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Changes in forest soil organic matter pools after a decade of elevated CO_2 and O_3

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ABSTRACT

The impact of rising atmospheric carbon dioxide (CO_2) may be mitigated, in part, by enhanced rates of net primary production and greater C storage in plant biomass and soil organic matter (SOM). However, C sequestration in forest soils may be offset by other environmental changes such as increasing tropospheric ozone (O_3) or vary based on species-specific growth responses to elevated CO_2. To understand how projected increases in atmospheric CO_2 and O_3 alter SOM formation, we used physical fractionation to characterize soil C and N at the Rhinelander Free Air CO_2–O_3 Enrichment (FACE) experiment. Tracer amounts of $^{15}NH_4^+$ were applied to the forest floor of *Populus tremuloides*, *P. tremuloides–Betula papyrifera* and *P. tremuloides–Acer saccharum* communities exposed to factorial CO_2 and O_3 treatments. The ^{15}N tracer and strongly depleted ^{13}C–CO_2 were traced into SOM fractions over four years. Over time, C and N increased in coarse particulate organic matter (cPOM) and decreased in mineral-associated organic matter (MAOM) under elevated CO_2 relative to ambient CO_2. As main effects, neither CO_2 nor O_3 significantly altered ^{15}N recovery in SOM. Elevated CO_2 significantly increased new C in all SOM fractions, and significantly decreased old C in fine POM (fPOM) and MAOM over the duration of our study. Overall, our observations indicate that elevated CO_2 has altered SOM cycling at this site to favor C and N accumulation in less stable pools, with more rapid turnover. Elevated O_3 had the opposite effect, significantly reducing cPOM N by 15% and significantly increasing the C:N ratio by 7%. Our results demonstrate that CO_2 can enhance SOM turnover, potentially limiting long-term C sequestration in terrestrial ecosystems; plant community composition is an important determinant of the magnitude of this response.

1. Introduction

A major challenge for accurately predicting the extent to which C storage in terrestrial ecosystems will counterbalance anthropogenic CO_2 emissions rests, in part, on our understanding of soil organic matter (SOM) dynamics. Elevated atmospheric CO_2 consistently stimulates plant growth (Norby et al., 2005), thereby increasing plant detritus inputs into soil and potentially increasing the formation of SOM. However, other environmental factors, such as elevated atmospheric O_3 concentrations and species-specific growth responses to elevated CO_2 may modify the long-term storage of anthropogenic CO_2 in SOM (Kubiske et al., 2007; Ren et al., 2007; Sitch et al., 2007; Zak et al., 2007). Therefore, the extent to which greater plant growth in a CO_2-enriched atmosphere will influence soil C storage remains uncertain.

Changes in plant litter production due to elevated CO_2 and O_3 may alter the formation and cycling of SOM. For example, forest communities exposed to elevated CO_2 exhibit greater net primary production (NPP), increased litter production, and enhanced rhizodeposition (Norby et al., 2005; Phillips et al., 2006). In contrast, elevated O_3 damages leaves, which can lead to declines in photosynthesis, above and belowground growth, and inputs of plant litter to SOM (Grantz et al., 2006; Zak et al., 2007). These changes in plant growth may feedback to affect decomposition and the distribution of C and N in SOM pools of varying composition and stability, as well as the potential for forest soils to sequester C (Jastrow et al., 2007). In addition, species-specific responses to elevated CO_2 and O_3 can alter competitive interactions among plants (Kubiske et al., 2007), as well as modify plant–soil interactions (Gill et al., 2006). Because plant species differ in litter biochemistry, in addition to their response to atmospheric CO_2 and O_3, the need to understand community-level responses further complicates our ability to forecast the consequences of these rising trace gases.

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Physical fractionation of SOM into uncomplexed and mineral-associated pools can provide a sensitive measure of changes in soil C and N dynamics, wherein uncomplexed pools can often display greater responses to environmental change than whole (unfractionated) SOM (Christensen, 2001; Gregorich et al., 2006). Physically isolated fractions based on particle size or density are widely used to gain insight into the characteristics of SOM and the nature of soil C and N cycling (Accoe et al., 2002; Liao et al., 2006). These fractionation approaches consider that microbial decay metabolized plant residues into smaller organic molecules (Tiessen and Stewart, 1983). Evidence from studies using radiocarbon and ^{13}C indicate that physically isolated soil fractions differ widely in their turnover rates, with turnover rates generally slowing with diminishing fraction size (Bird et al., 2002). Although elevated CO_2 can both increase and decrease soil C storage (Jastrow et al., 2005; Talhelm et al., 2009), interactions with nutrient availability (Carney et al., 2007; Peralta and Wander, 2008), or soil texture (Langley et al., 2009) can alter the response of SOM. Changes in C and N cycling within SOM pools or in the transfer of C and N between pools could have important implications for soil N availability as well as long-term C sequestration. However, it is largely unknown how changes in plant litter production under elevated CO_2 will influence physically isolated SOM pools, and how the response might be modified by plant species composition and elevated O_3.

The primary objective of this study was to evaluate how elevated CO_2 and O_3 influence the formation of SOM and the distribution of C and N in uncomplexed particulate organic matter (POM) and mineral-associated organic matter (MAOM) pools. Plants exposed to elevated atmospheric CO_2 have acquired additional C (King et al., 2005), N, and ^{15}N (Zak et al., 2007). This additional C, N and ^{15}N enters the soil via plant litter that decomposes to generate SOM. Therefore, we expect that increased plant N demand and enhanced litter inputs under elevated CO_2 would result in enhanced POM N and C stocks, and increased ^{15}N content in the POM, plus reduced N and C stocks in MAOM. By contrast, we expect elevated O_3 to counteract the effects of elevated CO_2, causing no changes in C, N, and ^{15}N in SOM pools compared to ambient CO_2 and O_3. Alternatively, even though trees grow faster under elevated CO_2 and acquire more N and ^{15}N by more fully exploring soil, changes in SOM dynamics respond more slowly and may remain undetectable at the decadal time scale. The second objective of this research was to evaluate the importance of species-specific interactions in determining the response of C and N cycling in physically isolated SOM pools under elevated atmospheric CO_2 and O_3.

