Effects of elevated CO_2 and nitrogen addition on soil organic carbon fractions in a subtropical forest

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Abstract

Aims The aim of this study was to investigate the effects of elevated CO_2 concentration and nitrogen addition on soil organic carbon fractions in subtropical forests where the ambient N deposition was high.

Methods Seedlings of typical subtropical forest ecosystems were transplanted in ten open-top chambers and grown under CO_2 and nitrogen treatments. The treatments included: 1) elevated CO_2 (700 μmol mol^{-1}); 2) N addition of 100 kg NH_4NO_3 ha^{-1} yr^{-1}; 3) combined elevated CO_2 and N addition; and 4) control. We measured soil total organic carbon (TOC), particulate organic carbon (POC), readily oxidizable organic carbon (ROC), and microbial biomass carbon (MBC).

Results Results showed that elevated CO_2 alone did not significantly affect soil TOC, POC and ROC after 4 years of treatment, but increased soil MBC and soil respiration compared to the control. N addition alone had no significant effect neither on soil TOC, POC and ROC, but decreased MBC and soil respiration over time. However, the elevated CO_2 and N addition together significantly increased soil POC and ROC, and had no significant effect on soil MBC.

Conclusions This study indicated that even in N-rich subtropical forest ecosystems, inputs of N are still needed in order to sustain soil C accumulation under elevated CO_2.

Keywords Carbon fraction · Elevated CO_2 · Global climate change · Nitrogen deposition · Soil organic carbon · Subtropical forest

Abbreviations

C	carbon
CK	control
HC	elevated CO_2 and ambient N deposition
HCHN	elevated CO_2 and N addition
HN	N addition and ambient CO_2
MBC	microbial biomass carbon (mg kg^{-1})
N	nitrogen
POC	particulate organic carbon (g kg^{-1})
ROC	readily oxidizable organic carbon (g kg^{-1})
SM	soil moisture (%)
SR	soil respiration (kg CO_2 m^{-2} yr^{-1})
TOC	soil total organic carbon (g kg^{-1})

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Introduction

Global changes such as elevated atmospheric CO_2 concentrations and nitrogen (N) deposition will have

dramatic impacts on ecosystem productivity and function in the coming decades (Dijkstra et al. 2005; Bradford et al. 2008). Several studies have shown that the changes in elevated CO_2 concentrations and N addition may alter soil microbial activity (Glaser et al. 2006; Carney et al. 2007), detrital production (Reich et al. 2006b), labile C input (Bradford et al. 2008), and soil respiration (Deng et al. 2010). But most previous studies of the interactive effects of elevated CO_2 and N deposition on soil carbon (C) dynamics have been restricted to the temperate forest and grassland ecosystems where N is limited (Treseder and Allen 2000; Hagedorn et al. 2001; Shaw et al. 2002; Luo et al. 2004; Dijkstra et al. 2005; Reich et al. 2006b; West et al. 2006). There is limited information regarding soil C responses to interactive effects of elevated CO_2 and N deposition in subtropical and tropical forest ecosystems where ambient N deposition is high (Luo et al. 2004, 2006).

Subtropical and tropical forest ecosystems play a significant role in global C cycling and store 46 and 11% of the world's living terrestrial C and total C, respectively (Brown and Lugo 1982). Atmospheric CO_2 concentration and N deposition are two primary factors that are concurrently changing in subtropical forests of China (Deng et al. 2010). The availability of soil N in the subtropical forests is considered to be high (Liu et al. 2010) and the response of subtropical forests to elevated CO_2 is still not clear. Several studies using meta-analysis (e.g. De Graaff et al. 2006; Van Groenigen et al. 2006) found that elevated CO_2 only increased soil C at N addition larger than 30 kg ha^{-1} yr^{-1}. However, in the temperate forests with limited N, elevated CO_2 enhanced C assimilation more than similar forests with high N fertility (Butnor et al. 2003). Thus, there is a need to investigate how elevated CO_2 and N deposition interactively influence C processes and C storage in forests with high N availability.

