Elevated atmospheric carbon dioxide concentration stimulates soil microbial activity and impacts water-extractable organic carbon in an agricultural soil

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Abstract Carbon dioxide (CO₂) enrichment and increased nitrogen (N) deposition can change microbial activity and dissolved organic carbon (DOC) turnover, consequently affecting carbon sequestration in soils. However, we do not have much available information on the relationship between soil DOC and microbial activity under CO₂ enrichment and N addition in semi-arid agroecosystems. Using free air CO₂ enrichment (FACE), soybean and winter wheat were grown in the

field under ambient CO₂ (350 μmol mol⁻¹) and elevated CO₂ (550 μmol mol⁻¹) conditions subjected to two N fertilizer regimes (132 and 306 kg N ha⁻¹ year⁻¹). Rhizosphere soils and bulk soils at three depths, 0–10, 10–20 and 20–40 cm, were collected to determine water extractable organic matter (WEOM) characteristics with fluorescence spectroscopy and parallel factor analyses of excitation/emission matrix, as well as five extracellular enzymes activities. All significant effects were observed in the topsoil (0–10 cm): elevated CO₂ decreased water extractable organic carbon concentration of the rhizosphere soils and bulk soils by 8.5 and 10.1 %, respectively. Furthermore, elevated CO₂ changed the composition and structure of soil WEOM by increasing the plant- and microbial-derived components in the rhizosphere and solubilizing soil organic matter (SOM). The activities of β-1,4-glucosidase, cellobiohydrolase, phenol oxidase, and peroxidase were stimulated by elevated CO₂ in the rhizosphere soils and bulk soils. Our findings suggest that the stimulation of microbial activity elicited by elevated CO₂ increased the turnover of labile WEOM and the solubilization of SOM in the topsoils, which could be adverse to the accumulation and stability of soil carbon in the semi-arid agroecosystems in northern China.

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Introduction

The atmospheric concentration of carbon dioxide (CO_2) has increased from $280 \mu\text{mol mol}^{-1}$ in 1,800–379 $\mu\text{mol mol}^{-1}$ in 2005, and is predicted to reach $550 \mu\text{mol mol}^{-1}$ in the middle of this century (Solomon 2007). Over the same period, the global nitrogen (N) deposition has increased from 34 to 105 Tg year^{-1} and is expected to double over the next 25 years (Galloway et al. 2008). Both CO_2 enrichment and N deposition stimulate plant photosynthesis resulting in increased C stocks in soils (De Graaff et al. 2006; Lu et al. 2011). However, both CO_2 enrichment and N deposition have also been shown to stimulate microbial activity and accelerate soil organic C (SOC) turnover (Hagedorn et al. 2008; Müller et al. 2009; Fang et al. 2012), leading to controversies regarding the question of whether or not soils may sequester more C under elevated CO_2 and N deposition (Norby et al. 2005; Carney et al. 2007; Liu and Greaver 2010; Lu et al. 2011). It is believed that CO_2 enrichment and N deposition will affect soil microbial communities and impose significant control on SOC accumulation in soils. However, this response is highly variable, related to for example, plant community (Handa et al. 2008), soil types (Guenet et al. 2012), and soil nutrient availability (Dawes et al. 2013).

As an easily accessible source of energy and nutrients for soil microbes, dissolved organic matter (DOM) contributes considerably to soil C and N mineralization (Kalbitz et al. 2003), C loss (Bengtson and Bengtsson 2007), and the accumulation and stability of soil organic matter (SOM) (Hagedorn et al. 2012). To elucidate the role of soils as C sinks/sources under elevated CO_2 and increased N deposition, dissolved organic C (DOC) in soils might represent a highly sensitive indicator for SOC dynamics (Hagedorn et al. 2008). Existing evidence from various terrestrial ecosystems indicates that experimental CO_2 enrichment can increase (Freeman et al. 2004; Finzi et al. 2006; Hagedorn et al. 2008; Dawes et al. 2013), decrease (Aitkenhead-Peterson and Kalbitz 2005), and not change (Hagedorn et al. 2002; Oh et al. 2007; Liu et al. 2010) DOC concentration in soils. Similarly controversial results are being reported from N application experiments, including positive (Gallo et al. 2005; Currey et al. 2011), negative (Hill et al. 2006), and neutral

(Cory et al. 2004; McDowell et al. 2004). So far, most relevant studies focused on the changes of soil DOC concentration, and relatively fewer studies have looked at the response of DOM composition and structure to elevated CO_2 , N application, and their interactions (Michel et al. 2006; Hagedorn et al. 2008), especially in semi-arid agroecosystems.

