Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂


Abstract
The earth’s future climate state is highly dependent upon changes in terrestrial C storage in response to rising concentrations of atmospheric CO₂. 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 CO₂ 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 CO₂ 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 CO₂, forest productivity, nitrogen.

INTRODUCTION
Predictions of the earth’s future climate state are highly sensitive to the C-cycle response of ecosystems to rising concentrations of atmospheric CO₂ (Friedlingstein et al. 2006; Meehl et al. 2007). Ecosystem responses to experimental increases in atmospheric CO₂ concentrations vary widely, from ecosystems in which soil-N availability precludes an enhancement of net primary productivity (NPP) in response to elevated CO₂ (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 CO₂ (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 CO₂, it is necessary to understand the mechanistic connection between plant physiological responses to elevated CO₂ and their attending effects on nutrient availability and uptake.

Among elevated CO₂ experiments, the Duke Forest free-air CO₂ enrichment (FACE) experiment is unique in that NPP in this N-limited system has remained consistently and significantly higher under elevated compared with ambient CO₂ 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-CO₂ response gradient raising the question of what processes sustain higher productivity under elevated CO₂ 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

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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 CO2-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 CO2 increase N uptake and sustain the long-term productivity response of a warm-temperate forest to elevated concentrations of atmospheric CO2 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 CO2 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 CO2 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 CO2 (Hendrey et al. 1999). All plots were 30-m-diameter with elevated CO2 plots maintaining an atmospheric CO2 concentration 200 μL L−1 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 CO2 was derived from the combustion of natural gas with a depleted 13C signature (δ13C = −43.0 ± 0.6‰) resulting in plant tissues with a δ13C value of −39‰, tissues produced under ambient CO2 conditions maintained their C3 signature of −28.0‰ (Jackson et al. 2009).

A CO2 × 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 CO2 in soil air spaces was calculated from measurements of CO2 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 CO2 diffusion out of the soil (i.e., soil CO2 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 CO2 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 Q10 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 = b20 × Q10(T−20)/10. The Q10 values were taken from a previous study at this site (Bernhardt et al. 2006); b20, 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 r2 of 0.59.

While soil CO2 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 CO2 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
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 $^\circ$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 fumigating 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$_2$O 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; GE 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):

$$TBCF = F_{efflux} + F_{leaching} + F_{transpiration} - F_{litter} + \Delta (CSOM + Croots) \quad (2)$$

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 $\Delta (CSOM + Croots)$ 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$\text{sum}$’ Table 1) based on the sum of all C inputs to the soil excluding litterfall (Table S2). TBCF$\text{sum}$ 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$\text{sum}$) 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...
Elevated CO₂ 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 RCBD design. When temporal data were available, a repeated-measure ANOVA was 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 CO₂ 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 1.500 µg C m⁻² year⁻¹ under ambient CO₂ to 1.750 µg C m⁻² year⁻¹ under elevated CO₂ (Fig. 1a; ANOVA, P < 0.05). TBCF increased 10% under elevated CO₂ (repeated-measures ANOVA, P < 0.01). The increase in C entering the soil under elevated CO₂ 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 CO₂ 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 N₂ 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 CO₂, 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 CO₂ (Fig. 2b; slopes differ significantly, ANOVA, 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 CO₂ relative to ambient CO₂ 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 CO₂ efflux under ambient and elevated CO₂ (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 CO₂ (Fig. 3c; ANOVA after log-linearization, intercept increased under elevated CO₂, P < 0.05). Net primary production was positively correlated with canopy N content (Fig. 4). The increase in NPP under elevated CO₂ 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 CO₂, Crous et al. 2008), although this effect was not evident in this data set (i.e. a significantly higher y-intercept under elevated CO₂ 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 CO₂ 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 CO₂, the increase in TBCF under elevated CO₂ 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 CO₂. 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
under elevated compared with ambient CO₂. 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 CO₂ 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 CO₂ – an additional 1000 g C 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 g C 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 CO₂ of 213 g C m⁻² year⁻¹ (McCarthy et al. 2010). Because of the depleted ¹³C composition of the fumigation gas, there is a tracer for C fluxes in the elevated CO₂ plots. This ¹³C-depleted label was incorporated into all soil C pools under elevated CO₂ (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-CO₂-treatment C must account for the large change in the isotopic composition of the soil C pool; if the C released as CO₂ 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,
ambient CO2, while filled symbols refer to plots fumigated with elevated CO2. 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 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 CO2 plot with its elevated CO2 block-part, and thus show the directional response of the forest to CO2-enrichment. The solid black line reflects ± 1 SE of the nine years of measurements. The solid-grey arrows connect each ambient CO2 plot with its elevated CO2 block-part, and thus show the directional response of the forest to CO2-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-CO2-treatment soil C by C fixed post-CO2 treatment without a concomitant increase in pool size strongly suggests accelerated rates of SOM turnover under elevated CO2. We recognize that forest responses to a gradual change in atmospheric CO2 may differ from an experimental manipulation of CO2. 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 CO2 concentration.

Mass balance calculations suggest that greater N uptake under elevated CO2 was the result of faster SOM decomposition in surface soils (to 30 cm depth). The C budget indicates that Rb was, on average, 100 gC m$^{-2}$ year$^{-1}$ higher under elevated than ambient CO2 (Fig. 1a, Table S2). Based on the turnover time of the surface organic horizon under elevated CO2 (Lichter et al. 2008), c. 40 of the additional 100 gC m$^{-2}$ year$^{-1}$ (as CO2) are likely to have originated from this horizon under elevated CO2. 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 $R_{bn}$, 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 & Schlisinger 2002). Hence the release of N from the decomposition of surface soil SOM under elevated CO2 is more than sufficient to account for the difference in soil-N uptake between CO2 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 15N tracer experiment in which they found greater rates of N uptake from surface soils under elevated CO2. 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 CO2, 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, García 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 NH4+ mineralization were significantly enhanced under elevated compared with ambient CO2 (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 CO2.

There is a continuum of NPP responses to experimental increases in atmospheric CO2 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 CO2 at the Duke FACE site (McCarthy et al. 2010) anchors one end of this continuum, as does the Florida scrub-oak elevated-CO2 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 CO2 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 CO2 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 CO2-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 CO2. This study points to the pivotal role of belowground C allocation in ecosystem response to elevated CO2 and suggests that fungal community composition may mediate positive- vs. negative feedback effects of elevated CO2 on NPP. Ultimately, the key to correctly incorporating these feedbacks in coupled climate-carbon cycle models.

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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 CO₂ enrichment.

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REFERENCES


**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 CO₂ enrichment (FACE) experiment in the absence of N fertilization. Open circles refer to ambient CO₂; filled circles refer to elevated CO₂. There was no difference in the slope of this relationship between CO₂ treatments (*ANCOVA, P > 0.5*), but there was a significantly higher intercept for the elevated CO₂ data (*ANCOVA, P < 0.001*).

**Table S1** Pools of carbon at the Duke free-air CO₂ enrichment (FACE) experiment. All units are g C m⁻². Fine roots and coarse root were summed for Fig. 1 and soil CO₂ was ignored. Values are mean (± 1 SE). *P*-values reflect the main effect of CO₂ 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 CO₂ enrichment (FACE) site. All units are g C m⁻² year⁻¹. Values are mean (± 1 SE). *P*-values reflect the main effect of CO₂ 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) CO₂ treatments in 2005 at the Duke free-air CO₂ 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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