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Carbon dynamics in subtropical forest soil: effects of atmospheric carbon dioxide enrichment and nitrogen addition

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Abstract
Purpose The levels of atmospheric carbon dioxide concentration ([CO_2]) are rapidly increasing. Understanding carbon (C) dynamics in soil is important for assessing the soil C sequestration potential under elevated [CO_2]. Nitrogen (N) is often regarded as a limiting factor in the soil C sequestration under future CO_2 enrichment environment. However, few studies have been carried out to examine what would happen in the subtropical or tropical areas where the ambient N deposition is high. In this study, we used open-top chambers to study the effect of elevated atmospheric [CO_2] alone and together with N addition on the soil C dynamics in the first 4 years of the treatments applied in southern China.
Materials and methods Above- and below-ground C input (tree biomass) into soil, soil respiration, soil organic C, and total N as well as dissolved organic C (DOC) were measured periodically in each of the open-top chambers. Soil samples were collected randomly in each chamber from each of the soil layers (0–20, 20–40, and 40–60 cm) using a standard soil sampling tube (2.5-cm inside diameter). Soil leachates were collected at the bottom of the chamber below-ground walls in stainless steel boxes.
Results and discussion The highest above- and below-ground C input into soil was found in the high CO_2 and high N treatment (CN), followed by the only high N treatment (N+), the only high CO_2 treatment (C+), and then the control (CK) without any CO_2 enrichment or N addition. DOC in the leachates was small for all the treatments. Export of DOC played a minor role in C cycling in our experiment. Generally, soil respiration rate in the chambers followed the order: CN treatment>C+treatment>N+treatment>the control. Except for the C+ treatment, there were no significant differences in soil total N among the CN treatment, N + treatment, and the control. Overall, soil organic C (SOC) was significantly affected by the treatments ($p<0.0001$). SOC for all the soil layers in the treatments followed the order: CN treatment>N+treatment>C+treatment=CK treatment. Compared with the control, the higher SOC in the CN and N+ treatment was due to the greater above- and below-ground C input. The increased soil respiration in the C+ treatment led to the lower SOC.
Conclusions Elevated atmospheric [CO_2] in the subtropical China accelerated soil C sequestration in this area; however, this increase would still need additional N input. The increased soil C pool was due to the enhanced tree growth. Special climatic condition in this area and the high density of tree planting might further accelerate soil C sequestration in this area.

Keywords Carbon dioxide enrichment · Nitrogen addition · Soil organic C · Soil respiration · Subtropical forest soil · Tree biomass

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1 Introduction

Understanding carbon (C) dynamics in soil is important for assessing the soil C sequestration potential in forest ecosystems (Xu and Chen 2006; Xu et al. 2008; 2009). Numerous studies have been done about soil C dynamics under elevated atmospheric carbon dioxide concentration ([CO_2]) and shown that the reserves of C pools in the soil do not change with the elevated [CO_2] (Niklaus et al. 2001; Gill et al. 2002; Hagedorn et al. 2003; Lichter et al. 2005). There is also much research done with different conclusions that the reserves of C pools in the soil would increase under the same condition as above (Williams et al. 2000; Jastrow et al. 2005; Luo et al. 2006). Using the combined methods of isotope ^{13}C and meta-analysis, Jastrow et al. (2005) found that the average input of C in the mineral soil of forest in the temperate zone was about 19 $g cm^{-2} year^{-1}$ with the elevated [CO_2]. With meta-analysis, Luo et al. 2006) also showed that most soils could accumulate C consistently with the elevated [CO_2]. However, most of the above research was carried out in temperate forests. Few experiments have been done in the subtropical or tropical environments.

