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LETTER

Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2

John E. Drake,[1,2] Anne Gallet-Budynek,[2,3] Kirsten S. Hofmockel,[4] Emily S. Bernhardt,[5] Sharon A. Billings,[6] Robert B. Jackson,[5,7] Kurt S. Johnsen,[8] John Lichter,[9] Heather R. McCarthy,[7,10] M. Luke McCormack,[11] David J. P. Moore,[12] Ram Oren,[7] Sari Palmroth,[7] Richard P. Phillips,[13] Jeffrey S. Pippen,[7] Seth G. Pritchard,[14] Kathleen K. Treseder,[15] William H. Schlesinger,[16] Evan H. DeLucia[1,17] and Adrien C. Finzi[2]*

Abstract

The earth's future climate state is highly dependent upon changes in terrestrial C storage in response to rising concentrations of atmospheric CO_2. Here we show that consistently enhanced rates of net primary production (NPP) are sustained by a C-cascade through the root-microbe-soil system; increases in the flux of C belowground under elevated CO_2 stimulated microbial activity, accelerated the rate of soil organic matter decomposition and stimulated tree uptake of N bound to this SOM. This process set into motion a positive feedback maintaining greater C gain under elevated CO_2 as a result of increases in canopy N content and higher photosynthetic N-use efficiency. The ecosystem-level consequence of the enhanced requirement for N and the exchange of plant C for N belowground is the dominance of C storage in tree biomass but the preclusion of a large C sink in the soil.

Keywords

Carbon sequestration, coupled biogeochemical cycles, coupled climate-carbon cycle models, elevated CO_2, forest productivity, nitrogen.

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INTRODUCTION

Predictions of the earth's future climate state are highly sensitive to the C-cycle response of ecosystems to rising concentrations of atmospheric CO_2 (Friedlingstein *et al.* 2006; Meehl *et al.* 2007). Ecosystem responses to experimental increases in atmospheric CO_2 concentrations vary widely, from ecosystems in which low soil-N availability precludes an enhancement of net primary productivity (NPP) in response to elevated CO_2 (Oren *et al.* 2001; Menge & Field 2007) to experiments that, in the absence of N fertilization, show only a transient response of NPP to elevated CO_2 (Reich *et al.* 2006; Seiler *et al.* 2009; Norby *et al.* 2010), to demonstrably N limited ecosystems where the enhancement in NPP is sustained through time (Langley *et al.* 2009; McCarthy *et al.* 2010). To understand why ecosystems respond differently to elevated CO_2, it is necessary to understand the mechanistic connection between plant physiological responses to elevated CO_2 and their attending effects on nutrient availability and uptake.

Among elevated CO_2 experiments, the Duke Forest free-air CO_2 enrichment (FACE) experiment is unique in that NPP in this N-limited system has remained consistently and significantly higher under elevated compared with ambient CO_2 for over a decade in the absence of nutrient amendment, even in the face of extreme climate events such as droughts and ice storms (McCarthy *et al.* 2010). This site is therefore at one end of the NPP-CO_2 response gradient raising the question of what processes sustain higher productivity under elevated CO_2 and how the responses here may apply to other ecosystems.

The results of an N-fertilization study in a prototype plot at the Duke FACE site provides some guidance as to where these processes might reside (Oren *et al.* 2001); the prototype plot is a single FACE plot established prior to the fully replicated experiment. With fertilization (i.e. a reduction in N limitation), the prototype plot and its nearby reference plot reduced the flux of C belowground by *c.* 25% (Palmroth *et al.* 2006). However, fine root production decreased by only *c.* 12% (Jackson *et al.* 2009) suggesting that the remaining 13% reduction in

[1]*Program of Ecology, Evolution, and Conservation Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61802, USA*
[2]*Department of Biology, Boston University, Boston, MA 02215, USA*
[3]*INRA, UMR1220 TCEM, F-33883 Villenave d'Ornon, France*
[4]*Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA*
[5]*Department of Biology, Duke University, Durham, NC 27708, USA*
[6]*Department of Ecology and Evolutionary Biology and Kansas Biological Survey, University of Kansas, Lawrence, KS 66047, USA*
[7]*Nicholas School of the Environment, Duke University, Durham, NC 27708, USA*
[8]*US Forest Service Southern Research Station, Durham, NC 27708, USA*
[9]*Biology and Environmental Studies, Bowdoin College, Brunswick, ME 04011, USA*
[10]*Department of Earth System Science, University of California, Irvine, CA 92697, USA*
[11]*Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA 16802, USA*
[12]*Department of Geography, King's College London, London WC2Rz 2LS, UK*
[13]*Department of Biology, Indiana University, Bloomington, IN 47403, USA*
[14]*Department of Biology, College of Charleston, Charleston, SC 29401, USA*
[15]*Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA*
[16]*Cary Institute for Ecosystem Studies, New York, NY 12545, USA*
[17]*Department of Plant Biology and Institute of Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*
**Correspondence: E-mail: afinzi@bu.edu*

belowground C flux was driven by declines in root exudation and C allocation to ectomycorrhizal fungi. By inference, this study suggests that C allocation to root exudation and mycorrhizal fungi and their effects on N availability in the prototype plot sustained the CO_2-induced increase in NPP in the absence of fertilization. The objective of this study is to test the idea that increases in belowground C flux under elevated CO_2 increase N uptake and sustain the long-term productivity response of a warm-temperate forest to elevated concentrations of atmospheric CO_2, using a fully replicated field experiment.

To address this idea, we constructed a belowground C budget based on 12-years of measurements from the fully replicated Duke FACE experiment, focusing on C fluxes and their relationship to ecosystem N pools, N availability and uptake by forest trees. The duration of experimental treatments and the comprehensive measurements of belowground processes provide an unparalleled opportunity to understand the coupled nature of the belowground cycles of C and N. We show that increases in the flux of C belowground with elevated CO_2 accelerated the rate of SOM decomposition and increased N uptake resulting in a long-term enhancement of a C sink in woody biomass but not in the mineral soil.

MATERIALS AND METHODS

Field site

The Duke free-air CO_2 enrichment (FACE) experiment was located in Orange County, North Carolina (35°97′ N, 79°09′ W). The forest was dominated by loblolly pine (*Pinus taeda*) trees that were planted as 3-year-old half-sib seedlings in a 2.4 × 2.4 m spacing in 1983. Measurements in a prototype FACE plot along with a paired, un-instrumented reference plot, began in 1994 (Oren *et al.* 2001). Measurements in six additional plots began in 1996, with three receiving elevated CO_2 (Hendrey *et al.* 1999). All plots were 30-m-diameter with elevated CO_2 plots maintaining an atmospheric CO_2 concentration 200 μL above ambient throughout the forest volume. The three fully instrumented control plots fumigated with ambient air only. This study presented results from the six plots that were operational in 1996. The fumigation CO_2 was derived from the combustion of natural gas with a depleted ^{13}C signature ($\delta^{13}C$ = −43.0 ± 0.6‰) resulting in plant tissues with a $\delta^{13}C$ value of −39‰; tissues produced under ambient CO_2 conditions maintained their C3 signature of −28‰ (Jackson *et al.* 2009).