The rhizosphere priming effect (RPE) is defined as the stimulation or suppression of SOM decomposition by live roots and associated rhizosphere organisms, and plays a crucial role in regulating SOM storage and turnover (Cheng et al. 2014). CO_2 enrichment strongly affects microbial communities through elevating water use efficiency, increasing rhizo-deposition of labile C, and accelerating plant growth (Burns et al. 2013). In general, CO_2 enrichment increases the activity of cellulose-degrading enzymes due to increase in root exudation or turnover, and much of the additional fixed C is partitioned to rapidly cycling C pools rather than into more recalcitrant fractions of SOM (Hungate et al. 1996; Henry et al. 2005). Moreover, the RPE caused by the addition of labile C may stimulate the breakdown of more resistant SOM due to the work of extracellular enzymes (Kuznyakov 2010). In contrast, N fertilizer application will inhibit the activity of lignin-degrading white-rot fungi, increasing the release of water soluble soft-rot products (Sinsabaugh et al. 2004; DeForest et al. 2004; Gallo et al. 2005). In the semi-arid agricultural ecosystem of northern China, elevated CO_2 significantly increased the rate of crop photosynthesis (Hao et al. 2013), the production of wheat and soybean roots (Hao et al. 2014), and the rate of soil respiration (Lam et al. 2011). Therefore, we hypothesized that elevated CO_2 would stimulate cellulose- and lignin-degrading enzyme activities in the rhizosphere due to greater root biomass and rhizo-deposition, while N fertilizer application would inhibit lignin-degrading enzyme activities in the N-rich agricultural soils (Ebersberger et al. 2003). We also hypothesized that the increase in extracellular enzyme activities caused by elevated CO_2 could stimulate the breakdown of more resistant SOM (Kuznyakov 2010), thereby changed the composition and stability of soil DOM.

The free-air carbon dioxide enrichment (FACE) experiment allows for exploration of the effects of elevated CO_2 in intact ecosystems under more or less native conditions. Within a FACE experiment, we

examined the intrinsic links between soil water extractable organic carbon (WEOC) dynamics and activity of microbial communities involved in soil C cycling in an agricultural ecosystem under CO₂ enrichment and N addition. The specific objectives of our study were to investigate: (1) the effects of CO₂ enrichment and N application on the amount and composition of soil WEOC in different soil layers and crop growth periods; (2) the effects of CO₂ enrichment and N application on soil extracellular enzyme activities (EEAs) that are responsible for SOM decomposition; and (3) the correlations of soil WEOC quantity and quality with microbial activities.

Materials and methods

Site description and experimental design

Our study was conducted in a wheat-soybean field at the Changping research station, Chinese Academy of Agricultural Sciences, located in northern Beijing, China (40.13°N, 116.14°E, Fig. 1). The climate is characterized by warm, moist summers and cold, dry winters, with mean annual precipitation of 572 mm and mean annual temperature of 11.8 °C. The soil at the study site is a Chromic Luvisols, with a pH (1:5 soil: water) of 8.4 and contained 8.18 g kg⁻¹ organic C, 0.82 g kg⁻¹ total N, 5.24 mg kg⁻¹ NH₄⁺-N and 14.61 mg kg⁻¹ NO₃⁻-N (Lam et al. 2011). Typically, soybean (*Glycine max* (L.) Merr) is sown in the middle of June and harvested in early October and winter wheat (*Triticum aestivum* L.) is sown in the middle of October and harvested in early June of the next year. The following four combined treatments were applied: ambient CO₂ (350 ± 40 μmol mol⁻¹) plus low N application (ALN, 132 kg urea-N ha⁻¹ year⁻¹), ambient CO₂ plus high N application (AHN, 306 kg urea-N ha⁻¹ year⁻¹), elevated CO₂ (550 ± 60 μmol mol⁻¹) plus low N (ELN), elevated CO₂ plus high N (EHN). The FACE rings were constructed on six of twelve plots to receive air with increased CO₂ concentrations (200 ± 40 μmol CO₂ mol⁻¹), and the other six rings receive ambient air without FACE apparatus. Each ring is 4 m in diameter, and the distance between two adjacent rings is larger than 17 m. CO₂ fumigation in FACE treatments was performed 24 h per day throughout the growing season. Urea fertilizer was applied in both wheat and soybean cropping seasons. This FACE experiment was conducted over 4 years

