Journal of Soil Science and Plant Nutrition (2020) 20:1203–1214
https://doi.org/10.1007/s42729-020-00205-1

ORIGINAL PAPER

Elevated CO_2 Affects the Soil Organic Carbon Fractions and Their Relation to Soil Microbial Properties in the Rhizosphere of *Robinia pseudoacacia* L. Seedlings in Cd-Contaminated Soils

Shuping Huang [1,2] · Xueshi Huang [3] · Ben Fang [4]

Received: 17 October 2019 / Accepted: 20 February 2020 / Published online: 5 March 2020

Abstract
As the global climates change, elevated CO_2 and soil contamination by heavy metal co-occur in natural ecosystems, which are anticipated to affect soil organic carbon fractions (SOC) and their relation to soil microbial activities, but this issue has not been extensively examined. We investigated the response of SOC and their relation with soil microorganisms and enzyme activities in rhizosphere soils of *Robinia pseudoacacia* L. seedlings to elevated CO_2 plus cadmium (Cd) contamination. We found that elevated CO_2 significantly ($p < 0.05$) stimulated total organic carbon (TOC) (8.6%), dissolved organic carbon (DOC) (32.6%), microbial biomass carbon (MBC) (13.5%), bacteria (11.6%), fungi (20.9%), actinomycetes (15.3%), urease (20.1%), dehydrogenase (15.8%), invertase (11.1%), and β-glucosidase (11.9%), and DOC, MBC, bacteria, actinomycetes, urease, and invertase presented smaller growth trend in the range of 500–700 μmol mol^{-1} CO_2 than in the range of 385–500 μmol mol^{-1} CO_2. Cd decreased DOC (30.1%), MBC (24.9%), bacteria (21.5%), actinomycetes (15.9%), and enzyme activities. Elevated CO_2 offsets the negative effect of Cd on SOC and microbial activities (except for TOC and L-asparaginase). Procrustes rotation test was used to determine the drivers (elevated CO_2, Cd, and CO_2 + Cd) of the relation between SOC and microbial activities, revealing the correlations between SOC, soil microorganisms, and enzyme activities were higher under elevated CO_2 than under elevated CO_2 + Cd. Our results suggest elevated CO_2 could stimulate soil fertility and microecological cycle in the rhizosphere microenvironment exposed to heavy metal by affecting the relationship between SOC and soil microbial properties.

Keywords Elevated atmospheric CO_2 · Cd-contaminated soil · Soil organic carbon fractions · Rhizosphere microbial properties · *Robinia pseudoacacia* L. seedlings

1 Introduction

The atmospheric concentration of carbon dioxide (CO_2) has been steadily increased during the last 12 years at the rate of 1.9 μmol mol^{-1} $year^{-1}$ and is anticipated to be as high as 550 μmol mol^{-1} by the middle of the twenty-first century (IPCC 2007). The increased root and shoot biomass by greater photosynthetic assimilation rates under elevated CO_2 could lead to a fraction of the additional fixed carbon (C) releasing into the rhizosphere by root exudation (Allard et al. 2006; De Costa et al. 2003a, b, 2006). The soil organic carbon (SOC) contributes to enhancing mineral weathering, nutrient mobilization, and assimilation by the soil microbial biomass, which may play an important role in stimulating microbial and enzyme activity in rhizosphere soils.

Soil contamination by with heavy metals is another widespread and serious issue and is on account of both natural and anthropogenic activities. The presence of toxic metals in soil increases the risk of adverse effects on its health and physicochemical properties. In China, more than 2.0×10^9 ha of land is contaminated with heavy metals (Guo et al. 2011). Among heavy metals, cadmium (Cd) is a heavy metal toxic at very

✉ Shuping Huang
huangshuping8808@126.com

[1] School of Geographic Sciences, Xinyang Normal University, Xinyang 464000, People's Republic of China

[2] Henan Key Laboratory for Synergistic Prevention of Water and Soil Environmental Pollution, Xinyang Normal University, 237 Changan Road, Xinyang 464000, People's Republic of China

[3] Faculty of Resources & Safety Engineering, China University of Mining & Technology, Beijing 100000, People's Republic of China

[4] Scholl of Environment Science and Engineering, Chang'an University, Xi'an 710054, People's Republic of China

low exposure level that provokes acute and chronic effects on health and environment (NCMWHO2003) and is often present in contaminated soils. It has been widely reported that Cd adversely impact on soil biological functions, including the activity of enzymes (Hassan et al. 2013; Renella et al. 2004) and the diversity of soil microbial community (Pan and Yu 2011; Yang et al. 2019).

It is reported that the carbon imported to soils would be up to 40% through root exudation (Lynch and Whipps 1991), a process that is influenced by plant growth response to elevated CO_2, which led to altering the bioavailability and mobility of heavy metals for plants and microorganisms (Wang et al. 2006). As a result, the combination of elevated CO_2 and heavy metals would affect the rhizosphere microenvironment of plants. Some studies suggested that elevated CO_2 changes the content of SOC and microbial activity in rhizosphere of wheat and pine seedlings exposed to heavy metal pollution (Jia et al. 2014; Kim and Kang 2011). We previously implied, in a short-term pot experiment, elevated CO_2 combined with Cd/Pb contamination led to varied soil organic compounds and enzyme activities in rhizosphere of wheat and *R. pseudoacacia* seedlings (Huang et al. 2016; Jia et al. 2016a), all of which revealed the various responses of rhizosphere microenvironment among different plant species under elevated CO_2 plus heavy metal contamination. To our knowledge, many studies have investigated the correlations between the SOC and microbial activities in the rhizosphere microenvironment under stress of elevated CO_2 or heavy metals (Khan et al. 2018a, b; Sardar et al. 2007), which much less focus on the interactions of multiple factors. In fact, elevated CO_2 and heavy metal pollution generally occur simultaneously with the development of industrialization and urbanization (Sun et al. 2010). Thus, the interactive effects of multiple environmental factors on the correlations between the SOC and microbial activities in the rhizosphere microenvironment should be studied.

Robinia pseudoacacia L. is a promising woody species which can be applied to restoration of degraded ecosystems due to its fast growth, deep root system, and a tolerance of low nutrient levels and heavy metals (Liu et al. 2013; Vlachodimos et al. 2013; Yang et al. 2015). On account of these advantages, *R. pseudoacacia* is frequently used for phytoremediation in heavy metal-polluted soils (Yang et al. 2015). It is thus significant to investigate the effects of elevated CO_2 and Cd pollution on SOC and their relationship to microbial properties in the rhizosphere of this plant species.

Therefore, the main aims of this study were to explore the interactive effect of rising atmospheric CO_2 concentration and Cd contamination on soil organic carbon fractions and microbial properties in rhizosphere soils of *Robinia pseudoacacia* L. seedlings. To address this issue, we designed an open-top field chamber experiment with four treatments: control, elevated CO_2, Cd treatment, and elevated CO_2 + Cd. And we measured SOC concentration (TOC, DOC, and MBC) and soil microbial activities (soil microorganisms and enzyme activities) in the rhizosphere soils of *Robinia pseudoacacia* L. seedlings. We hypothesized that (1) Cd treatment would inhibit SOC concentration and soil microbial activities. (2) The negative effects of Cd treatment would be essentially offset by the elevated CO_2 condition. (3) Elevated CO_2 would stimulate the correlation between SOC and microbial activity under Cd treatment.

2 Materials and Methods

2.1 Soils Preparation and Plant Species

The experimental soils were collected from the surface layer (0–20 cm) of cultivated land in Central Shaanxi, China (34°16′N, 108°54′E). The type and basic chemical characteristics of soil are shown in Table 1. Fresh soils were passed through a 5-mm sieve and pretreated with Cd using a dissolved solution of $CdCl_2 \cdot 2H_2O$. Three levels of Cd were selected to contamination soils on the basis of current environmental quality standard GB 15168-2018 in China: 0.2 (the control, Cd0, with no added Cd), 1.2 (Cd1), and 5.2 mg Cd kg^{-1} (Cd5) dry soils.

