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Enhanced litter input rather than changes in litter chemistry drive soil carbon and nitrogen cycles under elevated CO_2: a microcosm study

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Abstract

Elevated CO_2 has been shown to stimulate plant productivity and change litter chemistry. These changes in substrate availability may then alter soil microbial processes and possibly lead to feedback effects on N availability. However, the strength of this feedback, and even its direction, remains unknown. Further, uncertainty remains whether sustained increases in net primary productivity will lead to increased long-term C storage in soil. To examine how changes in litter chemistry and productivity under elevated CO_2 influence microbial activity and soil C formation, we conducted a 230-day microcosm incubation with five levels of litter addition rate that represented 0, 0.5, 1.0, 1.4 and 1.8 × litterfall rates observed in the field for aspen stand growing under control treatments at the Aspen FACE experiment in Rhinelander, WI, USA. Litter and soil samples were collected from the corresponding field control and elevated CO_2 treatment after trees were exposed to elevated CO_2 (560 ppm) for 7 years. We found that small decreases in litter [N] under elevated CO_2 had minor effects on microbial biomass carbon, microbial biomass nitrogen and dissolved inorganic nitrogen. Increasing litter addition rates resulted in linear increase in total C and new C (C from added litter) that accumulated in whole soil as well as in the high density soil fraction (HDF), despite higher cumulative C loss by respiration. Total N retained in whole soil and in HDF also increased with litter addition rate as did accumulation of new C per unit of accumulated N. Based on our microcosm comparisons and regression models, we expected that enhanced C inputs rather than changes in litter chemistry would be the dominant factor controlling soil C levels and turnover at the current level of litter production rate ($230\,g\,C\,m^{-2}\,yr^{-1}$ under ambient CO_2). However, our analysis also suggests that the effects of changes in biochemistry caused by elevated CO_2 could become significant at a higher level of litter production rate, with a trend of decreasing total C in HDF, new C in whole soil, as well as total N in whole soil and HDF.

Keywords: $\delta^{13}C$, decomposition, DIN, EMMA, global change, MBC, MBN, new soil C, old soil C, stable isotope

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Introduction

Atmospheric CO_2 concentration has increased during the past 250 years, with the rate of increase actually accelerating during the past 10 years (IPCC, 2007). Elevated CO_2 is known to stimulate plant growth as

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long as other factors are not limiting. Norby *et al*. (2005) analyzed the response of net primary production (NPP) to elevated CO_2 in four forest FACE experiments and found a median stimulation of $23 \pm 2\%$. A number of studies have shown that increases in plant growth and productivity under elevated CO_2 were associated with decreased litter N concentration (Norby *et al*., 2001; King *et al*., 2005b; Liu *et al*., 2007). Because soil microbial communities are generally more competitive for existing soil N than plants (Sylvia *et al*., 1998), it has been hypothesized that increased inputs of litter with higher C/N under elevated CO_2 may decrease litter decomposition rate while increasing microbial N immobilization. Together, these changes could decrease N availability for plant growth and produce a negative feedback on NPP enhancement (Strain & Bazzaz, 1983; Luo *et al*., 2004; Hu *et al*., 2006). Experimental results have been inconclusive in either forest or agricultural ecosystems (Torbert *et al*., 2000; Norby *et al*., 2001). Several studies have shown that small reductions in litter [N] under elevated CO_2 will have little impact on mass loss rate for litter (Torbert *et al*., 2000; King *et al*., 2001; King *et al*., 2005b; Booker *et al*., 2005). In addition, rates of gross and net N mineralization have, generally, not been altered by elevated CO_2 across forest FACE experiments despite changes in leaf tissue chemistry (Finzi *et al*., 2001; Zak *et al*., 2003; Johnson *et al*., 2004; de Graaff *et al*., 2006).

Independent of whether the observed enhancement in NPP is persistent, it is uncertain if increased NPP will lead to significant and long-term increases in soil C storage (Lichter *et al*., 2005). In particular, we know little about how increased detrital inputs to soils might affect long-term storage of soil organic C (SOC) in natural or agricultural ecosystems (Torbert *et al*., 1998; Lichter *et al*., 2005; de Graaff *et al*., 2006). Research results on the effects of elevated CO_2 on soil C formation have been mixed. Studies conducted in open-top chambers on native prairie in Kansas showed that soil C stocks increased under elevated CO_2, with more C accumulation in the physically protected SOC (Williams *et al*., 2000). In contrast, Carney *et al*. (2007) found that elevated CO_2 increased phenol oxidase activity and fungal abundance in the soils in a scrub oak ecosystem, as well as SOC decomposition rates.

Our previous work has shown that a 150 ppm increase in atmospheric CO_2 concentration increased aboveground litter production in aspen and birch stands by 33%, which significantly increased the flux of labile and nonlabile C to the soil at the Aspen FACE experiment (Liu *et al*., 2005). Because soil microbes preferentially utilize simple organic compounds over complex polymers, an increase in litter inputs to the soil could slow the decomposition of older or more resistant C, resulting in an increase in sequestered soil C (Sylvia *et al*., 1998; Cardon *et al*., 2001). On the other hand, soil microbes are generally C limited (Anderson & Domsch, 1978), and a higher flux of labile C to the soil could result in a 'priming effect', whereby decomposition of older SOC is enhanced (Torbert *et al*., 1997; Sayer *et al*., 2007). However, surprisingly few studies have directly investigated the effects of changes in litter mass inputs under elevated CO_2 on litter decomposition and N mineralization (Torbert *et al*., 1998, 2000). To address this knowledge gap, we conducted a 230-day microcosm study designed to examine separately the effects of changes in litter production and changes in litter chemistry on microbial metabolism and SOC formation. We used leaf litter and soil samples collected from the aspen community in control and elevated CO_2 treatments at the Aspen FACE experiment in Rhinelander, WI, USA. We hypothesized that (1) at equal litter addition rate, litter from the elevated CO_2 treatment would result in lower respiration rates due to its lower N concentration compared with control litter; (2) increased litter addition would not only stimulate soil respiration and C mineralization but also increase SOC due to increasing inputs of slowly decomposing C.

