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Response of *Robinia pseudoacacia* L. rhizosphere microenvironment to Cd and Pb contamination and elevated temperature

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ABSTRACT

Elevated air temperature and contamination of soil with heavy metals co-occur in natural ecosystems and have important effects on the soil microenvironment by influencing plant physiology. Effects of elevated temperature and cadmium (Cd) pollution on the rhizosphere microenvironment have been examined for wheat seedlings, but rhizosphere responses to elevated temperature might differ among plant species and for other heavy metals. This study examined the response of the black locust rhizosphere microenvironment to slightly elevated temperature in combination with Cd and lead (Pb) contamination. Elevated temperature combined with Cd and Pb exposure corresponded to an increase in soluble sugars, free amino acids, and organic acids and to a decrease in soluble phenolic acids in rhizosphere soils. These combined variables also corresponded to a decrease in microbial abundance, microbial biomass carbon, fluorescein diacetate hydrolysis, and the ratio of microbial biomass carbon (C) to total organic C compared to elevated temperature alone or to individual metals. The combination of elevated temperature plus exposure to heavy metals had varying effects on the different enzymes relative to the individual stress factors. The removal rate of Cd and Pb in rhizosphere soils increased significantly under elevated temperature and was greater for Cd than for Pb. Generally, elevated temperature led to increased microbial activity in rhizosphere soil of black locust seedlings exposed to heavy metals by stimulating microbial abundance, microbial biomass C, and enzyme activity. Variability in plant species and heavy metals might lead to different responses of phenolic acids to elevated temperature + heavy metals. Increased removal of heavy metals indicated that elevated temperature might improve the phytoremediation efficiency of heavy metal-contaminated soils by black locust seedlings.
A capsule abstract: Elevated temperature led to increased microbial activity in rhizosphere soil of black locust seedlings exposed to heavy metals.

1. Introduction

According to the Intergovernmental Panel on Climate Change (IPCC, 2007), global air temperature is predicted to increase by 1.4–5.8 °C by the end of the 21 st century as a result of increasing emissions of carbon dioxide and other greenhouse gases (GHG). Temperature influences nutrient cycling and organic matter in terrestrial ecosystems, mainly through plant-mediated responses, such as increased and qualitatively altered rhizodepositions (Zhang et al., 2015). Root exudates sustain a larger and more active microbial community in the rhizosphere than occurs in bulk soil; microorganisms utilize these low-molecular-weight organic compounds as an energy source for proliferation and enzyme synthesis (Renella et al., 2006; Baudoin et al., 2003; Berg and Smalla, 2009). It has been shown that increased air temperature has variable effects on the composition and activity of the rhizosphere microbial community composition and enzyme activity, depending on plant type (herbaceous, woody) and species, soil type, and other ecosystem characteristics (Uselman et al., 2000; Maksymiec, 2007; Frey et al., 2008; Hayden et al., 2012; Jia et al., 2015; Schindlbacher et al., 2011; Wang et al., 2013; A'Bear et al., 2014). The rhizosphere is a critical

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environmental compartment in which fundamental processes responsible for ecosystem functioning and crop production occur (Coleman et al., 1992), and any change in soil quality, including metal pollution, can impact the cycling of carbon and other nutrients.

Soil pollution by heavy metals is widespread, and its impacts on soil microbial diversity and soil ecological functions have been widely reported (D'Aascoli et al., 2006; Gomes et al., 2010). Heavy metal pollution is often associated with the same anthropogenic activities (e.g. heavy industry, intensive agriculture) that cause greenhouse gas emissions and corresponding increases in air temperature, particularly in the emerging economies. Among heavy metals, cadmium (Cd) and lead (Pb) are highly toxic and are often both present in contaminated soils (Meng, 2000; Mleiki et al., 2015). In China, more than 2.0×10^9 ha of land is contaminated with heavy metals (Guo et al., 2011). The effect of metals, including Cd and Pb, on microbial biomass and enzyme activity in the rhizosphere have mostly been examined using model studies without plants (Liao et al., 2006; Renella et al., 2006; Xu et al., 2006). These studies have shown reduced microbial mineralization capacity of organic compounds in root exudates (Renella et al., 2006), but this negative impact may be balanced by the increased concentration of organic compounds in the rhizosphere of plants exposed to Cd pollution and increased air temperature (Jia et al., 2015). Currently, information about the interactive effects of soil heavy metal pollution and elevated air temperature in the rhizosphere of real plant/soil systems is still scarce.