Several different forms of soil organic carbon (SOC) exist in forest soils (Ågren and Bosatta 2002; Kirschbaum 2004; Eliasson et al. 2005; Knorr et al. 2005; Bradford et al. 2008). Individual SOC fractions have different responses to change in soil N availability (Hagedorn et al. 2003) and elevated CO_2 (Luo et al. 2004). The labile soil carbon fractions with short turnover times, such as particulate organic carbon (POC), readily oxidizable organic carbon (ROC) and microbial biomass carbon (MBC) are considered biologically available sources of C and respond faster to environment

changes than total SOC (Blair et al. 1995; Yang et al. 2009). Consequently, SOC change may be revealed through soil labile carbon fractions in the short-time experiment.

In this study, we used open-top chambers to study the effects of elevated CO_2 and N deposition on soil organic carbon fractions in forest ecosystems in Southern China. We grew 48 seedlings of 6 typical subtropical tree species in the chambers and measured soil organic C and its fractions over 4 years. We hypothesized that: 1) elevated CO_2 and nitrogen addition treatments would have no significant effect on soil total organic carbon (TOC), because change in TOC was difficult to detect in the short term; 2) elevated CO_2 would stimulate labile carbon fractions (POC and ROC) accumulation rates, due to the high ambient N availability in the forest ecosystems; and 3) combined elevated CO_2 and N addition treatment would not enhance labile carbon fractions compared to elevated CO_2 alone.

Materials and methods

Study site

The experiment was conducted at South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China (23°20′ N and 113°30′ E). The area has a typical south subtropical monsoon climate, with annual precipitation ranging from 1600 mm to 1900 mm, of which nearly 80% falls in the hot-humid wet/rainy season (April-September) and 20% in the dry season (October-March) (Liu et al. 2008). The annual total solar radiation in the visible waveband reached 4,367.2-4,597.3 MJ m^{-2} and mean annual temperature was 21.5°C. The N deposition in Guangzhou was 46–73 kg N ha^{-1} year^{-1} in the late 1990s (Ren et al. 2000).

Design of open-top chambers

Ten open-top chambers were built for this experiment. Each chamber was 3 m in diameter, 3 m tall and inserted 0.7 m deep into the soil. The above-ground part was wrapped with impermeable and transparent plastic sheets, leaving the top of the chamber totally open. Light intensity in the chamber was 97% of that in open space. Measured rainfall intensity was identical inside and outside of the chambers and the air

temperature was not significantly different. The below-ground part was delimited by concrete wall filling with the brick that prevented any lateral or vertical water and/or element fluxes with the outside surrounding soil. Three holes at the bottom of the wall were connected to a stainless steel water collection box. Holes were capped by a 2 mm net to prevent losses other than those of leachates. In the treatment chambers with elevated CO_2, the additional CO_2 was distributed from a CO_2 tank by a transparent PVC pipe with pinholes. A big fan was connected to the pipe to ensure equal distribution of CO_2 in the entire chamber. Air was introduced into chambers via the fan at an exchange rate of about 1.5 chamber volumes per minute. The CO_2 flux from the tank was controlled by a flow meter and the CO_2 concentrations in the chambers were periodically checked using a Li-Cor 6400 (Li-Cor Inc., Lincoln, Nebraska, USA).

The soils used in the experiment were collected from a nearby evergreen broad-leaved forest after harvesting from 26 February 2005 to 3 March 2005. Simultaneously, the soil was collected at three different layers (0–20, 20–40 and 40–70 cm depth) that were homogenized separately and used to fill the below-ground part of the chambers. The lateritic soil with its chemical properties (before the soil was collected) was shown by Liu et al. (2008). On 10 March 2005, one to two years old seedlings grown in a nursery were transplanted into the chambers without damaging the roots. All the chambers were planted with 48 randomly selected seedlings with 8 seedlings each of the following 6 species: *Castanopsis hystrix* Hook.f. & Thomson ex A.DC, *Syzygium hancei* Merr. et Perry, *Pinus massoniana* Lambert, *Schima superba* Gardn. and Champ., *Acmena acuminatissima* (Blume) Merr. et Perry, and *Ormosia pinnata* (Lour.) Merr. These 6 species were selected because they are all native and all widely distributed in southern China. No significant difference in height and basal diameter of the plant seedling was found among all treatments at the beginning of the experiment (Deng et al. 2010).