Methods

Study site

This study took place at the Aspen FACE experiment, which has factorial treatments of elevated and ambient CO_2 and O_3 organized in a randomized complete block design replicated three times (Dickson *et al*., 2000). Each plot is split into three species assemblages: aspen (*Populus tremuloides* Michx), aspen/birch (*Betula papyrifera* Marsh) and aspen/maple (*Acer saccharum* Marsh). To clearly target the negative feedback hypothesis, litter and soil were collected only from the aspen community in all control (ambient CO_2, ambient O_3) and elevated CO_2 (560 ppm CO_2, ambient O_3) treatment plots. Fumigation at Aspen FACE began in May 1998, soon after planting, and has since continued during daylight hours of the growing season.

Soil and litter sampling

In July 2005, three soil cores (10 cm diameter $\times$ 25 cm deep) were collected from the aspen section of each plot with a Giddings soil corer (Giddings Corp., Fort Collins, CO, USA) and immediately frozen. Forest floor material was removed before sampling. The cores were shipped frozen to North Carolina State University, where they were thawed individually and cleaned of all root material and coarse organic matter (OM). Soils were then

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composited by CO_2 treatment, homogenized and sieved (1 mm mesh) to remove rocks and additional roots and debris.

Naturally senesced aspen leaf litter was collected using litter traps (43 cm diameter) from the aspen community in control and elevated CO_2 plots from June to October in 2004. After removing litter from understory plants and coarse woody material, aspen leaf litter was also composited by CO_2 treatment, air dried and ground in liquid nitrogen. Initial litter biochemistry (soluble sugars, lipids, condensed tannins, phenolics, hemicellulose, lignin, %C and %N) was analyzed according to the procedures detailed in Liu *et al*. (2005).

Microcosm design and litter addition rate

Microcosms were constructed of plastic jars which were filled with soils from the Aspen FACE experiment to a depth of 10 cm. The base level of litter addition rates was determined according to litter : soil ratio as described by Randlett *et al*. (1996). Specifically, 1.0 and 1.4 g of pulverized litter were each mixed into 40 g of soil, which was equivalent to, respectively, litterfall under control ($230\,g\,C\,m^{-2}\,yr^{-1}$) and elevated CO_2 ($302\,g\,C\,m^{-2}\,yr^{-1}$) treatments in 2004 at the Aspen FACE experiment.

To better characterize microbial responses to substrate forcing, we also included 0, 0.5 and 1.8 g litter addition rates for both control and elevated CO_2 treatments. This resulted in 10 treatment combinations with 11 replicates per treatment combination (5 levels of litter addition rate $\times$ 2 CO_2 levels $\times$ 11 replicates = 110 microcosms). Litter was well mixed with 40 g soil and placed into a 120 mL jar with a surface area of 20 cm^2. Septa were fitted on lid to allow sampling of headspace gas.

Laboratory incubation of microcosms

Microcosms were incubated in dark at 28 °C. Microbial respiration rates were measured at least weekly throughout the 230-day incubation. Headspace gas was sampled from five jars for each mass addition treatment and analyzed for CO_2 concentration using an infrared gas analyzer (EGM-4; PP Systems, Hitchin, UK). To avoid excessive CO_2 accumulation in the headspace, jars were sealed only for 2 h before gas sampling. DI water (2 mL) was added into each jar every day to maintain constant soil moisture.

For each treatment combination, three jars were sampled at day 26, 120 and 230 for determinations of soil microbial biomass C (MBC), microbial biomass N (MBN), dissolved organic C (DOC) and dissolved inorganic N (DIN) (NO_3^- and NH_4^+). Both MBC and MBN were measured using the chloroform fumigation extraction method (Vance *et al*., 1987). For each soil sample, two subsamples (10 g dry equivalent) were prepared. One was extracted by shaking for 45 min with 35 mL of 2 M KCl and filtered through No. 1 Whatman filter paper. The second subsample was fumigated with chloroform for 48 h in the dark, followed by the same KCl extraction as the first subsample. All the extracts were stored frozen at −20 °C until analysis. DOC in the extracts was measured on a TOC analyzer (TOC-5050A; Shimadzu Corporation, Kyoto, Japan). Nitrogen in the extracts was quantified using a Lachat Automated Ion Analyzer (Lachat Quickchem Systems, Milwaukee, WI, USA). MBC was estimated by subtracting the total DOC of nonfumigated subsamples from the fumigated subsamples, using a conversion factor of 0.33 (Tu *et al*., 2006 b). Using a conversion factor of 0.45 (Tu *et al*., 2006 b), we calculated MBN as the difference in extractable N between the fumigated and nonfumigated samples after alkaline persulfate digestion (Cabrera & Beare, 1993). DIN was determined as dissolved N concentration from nonfumigated extracts without alkaline persulfate digestion.

Density fractionation and C determination

Density fractionation of soil samples was performed at the time of microcosm construction and at the end of the 230-day incubation, following a procedure adapted from Sollins *et al*. (1984) and Tu *et al*. (2006 a). Soil samples (10 g) were placed in 50 mL polycarbonate centrifuge tubes and filled with 45 mL of $1.6\,g\,mL^{-1}$ KI solution. The tubes were shaken by hand and left at room temperature for 2 h. The supernatant containing OM with density $<1.6\,g\,mL^{-1}$ (the light fraction) was gently removed from tubes by pipetting. The residual material with a density $>1.6\,g\,mL^{-1}$ [the high density fraction (HDF)] was then washed with 10 mL of DI water by centrifugation (3 $\times$), oven dried at 60 °C and ground to a fine powder for %C, %N and stable isotope analysis. Typically, the light fraction ($d<1.6\,g\,mL^{-1}$) is considered to be mineral-free particulate organic matter (POM), whereas the HDF ($d>1.6\,g\,mL^{-1}$) contains completely humified fine POM, relatively OM-free sand and OM-rich clays (Baisden & Amundson, 2002). Litter, the whole soil and HDF ($d>1.6\,g\,mL^{-1}$) were analyzed for %N, %C, $\delta^{15}N$ and $\delta^{13}C$ by a Thermo Finnigan DELTA Plus mass spectrometer (Analytical Services Lab, Department of Soil Science, North Carolina State University). The signature of ^{13}C and $\delta^{15}N$ of litter and soil used for microcosm construction are listed in Table 1.

