Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO₂

Abstract
A common finding in multiple CO₂ enrichment experiments in forests is the lack of soil carbon (C) accumulation owing to microbial priming of ‘old’ soil organic matter (SOM). However, soil C losses may also result from the accelerated turnover of ‘young’ microbial tissues that are rich in nitrogen (N) relative to bulk SOM. We measured root-induced changes in soil C dynamics in a pine forest exposed to elevated CO₂ and N enrichment by combining stable isotope analyses, molecular characterisations of SOM and microbial assays. We find strong evidence that the accelerated turnover of root-derived C under elevated CO₂ is sufficient in magnitude to offset increased belowground inputs. In addition, the C losses were associated with accelerated N cycling, suggesting that trees exposed to elevated CO₂ not only enhance N availability by stimulating microbial decomposition of SOM via priming but also increase the rate at which N cycles through microbial pools.

Keywords
Exudation, plant-microbial feedbacks, priming effects, rhizodeposition, rhizosphere.

INTRODUCTION
Forest ecosystems represent the primary terrestrial sink for atmospheric CO₂ globally, with the majority of this C stored in soil pools (Dixon 1994). Long-term carbon (C) storage in forests, thus, depends on the balance between C gains from net primary productivity (NPP) and C losses from soil organic matter (SOM) decomposition and leaching. Although the factors that control C uptake and storage in biomass under rising CO₂ are relatively well understood (Ainsworth & Long 2005), there is considerable uncertainty regarding the factors which control C retention and loss in forest soils.

Most current ecosystem models represent soil C losses using temperature-dependent first-order rate constants for a limited number of SOM pools, with little if any representation of root-derived C fluxes or rhizosphere processes. This omission may be critical, as a large fraction of the C used to support biological activity in forest soils results from the release of recently fixed C from tree roots (Grayston et al. 1997) which disproportionately affects SOM decomposition and nutrient release (Drake et al. 2011; Phillips et al. 2011). Roots and mycorrhizal fungi, for example, stabilise SOM by promoting aggregation and releasing recalcitrant biomolecules to soil, such that much of the C that is stored in soils is believed to be root-derived (Schmidt et al. 2011). However, roots and mycorrhizal fungi can also enhance SOM decomposition. Roots release exudates to soil and provide carbohydrates to mycorrhizal fungi, and both processes provide heterotrophic microbes with the energy needed to synthesise extracellular enzymes to degrade SOM (Cheng & Kuzyakov 2005). Rhizosphere processes are more biogeochemically active and appear to be more responsive to increases in CO₂ and temperature than are bulk soil processes (Phillips et al. 2011; Zhu & Cheng 2011), thus understanding rhizosphere SOM dynamics is critical to predicting how elevated SOM content and composition will respond to rising atmospheric CO₂ (Norby & Zak 2011).

Multiple forest CO₂ enrichment experiments have reported little change in soil C stocks despite large increases in both above and belowground NPP (Hoosbeek et al. 2004; Hoosbeek & Scarascia-Mugnozza 2009; Langley et al. 2009; Talhelm et al. 2009; Drake et al. 2011; but see Iversen et al. 2012). These findings challenge long-standing assumptions about the controls on soil C accumulation in aggrading forests (Richter et al. 1999). Many have suggested that elevated CO₂ leads to increases in young recently-fixed plant-derived C which then stimulates microbial decomposition of old stable SOM (Hoosbeek et al. 2004; Carney et al. 2007; Langley et al. 2009; Sayer et al. 2011). This explanation, referred to as the ‘soil priming hypothesis’ (sensu Cheng 1999) could explain a lack of soil C accumulation under elevated CO₂. An alternative, and not mutually exclusive, explanation is that elevated CO₂ may lead to the accelerated turnover of the recently-fixed C that is deposited in the rhizosphere under elevated CO₂. Roots release C to soil in the form of rhizodeposits, soluble exudates and mycorrhizal tissues, and such fluxes are generally enhanced under elevated CO₂ (Phillips 2007). However, little of this new C may become SOM if the substrates are decomposed preferentially by fast-turnover rhizosphere microbes (Cheng 1999). Both hypotheses, soil priming and accelerated turnover of root deposits and rhizosphere microbes, would likely have similar consequences for soil C balance, but different consequences for N availability and forest productivity. Rhizosphere microbes are generally rich in N rela-
Elevated CO2