2. Materials and methods

2.1. Research site

Our study was conducted at the Rhinelander FACE (Free Air CO_2–O_3 Enrichment) experiment located near Rhinelander, Wisconsin, USA (49°40.5′ N, 89°37.5′ E, 490 m elevation; Karnosky et al., 1999). In 1997, twelve 30-m diameter FACE rings were established and planted with trembling aspen (*Populus tremuloides* Michx.), sugar maple (*Acer saccharum* Marsh.), and paper birch (*Betula papyrifera* Marsh.). Half of each FACE ring was planted with five genotypes of trembling aspen, which differ in CO_2 and O_3 sensitivity. One ring quarter was planted at equivalent densities of aspen and sugar maple; the remaining quarter was planted with aspen and paper birch. We refer to the split-plots of differing plant species composition as aspen, aspen–birch and aspen–maple. Each ring was assigned to factorial CO_2 ($n = 2$) and O_3 ($n = 2$) treatments in a randomized complete-block design (Dickson et al., 2000). Elevated CO_2 and O_3 concentrations were applied during the daylight hours throughout the growing season (May through October). Elevated CO_2 was maintained at 200 μL/L above ambient concentrations (~560 μL/L) and elevated O_3 was maintained at 30–40 nL/L above ambient (50–60 nL/L; Karnosky et al., 2005). Soils were Alfic Haplorthods with a sandy loam Ap horizon overlaying a sandy clay loam Bt horizon. The site was formerly under agricultural production and was planted with hybrid poplar and larch beginning in 1972, prior to creation of the FACE experiment. Initial soil physical and chemical properties were summarized by Dickson et al. (2000).

2.2. Isotope labeling and soil sampling

In late June 2003 backpack sprayers were used to evenly dispense (0.034 L/m^2) a dilute solution of $^{15}NH_4Cl$ (99.98% ^{15}N) over the forest floor of each FACE ring. The isotope was applied at the rate of 15 mg $^{15}N/m^2$ (3% of the extractable inorganic N pool, 0–10 cm depth) followed by 1.6 L/m^2 of water to move the tracer into the mineral soil (Zak et al., 2007).

Soils were collected prior to isotope labeling in 2003, and following isotope addition in early July 2004 and early October 2007. In each plant community of each ring, we collected and composited randomly located soil cores (4.8 cm diameter) to a depth of 20 cm (5 cores per community, totaling15 cores per ring in 2003 and 2004; 3 cores per community, totaling 9 per ring in 2007). In the field, samples were homogenized through an 8-mm sieve to remove roots and coarse fragments (rocks and organic debris) while minimizing disruption of soil aggregates. Root-free, sieved samples were stored at 4 °C, and transported to the lab, where they were air-dried. Subsamples of the whole soil were thoroughly hand picked to remove remaining visible roots, ground and analyzed for C and N concentration, and $\delta^{13}C$ and $\delta^{15}N$ using a Delta plus isotope ratio mass spectrometer (Thermo-Finnigan, San Jose, California, USA) interfaced to an NC2500 elemental analyzer (CE Elantech, Lakewood, New Jersey, USA).

Soil bulk densities were determined in 2004 and 2007; soil mass was divided by core volume, with appropriate corrections for soil moisture and coarse fragments (Talhelm et al., 2009). Because bulk density did not change significantly between 2004 and 2007 ($R^2 < 0.1$, $P > 0.5$; Talhelm et al., 2009), the 2004 bulk densities were used for all calculations of C and N stocks.

2.3. Soil fractionation

Soil organic matter (SOM) was physically fractionated according to a modified version of Cambardella and Elliott (1992) to isolate three particle-size classes, including coarse POM (cPOM, >250 μm), fine POM (fPOM, 250–53 μm), and MAOM (<53 μm). Soils were passed through an 8-mm sieve in the field and air-dried, to enable future analysis of the size distribution of water stable aggregates. After hand-picking to remove any remaining visible roots and organic debris >8 mm long, a 10-g subsample of soil was shaken with 40 mL of deionized water on a reciprocal shaker for 16 h to disperse aggregates. Because of the relatively high sand content and weak aggregate stability of the soil, deionized water was used rather than a solution of sodium hexametaphosphate, and this provided sufficient dispersion of soil particles. The soil was then transferred to a 250-μm sieve stacked on top of a 53-μm sieve and rinsed thoroughly with deionized water to separate and collect cPOM and fPOM, respectively. The slurry of material that passed the 53-μm sieve was transferred to a 1-L centrifuge bottle. Three mL of a solution containing 0.25 M $MgCl_2$ and 0.25 M $CaCl_2$ was added to flocculate clays, and the sample was centrifuged at 2000 *g* for 10 min at 22 °C. Soil fractions were dried at 65 °C, weighed, ground, and analyzed for C and N concentration, and $\delta^{13}C$ and $\delta^{15}N$ as previously described for whole soil. On average, 99.5 ± 0.1% of the whole soil mass was recovered during the size fractionation procedure.

Summation of the fractions recovered 88.4 ± 1.3% of the whole soil C. Nitrogen recovery determined from summation was 111.6 ± 2.4%, which was likely due to slight overestimation of N concentration values from the elemental analyzer caused by the low amounts of N in the POM fractions. The fractionation method isolates both POM and sand, which contributes to the low N concentrations in POM. In this study, cPOM included recent inputs that are a source for new soil C (<2 mm). To quantify how much of the cPOM fraction included live fine roots and organic debris, we hand picked roots and litter that were 2–8 mm in size from a subset of cPOM samples ($n = 16$) and calculated their contribution to cPOM stocks. Using concentrations of 500 mg C g^{-1} and 11 mg N g^{-1} for litter (Liu et al., 2009), and 1.1 mg N g^{-1} with a C:N of 44 for fine roots (Chapman et al., 2005), we determined that the total contribution of 2–8 mm plant roots and litter to the cPOM fraction were minimal, comprising only 4.2% ± 1.5 of the cPOM C stock and 2.1% ± 0.6 of the cPOM N stock.

We used the N concentration and ^{15}N contents of whole soil and soil fractions to calculate recovery of the ^{15}N tracer. We calculated the atom % excess ^{15}N (APE ^{15}N) of each sample as the difference in atom% ^{15}N of the fraction collected after labeling and the natural abundance measured for that fraction at the start of the study. The total quantity of ^{15}N in each pool (μg ^{15}N excess g^{-1}) was then estimated as the atom% ^{15}N excess of that pool multiplied by the N content of that pool divided by 100. To evaluate differences between soil fractions, the quantity of ^{15}N was normalized by the particle-size distribution (multiply by g fraction × g^{-1} whole soil).