End member mixing analysis was used to determine the contributing sources of soil OM (Christophersen *et al*., 1990). Litter and soil were selected as end members and $\delta^{15}N$ and $\delta^{13}C$ were used as conservative

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Table 1 Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) signature of litter and soil collected from pure aspen community under ambient and elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA

	Soil	Litter
$\delta^{15}N$		
Ambient	8.8 ± 0.5^{a}	16.1 ± 0.6^{b}
Elevated	10.1 ± 01^{a}	16.3 ± 0.1^{b}
$\delta^{13}C$		
Ambient	-27.5 ± 0.2^{b}	-29.1 ± 0.0^{a}
Elevated	-29.6 ± 0.2^{b}	-42.9 ± 0.1^{a}

Values are means ± SE. The differences of means between ambient and elevated CO_2 were determined by paired *t*-test. Within a row, means with different letters are significantly different ($P < 0.05$, $n = 3$).

tracers. The proportion of end members contributing to soil C after 230 days incubation (P_l: %C derived from litter and P_s: %C derived from initial soil) were estimated by the least-squared procedures developed by Christophersen *et al*. (1990).

Calculations and statistical analyses

The cumulative C lost by respiration during incubation was calculated as

$$\text{cumulative C loss} = \sum_{i=0}^{n} R_i T_i$$

where n is the number of incubation days, R_i is the mean respiration rate ($g\,C\,h^{-1}\,kg^{-1}$ soil) between two successive respiration measurements, T_i is the hours between two successive respiration measurements.

New C was defined as soil C derived from litter added during this experiment, and its concentration was estimated by:

$$c_{new} = P_l \times c_{total}.$$

Old C was defined as soil C derived from initial soil and was estimated as:

$$c_{old} = P_S \times c_{total},$$

where c_{total} is total C concentration in soil.

The initial concentrations of chemical constituents and signatures of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) of litter and soil were analyzed for the differences of means between ambient and elevated CO_2 by paired *t*-test ($P < 0.05$). Effects of litter addition rate and CO_2 treatments on microbial respiration rate were analyzed using repeated measured analysis of variance. MBC, MBN, DOC and DIN were analyzed using an ANOVA with microcosm harvest time included as a split plot treatment. The effect of litter addition rate (Y) on C or N concentrations (X) in whole soil and HDF were assessed by a linear regression function, $Y = a + bX$, and the difference of slopes between ambient and elevated CO_2 were compared by analysis of covariance. Data were transformed to meet the assumptions of normality and homogeneity of variances when necessary. All the statistical analyses were done using SAS (Statistical Analysis System, Version 9; SAS Institute Inc., Cary, NC, USA).

Results

Initial soil C and N contents and litter chemistry

Elevated CO_2 decreased litter N concentration by 13%, but did not alter the concentrations of soluble sugars, lipids, condensed tannins, soluble phenolics, hemicellulose or lignin (Table 2). The concentrations of C and N in soil samples were similar under ambient and elevated CO_2 treatment (Table 2).

Microbial respiration

Effects of litter addition rate. The microbial respiration rate of samples from the ambient and elevated CO_2 treatments showed a similar temporal pattern, with the first and highest peak occurring during day 1–3, and the second peak occurring during day 25–75 (Fig. 1). Increasing litter addition rate resulted in initially higher microbial respiration rates ($P < 0.01$), with a convergence among treatments over time (Fig. 1). Greater than 78% of total C mineralized from soils was lost in the first half of the incubation period

Table 2 Concentrations of chemical constituents ($mg\,g^{-1}$) of litter and soil collected from pure aspen community under ambient and elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA

	Ambient CO_2	Elevated CO_2
Soluble sugars	15.4 ± 1.8^{a}	22.8 ± 4.7^{a}
Lipids	56.5 ± 2.5^{a}	59.8 ± 4.1^{a}
Condensed tannins	18.5 ± 1.0^{a}	15.5 ± 2.2^{a}
Phenolics	16.4 ± 0.6^{a}	15.9 ± 0.8^{a}
Hemicelluose	194.6 ± 11.0^{a}	211.0 ± 3.04^{a}
Lignin	203.9 ± 4.6^{a}	200.7 ± 13.4^{a}
C (litter)	500.7 ± 15.0^{a}	489.2 ± 24.0^{a}
N (litter)	11.1 ± 0.0^{a}	9.5 ± 0.2^{b}
C (soil)	10.6 ± 0.2^{a}	10.7 ± 0.7^{a}
N (soil)	0.90 ± 0.01^{a}	0.87 ± 0.01^{a}

The differences of means between ambient and elevated CO_2 were determined by paired *t*-test. Values are means ± SE. Within a row, means with different letters are significantly different ($P < 0.05$, $n = 3$).

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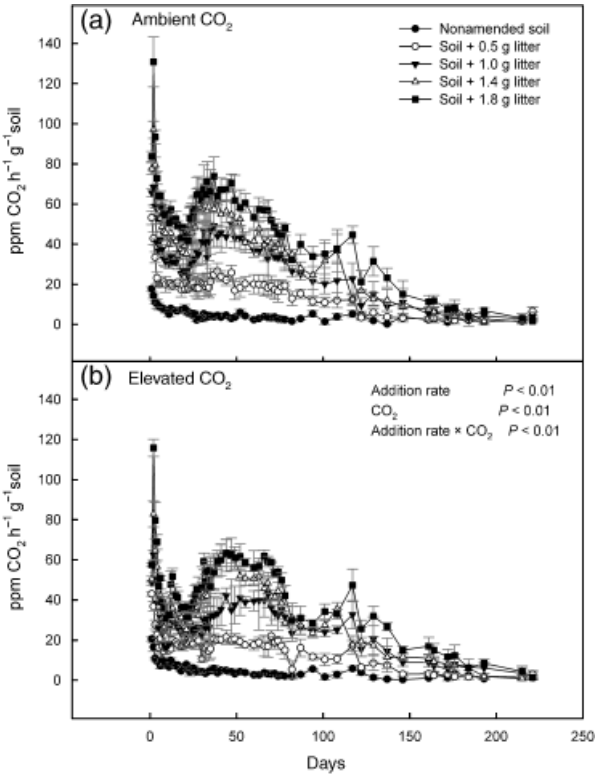

Fig. 1 Responses of microbial respiration rate to litter addition rate and CO_2 treatments. Microcosms were constructed from litter and soil collected from either ambient or elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA. Values are means ($n = 5$) ± SE.