Ambient CO2

tated under elevated CO2, and that such effects would be governed by plant and microbial demand for N. Hence, it is critical to quantify both the degree to which roots influence SOM dynamics and the mechanisms responsible for such effects in order to develop robust projections of ecosystem C storage and potential forest C cycle-climate feedbacks (Norby & Zak 2011).

At the Duke Forest Free Air CO2 Enrichment (FACE) site, NC – the longest running CO2 enrichment experiment in a forested ecosystem – there has been no C accumulation in the mineral soil over the course of 14 years of CO2 enrichment, despite the addition of over 1000 g C m$^{-2}$ to soils from greater belowground production (Drake et al. 2011). Given reports of enhanced root exudation and accelerated rhizosphere N cycling under elevated CO2 at this site (Phillips et al. 2011), we hypothesised that root-induced changes in SOM may be both limiting soil C accumulation and increasing N availability (Drake et al. 2011). We predicted that the turnover of root-derived C and rhizosphere microbial tissues would be accelerated under elevated CO2, and that such effects would be governed by plant and microbial demand for N. Our overall objective was to examine the mechanisms by which root-microbe interactions influence SOM and long-term soil C dynamics under conditions of varying N availability.

MATERIALS AND METHODS

Field site

The Duke FACE was established in loblolly pine (Pinus taeda L.) plantation planted in 1983 in Orange County, NC. From 1996 to 2010, eight 30 m diameter plots containing ~100 trees in each plot were fumigated with exogenous CO2 to maintain an atmospheric concentration ~200 ppmv above ambient levels (i.e. ~585 ppmv), whereas four plots were fumigated with air only. The mean annual temperature of the site is 15.5 °C and mean annual precipitation at the site is 1140 mm year$^{-1}$. Soils are highly-weathered clay loams (mixed thermic Ultic Hapludalfs) and moderately acidic (pH = 5.6). Ammonium nitrate was hand-broadcast to half of each plot at a rate of 11.2 g N m$^{-2}$ year$^{-1}$ in two applications in 2005 (half in March, half in April), and in a single application from 2006 to 2010. The unfertilised half of each plot was separated from this treatment by a 70 cm deep vertical impenetrable tarp to prevent fertiliser from reaching the unfertilised side of each plot.

Ingrowth cores

Net C inputs to soil from roots and mycorrhizal fungi were quantified using a modification of the $^{13}$C natural abundance technique. The exogenous source of CO2 at the FACE site contains a distinct $^{13}$C signature such that plants and surface soils receiving CO2 enrichment are depleted in $^{13}$C relative to plants and soils exposed to ambient CO2. We reciprocally transplanted soils with unique $^{13}$C isotopic signatures into plots, such that elevated CO2 soils were placed in ambient plots, and ambient soils were placed in elevated plots in a replicated design. As roots and mycorrhizal fungi have $^{13}$C signatures that reflect recently-fixed C, which are distinct from the signatures of transplanted soils (Table 1), inputs of root and fungal C can be calculated using a two end-member mixing model. Importantly, our design enabled us to use soils with unique isotopic signatures but similar soil properties (Phillips et al. 2011).