Robinia pseudoacacia L. seeds were supplied by Northwest A&F University, China. "A&F" is the abbreviation for the "Agriculture and Forestry".

2.2 Experimental Site and CO_2 Concentration

The experiment site was located on the Weishui Campus of Chang'an University, Xi'an, China (34°15′N, 108°55′E) from

Table 1 Type and basic chemical characteristics of soil used in this study

Soil type	Leached brown soil (according to Chinese soil classification)
pH	7.1 ± 0.3
Organic matter content (g kg^{-1})	12.4 ± 0.6
Organic carbon (g kg^{-1})	6.2 ± 0.2
Total nitrogen (g kg^{-1})	1.3 ± 0.1
Soluble salts (mg kg^{-1})	373.4 ± 9.1
Available N (mg kg^{-1})	0.1 ± 0.01
Available P (mg kg^{-1})	41.9 ± 1.4
Available K (mg kg^{-1})	149.6 ± 5.9
Cation exchange capacity (meq 100 g^{-1})	27.2 ± 1.1
Total Cd (mg kg^{-1})	0.2 ± 0.0

June to September 2014. The climate was characterized by an annual precipitation fluctuates between 508 and 702 mm and an average temperature of 13.6 °C (1995–2010). Three levels of CO_2 concentration were adjusted by an automatic control system which can regulate the influx rate of CO_2 or air: 385 ± 19 μmol mol^{-1} (the control, ACO_2), 500 ± 20 μmol mol^{-1} (E_1CO_2), and 700 ± 23 μmol mol^{-1} (E_2CO_2), respectively. All treatments were arranged in a randomized block design with three replicates to test for homogeneity of treatments. Three hexagonal OTCs (4.4 m dia × 1.6 m tall) were established under ACO_2 (the control), three chambers were maintained under E_1CO_2, and the other three chambers were established under E_2CO_2. These chambers kept similar light exposure and microhabitat characteristics. The humidity, temperature, and soil water content were measured automatically every 60 s, and air temperature was noted at every 10 min during experiment period. Average temperature and humidity in the ACO_2, E_1CO_2, and E_2CO_2 chambers throughout the experiment were 30.5 °C (77.1%), 30.0 °C (76.6%), and 29.9 °C (76.3%), respectively. The effects of temperature and humidity were negligible as the air temperature and humidity were almost identical across all chambers.

2.3 Pot Experiment

Experiment was conducted in plastic pots (70 cm long × 50 cm tall × 40 cm wide) and each pot contained 25 g soils (a control with no Cd was established). Each treatment was prepared with three replicates. On 5 June, *Robinia pseudoacacia* L. seeds were planted in each pot to obtain 40 seedlings per pot after emergence. The pots were then placed in the open-top chambers. Soil water content was maintained at 60 ± 2.3% of field capacity by watering as needed and was measured with a hand-held probe (IMKO, Germany) throughout the experiment period to exclude influence of soil moisture. The treatments consisted of (1) ACO_2 + Cd0, Cd1, Cd5; (2) E_1CO_2 + Cd0, Cd1, Cd5; (3) E_2CO_2 + Cd0, Cd1, Cd5. The measurements of these parameters were performed in triplicate. Weeds and litter were monitored and removed from pots by hand to reduce their impact on seedling growth.

2.4 Rhizosphere Sampling

According to the method described by Jia et al. (2014), rhizosphere samples were collected in July, August, and September. Three samples were randomly extracted from each pot. These three soil samples from each pot were mixed to obtain one composite sample. Each composite sample was passed through a 2-mm mesh to remove visible living plant material and divided into two subsamples. One was air-dried to determine soil organic carbon fractions, and the other was stored at 4 °C prior to microbial analysis.

2.5 Soil Organic Carbon Fractions

Soil total organic carbon (TOC) was determined by the $K_2Cr_2O_7$-H_2SO_4 oxidation method as described by Nelson and Sommers (1982). Dissolved organic carbon (DOC) was measured by adding soil to water at a ratio of 1:10 (w/v) and assayed with TOC analyzer (TOC-5050A, Shimadzu, Japan). Microbial biomass carbon (MBC) was assayed by fumigation extraction method according to Vance et al. (1987). The measurements of these parameters were performed in triplicate.

2.6 Soil Microbial Analysis

The numbers of bacteria, fungi, and actinomycetes were determined by colony forming units (CFU) using modified plate-dilution technique which was based on meat peptone agar, Thayer-Martin agar, and Gause's starch agar, respectively (Yang et al. 2009). Five soil enzyme activities were assayed: for urease activity, the release of NH^+ was assayed during the hydrolysis of urea (as a substrate) in Tris buffer (reported as mg NH_4-N h^{-1} g dry soil $equivalent^{-1}$) (Tabatabai and Bremmer 1972). For dehydrogenase activity, the reduction of 2,3,5-triphenyl tetrazolium chloride to triphenylformazan (TPF) was measured after soil incubated at 24 h at 30 °C (reported as μg TPF h^{-1} g dry soil $equivalent^{-1}$) (Casida et al. 1964). For invertase activity, 5 g soil was incubated at 37 °C for 24 h with 15 mL of 8% (m/v) sucrose. The suspension was reacted with 3, 5-dinitrosalicylic acid for colorimetric assay, and absorbance was read at 508 nm (reported as μg glucose h^{-1} g dry soil $equivalent^{-1}$) (Xu and Zheng 1986). For β-glucosidase activity (μg *p*-nitrophenol day^{-1} g dry soil $equivalent^{-1}$), 1 g of air-dried soil was incubated for 1 h with *p*-nitrophenyl-β-D-glucoside at pH 6.0 and measured by spectrophotometric assay (Eivazi and Tabatabai 1999). L-Asparaginase was measured according to Frankenberger and Tabatabai (1991) and was expressed as (μg ammonia h^{-1} g dry soil $equivalent^{-1}$). The measurements of these parameters were performed in triplicate.

2.7 Statistical Analyses

A general linear model and type-II sum of squares were used to analyze the effects of CO_2, Cd, and their interactions. Two-way analysis of variance (ANOVA) was used to examine the individual and combined effects of CO_2 and Cd on different parameters (soil organic carbon fractions, microbial population, and enzyme activity). Tukey's multiple comparison post hoc tests were used to assess the significance of differences between treatments for each variable. These statistical tests were performed using SPSS (SPSS Inc., version 24.0). The association between SOC, soil microorganisms, and enzyme activities (subdivided into groups of SOC [TOC, DOC, and MBC],

soil microorganisms [bacteria, fungi, and actinomycetes], and enzyme activities [urease, dehydrogenase, invertase, β-glucosidase, and L-asparaginase]) was determined by Procrustes rotation using the "protest" function in "vegan" (Oksanen et al. 2013). The Procrustes rotation is a method for determining the similarity between multivariate datasets (Peres-Neto and Jackson 2001). One-way ANOVAs and pairwise *t* tests were used to identify significant differences between the Procrustes rotation results. These statistical analyses were carried out in R version 3.0 (R Core Team 2013).

3 Results

3.1 Soil Organic Carbon Fractions in Rhizosphere Soils

Elevated CO_2 significantly ($p < 0.05$) led to an increase in the concentration of TOC, DOC, and MBC by 8.6%, 32.6%, and 13.5% in the rhizosphere soil of *Robinia pseudoacacia* L. seedlings compared to ACO_2, while DOC and MBC presented smaller growth trend in the range of 500–700 μmol mol^{-1} CO_2 than in the range of 385–500 μmol mol^{-1} CO_2 (Fig. 1). Cd significantly ($p < 0.05$) reduced the DOC and MBC concentration by 30.1% and 24.9% but did not have a significant effect on soil TOC concentration. Compared to Cd only stress, ECO_2 + Cd increased the TOC, DOC, and MBC concentration by 1.6%, 21.4%, and 16.1% in the rhizosphere soils, respectively (Fig. 1). With no significant variation in soil TOC content, DOC was higher in August than in other months, while MBC increased over time. In addition, the interaction between of CO_2 and Cd on DOC and MBC was significant (Table 2).