Under elevated [CO_2], soil C pool change can be attributed to many factors. Luo et al. (2006) showed that the increases in soil C pool sizes vary with CO_2 experimental facilities, ecosystem types, and nitrogen (N) treatments. Additional N supply enhances effects of CO_2 on C accumulations in both plant and soil pools. We would need about 7.7–37.5 Gt N to accumulate about 350–980 Gt C in the land ecosystem according to the calculation of Hungate et al. (2003). Hessen et al. (2004) pointed out that 0.067 g N is needed to accumulate 1 g C in soil organic matter. In the temperate environment, N is often regarded as the plant growth limiting factor. However, in some subtropical areas, large N deposition was reported (Ren et al. 2000; Zheng et al. 2002). In Asia, the use and emission of reactive N increased from 14 Tg N $year^{-1}$ in 1961 to 68 Tg N year in 2000 and is expected to reach 105 Tg N $year^{-1}$ in 2030 (Zheng et al. 2002). Currently, this leads to high atmospheric N deposition (NH_4^+-N, NO_3^--N) in precipitation in some forests of southern China (30–73 kg N $ha^{-1} year^{-1}$; Ren et al. 2000; Mo et al. 2006). The N deposition in Guangzhou city of southern China increased from 46 kg N $ha^{-1} year^{-1}$ in 1988 to 73 kg N $ha^{-1} year^{-1}$ in 1990 (Ren et al. 2000). High N deposition in this area leads to higher N availability in most forest ecosystems. Up to date, most studies about soil C sequestration responses to elevated [CO_2] have been performed in temperate areas, which are often N-limited under natural conditions and with low ambient N deposition. There have been no reports about whether high N deposition would facilitate soil C sequestration and effects of the N deposition on soil C sequestration potential under elevated [CO_2] in subtropical China.

In this study, we used open-top chambers to study the effect of elevated atmospheric [CO_2] alone and together with N addition in southern China on the soil C dynamics over 3.5 years after the treatments started. We hypothesized: (1) elevated atmospheric [CO_2] would increase soil C pool size in the subtropical area and (2) additional N input would not increase soil C pools under elevated atmospheric [CO_2] as the ambient N deposition was high in this area.

2 Materials and methods

2.1 Study site and experimental setup

The experimental site was located in Guangzhou city, Guangdong Province, China (23°20′N and 113°30′E). The area has a monsoon climate characterized by mean annual total solar radiation of 4,367.2–4,597.3 MJ m^{-2} in the visible waveband and a mean annual temperature of 21.5°C. The annual precipitation ranges from 1,600 to 1,900 mm, and the mean relative air humidity is 77%. There are two distinct seasons, a wet/rainy season from April to September and a dry season from October to March.

The experiment was carried out in ten open-top chambers. Each cylindrical chamber had a diameter of 3 m, a 3-m high above-ground section and a 0.7-m below-ground section. The open chambers were located in an open space where they all were exposed to full light and rain. From April 2005, chambers were exposed to different treatments. Three chambers received a high CO_2 and high N treatment (CN), three chambers a high CO_2 treatment (C+), two chambers a high N treatment (N+), and finally, two chambers were used as a control (CK) which did not receive high CO_2 or high N treatment. The high CO_2 treatments were achieved by supplying additional CO_2 from a tank until a concentration of ca. 700 ppm CO_2 was reached in the chambers. The high N addition treatments were achieved by spraying seedlings once a week for a total amount of NH_4NO_3 at 100 kg N $ha^{-1} year^{-1}$. No other fertilizer was used. The soils in all chambers were collected from a nearby evergreen broadleaved forest. One- to 2-year-old seedlings grown in a nursery were transplanted in the chambers with minimal damage to the roots. In order to assess the responses of different tree species to the elevated [CO_2] and N treatments in the short-term period, we specially planted high-density seedlings in each chamber. All the chambers were planted with 48 randomly located labeled seedlings with eight seedlings for each of the following six species: *Castanopsis hystrix* Hook.f. & Thomson ex A. DC, *Schima superba* Gardn. and Champ., *Acmena acuminatissima* (Blume) Merr. et Perry, *Ormosia pinnata* (Lour.) Merr., *Syzygium hancei* Merr. et Perry, and *Pinus massoniana* Lambert. These species are native, and the most widely spread tree species in southern China. As trees were growing fast, one tree per species was harvested at the end of each year to avoid excessive crowding in each chamber. The open-top chamber and the experiment design have been described in detail elsewhere (Liu et al. 2008).