A CO_2 × N-fertilization study was initiated in 2005 when ammonium nitrate was hand-broadcast to half of each plot at a rate of 11.2 gN m^{-2} $year^{-1}$. This amount was applied in two applications in the first year, half in March and half in April; fertilizer was applied once in March in subsequent years. The fertilizer was prevented from moving between half-plots by a 70-cm deep polyvinyl tarp.

Some of the data used to construct the carbon (Fig. 1a, Tables S1–S2) and N budgets (Fig. 1b, Table S3) were drawn from previous analyses, while other data came from previously unpublished and new observations. Here we briefly describe the methodologies for all of the pools and fluxes used in this analysis.

Pools

The standing pool of fine root biomass was measured every 3 months by taking three 4.76 cm diameter soil cores per plot (AMS; Forestry Suppliers, Jackson, MS, USA) to a depth of 15 cm. The soil was brought to the laboratory, where roots were picked by hand, dried, weighed, and analysed for C-content using an elemental analyser (ECS 4010; Costech Analytical, Valencia, CA, USA). Once a year, this process was also performed for the 15–30 cm depth (Jackson *et al.* 2009).

Pools of coarse roots were estimated from regional allometric relationships between diameter-at-breast-height (1.45 m) and coarse root biomass (McCarthy *et al.* 2010). The total root pool was calculated as the sum of fine and coarse root pools (Fig. 1a). Carbon stored in the litter layer on the forest floor and in SOM was measured from 12 soil cores 4.76 cm in diameter to a depth of 30 cm. Total C in the organic and mineral soil horizons was calculated using standard methods (Lichter *et al.* 2008).

The amount of C stored as gaseous CO_2 in soil air spaces was calculated from measurements of CO_2 concentration in soil gas wells (Andrews & Schlesinger 2001; Jackson *et al.* 2009). These measurements were converted to gC m^{-2} using the ideal gas law while accounting for the temperature sensitivity of air density using thermocouples buried next to each gas well.

Microbial biomass-N was measured using the chloroform-fumigation extraction method (Allen *et al.* 2000). Microbial biomass-N was converted into microbial biomass-C by multiplying by the measured C/N ratio of microbes at this site (C/N = 7.1).

Fluxes-soil C outputs

The rate of CO_2 diffusion out of the soil (i.e. soil CO_2 efflux or soil respiration) was measured with a closed IRGA system (PP-systems, Amesbury, MA, USA) monthly at 12 permanently installed PVC collars (10 cm diameter) per plot (Jackson *et al.* 2009). Measurements were made from 1100 to 1700 EST, which bracketed the time of maximum soil CO_2 efflux. Soil temperature was concurrently measured at a depth of 3 cm directly adjacent to each collar. These instantaneous measurements were scaled to annual fluxes by fitting Q_{10} temperature response curves and interpolating these curves with soil temperatures measured using thermistors in each plot measured every 30 min from 1997 to 2007. The temperature response curves were of the form: efflux = $b_{20} \times Q_{10}^{(T-20)/10}$. The Q_{10} values were taken from a previous study at this site (Bernhardt *et al.* 2006); b_{20}, the rate of efflux at 20 °C, was fit seasonally (spring, summer, fall and winter) for every plot in all years and for every subplot of all years after N-fertilization; T refers to the measured soil temperature in degrees C. A regression of predicted efflux vs. measured efflux had a slope of 1.01, an intercept of −0.01 and an r^2 of 0.59.

While soil CO_2 efflux is the dominant flux for soil C loss, two other fluxes of dissolved inorganic carbon (DIC) remove C from the soil. DIC leaching was measured with lysimeters at 2 m depth (Andrews & Schlesinger 2001). Soil DIC also is dissolved into soil water and transported up the stems of trees. This rate was calculated from measurements of soil CO_2 concentrations and sap flow (Schafer *et al.* 2002). The amount of DIC dissolved in soil water was predicted using Henry's law after correcting for temperature effects on the solubility of DIC (Butler 1982). This concentration of DIC was then multiplied by the rate of tree water uptake and summed annually.

Fluxes-soil C inputs

Fine root production was measured monthly using 12 minirhizotrons per plot to document changes in fine root length to a depth of 30 cm (Pritchard *et al.* 2008a). Root length increments were converted to