Black locust (*Robinia pseudoacacia* L.) is a woody species that can be used for restoration of degraded ecosystems because of its fast growth and a tolerance of low nutrient levels and heavy metals (Vlachodimos et al., 2013; Liu et al., 2013; Yang et al., 2015). Because elevated air temperature and metal pollution can both affect black locust physiology (Blankinship et al., 2011; Tzvetkova and Petkova, 2015), we investigated the effects of elevated air temperature and high Cd and Pb concentrations on microbial biomass and activity in the rhizosphere of this plant species. We hypothesized that: i) the concentration of organic compounds in rhizosphere soils of black locust seedlings would be greater under elevated than ambient temperature when combined with Cd and Pb concentrations and ii) increased concentrations of organic compounds under elevated temperature would inhibit microbial and enzyme activity in rhizosphere soils of black locust seedlings by increasing the bioavailability of Cd and Pb. We tested our hypotheses in a field trial in which air temperature could be controlled and Cd and Pb concentrations could be increased to specific concentrations. Our results will aid in understanding the impact on rhizosphere microbial communities and activity caused by two co-occurring environmental change scenarios such as metal pollution and global warming.

2. Materials and methods

2.1. Plant material and soils

Black locust seeds were obtained from Northwest A&F University, China. The experimental soil was collected from the surface layer (0–20 cm) of cultivated land in Central Shaanxi, China (34°16′N, 108°54′E). The soil chemical characteristics and type are shown in Table 1. Fresh soil was passed through a 5-mm sieve, and the sieved soils were pretreated with Cd using a dissolved solution of $CdCl_2 \cdot 2H_2O$ thoroughly mixed into the soil at rates of 0.2 (Cd0 [the control], no added Cd or Pb), 1.2 (Cd1), and 5.2 (Cd5) mg Cd kg^{-1} soil dry weight. The Pb treatments consisted of a control (Pb0, 15.6 mg kg dry $soil^{-1}$, no added Pb or Cd) and 515.6 mg Pb kg^{-1} soil dry weight (Pb), obtained by adding Pb $(NO_3)_2$ solution. The Cd0 (with no added Cd or Pb) also represented the Pb0 treatment; therefore, the "Pb0" treatments is labeled "Cd0". These concentrations were selected according to Chinese environmental quality standard (GB 15168–1995) and on the basis of Cd concentrations observed in some farmland areas in China (Song et al., 2006). In addition to the control, Cd-only and Pb-only treatments were combined into the following treatments: Cd0/Pb, Cd1/Pb, and Cd5/Pb. The Cd0/Pb represents the Pb-only treatment. The contaminated soils were incubated for 30 days (Liao et al., 2010).

2.2. Experimental site and air temperature

The study was conducted on the Weishui Campus of Chang'an University, Xi'an, China (34°15′N, 108°55′E) from June to September 2014. The mean annual temperature (1995–2010) and precipitation (1995–2010) in the study region are 13.6 °C and 508–720 mm. The temperature treatments consisted of elevated and ambient temperature. Open-top chambers (OTCs) made of translucent synthetic glass (which has ~90% solar transmittance in the visible wavelengths) were used as a passive warming devices to generate an artificially warmed environment. Three hexagonal OTCs (4.4 m dia × 1.6 m tall) were established as the elevated temperature (ET) treatment, and the control consisted of three open plots under ambient temperature (AT). The OTCs were located 4–5 m distance from the open plots, and were separated by 3–4 m. During the seedling growth period, the average air temperature in the OTCs was 2.7 °C higher than the ambient temperature, which led to an average increase in soil temperature of 1.1 °C above ambient. The targeted 2.7 °C temperature increase was based on general circulation models that predict warming from 2 to 5 °C within the next century (Solomon et al., 2007). The ambient-temperature treatment followed changes in the natural temperature. Automated measurements of humidity, temperature, and soil water content were taken every 60 s throughout the

Table 1
Type and chemical characteristics of the used soil.

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
Total Pb (mg kg^{-1})	15.6 ± 0.8

experiment; the average temperature and humidity in elevated-temperature chambers and ambient plots during the growth stage were 30.5 °C (77.1%) and 27.8 °C (75.7%), respectively.