2.2 Sample collection and measurements

2.2.1 Soil organic C and total N

Soil samples were collected in July and November of 2005 and April, August, and November of 2006, 2007, and 2008, respectively. Three samples of nine cores were collected randomly in each chamber from each of soil layers (0–20, 20–40, and 40–60 cm, respectively) using a standard soil sampling tube (2.5-cm inside diameter). The composite samples were gently mixed. Soil samples were air-dried and sieved to pass a 2-mm mesh. Dead roots and plant residues were picked out. Soil organic C (SOC) was determined following the Walkley-Black's wet digestion method (Nelson and Sommers 1982). Soil total N was measured using the micro-Kjedahl method (Jackson 1964).

2.2.2 Dissolved organic C

Leaching water sample collection started in 2007 in the third year of the treatment applications and continued for 2 years. Soil leachates were collected at the bottom of the chamber below-ground walls in stainless steel boxes. The below-ground section was delimited by a brick wall preventing any lateral or vertical water movement and/or element flux to or from the outside surrounding soil. Three holes at the bottom of the cylinder were connected to stainless steel water collection boxes. Holes were capped by a 2-mm net to prevent losses other than those of leachates. During the dry season, they were collected after each rainfall. During the wet season, they were collected once a week. The method of collecting the leaching water samples was shown in detail by Liu et al. (2008). Each time, the exact volume of total leachates was measured, and 100 ml per box was collected for chemical analysis. The dissolved organic C (DOC) concentrations in the solutions were measured using a TOC-VCSH analyzer. In order to determine C input from rainfall, rain water was collected in an open area near the chambers and subjected to the same analyses as for leachates.

2.2.3 Soil respiration measurement

In order to measure soil respiration, in April 2006, four PVC circular tubes (10-cm diameter) were permanently installed in each chamber, each inserted 5 cm into the mineral soil between the growing plants. Since 26 May 2006, soil respiration measurements were made once a week using an infrared gas analysis system (Licor 6400, LiCor Inc, Lincoln, NE, USA). To avoid extremely high temperatures at noon, soil CO_2 fluxes were determined in the morning (09:00–12:00). The LICOR-6400 chamber (with a foam gasket) was placed on the PVC tubes making an airtight seal. Soil respiration of a tube was determined three times repeatedly by measuring the rate of CO_2 increase in the LICOR 6400 respiration chamber. And the chamber soil respiration was the mean of the data from the four locations in each chamber (they differed by less than 5% at any measurement period).

2.2.4 Tree biomass

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.

2.3 Data analysis

Data analyses were carried out using the SAS software. For all statistical tests, we chose the probability level to reject the null hypothesis to be inferior or equal to 0.05. In order to know the effects of CO_2 and N treatments on soil organic C, soil total N, soil respiration, dissolved organic C in the leachates, and tree biomass at different measurement occasions, repeated measures of ANOVA were used. When the effects were significant, they were further analyzed using Tukey multiple comparison test (HSD). In order to obtain a global picture of the effect of treatments on soil organic C, soil total N, soil respiration, and tree biomass over the whole experiment, we compared the treatments using a mixed model.

3 Results

3.1 Tree biomass

The total above- and below-ground tree biomass was significantly affected by both high CO_2 and N treatments ($p<0.0001$ for both). In general, the CN treatment had the highest above-ground and below-ground biomass, followed by the N+ treatment, the C+ treatment, and then the control (Table 1). In January 2009, the above-ground biomass increased by 66%, 65%, and 15% in the CN treatment, N treatment, and C+ treatment chambers, respectively, when it was compared with the control chambers. At the same time, the below-ground biomass increased by 48%, 46%, and 14% in the CN treatment, N+ treatment, and C+ treatment chambers, respectively, compared with the control.