In July 2008, we collected soils from the 0–5 cm depth of the mineral soil in ambient and elevated CO2 soils for placement into the ingrowth cores. We used soils from shallow depths only to ensure that soil collected from the elevated CO2 plots was isotopically unique owing to the depleted signature of the $^{13}$C gas used for the CO2-enrichment (Table 1). Soils were stored at 22 °C for 2–3 months prior to deployment in the field to reduce the likelihood that changes in the isotopic signatures of soils would result from the preferential decomposition of isotopically depleted labile C in the soils. All soils were sieved and mixed with 50% sand by volume such that the C content of ingrowth core soil was <2%. Soils were packed into mesh-lined pvc cores (5 cm diameter; 15 cm length) from which large windows of the pvc (~80% of the surface area) were removed. To partition the contribution of roots and fungi, we placed soils into three types of ingrowth cores: one with a mesh permitting the penetration of both roots and fungal mycelium (‘root accessible’; 2 mm diameter), one with a mesh size allowing for penetration by mycelium only (‘root exclusion’; 50 μm), and a second root exclusion core which was rotated approximately monthly (15 times during the incubation) to reduce mycelial ingrowth. Although our initial goal was to completely exclude mycelial ingrowth from the ‘spin cores’ (Langley & Hungate 2003), nearly all of these cores contained some visible mycelia at the time of harvest; hence, we consider the spin cores to represent reduced mycelial production and turnover.

Cores were placed in the upper mineral soil where most roots and mycorrhizal hyphae proliferate (Parrent & Vilgalys 2007). We used a reciprocal transplant design (i.e. ambient CO2 cores into elevated CO2 plots, elevated CO2 cores into ambient CO2 soils) to exploit differences in the isotopic signatures between the fumigated and control plots. Within each plot, all three core types were established. In one ambient CO2 and one elevated CO2 plot, we included four replicates cores of each type to quantify within-plot spatial variability. All cores were placed in the ground in fall 2008 and harvested approximately 1 year later. At harvest, cores were transported to the lab on ice and stored at 4 °C until processing within 48 h of collection. Each core was split into 0–7.5 and 7.5–15 cm depths. We report the results from the 0–7.5 cm depth only here, as this zone of soil contained the majority of the fine root (66% in the root accessible cores) and microbial (67% in the root exclusion cores) inputs in each core. Roots were picked by hand and analysed for fine root length using a flat-bed scanner and Win RHIZO

Table 1 $^\delta$13C values of tissue and soils used in the two end-member mixing model. Asterisks indicate significant differences at $P < 0.05$

<table>
<thead>
<tr>
<th></th>
<th>Roots and hyphae (growing into cores)</th>
<th>Soil C pre-ingrowth</th>
<th>Soil C post-ingrowth</th>
<th>Change from initial</th>
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<tr>
<td>Ambient CO2</td>
<td>$-27.5$ (0.21)</td>
<td>$-33.2$ (0.17)</td>
<td>$-32.3$ (0.07)</td>
<td>0.9*</td>
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<tr>
<td>Elevated CO2</td>
<td>$-37.5$ (0.60)</td>
<td>$-27.6$ (0.06)</td>
<td>$-28.3$ (0.07)</td>
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Pyrolysis-gc-ms

The molecular structure of SOM was analysed using pyrolysis gas chromatography/mass spectrometry (py-GCMS), a technique that provides a broad molecular profile of the organic composition of SOM (Wickings et al. 2011). Air-dried, pulverised soil samples were pyrolysed at 600 °C for 20 s on a CDS Pyroprobe 5150 pyrolyser (CDS Analytical Inc, Oxford, PA, USA). Pyrolysis products were transferred to a Thermo Trace GC Ultra (Thermo Fisher Scientific, Austin, TX, USA) via a 300 °C transfer line and passed through a 60 m fused silica capillary column (SGE Incorporated, Austin, TX, USA) (0.25 μm i.d.) over 60 m. The GC oven temperature was increased from 40 to 270 °C at a ramp rate of 5 °C min⁻¹ with a final ramp from 270 to 300 °C (30 °C min⁻¹). Compounds were then transferred to a Thermo Polaris Q ion trap mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA) and ionised with a heated electron source (200 °C). Peaks were analysed using the Automated Mass Spectral Deconvolution and Identification System (V 2.65) with the National Institute of Standards and Technology compound library. Compound relative abundance was determined relative to the total identified ion signal and all compounds were grouped into chemical classes (determined relative to the total identified ion signal and all compound classes; sensu Wickings et al. 2011; Tables S1 and S2). Compound class ‘profiles’ were identified using non-metric multidimensional scaling (NMS). Given initial differences in chemistry between soils collected from the ambient and elevated CO₂ plots, we calculated the change in abundance of each compound (relative to the initial abundances) over the course of the 1-year incubation period. For example, soils placed into cores in the ambient CO₂ plots had phenol abundances of 19% initially and 31% at harvest – a 12% increase in the proportion of phenols. Compound class data were plotted using NMS (Figure S5).

