Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation

Abstract
The degree to which rising atmospheric CO₂ will be offset by carbon (C) sequestration in forests depends in part on the capacity of trees and soil microbes to make physiological adjustments that can alleviate resource limitation. Here, we show for the first time that mature trees exposed to CO₂ enrichment increase the release of soluble C from roots to soil, and that such increases are coupled to the accelerated turnover of nitrogen (N) pools in the rhizosphere. Over the course of 3 years, we measured in situ rates of root exudation from 420 intact loblolly pine (Pinus taeda L.) roots. Trees fumigated with elevated CO₂ (200 p.p.m. v. over background) increased exudation rates (µg C cm⁻¹ root h⁻¹) by 55% during the primary growing season, leading to a 50% annual increase in dissolved organic inputs to fumigated forest soils. These increases in root-derived C were positively correlated with microbial release of extracellular enzymes involved in breakdown of organic N (R² = 0.66; P = 0.006) in the rhizosphere, indicating that exudation stimulated microbial activity and accelerated the rate of soil organic matter (SOM) turnover. In support of this conclusion, trees exposed to both elevated CO₂ and N fertilization did not increase exudation rates and had reduced enzyme activities in the rhizosphere. Collectively, our results provide field-based empirical support suggesting that sustained growth responses of forests to elevated CO₂ in low fertility soils are maintained by enhanced rates of microbial activity and N cycling fuelled by inputs of root-derived C. To the extent that increases in exudation also stimulate SOM decomposition, such changes may prevent soil C accumulation in forest ecosystems.

Keywords
Fine roots, plant-microbial feedbacks, priming effects, rhizodeposition, rhizosphere.

INTRODUCTION
Aggrading forests currently provide the largest terrestrial sink for atmospheric CO₂ globally, but uncertainty regarding the persistence of this sink has hindered efforts to predict biotic feedbacks to climate change (Norby et al. 2005). Numerous studies have reported that CO₂ enrichment increases tree seedling biomass (Curtis & Wang 1998), net primary production (NPP) in young forest stands (Norby et al. 2005; Finzi et al. 2007) and carbon (C) assimilation rates in mature trees (Korner 2005). However, most trees are limited by nutrient availability, and thus, any sustained CO₂ enhancement of forest ecosystem NPP under elevated CO₂ will require either the additional supply of limiting resources from exogenous sources or physiological adjustments that allow trees to increase uptake of soil resources (Johnson 2006).

Current conceptual models of nutrient limitation suggest that trees exposed to rising CO₂ should allocate a greater amount of photosynthate to roots to ‘scavenge’ or ‘mine’ nutrients from soil (Johnson 2006). In forests with low nitrogen (N) availability, rising atmospheric CO₂ can exacerbate N limitation, an inference supported by increased belowground C allocation and fine root production in multiple CO₂ enrichment experiments (Lukac et al. 2003; Norby et al. 2004; Pritchard et al. 2008a). However, the vast majority of N in forest soils is stored in organic forms requiring depolymerization by microbially derived extracellular enzymes prior to root uptake (Schimel & Bennett 2004). Thus, increased root exploration alone is unlikely to sustain plant N requirements under rising CO₂ unless accompanied by the concomitant stimulation of soil microbial activity and the release of nutrients from soil organic matter (SOM).

Root exudation – the release of soluble C compounds from roots to soil – has long been hypothesized to influence forest productivity responses to elevated CO₂ (Strain & Bazzaz 1983). Root exudates are low molecular weight organic substances that strongly influence nutrient availability due to their preferential use as substrates by soil microbes involved in nutrient transformations (symbiotic and nonsymbiotic) and their chelating properties. Root exudates are estimated to represent 1–5% of photoassimilate in trees (Grayston et al. 1996), and this proportion may increase in trees in response to elevated CO₂ (Phillips et al. 2009). In recent years, several high-profile CO₂ enrichment studies have invoked increases in exudation to explain changes in ecosystem C dynamics (Supporting Information, Table S1). Despite the presumed importance of exudates, few studies have directly measured exudation rates in field-grown plants exposed to elevated CO₂ and no studies have quantified the effects of CO₂ enrichment on exudation by mature trees. Thus, we know little about how this widely hypothesized but rarely quantified process will influence the capacity of forest ecosystems to sequester additional C under rising atmospheric CO₂.