3.2 Microbial Population and Enzyme Activity in Rhizosphere Soils

The population of bacteria, fungi, and actinomycetes increased by 11.6%, 20.9%, and 15.3% significantly ($p < 0.05$) with increasing CO_2 levels, while bacteria and actinomycetes had smaller growth trend in the range of 500–700 μmol mol^{-1} CO_2 than in the range of 385–500 μmol mol^{-1} CO_2 (Fig. 2). Relative to the control, bacteria and actinomycetes were significantly ($p < 0.05$)

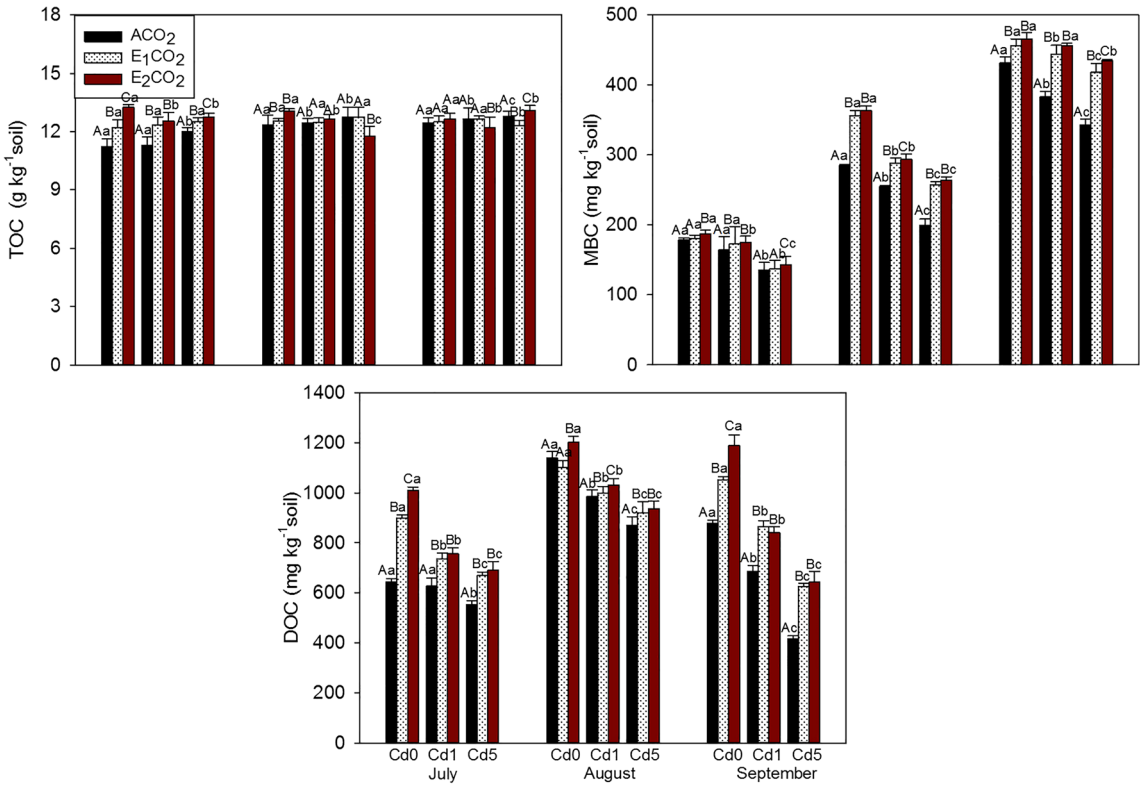

Fig. 1 Concentration of TOC (total organic carbon), MBC (microbial biomass carbon), and DOC (dissolved organic carbon) in rhizosphere soils of *Robinia pseudoacacia* L. seedlings under different treatments. (Date are means ± SE; $n = 9$). Cd0, Cd1, and Cd5 in figures represent 0.0, 1.0, and 5.0 mg Cd was added to per kilogram dry soil, respectively. ACO_2, E_1CO_2, and E_2CO_2 in table represent 385 ± 19 μmol mol^{-1}, 500 ± 20 μmol mol^{-1}, and 700 ± 23 μmol mol^{-1} CO_2 were set in this study, respectively. Different capital letters indicate a significant difference ($p < 0.05$) between elevated CO_2 and ambient CO_2 for the same heavy metal level (within the same period); different lowercase letters indicate significant differences ($p < 0.05$) between treatments for the same CO_2 level (within the same period). (The same below)

Table 2 Summary of the results of factorial analysis (ANOVA) (*F* values) for the effects of CO_2, Cd, and their interactions on TOC (total organic carbon), MBC (microbial biomass carbon), and DOC (dissolved organic carbon), microbial population, and enzyme activity in rhizosphere soils

Variables	July			August			September		
	CO_2	Cd	$CO_2 \times Cd$	CO_2	Cd	$CO_2 \times Cd$	CO_2	Cd	$CO_2 \times Cd$
TOC	35.6**	2.8 ns	0.7 ns	164.1**	2.6 ns	22.7**	4.5*	5.6*	1.2 ns
DOC	73.6**	17.6**	6.6**	89.4**	40.6**	9.1**	65.1**	4.7*	12.6**
MBC	129.2**	53.4**	8.7**	43.5**	12.5 **	24.1**	35.8**	53.1**	16.3**
Bacteria	31.3**	58.2**	24.1**	35.9**	17.6**	6.7**	121.8**	40.1**	24.5**
Fungi	16.4**	9.9**	0.9 ns	26.2**	4.4*	2.7 ns	57.6**	2.9 ns	12.2**
Actinomycetes	22.8**	30.8**	10.4**	68.1**	37.3**	21.7**	43.4**	7.5**	5.1*
Urease	16.3**	34.5**	9.8**	84.6**	147.5**	31.7**	66.3**	179.1**	49.8**
Dehydrogenase	89.1**	2.3 ns	14.2**	5.3*	51.3**	6.8**	11.3**	44.8**	15.7**
Invertase	65.6**	12.4**	6.6**	32.6**	45.2**	21.8**	72.7**	54.5**	18.1**
β-glucosidase	11.3**	24.7**	3.2 ns	22.4*	26.3**	2.5 ns	47.9*	57.3**	35.2**
L-asparaginase	23.4**	14.9**	9.4**	13.8**	34.1**	3.4 ns	34.6**	9.5**	1.4 ns

ns not significant

**$p < 0.01$; *$p < 0.05$

decreased by 21.5% and 15.9% with increasing Cd, while fungi did not vary significantly (Fig. 2). Bacteria and actinomycetes increased significantly ($p < 0.05$) from July to September, while the lowest population of fungi occurred in August. Under ECO_2 + Cd, bacteria, fungi, and actinomycetes increased by 8.5%, 13.7%, and 11.2% relative to ACO_2 + Cd and decreased compared to ECO_2 alone (Fig. 2). Significant interactive effects of CO_2 and Cd on bacteria and actinomycetes were observed (Table 2).