The depleted ^{13}C signature of the fossil fuel-derived gas used in the elevated CO_2 treatments (−18.7 ± 1.0‰ compared to −8.6 ± 0.1‰ for control and $+O_3$ plots) allowed us to employ an isotope mixing model to calculate the proportions of "new" C fixed since the beginning of the experiment and "old" pre-treatment C remaining in SOM fractions and whole soil. We used a method similar to earlier reports from the Rhinelander FACE site (Loya et al., 2003; Pregitzer et al., 2006; Talhelm et al., 2009) to calculate the proportion of new C (f) from the equation:

$$f = (\delta_t - \delta_0)/(\delta_i - \delta_0)$$

where δ_t is the $\delta^{13}C$ of the whole soil or fraction at sampling, δ_0 is the mean $\delta^{13}C$ for the corresponding soil or fraction in the control plots, and δ_i is the $\delta^{13}C$ signature of the root and leaf litter inputs. Estimates of ^{13}C signatures of root and leaf litter inputs for each year (δ_i) were derived from data and assumptions reported by (Talhelm et al., 2009), where contributions from roots and leaf litter were assumed to be equal and estimates were calculated as the mean of all input signatures from each growing season prior to the sampling year. Because the average difference in the $\delta^{13}C$ between leaves and roots was <0.5‰, altering the proportion of new C has minimal effects on subsequent analyses (Talhelm et al., 2009). New C was

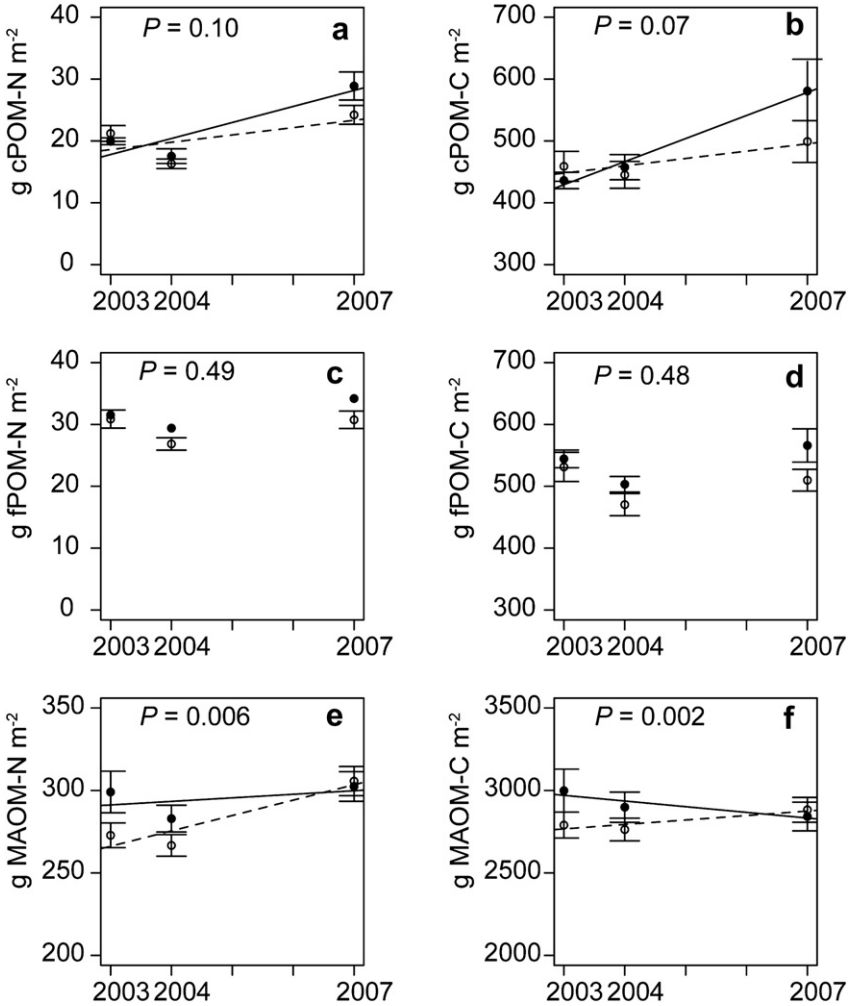

Fig. 1. C and N in cPOM, fPOM, and MAOM fractions under ambient (open circles) and elevated (solid circles) CO_2. Values are CO_2 by time interaction means, and error bars are ±1 SE. Reported P values are for CO_2 by time interactions.

calculated by multiplying the proportion new (f) by the mass of total C in the whole soil or fraction; old C was calculated by subtracting new C from total C in whole soil or fraction.

2.4. Statistical analyses

The effects of elevated atmospheric CO_2 and O_3 concentrations and community type on C and N stocks, ^{15}N recovery, new and old C, and C:N ratio of soil size fractions were evaluated using a repeated-measures ANOVA for a split-plot, randomized complete-block design; separate ANOVAs were used for each fraction. Data were natural log-transformed as needed prior to statistical analysis to fulfill the assumption of normality, but the untransformed means and standard errors are reported in figures. Tukey's HSD was used to determine significant differences among means. We used SAS version 9.1 for all statistical tests (SAS Institute, Cary, North Carolina). All effects and comparisons were considered significant at $\alpha \leq 0.1$ because of constraints on replication, and the desire to minimize the chances of failing to detect differences due to Type II error.

3. Results

3.1. N and C stocks

Time was significant as a main effect ($P \leq 0.001$), wherein cPOM N (2007 > 2003 > 2004), and cPOM C (2007 > 2004 = 2003) generally increased over time. Similarly the fPOM N (2003 = 2007 > 2004), fPOM C (2004 > 2003 = 2007) and MAOM N (2007 > 2003 = 2004) varied significantly over time. Time as a main effect did not influence MAOM C.

As a main effect, CO_2 concentration did not influence the N ($P \geq 0.28$) and C ($P \geq 0.36$) of SOM fractions. Nonetheless, cPOM N and C increased faster over time under elevated CO_2, with 19% more N and 16% more C compared to ambient CO_2 in 2007 ($CO_2 \times$ time $P = 0.10$ for N; $P = 0.07$ for C; Fig. 1a,b). The N and C of MAOM had the opposite trajectory. In 2003, MAOM N and C were 10 and 7% higher, respectively, under elevated CO_2 but by 2007 were 1% lower than those in ambient CO_2 ($CO_2 \times$ time $P = 0.006$ for N; $P = 0.002$ for C; Fig. 1e,f). No significant main or interaction CO_2 effects were detected in fPOM N or C (Fig. 1c,d).