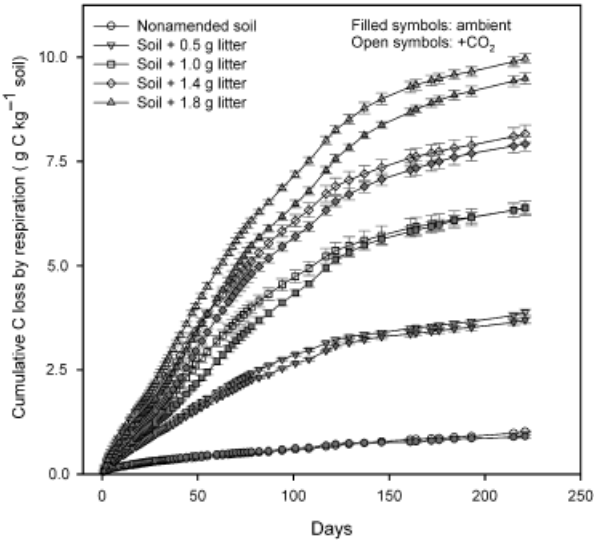

Fig. 2 Effect of litter addition rate on cumulative C loss by microbial respiration under ambient (filled symbols) and elevated CO_2 (open symbols) treatments. Microcosms were constructed from litter and soil collected from either ambient or elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA. Values are means ($n = 5$) ± SE.

(before day 115). The cumulative C lost by microbial respiration increased with litter addition rate (Fig. 2).

After 230 days incubation, about 22–30% initial C (soil + litter) had been lost by respiration (Table 3). The ratios of C loss by respiration and C input by litter amendments were 0.63 for 0.5 g, 0.52 for 1 g, 0.47 for 1.4 g and 0.44 for 1.8 g litter, respectively (Table 3).

CO_2 effects. The nonamended soil from the elevated CO_2 treatment had significantly higher cumulative C loss by respiration during the first 70 days compared with the nonamended soil from the ambient CO_2 treatment (Fig. 2). For the other four litter addition rates, soils from the elevated CO_2 treatment had significantly lower cumulative C losses in the first 100–120 days. This difference persisted beyond 100–120 days only in the 1.8 g litter-addition treatment (Fig. 2).

MBC, MBN, DOC and DIN

Effects of litter addition rate. In general, increasing litter addition rate increased MBC, but the differences were significant only at 120 and 230 days, which resulted in a significant addition rate × time interaction ($P = 0.05$) (Fig. 3a and b). MBN increased with litter addition rate but the changes of MBN were not proportional to addition rate (Fig. 3c and d). The significant addition rate × time interaction ($P = 0.01$) on DOC occurred because DOC increased with increasing litter addition rate, but tended to converge to a similar value by the end of the incubation period (Fig. 4a and b).

CO_2 effects. The significant CO_2 × time effect ($P = 0.01$) for MBN occurred because MBN increased over incubation time in the ambient CO_2 samples, but showed no significant change at elevated CO_2 (Fig. 3c and d). Elevated CO_2 had no effect on DOC at day 26, but significantly reduced DOC after 120 and 230 days, resulting in a significant CO_2 × time interaction ($P = 0.01$; Fig. 4a and b).

Compared with nonamended soils, litter additions significantly reduced DIN in soils after 26 and 120 days for both CO_2 treatments (Fig. 4c and d). However, litter-amended soils showed a rapid DIN increase over time, such that by 230 days DIN levels in amended soils were actually higher than that in nonamended soils under ambient treatment, and the difference between amended and nonamended soils were no longer significant under elevated CO_2 treatments, resulting in a significant CO_2 × addition rate × time interaction ($P < 0.01$; Fig. 4c and d).

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Table 3 Carbon budget (g C) of microcosms (litter amendments + 40 g soil) at the time of construction and at the end of the 230-day incubation

		Litter amendments				
Parameter	CO_2 treatment	0 g	0.5 g	1 g	1.4 g	1.8 g
Litter C added into microcosm	Ambient		0.25 ± 0.01	0.50 ± 0.02	0.70 ± 0.02	0.90 ± 0.03
	Elevated		0.24 ± 0.01	0.49 ± 0.02	0.68 ± 0.03	0.88 ± 0.04
Before incubation						
Total C in microcosm (litter C + soil C)	Ambient	0.42 ± 0.01	0.67 ± 0.01	0.92 ± 0.02	1.11 ± 0.03	1.41 ± 0.03
	Elevated	0.43 ± 0.01	0.68 ± 0.00	0.93 ± 0.01	1.13 ± 0.01	1.44 ± 0.02
After 230-day incubation						
C loss by respiration	Ambient	0.04 ± 0.00	0.16 ± 0.00	0.26 ± 0.01	0.33 ± 0.01	0.40 ± 0.00
	Elevated	0.04 ± 0.00	0.15 ± 0.00	0.26 ± 0.01	0.32 ± 0.01	0.38 ± 0.01
C remaining in microcosm	Ambient	0.42 ± 0.01	0.54 ± 0.00	0.68 ± 0.01	0.74 ± 0.01	0.86 ± 0.04
	Elevated	0.43 ± 0.03	0.52 ± 0.01	0.67 ± 0.02	0.73 ± 0.02	0.82 ± 0.01

Values are means ($n = 5$) ± SE. Litter and soil were collected from either ambient or elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA.