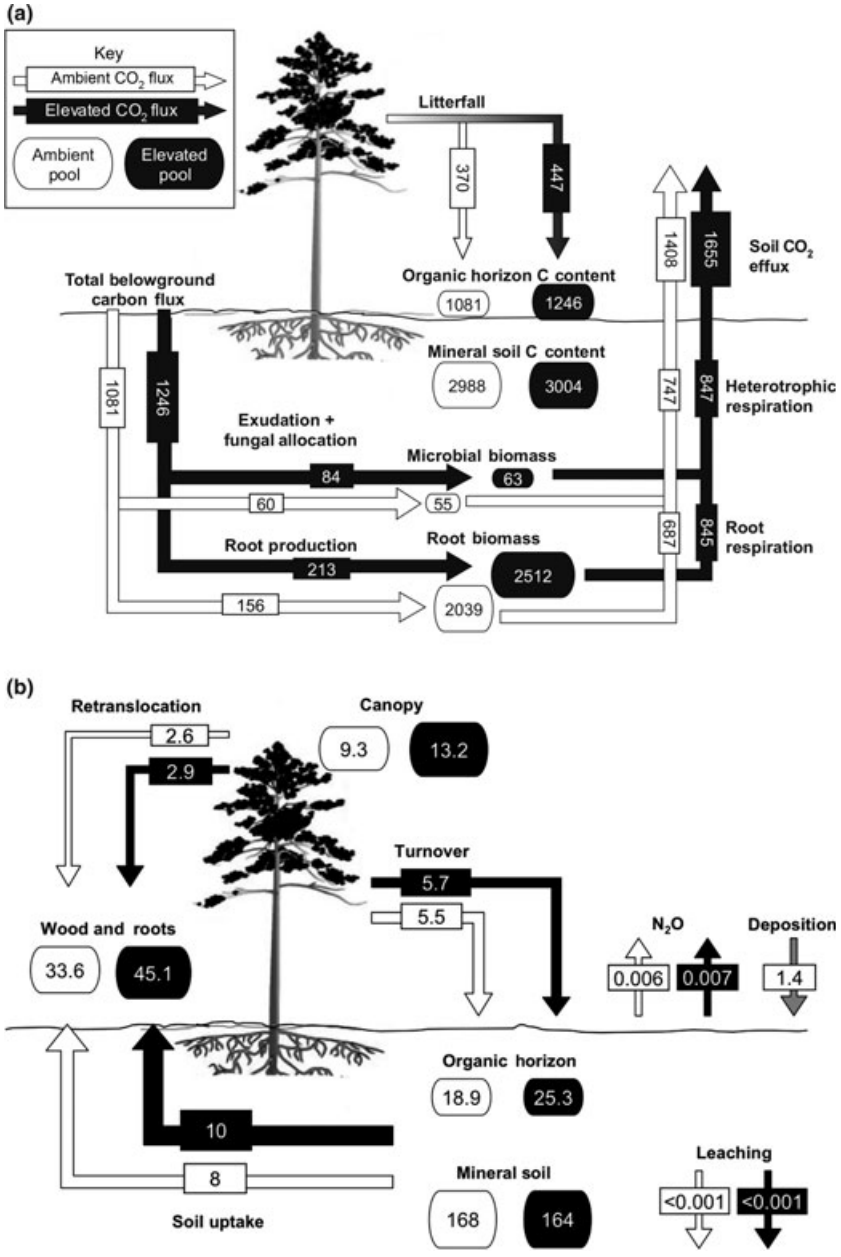

Figure 1 Belowground carbon (a) and ecosystem N (b) budgets for a warm-temperate forest exposed to elevated atmospheric CO_2 at the Duke free-air CO_2 enrichment (FACE) site. Data were compiled across multiple years and largely reflect 2003–2007. Ambient CO_2 is shown in white while elevated CO_2 is shown in black. Ovals reflect pools and have units of g (C or N) m^{-2}; squares within arrows reflect fluxes of C and have units of g (C or N) m^{-2} $year^{-1}$. All terms in (a) were significantly different between ambient and elevated CO_2 (ANOVA, randomized complete block design, repeated measures when available, $P < 0.05$) with the exception of mineral soil C content. All terms in (b) were significantly different with the exceptions of turnover, organic horizon N, mineral soil N, N_2O and leaching. Deposition was only measured under ambient CO_2 but is thought to reflect both treatments and is thus presented as a single grey arrow. These budgets were simplified for clarity from Tables S1–S3.

gC m^{-2} $year^{-1}$ using specific root length (gC cm^{-1}) measured on roots obtained from soil cores. Coarse root production was calculated as the annual change in coarse root C (described above). The rate of C exuded from fine roots was measured *in-situ* with a recently developed chamber based method (Phillips *et al.* 2008, 2010).

Production of ectomycorrhizal (*ECM*) fungi was estimated using microscopy methods to estimate the biovolume of fungal sheaths surrounding root tips (Garcia *et al.* 2008) and dividing this measure of the ECM pool size by the mean residence time (MRT) of ECM-C. This approach assumes that pools sizes are at steady state. The MRT of ECM-C was estimated to be 0.41 years from ^{14}C analysis of ECM tips isolated from the ambient CO_2 plots, which corresponds well with minirhizotron estimates (Pritchard *et al.* 2008b). We assumed a fresh tissue density of 1.1 g cm^{-3}, a solids content of 40%, and a carbon content of 40% (Paul & Clark 1996) for all mycorrhizal tissues. Production by other fungal types such as arbuscular mycorrhizal (AMF) and non-mycorrhizal (NM) were also estimated from microscopic methods, and the production of glomalin was estimated as easily extractable immunoreactive soil protein (EE-IRSP). The MRT of glomalin was estimated to be 17.6 years

based on ^{14}C analysis of glomalin from the ambient CO_2 plots. Intraradical AMF fungi were assumed to have the same MRT as pine fine roots (Matamala *et al.* 2003), while the MRT of AMF and NM extraradical hyphae was assumed to be 7 days based on a pot study (Staddon *et al.* 2003). These fluxes and the exudation of C by fine roots were summed to obtain the 'Exudation + Fungal Allocation' value (Fig. 1a).

Ecosystem fine root respiration (R_{fr}) was estimated from *in situ* measurements of the tissue-specific rate of R_{fr} at 20 °C and the temperature dependence of R_{fr} (Drake *et al.* 2008). These measurements were scaled to an annual flux using daily measurements of average soil temperature at 15 cm depth and fine root biomass (Jackson *et al.* 2009). To estimate coarse root respiration (R_{cr}), we assumed that the tissue-specific rate of respiration was the same for aboveground wood and coarse roots (Hamilton *et al.* 2002). We scaled this rate to an annual flux assuming a Q_{10} of 2.0 using soil temperature and coarse root biomass.

Litterfall was collected monthly from January through September and biweekly from October through December from twelve litter baskets (0.218 m^2 each) per plot (Finzi *et al.* 2001). Litter was sorted into components, dried, weighed and a subsample was measured for C-content using an elemental analyser (ECS 4010; Costech Analytical). Woody debris was collected from two additional 0.49 m^2 collectors per plot.

Fluxes-internal C cycling

Fine root mortality was estimated using the same minirhizotron approach as was used to estimate fine root production. Litter decomposition was estimated using an MRT approach; litter mass was divided by MRTs measured at this site (Lichter *et al.* 2008) to estimate the loss of litter to decomposition. The turnover of C in SOM was estimated from the rate at which the isotopic composition of the fumigation gas was incorporated into the bulk SOM pool in the elevated CO_2 plots (Lichter *et al.* 2008). There was no isotopic tracer in the ambient CO_2 plots, so SOM exchange was only estimated for the elevated CO_2 treatment.

The rate of heterotrophic respiration (R_h) was estimated by mass balance; the sum of fine (R_{fr}) and coarse root respiration (R_{cr}) was subtracted from the sum of all ecosystem C outputs-soil CO_2 efflux (F_{efflux}), DIC leaching ($F_{leaching}$) and DIC transpiration ($F_{transpiration}$)-to calculate the amount of CO_2 produced in the soil that was not accounted for by autotrophic respiration.

$$R_h = (F_{efflux} + F_{leaching} + F_{transpiration}) - (R_{fr} + R_{cr}) \quad (1)$$

Nitrogen measurements

Biomass N pools were measured annually beginning in 1996 using standard methods (Finzi *et al.* 2002, 2007). The annual rate of soil N uptake and N retranslocation prior to foliage senescence were calculated from measurements of biomass increments and turnover and the concentration of N in each component (Finzi *et al.* 2002, 2007). N stored in the organic and mineral soil horizons was measured as described above for the measurements of soil C. The concentration of inorganic N in the top 15 cm of mineral soil was measured two to four times per growing season as the beginning of the Duke FACE experiment. The rate of N-deposition, leaching and gaseous N_2O loss were reported previously (Finzi *et al.* 2002; Sparks *et al.* 2008).

NPP and Canopy N

Measurements of NPP and standing pools of biomass C were published previously (McCarthy *et al.* 2010). Nitrogen concentrations of live pine needles and deciduous leaves were measured in September of each year at peak canopy N content (Finzi *et al.* 2004) by the Kjeldahl digestion method or with an elemental analyser (Model NC2500; CE Instruments, Rodano, Italy). To account for the effects of canopy position and leaf age, we collected needles of both age classes in the bottom 25%, middle 50% and top 25% of the crown; we sampled a total of 12 pine trees per plot, removing 8–10 needles from each tree, needle age class and height. We also collected mature foliage samples of the most abundant hardwood tree species in each plot.