Elevated CO_2 caused significantly ($p < 0.05$) an increase in urease, dehydrogenase, invertase, and β-glucosidase by 20.1%, 15.8%, 11.1%, and 11.9% relative to ambient CO_2, irrespective of the decreased L-asparaginase by 19.9%. In addition, urease and invertase showed smaller growth trend in the range of

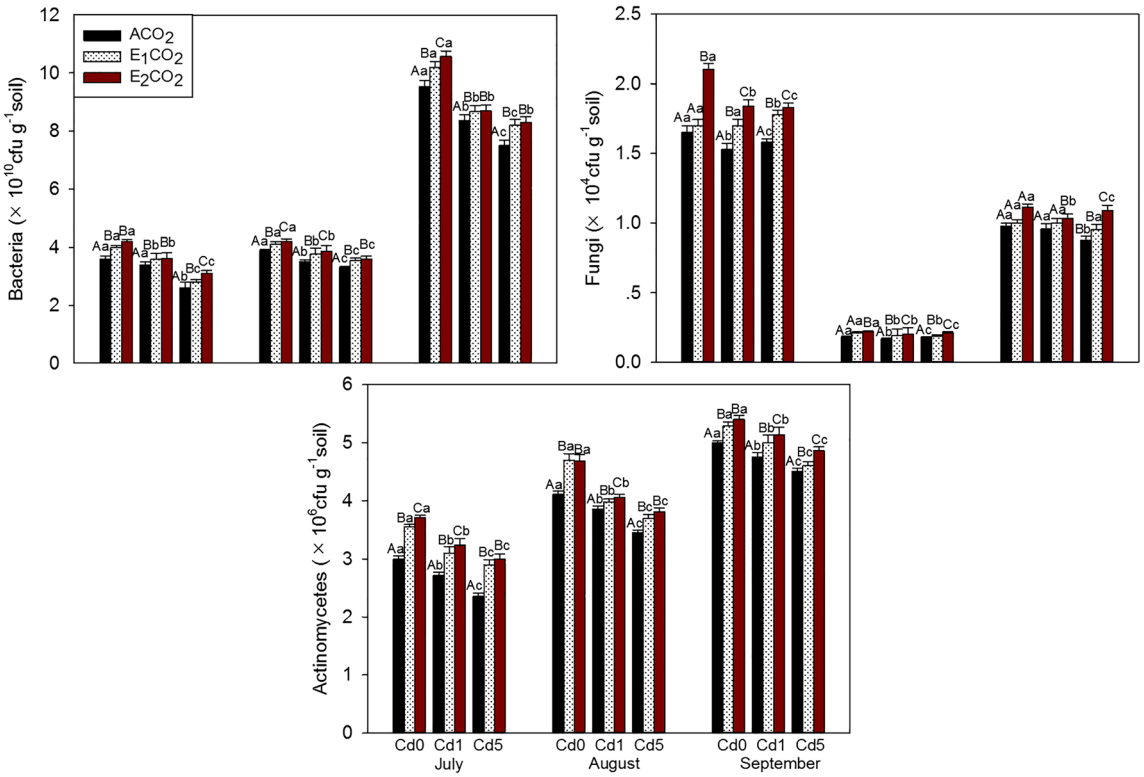

Fig. 2 Microbial population in rhizosphere soils under different treatments. (Date are means ± SE; $n = 9$)

500–700 μmol mol^{-1} CO_2 than in the range of 385–500 μmol mol^{-1} CO_2 ($p < 0.05$) (Fig. 3). Except for L-asparaginase, most soil enzyme activities increased from July to September. Under Cd treatment, urease, dehydrogenase, invertase, β-glucosidase, and L-asparaginase activity decreased by 24.4%, 22.0%, 19.5%, 34.0%, and 29.4% with increasing Cd concentration (Fig. 3). Except for L-asparaginase, elevated CO_2 plus Cd treatment was associated with significantly higher enzyme activities by 12.5%, 17.3%, 15.1%, and 8.3% compared to ambient CO_2 + Cd (Fig. 3). Interactive effects of CO_2 and Cd on urease, dehydrogenase, and invertase were significant (Table 2).

3.3 Relationship Between Soil Organic Carbon Fractions and Microbial Properties

In order to evaluate the relationship between soil organic carbon fractions (TOC, DOC, and MBC), soil microorganisms (bacteria, fungi, and actinomycetes), and enzyme activities (urease, dehydrogenase, invertase, β-glucosidase, and L-asparaginase), the Procrustes rotation test was used, and the results are shown in Table 3. We found that the relationship between soil organic carbon fractions and the group of bacteria, fungi, and actinomycetes was strongest (20 out of 27 treatments in 3 months showed a significant correlation and the strongest correlations were found here), followed by that of soil microorganisms and enzyme activities (18 out of 27 treatments showed a significant correlation). Significant correlations between the SOC matrix and the enzyme activities matrix were found in only 12 out of the 27 treatments. Pairwise *t* tests on the Procrustes rotation results revealed that the SOC group and the group of enzyme activities were more significantly related to the soil microorganisms group ($P = 0.006$ and $P = 0.02$, respectively) than the relationship

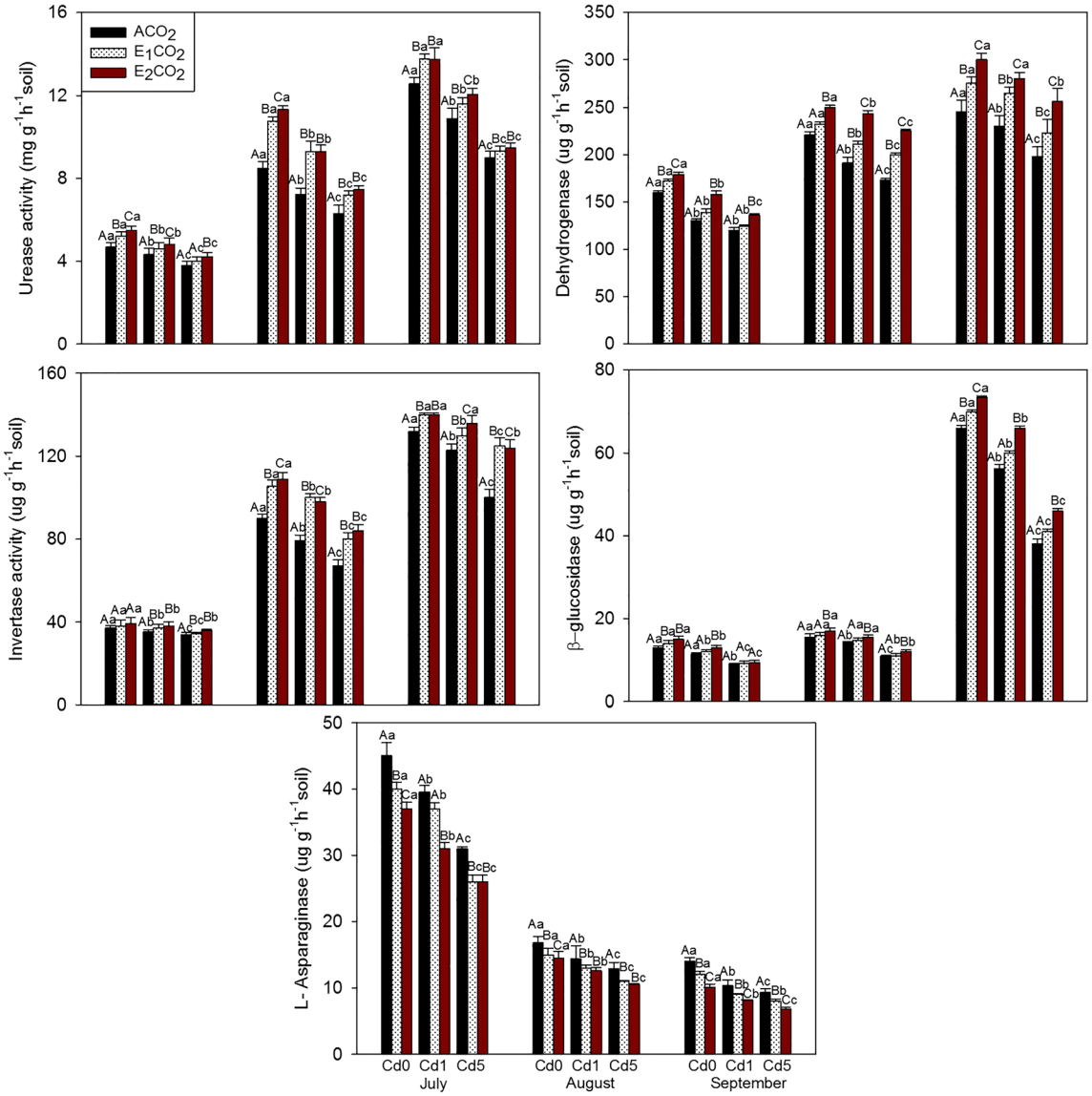

Fig. 3 Enzyme activity in rhizosphere soils under different treatments. (Date are means ± SE; $n = 9$)