3.2 DOC leaching

DOC via leachates was very low. It was not significantly affected by both CO_2 and N treatments (Fig. 1). Generally, there was higher DOC leaching loss in 2007 than in 2008. In 2007, net annual total DOC loss via the leaching water in the CN, C+, N+, and CK chambers reached 10.9, 14.1, 10.2, and 10.3 kg $ha^{-1} year^{-1}$, respectively. While they were 6.60, 7.51, 8.90, and 9.30 kg $ha^{-1} year^{-1}$, respectively, in 2008.

3.3 Soil respiration

Soil respiration rates varied with the different sampling months, with higher values in the wet season as soil respiration rates were positively related to soil moisture ($p<0.0001$). The CN treatment had the highest soil respiration rates, followed by the C+ treatment, and then the N+ and CK treatments (Table 2). Both CO_2 and N treatments positively affected soil respiration rates ($p<0.0001$ for both). Among the years, the highest soil respiration rates were in 2008, and the lowest were in 2007.

3.4 Soil total N

Soil total N was related to soil depth ($p<0.0001$), sampling month ($p<0.0001$), N treatment ($p<0.0001$), and the interaction between the CO_2 treatment and the N treatment ($p=0.0004$). The CO_2 treatment alone did not affect soil total N in all soil depths. The 0–20 cm soil depth had the highest soil total N, while 40–60 cm soil depth had the lowest soil total N (Fig. 2). Although greater soil total N was found in the CN treatment, followed by the N+ treatment, the control, and then the C+ treatment, only soil total N in the CN treatment and the C+ treatment showed the statistically significant difference.

3.5 Soil organic C

Overall, SOC was significantly affected by the soil depth ($p<0.0001$), sampling month ($p<0.0001$), and treatments ($p<0.0001$). The 0–20 cm soil depth showed the higher SOC than the 20–40 cm soil depth. The 40–60 cm soil depth had the lowest SOC. With time, SOC increased for all the treatments, which is more obvious in the depth of 0–20 cm (Fig. 3). Overall, SOC for all the soil layers in the treatments followed the order: CN treatment>N+treatment>C+treatment=CK treatment. In November 2008, SOC in the 0–20 cm soil depth was 4,158, 3,645, 3,410, and 3,287 gm^{-2} for the CN, N+, CC, and CK treatments, respectively. The detailed information about the effects of CO_2 treatment, N treatment, sampling months, and their interactions on SOC for each soil depth was shown in Table 3.

Table 1 Tree above-ground biomass and below-ground biomass (means±standard errors) under different CO_2 and N treatments

Sampling month	CN	C+	N+	CK
Above-ground biomass (kg m^{-2})				
Aug 05	0.22±0.01	0.15±0.00	0.18±0.01	0.21±0.00
Jan 06	0.48±0.00a	0.31±0.01b	0.36±0.04b	0.37±0.01b
May 06	0.75±0.03	0.51±0.04	0.57±0.07	0.53±0.01
Jan 08	4.38±0.18a	3.11±0.29b	3.42±0.42ab	2.62±0.15b
Sep 08	6.59±0.15a	4.77±0.20bc	6.00±0.10ab	4.07±0.08c
Jan 09	7.98±0.21a	5.55±0.09b	7.94±1.26a	4.82±0.02b
Below-ground biomass (kg m^{-2})				
Aug 05	0.07±0.00	0.06±0.00	0.06±0.01	0.07±0.00
Jan 06	0.15±0.00	0.13±0.00	0.13±0.02	0.13±0.00
May 06	0.23±0.01	0.22±0.02	0.19±0.03	0.20±0.00
Jan 08	1.30±0.04a	1.08±0.07b	1.08±0.11b	0.93±0.03b
Sep 08	1.93±0.04a	1.59±0.11b	1.88±0.28ab	1.39±0.12b
Jan 09	2.39±0.12a	1.85±0.10b	2.37±0.15a	1.62±0.09b