The consequences of increased exudation on ecosystem C storage depend on soil microbial processes and nutrient availability. Microbial activity is generally limited by the availability of labile C in soil (Tate 1995). In response to exudates, increases in microbial activity and population growth may stimulate microbial demand for nutrients, which can be met by increasing enzyme synthesis and the depolymerization of N from SOM (Cheng & Kuzyakov 2005). Although rhizosphere microbes may initially immobilize this newly mineralized N in biomass, the continuous pulsing of C from exudation may
increase soil N availability as microbial populations grow, turnover and release nutrients into the rhizosphere (Badalucco & Kuikman 2001). Such ‘priming effects’ have been recently invoked as a mechanism to explain increased N uptake by tree seedlings grown in the greenhouse (Dijkstra et al. 2009), but the degree to which such effects influence N availability and feedbacks to forest productivity is unknown (Cheng & Kuzyakov 2005).

Forest ecosystem ecologists have understood for some time that trees and soil microbes are linked through litter supply of C and microbial mineralization of organic nutrients (McLaugherty et al. 1985; Aber et al. 1990). Recent work has suggested that increased leaf litter produced by trees under elevated CO₂ can stimulate SOM decomposition in upper surface soils through priming effects and drive soil C loss (Carney et al. 2007; Talhelm et al. 2009). To the extent that belowground C inputs fuel microbial activity and SOM mineralization (Zak et al. 2000; Cheng & Kuzyakov 2005), enhanced exudation under elevated CO₂ may induce similar soil C losses, but with consequences for soil C storage throughout the entire rooting zone. Unfortunately, there are no field-derived empirical datasets available to test the hypothesis that tree roots stimulate SOM decomposition in mature forest ecosystems through the release of exudates.

We examined plant–microbe interactions in the rhizosphere and bulk soils of the longest running forest free-air CO₂ enrichment (FACE) experiment. We predicted that trees growing under elevated CO₂ exude more C into the rhizosphere than trees under ambient CO₂, and that enhanced rates of exudation would be associated with increased microbial activity and the accelerated turnover of N from SOM. We predicted that the strength of these CO₂ effects would be reduced for plots fertilized with inorganic N. To test this hypothesis, we measured differences in root exudation rates and rhizosphere microbial activity in forest plots exposed to elevated CO₂ and/or N fertilization.

**MATERIAL AND METHODS**

**Field site**

The Duke Forest FACE experiment was established in a loblolly pine (Pinus taeda L.) plantation in Orange County, North Carolina (35°58’ N, 79°05’ W). The site is dominated by pine (> 90% of the basal area), although other hardwoods have developed in the understory since the initial planting in 1983. In 1996, eight 30 m diameter plots containing c. 100 trees were established. Four experimental plots are fumigated with exogenous CO₂ to maintain an atmospheric concentration of 200 p.p.p.v. above ambient levels (i.e. c. 585 p.p.m.v.), whereas four plots are fumigated with air only. The mean annual temperature is 15.5 °C and mean annual precipitation is 1140 mm year⁻¹. Soils are highly weathered clay loams (mixed thermic Ultic Hapludalfs) and moderately acidic (pH = 5.6). A detailed description of the site can be found in Lichter et al. (2008). Ammonium nitrate was hand-broadcast to half of each plot during each sampling period. We predicted that the primary growing season (April–October) as the months during which the average monthly net ecosystem production (NEP) exceeded the yearly NEP average for 2007–2008; non-growing season months had average monthly NEP values less than the yearly NEP. We used gap-filled data collected at 30 min intervals to estimate the average monthly NEP for the site on the approximate dates of exudate collection and soil sampling. Average monthly NEP values ranged from 0.14 to 3.12 g C m⁻² day⁻¹, and values exceeding mean annual NEP (2.19 g C m⁻² day⁻¹ for 2007–2008) were considered