beginning in May 2006 and ending in October 2010. For the HN and LN treatments, a 50 % of N added was applied as basal fertilizer, and 10 and 40 % was top-dressed at early- and late-tillering, respectively. Phosphorus and potassium fertilizers were applied in all plots at rates of 65 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹ in either soybean or wheat season. The plots were randomly arranged with three replications (Fig. 1).

Soil sampling and WEOC determination

Soil WEOM mainly derives from rhizo-deposition and degradation of litter and humus, and thereby is significantly affected by crop types and growth stages. In 2009–2010, soil samples were collected in the seeding (June), filling (August), and harvest stages of soybean (October) and booting stage of wheat (April), respectively. At each sampling time, rhizosphere soil adhering to the plant roots was taken, whereas bulk surface (0–10 cm) and subsurface (10–20 and 20–40 cm) soils were taken from the space between crop rows using a core sampler (2.5 cm in diameter). Both rhizosphere soils and bulk soils were taken in five replicates from each core and were composited and mixed to ensure homogeneity in the field. The soil samples were then transported to the lab in chilled polystyrene boxes and stored at 4 °C.

The WEOM of soil samples was extracted with deionized water (solid to water ratio of 1: 2.5 w/v) by shaking fresh soil samples for 2 h on a horizontal shaker at room temperature. The soil supernate was filtered using 0.45 μm polytetrafluoroethylene filters, and the extracts were immediately analyzed for WEOC concentration using the TOC analyzer (Liqui TOCII, Elementar, Germany).

Soil WEOM composition analysis

A subset of extracts from August 2009 samples was analyzed the composition and structure of WEOM using three-dimensional excitation/emission matrix fluorescence spectroscopy (EEM) technique. The EEM spectra of WEOM were recorded using a fluorescence spectrophotometer (Hitachi, Model F-4600) equipped with a 150 W xenon arc lamp as the excitation source. Prior to fluorescence analysis, all sub-samples for fluorescence analysis were diluted to the uniform concentration of 10 mg L⁻¹ to reduce inner filter effects. Spectra were recorded using 5 nm

Fig. 1 Illustration of field plots in the Free Air CO₂ Enrichment (FACE) experiment

excitation and 10 nm emission band-pass widths, and the scan speed was 2,400 nm min⁻¹ (Fang et al. 2014). The scanning wavelength range was set as: excitation wavelength (Ex) 200–400 nm, emission wavelength (Em) 300–550 nm. Data in the two triangular areas corresponding to the Rayleigh and Raman scattering peaks were eliminated to acquire better mathematical results. To reduce the impact of Raman scatter, an EEM spectrum of deionized water was subtracted from the EEM spectrum of each sample.

The parallel factor analysis (PARAFAC) provides a statistical model considering that fluorescence emission of one component measured at a certain pair of excitation/emission wavelengths is a tri-linear function of the concentration of this component and its specific absorption/emission properties (Stedmon and Bro 2008). Stedmon and Bro (2008) have illustrated the detail of this method and the procedures for EEMs analysis. We conducted the PARAFAC modeling using MATLAB (MathWorks R2010b) and DOMFluor Toolbox (<http://www.models.kvl.dk/search/node/DOMfluor>). A non-negativity constraint was applied to the parameters to allow only statistically relevant results. A split-half analysis was performed in order to choose the meaningful number of fluorescent components which varied from two to five. The intensity of the n th component in a given sample, I_n , was calculated as the fluorescence intensity at the peak excitation and emission maximum of the n th component using the following equation (Kowalczyk et al. 2010):

$$I_n = \text{Score}_n * \text{Ex}_n(\lambda_{\max}) * \text{Em}_n(\lambda_{\max}) \quad (1)$$

where Score_n is the relative intensity of the n th component, $\text{Ex}_n(\lambda_{\max})$ is the maximum of the excitation loading of the n th component, and $\text{Em}_n(\lambda_{\max})$ is the maximum of the emission loading of the n th component derived from the model. The total fluorescence intensity of a given sample was calculated as the sum of the components present in the sample:

$$I_{\text{Total}} = \sum_1^n I_n \quad (2)$$

The percent fluorescence response of each component was calculated by the Eq. (3).