Estimating TBCF and C budget closure

Total belowground carbon flux (TBCF) was calculated for all plots in all years from 1997–2007. TBCF was calculated by mass balance according to Litton *et al.* (2007):

$$TBCF = F_{efflux} + F_{leaching} + F_{transpiration} - F_{litter} + \Delta(C_{SOM} + C_{roots}) \quad (2)$$

where F_{efflux} is soil CO_2 efflux, $F_{leaching}$ is DIC leeched from the soil, $F_{transpiration}$ is DIC transpired by the trees, F_{litter} is litterfall and Δ (C_{SOM} + C_{roots}) reflects the change in C stored in SOM and roots on an annual time step. It is this estimate of TBCF on which we based our analyses in the article. A treatment average was used for SOM because of its inherent variability, but plot averages were used for all other terms.

Total belowground carbon flux was a critical component of the data analysis and interpretation in this study. To assess whether our use of this estimate on a yearly basis was justifiable, we compared our average annual estimate of TBCF in [1] with a bottom-up estimate of TBCF (herein called '$TBCF_{sum}$' Table 1) based on the sum of all C inputs to the soil excluding litterfall (Table S2). $TBCF_{sum}$ was 15% and 7% lower than TBCF under ambient and elevated CO_2, respectively, but the two estimates were not statistically different from one another under either CO_2 treatment (ANOVA, $P > 0.5$). Given spatial and temporal variations in belowground C allocation, the concordance between the two estimates of belowground C flux (TBCF vs. $TBCF_{sum}$) supported our use of yearly estimates of TBCF based on eqn 1.

We estimated lack of closure for the soil C budget as the difference between total C outputs from and inputs to the soil pool (Tables 1, S2). Outputs exceeded inputs by 10% and 6% under ambient and elevated CO_2, respectively, suggesting that soils were a net source of CO_2 to the atmosphere. However, repeated measurements of soil C pools indicate that soils are not a net source of C to the atmosphere; the surface organic horizon is a small sink for C and the mineral soil horizon to a depth of 30 cm is C neutral (Lichter *et al.* 2008). The lack of closure was therefore most likely the result of an underestimate of C inputs to the soil.

Statistical analysis

The original publications from which some of the data were obtained used a range of statistical designs, often with covariates. We re-analysed all of the data presented here using a common statistical approach that treated each plot as a replicate (n = 3 for ambient and

Table 1 Evaluation of the carbon budget at the Duke free-air CO_2 enrichment (FACE) site

Analysis	Flux	Ambient CO_2	Elevated CO_2	*P*-value
Estimates of TBCF	*TBCF	1081 (68)	1246 (70)	< 0.05
	†$TBCF_{sum}$	922 (72)	1156 (84)	< 0.05
	Underestimate of inputs ($TBCF - TBCF_{sum}$)	159 (99)	90 (109)	> 0.1
Soil C balance	‡Sum of all C outputs	1434 (49)	1692 (129)	< 0.05
	§Sum of all C inputs	1292 (83)	1603 (92)	< 0.05
	¶Lack of closure (outputs–inputs)	142 (96)	89 (158)	> 0.1

All units are g C m^{-2} $year^{-1}$. Values are mean (± 1 SE). *P*-values reflect the main effect of CO_2 treatment in a repeated-measures RCBD. The first analysis checks the sum of belowground soil C inputs and the second analysis checks the net soil C balance. Total belowground carbon flux (TBCF) should theoretically equal the sum of all inputs of C into soils except litterfall ($TBCF_{sum}$; Table S2).

*$TBCF = F_{efflux} + F_{leaching} + F_{transpiration} - F_{litter} + \Delta (C_{soil} + C_{roots})$; where F_{efflux} is soil CO_2 efflux, $F_{leaching}$ is DIC leached from the soil, $F_{transpiration}$ is DIC transpired by the trees, F_{litter} is litterfall, and $\Delta (C_{soil} + C_{roots})$ reflects the change in C stored in soils and roots on an annual time step.

†$TBCF_{sum}$ = fine and coarse root production + fine and coarse root respiration + exudation + fungal production + throughfall leaching.

‡Outputs = Soil CO_2 efflux + dissolved inorganic carbon (DIC) leaching + transpiration of DIC.

§Inputs = All the inputs listed in (b) + litterfall.

¶The underestimate of inputs and lack of soil-C budget closure are based on the same underlying budget terms and are thus similar in magnitude.

elevated CO_2) in a randomized complete block design (RCBD), with blocks defined by initial measurements of annual rates of net N mineralization (Finzi & Schlesinger 2002; Finzi *et al.* 2002). Blocks were treated as random variables. Analyses on data gathered after the N-fertilizer treatment began in 2005 were performed using a split-plot RCBD design. When temporal data were available, a repeated-measure RCBD was used with the covariance between years modelled in the first order auto-regressive framework. We defined significant differences at the $P < 0.1$ level. Given the small number of plots, we treated annually repeated measurements as replicates for the correlations involving soil N (Fig. 3a,c); initial statistical analysis of these datasets demonstrated low temporal covariation enabling us to use years as independent observations in our analyses. The one exception was the substantial temporal covariation in NPP and canopy N content, so we used the long-term average value of NPP and canopy N in each plot prior to regression analysis (Fig. 4). All analyses were performed using the MIXED and REG procedures of the SAS system (SAS 9.1; SAS Institute, Cary, NC, USA). The assumptions of homoskedasticity and normality of residuals were checked for all analyses using the UNIVARIATE and REG procedures.

RESULTS

Elevated CO_2 increased the rate of C-cycling through the soil. The total quantity of C entering the soil via litterfall and all belowground C inputs increased 17% from *c.* 1500 gC m^{-2} $year^{-1}$ under ambient CO_2 to *c.* 1750 gC m^{-2} $year^{-1}$ under elevated CO_2 (Fig. 1a; ANOVA, $P < 0.05$). TBCF increased 16% under elevated CO_2 (repeated-measures ANOVA, $P < 0.01$). The increase in C entering the soil under elevated CO_2 was matched by increased C loss attributable to significant increases in fine and coarse root respiratory fluxes (i.e. autotrophic respiration; ANOVA, $P < 0.05$), and a significant increase in heterotrophic respiration (Fig. 1a; repeated-measures ANOVA, $P < 0.05$). There also were smaller but significant increases in belowground C allocation to mycorrhizal fungi (Table S2, ANOVA, $P = 0.06$) and the exudation of C from roots into the soil (Fig. 1a, Table S2; ANOVA, $P < 0.05$).

The additional N required to support higher rates of NPP under elevated CO_2 was largely supplied by increased soil N uptake, not by increases in the retranslocation of nutrients prior to tissue senescence (Figs 1b and 2a) nor by increases in atmospheric-N deposition (Lichter *et al.* 2000; Sparks *et al.* 2008) or N_2 fixation (Hofmockel & Schlesinger 2007). The retranslocation of N prior to tissue senescence was small relative to soil N uptake and increased by only 11% (repeated-measures ANOVA, $P < 0.01$). N uptake from the soil increased by an average of 25%, with annual average uptake rates of 8 and 10 gN m^{-2} $year^{-1}$ under ambient and elevated CO_2, respectively (Fig. 2a; repeated-measures ANOVA, $P < 0.01$). From 1997 through 2005, an additional 18 gN m^{-2} were taken up from the soil under elevated CO_2 (Fig. 2b; slopes differ significantly, ANCOVA, $P < 0.001$), which largely accumulated in the standing pools of N in pine foliage and wood (Fig. 1b, Table S3), although a marginally significant increase was observed in the wood of understory hardwood trees (Table S3).