2.3. Pot experiment

The experiment was performed in plastic pots (70 cm long × 40 cm wide × 50 cm tall). Soil (25 kg) was added to each pot and the controls with no Cd or Pb were established. Three replicates of each metal treatment were prepared. Forty black locust seedlings were obtained per pot after emergence. The pots were placed in open plots and OTCs for 90 days, using a randomized complete block design for OTCs. Soil water content in each pot was monitored with a manual probe (IMKO, Germany), and the pots were maintained at 60% field capacity by watering as needed during the seedling growth stage to exclude effects of soil moisture. The treatments consisted of: (1) AT + Cd0, Cd1, or Cd5; (2) AT + Pb-contaminated soils; (3) AT + Cd + Pb-contaminated soils; (4) ET + Cd0, Cd1, or Cd5; (5) ET + Pb-contaminated soils; and (6) ET + Cd + Pb-contaminated soils. Weeds and litter were monitored and removed from pots by hand to reduce their effect on plant growth and soil characteristics. With the exception of the wind, microhabitat characteristics in the OTCs and open plots were similar based on the use of translucent synthetic glass with high solar transmittance in visible wavelengths for OTCs, a randomized complete block design of OTCs, and controlling for soil moisture. Thus, microhabitat characteristics such as light exposure and soil moisture in the OTCs and open plots were similar, and these variables had little effect on seedlings growth, allowing us to isolate the effects of elevated temperature.

2.4. Rhizosphere sampling

Rhizosphere samples were collected at 30, 60, and 90 days using the method described by Jia et al. (2014). The soils were mixed, homogenized, and divided into three subsamples. One subsample was air dried to determine pH, total Cd, and Pb. Another subsample was stored at −20 °C for analysis of organic compounds. The third subsample was stored at 4 °C for analysis of microorganism abundance, microbial biomass C (MBC), and enzyme activity.

2.5. Soil organic compounds

Total soluble sugars were determined by colorimetry using the phenol–sulfuric acid method (Dubois et al., 1956). Soluble phenolic acids were determined by the Folin–Ciocalteu method (DeForest et al., 2005). Free amino acids were assessed colorimetrically by the ninhydrin method described by Moore and Stein (1954). Organic acid concentrations were determined according to Jia et al. (2015), as follows: organic acids were extracted from 5 g of sieved soil (2 mm) for 1 h using 20 mL of purified water followed by centrifugation at 10,000 × g for 10 min. Ten milliliters of the supernatant was mixed with 0.25 mL of 0.2 mol L^{-1} NaOH and dried in a Termovap Sample Concentrator, and 2.5 mL of acid ethylene glycol (47:3 ratio of ethylene glycol to sulfuric acid) was added and the mixture was placed in an 80 °C water bath for 8 min to dissolve the salt. After cooling at room temperature, 1 mL of 10% (m/v) hydroxylamine hydrochloride and 4 mL of 4.5 mol L^{-1} NaOH were added and the mixture was homogenized. After 3 min, 12 mL of 0.15 mol L^{-1} $FeCl_3$ was added and thesolution was diluted to a final volume of 50 mL using purified water. After 20 min, absorbance of the solution was read at 500 nm. The measurements of organic compound concentration were performed in triplicate.

2.6. Soil biochemical properties

Colony-forming units of bacteria, fungi, and actinomycetes were analyzed by a modified plate-dilution technique on meat peptone agar, Thayer–Martin agar, and Gause's starch agar, respectively (Yang et al., 2009). Soil MBC was measured by fumigation–extraction (Vance et al., 1987). Total organic carbon (TOC) was measured using the $K_2Cr_2O_7$–H_2SO_4 oxidation method (Nelson and Sommers, 1982).

The ratio of soil microbial biomass C to TOC (C_{mic}-to-C_{org}) was calculated by determining soil MBC/TOC. Fluorescein diacetate (FDA) hydrolysis was evaluated according to Mora et al. (2005), and enzyme activity (U) was expressed as the quantity of fluorescein released (mg fluorescein min^{-1} g dry soil equivalent^{-1}).