$$P_n = I_n / I_{\text{Total}} \quad (3)$$

The humification index (HIX) value was calculated as:

$$\text{HIX} = \sum I_{435 \rightarrow 480} / \sum I_{300 \rightarrow 345} \quad (4)$$

where I is the fluorescence intensity at each wavelength using an excitation wavelength of 254 nm (Ohno 2002).

Enzyme assays

Parallel to the determination of WEOC concentration, enzyme assays were performed on all collected soils. Microbial functional activity was evaluated by determining enzymes involved in labile and recalcitrant C

transformations. β -1,4-glucosidase and cellobiohydrolase are associated with the breakdown of cellulose. N-acetylglucosaminidase is involved in the degradation of chitin and phenol oxidase and peroxidase are involved in the decomposition of lignin (Billings and Ziegler 2008). The cellulose-degrading and chitin-degrading enzymes were assessed using 96-well black plates and fluorescently labeled substrates (Sinsabaugh et al. 2003). Simply, each soil sample (1.0 g fresh soil) was homogenized in 125 μ L of 50 μ M sodium acetate buffer (pH 5.5) for 30 s with a hand blender. Each 96-well plate contained a background sample as well as three soil samples. We used eight wells of 50 μ L substrate and 200 μ L buffer as negative controls and eight wells of 50 μ L standard [10 μ M 4-methylumbelliferone (MUB)] and 200 μ L buffer to calculate the emission coefficient. For each soil sample, we used eight replicate wells of 200 μ L soil slurry and 50 μ L of substrate for the assay, eight wells of 200 μ L soil slurry and 50 μ L of standard to calculate the quench coefficient, and eight sample controls containing 200 μ L soil slurry only. After incubation at 20 °C for 2 h (cellulase) and 0.5 h (chitinase), plates were scanned at an excitation of 365 nm and an emission of 450 nm on an f-Max fluorometer (Molecular Devices Corp., Sunnydale, CA, USA). To raise the MUB emission coefficients to readable levels in all plates, we added 10 μ L of 0.5 M NaOH to each well. For the phenol oxidase and peroxidase assay, the substrates of L-3,4-dihydroxyphenylalanine (DOPA) and 96-well clear plates were used for spectrophotometric analysis. Phenol oxidase assays were conducted by adding 50 μ L of 25 μ M DOPA to each sample well and peroxidase assays received an additional 10 μ L of 0.3 % H_2O_2 . Following an 18 h incubation at 20 °C, absorbance was read at 450 nm on an EL-800 plate reader (Biotek Instruments, Inc., Winooski, VT, USA). All enzyme activities are expressed as $\mu\text{mol h}^{-1} \text{g}^{-1}$ soil.

Data analysis

We used a repeated measures analysis of variance (ANOVA) with a Tukey's Honestly Significant Difference (HSD) to evaluate the effects of treatment, month, soil layer, and their interaction on soil WEOC and EEAs. We performed a three-way ANOVA to examine the effects of CO_2 enrichment, N application, soil layer and their interactions on the components and

Fig. 2 The variation of soil WEOC concentration under different treatments. The soil WEOC concentration at each layer/month is the mean of 4 months/three soil layers. The significance of differences among the four treatments is indicated by different lower case letters in each soil layer or each month

HIX of WEOM. Also, a correlation analysis was used to test the relationships of soil WEOC concentration, each WEOM component, and HIX of WEOM with soil EEAs.

