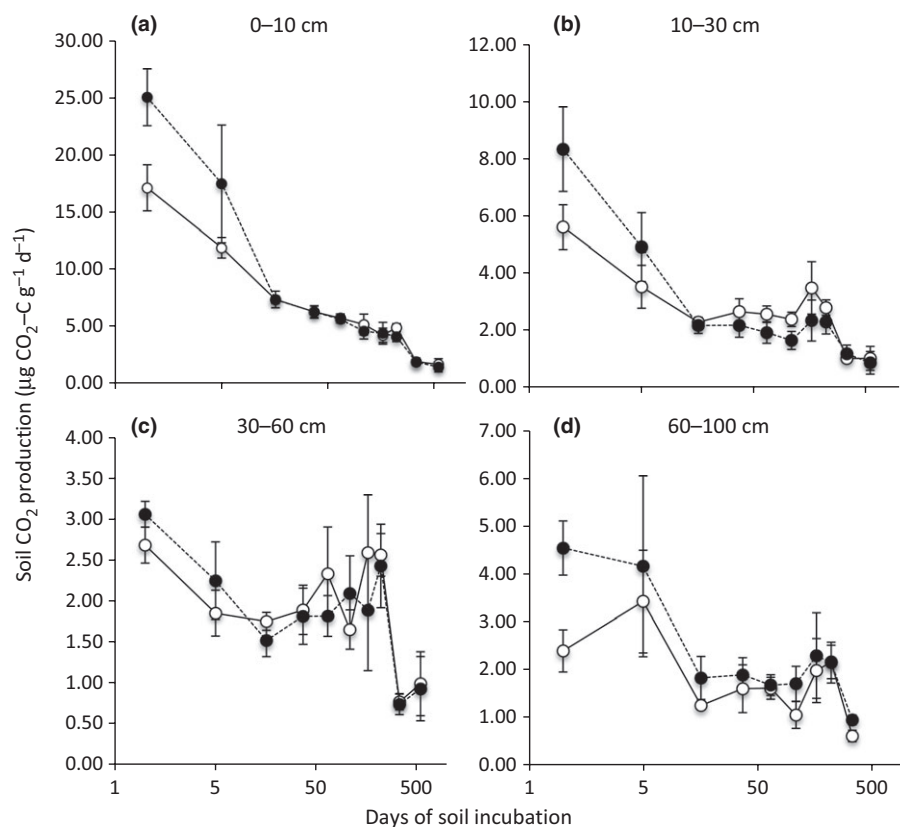


Fig. 1 CO₂ production during soil incubations for four soil depths (a, 0–10 cm; b, 10–30 cm; c, 30–60 cm; d, 6–100 cm) in a subtropical oak woodland exposed to 11 yr of increased CO₂. Ambient CO₂, open circles; elevated CO₂, closed circles. Bars show ± 2 SEM.

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 CO₂ 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 CO₂ 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 CO₂ caused a compensatory response, tending to reduce soil C : N. Our finding that elevated CO₂ 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 CO₂ to decrease soil C : N.

Some previous measurements at this site indicated that elevated CO₂ 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 CO₂. The measurements reported here of microbial biomass, the size of the labile soil C pool, and the distribution and retention of ¹⁵N 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 CO₂ 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 CO₂ 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 CO₂, causing no change in soil C accumulation (Marhan *et al.*, 2010). In the scrub-oak experiment reported here, elevated CO₂ slightly

