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JOURNAL OF ENVIRONMENTAL SCIENCES <u>ISSN 1001-0742</u> CN 11-2629/X www.jesc.ac.cn

Journal of Environmental Sciences 21(2009) 1221-1224

# Four years of free-air CO<sub>2</sub> enrichment enhance soil C concentrations in a Chinese wheat field

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Received 24 October 2008; revised 23 January 2009; accepted 09 February 2009

#### Abstract

Elevated atmospheric  $CO_2$  can influence soil C dynamics in agroecosystems. The effects of free-air  $CO_2$  enrichment (FACE) and N fertilization on soil organic C ( $C_{org}$ ), dissolved organic C (DOC), microbial biomass C ( $C_{mic}$ ) and soil basal respiration (SBR) were investigated in a Chinese wheat field after expose to elevated  $CO_2$  for four full years. The results indicated that elevated  $CO_2$  has stimulative effects on soil C concentrations regardless of N fertilization. Following the elevated  $CO_2$ , the concentrations of  $C_{org}$  and SBR were increased at wheat jointing stage, and those of DOC and  $C_{mic}$  were enhanced obviously across the wheat jointing stage and the fallow period after wheat harvest. On the other hand, N fertilization did not significantly affect the content of soil C. Significant correlations were found among DOC,  $C_{mic}$ , and SBR in this study.

**Key words**: dissolved organic C; free air CO<sub>2</sub> enrichment; microbial biomass C; N fertilization; soil basal respiration; soil organic C **DOI**: 10.1016/S1001-0742(08)62407-9

# Introduction

With increasing global industrialization, the concentration of atmospheric CO<sub>2</sub> has increased by 32% in last 250 years (Nowak *et al.*, 2004), which have significant effects on the C cycling in terrestrial ecosystems (van Groenigen *et al.*, 2006; Prior *et al.*, 2008). Understanding the response of soil C dynamics to high atmospheric CO<sub>2</sub> concentrations is critical for evaluating the potential for soil C sequestration on time scales of decades to centuries (Pendall *et al.*, 2001).

Since free-air CO<sub>2</sub> enrichment (FACE) exposure systems nearly completely overcome the problem of microclimatic artifacts during exposure (Erbs and Fangmeier, 2006), most recent studies focused on the effects of elevated CO<sub>2</sub> on soil C and N dynamics by means of FACE technology. The studies indicated that elevated CO<sub>2</sub> increased the concentrations of total organic C, microbial biomass C ( $C_{mic}$ ) and stimulated soil respiration in cropland, grassland and forest ecosystems (Pendall *et al.*, 2001; Dijkstra *et al.*, 2005; Moscatelli *et al.*, 2005; Prior *et al.*, 2008). Using meta-analytic techniques, van Groenigen *et al.* (2006) studied the effect of elevated CO<sub>2</sub> on soil C dynamics between several levels of ecosystem management, and concluded that soil organic C ( $C_{org}$ ), soluble C,  $C_{mic}$  and microbial respiration at elevated CO<sub>2</sub> increased by

4.1%, 9.4%, 8.5% and 18.0%, respectively, compared to those at ambient. So far, there is little information about the effect of elevated  $CO_2$  and N fertilization on soil C dynamics of rice-wheat rotation ecosystems.

Therefore, the aim of this study was to investigate the responses of  $C_{org}$ , dissolved organic C (DOC) and  $C_{mic}$  to free-air CO<sub>2</sub> enrichment and N fertilization in the wheat growing season of a rice-wheat rotation ecosystems (transplanting rice in mid-June and harvest in October, then sowing wheat in early November and harvest in early June in the following year), which has been fumigated with elevated atmospheric CO<sub>2</sub> for four full years.

## 1 Materials and methods

#### 1.1 Experimental site and design

The experimental site is located in a suburb of Jiangdu in Jiangsu of China ( $32^{\circ}35'N$ ,  $119^{\circ}42'E$ ). The soil at the study site is Shajiang-Aquic Cambosols, with 18.4 g/kg total C, 1.5 g/kg total N, pH (H<sub>2</sub>O) 7.2 (Yang *et al.*, 2009), 13.7% clay and bulk density 1.16 g/cm<sup>3</sup>. A randomized complete block design was established with two levels of target atmospheric CO<sub>2</sub> concentration over the ricewheat rotation system. It consisted of three replicate rings for the elevated CO<sub>2</sub> (hereinafter referred to as FACE) and three for the ambient CO<sub>2</sub> (hereinafter referred to as ambient). The atmospheric CO<sub>2</sub> of each FACE ring was

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enriched by 200 µmol CO<sub>2</sub>/mol on average the ambient (370 µmol CO<sub>2</sub>/mol). In each FACE and ambient plot, two levels of N fertilization were applied in each sub-plot, and ammonium-based nitrogen fertilizer was applied in wheat season at the rates of 350 and 174 kg N/ha (Li et al., 2009). The FACE experiment including four different treatments was established in 2004. Our study was conducted during the wheat growing season of 2008.