Results

Soil WEOC concentration

Soil WEOC concentration decreased with soil depth, ranging from 127.77 mg kg^{-1} in 0–10 cm to 109.36 mg kg^{-1} in 20–40 cm depth (Fig. 2a; Table 1, $P < 0.001$). WEOC concentration was higher in rhizosphere soils than in bulk soils (Fig. 2a). Elevated CO_2 tended to decrease WEOC concentration in the rhizosphere and bulk soils (0–10 cm). Compared with AHN treatment, EHN treatment significantly decreased WEOC concentration by 8.9 and 8.5 % in the rhizosphere and bulk soils in 0–10 cm depth, respectively (Fig. 2a). However, the difference in WEOC concentration was not significant among the four treatments below 10 cm depth (Fig. 2a).

Table 1 Summary of repeated measures ANOVA evaluating the effects of month, soil layer, treatment and their interactions on soil WEOC concentration and EEAs

Source of variation	WEOC ^{a, b}	Soil EEAs ^{a, b}				
		bG	CBH	NAG	PhOx	PerOx
Between subjects						
Treatment	0.031	0.016	0.019	0.11	0.021	0.034
Soil layer	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment × Soil layer	0.37	0.18	0.27	0.81	0.34	0.63
Within subjects (Multivariate)						
Month	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Month × Treatment	0.39	0.023	0.026	0.63	0.032	0.044
Month × Soil layer	0.23	0.079	0.099	0.65	0.15	0.31
Month × Treatment × Soil layer	0.85	0.31	0.69	0.97	0.78	0.95

^a EEAs are extracellular enzymes activities. bG, CBH, NAG, PhOx, PerOx are the abbreviation of β -1,4-glucosidase, cellobiohydrolase, N-acetylglucosaminidase, phenol oxidase and peroxidase, respectively. WEOC is water extractable organic carbon

^b Statistical significant differences were set with *P* values <0.05 unless stated otherwise

The average WEOC concentration across soil profiles was significantly different between months (Table 1, *P* < 0.001). The peak average WEOC concentration occurred in August while June experienced the lowest one (Fig. 2b), which was corresponding to the filling and seeding stages of soybean, respectively. N application did not significantly change soil WEOC concentration at any soil depth (Fig. 2b).

DOM composition and structure

The core consistency diagnostic scores indicate that the three component model provided the best spectral resolution of components and was likely the most appropriate model for this soil WEOM data set. The excitation and emission spectra and their spectral loadings of a given fluorescent component, obtained using the split half analysis, were displayed in Fig. 3. The maximas were (270 nm excitation/442 nm emission) for component 1 (210 nm excitation/372 nm emission) for component 2, and (240 nm excitation/422 nm emission) for component 3 (Fig. 3b, d, f). The three components had been assigned in the literature to humic-like, protein-like and fulvic acid-like molecules (Graeber et al. 2012; Ishii and Boyer 2012).

Elevated CO₂ significantly changed the composition and structure of WEOM in the rhizosphere and bulk soils (Fig. 4, Appendix; Table 2), and the effects varied with soil layer (Table 2). For example,

ELN and EHN treatments increased the intensity of fluorescence peaks of protein-like compounds (Ex/Em = 200–220/304–416 nm) in the rhizosphere, 10–20 and 20–40 cm soils, but decreased them in the 0–10 cm soil layer (Appendix). Overall, elevated CO₂ (ELN and EHN) tended to decrease the percentages of humic-like (P₁) and fulvic acid-like (P₃) compounds in the rhizosphere and bulk soils below 10 cm, but increased them in 0–10 cm depth (Fig. 4). However, the percentage of protein-like (P₂) compound inversely responded to elevated CO₂, with increase in the rhizosphere and bulk soils below 10 cm depths but decrease in 0–10 cm depth. Furthermore, elevated CO₂ significantly decreased the HIX of WEOM in the rhizosphere and bulk soils below 10 cm depths, but increased them in 0–10 cm depth (Fig. 4d). N application did not have any significant impacts on these variables at any soil depth.

Soil extracellular enzymes activities

The five EEAs significantly declined with soil depth (Fig. 5; Table 1, *P* < 0.001). Compared with the ambient CO₂ treatments (ALN and AHN), elevated CO₂ treatments (ELN and EHN) increased the activities of β -1,4-glucosidase, cellobiohydrolase, phenol oxidase and peroxidase in the rhizosphere soils and bulk soils in 0–10 cm depth (Fig. 5). However, the four treatments did not affect soil N-acetylglucosaminidase activity (Fig. 5e).