Table 3 Correlations between SOC (soil organic carbon fractions), Mic. (microbial population), and Enz. (enzyme activity) as derived from the Procrustes test

Time	Items	ACO_2	E_1CO_2	E_2CO_2	Cd1	Cd5	E_1CO_2* Cd1	E_1CO_2* Cd5	E_2CO_2* Cd1	E_2CO_2* Cd5
July	SOC vs. Mic.	0.572***	0.408**	0.344**	0.452**	0.196	0.262*	0.285*	0.216	0.220
	SOC vs. Enz.	0.588***	0.232	0.166	0.529***	0.374**	0.279*	0.129	0.162	0.191
	Mic. vs. Enz.	0.214	0.461**	0.310**	0.175	0.637***	0.238	0.373**	0.162	0.384**
August	SOC vs. Mic.	0.216	0.463**	0.512***	0.542***	0.762***	0.384**	0.390**	0.571***	0.292*
	SOC vs. Enz.	0.193	0.165	0.228	0.497***	0.461**	0.363**	0.231	0.218	0.382**
	Mic. vs. Enz.	0.376**	0.243	0.350**	0.468**	0.558***	0.263**	0.145	0.285*	0.413***
September	SOC vs. Mic.	0.307**	0.511***	0.165	0.438**	0.579***	0.445**	0.192	0.392**	0.217
	SOC .vs. Enz.	0.145	0.510***	0.213	0.287*	0.154	0.442**	0.319**	0.129	0.233
	Mic. vs. Enz.	0.310**	0.453**	0.582***	0.461**	0.127	0.208	0.275*	0.281*	0.188

ACO_2, E_1CO_2, and E_2CO_2 in table represent 385 ± 19 μmol mol^{-1}, 500 ± 20 μmol mol^{-1}, and 700 ± 23 μmol mol^{-1} CO_2 set in this study, respectively. Cd1 and Cd5 in table representing 1.0 and 5.0 mg Cd were added to per kilogram dry soil, respectively. Numbers give the correlation coefficient of rotation (CoR)

ns not significant

*$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; correlations are considered weak if $0.3 < CoR < 0.5$ and strong if $CoR > 0.5$

between SOC group and enzyme activities group. Overall, the relationship between the groups of SOC, soil microorganisms, and enzyme activities was more significant under elevated CO_2 or Cd alone than under elevated CO_2 plus Cd treatments.

4 Discussion

4.1 Soil Organic Carbon Fractions in Rhizosphere Soils

Our results demonstrated that elevated CO_2 alone corresponded with increased TOC, DOC, and MBC concentrations (Fig. 1). Uselman et al. (2000) and Jia et al. (2016b) reported that the growth, photosynthesis, and root biomass of *Robinia pseudoacacia* L. can be enhanced by elevated CO_2. This might lead to increased net primary production to the root system, which may explain why the soil organic carbon fractions were higher under elevated CO_2 conditions. Similar to our results, Wang et al. (2017) suggested that elevated CO_2 probably MBC and DOC content, which was on account of the increased root carbon exudation driven by CO_2 as indicated in the literature (Chen et al. 2012; Klamer et al. 2002; Koyama et al. 2019). The content of soil organic carbon fractions has been found to be either increased (Deng et al. 2016; Luo et al. 2006), decreased (Carney et al. 2007; Langley et al. 2009), or unaffected (Keiluweit et al. 2015; Koyama et al. 2018) by CO_2 enrichment. Additionally, the smaller growth trend of DOC and MBC concentration in the range of 500–700 μmol mol^{-1} CO_2 than in the range of 385–500 μmol mol^{-1} CO_2 suggested that the decreased growth rate of soil organic carbon content under higher CO_2 levels.

Kuzyakov (2001) and Walker et al. (2003) suggested that aboveground litter, root residues, and root exudates should be the main source of soil organic carbon fractions in rhizosphere soils. Because weeds and litter were removed from the experiment pots during our study, the variation in organic carbon in rhizosphere soils could be related to the effect of Cd on *Robinia pseudoacacia* L. growth. Soil microbial biomass, which plays an important role in nutrient cycling and ecosystem sustainability, has been found to be sensitive to increased heavy metal concentrations in soils (Giller et al. 1998; Vig et al. 2003). As hypothesized, Cd addition decreased DOC and MBC in rhizosphere soils, which could be due to the microorganisms under Cd stress diverting energy from growth to cell maintenance functions (Killham 1985). As hypothesized, we observed that elevated CO_2 results in higher DOC and MBC in the rhizosphere of *Robinia pseudoacacia* L. seedlings grown in Cd-contaminated soils. The threat of heavy metals to plants grown in Cd-contaminated soils can be relieved by elevated CO_2 through stimulating shoot and root growth, and overall biomass production (Kim and Kang 2011; Li et al. 2012). The increase in growth and biomass production under elevated CO_2 favored organic compound diffusion from roots into rhizosphere soils, leading to increased DOC and MBC content in Cd-polluted soils. Similarly, an increase in microbial biomass C under elevated CO_2 + Cd (copared to Cd stress only) was demonstrated by Luo et al. (2019). So, elevated CO_2 significantly offset the negative impact of Cd addition on soil organic carbon concentration, due to elevated CO_2 stimulating plant growth and productivity or altered carbon allocation belowground, mainly as a result of stimulation of photosynthesis (Kassem et al. 2008; Kim and Kang 2011).

4.2 Microbial Properties in Rhizosphere Soils

Microbes need nutrients from decomposing soil organic matter that they can use to construct themselves; therefore, the increased TOC, DOC, and MBC concentrations observed under elevated CO_2 contributed to higher microbial population in rhizosphere soils (Fig. 2). The greater microbial activities on account of elevated CO_2 was consistent with previous observations in rice and grass soils (Bhattacharyya et al. 2013; Luo et al. 2014). Extracellular enzyme activity is, in general, positively related to microbial activity measured via respiration (Frankenberger and Dick 1983). Kandeler et al. (2006) have showed that elevated CO_2 presented directly or indirectly effect on enzyme activities. We found that the microorganism activity increased under elevated CO_2 (Fig. 2), resulting in higher enzyme activities examined here (Kools et al. 2005). In addition, changes in soil organic carbon fractions under elevated CO_2 can be associated with the variations in microbial biomass and activities in rhizosphere soils (Bhattacharyya et al. 2013; Luo et al. 2014); Compared to 385–500 μmol mol^{-1} CO_2, bacteria, actinomycetes, urease, and invertase presented smaller growth trend in the range of 500–700 μmol mol^{-1} CO_2 (Figs. 2 and 3), which could be associated with the same variation tendency in DOC and MBC (Fig. 1). However, the decreased L-asparaginase with increasing CO_2 indicated that elevated CO_2 may show different behaviors in their ability to affect different soil enzyme activities. Yuan et al. (2006) also reported the elevated CO_2 increased soil urease activity and decreased the β-glucosidase, invertase, acid phosphates, and β-glucosaminidase activities. In addition, Ebersberger et al. (2003) suggested that the stimulation of elevated CO_2 in invertase, xylanase, urease, protease, and alkaline phosphatase activities revealed that the larger biomass of microbes was accompanied by an increase in their activity. It is possible that the variation of soil organic carbon composition could lead to a decrease in bacteria and actinomycetes population with increasing Cd levels. Several authors have pointed out that heavy metals can affect microbial biomass and specific microbial groups (D'Aascoli et al. 2006; Gomes et al. 2010; Shen et al. 2005). As hypothesized, we observed bacteria and actinomycetes decreased with increasing Cd levels, which was consistent with previous studies (Jia et al. 2016a; Pan and Yu 2011). Furthermore, we did not find significant variation in fungi population under Cd stress. Previous studies have suggested that fungi are more resistant than bacteria under the long-term heavy metal contamination stress (Fliessbach et al. 1994; Frostegård et al. 1996). Gao et al. (2010) also reported that the order of sensitivity reaction of soils microbe population responding to Cd polution was actinomycetes > bacteria > fungi. We also found that the response of fungi to Cd differed from bacteria and actinomycetes, indicating that heavy metals could present different behaviors in their ability to affect soil microorganisms.