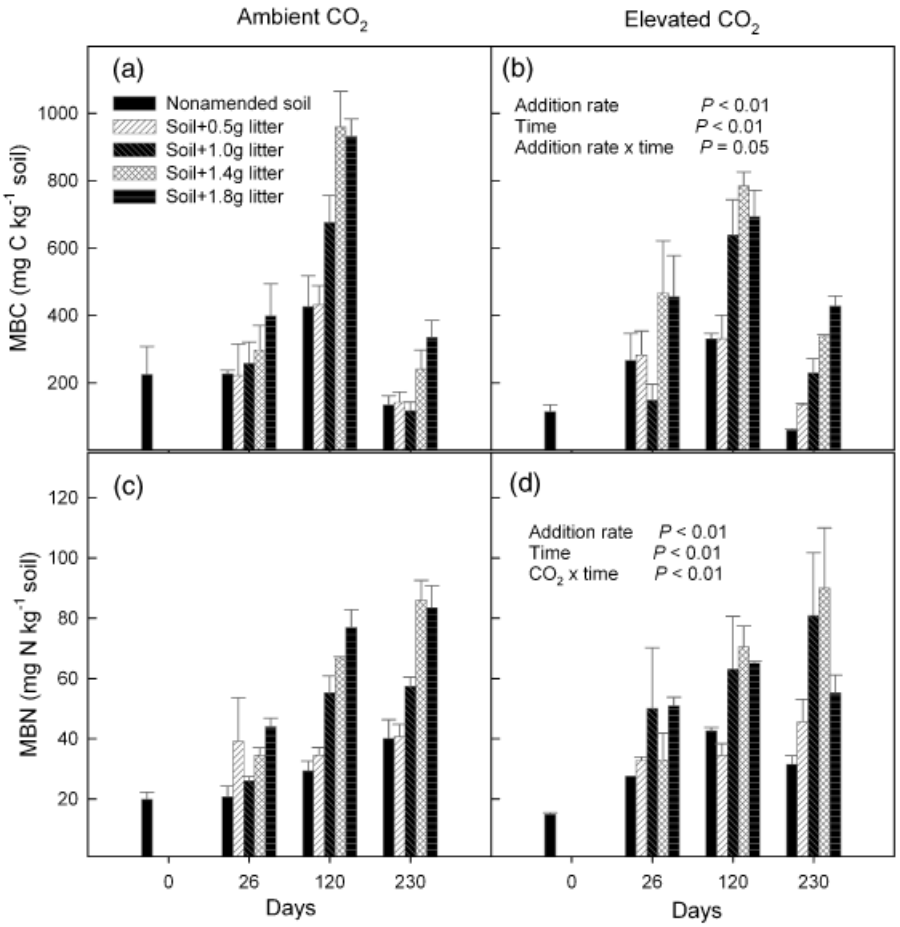

Fig. 3 Responses of microbial biomass C (MBC; a and b) and microbial biomass N (MBN; c and d) to litter addition rate and CO_2 treatments. Litter and soil were collected from either ambient or elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA. Values are means ($n = 3$) ± SE.

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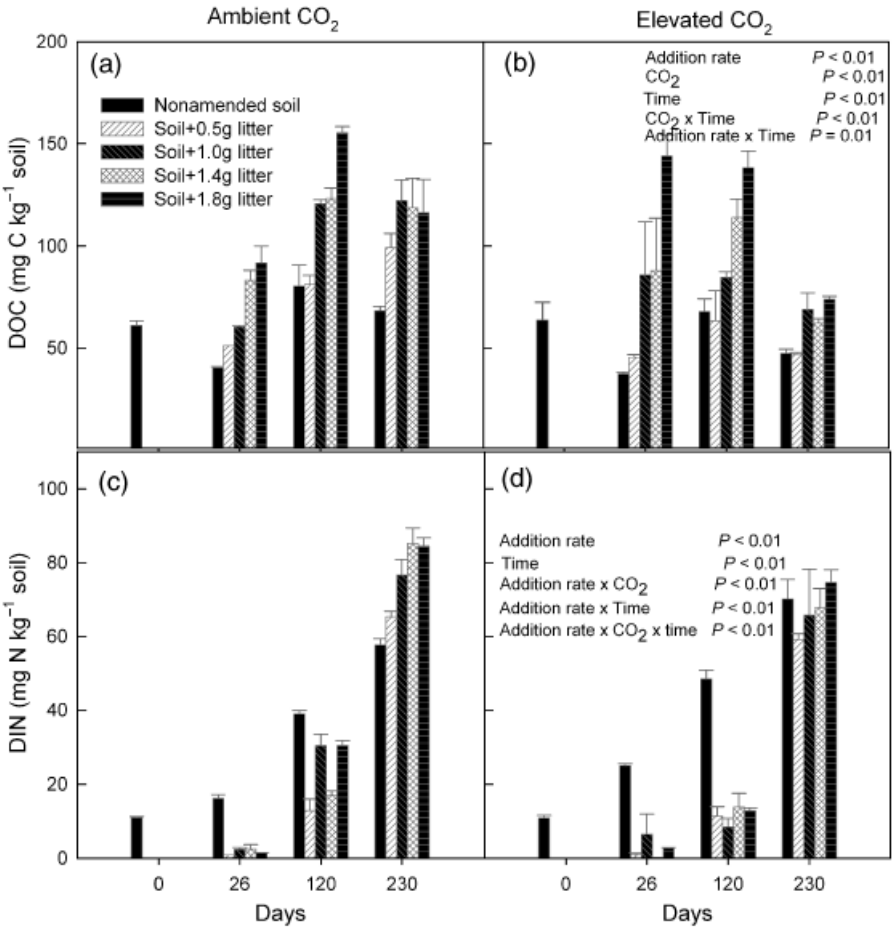

Fig. 4 Responses of dissolved organic carbon (DOC; a and b) and dissolved inorganic nitrogen (DIN; c and d) to litter addition rate and CO_2 treatments. Litter and soil were collected from either ambient or elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA. Values are means ($n = 3$) ± SE.

Soil C and N

Across CO_2 and litter addition treatments, the HDF contained 93 ± 4% of total soil C and 87 ± 2% of total soil N, with neither litter addition rate nor CO_2 level significantly affecting this proportion.

Effects of litter addition rate. The effects of litter addition rate on soil C and N parameters were examined by linear regression (Fig. 5). Higher litter addition rates resulted in higher C loss by respiration (Fig. 5a) and higher total soil C, new C and N concentrations in both whole soil and HDF (Fig. 5b–g). Increasing litter addition rate had no significant impacts on old C concentrations in both whole soil and HDF (Fig. 5h and i).

CO_2 effects. The linear regression slopes of C loss by respiration and total C in whole soil were similar under ambient and elevated CO_2 treatment (Fig. 5a and b). However, elevated CO_2 significantly decreased the slopes of total C in HDF, new C in whole soil and total N in both whole soil and HDF (Fig. 5c–f).

Microcosm comparisons using litter and litterfall rates observed at Aspen FACE. In the microcosms, the 1.0 and 1.4 g litter addition treatments were equivalent to litter production under control (230 g C m^{-2} yr^{-1}) and elevated CO_2 (302 g C m^{-2} yr^{-1}) treatments at the Aspen FACE experiment, respectively. To better understand how elevated CO_2 may affect mineral soils in the field, we constructed the following comparisons: (1) leaf litter chemistry change/no change in litterfall: 1.0 g elevated CO_2 litter vs. 1.0 g ambient CO_2 litter; (2) no leaf litter chemistry change/litterfall change: 1.4 g ambient CO_2 litter vs. 1.0 g ambient CO_2 litter and (3) leaf litter chemistry change/litterfall change: 1.4 g elevated CO_2 litter vs.