Fig. 3 Split-half analysis of the three component PARAFAC model. Excitation and emission spectral loadings obtained from the whole dataset and separately from two random halves of the dataset are shown for the components 1, 2, and 3

Fig. 4 The percentage of three WEOM components and the HIX of WEOM in the rhizosphere soils and bulk soils under different treatments. The significance of differences among the four treatments is indicated by different lower case letters in each soil layer

Table 2 Results of three-way ANOVA of the effects of CO₂ enrichment, N application, soil layer and their interactions on the percentages of WEOM components and HIX of WEOM

Source of variation	P ₁ ^a		P ₂ ^a		P ₃ ^a		HIX of WEOM	
	F	P	F	P	F	P	F	P
CO ₂ enrichment	8.13	0.008	6.09	0.019	7.80	0.009	0.88	0.021
N application	0.60	0.45	0.31	0.58	1.04	0.32	0.60	0.45
Soil layer	2.20	0.11	1.56	0.22	1.13	0.35	2.55	0.073
CO ₂ enrichment × N application	0.001	0.98	0.17	0.68	0.48	0.50	0.044	0.84
CO ₂ enrichment × Soil layer	3.97	0.016	3.86	0.018	11.51	<0.001	3.01	0.045
N application × Soil layer	0.17	0.91	0.033	0.99	0.097	0.96	0.13	0.94
CO ₂ enrichment × N application × Soil layer	1.02	0.40	0.74	0.54	0.26	0.86	0.90	0.45

^a P₁, P₂ and P₃ are the percentages of component 1, 2 and 3 of soil WEOM

Fig. 5 The responses of five soil EEAs to CO₂ enrichment and N application treatments in different soil layers. The significance of differences among the four treatments is indicated by different lower case letters in each soil layer

Soil enzyme activities significantly varied across months (Table 1, $P < 0.001$). The lowest and highest values of five soil EEAs occurred in June and August, respectively (Fig. 6), which was corresponding to the seeding and filling stages of soybean. Elevated CO₂

treatments (ELN and EHN) tended to increase the activities of β -1,4-glucosidase, cellobiohydrolase, phenol oxidase and peroxidase but not N-acetylglucosaminidase in soils in April and August. There was no significant difference in five soil EEAs between

Fig. 6 The responses of averaged soil EEAs to CO₂ enrichment and N application treatments in different month. The significance of differences among the four treatments is indicated by *different lower case letters* in each month

low N and high N treatments under any CO₂ concentration (Fig. 6).

Correlation between soil WEOC dynamics and enzyme activities

The significant response of microbial processes to CO₂ enrichment and N application was only detected in the topsoils. So, only the rhizosphere and the bulk soils in 0–10 cm depth were used for further correlation analysis. The result showed that soil WEOC was significantly and positively correlated with the activities of soil β -1,4-glucosidase, cellobiohydrolase, phenol oxidase and peroxidase (Table 3). Among the five enzyme activities examined, only phenol oxidase and peroxidase activities were correlated with the percentage of fulvic acid-like compound (P₃) (Table 3). Furthermore, the HIX of WEOM was also related to soil peroxidase activity (Table 3).

Discussion

Soil C degradation under CO₂ enrichment

Five soil EEAs decreased with soil depth and their peak values occurred in the booting stage of wheat and the filling stage of soybean (Figs. 5, 6), indicating that plant growth is an important control on temporal patterns of soil EEAs (Phillips et al. 2011). CO₂ enrichment tended to stimulate cellulose-degrading (β -1,4-glucosidase and cellobiohydrolase) and lignin-degrading (phenol oxidase and peroxidase) enzyme activities in the rhizosphere and the upper 10 cm bulk soils. These results, to a certain extent, confirm our first hypothesis that elevated CO₂ would stimulate cellulose- and lignin-degrading enzyme activities in the rhizosphere due to greater root biomass, greater fine root turnover and greater rhizo-deposition (Hao et al. 2013, 2014). Kandeler et al. (2006) also

Table 3 Pearson's correlation coefficients of soil WEOC concentration, composition and structure with microbial activities