Total belowground carbon flux increased under elevated CO_2 relative to ambient CO_2 and was inversely and asymptotically correlated with the availability of soil N in both treatments, with substantial increases in TBCF as N availability declined (Fig. 3a). Consistent with the role of N supply affecting TBCF, experimental additions of N reduced surface soil CO_2 efflux under ambient and elevated CO_2 (Fig. 3b; Butnor *et al.* 2003), a reduction driven by declines in root production, respiration and exudation (Drake *et al.* 2008; Jackson *et al.* 2009; Phillips *et al.* 2010). The inverse relationship between TBCF and N availability was not exclusively a consequence of N fertilization; a similar relationship holds when data points from the fertilized halves of each FACE plot were excluded (Fig. S1). N uptake per unit fine root production was significantly higher under elevated compared with ambient CO_2 (Fig. 3c; ANCOVA after log-linearizaton, intercept increased under elevated CO_2, $P < 0.05$).

Net primary production was positively correlated with canopy N content (Fig. 4). The increase in NPP under elevated CO_2 was the result of greater canopy N content. There is an increase in photosynthetic N-use efficiency at this site (i.e. an increase in C-uptake per unit foliar N under elevated CO_2, Crous *et al.* 2008), although this effect was not evident in this data set (i.e. a significantly higher y-intercept under elevated CO_2) presumably because the sample size for this test was small (i.e. $n = 3$ for each treatment).

DISCUSSION

The long-term increase in forest productivity under elevated CO_2 at the Duke FACE site appears to be maintained by a belowground exchange of tree C for soil N, with the quantity of C allocated belowground set by the availability of N in the soil and the demand for N to meet growth requirements. Compared with ambient CO_2, the increase in TBCF under elevated CO_2 accelerated the rate of SOM decomposition and increased the rate of N uptake by trees. This process set into motion a positive feedback maintaining greater C gain under elevated CO_2. Greater N uptake from the soil resulted in greater canopy N content, which in combination with higher photosynthetic N-use efficiency (Crous *et al.* 2008), stimulated higher rates of NPP

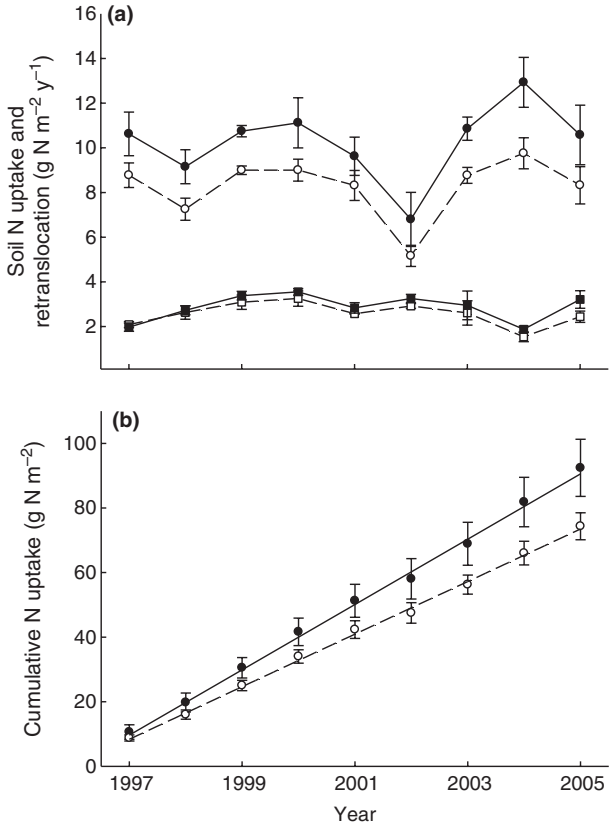

Figure 2 Ecosystem nitrogen fluxes at the Duke free-air CO_2 enrichment (FACE) site. Open symbols refer to ambient CO_2 while filled symbols refer to elevated CO_2. Circles in (a) refer to soil N uptake and squares refer to N retranslocation before leaf abscission. Cumulative soil N uptake (b) was the running sum of soil N uptake values in (a). Values are the mean of three replicates; error bars reflect ± 1 SE.

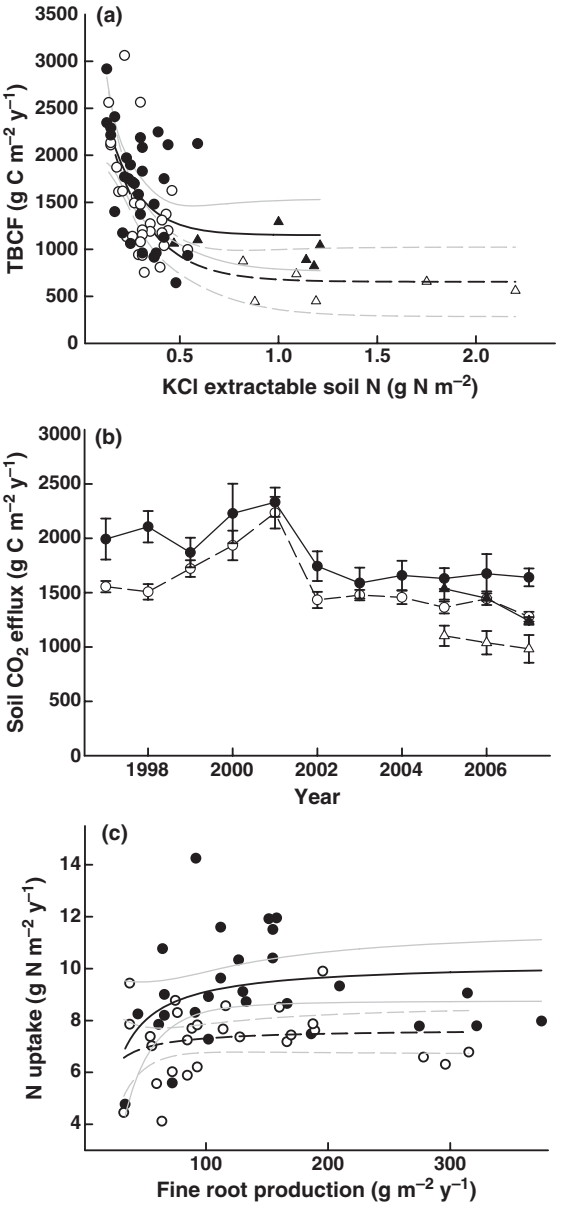

Figure 3 Results of long-term free-air CO_2 enrichment (FACE) of a loblolly pine (*Pinus taeda*) forest. Open symbols and dashed lines refer to ambient CO_2; filled symbols and solid lines refer to elevated CO_2; triangles refer to N-fertilized subplots. Grey lines refer to 95% confidence intervals. (a) Total belowground carbon flux (TBCF) in relation to KCl-extractable mineral N in the mineral soil A horizon (0–15 cm). Lines are of the form $y = c + a \times \exp(-bx)$; a = 2012 and 1408, b = 3.61 and 3.56, c = 740 and 1163 for ambient and elevated CO_2, respectively, and the total r^2 for the fitting procedure was 0.44. (b) Annual soil CO_2 efflux from 1997 to 2007; error bars reflect ± 1 SE. (c) Soil N uptake into foliage and wood as a function of fine root production. Lines are of the form $y = a/x + c$; a = −78.4 and −144.6, c = 7.6 and 9.8 for ambient and elevated CO_2, respectively, and the total r^2 for the fitting procedure was 0.29.

under elevated compared with ambient CO_2. The consequence of the belowground trade of C for N is a sustained sink for C in biomass, but the preclusion of a large soil C sink.