### 1.2 Soil sampling and analysis

Soil samples were collected from 0-15 cm depth in each plot on March 18 at wheat jointing stage (JS) and on June 8 during the fallow period after wheat harvest (FP) in 2008. Each soil sample pooled from five soil cores (2.5 cm diameter) was stored in individual plastic bag. Thereafter, two sub-samples were prepared. One subsample was air-dried and then passed through a 2-mm sieve for soil pH, Corg and total N measurements, the other was kept at 4°C until the analysis of DOC,  $C_{\text{mic}}$  and soil basal respiration (SBR). Corg and total N contents were determined by an Element Analyzer (Elementar, Vario EL III, Germany). Soil pH was measured at a 1:2.5 (W/V) ratio of soil to distilled water. Cmic was determined according to chloroform-fumigation-extraction (CFE) method (Vance et al., 1987). The fumigated and non-fumigated soils were extracted with 0.5 mol/L K<sub>2</sub>SO<sub>4</sub> and the extracts were analyzed for C using an automated TOC analyzer (Multi N/C 3000, Analytik Jena AG, Germany). Cmic was determined based on the difference between C extracted from fumigated and unfumigated soil samples, using a conversion factor  $(K_e)$  of 0.45. Basal respiration was determined by placing 30 g of field moist soil in a 50 mL beaker and incubating the sample for 10 d in the dark at 25°C in a 1 L airtight sealed jar with 10 mL of 1 mol/L NaOH. The CO<sub>2</sub>-C evolved was determined after 2, 5 and 10 d by titration (Anderson, 1982). Basal respiration rate was calculated based on cumulative CO<sub>2</sub> evolution over the 10-d period.

Two two-way ANOVA were applied separately for

the two sampling dates to test the effects of elevated atmospheric CO<sub>2</sub> and N fertilization on soil C. Statistical analyses were performed using SPSS statistical software (SPSS Inc., Chicago, USA). Differences with P < 0.05were considered significant.

# 2 Results and discussion

## 2.1 Soil organic carbon

Soil organic C is not only one of important indicators of soil fertility, but its sequestration also plays an important role in alleviating increasing atmospheric CO<sub>2</sub> concentrations (Post et al., 2004). In our study, significant elevated CO<sub>2</sub> effects on C<sub>org</sub> were only found at JS (P < 0.01), and no significant effect of N fertilization was observed during two periods (Table 1). The values of C:N ratio of the soil were significantly higher under FACE than under ambient at JS (P < 0.05), but significantly lower under high nitrogen (HN) than under low nitrogen (LN) in both periods (P< 0.05) (Table 1). Under LN and HN, C<sub>org</sub> increased by 11.4% and 9.8% at elevated CO<sub>2</sub>, respectively (Fig. 1). The result was consistent with previous study by Prior et al. (2008), in which  $C_{org}$  at the 5–10 cm depth under dry treatment increased significantly by 11.8% at elevated CO2 in an American sorghum field.

 $C_{org}$  was negatively correlated with pH (r = -0.469, P < 0.05), and positively with soil C:N ratio (r = 0.771, P <0.01). The obtained results indicated that soil Corg and pH were synchronously affected by the elevated  $CO_2$  at JS. An elevated  $CO_2$  can increase soil  $C_{org}$  content mainly via increasing plant photosynthesis, production and allocation of photosynthate to below ground components, and thus increasing C input into soils (Xie et al., 2005) and reducing soil pH by increasing CO<sub>2</sub> partial pressure in soil solution (Wang et al., 2008) or by enhancing plant root exudates containing certain acid substances especially organic acids (Diaz et al., 1993; Berntson and Bazzaz, 1996).