Elevated CO_2-induced increases in cPOM N were greatest beneath the aspen–birch (+26%) and aspen–maple (+9%) communities, whereas N decreased (−8%) beneath aspen ($CO_2 \times$ species $P = 0.009$; Fig. 2). Community responses to elevated CO_2 for N in fPOM and MAOM were not statistically significant. Species-specific CO_2 responses in C of measured SOM fractions paralleled the changes in N (Fig. 2). The CO_2 response in cPOM C was greatest in aspen–birch (+23%) with no changes in aspen–maple and 6% decrease beneath aspen ($CO_2 \times$ species $P = 0.04$). As with N, the species-specific response of fPOM and MAOM C to elevated CO_2 was not significant.

As a main effect, elevated O_3 reduced cPOM and fPOM N by 14% relative to ambient O_3 ($P = 0.04$ for cPOM and 0.07 for fPOM) with no significant effects on the MAOM fraction. In contrast, O_3 had no significant main effects on the C in any measured SOM fraction

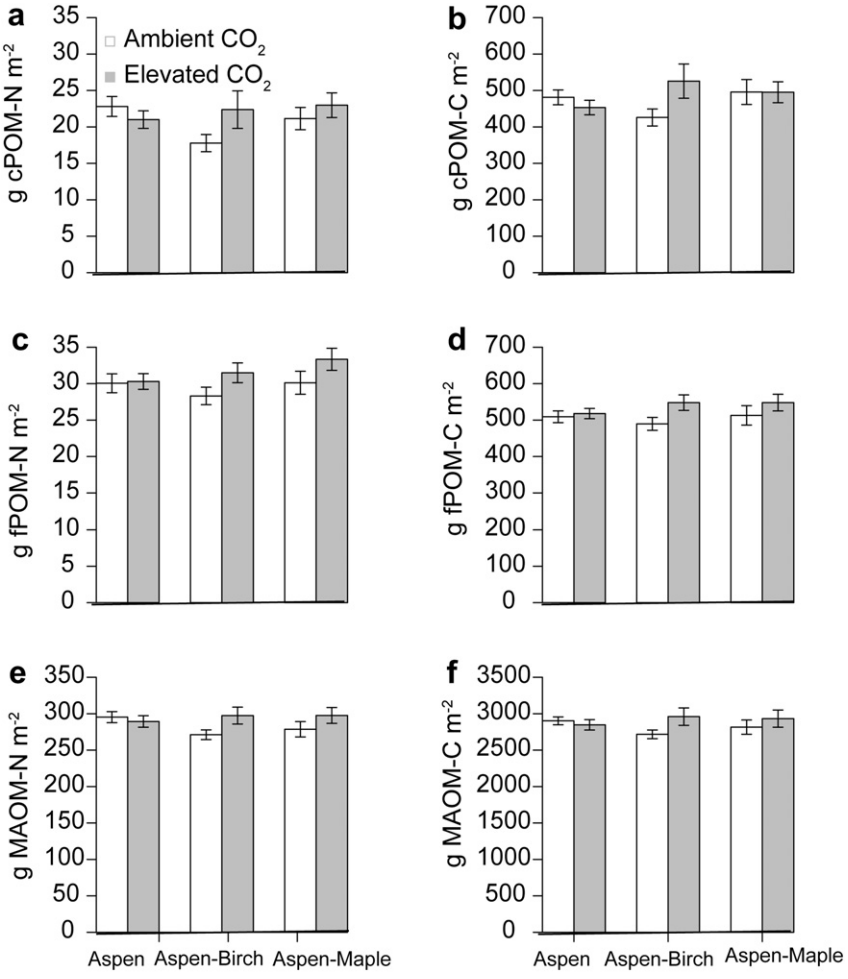

Fig. 2. C and N in cPOM, fPOM, and MAOM fractions under ambient and elevated CO_2. Values are CO_2 by species interaction means, and error bars are ±1 SE.

($P \geq 0.20$). Further, no statistically significant CO_2 by O_3 ($P \geq 0.29$) or O_3 by species ($P \geq 0.31$) interactions were detected for the N or C of any SOM fraction.

3.2. C:N ratios

Atmospheric O_3 exerted significant main effects, and interacted with CO_2 to increase the C:N ratio of measured SOM fractions. For example, the C:N of cPOM increased by 7% under elevated O_3, compared to ambient O_3 ($P = 0.04$; Fig. 3). However, CO_2 moderately interacted with O_3, where on average, elevated CO_2 alone caused a 7% decrease in the C:N of cPOM, and O_3 counteracted this effect, inducing a 5% increase in the cPOM C:N ratio in elevated $CO_2 + O_3$ treatment ($CO_2 \times O_3$, $P = 0.10$). In the fPOM, O_3 increased the C:N ratio by almost 6% relative to ambient O_3 ($P = 0.007$; Fig. 3). The O_3 effect on fPOM C:N was greatest in 2003 (7.6% increase) and diminished with time (3% increase in 2007; $O_3 \times$ time $P = 0.01$). The C:N of the MOAM under elevated O_3 increased by ~2% compared to ambient conditions ($P = 0.07$; Fig. 3), with no significant O_3 by time interactions.

Plant community composition significantly altered the C:N ratio of cPOM and fPOM, with aspen–birch communities producing higher C:N ratios (Table 1). The C:N of the cPOM was significantly greater in soils beneath aspen–birch compared to soils beneath aspen or aspen–maple (main effect; $P = 0.007$). Similarly for fPOM, greater C:N ratios occurred under aspen–birch and aspen compared to aspen–maple ($P = 0.03$). No species-specific changes in the C:N of MAOM were detected.