Experimental design

The experimental treatments started in April 2005. Three chambers received elevated CO_2 and ambient N deposition treatment (HC), two chambers received N addition and ambient CO_2 treatment (HN), three chambers received elevated CO_2 and N addition

treatment (HCHN), and two chambers were used as controls (CK) without elevated CO_2 or N addition. The HC and HCHN chambers were parallel arranged, and the distance between the adjacent chambers was 5 m. The HN and CK chambers were 50 m from the HC and HCHN chambers, and the distance between the adjacent chambers was 5 m. The elevated CO_2 chambers were controlled at ca. 700 μmol mol^{-1}. The N addition treatments were achieved by spraying liquid fertilizer on seedlings once a week for a total amount of NH_4NO_3 at 100 kg ha^{-1} yr^{-1}. No other fertilizer was used. Since the walls of the chambers below-ground blocked lateral and vertical water fluxes, the seedlings were watered with tap water. About 600 mm extra water was applied in each chamber per year. All the chambers received the same amount of water as the control (CK) chambers.

Sample collection and measurements

Soil samples were collected in July of 2009. Twelve cores were collected randomly in each chamber from 0–20 cm soil layers using a standard soil sampling tube (2.5 cm inside diameter). One sample consisted of four cores, and 3 samples were collected in one chamber. Total of 30 samples were collected in this study. The fresh soil samples were passed through a 2 mm sieve to remove rocks and plant roots. Portions of fresh soils were used for microbial biomass carbon measurements. Sub-samples were air-dried and ground 2 mm for TOC and other C fractions measurements.

Particulate organic carbon (POC 53–2000 μm) was measured using the method described by Cambardella and Elliott (1992). Ten grams of the <2 mm air-dried soil was dispersed with 50 mL of 5 g·L^{-1} sodium hexametaphosphate solution by hand shaking the mixture for 5 min then set on a reciprocal shaker (90 r ·min^{-1}) for 18 h. The dispersed soil sample was passed subsequently through 53 μm stainless steel sieve and rinsed thoroughly with distilled water. The material remaining on the sieve, defined as the POC fraction, was dried at 50°C for 12 h, weighed and finely ground 0.15 mm for carbon measurement.

Readily oxidizable organic carbon (ROC) was determined with 333 mmol L^{-1} $KMnO_4$ oxidation (Blair et al. 1995, 1997). The air-dried soil, passed through a 2-mm sieve, was taken and passed through a 149 μm sieve. Sub-samples of soil containing 15–30 mg C were weighed into 100 mL plastic centrifuge tubes

and 25 mL of 333 mmol $KMnO_4$ were added to each tube. The tubes were tightly sealed and after shaking for 1 h at 250 r·min^{-1}, were centrifuged for 5 min at 2000 r·min^{-1} and the supernatants diluted 1:250 with deionized water. The absorbance of the diluted samples and standards were read on a UVspectrophotometer at 565 nm. The change in the concentration of $KMnO_4$ was used to estimate the amount of carbon oxidized, assuming that 1 mmol MnO_4 is consumed in the oxidation of 9 mg carbon (Tirol-Padre and Ladha 2004).

Microbial biomass carbon (MBC) was determined with the chloroform fumigation-extraction method (Joergensen and Brookes 1990). Soil TOC was measured following the Dichromate (0.8 mol·L^{-1}) heating-oxidation (Liu 1996). Soil pH was determined by mixing 10 g soil with 25 ml of deionized CO_2-free water for 5 min, leaving it for 30 min for the soil and water to separate and then measuring the pH of supernatant using a glass electrode (Liu 1996).

Soil respiration was measured once a week using an infrared gas analyzer Li-Cor 6400 (Li-Cor Inc, Lincoln, NE, USA) connected with soil chamber from 26 May 2006 to December 2008 (Deng et al. 2010). The measurements were made between 9:00 am and 12:00 pm local time. The soil respiration chamber (with a foam gasket) was put on the soil collars making an air-tight seal. Soil respiration was measured three times for each soil collar. Soil respiration in a treatment chamber was calculated as the mean of four collar measurements. Soil moisture of the top 5 cm soil layer was measured on five random locations within a treatment chamber using a PMKit at the same time when the soil respiration measurements were being taken.