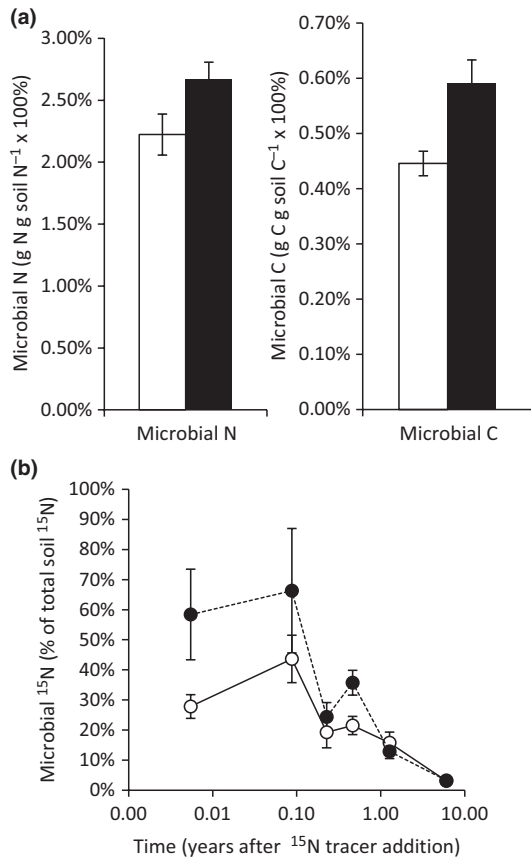


Fig. 2 (a) Soil microbial biomass nitrogen (N) and carbon (C) from the ambient (open bars) and elevated (closed bars) CO₂ treated plots. Microbial C and N (as a proportion of total soil C and N) are shown as means across seven sample dates spanning 1997 to 2004, years 2–9 of CO₂ exposure. (b) Tracer ¹⁵N in the microbial biomass (as a proportion of tracer ¹⁵N in total soil) over time after label addition (log scale). 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 CO₂ 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 CO₂ 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 CO₂ 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 O₂ consumption assay indicates that C derived from the litter and roots is more labile in the elevated CO₂ 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).

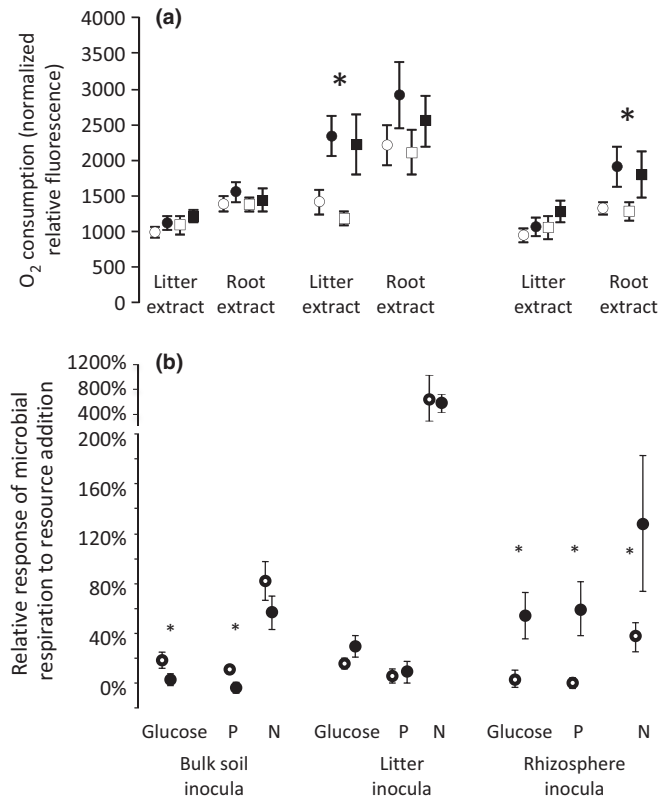


Fig. 3 (a) Total respiration (O₂ consumption, expressed as normalized relative fluorescence) of microbial inocula from three soil habitats (bulk soil, litter, rhizosphere) on extracts of litter and roots. Circles, inocula from ambient CO₂; squares, inocula from elevated CO₂. Open symbols, substrates produced in the ambient CO₂ treatment; closed symbols, substrates produced in the elevated CO₂ treatment. Significant differences between substrates produced under ambient and elevated CO₂ 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) CO₂ treatments. *Significant differences in resource limitation for individual comparisons (*t*-tests) of inocula from the ambient and elevated CO₂ treatments. For full statistical results, see Supporting Information Tables S1 and S2. Bars show ± 2 SEM.