**Exudation measurements**

Exudates were collected every 4–8 weeks from April 2007 to October 2009 using a culture-based cuvette system developed specifically for loblolly pine roots at this site (Phillips et al. 2008). Terminal fine roots (< 1 mm average diameter with laterals) attached to pine trees were excavated from the upper 10 cm of soil below the organic horizon. Soil particles adhering to roots were carefully rinsed off with a squirt bottle and fine forceps were used to dislodge SOM aggregates. Although the vast majority of microbes were removed during this step, some rhizoplane bacteria likely remained – a inevitable tradeoff in trying to minimize root injury (Neumann & Romheld 2001). Intact roots were placed into glass cuvettes, filled with glass beads (c. 750 μm diameter) and sealed. Cuvettes (including controls with beads only) were covered in foil and reburied in the excavated area in soil. After a short equilibration period, fresh nutrient solution was flushed through each cuvette to remove soluble C. After 24 h, ‘trap solutions’ (Neumann & Romheld 2001) containing exudates were collected from each cuvette, placed on ice and filtered through sterile 0.22 µm syringe filters (Millex PVDF; Millipore Co., Billerica, MA, USA) within 2–5 h of collection. A detailed description of the method is available in Phillips et al. (2008).

Exudates were collected from two to three root systems in control and fertilized plots of each ring during each sampling period. We collected exudates in two rings (1 ambient + 1 elevated CO₂ ring) in April 2007, four rings (2 ambient, 2 elevated) in July 2007, six rings (3 ambient, 3 elevated) from August 2007 to March 2008 and eight rings (4 ambient, 4 elevated) from May 2008 to October 2009. For each sampling period, exudates were collected over five consecutive days (rather than on a single day) and from new root systems on each sampling date. All roots were harvested following the final exudate collection for each root and scanned so that morphological variables (fine root length, surface area, etc.) could be quantified. All scanned images were visually inspected, calibrated using materials of known size and analysed using WinRhizo software (Regents Instruments Inc., Québec, Canada).

Trap solutions were analysed for non-particulate organic C on a TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Control cuvettes (beads only) were used to account for C contamination resulting from non-exudate sources. Exudation rates were calculated as the mass of C (μg) flushed from each root system (minus the average C concentration in control cuvettes) over the 24 h incubation period. Exudation rates (μg C cm⁻² root h⁻¹) were calculated by dividing the total amount of C flushed by the total fine root length within each cuvette. Annual exudation rates were calculated individually for each plot, by multiplying the average mass-specific exudation rate (μg C g⁻¹ root h⁻¹) in each plot from 2007 to 2009 by the average standing crop fine root biomass (< 2 mm diameter) in each plot from 2007 to 2008 (Jackson et al. 2009). Fine root biomass was measured six times over the 2-year interval using a 5-cm diameter soil core (four in each plot for each sampling date).

**Defining the primary growing season**

We defined the primary growing season (April–October) as the months during which the average monthly net ecosystem production (NEP) exceeded the yearly NEP average for 2007–2008; non-growing season months had average monthly NEP values less than the yearly NEP. We used gap-filled data collected at 30 min intervals to estimate the average monthly NEP for the site on the approximate dates of exudate collection and soil sampling. Average monthly NEP values ranged from 0.14 to 3.12 g C m⁻² day⁻¹, and values exceeding mean annual NEP (2.19 g C m⁻² day⁻¹ for 2007–2008) were considered
primary growing season months. NEP was calculated using the eddy covariance system which estimates the net exchange of CO$_2$ between the atmosphere and forest canopy above a single ambient CO$_2$ ring.