3.3. ^{15}N recovery

On average ^{15}N recovery was greatest in the MAOM (34.0 ± 1.9%) followed by fPOM (11.5 ± 0.77%) and cPOM (10.3 ± 0.94%). For all SOM fractions ^{15}N recovery increased over time as labeled plant litter entered the soil and was incorporated into SOM fractions ($P < 0.0001$; Fig. 4). Soils exposed to elevated CO_2 tended to contain more ^{15}N in cPOM (+28%) and fPOM (+10%), and less ^{15}N (−5%) in MAOM than did the same soil fractions under ambient CO_2; however, only the increase in fPOM recovery was statistically significant ($P = 0.09$). As a main effect, O_3 did not affect the recovery of ^{15}N among any of the SOM fractions ($P \geq 0.17$). In the MAOM fraction a moderately significant CO_2 by time interaction was detected, where ^{15}N recovery under elevated CO_2 was greater than for ambient in 2004 (+5%) and less in 2007 (−10%; $P = 0.08$). No significant CO_2 or O_3 by time interactions were detected for the other SOM fractions ($P \geq 0.13$). Elevated CO_2 tended to increase the ^{15}N recovery to a greater extent under aspen–birch compared to aspen or aspen–maple communities; however CO_2 by species interactions on ^{15}N recovery were not statistically significant.

Plant community type exerted a significant main effect on the ^{15}N recovery in SOM fractions, independent of atmospheric gas concentrations. For example, almost twice as much ^{15}N was recovered in the cPOM beneath aspen–maple communities compared to aspen communities, and 44% more ^{15}N was recovered in the cPOM fraction in the aspen–maple soil compared to that in the aspen–birch community ($P = 0.006$). Similarly, the fPOM from beneath the aspen–maple community contained more ^{15}N than fPOM beneath aspen (24%) or aspen–birch (41%) communities ($P = 0.010$). We found no significant O_3 or $CO_2 \times O_3$ effects ($P \geq 0.17$).

Table 1
The C:N ratio for bulk soil and soil size fractions in aspen, aspen–birch and aspen–maple plots at the Rhinelander FACE experiment.

Particle-Size Fraction	Plant Community		
	Aspen	Aspen–Birch	Aspen–Maple
Bulk Soil	11.77 ± 0.31a	11.92 ± 0.33a	11.66 ± 0.35a
cPOM	21.91 ± 0.65a	24.53 ± 0.69b	23.05 ± 0.65a
fPOM	17.14 ± 0.19b	17.42 ± 0.13b	16.78 ± 0.15a
MAOM	9.87 ± 0.08a	9.99 ± 0.07a	9.99 ± 0.08a

Values are averages of 36 field replicates and standard errors. Values followed by the same letter are not significantly different between plant communities at $P \leq 0.05$ (according to repeated-measures ANOVA and Tukey's test).

3.4. Isotopic partitioning of C stocks under elevated CO_2

Overall, "new" C (fixed under elevated CO_2 since the experiment's inception) significantly increased in all SOM fractions and whole soil over the duration of our study, whereas "old" C (pretreatment C, not necessarily recalcitrant C) significantly decreased in fPOM, MAOM and whole soil (Fig. 5; $P \leq 0.001$). Thus, for cPOM, the overall increase in total cPOM C ($P = 0.06$) under elevated CO_2 (Fig. 5a) was due to the addition of new C ($P = 0.0003$) to a stable old C pool. In contrast, old C was lost from the fPOM pool (Fig. 5b; $P = 0.0009$), but new C accumulated ($P < 0.0001$) at a rate that outpaced the loss of old C, leading to an increase in total fPOM C over time ($P = 0.01$). For the MAOM fraction (Fig. 5c), however, the significant accumulation of new C in this pool ($P < 0.0001$) merely compensated for the loss of old C ($P = 0.0006$), thereby maintaining constant total MAOM C stocks during the course of this study ($P = 0.85$). Similarly, for whole soil in the elevated CO_2 treatments

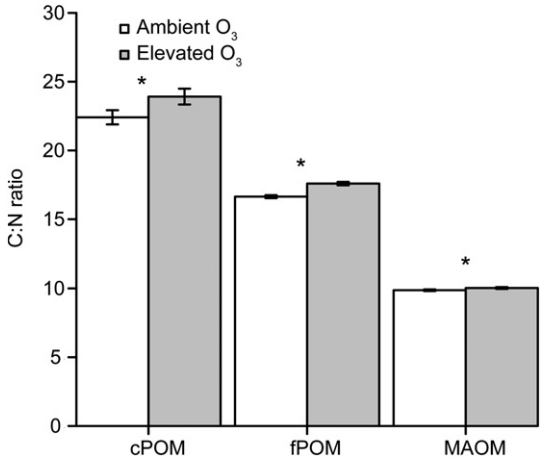

Fig. 3. Main effect of O_3 on the C:N ratio of SOM fractions. Values are means of three sampling dates, and error bars are ±1 SE. Asterisks denote the significant O_3 main effect at $P < 0.10$.

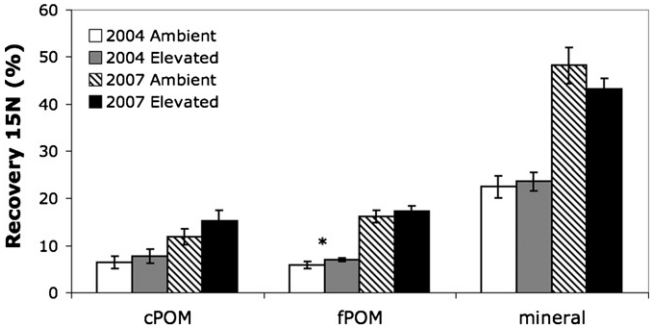

Fig. 4. The effect of CO_2 in 2004 (ambient white, elevated gray) and 2007 (ambient hatched, elevated black) on % recovery of ^{15}N in SOM fractions, including cPOM, fPOM and MAOM. Values are means across three communities, and error bars are ±1 SE.