Tree height and basal diameter were measured at the time of planting in early March 2005 and then assessed six times in August 2005, January 2006, May 2006, January 2008, September 2008, and January 2009. Tree height was measured from the soil-stem surface to the tip of the apical bud, and the basal diameter was assessed at the soil surface. To measure tree biomass, one plant of each species in every chamber was harvested in January in 2006, 2007, 2008, and 2009, respectively. The plant was separated into roots, stems, and leaves. Plant samples were oven-dried at 60°C before weighing. A traditional plant growth function was developed for different component biomass estimation (Whittake and Woodwell 1986; Wen

et al. 1997): $W=a(D^2H)^b$, where W is dry biomass of plant components including roots, stems, and leaves; D is plant basal diameter; H is height; and a and b are regression coefficients. The above-ground tree biomass in each chamber was the sum of all tree stem and leave biomass. The below-ground tree biomass was the sum of all tree root biomass.

Statistical analysis

The three soil samples per chamber were averaged before statistical analyses. Two-way analysis of variance (ANOVA) was used to examine CO_2, N and their interactive effects on soil organic carbon concentration (TOC), particulate organic carbon (POC), readily oxidizable organic carbon (ROC), microbial biomass carbon (MBC) and pH. One-way ANOVA with Tukey's HSD test was used to examine different treatments effects on soil TOC, POC, ROC and pH. Repeated measures ANOVA was used to examine the effects of different treatments on soil respiration, moisture and tree biomass at different measurement occasions. All the tests were performed using SPSS software (version 11.5; SPSS Inc).

Results

Soil pH

Results of two-way ANOVA showed that CO_2 and N significantly influenced soil pH, but no interaction effect of CO_2 and N was detected (Table 1). The elevated CO_2 only (HC), N addition only (HN), and combined elevated CO_2 and N addition (HCHN) treatments significantly decreased soil pH in the 0–20 cm layers compared to the control (CK) (Fig. 1). Soil pH under the combined elevated CO_2 and N addition treatment was significantly lower than that under elevated CO_2 only and N addition only treatments. There was no significant difference between HC and HN treatments.

Soil total organic carbon (TOC)

There was no significant difference among the four treatments (Fig. 2a). Averaged TOC for the 0–20 cm depth showed a trend of decline in the treatment order: HN (10.126±1.936 gkg^{-1}) > HCHN (9.340±0.786 gkg^{-1}) > HC (8.942±0.823 gkg^{-1}) > CK (7.733±2.011 gkg^{-1}).

Table 1 Significance of the effects of CO_2, N, and CO_2*N interaction on soil pH, total organic carbon (TOC), particulate organic carbon (POC), readily oxidizable organic carbon (ROC) and microbial biomass carbon (MBC) in the two-way ANOVA

	pH		TOC (g kg⁻¹)		POC (g kg⁻¹)		ROC (g kg⁻¹)		MBC (mg kg⁻¹)	
	F	p	F	p	F	p	F	p	F	p
CO_2	11.35	0.015	0.29	0.612	0.19	0.689	2.81	0.145	28.12	0.002
N	7.74	0.032	0.80	0.407	7.05	0.057	48.71	<0.001	6.65	0.045
CO_2*N	0.06	0.819	0.01	0.929	8.79	0.041	8.47	0.027	2.67	0.154

Soil particulate organic carbon (POC)

The interaction of elevated CO_2 and N addition affected the soil POC significantly ($p<0.05$) (Table 1). The elevated CO_2 alone (HC) or N addition alone (HN) treatment had no significant effect on soil POC compared to the control. But the POC was significantly higher in the combined elevated CO_2 and N addition treatment compared to elevated CO_2 only treatment (Fig. 2b). In the top soil layer (0–20 cm), the HCHN treatment had the highest POC, followed by the HN and CK treatments, and the HC treatments had the least POC concentration.

Soil readily oxidizable organic carbon (ROC)

There was significant effect on soil ROC among the four treatments (Fig. 2c). In the 0–20 cm soil layer, the average soil ROC followed the order: HCHN (1.904 ± 0.249 g kg⁻¹) > HN ($1.547\pm$

0.160 g kg⁻¹) > CK (1.261 ± 0.113 g kg⁻¹) > HC (1.169 ± 0.186 g kg⁻¹). The combined elevated CO_2 and N addition (HCHN) treatment had the highest ROC content and was significantly different from the control. The N addition alone (HN) and elevated CO_2 alone (HC) had no significant effect on soil ROC, but the HN treatment had higher ROC than HC treatment.