Calculation of root-derived C

Dried samples were ground and the C isotope ratios were analysed by continuous-flow isotope ratio mass spectrometry at the Duke Environmental Stable Isotope Laboratory (Durham, NC, USA). Because of the unique isotopic ¹³C signatures in the soil and roots from ambient and elevated CO₂ plots, we calculated net inputs of C from roots and fungi as:

\[ \text{Root-derived } C = \frac{C_{\text{root}} * (\delta^{13}C_{\text{root}} - \delta^{13}C_{0})}{(\delta^{13}C_{\text{root}} - \delta^{13}C_{0})} \]

where \( C_{\text{root}} \) is the concentration of C in the core, \( \delta^{13}C_{\text{root}} \) is the ¹³C of the SOM at the end of the experiment, \( \delta^{13}C_{0} \) is the ¹³C of the SOM at the beginning of the experiment (i.e. prior to ingrowth) and \( \delta^{13}C_{\text{root}} \) is the ¹³C of the root- or mycorrhizal-derived fungi.

To examine the extent to which the accelerated turnover of root-derived C contributes to the lack of soil C accumulation under elevated CO₂ at this site (Lichter et al. 2008; Drake et al. 2011), we multiplied the percentage of new C by the C content of the cores at the time of harvest, and calculated the difference in the amount of C stored between ambient and elevated CO₂ plots.

Sensitivity analysis

In our initial analysis, we assumed that all inputs to the cores were derived from fixed C using the ¹³C values in Table 1. If hyphae from saprotrophic fungi – which have different ¹³C signatures relative to mycorrhizal fungi – grew into the cores, then our calculation of root-derived C would be incorrect. To test the sensitivity of this assumption, we increased the percentage of saprotrophic fungal inputs from 0 to 60%, and quantified the CO₂ effect on root-derived C stored (Figure S2). To do this, we assumed that the mycelium of saprotrophic fungi would be enriched in ¹³C by 2‰ relative to SOM (Högberg et al. 1999). Negative values indicate less C storage in elevated CO₂ plots relative to ambient CO₂ plots.

Experimental design and statistical analyses

We used a split-plot within a randomised complete block design for root and soil analyses. The split-plot design accounted for the fertilisation treatment (half of each ring was fertilised) and the blocking accounted for an NPP gradient across the site. Each block contained one ambient and one elevated CO₂ ring. We used PROC MIXED (SAS 2003) to examine the interaction between block, CO₂, N, type of ingrowth core and core group location (i.e. the spatial location of each group of three cores). Linear regression was used to examine the relationship between C stored and changes in phenolic abundances and N availability. NMS was conducted using PC-ORD (MJM Software Design, Gleneden Beach, OR, USA) on chemical class data determined using py-GCMS following the methods outlined previously. For the ordination, a Sørensen distance matrix was used with a random starting configuration, and 20 and 50 runs with real and randomised data respectively. The ordination was run initially by requesting a six-dimensional solution followed by a step down in dimensionality until an adequate number of dimensions was determined. The number of dimensions in the final solution was chosen based on an instability criterion of 0.0005. Correlative relationships between individual chemical classes and ordination scores were assessed using Pearson correlation.