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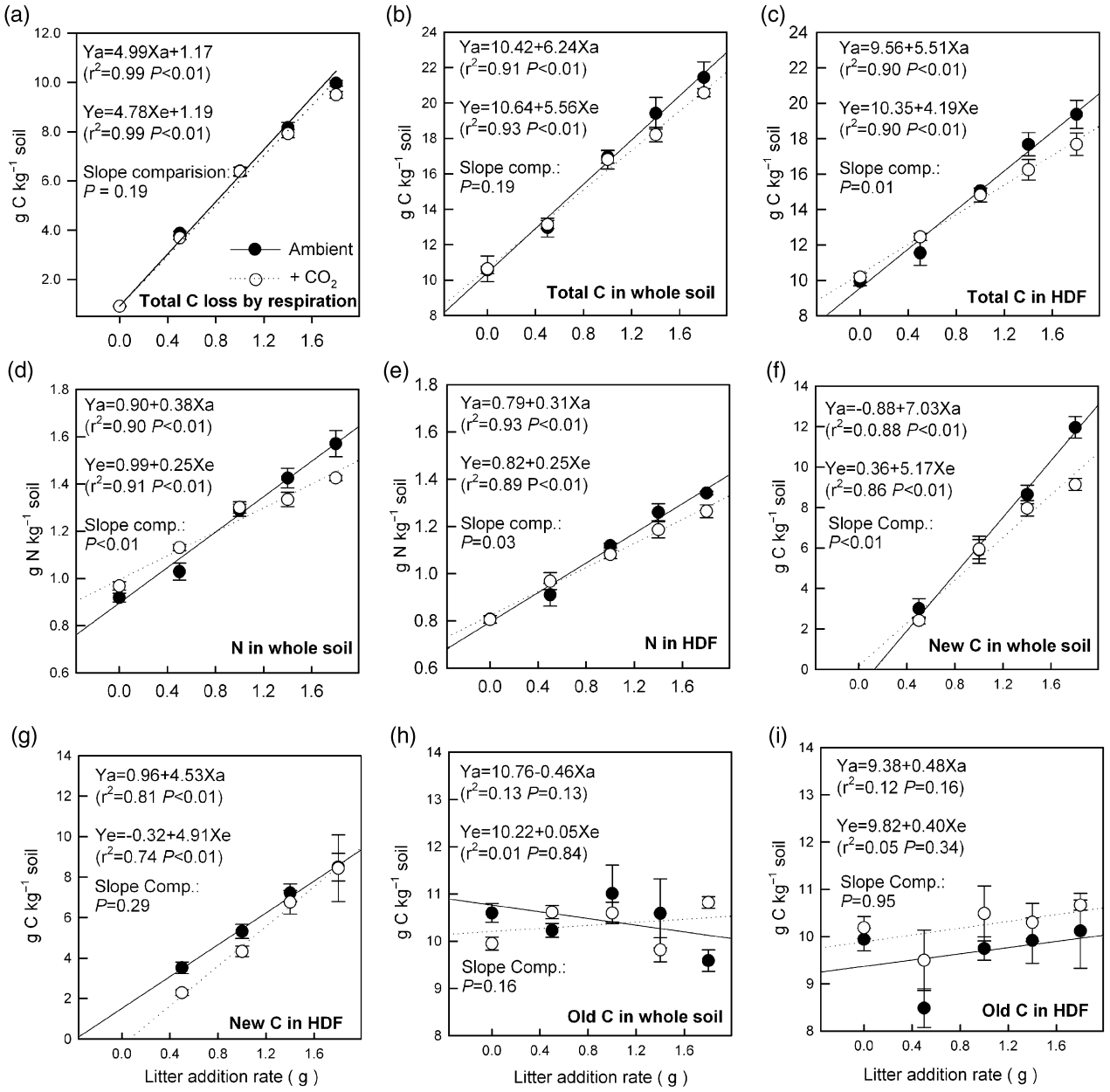

Fig. 5 Relationships between litter addition rate (Y) and soil carbon (g C kg^{-1} soil) or N (g N kg^{-1} soil) concentrations (X) in whole soil and high density fraction (HDF) after incubation for 230 days. Values are means ($n = 5$) ± SE under ambient (filled symbols) and elevated CO_2 (open symbols) treatments. Litter and soil were collected from either ambient or elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA. The regression models for ambient (Y_a vs. X_a) and elevated CO_2 (Y_e vs. X_e) treatments and the pairwise comparison results of the regression slopes (ambient vs. elevated CO_2, $P < 0.05$) are shown in each panel.

1.0 g ambient CO_2 litter (Fig. 6). The results show that altered tissue chemistry alone has no significant effects on soil C and N parameters except for reducing new C formation in HDF (Fig. 6). In contrast, all parameters except the retention of old C were significantly affected by the increase in litter addition rate from 1.0 to 1.4 g, with increases of C loss by respiration, accumulation of total C and total N, and formation of new C in both whole soil and HDF (Fig. 6). The mean percent change for all parameters under the combination of altered

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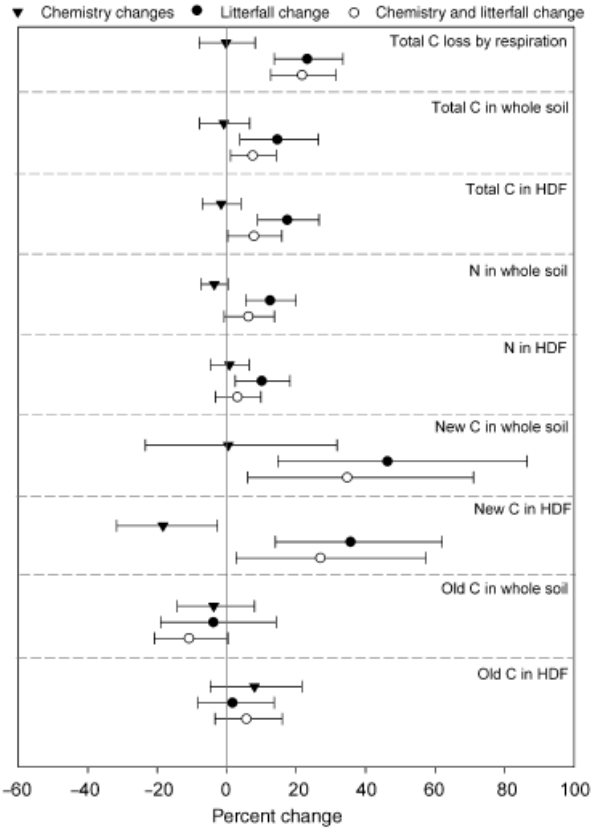