The variation in TBCF observed here is consistent with a previous cross-site synthesis of forest FACE experiments showing that TBCF varies inversely with canopy leaf area (Palmroth *et al.* 2006). Similarly, canopy leaf area was positively correlated with spatial variation in soil N availability at the Duke FACE site (McCarthy *et al.* 2006). Together with the data presented here, these results indicate that the long-term stimulation in NPP under elevated CO_2 in this N limited ecosystem (Finzi *et al.* 2002; McCarthy *et al.* 2010) is enabled by increases in TBCF that stimulate N uptake and enable increases in canopy leaf area and N mass.

Despite 12 years of increased C inputs to the soil under elevated CO_2 – an additional 1000 gC m^{-2} or 33% of the soil C pool to a depth of 30 cm – there was no net accumulation of C in the mineral soil pool during the experiment. There was, however, an additional accumulation of C as litter in the organic horizon of *c.* 30 gC m^{-2} year^{-1} (Fig. 1a, Table S1; Lichter *et al.* 2008). This C sink was small (14%) relative to the increase in biomass accumulation under elevated CO_2 of 213 gC m^{-2} year^{-1} (McCarthy *et al.* 2010). Because of the depleted ^{13}C composition of the fumigation gas, there is a tracer for C fluxes in the elevated CO_2 plots. This ^{13}C-depleted label was incorporated into all soil C pools under elevated CO_2 (Lichter *et al.* 2008) demonstrating that C fixed since the experiment began in 1996 replaced some of the C initially present in the soil (Table S2). Increases in microbial activity and the decomposition of pre-CO_2-treatment C must account for the large change in the isotopic composition of the soil C pool; if the C released as CO_2 by microbes was solely from organic materials fixed since the experiment began, the isotopic composition of the SOM pools could not have changed given that there was no accumulation of soil C (Fig. 1a,

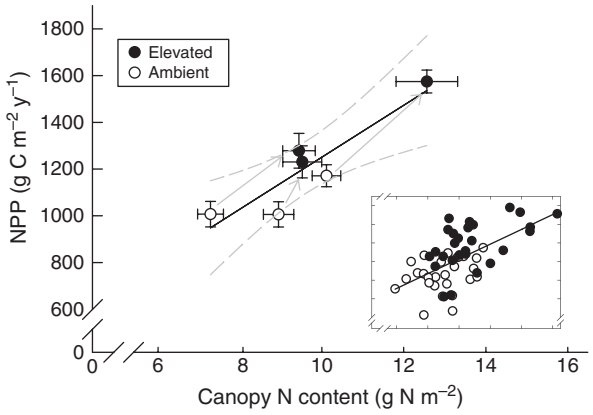

Figure 4 Net primary production (NPP) at the Duke free-air CO_2 enrichment (FACE) site in relation to total canopy nitrogen. Open symbols refer to plots at ambient CO_2, while filled symbols refer to plots fumigated with elevated CO_2. Each symbol refers to the average value for a single plot from 1997 to 2005; error bars reflect ± 1 SE of the nine years of measurements. The solid-grey arrows connect each ambient CO_2 plot with its elevated CO_2 block-pair, and thus show the directional response of the forest to [CO_2]-enrichment. The solid black line reflects the best regression fit: $y = 111x + 140.5$, $r^2 = 0.83$. The dashed lines are the 95% confidence intervals for the regression. The inset shows the raw data used to derive the plot-level means in the main panel – each point represents a plot-year pair. The scaling of the inset axes are the same as the main plot. The solid line in the inset reflects the best regression fit: $y = 79.35x + 434.2$, $r^2 = 0.35$.

Table S2). The replacement of pre-CO_2-treatment soil C by C fixed post-CO_2 treatment without a concomitant increase in pool size strongly suggests accelerated rates of SOM turnover under elevated CO_2. We recognize that forest responses to a gradual change in atmospheric CO_2 may differ from an experimental manipulation of CO_2. However, the duration of the experimental treatments suggests that this conclusion is likely to reflect the response of soil C pools to a long-term rise in atmospheric CO_2 concentration.

Mass balance calculations suggest that greater N uptake under elevated CO_2 was the result of faster SOM decomposition in surface soils (to 30 cm depth). The C budget indicates that R_h was, on average, 100 gC m^{-2} year^{-1} higher under elevated than ambient CO_2 (Fig. 1a, Table S2). Based on the turnover time of the surface organic horizon under elevated CO_2 (Lichter *et al.* 2008), *c.* 40 of the additional 100 gC m^{-2} year^{-1} (as CO_2) are likely to have originated from this horizon under elevated CO_2. The organic horizon has a C : N ratio of 45 (Finzi *et al.* 2001; Lichter *et al.* 2008), so the decomposition of 40 gC m^{-2} year^{-1} released on average an additional 0.9 gN m^{-2} year^{-1} to the available pool. The remaining C lost through R_h, 60 gC m^{-2} year^{-1}, must therefore have originated from mineral soils, which have an average C : N ratio of 20 (Lichter *et al.* 2008), which likely released an additional 3 gN m^{-2} year^{-1}. The proportional mineralization of C and N from the organic and mineral soil horizons assumed in our calculations is supported by a litter decomposition study that found proportional rates of C and N mineralization from pine litter over the first year of decomposition (Finzi & Schlesinger 2002). Hence the release of N from the decomposition of surface soil SOM under elevated CO_2 is more than sufficient to account for the difference in soil-N uptake between CO_2 treatments (Figs 1b and 2a).

In addition to these mass-balance calculations, other studies at the Duke FACE site also support increased N uptake from surface soils. Hofmockel, K.H., Budynek, A., Currie, W.S., Jackson, R.B. and Finzi, A.C. (unpublished data) presented the results of a whole-plot ^{15}N tracer experiment in which they found greater rates of N uptake from surface soils under elevated CO_2. Additionally, a host of studies show increases in microbial activity that are consistent with inputs of labile C stimulating SOM decomposition in the mineral soil horizon. For example, all of the belowground C fluxes thought to increase decomposition rates (Kuzyakov *et al.* 2000) increased under elevated CO_2, including root production and mortality (Pritchard *et al.* 2008a), root exudation (Phillips *et al.* 2010), fungal rhizomorph production (Pritchard *et al.* 2008b) and allocation of C to mycorrhizal fungi (Table S2, Garcia *et al.* 2008). These C inputs stimulated microbial respiration and the activity of extracellular enzymes that decompose SOM (e.g. glucosidase, N-acetylglucosaminidase, phenol oxidase; Finzi *et al.* 2006; Phillips *et al.* 2010; Fig. 1a). All of these processes were enhanced in rhizosphere soils where gross rates of NH_4^+ mineralization were significantly enhanced under elevated compared with ambient CO_2 (Phillips *et al.* 2010). Thus the preponderance of the evidence points to increased decomposition in surface soils as the primary source of additional N taken up by the trees growing under elevated CO_2.