RESULTS

Root- and hyphal-derived inputs of C into the ingrowth cores resulted in significant changes in the ¹³C of soils within the transplanted cores after 1 year (Table 1). Such changes were apparent in both types of root exclusion cores, with inputs of new root-derived C in the spin cores likely resulting from rapid proliferation of ectomycorrhizal mycelium which can grow ~1 cm day⁻¹ (Donnelly et al. 2004). Fine root growth within the root accessible cores was greater under elevated CO₂ (Figure S1), yet less root-derived C was sequestered in all ingrowth cores in the elevated CO₂ plots (Fig. 1; \( P < 0.0001 \)). The magnitude of this difference depended on soil
nitrogen availability (Fig. 1; CO₂ × N: P = 0.002). In the root accessible cores in unfertilised plots, 42% less C was sequestered under elevated CO₂ whereas in the fertilised plots the decline in C sequestration was similar in magnitude but not statistically significant (Fig. 1). Even less recently fixed C was stored in the root exclusion cores under elevated CO₂: CO₂ enrichment decreased C storage of hyphal-derived C by 82% in the unfertilised root exclusion cores and by 59% in fertilised cores (Fig. 1; P < 0.0001). Overall, less root-derived C was stored in the fertilised cores (P < 0.0001).

We examined the robustness of the CO₂ effect by testing the sensitivity of the mixing model. Our tests indicate that the ingrowth of saprotrophic fungi would need to represent > 30% of all hyphal biomass in the root accessible cores or > 70% of all hyphal biomass in the root exclusion cores to provide an alternative explanation for the observed CO₂ treatment effects on C (Figure S2). Such a scenario is highly unlikely given previous ingrowth core studies – including one from this site (Parrent & Vilgalys 2007) – which indicate that mycorrhizal hyphae comprise over 85% of the active mycelial growth in low C soils (Hendricks et al. 2006).

Both root and mycorrhizal activities altered SOM composition relative to the initial pre-ingrowth soils (Table S2), but the magnitude and direction of these effects depended on source of the belowground inputs (Figure S3; Table S1). Of the identified compounds that could be assigned to a chemical class (> 65% of all identified compounds), phenolic compounds were most abundant (35% of the total), followed by polysaccharides (26%) and proteins (21%). Notably, phenolic abundances increased in cores in the ambient CO₂ plots but decreased in soils in the elevated CO₂ plots (Fig. 2a; CO₂ effect: P < 0.0001). These changes in the relative abundance of phenolics were positively correlated with the amount of C stored in the cores at the time of harvest (r² = 0.43; P = 0.003; Fig. 2b).

The lower amount of root-derived C stored under elevated CO₂ was also linked to available N in soil at the time of harvest. Net N mineralisation potential in unfertilised cores was 75% greater under elevated CO₂ relative to ambient CO₂, and was negatively correlated with the amount of root-derived C sequestered in soil. All three core types are included. White triangles refer to ambient CO₂, black triangles refer to elevated CO₂. The best fit line for all cores is described by the linear equation y = 0.04x – 0.033 (r² = 0.39; P < 0.001).

**DISCUSSION**

Multiple CO₂ enrichment experiments in forests have reported little or no C accumulation in mineral soils (Lichter et al. 2008; Talhelm et al. 2009; Drake et al. 2011; Hoosbeek et al. 2011) – and in some cases have documented enhanced soil C losses (Carney et al. 2007) – despite large increases in aboveground and belowground productivity. To date, these patterns have been attributed to priming
Figure 3 The relationship between N availability and root-derived C sequestered in unfertilised and fertilised soils under ambient and elevated CO2 for all three core types. In (a) net N mineralisation rates (n = 4 replicate cores) decline as the amount of root-derived C stored increases (\( y = -0.06x + 0.19; r^2 = 0.49; P = 0.01 \)). In (b), the abundance of N-bearing compounds (n = 4 replicate cores) decrease as the amount of root-derived C increases indicating reduced N availability (\( y = -0.01x - 0.004; r^2 = 0.66; P = 0.001 \)). White triangles refer to ambient CO2, black triangles refer to elevated CO2.

effects, defined broadly as increases in the decomposition of old soil C owing to microbial utilisation of new C. The results presented here suggest that in addition to priming, the lack of soil C accumulation in forests exposed to elevated CO2 may result from the accelerated turnover of recently fixed C from rhizodeposits and mycorrhizal fungi – a process which also releases N. Hence, plants may be both enhancing the availability of N by stimulating microbial decomposition of SOM via priming and increasing the rate at which N cycles through the microbial pools owing to the rapid turnover of N-rich fungal tissues.