Soil microbial biomass carbon

The N addition alone (HN) treatment significantly decreased soil MBC (Table 1; Fig. 2d), compared to the control (CK). On the contrary, the elevated CO_2 alone (HC) treatment significantly increased soil MBC. Both HC and combined elevated CO_2 and N addition (HCHN) treatments had higher MBC concentrations than the HN treatment.

Above- and below- ground biomass, soil respiration and moisture

The elevated CO_2 alone (HC), N addition alone (HN), and combined elevated CO_2 and N addition (HCHN) treatments increased the above- and below-ground biomass (Fig. 3). Both above- and below-ground biomass was lower under the HC treatment than the HN and HCHN treatments.

From 2006 to 2008, the elevated CO_2 alone treatment had increased soil respiration and soil moisture (Table 2). The N addition alone (HN) treatment increased soil respiration in 2006, but had no significant effect on soil respiration in 2007 and 2008. The HN treatment decreased soil moisture. The N addition alone (HN) treatment increased soil respiration in three years, and only increased soil moisture in the first two years. In 2008, the HCHN had no significant effect on soil moisture.
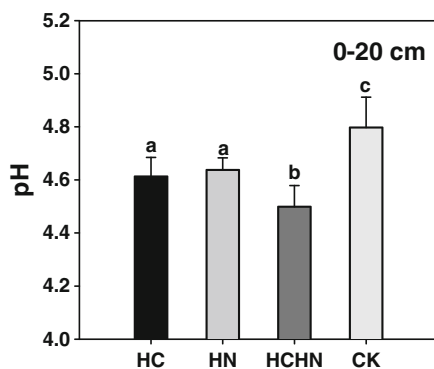
Fig. 1 Soil pH under different CO_2 and N addition treatments in July of 2009. HC: elevated CO_2 and ambient N deposition; HN: N addition and ambient CO_2; HCHN: elevated CO_2 and N addition; CK: control. Different letters across treatments denote significant difference at $p<0.05$

Fig. 2 Soil total organic carbon (TOC), particulate organic carbon (POC), readily oxidizable organic carbon (ROC) and microbial biomass carbon (MBC) under different CO_2 and N addition treatments in July of 2009. HC: elevated CO_2 and ambient N deposition; HN: N addition and ambient CO_2; HCHN: elevated CO_2 and N addition; CK: control. Different letters across treatments denote significant difference at $p < 0.05$
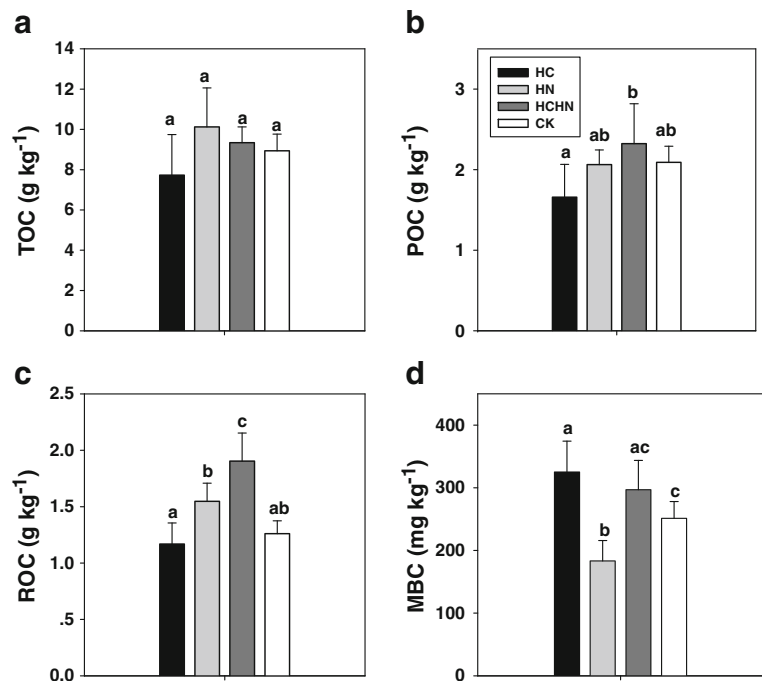