The activity of five enzymes was analyzed as follows. Urease activity was quantified as the amount of NH_4^+ released from the hydrolysis of urea in Tris buffer (expressed as mg NH_4^+-N h^{-1} g dry soil equivalent^{-1}) (Tabatabai and Bremmer, 1972). Dehydrogenase activity was estimated from the reduction of 2,3,5-triphenyl tetrazolium chloride to triphenyl formazan (TPF) in soil samples after incubation at 30 °C for 24 h (expressed as μg TPF h^{-1} g dry soil equivalent^{-1}) (Casida et al., 1964). Invertase activity (expressed as μg glucose h^{-1} g dry soil equivalent^{-1}) was determined by incubating 5 g soil with 15 mL of 8% (m/v) sucrose for 24 h at 37 °C; the suspension was reacted with 3,5-dinitrosalicylic acid for the colorimetric assay and absorbance was read at 508 nm (Xu and Zheng, 1986). Soil β-glucosidase activity was obtained using a spectrophotometric assays by incubating 1 g of air-dried soil for 1 h with *p*-nitrophenyl-β-D-glucoside at pH 6.0 (expressed as μg *p*-nitrophenol d^{-1} g dry soil equivalent^{-1}) (Eivazi and Tabatabai, 1999). Activity of l-asparaginase was estimated according to Frankenberger and Tabatabai (1991) (expressed as μg ammonia h^{-1} g dry soil equivalent^{-1}). Enzyme activity measurements were performed in triplicate.

2.7. Removal rate of Cd and Pb

Total Cd and Pb concentrations in rhizosphere soil were obtained by digesting dried, ground soil samples (0.5 g) with 6 mL HCl, 3 mL of 16 mol L^{-1} concentrated HNO_3, and 4 mL $HClO_4$ in a microwave digestion system (SINED, MDS-6). The concentrations of total Cd and Pb were determined by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher-X series). The removal rates (RR) of Cd and Pb were calculated as follows: RR (%) = 100 × (the treatment Cd or Pb content – residual Cd or Pb content in rhizosphere soil)/(treatment Cd or Pb content).

2.8. Statistical analyses

A general linear model and type-III sum of squares were used to analyze the effects of temperature, Cd, Pb, and their interactions. Three-way analysis of variance (ANOVA) was used to test the effects of temperature, Cd, and Pb on each variable (soil microorganism abundance, MBC, enzyme activity, organic compounds). Effects of elevated temperature, Cd, and Pb on these variables were tested using one-way ANOVA. Tukey's multiple-comparison post-hoc tests were used to assess the significance of differences between treatments for each variable. All statistical tests were performed using SPSS (SPSS Inc., version 19.0).

3. Results

3.1. Organic compounds in rhizosphere soils

Under elevated temperature, concentrations of total soluble sugars, free amino acids, and organic acids increased significantly

($p < 0.05$) relative to ambient temperature, and soluble phenolic acids decreased ($p < 0.05$). Moreover, soluble sugars and phenolic acids increased with time (Fig. 1). Concentrations of soluble sugars, free amino acids, and organic acids were significantly ($p < 0.05$) higher in treatments with individual metals relative to the control, and soluble phenolic acids were lower ($p < 0.05$). Sugars, amino acids, and organic acids were higher, and phenolic acids were lower, under elevated temperature with individual and combined metals than under any of the individual variables. The lowest concentrations of soluble phenolic acids ($p < 0.05$) occurred in the ET + Cd + Pb treatment (Fig. 1). There were significant interactive effects of temperature, Cd, and Pb on free amino acids and organic acids (Table 2).

3.2. Culturable microbial groups and microbial biomass C

The abundance of bacteria and actinomycetes increased significantly ($p < 0.05$) and fungal abundance decreased significantly ($p < 0.05$) in the elevated temperature treatment compared with ambient temperature (Fig. 2). Exposure to either Cd or Pb significantly ($p < 0.05$) reduced the abundance of bacteria and actinomycetes but did not have a significant effect on fungi. Bacteria and actinomycetes were more abundant under ET + Cd or Pb than under heavy metals alone and were less abundant than under elevated temperature alone (Fig. 2). Under ET + Cd + Pb, bacteria and actinomycetes increased compared to AT + Cd + Pb or ET + individual heavy metals and decreased relative to elevated temperature alone. There were significant interactive effects of temperature, Cd, and Pb on all microbial groups (Table 2).

Relative to the control, soil MBC, C_{mic}-to-C_{org}, and FDA were significantly ($p < 0.05$) greater under elevated temperature, and soil MBC and C_{mic}-to-C_{org} increased with time. Each of these variables was significantly lower ($p < 0.05$) under either Cd or Pb relative to the control (Fig. 2). In treatments with Cd + Pb or individual metals, soil MBC and C_{mic}-to-C_{org} were higher ($p < 0.05$) under elevated temperature than under ambient temperature (Fig. 2). In addition, soil MBC, C_{mic}-to-C_{org}, and FDA were lower under elevated temperature with the combined or individual metals than under elevated temperature alone. Interactive effects of temperature, Cd, and Pb on MBC, C_{mic}-to-C_{org}, and FDA were significant (Table 2).