We found significantly less new root-derived C sequestration in soils under elevated CO2 across all treatments (Fig. 1) despite measured CO2-induced increases in fine root production in our cores (Figure S1) and previous work documenting increases in fine root and mycelial production and turnover in response to elevated CO2 at this site (Pritchard et al. 2008a,b). Rather, the reduced storage of new C resulted from the accelerated turnover of root and fungal inputs – a finding consistent with the response of young trees exposed to elevated CO2 (Heath et al. 2005; Godbold 2006). Multiplying the percentage of new C (Fig. 1) by the C content of the cores at the time of harvest, and calculating the differences in C storage between ambient and elevated CO2 plots, we estimate that ~114 g C m\(^{-2}\) year\(^{-1}\) more root-derived C may be respired (i.e. lost) in unfertilised soils under elevated CO2. In a recent mass-balanced belowground C budget at the site, Drake et al. (2011) reported that heterotrophic soil microbes respired 100 g C m\(^{-2}\) year\(^{-1}\) more C under elevated CO2. Hence, the accelerated loss of new root-derived C reported here is sufficient in magnitude to account for the enhanced CO2 fluxes and associated lack of soil C accumulation in this forest. We recognise that the growth of roots and fungi into the artificial matrix of an ingrowth core may overestimate estimates of root-derived inputs and turnover (Hendricks et al. 2006); nevertheless, our estimates are comparable to those in other forests (Courty et al. 2010; Wallander et al. 2011) and are sufficient in magnitude to explain the missing source of C input to soils (89–142 g C m\(^{-2}\) year\(^{-1}\)) in the belowground C budget at the Duke FACE site (Table 1 in Drake et al. 2011).

Much of the decrease in the residence time of new C under elevated CO2 may be attributed to the rapid turnover of rhizodeposits and mycorrhizal structures that turn over in weeks to months (Pritchard et al. 2008b; Drigo et al. 2012; Fernandez & Koide 2012) in contrast with fine roots which turn over far more slowly (Matamala et al. 2003). Fine root lifespans at this site are 574 and 500 days in ambient and elevated CO2 plots respectively (Pritchard et al. 2008a). As such, changes in root mortality would have contributed little to the enhanced turnover of new C during the 1-year ingrowth period. This is further supported by our finding that the reduction in new C storage under elevated CO2 was similar in the root accessible (1.1 g C kg soil\(^{-1}\) year\(^{-1}\)) and root exclusion cores (1.2 g C kg soil\(^{-1}\) year\(^{-1}\)). We multiplied the standing crop of fine roots from the root accessible ingrowth cores at the time of harvest by in situ exudation rates measured previously in these same plots (Phillips et al. 2011). Our results indicate a strong negative correlation between exudation inputs and new root-derived C remaining in the cores, as the ingrowth cores receiving the largest root and fungal exudate inputs stored the least C (Figure S4). Thus, under elevated CO2 the increased root litter inputs are decomposed more quickly due to the enhanced release of labile C exudates which stimulates microbial decomposition of both the root and mycelial tissues.