There is a continuum of NPP responses to experimental increases in atmospheric CO_2 in N-limited ecosystems with no legacy of agricultural production (Oren *et al.* 2001; Reich *et al.* 2006; Menge & Field 2007; Langley *et al.* 2009; Seiler *et al.* 2009; McCarthy *et al.* 2010). The consistent, decadal-scale enhancement in NPP under elevated CO_2 at the Duke FACE site (McCarthy *et al.* 2010) anchors one end of this continuum, as does the Florida scrub-oak elevated-CO_2 experiment (Langley *et al.* 2009; Seiler *et al.* 2009). Interestingly, ectomycorrhizal fungi (EMF) colonize the roots of the plant communities at the Duke and Florida sites, whereas the down regulation of productivity under elevated CO_2 in sites without an agricultural history has been observed in plant communities where roots are colonized by arbuscular mycorrhizal fungi (AMF, Reich *et al.* 2006; Menge & Field 2007; Norby *et al.* 2010). Although the data are few, we speculate that the difference in mycorrhizal association may, in part, explain the differences in productivity among experiments. EMF have broad enzymatic capability (Chalot & Brun 1998), decompose labile and recalcitrant components of soil organic matter, access organic sources of N and transfer large amounts of N to host plants (Hobbie & Hobbie 2006). AMF also acquire N from SOM (Hodge *et al.* 2001), although they do not have as broad an N-based enzymatic capability and appear to transfer only a small fraction of the host plant's demand for N (Hodge & Fitter 2010). Consequently, belowground-C allocation in AMF ecosystems may not return sufficient N, and therefore generate enough C-fixation through the effect of additional N on photosynthesis, to offset the cost of the belowground C investment, thereby preventing long-term CO_2 fertilization of NPP in N limited, AMF dominated ecosystems. This is a critical area for future research.

Global-scale models used for climate projections typically include water and C limitations to the CO_2-fertilization response of the terrestrial biosphere (Friedlingstein *et al.* 2006; Meehl *et al.* 2007). These models are challenged by experiments demonstrating a diversity of nutrient-dependent ecosystem responses to elevated CO_2. This study points to the pivotal role of belowground C allocation in ecosystem response to elevated CO_2 and suggests that fungal community composition may mediate positive- vs. negative feedback effects of elevated CO_2 on NPP. Ultimately, the key to correctly incorporating these feedbacks in coupled climate-carbon cycle models

is to identify a simple framework for describing the most important belowground processes that affect N availability and ultimately C uptake and storage in the terrestrial biosphere; this analysis makes a first such attempt. Substantial, additional research is required to fully address the range of ecosystem responses to long-term CO_2 enrichment.

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REFERENCES

Allen, A.S., Andrews, J.A., Finzi, A.C., Matamala, R., Richter, D.D. & Schlesinger, W.H. (2000). Effects of free-air CO_2 enrichment (FACE) on belowground processes in a *Pinus taeda* forest. *Ecol. Appl.*, 10, 437–448.

Andrews, J.A. & Schlesinger, W.H. (2001). Soil CO_2 dynamics, acidification, and chemical weathering in a temperate forest with experimental CO_2 enrichment. *Global Biogeochem. Cycles*, 15, 149–162.

Bernhardt, E.S., Barber, J.J., Pippen, J.S., Taneva, L., Andrews, J.A. & Schlesinger, W.H. (2006). Long-term effects of free air CO_2 enrichment (FACE) on soil respiration. *Biogeochemistry*, 77, 91–116.

Butler, J.N. (1982). *Carbon Dioxide Equilibria and Their Applications*. Addison-Wesley, Reading, MA.

Butnor, J.R., Johnsen, K.H., Oren, R. & Katul, G.G. (2003). Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands. *Glob. Chang. Biol.*, 9, 849–861.

Chalot, M. & Brun, A. (1998). Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *FEMS Microbiol. Rev.*, 22, 21–44.

Crous, K.Y., Walters, M.B. & Ellsworth, D.S. (2008). Elevated CO_2 concentration affects leaf photosynthesis-nitrogen relationships in *Pinus taeda* over nine years in FACE. *Tree Physiol.*, 28, 607–614.

Drake, J.E., Stoy, P.C., Jackson, R.B. & DeLucia, E.H. (2008). Fine-root respiration in a loblolly pine (*Pinus taeda* L.) forest exposed to elevated CO_2 and N fertilization. *Plant Cell Environ.*, 31, 1663–1672.

Finzi, A.C. & Schlesinger, W.H. (2002). Species control variation in litter decomposition in a pine forest exposed to elevated CO_2. *Glob. Chang. Biol.*, 8, 1217–1229.

Finzi, A.C., Allen, A.S., DeLucia, E.H., Ellsworth, D.S. & Schlesinger, W.H. (2001). Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. *Ecology*, 82, 470–484.

Finzi, A.C., DeLucia, E.H., Hamilton, J.G., Richter, D.D. & Schlesinger, W.H. (2002). The nitrogen budget of a pine forest under free air CO_2 enrichment. *Oecologia*, 132, 567–578.

Finzi, A.C., Delucia, E.H. & Schlesinger, W.H. (2004). Canopy N and P dynamics of a southeastern US pine forest under elevated CO_2. *Biogeochemistry*, 69, 363–378.

Finzi, A.C., Sinsabaugh, R.L., Long, T.M. & Osgood, M.P. (2006). Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. *Ecosystems*, 9, 215–226.

Finzi, A.C., Norby, R.J., Calfapietra, C., Gallet-Budynek, A., Gielen, B., Holmes, W.E. *et al.* (2007). Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2. *Proc. Natl. Acad. Sci. USA*, 104, 14014–14019.

Friedlingstein, P., Cox, P., Betts, R., Bopp, L., Von Bloh, W., Brovkin, V. *et al.* (2006). Climate-carbon cycle feedback analysis: results from the (CMIP)-M-4 model intercomparison. *J. Clim.*, 19, 3337–3353.

Garcia, M.O., Ovasapyan, T., Greas, M. & Treseder, K.K. (2008). Mycorrhizal dynamics under elevated CO_2 and nitrogen fertilization in a warm temperate forest. *Plant Soil*, 303, 301–310.

Hamilton, J.G., DeLucia, E.H., George, K., Naidu, S.L., Finzi, A.C. & Schlesinger, W.H. (2002). Forest carbon balance under elevated CO_2. *Oecologia*, 131, 250–260.

Hendrey, G.R., Ellsworth, D.S., Lewin, K.F. & Nagy, J. (1999). A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Glob. Chang. Biol.*, 5, 293–309.

Hobbie, J.E. & Hobbie, E.A. (2006). N-15 in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Ecology*, 87, 816–822.