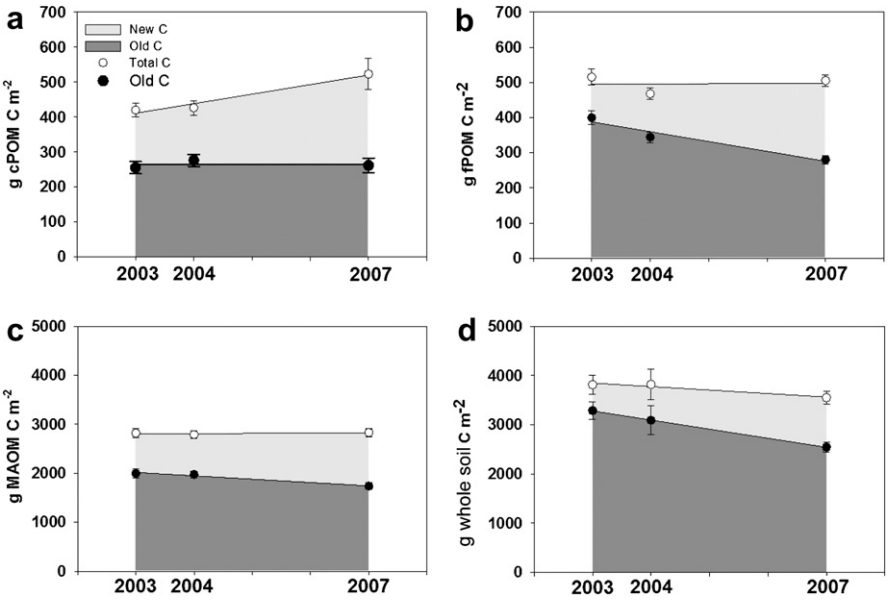

Fig. 5. Incorporation of free air CO_2 enrichment (FACE)-derived CO_2 into SOM fractions and whole soil over time. Values for old (pre-treatment) C (dark circles, dark gray shading), total C (white circles), and new C (light gray shading) are means from both ambient and elevated O_3 combined, and error bars are ±1 SE.

(Fig. 5d), the overall accumulation of new C ($P = 0.0005$) simply replaced declining old C ($P = 0.0002$), with no net change in total soil organic C during the course of this study ($P = 0.27$).

For the SOM fractions and whole soil, there were no significant O_3 by time effects on new C or old C. The addition of O_3 to elevated CO_2 significantly reduced new C stocks in whole soil ($P = 0.03$). Because none of the individual SOM fractions showed a significant reduction in new C ($P \leq 0.43$), but all slightly decreased, we conclude that the CO_2 by O_3 interaction effect on the whole soil was likely distributed across several, if not all the SOM fractions (Fig. 6a), but any individual changes were too small be statistically significant. Some C could also have been lost with the unmeasured soluble fraction. This is a common and well-documented problem for physical SOM fractionation procedures (Chan, 2001; Crow et al., 2007; Moran et al., 2005). In contrast, old POM C stocks were measurably greater under elevated $CO_2 + O_3$ (Fig. 6b; $P \leq 0.10$). However, O_3 had no detectable effect on the total C stocks of any SOM fraction or whole soil ($P \geq 0.36$). The negative effect of O_3 on new C occurred largely in the aspen–maple community, but only MAOM exhibited a significant O_3 by species interaction ($P = 0.10$). No significant O_3 by species interactions or main effects of community were found on old C. Overall, new C in whole soil was greatest under aspen and least in aspen–maple ($P = 0.03$). This trend was not consistent for the SOM fractions, and only fPOM had a significant community effect – with less new C under aspen–maple than the other two communities ($P = 0.09$).

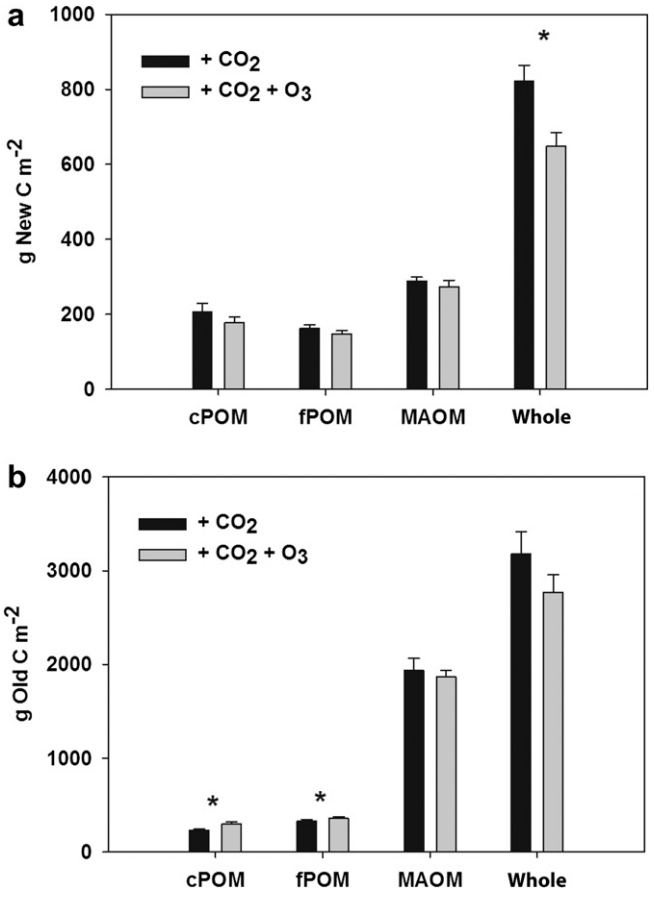

Fig. 6. Effect of ambient and elevated O_3 on new C incorporated from free-air CO_2 enrichment (FACE)-derived CO_2 into SOM fraction and whole soil C stocks. Values are means of three sample times and error bars are ±1 SE.

4. Discussion

4.1. Elevated CO_2 effects on SOM cycling

The rising atmospheric CO_2 concentration has the potential to increase plant growth, enhancing inputs of C in terrestrial ecosystems, and thereby increasing soil organic matter; however, projected O_3 concentrations and species-specific responses to CO_2 may diminish these inputs (Kubiske et al., 2007; Talhelm et al., 2009). Previous studies have found no effects of elevated CO_2 or elevated O_3 on the N concentration of total SOM, despite increased rates of

soil N cycling (Holmes et al., 2006; Zak et al., 2007). Similarly no significant main effects of CO_2 or O_3 on whole soil C have been detected after a decade of fumigation, despite slower SOM formation rates within the aspen community under elevated CO_2 (Talhelm et al., 2009). In contrast, this study provides evidence that, over time, the C and N in SOM fractions are sensitive to changes in atmospheric trace gas concentrations. Further, it appears that the processing of SOM has been altered under elevated CO_2, as indicated by more rapid increases in cPOM stocks and concurrent decrease in MAOM, relative to ambient CO_2 dynamics over the duration of our study (Fig. 1).