Discussion

Effect of elevated CO_2 on soil organic carbon fractions

Our results showed that elevated CO_2 alone (HC) had no significant effect on soil particulate organic carbon (POC) and readily oxidizable organic carbon (ROC) (Fig. 2b, c). This was a little surprising, as we had hypothesized that POC and ROC may be enhanced by elevated CO_2, since they are labile organic carbon fractions and have short turnover times (Yang et al. 2009). Previous studies showed that elevated CO_2 may stimulate plant-derived C (Baldock et al. 1992; Tirol-Padre and Ladha 2004; Grandy and Neff 2007). Absence of significant changes in POC and ROC in this study may be due to the balance of carbon inputs and outputs. We found that the above- and below-

ground biomass increased under elevated CO_2 treatment (Fig. 3). These increases may have promoted an accumulation of particulate organic matter, as shown in a grazed grassland (Allard et al. 2005). However, new carbon inputs can also accelerate the decomposition of SOC and increase soil carbon turnover (Taneva et al. 2006; Fontaine et al. 2004). Indeed, the elevated CO_2 treatment in this study significantly enhanced soil respiration and increased soil microbial biomass carbon (Table 2, Fig. 3d). Enhanced soil moisture in the elevated CO_2 treatment may also contribute to the higher litter decomposition and nutrient mineralization rates (Liu et al. 2008; Deng et al. 2010). As a result, elevated CO_2 reduced the new-C residence times leading to no significant effect on POC and ROC. Similar results were also reported by Lichter et al. (2005) who found that elevated CO_2 treatment produced no

Fig. 3 Ecosystem above-ground biomass and below-ground biomass (mean ± standard error) under different CO_2 and N addition treatments. HC: elevated CO_2 and ambient N deposition; HN: N addition and ambient CO_2; HCHN: elevated CO_2 and N addition; CK: control
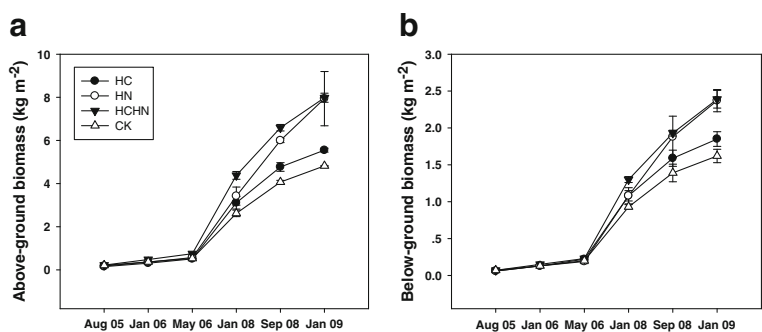
Table 2 Annual mean soil respiration, mean soil moisture of the top 5 cm soil layer under different CO_2 and N addition treatments. Different letters across treatments denote significant difference at $p < 0.05$

Year		HC	HN	HCHN	CK
2006	SR (kg CO_2 m^{-2} yr^{-1})	2.22±0.12a	1.97±0.10b	2.72±0.9c	1.83±0.03 d
	SM (%)	21.97±4.77a	19.67±5.01b	22.50±4.99a	20.62±4.50b
2007	SR (kg CO_2 m^{-2} yr^{-1})	4.43±0.18a	3.53±0.10b	5.16±0.10c	3.45±0.07b
	SM (%)	22.00±5.59a	18.31±6.57b	21.47±5.82a	20.17±6.60c
2008	SR (kg CO_2 m^{-2} yr^{-1})	4.65±0.06a	3.60±0.05b	5.48±0.12c	3.62±0.01b
	SM (%)	22.45±6.93a	18.49±7.07b	21.55±6.78ab	20.58±6.88b

SR: Soil respiration (kg CO_2 m^{-2} yr^{-1}); SM: soil moisture (%); HC: elevated CO_2 and ambient N deposition; HN: N addition and ambient CO_2; HCHN: elevated CO_2 and N addition; CK: control

detectable effect on the C content of any particulate organic matter size fraction.

There was no significant effect of elevated CO_2 alone (HC) on TOC (Fig. 2a). This was consistent with our hypothesis. Since no change in ROC or POC was found as discussed above, it was understandable that elevated CO_2 alone did not significantly affect soil TOC in the short term. However, in several studies elevated CO_2 reduces soil carbon content (Carney et al. 2007; Langley et al. 2009). The controversy may be caused by the difference in N availability. Due to the high ambient N availability in subtropical forests, N supply may meet the need of enhanced plant growth under elevated CO_2 in the short term. In the long term, however, the increasing soil respiration and microbial activity under elevated CO_2 treatment may alter the rate the total organic carbon decomposition (Fontaine et al. 2004) which could reduce soil carbon stock.