Soil sampling

Soils were sampled in the upper 15 cm of the mineral soil in March 2008, July 2008, April 2009, July 2009 and October 2009. We selected these dates to span the primary growing season and to coincide with a subset of the exudation sampling dates. Soils were sampled within 3 weeks of an exudation measurement. We used a large diameter soil core (15-cm diameter) to ensure that fine roots would have a sufficient mass of adhering rhizosphere soil. We collected replicate cores from control and fertilized plots in six rings (3 ambient, 3 elevated) in March 2008 and eight rings (4 ambient, 4 elevated) on the other four dates. Soils were transported to the laboratory on ice, and fine roots with adhering soil were separated from non-adhering soil within 4 h of collection. Soil adhering to roots was dislodged using fine forceps; this fraction was operationally defined as rhizosphere soil (Wollum 1994) and non-adhering soil was considered bulk soil. All soils were sieved to 2 mm.

Laboratory assays

Soil microbial biomass was measured within 24 h of soil collection from the field using a modification of the substrate-induced respiration method (Anderson & Domsch 1978). This assay measures the maximum respiratory response of microbes prior to exponential growth and thus reflects the active microbial biomass. Two grams of the maximum respiratory response of microbes prior to exponential respiration method (Anderson & Domsch 1978). This assay measures from the field using a modification of the substrate-induced covariance system which estimates the net exchange of CO$_2$ between the atmosphere and forest canopy above a single ambient CO$_2$ ring. Laboratory assays

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of dry weight mass in each fraction. Hence, treatment effects on soil microbes reflected changes in soil process rates (i.e. per g soil) and changes in the spatial extent of the process. All data presented are presented as least squares and error bars are ± 1 SE. Given the limited number of experimental plots receiving CO2 enrichment (n = 4), mean values were considered statistically different at α = 0.10.

RESULTS

CO2 and N fertilization effects on exudation

Over the 3-year period, exudation rates ranged from 0.1–6 µg C cm⁻¹ fine root h⁻¹ and were strongly influenced by CO2 enrichment, N fertilization and time of year. CO2 enrichment increased exudation rates (µg C cm⁻¹ fine root h⁻¹) by 55% during the primary growing season (i.e. April–October) in non-fertilized soils (P = 0.004; Fig. 1). In contrast, CO2 enrichment had no effect on exudation in fertilized plots from April to October (P = 0.518), or in fertilized and non-fertilized plots from November to March (P = 0.294 and P = 0.667, respectively; Fig. 1b). Annual exudation fluxes ranged from 17 to 43 g C m⁻² year⁻¹ across the eight plots. As a result of higher fine root biomass and higher exudation rates, we estimate that elevated CO2 increased labile C supply from roots by 50% annually in unfertilized plots (P = 0.020; Table 1).

The relationship between exudation rate and NAGase activity was affected by N fertilization but not by CO2. Rhizosphere NAGase activities (µmol g⁻¹ soil h⁻¹) were strongly correlated with exudation rates (µg C cm⁻¹ fine root h⁻¹) in non-fertilized soils (r = 0.79; P = 0.006; Fig. 2a). In contrast, exudation rates and NAGase activities were not correlated in fertilized soils (r = 0.42; P = 0.22; Table 1).

Table 1 Effects of elevated CO2 and N fertilization on root exudation at the Duke Forest FACE site, NC from 2007 to 2009

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exudation flux (mg C g root⁻¹ year⁻¹)</th>
<th>Fine root biomass (g m⁻² year⁻¹)</th>
<th>Annual flux (g C m⁻² year⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-fertilized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient CO2</td>
<td>11.1 (1.6)</td>
<td>235 (15)</td>
<td>23.2 (4.4)</td>
</tr>
<tr>
<td>Elevated CO2</td>
<td>13.2 (1.7)</td>
<td>302 (21)</td>
<td>34.8 (4.7)*</td>
</tr>
<tr>
<td>Fertilized (+N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient CO2</td>
<td>13.3 (0.6)</td>
<td>207 (9)</td>
<td>23.9 (0.8)</td>
</tr>
<tr>
<td>Elevated CO2</td>
<td>12.3 (1.1)</td>
<td>261 (29)</td>
<td>28.1 (3.6)</td>
</tr>
</tbody>
</table>

Exudation fluxes and fine root biomass values represent the average values from 12 and 6 sampling dates, respectively. Annual fluxes were calculated by multiplying the average mass-specific exudation flux by the average fine root biomass for each individual plot (n = 4 replicate plots per treatment).