Fig. 6 Percentage changes in soil C and N concentrations in 1.0 g elevated CO_2 litter (–▼– chemistry change), 1.4 g ambient litter (– ● – litterfall change) and 1.4 g elevated CO_2 litter (– ○ – chemistry and litterfall changes) compared with 1.0 g ambient litter after incubation for 230 days. Litter and soil were collected from either ambient or elevated CO_2 treatments at the Aspen FACE experiment, Rhinelander, WI, USA. Results are grouped by parameters. Symbols are means ± 95% confidence intervals ($n = 5$). The percentage changes were significantly different from 0 as the confidence interval did not overlap with 0.

litter chemistry and increased litter addition rate fell between the means of the two individual changes, indicating that chemistry change tended to offset the response magnitude induced by increased inputs. Specifically, altered chemistry plus increased litter inputs caused significant increases in total C loss by respiration (+ 22%), total C in whole soil (+ 7%), total C in HDF (+ 8%), new C in whole soil (+ 35%) and new C in HDF (+ 27%).

Discussion

We found that increasing the litter addition rate exerted a strong influence on the cycling and storage of C and N in microcosm soils. In contrast, litter chemistry changes due to elevated CO_2 had only a minor influence when compared with litter and litterfall rates observed at Aspen FACE. SOC content increased with increasing litter mass for several possible reasons. First inputs exceeded losses to soil respiration across litter additions rates. Second, because identical litter type was used for all microcosms, higher litter addition rate corresponded to greater inputs of slowly degrading components, such as lignin, to the microcosms, which could increase SOC. Third, litter additions appeared to stimulate microbial activity and enhance humification processes arising from increased labile litter input, as evidenced by increased MBC levels and total C in HDF. The HDF contains completely humified fine POM, suggesting that humification processes increased with higher litter addition rate (Martin & Haider, 1971; Zech *et al*., 1997; Hoosbeek *et al*., 2007).

Microbial respiration

In our study, microbial respiration rates of microcosms amended with 1.4 g litter from the elevated CO_2 treatment was 8–24% higher than that of microcosms amended with 1.0 g of litter from the ambient CO_2 treatment. This stimulation is similar to the values measured in the field by Pregitzer *et al*. (2006), who found that elevated CO_2 increased soil respiration by 8–26% at the Aspen FACE experiment. Pregitzer et al.'s estimates also included root and mycorrhizal respiration, and part of the increase was due to larger total C inputs belowground in the form of greater root production and exudation (Giardina *et al*., 2005; Sulzman *et al*., 2005). While our microcosms did not include these components, which should be considered in overall evaluations of elevated CO_2 effects on soil C mineralization, the similar magnitudes of the stimulation effect suggest that our study provides insights into the mechanisms behind how elevated CO_2 effects on litter quantity and quality affect soil respiration. Overall, while the goal of our study was to assess how changes in litter production and chemistry at elevated CO_2 influence soil C and N cycling, aboveground leaf litter is not the only source of detrital C for SOC formation. Belowground litter inputs, such as dead roots, mycorrhizae and exudates, exert a large influence on soil C formation (Giardina *et al*., 2004; Russell *et al*., 2007). However, our study suggests that increased litter C production at elevated CO_2 will not all be released to the atmosphere through higher rates of C mineralization but is likely an important contributing factor in the processes controlling SOC formation.

At equal litter additions, elevated CO_2 decreased microbial respiration slightly in the first 100–120 days. Similarly, Torbert *et al*. (1998) found that litter quality change caused by elevated CO_2 decreased microbial respiration by 20% in their 60 days incubation experi-

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ment in an agricultural ecosystem. However, we found that this negative effect diminished quickly with incubation time and no significant differences in cumulative C loss, except higher losses at the 1.8 g litter addition rate, were found by the end of the incubation period. This pattern could be related to transient N limitations to soil microbial biomass early in the decomposition process. For example, following the input and initial metabolism of fresh litter, C limitations to microbial growth could shift to N limitations (Berg & Laskowski, 2006). This N limitation could be stronger initially for litter from the elevated CO_2 treatment, which had a lower [N], and initially low [N] could stimulate the decomposition of recalcitrant C due to its positive effects on lignolytic enzymes (Berg & Laskowski, 2006), as well as possible shifts from bacterial to fungal microbial communities. In the field, these elevated CO_2-induced changes could lead to enhanced C release throughout the decomposition process. Carbon would eventually become limiting again after available C in the litter was metabolized, with higher [N] in this case inhibiting decomposition of recalcitrant C. Critically, annual inputs of fresh litter, increased belowground C inputs under elevated CO_2, strong seasonality of temperature and moisture, and other environmental factors would complicate extrapolation of our results to the field.

MBC and MBN

In the current study, litter addition showed a significant positive influence on MBC. Similarly, Lagomarsino *et al*. (2006) found that elevated CO_2 significantly increased MBC, which was associated with greater labile C inputs to the forest floor at the POPFACE experiment. However, in a litter manipulation study in tropical rainforests, Sayer *et al*. (2007) found that microbial biomass decreased in litter removal treatment, but not changed in litter addition treatment.

We found that MBN in litter-amended soil was significantly higher than nonamended soil during the whole incubation and across CO_2 treatments. High MBN through 230 days most likely was caused by higher N availability, as indicated by the relatively higher DIN concentration over this period. However, there was no clear relationship between MBN and litter addition rate among the four litter-amended levels. Elevated CO_2 caused a small decrease in leaf litter [N], but did not significantly affect the concentrations of C constituents. Comparisons at equal litter addition rate reveal that MBC, MBN and microbial C/N were not significantly altered by elevated CO_2 treatments (Fig. 3), which indicates that small changes in litter chemistry (e.g. decrease in litter [N]) are unlikely to induce a detectable change in microbial communities.