Period	Indicator	Low nitrogen (LN)		High nitrogen (HN)		Effect				
		Ambient	FACE	Ambient	FACE	С	Ν	C×N		
JS	рН	$6.89 \pm 0.06$	$6.47 \pm 0.07$	$6.55 \pm 0.05$	$6.26 \pm 0.16$	**	**	ns		

Table 1 Soil C dynamics under ambient or elevated CO<sub>2</sub> of two nitrogen levels at the wheat jointing stage (JS) and the fallow period after wheat harvest (FP)

12	рп	$0.09 \pm 0.00$	$0.47 \pm 0.07$	$0.55 \pm 0.05$	$0.20 \pm 0.10$	•••		118	
	$C_{org}$ (mg/g)	$11.13 \pm 0.31$	$12.60 \pm 0.50$	$11.07 \pm 0.47$	$12.27 \pm 0.32$	**	ns	ns	
	Total N (mg/g)	$1.22 \pm 0.07$	$1.24 \pm 0.06$	$1.32 \pm 0.04$	$1.35 \pm 0.04$	ns	**	ns	
	C:N	$9.17 \pm 0.71$	$10.20 \pm 0.78$	$8.33 \pm 0.15$	$9.10 \pm 0.36$	*	**	ns	
	DOC $(\mu g/g)$	$120.62 \pm 9.49$	$149.27 \pm 2.28$	$118.56 \pm 12.99$	$146.66 \pm 9.13$	**	ns	ns	
	$C_{mic}$ (µg/g)	$268.60 \pm 28.38$	$329.38 \pm 19.82$	$254.28 \pm 21.42$	$344.15 \pm 51.35$	**	ns	ns	
	SBR ( $\mu g \operatorname{CO}_2/(g \cdot d)$ )	$5.30 \pm 0.97$	$6.57 \pm 0.54$	$5.20 \pm 0.93$	$6.56 \pm 0.45$	*	ns	ns	
FP	pH	$6.68 \pm 0.15$	$6.31 \pm 0.05$	$6.33 \pm 0.02$	$6.21 \pm 0.07$	**	**	*	
	$C_{org} (mg/g)$	$11.13 \pm 0.81$	$12.20 \pm 0.60$	$11.13 \pm 0.89$	$12.10 \pm 1.02$	ns	ns	ns	
	Total N (mg/g)	$1.17 \pm 0.02$	$1.21 \pm 0.03$	$1.31 \pm 0.02$	$1.31 \pm 0.03$	ns	**	ns	
	C:N	$9.50 \pm 0.80$	$10.13 \pm 0.67$	$8.50 \pm 0.61$	$9.23 \pm 0.67$	ns	*	ns	
	DOC $(\mu g/g)$	$222.54 \pm 30.14$	$281.04 \pm 22.82$	$205.36 \pm 16.98$	$286.15 \pm 23.25$	**	ns	ns	
	$C_{mic}$ (µg/g)	$686.99 \pm 30.45$	$796.36 \pm 19.82$	$684.17 \pm 25.91$	$808.85 \pm 70.19$	**	ns	ns	
	SBR ( $\mu g \operatorname{CO}_2/(g \cdot d)$ )	$16.61 \pm 3.02$	$16.49 \pm 0.87$	$16.62 \pm 2.72$	$16.63 \pm 1.08$	ns	ns	ns	(

 $\frac{10.02 \pm 2.12}{\text{C}_{\text{org}}: \text{ soil organic C; DOC: dissolved organic C; C_{\text{mic}}: \text{microbial biomass C; C_{\text{mic}}/C_{\text{org}}: \text{microbial quotient; SBR: soil basal respiration. C: effect of elevated CO<sub>2</sub>; N: N fertilization effect; C×N: the interactions of elevated CO<sub>2</sub> and N fertilization. Data are expressed as mean <math>\pm$  SD. \*\*P < 0.01; \*P < 0.05; ns: non-significant (P > 0.05).

#### 2.2 Soil dissolved organic C

Soil dissolved organic C (DOC) is thought to be an important labile C fraction since it is the main energy source for soil microorganisms and a primary source of mineralizable N, P and S, and it commonly responds rapidly to environmental changes (Zhang et al., 2007). In this study, significant elevated CO<sub>2</sub> effects on DOC were found at both JS and FP (P < 0.01), but no significant N fertilization effect was observed. Under LN and HN, DOC increased significantly by 25.0% and 31.5% at elevated  $CO_2$ , respectively (Fig. 1). With larger change magnitude than total organic C, the DOC tended to be more sensitive to elevated CO<sub>2</sub>. These positive effects were lower than that determined by Kang et al. (2001) who observed 49.1% increase of DOC at elevated CO2 in a peatland of north Wales. The differences may be caused by the different soil types, crop species and  $CO_2$  exposure duration.