*P < 0.05.
Rhizosphere and bulk soil response to treatments

Across treatments and dates, rhizosphere soil made up 5–15% of the mass in the top 15 cm of soil (Table S1). Despite greater exudation rates under elevated CO2 (Fig. 1), there was no significant effect of CO2 on rhizosphere soil mass (P = 0.342; Table S1). However, rhizosphere soil mass decreased by 28% in N-fertilized soils (P = 0.021). In general, area-weighted estimates of soil processes (e.g. activity m\(^{-2}\) h\(^{-1}\)) were influenced by treatment effects on mass-specific rates and to a lesser degree, changes in rhizosphere soil mass (Table S2).

Soil microbial biomass in the rhizosphere increased by 40% with CO2 enrichment (P = 0.038) and decreased by 44% with N fertilization (P = 0.038), though the magnitude of these effects were dependent on season (CO2 × N × season interaction; P = 0.044; Fig. 2a). In the middle of the growing season (i.e. July), rhizosphere microbial biomass was 56% higher under CO2 enrichment in non-fertilized plots (P = 0.088) but was not different between CO2 treatments in fertilized plots (P = 0.733). Moreover, the microbial response in the rhizosphere tracked exudation patterns in July of 2008 and 2009, when exudation rates increased by 44% in response to elevated CO2 in non-fertilized soils but were unaffected by CO2 in fertilized soils (data not shown). These effects were not apparent early (i.e. April) or late (i.e. October) in the growing season. In contrast, neither elevated CO2 nor N fertilization had a significant effect on microbial biomass in the bulk soil (P = 0.972 and 0.133, respectively).

Soil enzyme activities and N cycling

Similar to the response of the microbial biomass, enzyme activities in rhizosphere soil responded more strongly to experimental treatments than activities in the bulk soil. Over the 2-year period, NAGase activities in the rhizosphere were 66% greater than in bulk soil. Nitrogen fertilization reduced rhizosphere NAGase activities by 52% (P < 0.0001) but by only 18% in bulk soil (P < 0.013). During the middle of the growing season, rhizosphere NAGase activities increased with CO2 enrichment in non-fertilized plots (P = 0.073), while there was no evidence for a CO2 effect in fertilized plots (P = 0.754; Fig. 2a). During the early-late portion of the growing season, NAGase activities were significantly lower under elevated CO2 in the non-fertilized plots (P < 0.032). None of these effects were significant in bulk soil.

Phenol oxidase activities in the rhizosphere increased by 47% with CO2 enrichment and decreased by 40% with N fertilization (P = 0.023 and 0.003, respectively; Fig. 3c). Unlike NAGase activities, CO2 effects on rhizosphere phenol oxidase activities occurred primarily during the early-late season, with increased activities in both fertilized and non-fertilized soils (P = 0.062 and 0.097, respectively; Fig. 3c). Phenol oxidase activities in bulk soil decreased by 27% with N fertilization (P = 0.038) but were unaffected by elevated CO2 (P = 0.745).