3.3. Enzyme activity in rhizosphere soils

The activity of dehydrogenase, invertase, β-glucosidase, and L-asparaginase was significantly ($p < 0.05$) higher under elevated temperature than under ambient temperature, and urease activity was lower ($p < 0.05$); moreover, dehydrogenase, invertase, and urease activity increased gradually with time (Fig. 3). Urease activity was lower under ET + either Cd or Pb than under the individual metals or ET. Generally, dehydrogenase, invertase, β-glucosidase, and L-asparaginase activity were higher ($p < 0.05$) under ET + either Cd or Pb stress than under individual metals (Fig. 3). Compared to AT + Cd + Pb, urease activity decreased ($p < 0.05$) and dehydrogenase, invertase, β-glucosidase, and L-asparaginase activity increased ($p < 0.05$) under ET + Cd + Pb; the activity of all enzymes except for L-asparaginase was lower under ET + Cd + Pb than ET + the individual metals (Fig. 3).

3.4. Removal rate of Cd and Pb in rhizosphere soils

The removal rate of Cd and Pb was significantly higher ($p < 0.05$) under elevated temperature compared to ambient temperature (Fig. 4), and metal removal rates increased with time and with increasing of the Cd concentration. Under elevated temperature, the average increase in Cd removal was greater than that of Pb (Fig. 4).

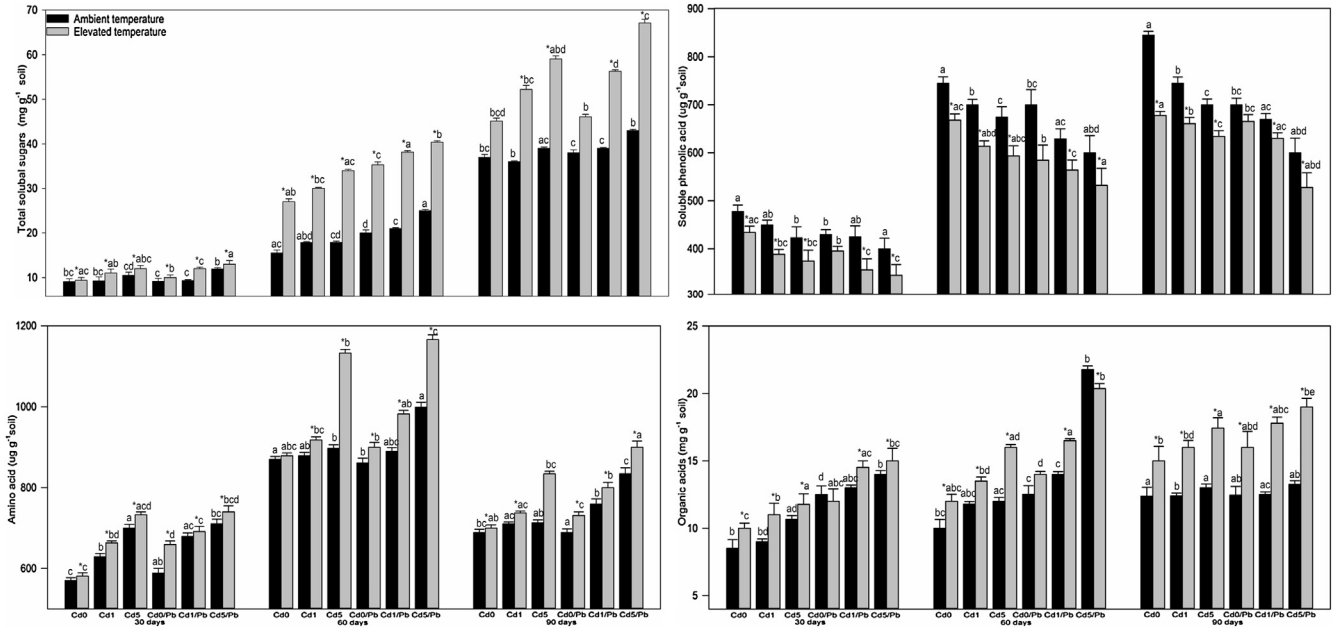

Fig. 1. Concentrations of total soluble sugars, soluble phenolic acids, amino acids, and organic acids in rhizosphere soils of black locust under different treatments. (Date are means ± SE; n = 3). Cd0, Cd1, and Cd5 in figures represent 0.2 (The control with no added Cd or Pb), 1.2, and 5.2 mg Cd Kg^{-1} dry soil weight, respectively. Pb represents 515.6 mg Pb kg^{-1} dry weight soil. Cd0 also represents the control for Pb0 treatment (15.6 mg Pb kg^{-1} dry weight soil, no added Cd or Pb); therefore, the label "Pb0" in the figures was replaced by "Cd0". The Pb-only treatment is labeled "Cd0/Pb" because no Cd0 was added. Asterisks indicate a significant difference ($p < 0.05$) between elevated temperature and ambient temperature at the same heavy metal level (within the same period); different lowercase letters indicate significant differences ($p < 0.05$) between treatments at the same temperature level (within the same period). (The same below).