Heavy metals reduce enzyme activity by masking catalytically active groups and interacting with the complex enzyme-substrate, which generates the impact of denaturation toward active proteins of enzymes (Gianfreda et al. 2005; Zaborowska et al. 2006). The lower urease, dehydrogenase, invertase, and β-glucosidase were also consistent with previous studies (Ma et al. 2015; Tripathy et al. 2014), suggesting that soil C and N cycling can be affected by heavy metals. In addition, it has been manifested that heavy metals have an impact on the biosynthesis of enzymes performed by microorganisms. Therefore, a decrease in microbial abundance derived from higher Cd stress (Fig. 2) would lead to adverse effects on enzyme activity in rhizosphere soils.

The increased microbial population under elevated CO_2 plus Cd treatment indicated that an increase in CO_2 concentration could counteract the negative effects of Cd on microbial abundance in rhizosphere soils. The opposite effects of elevated CO_2 and Cd on microorganisms observed here are consistent with previous studies (Chen et al. 2014; Wu et al. 2009). In addition, the adaption of microorganism could lead to selecting specific species that can utilize a broad spectrum of carbon sources via root exudation when a limited amount of carbon is added to the soil through root turnover (Farrar et al. 2003). Thus, we proposed that the increased organic carbon fractions in rhizosphere soils under elevated CO_2 + Cd condition (Fig. 1) had a stimulatory effect on soil microbial population on account of the greater substrate availability. Soil enzymes are mainly produced by a diverse group of heterotrophic microbes (Sinsabaugh et al. 2009). Therefore, an increase in enzyme activity (except for L-asparaginase) under elevated CO_2 in combination with Cd stress resulted from the stimulation of microbial abundance and production (Fig. 2), suggesting that the stimulatory effect of elevated CO_2 might be greater than the inhibitory action derived from Cd contamination, which demonstrated our hypothesis. Similar with our study, Luo et al. (2019) also reported that elevated CO_2 strongly alleviated the negative impact on C-degrading enzyme, to promote the β-glucosidase, cellobiohydrolase, polyphenol oxidase, and peroxidase activities and microbial biomass C concentration in soils, indicating elevated CO_2 could stimulate the microbial activities and offset the inhibition of Cd toxicity. In generally, our results showed that elevated CO_2 significantly increased the microbial population and soil enzyme activity under Cd stress. Our results support the view that elevated CO_2 may influence the soil microbial activity (Kim and Kang 2011) and may offset the inhibition of Cd toxicity on soil microbial activity (Luo et al. 2019). Soil enzyme activity was significantly positively correlated with soil microbes (Groffman et al. 2001); the increased microbial populations stimulated by the large quantities of SOC under elevated CO_2 contributed to higher soil enzyme activity. In addition, DOC contains macromolecules including acidic groups such as phenolic OH and carboxyl functional groups

(Hofrichter and Fakoussa 2001), and these molecules have crucial impact on the solubility, transport, and bioavailability of heavy metals (Kim and Kang 2011). Therefore, the increased DOC concentration under elevated CO_2 plus Cd stress (in comparison with Cd stress alone) influenced soil microbial activity by affecting the environmental behavior of Cd.

4.3 Relationship Between Soil Organic Carbon Fractions and Microbial Properties

Soil organic carbon and microbial properties could be stimulated by various environment factors, such as substrate and oxygen availability, soil moisture, temperature, and soil texture (A'Bear et al. 2014; Ma et al. 2016; Maenhout et al. 2018; Xu et al. 2018). To our knowledge, few studies have considered the relationships between the groups of SOC, soil microorganisms, and enzyme activities in the rhizosphere microenvironment under elevated CO_2 and Cd stress. In our study, the Procrustes rotation revealed that the soil organic carbon group and the soil enzyme activities group were more significantly related to the soil microorganisms group ($P = 0.006$ and $P = 0.02$, respectively) than the relationship between SOC group and enzyme activities group. Similar with our results, many studies have demonstrated that soil microbial diversity and community structure are correlated with the changes of soil organic carbon (Cookson et al. 2005; Marschner et al. 2003). Xiao et al. (2015) also found that in the soil microbe composition, soil organic carbon fractions (e.g., DOC and MBC) were positively correlated with microbial populations in the *Calamagrostis angustifolia* wetland. The variation of soil microbes could affect soil carbon loss and soil CO_2 emissions (Allison et al. 2010; Carney et al. 2007; Khalid et al. 2019), further to stimulate carbon sequestration in soil (Six et al. 2006). In the respect of the correlation between soil enzyme activity and microorganisms, the significantly positive correlation between them has demonstrated by Groffman et al. (2001). The population and composition of microbes could change the magnitude and quality of enzyme activity, further to stimulate the soil metabolic processes (Ushio et al. 2010). In addition, previous studies confirmed soil enzyme activities were related with the soil organic carbon fractions (Acosta-Martínez et al. 2007; Brzezińska et al. 2005), which due to the soil enzymes produced by microorganisms and play key parts in SOC mineralization process (Ahn et al. 2009). Summing up the above, previous studies have reported the relationships between the SOC, soil microorganisms, and enzyme activity. Soil organic carbon inputs used as substrates by microorganisms can be characterized by the abundance and diversity of microbial communities (Kassem et al. 2008; Wang et al. 2008) as well as the variations in enzyme activities (Kandeler et al. 2006), which demonstrated the tight correlation between SOC, soil microbes, and enzyme activity.

In addition, we revealed that the relationships between the groups of SOC, soil microorganisms, and enzyme activities were more significant under elevated CO_2 or Cd alone than under elevated CO_2 + Cd, which contrasted with our hypothesis. The data obtained here indicated that elevated CO_2 might decrease the correlation between soil organic carbon fractions and microbial activities in the rhizosphere of *Robinia pseudoacacia* L. seedlings in Cd-contaminated soils. A possible explanation is that elevated CO_2 would offset the negative effect on the microbial characteristics, to increase the microbial populations and enzyme activities, microbial biomass C in soil, indicating that elevated CO_2 can promote the microbial activities and alleviate the inhibition of Cd toxicity.

5 Conclusion

Both elevated CO_2 and Cd contamination could affect soil organic carbon fractions and microbial activities in the rhizosphere microenvironment of *Robinia pseudoacacia* L. seedling. Our study demonstrated that elevated CO_2 offsets the negative effect of Cd on soil microbial activities by stimulating soil microorganisms and enzyme activities. We found that the relationship between soil organic carbon fractions and soil microbial activities were higher under elevated CO_2 alone than under elevated CO_2 plus Cd, revealing that elevated CO_2 could stimulate soil fertility and microecological cycle in the rhizosphere microenvironment exposed to heavy metal.

Funding Information This study was jointly financed by National Natural Science Foundation of China (grant no. 41807038) and Nanhu Scholars Program for Young Scholars of XYNU.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

A'Bear AD, Jones TH, Kandeler E, Boddy L (2014) Interactive effects of temperature and soil moisture on fungal-mediated wood decomposition and extracellular enzyme activity. Soil Biol Biochem 70:151–158

Acosta-Martínez V, Cruz L, Sotomayor-Ramírez D, Pérez-Alegría L (2007) Enzyme activities as affected by soil properties and land use in a tropical watershed. Appl Soil Ecol 35:35–45

Ahn MY, Zimmerman AR, Comerford NB, Sickman JO, Grunwald S (2009) Carbon mineralization and labile organic carbon pools in the sandy soils of a North Florida watershed. Ecosystems 12:672–685

Allard V, Robin C, Newton PCD, Lieffering M, Soussana JF (2006) Short and long-term effects of elevated CO_2 on *Lolium perenne* rhizodeposition and its consequences on soil organic matter turnover and plant N yield. Soil Biol Biochem 36:1178–1187

Allison SD, Wallenstein MD, Bradford MA (2010) Soil-carbon response to warming dependent on microbial physiology. Nat Geosci 3:336–340