Effect of N addition on soil organic carbon fractions

For the similar reasons as the elevated CO_2 alone (HC) treatment, N addition alone (HN) treatment also had no significant effect on soil POC, ROC, and TOC (Fig. 2b, c). We found that the N addition alone treatment enhanced above- and below-ground biomass (Fig. 3) as well as promoted soil respiration in the first 2 years of the experiment (Table 2). This was inconsistent with some other studies that reported increases in soil POC and ROC with N deposition treatments (Diekow et al. 2005; Li et al. 2010). The possible reason could be that N addition treatment experiments in these studies were implemented at least 6 years

(Diekow et al. 2005; Li et al. 2010), and our experiment just was carried out for only 4 years.

One thing worth mentioning is that the effect of the N addition alone treatment on soil respiration had been weakened over time (Table 2, Deng et al. 2010). This was consistent with our result that the N addition alone treatment decreased the soil microbial biomass carbon (MBC) (Fig. 2d). The N addition often lead to soil acidification (Fig. 1) especially in tropical forests where the acidic soils often will inhibit soil microbial activity (Waldrop et al. 2004). Meanwhile, soil microbial activity was closely related to soil moisture (Deng et al. 2010). In our study, N addition significantly decreased soil moisture (Table 2), which would suppress the microbial activity. Hence, we speculate that the POC and ROC may be increased under N addition in the long-term.

Effect of combined elevated CO_2 and N addition treatment on soil organic carbon fractions

Soil ROC and POC contents under the combined elevated CO_2 and N addition treatment were higher than those under other treatments (Fig. 2c). The combined elevated CO_2 and N addition treatment enhanced labile carbon derived-plant accumulation compared to the elevated CO_2 alone treatment. An increase in plant growth under elevated CO_2 caused N to accumulate in plant biomass. Soil N availability was expected to constrain sustainability of plant growth response to elevated CO_2 in the long term (Oren et al. 2001; Finzi et al. 2002; Reich et al. 2006a). Although the subtropical soil had a high N availability,

the young seedlings used in this experiment grew quickly and required more soil N (Deng et al. 2010). Elevated CO_2 together with N addition increased above- and below-ground biomass more than elevated CO_2 alone (Fig. 3). This also indicated that more N is needed to meet high N demand under elevated CO_2 treatment in the long-term (Luo et al. 2004; Reich et al. 2006a).

Furthermore, the combined elevated CO_2 and N addition treatment had no significant effect on soil MBC (Fig. 2d). The combined elevated CO_2 and N addition treatment had increased soil moisture in the first 2 years, but had no significant effect in 2008. The likely reason was that the effect of elevated CO_2 on soil moisture was reduced by N addition treatment. MBC has been found to be closely related to soil moisture (Kucera and Kirkham 1971; Deng et al. 2010).

The combined elevated CO_2 and N addition treatment had no significant effect on TOC in the short-term experiment (Fig. 2a). Although the combined elevated CO_2 and N addition treatment increased the new-C (root biomass and labile soil organic carbon) inputs to the soil, the soil respiration was also enhanced. No significant change in TOC was detected in this study over 4 years. Since the combined elevated CO_2 and N addition treatment was more favorable for soil C accumulation than elevated CO_2 alone treatment, we think that even in the subtropical forest where soil N availability is high, N addition is still needed to meet the enhanced N demand by elevated CO_2 treatment.

Conclusions

Both elevated CO_2 and N addition alone had no significant effect on soil labile organic carbon (POC and ROC). While the elevated CO_2 alone increased the microbial activity (MBC and soil respiration), N addition alone decreased the microbial activity. However, combined elevated CO_2 and N addition treatment had stronger effects on accumulation rates of labile organic carbon (POC and ROC) than elevated CO_2 or N addition alone, but had no significant influence on MBC. Our results demonstrated that even in the N-rich subtropical forest ecosystems, more exogenous N is needed to maintain higher C inputs to soil under CO_2 enrichment.

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