DIN

Compared with nonamended soil, DIN was significantly lower in litter-amended soil on day 26 and day 120, which indicates that litter additions favored gross N immobilization over gross N mineralization. We did not observed any significant effect of elevated CO_2 on DIN among the four litter-amended treatments, suggesting that the small change in litter [N] is not adequate to modify net N immobilization/mineralization in soils. We also found no significant difference in DIN between the 1.0 and 1.4 g litter additions that most closely approximate litterfall rates in the field under the ambient and elevated CO_2 treatments, respectively. Our results are consistent with Zak *et al*. (2003) for three forest FACE experiments showing that elevated CO_2 did not affect microbial N cycling, pools or processes. However, Holmes *et al*. (2006) reported that gross N mineralization and gross NH_4^+ immobilization were equivalently enhanced under elevated CO_2 at the Aspen FACE experiment. The reasons behind the inconsistent findings are unclear.

Soil carbon formation

Changes in litter production and litter chemistry under elevated CO_2 have the potential to modify soil C turnover and storage by altering inputs relative to decomposition losses. Above- and belowground inputs have consistently increased in elevated CO_2 experiments (Norby *et al*., 2001, 2005; Giardina *et al*., 2005; King *et al*., 2005a). Decomposition losses are less well understood, but may be a function of microbial community composition or activity (Larson *et al*., 2002; Phillips *et al*., 2002; Carney *et al*., 2007). Critically, no consistent response of SOC under elevated CO_2 has been reported from FACE experiments, and the underlying mechanisms have been difficult to ascertain because control treatments cannot take advantage of the C label created through fumigation with ^{13}C-depleted CO_2.

Effects of litter addition rates. We found total soil C and new C in whole soil and in the HDF all increased linearly with increasing litter addition rate. These results indicate that although adding litter-stimulated microbial respiration, thus increasing C loss from soil, the increases in litter addition rate more than offset enhanced respiration, which resulted in increased new C formation and total C in whole soil and the HDF. Our microcosm results were contrasted with field observations at several FACE experiments. Although more litter accumulated in the forest floor, C content in the soil was not changed at the Duke FACE experiment after 6 years of elevated CO_2 treatment (Lichter *et al*.,

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2005) nor at the POPFACE experiment after 3 years of CO_2 enrichment (Gielen *et al*., 2005). In an open top chamber study within an agro-ecosystem, Torbert *et al*. (1997) found that, after 2 years CO_2 treatment, new soil C content increased in grain sorghum community but decreased in soybean community, although total SOC contents were not changed for both communities. A meta-analysis of 65 studies to determine CO_2 effects on soil C contents found that elevated CO_2 increased soil C by 1.2% per year, and such changes was significant for herbaceous species but not woody species (de Graaff *et al*., 2006). The mixed results from those studies could be due to the difficulty of detecting small changes in the large, spatially and chemically heterogeneous SOC pool.

Biogeochemical processes in our microcosm study proceeded under controlled environmental conditions (e.g. pulverized litter mixed with homogeneous soil, constant temperature and moisture, no faunal disturbance), and so our ability to detect small changes was greatly enhanced relative to field studies. Further, the differences in stable isotopic composition among microcosm components allowed us to precisely separate litter chemistry and quantity effects on soil C formation. Although an artificial system, our results indicate that soil C and N cycles strongly respond to changes in litter inputs, but responses to CO_2-induced changes in chemistry were weaker than responses to increases in litter addition rate. However, SOC formation rates under field conditions may well differ from the findings in the current study. Future field studies are necessary to test the relevance of our results to natural forests.

CO_2 effects. The regression slopes for total C in whole soil were not significantly different between ambient and elevated CO_2 treatment (Fig. 5a), indicating soil C levels were controlled by litter input rates rather than changes in litter chemistry. We also found that the regression slopes of total C in HDF and new C in whole soil were significantly altered by elevated CO_2, with the strength of this effect increasing with litter addition rate (Fig. 5b–h). These results indicated that the changes in litter production may be the key factor driving soil C and N process rates where litterfall rates are close to those observed at our site ($230\,g\,C\,m^{-2}\,yr^{-1}$, equaling a 1.0-g litter addition rate), but the effects of litter chemistry changes could become pronounced if litterfall rates increase ($>414\,g\,C\,m^{-2}\,yr^{-1}$, equaling a >1.8 g litter addition rate).

Elevated CO_2 significantly lowered the slope of total C in HDF (Fig. 5c). C compounds in HDF are mainly minerally bound C and completely humified fine POM (Baisden & Amundson, 2002). Our results suggested that increasing litter inputs increased humification in the mineral soil (Hoosbeek *et al*., 2007), but chemistry changes caused by elevated CO_2 might offset this tendency.

Fontaine *et al*. (2007) found that fresh C input provided energy to sustain microbial activity and thereby enhanced decomposition of recalcitrant SOC. Our results indicated that litter input stimulated microbial activity and C loss by microbial respiration increased with increasing litter addition rate. We expected that higher microbial activity may lead to a greater 'priming effect'. In contrast, litter addition rates had no impact on old C decomposition in our microcosms, as the regression slopes were not significantly different from 0 (Fig. 5h and i). Our findings suggest that increasing litterfall under elevated CO_2 may not affect the stability of SOC.

Soil N process rates. To predict if elevated CO_2 will induce a negative feedback between plant growth and soil nutrient availability, it is essential to examine whether soil N availability decreases progressively with time since exposure to elevated CO_2 conditions (Oren *et al*., 2001; Luo *et al*., 2004). We found that total N in whole soil and HDF showed positive linear correlation with litter addition rate. We also found that soil under elevated CO_2 had higher C/N averaged across litter addition treatments. Taken together, our results indicate that higher litter production and lower litter [N] should increase SOC per unit N retuned in litterfall. To better understand the effects of elevated CO_2 on soil C and N turnover rates, new studies are needed to investigate how changes in aboveground tissue chemistry and production interact with belowground chemistry and production (e.g. roots and mycorrhizae) to alter ecosystem N cycling.

Conclusion

Our study showed that microbial respiration, DIN, MBC and MBN respond strongly to changes in litter production and to a lesser extent, to changes in litter chemistry caused by elevated CO_2. Soil C and N cycles were significantly influenced by litter production changes, whereas the impacts due to chemistry changes were pronounced only at high levels of litter addition. Overall, litter quantity appeared to have a greater influence on microbial activity, and soil C and N turnover rates than changes in litter chemistry. Our results suggest that changes in litter inputs under elevated CO_2 should lead to higher long-term C storage in soil despite higher rates of soil respiration, but that CO_2-related effects of chemistry may somewhat offset the effects of increased litter inputs.

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