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 CO₂ (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

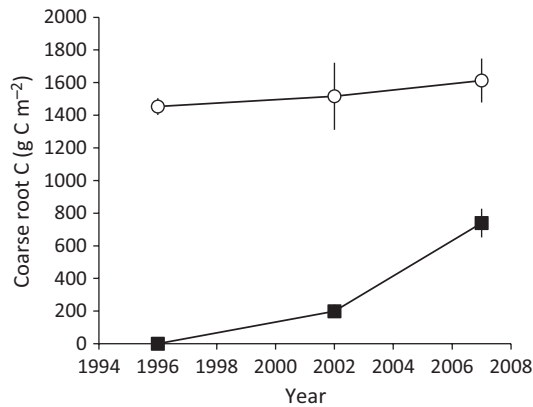


Fig. 4 Coarse root carbon (C) over time in the scrub-oak experiment, showing 'old' (open circles) and 'new' (closed squares) carbon for the elevated CO₂ plots, where new is defined as carrying a ¹³C isotopic signature of the CO₂ added to the elevated CO₂ plots. Modeling % old C as exponential decay over 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.

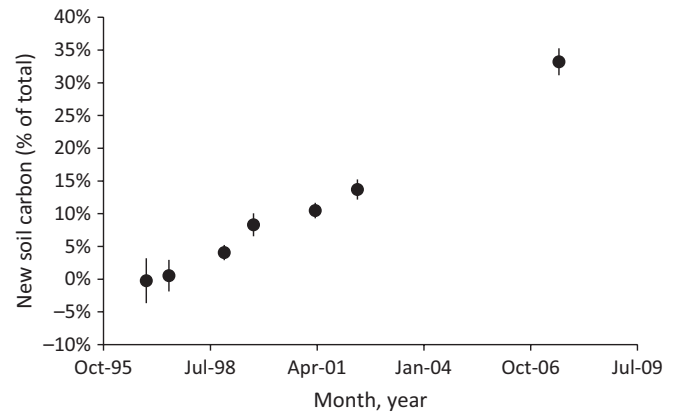


Fig. 5 New carbon in surface mineral soils over time. Bars show ± 2 SEM.

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 increase nutrient availability to plants (Zak *et al.*, 1993). Observations elsewhere that elevated CO₂ 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 CO₂ stimulated plant N uptake and ¹⁵N 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