Treatments with the same letter are not significantly different from each other ($p>0.05$). The treatments were: *CK* control, *N+* high N, *C+* high CO_2, and *CN* high CO_2+high N

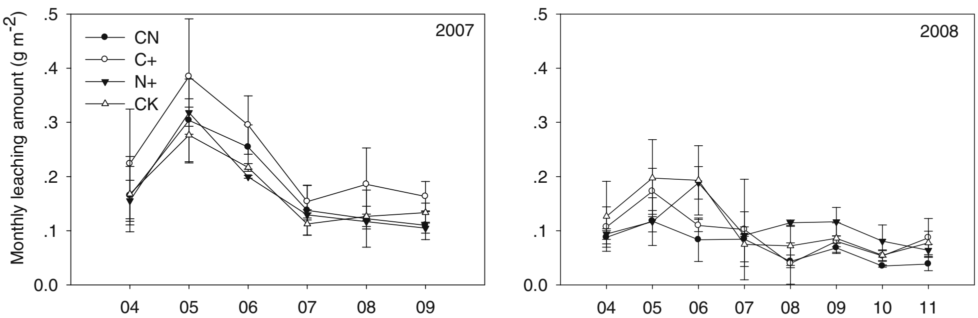

Fig. 1 Monthly leaching amount of total organic C (TOC) in the leachates under different CO_2 and N treatments. *Error bars* are standard deviations. The treatments were: *CK* control, *N+* high N, *C+* high CO_2, and CN high CO_2+high N

4 Discussion

Using meta-analysis methods, de Graaff et al. (2006) have summarized the results of 105 studies on plant biomass production from free air CO_2 enrichment and open-top chamber experiments. They concluded that, on average, elevated CO_2 stimulates the above- and below-ground plant biomass production by 20% and 30%, respectively. The greater above- and below-ground tree biomasses were also found in our experiment under elevated CO_2 treatment. Most of the studies focused on biomass changes under N addition have demonstrated that C pool sizes in shoots, roots, and whole plants increase under N addition compared with the control plants (Luo et al. 2006). The significantly increased growth was also shown in the N addition chambers in our experiment. Studies have shown that increases in SOC levels are directly linked to the return of fresh organic material to the soil (Kong et al. 2005; Gulde et al. 2008; Stewart et al. 2008). The greater tree biomass measured in the high CO_2 and N treatment chambers might increase the C input in the soil in these chambers in our experiment.

DOC is produced principally from microbial activity, root exudates, and leaching from soil organic matter (Huang et al. 1998). Both CO_2 and N treatments did not affect DOC leaching in our experiment. Hagedorn et al. (2002) also showed that the effects of elevated CO_2 and increased N deposition on DOC concentration, properties, and fluxes were small. In our subsoil, DOC leaching was very low. It was smaller than the continental export of DOC, which is equivalent to an average of 20 kg ha^{-1} $year^{-1}$ (Moore 1997). Export of DOC played only a minor role in the C cycling in our experiment.

The increased soil respiration rates induced by the CO_2 treatment were found in many studies (King et al. 2001; Pendall et al. 2001; Gill et al. 2002; Suwa et al. 2004; Bernhardt et al. 2006). Increased root biomass and soil organic matter increased root and microbial respiration under elevated [CO_2] (de Graaff et al. 2006). The greater soil organic matter (see Fig. 1) and increased root growth (see Table 2) were also shown in the CN treatment in our experiment, which led to the increased soil respiration in this treatment. The N treatment increased N availability in soils where plants had a faster growth, which led to the greater soil organic matter in the CN treatment than the C+ treatment chamber. This would also translate to greater soil respiration rates in the CN treatment than the C+ treatment. The N+ treatment chambers showed a faster root growth when compared with the C+ treatment chambers; however, the lower soil respiration was found in this treatment. The decreased microbial activities induced by the N addition would be the reason (Wallenstein et al. 2006).