Table 2
Results of three-way ANOVA (F values) examining the effects of temperature (T), Cd, and Pb on organic compounds, microorganisms, and enzyme activity in rhizosphere soils.

Variables	30 days							60 days							90 days						
	T	Cd	Pb	Cd × Pb	T × Cd	T × Pb	T × Cd × Pb	T	Cd	Pb	Cd × Pb	T × Cd	T × Pb	T × Cd × Pb	T	Cd	Pb	Cd × Pb	T × Cd	T × Pb	T × Cd × Pb
Soluble sugars	25.9**	17.5**	8.9**	5.3*	17.0**	3.1 ns	4.7*	15.5**	39.6**	21.4**	4.8*	1.8 ns	5.1*	2.2 ns	14.7**	15.3**	33.5**	12.4**	6.1*	0.9 ns	4.3*
Phenolic acids	28.6**	1.1 ns	5.6*	56.3**	12.6**	19.1**	3.2 ns	71.5**	5.4*	4.1*	22.3**	5.7*	3.2 ns	8.1**	89.3**	4.5*	5.2*	3.2 ns	2.1 ns	1.4 ns	21.1**
Amino acids	68.9**	48.4**	86.8**	23.8**	88.2**	52.9**	33.4**	34.9**	52.7**	28.7**	42.3**	45.3**	33.5**	16.6**	56.7**	27.5**	16.4**	12.5**	32.4**	13.4**	47.8**
Organic acids	114.5**	32.1**	44.6**	34.5**	23.2**	70.7**	38.2**	59.6**	54.7**	37.1**	21.5**	59.3**	166.9**	280.7**	78.8**	12.2**	53.4**	128.3**	65.1**	14.6**	25.2**
Bacteria	25.7**	9.7**	52.1**	25.9**	48.1**	13.0**	7.9**	81.4**	27.8**	83.1**	32.2**	142.8**	334.4**	94.6**	34.8**	51.7**	29.1**	18.4**	91.6**	57.0**	191.8**
Fungi	4.2*	4.7*	1.4 ns	13.3**	14.0**	23.3**	16.3**	4.8*	12.3**	2.5 ns	23.6**	13.9**	12.4**	25.7**	5.1*	1.6 ns	5.5*	9.3**	48.3**	26.0**	44.5**
Actinomycetes	36.8**	17.1**	89.3**	45.3**	54.3**	86.0**	67.7**	77.7**	47.5**	54.6**	78.3**	167.4**	172.5**	41.8**	30.1**	48.8**	70.2**	34.2**	112.9**	45.1**	78.7**
MBC	4.8*	81.8**	9.9**	23.4**	22.8**	55.0**	41.1**	8.5**	44.9**	47.9**	17.3**	121.1**	26.6**	166.0**	13.4**	45.5**	17.7**	43.7**	31.2**	55.6**	23.5**
C_{org}-to-C_{mic}	24.7**	20.7**	20.1**	23.5**	8.1**	15.2**	59.0**	42.4**	48.9**	52.5**	13.5**	83.8**	231.3**	14.1**	14.8**	5.8*	36.5**	9.2**	17.6**	5.7*	15.4**
FDA	19.7**	38.3**	2.6 ns	51.4**	65.5**	156.4**	46.6**	24.1**	14.9**	58.1**	11.8**	106.4**	97.6**	16.5**	50.8**	11.3**	16.4**	16.4**	21.8**	10.3**	32.6**
Urease	5.7**	18.9**	62.5**	43.1**	45.3**	272.7**	71.0**	5.5*	89.8**	57.1**	5.2*	83.2**	35.7**	47.6**	4.9*	30.1**	16.8**	12.5**	218.1**	90.1**	18.6**
Dehydrogenase	9.5**	5.3*	16.7**	71.3**	24.5**	6.1*	28.8**	13.6**	76.6**	45.6**	45.7**	96.6**	13.2**	150.7**	87.8**	33.7**	47.3**	38.6**	54.1**	61.9**	17.4**
Invertase	68.9**	80.4**	56.7**	44.5**	11.1**	137.4**	103.8**	170.1**	26.7**	23.1**	16.9**	51.3**	32.5**	16.8**	77.3**	2.3 ns	20.4**	52.7**	80.4**	18.5**	41.8**
β-glucosidase	15.2**	46.4**	1.4 ns	19.3**	23.1**	57.6**	95.3**	30.2**	22.8**	12.9**	23.5**	14.8**	24.6**	23.2**	87.8**	39.5**	14.7**	28.3**	26.9**	23.2**	32.4**
L-asparaginase	33.7**	67.6**	8.9**	63.2**	115.3**	27.1**	42.8**	105.5**	21.1**	13.7**	12.1**	45.3**	54.5**	19.2**	45.7**	67.8**	34.2**	23.1**	67.1**	328.2**	72.7**