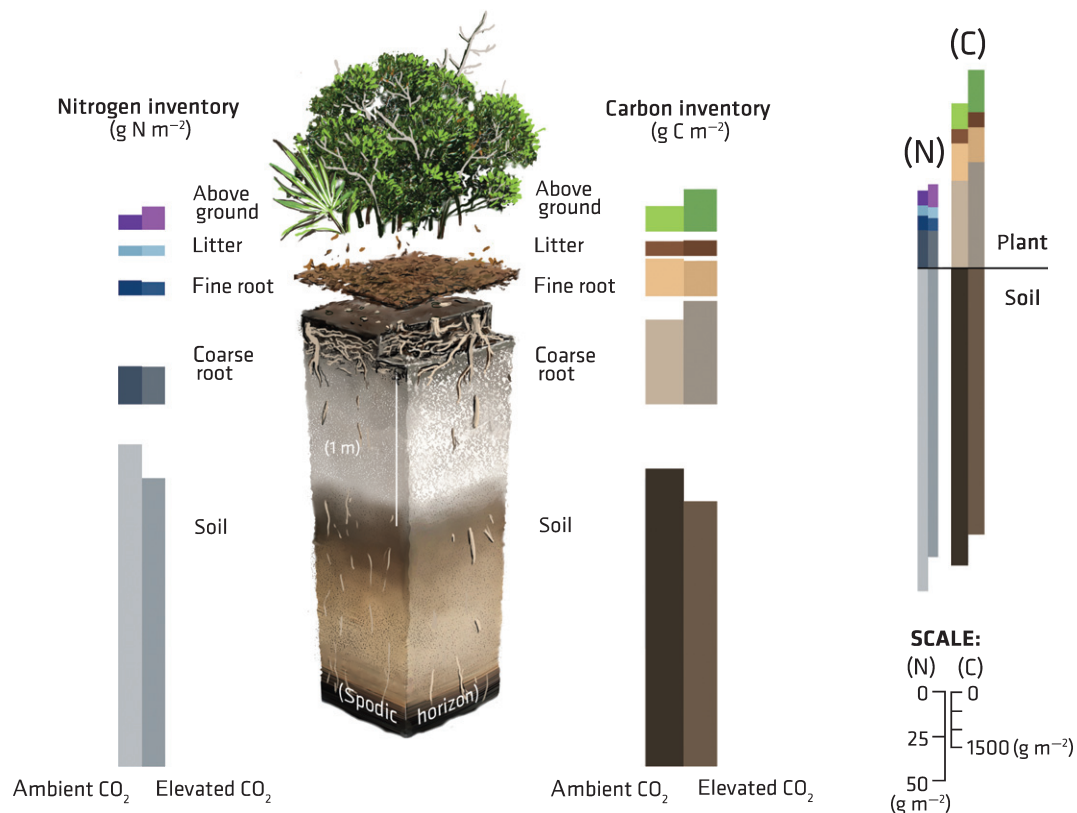


Fig. 6 Summary of ecosystem carbon (C) and nitrogen (N) inventories in a subtropical woodland after 11 yr of exposure to elevated CO₂.

likely promoted N losses, accounting for our finding that elevated CO₂ reduced recovery of added tracer ¹⁵N (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 CO₂ (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 CO₂ 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 CO₂ did not increase soil C, and in fact tended to decrease it, likely a consequence of increased microbial activity. Elevated CO₂ 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 ¹⁵N tracer, likely indicating enhanced ecosystem N losses. Thus, CO₂ 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 CO₂ 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

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References

- Adair EC, Reich PB, Hobbie SE, Knops JMH. 2009. Interactive effects of time, CO₂, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. *Ecosystems* 12: 1037–1052.
- Arnell 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 CO₂ 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 CO₂, 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 CO₂ concentrations: an invariant NPP:GPP ratio? *Global Change Biology* 6: 931–941.
- Cotrufo MF, Ineson P, Scott A. 1998. Elevated CO₂ 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 CO₂ 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 CO₂ 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, Luysaert 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 CO₂-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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂. *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 CO₂. *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 CO₂ 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, Croturo MF, Godbold DL *et al.* 2005. Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO₂ 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 CO₂. *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 CO₂: 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 CO₂. *Nature* 475: 214–216.
- Hagedorn F, van Hees PAW, Handa IT, Hättenschwiler S. 2008. Elevated atmospheric CO₂ 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 CO₂ 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 CO₂ enrichment experiments. *Plant and Soil* 187: 135–145.
- Hungate BA, Johnson DW, Dijkstra P, Hymus G, Stiling P, Megonigal JP, Pageal AL, Moan JL, Day F, Li JH *et al.* 2006. Nitrogen cycling during seven years of atmospheric CO₂ enrichment in a scrub oak woodland. *Ecology* 87: 26–40.
- Hungate BA, Lund CP, Pearson HL, Chapin FS. 1997c. Elevated CO₂ 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 CO₂ on net ecosystem CO₂ 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 CO₂-enrichment. *Global Change Biology* 18: 1684–1697.
- Iversen CM, Ledford J, Norby RJ. 2008. CO₂ 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 CO₂ 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 CO₂ and nitrogen fertilization. *Biogeochemistry* 77: 157–175.
- Johnson DW, Norby RJ, Hungate BA. 2001. Effects of elevated CO₂ 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 CO₂ 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 CO₂. *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 CO₂. *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 CO₂ 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 CO₂ exchange and interactions with elevated CO₂. *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 CO₂ 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 CO₂. *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 CO₂? *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 CO₂ 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 CO₂ 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 CO₂. *Oecologia* 140: 11–25.
- Nadelhoffer KJ, Emmett BA, Gunderson P, Kjonnas OJ, Koopmans CJ, Schleppel 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 CO₂ 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 CO₂, 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 CO₂ 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. CO₂ 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 CO₂ 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 CO₂ 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 CO₂. *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 CO₂. *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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ fertilization and climate variability. *Global Biogeochemical Cycles* 21: GB4018.
- Tingey DT, Phillips DL, Johnson MG. 2000. Elevated CO₂ and conifer roots: effects on growth, 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 CO₂ 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.
- Zabalay 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 CO₂ 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 pCO₂ 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 CO₂ treatment, habitat, and substrate

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