As the ambient N deposition is high in subtropical China and the soil might have been N-saturated, additional N input did not change the total N in the soil, which would translate into that there were no significant differences in

Table 2 Effects of treatments on soil respiration (means±standard deviations, μmol CO_2 $m^{-2}s^{-1}$)

	2006				2007					2008		
	CN	C+	N+	CK	CN	C+	N+	CK	CN	C+	N+	CK
Year	3.72a ±1.41	3.33b ±1.19	2.80c ±0.97	2.53d ±0.76	3.51a ±1.48	3.18b ±1.54	2.51c ±1.61	2.45c ±0.92	4.11a ±1.88	3.43b ±1.65	2.67c ±1.51	2.61c ±1.16
Wet	4.60a ±1.31	4.09b ±1.05	3.02c ±0.97	2.89c ±0.74	4.25a ±1.65	4.12a ±1.61	2.95b ±1.09	2.80b ±1.07	5.21a ±1.73	4.53b ±1.56	3.75c ±1.46	3.27d ±1.25
Dry	2.85a ±0.85	2.51b ±0.70	2.57b ±0.92	2.16c ±0.57	2.76a ±0.74	2.29b ±0.72	2.11c ±1.88	2.14c ±0.62	3.09a ±1.37	2.43b ±0.96	1.72c ±0.70	2.02d ±0.65

Treatments with the same letter are not significantly different from each other ($p>0.05$). The treatments were: *CK* control, *N+* high N, *C+* high CO_2, and *CN* high CO_2+high N, *year* the means of the whole year, *wet* the means in the wet season, and *dry* the means in the dry season