ns; not significant.
* $p < 0.05$.
** $p < 0.01$.

4. Discussion

4.1. Impact of temperature

The increased soluble sugars, amino acids, and organic acids in rhizosphere soils (Fig. 1) could be the result of a stimulatory effect of elevated temperature on black locust photosynthesis, growth, and synthesis of secondary metabolites, as previously reported (Uselman et al., 2000; Zhang et al., 2013a,b; Zhao et al., 2016). Increased photosynthesis and productivity stimulate the synthesis and root exudation of organic compounds (Yin et al., 2008; Hollister and Flaherty, 2010). Although elevated temperature increased phenolic acids in black locust seedlings in our previous study (Zhao et al., 2016), in this study we observed a decrease in phenolic acids concentration in rhizosphere. Microorganisms can decompose phenolic acids (Zhang et al., 2009), and the observed decrease in these compounds under elevated temperature could be related to increased microbial abundance and biomass carbon in rhizosphere (Fig. 2). However, soluble sugar concentration increased irrespective of increased microorganisms in rhizosphere soils, indicating the mechanisms by which phenolic acids decreased under elevated temperature are not clear.

The average increase in soil temperature of 1.1 °C was slightly within the range of daily or seasonal fluctuations (Jia et al., 2015). The temperature optima of heterotrophic soil microorganisms are sufficiently broad to tolerate such small changes in soil temperature (Machair et al., 2010). Consequently, this 1.1 °C temperature increase had limited influence on microbial activity compared to the influence of organic compounds in the rhizosphere soils. Increased availablity of organic substrates under elevated likely contributed to stimulation microbial growth and activity in these soils (Luo et al., 2014). Increase microbial activity under elevated temperature was cosistent with previous observations in wheat seedlings (Jia et al., 2015), but contradicted observations in temperate mountain forest and grassland soils (Schindlbacher et al., 2011; Hayden et al., 2012). The global effects of warming on soil microbial activity may depend on the interplay of key factors such as substrate availability, moisture content, plant species, and soil type.

Extracellular enzyme activity is, in general, positively related to microbial activity measured via respiration (Frankenberger and Dick, 1983); therefore, the increased microorganism activity observed under elevated temperature contributed to increased enzyme activity in rhizosphere soils. In addition, the interactions between enzymes and organic substrates can influence enzyme biophysical properties including kinetics and temperature sensitivity (Bárta et al., 2014), who suggests that the increased concentrations of organic compounds in rhizosphere soils can lead to increased enzyme activity. The positive effect of elevated air temperature on dehydrogenase, invertase, β-glucosidase, and L-asparaginase activity (Fig. 3) contrasted with previous studies (Allison and Treseder, 2008; Cusack et al., 2010; Kardol et al., 2010), possibly as a result of different conditions in the rhizosphere.

4.2. Impact of heavy metals

Migration of Cd and Pb in soils was restricted to the top 10 cm in the first year; furthermore, Cd and Pb pollution mainly affected the upper 30 cm of topsoil in other studies that examined these gynamics for up to 4 years (Abbaspour et al., 2007; Zhang et al., 2013a,b; Kraus and Wiegand, 2006). Therefore, the migration of heavy metals in soils is limited in long time, indicating that the observed removal of Cd and Pb here was a result of absorption by black locust (Zhao et al., 2016).