The continuous elevated CO<sub>2</sub> will possibly lead to significant effects on DOC. Newton et al. (1994) found considerably more root biomass at elevated CO<sub>2</sub>. Other observations (van Ginkel et al., 1996) further confirmed that more root material input might increase the process of assimilatory under elevated CO<sub>2</sub>. Soil with an actively growing root system and a highly active soil microbial biomass may stimulate DOC. Hussain et al. (1999) found that N supply of 120 kg N/ha is sufficient for growth and development of wheat. Therefore, the two N levels, 350 and 174 kg N/ha, designed as normal and low N supply in our experiments, are practically super-optimal for local wheat growth and development. No significant N fertilization effect on DOC (Fig. 1) may be due to that N nutrient is practically excessive for growth and development of wheat under normal N supply.

## 2.3 Soil microbial biomass C

Soil microbes play a key role in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and the control of plant pests and plant growth (Elsgaard *et al.*, 2001; Filip, 2002). Many studies have reported that changes in soil microbial biomass and activity gave early indication of environmental change (Livia and Frank, 2006). An understanding of microbial biomass and activity in response to belowground processes induced by elevated  $CO_2$  is thus crucial to predict the long-term response of ecosystems to climatic changes

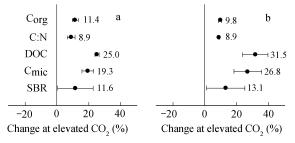


Fig. 1 Effect of elevated  $CO_2$  on several indicators of soil C cycling in JS and FP in 2008. (a) LN; (b) HN. The percentage change at elevated  $CO_2$  is calculated as (FACE-ambient)/ambient × 100%. Data are presented as mean  $\pm$  SE.

(Moscatelli *et al.*, 2005). According to our results,  $C_{mic}$  showed a similar trend with DOC during the study period. Under LN and HN,  $C_{mic}$  increased significantly by 19.3% and 26.8% at elevated CO<sub>2</sub>, respectively (Fig. 1). These results were in accordance with those of Moscatelli *et al.* (2005), who reported that in an Italian poplar plantation soil  $C_{mic}$  increased by a 16% under elevated CO<sub>2</sub>.

Significant elevated CO<sub>2</sub> effect on SBR was observed at JS (P < 0.01), but not at FP. No significant N fertilization effect on SBR was observed during the wheat growing season. Under LN and HN, SBR increased by 11.6% and 13.1% at elevated CO<sub>2</sub>, respectively (Fig. 1). These results were lower than those observed by Pendall *et al.* (2001), who found that FACE increased soil respiration rate by 38% during the peak of wheat growth in USA in 1997.

The main reason for above results might be that elevated  $CO_2$  can significantly increase root biomass and root tissue turnover, thereby increase the flux of energy and nutrients available for microbial biosynthesis (Zak *et al.*, 2000). As reported by Körner and Arnone (1992), elevated  $CO_2$  may stimulate microbial biomass production because of increasing C input into the rhizosphere, which will enhance the decomposition of soil organic matter, with a positive feedback on plant growth by releasing the necessary nutrients. Bernhardt *et al.* (2006) also found that elevated  $CO_2$  increase the C allocation rate to the roots, which led to the activity of soil microorganisms and enhance SBR significantly.

 $C_{mic}$  was positively correlated with SBR (r = 0.887, P < 0.01) and DOC (r = 0.941, P < 0.01) which was in line with those of Sowerby *et al.* (2000), who reported that the increase in microbial biomass was responsible for the increase in respiration of soils exposed to elevated CO<sub>2</sub> concentration. DOC was proposed as an indicator of the C available to soil microorganisms (Smolander and Kitunen, 2002). The close relationship between C<sub>mic</sub> and DOC was reported by many researchers, such as Zhang *et al.* (2007), who observed that C<sub>mic</sub> was positively correlated with DOC with a correlation coefficient of 0.99 under an abandoned cultivated wetland in Northeast China.

# **3** Conclusions

Results from this study demonstrated that elevated  $CO_2$  significantly increased  $C_{org}$  and stimulated SBR at wheat jointing stage after a 4-year exposure. Elevated  $CO_2$  significantly enhanced the concentrations of DOC and  $C_{mic}$  at wheat jointing stage and during the fallow period after wheat harvest. The N fertilization exhibited no significant effect on soil C dynamics during the study period. These results suggested that elevated  $CO_2$  has stimulative effects on soil C concentrations regardless of N fertilization.

#### Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 30770400, 40231003), the Knowledge Innovation Program of Chinese Academy of Sciences (No. KZCX2-408). The Chinese Rice/Wheat FACE Project is a research program within China-Japan Science and Technology Cooperation Agreement. The main instruments and apparatus of the system were supplied by Japan National Institute for Agro-Environmental Sciences (NIAES) and Japan Agricultural Research Centre for Tohoku Region (NARCT).

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