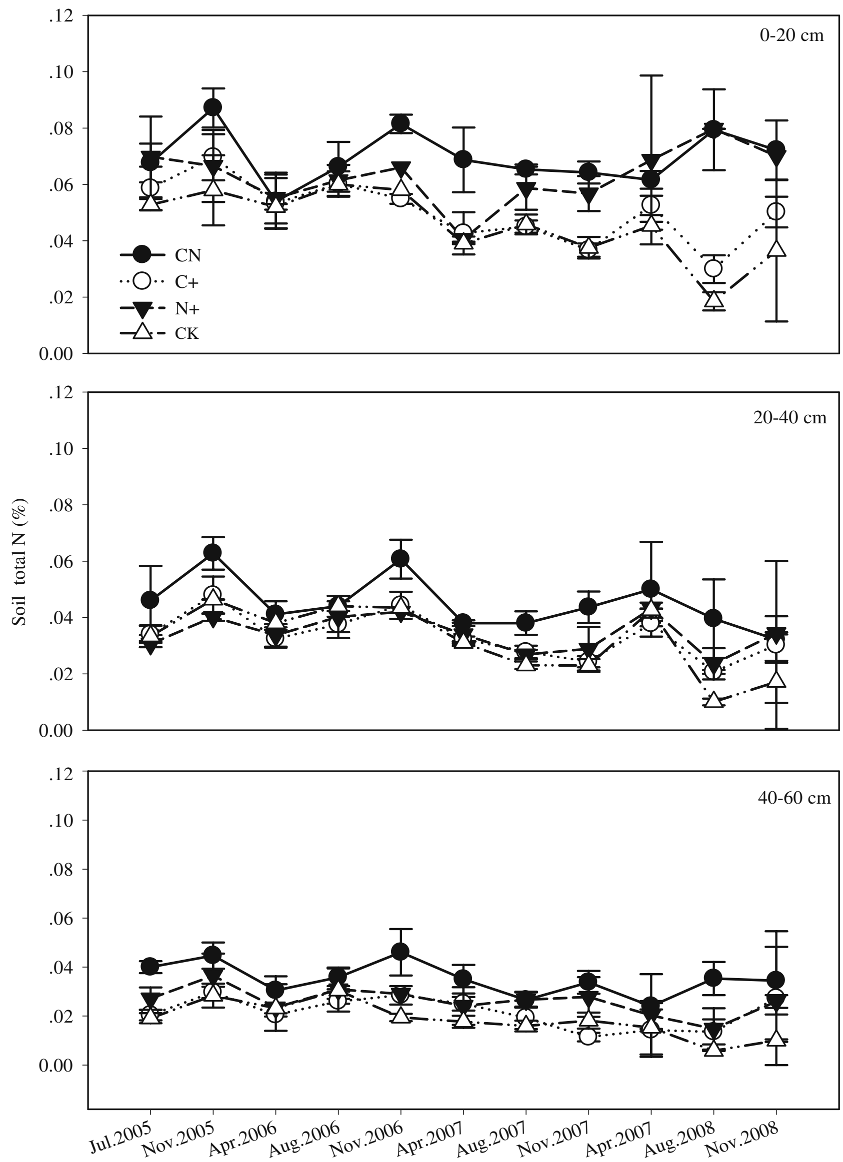

Fig. 2 The dynamics of soil total N over the 3.5 years of exposure to various CO_2 and N treatments. *Error bars* are standard deviations. The treatments were: *CK* control, *N+* high N, *C+* high CO_2, and CN high CO_2+high N

soil total N among the CN treatment, N+ treatment, and the control in our experiment. The additional N might be in part taken up by the roots and in part was leached out via soil water in the CN and N+ treatments. The relatively low soil total N in the C+ treatment should be due to the increased tree growth compared with the control.

Compared with the other treatments, although the greater soil respiration was found in the CN treatment, the increased organic matter input (including the above and below tree biomass) in this treatment still led to the higher soil organic C in this treatment. The C+ treatment had the increased tree biomass when compared with the control; however, the much greater soil respiration was measured in this treatment, which led to the similar organic C in these two treatments. This suggests that more C input in the soil under CO_2 enrichment in subtropical China would still need additional N input in the soil. This is against our hypothesis. This result is in agreement with some research carried out in temperate environment (Hungate et al. 2003; Hessen et al. 2004). The N+ treatment had higher biomass and relatively low soil respiration when compared with the C+ treatment, which resulted in the higher soil organic C in this treatment, too. There is lot of research which also showed that N inputs could increase the accumulation of soil C pools (Bowden et al. 2004; Deforest et al. 2004).

Some studies pointed out that, even in experiments with a dramatic manipulation like CO_2 doubling, detecting changes in SOC is difficult since pools of C in the soil are large, vary spatially within ecosystems, and turnover was slow compared with the duration of the most field experiments (1–5 years; Hungate et al. 1996).