Soil N dynamics responded to experimental treatments in the rhizosphere but not in the bulk soil, and there were no significant interactions between date, CO2 and N fertilization within each soil fraction. In the rhizosphere, gross mineralization rates were greater under CO2 enrichment (2.2 ± 0.4 mg N m\(^{-2}\) h\(^{-2}\)) than under ambient CO2 (1.5 ± 0.2 mg N m\(^{-2}\) h\(^{-2}\); P = 0.04), with much of this effect resulting from increased gross mineralization in the fertilized soils. In addition, the mean residence of the NH\(_4\)\(^{+}\) pool in rhizosphere soil under elevated CO2 (8.1 ± 1.6 h) was significantly lower than under ambient CO2 (21.0 ± 6.2 h; P = 0.051). Hence, the NH\(_4\)\(^{+}\) pool in the rhizosphere turned over 2.6 times more quickly under elevated compared to ambient CO2. In the bulk soil, CO2 enrichment and N fertilization had no effect on gross N mineralization or the mean residence time of the NH\(_4\)\(^{+}\) pool.

**DISCUSSION**

Forest ecosystems account for 50–60% of the annual NPP in terrestrial ecosystems across the globe (Gower 2003) and play a central role in global carbon and nitrogen cycles.
role in mitigating the impact of rising concentrations of atmospheric CO₂ on the climate system by sequestering C in biomass and soil (IPCC 2007). Understanding the mechanisms by which trees allocate C and cycle nutrients under elevated CO₂ is critical for predicting biotic feedbacks to climate change. Here, we show that increases in the flux of labile C from roots to soil under elevated CO₂ (Fig. 1) are coupled to enhanced rates of N cycling and SOM decomposition in the rhizosphere (Figs 2 and 3), a mechanism which may contribute to long-term stimulation of forest productivity but also reduce soil C accumulation in forests under elevated CO₂.

Our results indicate that exudation rates from mature field-grown trees are strongly influenced by elevated CO₂, but that such effects depend on season and soil fertility (Fig. 1). Although previous studies have reported CO₂-induced stimulation of exudation in trees, these studies were conducted with seedlings grown in artificial medium (Uselman et al. 2000; Phillips et al. 2009; Fransson & Johannsson 2010) making extrapolation of these results to forest ecosystems problematic (Phillips et al. 2008). Exudation rates responded to CO₂ enrichment during the primary growing season in non-fertilized soils only; hence, exudation rates appear to track C assimilation and allocation patterns in these forests. At the Duke FACE site, elevated CO₂ increases C allocated to fine root production (Pritchard et al. 2008a), with greater total belowground C allocation occurring in low fertility soils (Palmroth et al. 2006). Thus, much of the additional C assimilated under elevated CO₂ in low nutrient soils is released to soil as labile C, which cycles rapidly through the plant–soil system.

On an annual basis, CO₂ effects on exudation increased by c. 50% for trees enriched with CO₂ in non-fertilized plots but were unaffected by CO₂ enrichment in fertilized plots (Table 1). We recognize that scaling exudation estimates must be done cautiously given the potential for measurement and extrapolation errors associated with both the exudation and root sampling (Phillips et al. 2008). However, scaling issues are common to all ecosystem estimates of belowground processes given the substantial spatial and temporal heterogeneity of soil C pools (Hinsinger et al. 2005). Our estimates of mass-specific exudation rates in this study (8–17 µg g fine root h⁻¹) are similar to rates in loblolly pine seedlings from a greenhouse experiment (2–40 µg g fine root h⁻¹; Phillips et al. 2009), where exudates were collected using a similar culture-based trap system. At the Duke FACE site, we measured exudation rates from roots in the upper 15 cm of soil where c. 60% of fine roots reside. As much of the increased root production in response to CO₂ enrichment occurs at 15–30 cm depth at the site (Pritchard et al. 2008a), our estimate of the additional input of C released from roots under elevated CO₂ is likely to be conservative. Nevertheless, our estimates of the additional flux of C should be considered a rough approximation and used in addition to estimates derived from mass-balance and modelling approaches.