Hodge, A. & Fitter, A.H. (2010). Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proc. Natl. Acad. Sci. USA*, 107, 13754–13759.

Hodge, A., Campbell, C.D. & Fitter, A.H. (2001). An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, 413, 297–299.

Hofmockel, K.S. & Schlesinger, W.H. (2007). Carbon dioxide effects on heterotrophic dinitrogen fixation in a temperate pine forest. *Soil Sci. Soc. Am. J.*, 71, 140–144.

Jackson, R.B., Cook, C.W., Pippen, J.S. & Palmer, S.M. (2009). Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, 90, 3352–3366.

Kuzyakov, Y., Friedel, J.K. & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.*, 32, 1485–1498.

Langley, J.A., McKinley, D.C., Wolf, A.A., Hungate, B.A., Drake, B.G. & Megonigal, J.P. (2009). Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. *Soil Biol. Biochem.*, 41, 54–60.

Lichter, J., Lavine, M., Mace, K.A., Richter, D.D. & Schlesinger, W.H. (2000). Throughfall chemistry in a loblolly pine plantation under elevated atmospheric CO_2. *Biogeochemistry*, 50, 73–93.

Lichter, J., Billings, S.A., Ziegler, S.E., Gaindh, D., Ryals, R., Finzi, A.C. *et al.* (2008). Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. *Glob. Chang. Biol.*, 14, 2910–2922.

Litton, C.M., Raich, J.W. & Ryan, M.G. (2007). Carbon allocation in forest ecosystems. *Glob. Chang. Biol.*, 13, 2089–2109.

Matamala, R., Gonzalez-Meler, M.A., Jastrow, J.D., Norby, R.J. & Schlesinger, W.H. (2003). Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science*, 302, 1385–1387.

McCarthy, H.R., Oren, R., Finzi, A.C. & Johnsen, K.H. (2006). Canopy leaf area constrains $[CO_2]$-induced enhancement of productivity and partitioning among aboveground carbon pools. *Proc. Natl. Acad. Sci. USA*, 103, 19356–19361.

McCarthy, H.R., Oren, R., Johnsen, K.H., Gallet-Budynek, A., Pritchard, S.G., Cook, C.W. *et al.* (2010). Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric $[CO_2]$ with nitrogen and water availability over stand development. *New Phytol.*, 185, 514–528.

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M. *et al.* (2007). Global climate projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. & Miller, H.L.). Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 747–846.

Menge, D.N.L. & Field, C.B. (2007). Simulated global changes alter phosphorus demand in annual grassland. *Glob. Chang. Biol.*, 13, 2582–2591.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E. & McMurtrie, R.E. (2010). CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proc. Natl. Acad. Sci. USA*, 107, 19368–19373.

Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C. *et al.* (2001). Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. *Nature*, 411, 469–472.

Palmroth, S., Oren, R., McCarthy, H.R., Johnsen, K.H., Finzi, A.C., Butnor, J.R. *et al.* (2006). Aboveground sink strength in forests controls the allocation of carbon below ground and its $[CO_2]$ – induced enhancement. *Proc. Natl. Acad. Sci. USA*, 103, 19362–19367.

Paul, E.A. & Clark, F.E. (1996). *Soil Microbiology and Biochemistry*. Academic Press, San Diege.

Phillips, R.P., Erlitz, Y., Bier, R. & Bernhardt, E.S. (2008). New approach for capturing soluble root exudates in forest soils. *Funct. Ecol.*, 22, 990–999.

Phillips, R.P., Finzi, A.C. & Bernhardt, E.S. (2010). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecol. Lett.*, 14, 187–194.

Pritchard, S.G., Strand, A.E., McCormack, M.L., Davis, M.A., Finz, A.C., Jackson, R.B. *et al.* (2008a). Fine root dynamics in a loblolly pine forest are influenced by free-air-CO_2-enrichment: a six-year-minirhizotron study. *Glob. Chang. Biol.*, 14, 588–602.

Pritchard, S.G., Strand, A.E., McCormack, M.L., Davis, M.A. & Oren, R. (2008b). Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-CO_2-enrichment. *Glob. Chang. Biol.*, 14, 1252–1264.

Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D. *et al.* (2006). Nitrogen limitation constrains sustainability of ecosystem response to CO_2. *Nature*, 440, 922–925.

Schafer, K.V.R., Oren, R., Lai, C.T. & Katul, G.G. (2002). Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO_2 concentration. *Glob. Chang. Biol.*, 8, 895–911.

Seiler, T.J., Rasse, D.P., Li, J.H., Dijkstra, P., Anderson, H.P., Johnson, D.P. *et al.* (2009). Disturbance, rainfall and contrasting species responses mediated aboveground biomass response to 11 years of CO_2 enrichment in a Florida scrub-oak ecosystem. *Glob. Chang. Biol.*, 15, 356–367.

Sparks, J.P., Walker, J., Turnipseed, A. & Guenther, A. (2008). Dry nitrogen deposition estimates over a forest experiencing free air CO_2 enrichment. *Glob. Chang. Biol.*, 14, 768–781.

Staddon, P.L., Ramsey, C.B., Ostle, N., Ineson, P. & Fitter, A.H. (2003). Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of C-14. *Science*, 300, 1138–1140.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 The relationship between total belowground carbon flux (TBCF) and the availability of mineral soil N for plots at the Duke free-air CO_2 enrichment (FACE) experiment in the absence of N fertilization. Open circles refer to ambient CO_2; filled circles refer to elevated CO_2. There was no difference in the slope of this relationship between CO_2 treatments (ANCOVA, $P > 0.5$), but there was a significantly higher intercept for the elevated CO_2 data (ANCOVA, $P < 0.001$).

Table S1 Pools of carbon at the Duke free-air CO_2 enrichment (FACE) experiment. All units are g C m^{-2}. Fine roots and coarse root were summed for Fig. 1 and soil CO_2 was ignored. Values are mean (± 1 SE). *P*-values reflect the main effect of CO_2 treatment in a repeated-measures RCBD. Fine roots were defined as roots with diameter < 2 mm and coarse roots were > 2 mm.

Table S2 Fluxes of carbon at the Duke free-air CO_2 enrichment (FACE) site. All units are g C m^{-2} $year^{-1}$. Values are mean (± 1 SE). *P*-values reflect the main effect of CO_2 treatment in a repeated-measures RCBD. Fluxes were classified into three types-outputs of C from soil, inputs of C to soil and internal cycling of C within the soil. DIC is dissolved inorganic C, ECM is ectomycorrhizal fungus and SOC is soil organic C. Some of these fluxes were summed to obtain the values in Fig. 1(a); autotrophic respiration is the sum of fine and coarse root respiration and the exudation and fungal allocation term is the sum of exudation, ECM production and other fungal production.

Table S3 Nitrogen pools under ambient (A) and elevated (E) CO_2 treatments in 2005 at the Duke free-air CO_2 enrichment experiment. Values reflect the mean and SE of three replicates per treatment. Statistics refer to standing N in 2005 and included pre-treatment (1996) values as a covariate. Biomass %N (± SE) was calculated as a weighted average of all biomass pools.

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