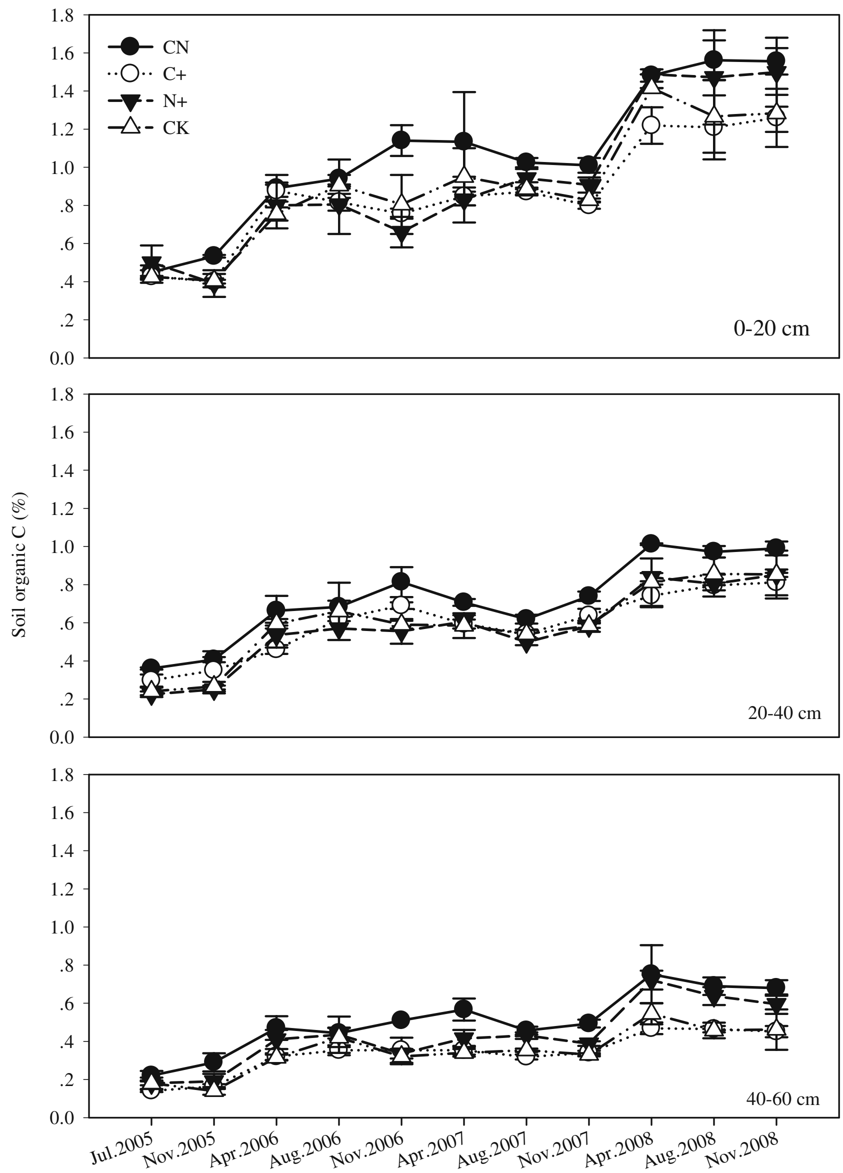

Fig. 3 The dynamics of soil organic C over the 3.5 years of exposure to various CO_2 and N treatments. *Error bars* are standard deviations. The treatments were: *CK* control, *N+* high N, *C+* high CO_2, and *CN* high CO_2+high N

However, the significant increase in soil C was determined in the chambers exposed to both high CO_2 and N treatments in our experiment. This might be due to the special climate in this area and the high density of tree planting in our chambers. We can conclude that elevated atmospheric CO_2 concentration and high N deposition in the subtropical China would benefit soil C sequestration in this area.

Table 3 Effects of CO_2 treatment (C), N treatment (N), sampling time (Time), and their interactions on soil organic C

Soil depth (cm)	C	N20	C*N	Time	C*Time	N*Time	Time*C*N	R-square
0–20	5.61*	51.37***	12.49***	59.79***	1.51	1.13	1.79	0.81
20–40	32.60***	13.28***	23.18***	42.62***	1.15	1.38	0.82	0.76
40–60	14.71***	156.07***	12.01***	21.63***	1.31	1.91*	0.31	0.72

Numbers are *F* values. *Stars* indicate the level of significance (*no star* not significant)

*$p<0.05$

**$p<0.01$

***$p<0.001$

5 Conclusions

We used open-top chambers to study the effect of elevated atmospheric [CO_2] alone and together with N addition in southern China on the soil C dynamics over 3.5 years after the treatments started. We found that the elevated atmospheric [CO_2] increased soil C pool size, but the increase would need additional N input. The N is also a soil C sequestration limiting factor under future CO_2 enrichment environment in this subtropical area. The N addition alone can also increase tree growth, which led to higher soil organic C in the chambers. We can conclude that elevated atmospheric CO_2 concentration and high N deposition in the subtropical China would benefit soil C sequestration in this area. In short-term, the significant increase in soil C pool was determined in the CN and N+ treatments in our experiment. This might be due to the special climate in this area and the high density of tree planting in our chambers.

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