Bhattacharyya P, Roy KS, Neogi S, Manna MC, Adhya TK, Rao KS, Nayak AK (2013) Influence of elevated carbon dioxide and temperature on belowground carbon allocation and enzyme activities in tropical flooded soil planted with rice. Environ Monit Assess 185: 8659–8671

Brzezińska M, Włodarczyk T, Stępniewski W, Przywara G (2005) Soil aeration status and catalase activity. Acta Agrophys 5:555–565

Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. Proc Natl Acad Sci 104:4990–4995

Casida LE, Klein D, Santoro T (1964) Soil dehydrogenase activity. Soil Sci 98:371–376

Chen XM, Liu JX, Deng Q, Yan JH, Zhang DQ (2012) Effects of elevated CO_2 and nitrogen addition on soil organic carbon fractions in a subtropical forest. Plant Soil 357:25–34

Chen P, Liu Q, Liu JY, Jia FA, He XH (2014) Response of soil microbial activity to cadmium pollution and elevated CO_2. Sci Rep 4:4287

Cookson WR, Abaye DA, Marschner P, Murphy DV, Stockdale EA, Goulding KW (2005) The contribution of soil organic matter fractions to carbon and nitrogen mineralization and microbial community size and structure. Soil Biol Biochem 37:1726–1737

D'Aascoli R, Rao MA, Adamo P, Renella G, Landi L, Rutigliano FA et al (2006) Impact of river overflowing on trace element contamination of volcanic soils in South Italy: part II. Soil biological and biochemical properties in relation to trace element speciation. Environ Pollut 144:317–326

De Costa WAJM, Weerakoon WMW, Abeywardena RMI, Herath HMLK (2003a) Response of photosynthesis and water relations of rice (*Oryza sativa*) to elevated atmospheric carbon dioxide in the subhumid zone of Sri Lanka. J Agron Crop Sci 189:71–82

De Costa WAJM, Weerakoon WMW, Herath HMLK, Abeywardena RMI (2003b) Response of growth and yield of rice (*Oryza sativa*) to elevated atmospheric elevated atmospheric carbon dioxide in the subhumid zone of Sri Lanka. J Agron Crop Sci 189:83–95

De Costa WAJM, Weerakoon WMW, Herath HMLK, Amaratunga KSP, Abey-wardena RMI (2006) Physiology of yield determination of rice under elevated carbon dioxide at high temperatures in a subhumid tropical climate. Field Crops Res 96:336–347

Deng Q, Cheng XL, Bowatte S, Newton PCD, Zhang QF (2016) Rhizospheric carbon-nitrogen interactions in a mixed-species pasture after 13 years of elevated CO_2. Agric Ecosyst Environ 235:134–141

Ebersberger D, Niklaus PA, Kandeler E (2003) Long term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. Soil Biol Biochem 35:965–972

Eivazi F, Tabatabai MA (1999) Glucosidases and galactosidases in soils. Soil Biol Biochem 20:601–606

Farrar J, Hawes M, Jones DL, Lindow S (2003) How roots control the flux of carbon to the rhizosphere. Ecology 84:827–837

Fliessbach A, Martens R, Reber HH (1994) Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol Biochem 26:1201–1205

Frankenberger WT, Dick WA (1983) Relationships between enzyme activities and microbial growth and activity indices in soil. Soil Sci Soc Am J 47:945–951

Frankenberger WT Jr, Tabatabai MA (1991) L-Asparaginase activity of soils. Biol Fertil Soils 11:6–12

Frostegård Å, Tunlid A, Bååth E (1996) Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biol Biochem 28:55–63

Gao Y, Zhou P, Mao L, Zhi YE, Zhang CH, Shi WJ (2010) Effects of plant species coexistence on soil enzyme activities and soil microbial community structure under Cd and Pb combined pollution. J Environ Sci 22:1040–1048

Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzymes activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci Total Environ 341:265–279

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol Biochem 30:1389–1414

Gomes NCM, Landi L, Smalla K, Nannipieri P, Brookes PC, Renella G (2010) Effects of Cd- and Zn-enriched sewage sludge on soil bacterial and fungal communities. Ecotoxicol Environ Saf 73:1255–1263

Groffman PM, Driscoll CT, Fahey TJ, Hardy JP, Fitzhugh RD, Tierney GL (2001) Colder soils in a warmer world: a snow manipulation study in northern hardwood forest. Biogeochemistry 56:135–150

Guo HY, Zhu JG, Zhou H, Sun YY, Ying Y, Pei DP et al (2011) Elevated CO_2 levels affects the concentrations of copper and cadmium in crops grown in soil contaminated with heavy metals under fully open-air field conditions. Environ Sci Technol 45:6997–7003

Hassan W, Akmal M, Muhammad I, Younas M, Zahaid KR, Ali F (2013) Response of soil microbial biomass and enzymes activity to cadmium (Cd) toxicity under different soil textures and incubation times. Aust J Crop Sci 7:674–680

Hofrichter M, Fakoussa R (2001) Microbial degradation and modification of coal. Biopolymers 1:393–429. https://doi.org/10.1007/s42729-020-00205-1

Huang SP, Jia X, Zhao YH, Bai B, Chang YF (2016) Elevated CO_2 benefits the soil microenvironment in the rhizosphere of *Robinia pseudoacacia* L. seedlings in Cd-and Pb-contaminated soils. Chemosphere 168:606–616

IPCC (2007) Climate change 2007: the physical science basis, contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

Jia X, Wang WK, Chen Z, He YH, Liu JX (2014) Concentrations of secondary metabolites in tissues and root exudates of wheat seedlings changed under elevated atmospheric CO_2 and cadmium-contaminated soils. Environ Exp Bot 107:134–143

Jia X, Liu T, Zhao YH, He YH, Yang MY (2016a) Elevated atmospheric CO_2 affected photosynthetic products in wheat seedlings and biological activity in rhizosphere soil under cadmium stress. Environ Sci Pollut Res 23:514–526

Jia X, Zhao YH, Liu T, Huang SP (2016b) Elevated CO_2 affects secondary metabolites in *Robinia pseudoacacia* L. seedlings in Cd- and Pb-contaminated soil. Chemosphere 160:199–207

Kandeler E, Mosier AR, Morgan JA, Milchunas DG, King JY, Rudolph S, Tscherko D (2006) Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. Soil Biol Biochem 38:2448–2460

Kassem II, Joshi P, Sigler V, Heckathorn S, Wang Q (2008) Effect of elevated CO_2 and drought on soil microbial community associated with *Andropogon gerardii*. J Integr Plant Biol 50:1406–1415

Keiluweit M, Bougoure JJ, Nico PS, Pett-Ridge J, Weber PK, Kleber M (2015) Mineral protection of soil carbon counteracted by root exudates. Nat Clim Chang 5:588–595

Khalid MS, Shaaban M, Hu RG (2019) N_2O, CH_4, and CO_2 emissions from continuous flooded, wet, and flooded converted to wet soils. J Soil Sci Plant Nutr 19:342–351

Khan MA, Ding X, Khan S, Brusseau ML, Khan A, Nawab J (2018a) The influence of various organic amendments on the bioavailability and plant uptake of cadmium present in mine-degraded soil. Sci Total Environ 636:810–817

Khan MA, Khan S, Ding X, Khan A, Alam M (2018b) The effects of biochar and rice husk on adsorption and desorption of cadmium on to soils with different water conditions (upland and saturated). Chemosphere 193:1120–1126

Killham K (1985) A physiological determination of the impact of environmental stress on the activity of microbial biomass. Environ Pollut 38:283–294

Kim S, Kang H (2011) Effects of elevated CO_2 and Pb on phytoextraction and enzyme activity. Water Air Soil Pollut 219:365–375

Klamer M, Roberts MS, Levine LH, Drake BG, Garland JL (2002) Influence of elevated CO_2 on the fungal community in a coastal scrub oak forest soil investigated with terminal-restriction fragment length polymorphism analysis. Appl Environ Microbiol 68:4370–4376

Kools SAE, van Roovert M, van Gestel CAM, van Straalen NM (2005) Glyphosate degradation as a soil health indicator for heavy metal polluted soils. Soil Biol Biochem 37:1303–1307