	Soil EEAs ^{a, b, c}				
	bG	CBH	NAG	PhOx	PerOx
WEOC ^a	0.84**	0.61*	0.29	0.72**	0.59*
P ₁ ^a	−0.35	0.076	0.20	0.44	−0.11
P ₂ ^a	0.20	−0.17	−0.20	−0.40	−0.26
P ₃ ^a	0.04	0.24	0.12	0.62*	0.77*
HIX ^a	−0.32	0.37	0.20	0.46	0.61*

^a EEAs are extracellular enzymes activities. WEOC is water extractable organic carbon. P₁, P₂ and P₃ are the percentages of component 1, 2 and 3 of soil WEOM. HIX is humification index of WEOM

^b bG, CBH, NAG, PhOx, PerOx are the abbreviation of β -1,4-glucosidase, cellobiohydrolase, N-acetylglucosaminidase, phenol oxidase and peroxidase, respectively

^c Correlation significance is represented by *, ** at $P < 0.05$ and $P < 0.01$, respectively

suggested that total belowground microbial biomass was significantly correlated with 1,4- β -glucosidase and cellobiohydrolase, plant production (especially belowground production) determined total cellulose input to soil. However, we did not find significant stimulation of N-acetylglucosaminidase activity under elevated CO₂ (Figs. 5e, 6e), which is different from other studies in N-limited forest or grassland ecosystems (Chung et al. 2006; Finzi et al. 2006; Jin and Evans 2007; Zheng et al. 2010; Guenet et al. 2012). This may be due to the annual N amendments of more than 132 kg ha^{−1} that repressed the synthesis of soil N-acetylglucosaminidase in the semi-arid farming ecosystem.

Under elevated CO₂, the increased cellulose-degrading enzymes accelerate the turnover of labile C from root and rhizo-deposition, which in turn promote soil CO₂ emission (Burns et al. 2013). One of our previous studies showed that elevated CO₂ increased soil CO₂ flux by 15 % compared with ambient CO₂ treatment (Lam et al. 2011). Moreover, the increase in phenol oxidase activity implies microbes demand more nutrients and thus degrades more litter and SOM (Xie et al. 2005; Finzi et al. 2006), which may promote the breakdown of other organic solutes (e.g., phenolic compounds). Similarly, soil peroxidase plays an important role in the degradation of “old”, protected organic matter and N

mineralization, too. Greater soil peroxidase activity under elevated CO₂ may accelerate the degradation of physically protected organic materials and thus changes the composition and structure of SOM (Tian et al. 2010). Our study also showed significant relationships between two lignin-degrading enzyme activities and WEOC concentration, as well as fulvic acid-like component (Table 3).

Soil WEOC dynamics under CO₂ enrichment

Most previous FACE studies indicate that elevated CO₂ does not change DOC concentrations (Siemens et al. 2012) or increase WEOC and DOC concentrations from greater inputs of labile throughfall DOC (Hagedorn et al. 2008), increased root exudation (Handa et al. 2008), and soil microbial shifts and associated soil “priming effects” (Carney et al. 2007; Hagedorn et al. 2008). But our study showed that elevated CO₂ decreased WEOC concentration in the rhizosphere and bulk soils in 0–10 cm depth, which could be related to semi-arid climate, low input of crop residues, and high N application. In the current study, the impacts of elevated CO₂ are controlled by multiple processes which could superimpose each other. First, the “rhizosphere priming effect” accelerates WEOC turnover and consumption in the rhizospheres (Lagomarsino et al. 2006; Carney et al. 2007). The higher input of labile C from crops will be compensated and occasionally overcompensated by higher decomposition rates and probably lower use efficiency (Zak et al. 1993). Second, the decrease of WEOC concentration in bulk soils under elevated CO₂ was the net effect of microbial solubilization and mineralization of WEOM. On the one hand, an increased phenol oxidase activity might increase WEOM concentrations through solubilizing SOM (Sinsabaugh 2010). On the other hand, enhanced microbial activity could also cause a net decrease in WEOM concentrations because of more WEOM degradation (Henry et al. 2005; Collins et al. 2008). Finally, the response of soil WEOC to elevated CO₂ largely relies on soil moisture status. Some evidences suggest more pronounced effects of CO₂ enrichment under drier soils due to the water saving effect (Hill et al. 2006; Guenet et al. 2012). Under semi-arid conditions, newly-formed DOC would be rapidly oxidized into CO₂ and difficult to be retained in soils (Hagedorn et al. 2008; Müller et al. 2009). Overall, these results indicate that elevated CO₂ will accelerate

DOC turnover and is adverse to C sequestration in the semi-arid arable soils.