We explored alternative explanations for the observed reductions in the storage of new root-derived C under elevated CO2. First, a portion of the C deposited into the ingrowth cores may have originated from sources other than roots. Dissolved organic carbon (DOC) leachate from decomposing leaf litter has the same isotopic signature as root-derived sources, and the lower amounts of new C stored under elevated CO2 could have resulted from reduced DOC leaching. However, this mechanism cannot explain the CO2 effects, as DOC losses from organic horizons at this site do not differ between ambient and elevated CO2 plots (Oh et al. 2007). Second, the isotopic signature of the ambient cores (which contained soil from the elevated CO2 plots) may become enriched due to the preferential decomposition of young, 13C-depleted C independent of ingrowth-mediated effects. Such effects would be strongest in cores containing significant amounts of 13C-depleted soil fungi which have a \( \delta^{13} \text{C} = -37_{\text{oom}} \) (Billings & Ziegler 2008). However, our initial pre-incubation step should have significantly reduced the amount of highly depleted C in the initial soils placed into the cores in the ambient plots. Furthermore, most of the microbial biomass in the elevated CO2 soils is dominated by microbes containing isotopic signatures enriched by 5–6_{\text{oom}} relative to fungi (Billings & Ziegler 2008). As such, the turnover of microbial pools would have
little effect on altering the $^{13}$C of the ingrowth core soils. Hence, the accelerated turnover of young root and hyphal-derived C provides the most parsimonious explanation for the reductions in C storage under elevated CO$_2$.

The most striking change we observed in the SOM chemical composition was the reduction in the relative abundance of phenolic compounds under elevated CO$_2$ in all core types. We also observed consistent increases in the proportional abundance of polysaccharides under elevated CO$_2$ and all cores exposed to elevated CO$_2$ had proportionally less lignin and protein through time. These represent unexpectedly large and rapid transformations in the chemistry of SOM with divergent trajectories under different CO$_2$ treatments. Notably, the cores with large reductions in phenolic compound abundance stored the least new C (Fig. 3b). Although many phenolic compounds in forest soils are relatively recalcitrant, extracellular enzymes released by rhizosphere microbes – including mycorrhizal fungi – can degrade these compounds (Chalot & Brun 1998). The results of our ingrowth core study suggest that increased mycorrhizal demand for N under elevated CO$_2$ may stimulate the synthesis of oxidative enzymes that depolymerise root-associated phenolics that bind N, consistent with previous reports of enhanced phenol oxidase activity in the rhizosphere at the Duke FACE site (Phillips et al. 2011) and in the bulk soil at the Rhinelander FACE site (Chung et al. 2006).

It is interesting to speculate on whether the reduced residence time of root-derived C has consequences for N availability and forest productivity. Ectomycorrhizal hyphae are 2–6% N by mass, with much of the N stored as amino sugars within chitin. Recent studies suggest that ectomycorrhizal mycelia turnover rapidly (e.g. 50% mass loss in 14 d; Drigo et al. 2012; Fernandez & Koide 2012), with the rates of decomposition depending on fungal N content (Koide & Malcolm 2009) and chitin content (Fernandez & Koide 2012). Given that the decomposition of fungal litter may be driven by the enzymatic activities of living ectomycorrhizal mycelium mining fungal necromass for N (Drigo et al. 2012), the accelerated turnover of hyphal tissues under elevated CO$_2$ may represent an important source of N to plants and microbes. Enzymes involved in the breakdown of chitin are often greater in forests exposed to elevated CO$_2$ (Larson et al. 2002; Phillips et al. 2011) and ectomycorrhizal fungal activities under elevated CO$_2$ are generally increased (Alberton et al. 2005). Whether increases in the turnover of these tissues can explain the accelerated N cycling observed at the Duke FACE site (Drake et al. 2011) and Rhinelander FACE site (Zak et al. 2011) is an intriguing question. Ectomycorrhizal mycelia has a C : N ranging from 14 to 29 (Wallander et al. 2003). Hence, the additional turnover of hyphal litter at the Duke FACE site (~114 g C m$^{-2}$ year$^{-1}$) could release 4–8 g N m$^{-2}$ year$^{-1}$ – a quantity of N sufficient in magnitude to account for the additional N taken up by trees since the onset of CO$_2$ enrichment (~4 g N m$^{-2}$ year$^{-1}$; Drake et al. 2011). At the Rhinelander FACE site, the additional turnover of 14–29 g C m$^{-2}$ year$^{-1}$ of hyphal litter would be required to balance the ‘extra’ N taken up by poplar trees under elevated CO$_2$ (~1 g N m$^{-2}$ year$^{-1}$, Zak et al. 2011). This flux is similar in magnitude to the amount of mycelial turnover in poplar trees exposed to elevated CO$_2$ at the POPEuro-FACE site (15–20 g C m$^{-2}$ year$^{-1}$; Godbold 2006). Whether such dynamics exist in forests dominated by arbuscular mycorrhizal trees, which produce less extramatrical hyphal biomass and have limited saprotrophic capabilities (Read & Perez-Moreno 2003), warrants further study.