The timing of sampling (mid-season in 2003 and 2004 compared to late season in 2007) may influence decomposition and affect temporal trends in C and N stocks over time, particularly in POM fractions. Time significantly affected C and N stocks, and interannual differences were more pronounced under elevated CO_2 compared to ambient CO_2. Elevated CO_2 has been shown to stimulate litter inputs (Liu et al., 2005), but the feedbacks on decomposition within the growing season cannot be elucidated, because studies have not documented seasonal variation in root turnover in our experiment. While we cannot rule out seasonal effects, our results indicate that fresh litter contributions to cPOM were minimal (<5%), suggesting that only the most rapidly decomposed material would contribute to seasonal augmentation of CO_2 effects on C and N stocks.

Change in the processing and distribution of C and N among SOM fractions has important implications for understanding the mechanisms controlling N availability and C sequestration in forest ecosystems, and also may serve as a sensitive indicator for future trends not yet apparent in whole soil. For example, under elevated CO_2, the increased partitioning of C and N into SOM pools with short residence times could diminish the net transfer of C from the atmosphere to the terrestrial biosphere (Gill et al., 2006). Our study demonstrates that elevated CO_2 can stimulate soil C and N partitioning into readily decomposable pools, and out of more stabilized mineral-associated fractions.

The POM fraction consists of plant and microbial debris that is generally of more recent origin (Ladd et al., 1977; Turchenek and Oades, 1979; Wick and Tiessen, 2008) and can be an early indicator of the effects of ecosystem changes on SOM quantity and chemistry (Gregorich et al., 1994; Haynes, 2000). Increased inputs of plant detritus and microbial immobilization together have likely caused increases under elevated CO_2 in C, N and ^{15}N in the cPOM fraction, which harbors relatively undecomposed material (Accoe et al., 2002). Because soil microbes are generally C limited (Anderson and Domsch, 1978; Bowen and Rovira, 1991; Darrah, 1996), higher inputs of labile C to the soil can induce a 'priming effect', whereby the addition of labile C to the soil stimulates microbial decomposition of older SOC (Blagodatskaya and Kuzyakov, 2008; Kuzyakov et al., 2000; Sayer et al., 2007; Torbert et al., 1998). Higher NPP under elevated CO_2 has increased the quantity of fresh C inputs entering the soil via fine root production and plant litter (Liu et al., 2005), which may include labile substrates that contribute to enhanced breakdown of more recalcitrant SOC. Contrasting results regarding the influence of labile C inputs on recalcitrant C decomposition (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2002) may be explained by the response of the extant microbial communities to the amount of labile C inputs (de Graaff et al., 2010). Although previous studies have shown that fresh C inputs can increase microbial activity and enhance recalcitrant SOC decomposition (Fontaine et al., 2007), Aspen FACE microcosm studies indicate that leaf litter additions alone do not increase the decomposition of old C (Liu et al., 2009). Because the microbial response is sensitive to the amount of C inputs, in combination leaf litter, root litter and root exudates may be stimulating the decomposition of SOM in Aspen FACE soils. For example, greater plant litter (leaf and fine root) production under elevated CO_2 (Liu et al., 2005; Zak et al., 2007) was accompanied by an increase in new C in both POM fractions (Fig. 5a and b) and whole soil (Fig. 5d), and a decrease in old C in fPOM, MAOM, and whole soil (Fig. 5b,c and d), suggesting that despite increased litter inputs, decomposition of old C from the most stable SOM pool studied is not being replenished by new C under elevated atmospheric CO_2.

As POM decomposes, it is expected to enter finer, mineral-associated organic matter pools, which may provide substrate for microbial mineralization (Ladd et al., 1977; Tiessen and Stewart, 1983). Our results are consistent with previous studies that demonstrate concurrent increases in N immobilization and mineralization under elevated CO_2 (Holmes et al., 2006), leading to more rapid rates of soil N cycling. These results suggest that N immobilized in the cPOM fraction under elevated CO_2 may be transferred into the MAOM, but this N and older mineral-associated C and N are rapidly mineralized, resulting in significant declines over time in MAOM relative to ambient CO_2 conditions (Fig. 1). Specifically, while MAOM N increased over time under ambient CO_2, N did not accrue in MAOM under elevated CO_2. In contrast, MAOM C actually decreased over time under elevated CO_2. The trend toward narrowing of MAOM C:N ratios (Fig. 1e,f) supports the idea that over time MAOM under elevated CO_2 is becoming more degraded, causing a shift in the quality of SOM (Moran and Jastrow, 2010).

At Rhinelander FACE new inputs to both coarse and fine POM (Fig. 5a,b) compensate for the loss of old C from fPOM and MAOM (Fig. 5b,c), and are responsible for maintaining the whole soil C in the elevated CO_2 treatments over time (Fig. 5d). Based on average published NPP values (Norby et al., 2005), during the course of our study, the fraction of NPP recovered in new soil C was 6%, 4% and 8% for cPOM, fPOM and MAOM. In contrast, at a poplar FACE site in Europe, C accrual in the most labile measured fraction resulted in an increase in whole soil C (Hoosbeek et al., 2006). Whether increased C accumulation in POM fractions leads to significant soil C storage depends on the extent to which these fractions are protected from decomposition in aggregates and how much of the organic matter in these pools gets transferred into mineral-associated fractions (Six and Jastrow, 2002; Tisdall and Oades, 1982).

Our results are consistent with the responses in grassland experiments in which elevated CO_2 resulted in an accumulation of SOM in labile pools and decline in the transformation of new litter into more stable SOM pools, as evidenced by an increase in POM and a decrease in mineral-associated fractions (Gill et al., 2006). In both the Rhinelander FACE study and the grassland CO_2 gradient study, no consistent increase in whole soil organic C occurred under elevated CO_2 (Gill et al., 2006, 2002; Talhelm et al., 2009). In fact, SOM is accumulating at a slower rate beneath aspen exposed to elevated CO_2 compared to ambient CO_2, despite a significant increase in above and belowground plant litter (Talhelm et al., 2009). Results from the Soy FACE experiment reveal similar findings, where POM C and N increased slightly and decomposition of SOM was accelerated in response to elevated CO_2 (Peralta and Wander, 2008). By contrast, no CO_2 effects on N cycling in SOM fractions were detected in similar studies in the sweetgum FACE experiment, in which ^{15}N-labeled tree sap traced belowground did not alter ^{15}N accumulation in POM or MAOM (Garten and Brice, 2009). Long-term data from the sweetgum FACE experiment supports the idea that N limitation is exacerbated under elevated CO_2, and ultimately dampens the CO_2-induced enhancement of NPP (Norby et al., 2010).