**Ecosystem consequences of enhanced exudation**

The progressive N limitation hypothesis states that in N limited ecosystems, the rapid uptake and immobilization of N in biomass under elevated CO₂ depletes pools of available N, resulting in only short-term increases in productivity under elevated CO₂ (Johnson 2006). Unlike the only other forest FACE experiment in a closed canopy forest (Norby et al. 2010), NPP has not down-regulated at the Duke FACE site (McCarthy et al. 2010), and increases in productivity have been accompanied by an increase in plant N uptake rather than increased N-use efficiency (Finzi et al. 2007). However, the mechanism by which these trees are acquiring N is unclear; the increase in uptake cannot be attributed to decreases in N losses via leaching (Finzi et al. 2002) or to increases in N inputs via N₂-fixation (Hofmockel 2007). Additionally, there have been no detectable changes in bulk soil net or gross N mineralization or organic N cycling between ambient and elevated CO₂ plots (Finzi & Schlesinger 2003).

Our results indicate that increased root C efflux from CO₂-enriched trees stimulates rhizosphere N cycling in low fertility soils at this site. Importantly, rhizosphere NAGase activity (an indicator of moderately fast N turnover) was positively correlated with measured rates of exudation in non-fertilized soils (Fig. 2a). We interpret these results as evidence that rhizosphere microbes such actinomycetes, which produce NAGase enzymes and respond strongly to CO₂ at this site (Billings & Ziegler 2008), are using energy derived from exudates to synthesize enzymes that release N from SOM (Cheng & Kuzyakov 2005). This was not the case in N-fertilized soils (Fig. 2b), where rhizosphere microbes appeared to use C-rich exudates for growth rather than for the production of enzymes to acquire N. This dramatic contrast between the fertilized and unfertilized treatments provides evidence that enhanced exudation is a mechanism trees employ for increasing N availability. Moreover, our results indicate that increased exudation stimulates recalcitrant SOM decomposition (Fig. 3c). Such effects would result in soil C losses throughout the rooting zone during the growing season, and offer a partial explanation for the lack of C accumulation in the Duke FACE soils despite 13 years of CO₂-induced increases in NPP and litter inputs (Drake et al. in press).

Our results indicate that increased C efflux from roots is an important physiological adjustment stimulating C cycling and SOM decomposition by CO₂-enriched plants growing in low fertility soil. Notably, other physiological adjustments by trees exposed to elevated CO₂ such as C allocation to ectomycorrhizal fungi can also stimulate N cycling in soil microsites. CO₂ enrichment has been reported to increase ectomycorrhizal production (Pritchard et al. 2008b; Talbot et al. 2008) and the flux of labile C from hyphae (Fransson and Johannsson 2010). Because these fungi have broad enzymatic capabilities, such changes would presumably accelerate N release from SOM pools (Read & Perez-Moreno 2003). The degree to which ectomycorrhizal fungi mediate exudation rates and priming effects in forest ecosystems exposed to elevated CO₂ warrants further study.

**CONCLUSIONS**

This study demonstrates that the enhanced C flux from roots to soil in low fertility forests exposed to elevated CO₂ creates hotspots for microbial activity that are associated with faster rates of SOM turnover and N cycling. Although the absolute magnitude of the C flux to the rhizosphere is a relatively small fraction of the total C budget, these fluxes appear to have important quantitative effects on long-term forest productivity and soil C accumulation in forests under elevated CO₂. Moreover, our analysis suggests that the spatial scale at which the soil is commonly sampled (i.e. rhizosphere excluded, or rhizosphere and bulk soil mixed together) may lack the sensitivity necessary to detect the effects of global changes on ecosystem function.

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AUTHOR CONTRIBUTIONS
R.P.P. and E.S.B designed the research; R.P.P., E.S.B and A.C.F. performed the research; R.P.P. analysed the data; and R.P.P. and E.S.B wrote the paper.

REFERENCES

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**SUPPORTING INFORMATION**

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

**Table S1** List of studies inferring the importance of exudation under CO₂ enrichment.

**Table S2** CO₂ enrichment and N fertilization effects on soil properties in rhizosphere and bulk soil.

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