Besides altering WEOC quantity, elevated CO_2 significantly changed the composition of WEOM. Elevated CO_2 significantly increased the percentage of protein-like compound (P_2) in the rhizosphere, while it decreased them in the bulk soil of 0–10 cm (Fig. 4b). The protein-like materials (e.g. tyrosine, tryptophan) are derived from bacteria (Dorodnikov et al. 2009) and belong to the fraction of biodegradable DOM (Chen et al. 2003). Although the percentage of aromatic protein materials is small, they contribute significantly to the CO_2 effluxes from soils because of their rapid turnover rates, usually within hours (van Hees et al. 2005; Fellman et al. 2008). On the contrary, elevated CO_2 decreased the percentages of humic-like materials (P_1 and P_3) in rhizosphere by promoting the release of plant-derived and microbial “protein-like” WEOM (Hill et al. 2006; Kalbitz and Kaiser 2008; Müller et al. 2009), while it increased them in the bulk soil of 0–10 cm by increasing the solubility of more humified, “older” material from SOM (Hagedorn et al. 2008). The changes in SOM solubilization caused a net shift of the WEOM composition in 0–10 cm topsoils to higher contents of “humic-like” materials, because less microbial residues were released compared with that in the rhizosphere. For bulk soils below 10 cm, the impact of CO_2 enrichment on SOM solubility was small probably due to insignificant effects on enzyme activities at these depths (Fig. 5). High fraction of “protein-like” WEOM could thus result from accumulation of plant-derived and microbial compounds from the rhizosphere or from increases of microbial biomass. Overall, elevated CO_2 significantly decreased the HIX of WEOM in the rhizosphere and subsurface soils below 10 cm, but showed a positive effect in the 0–10 cm depth (Fig. 4d). This indicates that elevated CO_2 tends to lower the humification of WEOM in the rhizosphere and subsurface soils, which is expected to be associated with a higher mineralization rate of DOC under CO_2 enrichment.

Soil WEOC dynamics in response to N application

N supply did not affect the responses of soil WEOC dynamics and enzyme activities to CO_2 enrichment (Figs. 2, 5, 6). A similar result was reported in a rice–

wheat rotational FACE system, China (Zhong et al. 2009). These results indicate that N may not be limiting in the semi-arid agroecosystem. The total N application rate of 132 and 306 kg N ha⁻¹ year⁻¹ could be excessive for the demands of crops and soil microbial communities (Lam et al. 2011). In general, insufficient N supply will limit N_2O emissions despite the greater C availability under elevated CO_2 , and vice versa (Baggs et al. 2003). However, elevated CO_2 increased soil N_2O emission by 60 % at the study site (Lam et al. 2011), which suggests that soil N was not limiting in this ecosystem. Hussain et al. (1999) also suggests that N supply of 120 kg N ha⁻¹ year⁻¹ is sufficient for growth and development of wheat. Therefore, the current N addition levels can be considered above-optimal for wheat and soybean growth and development in the study region.

Conclusions

By examining interactions among soil microbial activities and WEOM, our study provides new insights on the underlying mechanisms of microbial controls on soil WEOC dynamics in the semi-arid agroecosystems. CO_2 enrichment increased both cellulose-degrading and lignin-degrading enzyme activities in the rhizosphere and bulk soils in the upper 10 cm layer, which led to a significant decline in WEOC concentration in the topsoils. Simultaneously, an enhanced enzymatic activity under elevated CO_2 changed the composition of WEOM (i.e. HIX) in the surface and subsurface soils through accelerating the turnover of labile WEOM and solubilizing the recalcitrant SOM. Taken together, increasing atmospheric partial pressures of CO_2 accelerate the net mineralization and release of WEOC from plants, microbial biomass and SOM, which counteracts an increased storage of C in the semi-arid arable soils in the future.

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Appendix

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