Koyama A, Harlow B, Kuske CR, Belnap J, Evans RD (2018) Plant and microbial biomarkers suggest mechanisms of soil organic carbon accumulation in a Mojave Desert ecosystem under elevated CO_2. Soil Biol Biochem 120:48–57

Koyama A, Harlow B, Evans RD (2019) Greater soil carbon and nitrogen in a Mojave Desert ecosystem after 10 years exposure to elevated CO_2. Geoderma 355:113915. https://doi.org/10.1016/j.geoderma.2019.113915

Kuzyakov YV (2001) Tracer studies of carbon translocation by plants from the atmosphere into the soil (a review). Eurasian Soil Sci 34:28–42

Langley JA, McKinley DC, Wolf AA, Hungate BA, Drake BG, Megonigal JP (2009) Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. Soil Biol Biochem 41:54–60

Li TQ, Di ZZ, Han X, Yang XE (2012) Elevated CO_2 improves root growth and cadmium accumulation in the hyperaccumulator *Sedum alfredii*. Plant Soil 354:325–334

Liu XP, Fan YY, Long JX, Wei RF, Kjelgren R, Gong CM et al (2013) Effects of soil water and nitrogen availability on photosynthesis and water use efficiency of *Robinia pseudoacacia* seedlings. J Environ Sci 25:585–595

Luo Y, Hui DF, Zhang DQ (2006) Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87:53–63

Luo YQ, Zhao XY, Andren O, Zhu YC, Huang WD (2014) Artificial root exudates and soil organic carbon mineralization in a degraded sandy grassland in northern China. J Arid Land 6:423–432

Luo XZ, Hou EQ, Zang XW, Zhang LL, Yi YF, Wen DZ (2019) Effects of elevated atmospheric CO_2 and nitrogen deposition on leaf litter and soil carbon degrading enzyme activities in a Cd-contaminated environment: a mesocosm study. Sci Total Environ 671:157–164

Lynch JM, Whipps JM (1991) Substrate flow in the Rhizosphere. Kluwer Academic Publishers, Dordrecht, pp 15–24

Ma SC, Zhang HB, Ma ST, Wang R, Wang GX, Shao Y, Li CX (2015) Effects of mine wastewater irrigation on activities of soil enzymes and physiological properties: heavy metal uptake and grain yield in winter wheat. Ecotoxicol Environ Saf 113:483–490

Ma XY, Liu M, Li ZP (2016) Shifts in microbial biomass and community composition in subtropical paddy soils under a gradient of manure amendment. Biol Fertil Soils 52:775–787

Maenhout P, Van den Bulcke J, Van Hoorebeke L, Cnudde V, De Neve S, Sleutel S (2018) Nitrogen limitations on microbial degradation of plant substrates are controlled by soil structure and moisture content. Front Microbiol 9:1433

Marschner P, Kandeler E, Marschner B (2003) Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol Biochem 35:453–461

Nelson DW, Sommers LE (1982) Total carbon and organic matter (chapter 29). American Society of Agronomy, Inc., Madison, pp 539–577

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al (2013) Vegan: community ecology package. R package version 2.0–7

Pan J, Yu L (2011) Effects of Cd or/and Pb on soil enzyme activities and microbial community structure. Ecol Eng 37:1889–1894

Peres-Neto P, Jackson D (2001) How well do multivariate data sets match? The advantages of a procrustean superimposition approach over the Mantel test. Oecologia 129:169–178

Renella G, Mench M, Dvander L, Pietramellara G, Ascher J, Ceccherini MT et al (2004) Hydrolase activity, microbial biomass and community structure in long-term Cd-contaminated soils. Soil Biol Biochem 36:44–451

R Core Team (2013) R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Sardar K, Qing C, Hesham AEL, Yue X, Zheng H (2007) Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb. J Environ Sci 19:834–840

Shen GQ, Cao LK, Lu YT, Hong JB (2005) Influence of phenanthrene on cadmium toxicity to soil enzymes and microbial growth. Environ Sci Pollut Res 12:259–263

Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798

Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70:555–569

Sun F, Kuang YW, Wen DZ, Xu DZ, Li JL, Zuo WD, Hou EQ (2010) Long-term tree growth rate, water use efficiency, and tree ring nitrogen isotope composition of *Pinus massoniana* L. in response to global climate change and local nitrogen deposition in Southern China. J Soil Sediment 10:1453–1465

Tabatabai MA, Bremmer JM (1972) Assay of urease activity in soils. Soil Biol Biochem 4:479–487

Tripathy S, Bhattacharyya P, Mohapatra R, Som A, Chowdhury D (2014) Influence of different fractions of heavy metals on microbial ecophysiological indicators and enzyme activities in century old municipal solid waste amended soil. Ecol Eng 70:25–34

Uselman SM, Qualls RG, Thomas RB (2000) Effects of increased atmospheric CO_2, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). Plant Soil 222:191–202

Ushio M, Kitayama K, Balser TC (2010) Tree species effects on soil enzyme activities through effects on soil physicochemical and microbial properties in a tropical montane forest on Mt. Kinabalu. Borneo Pedobiol 53:227–233

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Vig K, Megharaj M, Sethunathan N, Naidu R (2003) Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. Adv Environ Res 8:121–135

Vlachodimos K, Papatheodorou EM, Diamantopouls J, Monokrousos N (2013) Assessment of *Robinia pseudoacacia* cultivations as a restoration strategy for reclaimed mine spoil heaps. Environ Monit Assess 185:6921–6932

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. Plant Physiol 132:44–51

Wang AS, Angle JS, Chaney RL, Delorme TA, Reeves RD (2006) Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*. Plant Soil 281:325–337

Wang D, Heckathorn SA, Barua D, Joshi P, Hamilton EW, LaCroix JJ (2008) Effects of elevated CO_2 on the tolerance of photosynthesis to acute heat stress in C3, C4, and CAM species. Am J Bot 95:165–176

Wang YH, Yan DH, Wang JF, Ding Y, Song XS (2017) Effects of elevated CO_2 and drought on plant physiology, soil carbon and soil enzyme activities. Pedosphere 27:846–855

Wu HB, Tang SR, Zhang XM, Guo JK, Song ZG, Tian S et al (2009) Using elevated CO_2 to increase the biomass of a *Sorghum vulgare* × *Sorghum vulgare var. sudanense* hybrid and *Trifolium pratense* L.

and to trigger hyperaccumulation of cesium. J Hazard Mater 170: 861–870

Xiao Y, Huang ZG, Lu XG (2015) Changes of soil labile organic carbon fractions and their relation to soil microbial characteristics in four typical wetlands of Sanjiang plain, Northeast China. Ecol Eng 82: 381–389

Xu GH, Zheng HY (1986) Handbook of analysis of soil microorganism. Agriculture Press, Beijing, pp 113–116 (249–91)

Xu W, Wang G, Deng F, Zou X, Ruan H, Chen HYH (2018) Responses of soil microbial biomass, diversity and metabolic activity to biochar applications in managed poplar plantations on reclaimed coastal saline soil. Soil Use Manag 34:597–605

Yang QX, Zhang J, Zhu KF, Zhang H (2009) Influence of oxytetracycline on the structure and activity of microbial community in wheat rhizosphere soil. J Environ Sci 7:954–959

Yang YR, Song YY, Scheller HV, Ghosh A, Ban YH, Chen H et al (2015) Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. Soil Biol Biochem 86:146–158

Yang BS, He F, Zhao XX, Wang H, Xu XH, He XH et al (2019) Composition and function of soil fungal community during the establishment of *Quercus acutissima* (Carruth.) seedlings in a Cd-contaminated soil. J Environ Manag 246:150–156

Yuan XX, Lin XG, Chu HY, Yin R, Zhang HY, Hu JL, Zhu JG (2006) Effects of elevated atmospheric CO_2 on soil enzyme activities at different nitrogen application treatments. Acta Ecol Sin 26:48–53

Zaborowska M, Wyszkowska J, Kucharski J (2006) Microbiological activity of zinc-contaminated soils. J Elem 11:543–557