Collectively, our results demonstrate that much of the root- and mycorrhizal-derived C entering forest soils under elevated CO$_2$ cycles rapidly, a process which limits soil C accumulation and increases rates of N cycling (Fig. 3). Hence, although greater

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**Figure 4** Conceptual model of Rhizo-Accelerated Mineralisation and Priming or RAMP. RAMP predicts that under elevated CO$_2$, greater inputs of root-derived C to soil via enhanced rhizodeposition (Rhizodep.), exudation and allocation to mycorrhizal fungi (myco. fungi) are offset by accelerated losses of C owing to greater microbial enzyme activities. This results in limited net C storage in soil despite enhanced belowground inputs. The attenuation of soil C gains increases N availability owing to the rapid turnover of N-rich fungal tissues and priming of SOM. Solid arrows refer to C fluxes from one pool to another while dotted lines refer to enzymatic activities that mediate the turnover of the litter (red) and SOM (blue) pools. The thickness of the arrows indicates the magnitude of the flux or activity.
amounts of C may enter soils from enhanced belowground production, less of this new C is stored owing to accelerated decomposition by microbes. These findings support the emerging view that root-induced changes in the rates of microbial processing of C and N are key mediators of long-term ecosystem responses to global change (Phillips 2007). We refer to this as the Rhizo-Accelerated Mineralisation and Priming or RAMP hypothesis (Fig. 4). RAMP predicts that under elevated CO₂, the amount of new C stored as stable SOM depends on the proportion of root and fungal necromass that becomes microbial residues or gets protected from enzymatic activity. In addition, RAMP predicts that under elevated CO₂, plants not only increase the rate at which N cycles through the microbial pools owing to the rapid turnover of N-rich fungal tissues but also enhance the overall availability of N by stimulating microbial decomposition of SOM via priming. The consequences of RAMP for long-term forest productivity will thus depend on the extent to which the N which is cycled through small, fast-turnover pools (e.g. amino acids in the soil solution, microbial biomass) is supplemented by N inputs from large, slow-cycling SOM pools. This is analogous to ocean systems where N released from slow-cycling pools induces ‘new production’ (sensu Eppley & Peterson 1979) – a process distinct from ‘regenerated production’ whereby N is released from fast-cycling pools.

After nearly two decades of research on forest ecosystem responses to global change, uncertainty about the role of roots and rhizosphere processes in soil C and N retention and loss has limited our ability to predict biogeochemical feedbacks to long-term forest productivity (Norby & Zak 2011). Recent efforts to incorporate mycorrhizal fungi into models suggest that ectomycorrhizal fungi may increase soil C storage by increasing plant productivity and reducing nutrient availability for saprotrophic fungi (Orwin et al. 2011). The results presented here indicate that decreases in the residence time of root-derived inputs and priming effects under elevated CO₂ (i.e. RAMP) may counterbalance this effect and have important consequences for N availability and forest productivity. Accordingly, ecosystem models which incorporate root and rhizosphere responses to global environmental change should lead to improved projections of long-term C storage in forests (Ostle et al. 2009).

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AUTHOR CONTRIBUTIONS

RPP and ESB designed the research; RPP and AF performed the research; SG and KW conducted the pyrolysis-gcms analysis; RPP, ICM and KW analysed the data; and RPP wrote the manuscript, with significant contributions from all co-authors.

REFERENCES