Similar to our study, increases in POM C under elevated CO_2 can be largely offset by loss of C from the older, mineral-bound fractions of SOM (Cardon et al., 2001; Gill et al., 2002). This represents a shift in partitioning to faster cycling SOM pools (Gill et al., 2002; Schlesinger and Lichter, 2001; Six et al., 1998; Van Kessel et al., 2000), which

parallels increased rates of N cycling (Holmes et al., 2006). Faster cycling of SOM may explain why increased storage of C and N in whole SOM has not been detected, despite greater C and N inputs to soil from plant detritus (Lichter et al., 2008, 2005; Liu et al., 2005; Talhelm et al., 2009) and greater C and N immobilization in the labile SOM fraction. Due to an increase in plant litter production, saprotrophic microorganisms may need to meet additional nutrient requirements by mineralizing older, mineral-associated SOM fractions (Gill et al., 2002). If short-term changes in the POM and MAOM provide a sensitive indicator of long-term alterations in the dynamics of SOM processing (Gregorich et al., 1994), then our results suggest that in a CO_2-enriched environment, movement of C and N from plant detritus into long-lived, mineral-bound pools will be delayed or perhaps even short-circuited. In this case, C sequestration would be limited by increased rates of SOM cycling that causes both C and N to be mineralized more rapidly.

4.2. Elevated O_3 effects on SOM cycling

Elevated O_3 tended to have the opposite effect of elevated CO_2, wherein the supply of N to plants was diminished due to decreased rates of gross N mineralization and equivalent rates of microbial immobilization (Holmes et al., 2006). Consistent with a significant ~15% increase in plant litter C:N ratio under elevated O_3 (Liu et al., 2005), we found significant decreases in the N stocks of cPOM and fPOM and significant increases in the C:N ratio of cPOM, fPOM, and MAOM (Fig. 3). Decreased soil N stocks and increased soil C:N ratio also are consistent with the lower mineralization rates. When combined with elevated CO_2, the O_3-induced trend toward increasing C:N persists in cPOM, but not in fPOM or MAOM. In addition, less new C inputs are being incorporated into SOM under elevated O_3 while more old POM C has been retained, compared to elevated CO_2 alone (Fig. 6). Given that the total C stocks of all fractions were unaffected by the O_3 treatment, it appears that elevated O_3 has the potential to reduce both inputs to SOM and cycling of soil C and N in northern hardwood forests.

4.3. Plant community effects

Consistent with decades of work, our study demonstrates that species-specific and community responses likely regulate CO_2 effects on soil C and N (Bazzaz and Garbutt, 1988; Gill et al., 2006; Hungate et al., 1996; Jackson et al., 1994; King et al., 2004; Talhelm et al., 2009; Tolley and Strain, 1984). At the Rhinelander FACE experiment, in soils beneath aspen–birch communities, ^{15}N recovery in both cPOM and fPOM tended to increase under elevated CO_2. The C:N ratios were greater for aspen–birch cPOM relative to aspen and aspen–maple and the C:N of aspen–birch fPOM was greater than aspen–maple (Table 1). These species-specific responses are likely not driven by the quantity of plant detritus, because aspen–birch communities have intermediate NPP and fine root biomass relative to aspen and aspen–maple communities (King et al., 2005; Pregitzer et al., 2008). Although elevated CO_2 has increased the concentration of condensed tannins in the aspen–birch community (Liu et al., 2005), there is no evidence that this has inhibited decomposition (Talhelm et al., 2009). Even so, CO_2 by species interactions were only significant for C and N in cPOM, and did not persist in fPOM or MAOM, suggesting that differences in litter decomposition rates among species are driving this species-specific response. A portion of the cPOM includes labile inputs that are not well protected and are most sensitive to changes in decomposition rates. Our results are consistent with previous research indicating that community composition may be a key factor regulating CO_2 effects on soil C content (Talhelm et al., 2009).

Under elevated CO_2, significant community effects were detected for new C, but not for old C. The whole soils of the aspen community had the greatest amount of new C overall, followed by aspen–birch then aspen–maple soils, confirming the findings of (Talhelm et al., 2009). In addition, the aspen–maple community differed from the other communities in its response to elevated $CO_2 + O_3$, with significantly lower amounts of new C being incorporated into the MAOM of this community. Previous studies indicate that community type does not interact with CO_2 or O_3 to influence the biomass or N concentration of roots or leaf litter (Zak et al., 2007). Therefore, community-specific C cycling responses to $CO_2 + O_3$ are likely driven by difference in microbial communities (Carney et al., 2007) rather than changes in the amount or chemical composition of inputs. In general, we found that community responses to O_3 differ from response to CO_2, where aspen–maple is more affected than aspen or aspen–birch communities. Identifying the precise microbial mechanisms driving species-specific changes in C and N partitioning among soil fractions is an important next step for extrapolating CO_2 and O_3 effects across a wide range of community types.

5. Conclusions

The capacity of terrestrial ecosystems to provide long-term C storage depends on the balance among NPP, the production and delivery of plant detritus to soil, and its subsequent decomposition into stable forms of SOM. Our study demonstrates that elevated O_3 generally diminished N in SOM fractions, whereas elevated CO_2 increased new C in all SOM fractions, and significantly decreased old C in fPOM and MAOM. Because elevated atmospheric CO_2 decreased C and N stabilization in mineral-associated SOM pools relative to ambient CO_2, our results suggest elevated CO_2 increased soil C and N cycling. While increased SOM cycling may support greater NPP, it may ultimately limit the ability of forest soils to sequester C.

The lack of a significant interaction between atmospheric CO_2 and O_3 on the C and N in POM and MAOM fractions may indicate that O_3 can elicit a negative effect of SOM cycling, regardless of atmospheric CO_2 concentration. While atmospheric CO_2 and O_3 are important modifiers of SOM pools, community-specific responses must be considered to accurately forecast the extent to which atmospheric chemistry will influence the C sequestration capacity of forest ecosystems. By understanding changes in plant–soil interactions under changing atmospheric conditions, we are beginning to understand the mechanisms controlling ecosystem C and N cycling. Accelerated SOM cycling under rising atmospheric CO_2 could have profound implications for the exchange of C from forest ecosystems to the atmosphere and the ability of soils to sequester C. Ultimately, if the C gained by increased plant productivity under elevated atmospheric CO_2 is lost through decomposition of relatively stable SOM, net C sequestration will be limited in many forest ecosystems.

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