Organic compounds in rhizosphere soils are mainly derived from root exudates, root residues, and aboveground litter

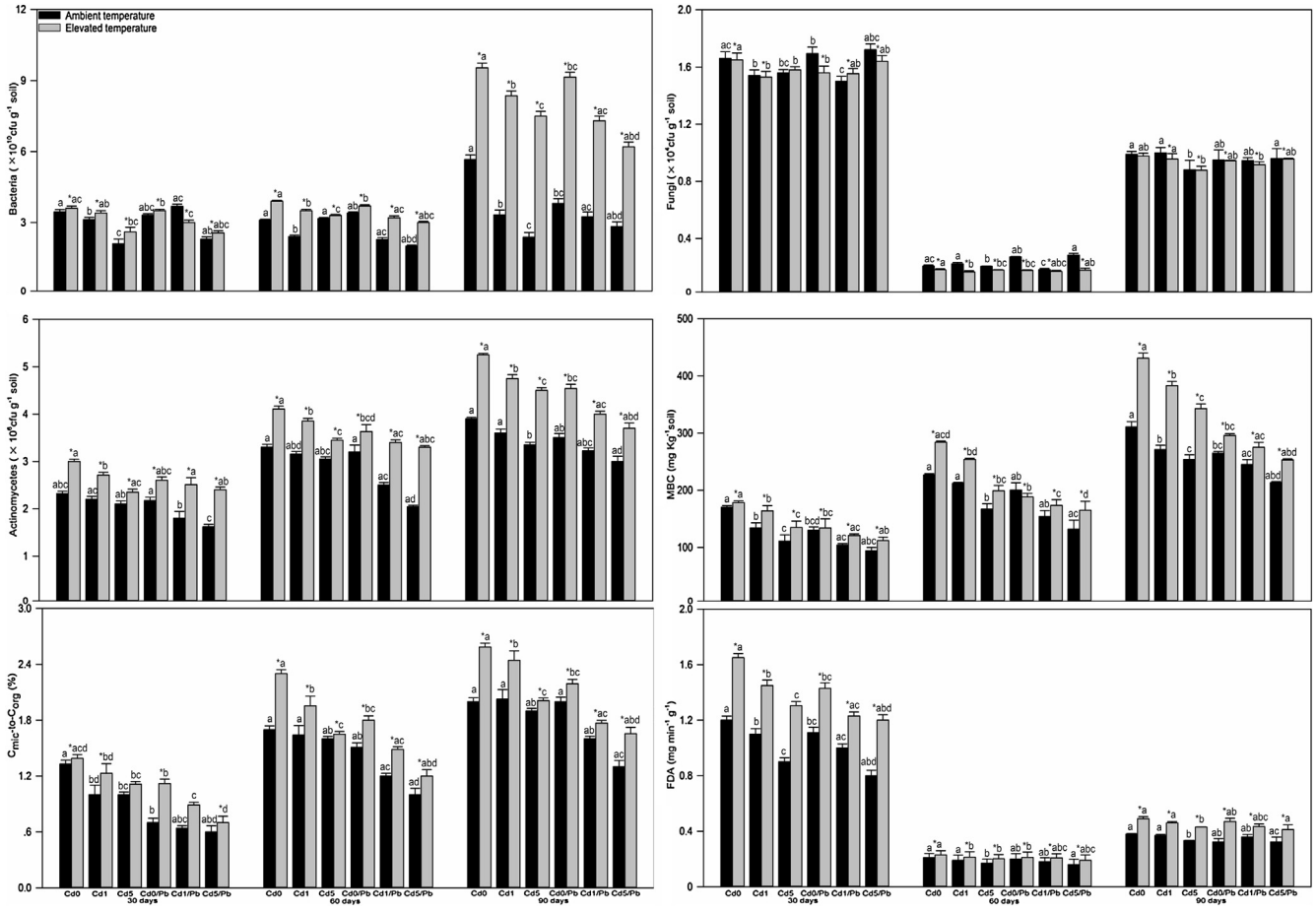

Fig. 2. The abundance of bacteria, fungi, actinomycetes, MBC, C_{mic}-to-C_{org}, and FDA in rhizosphere soils of black locust under different treatments. (Date are means ± SE; n = 3).

(Kuzyakov, 2001; Walker et al., 2003). Because in the present study litter and weeds were removed from the experimental pots, the increased concentration of organic compounds in the rhizosphere could be ascribed to the effect of Cd or Pb on black locust growth. Differently, the decrease in soluble phenolic acids could be related to plant uptake of heavy metals and to reduced root exudation of seedlings exposed to heavy metals. These changes in the composition of the organic compounds in soil could influence the microbial biomass and microbial activity in the rhizosphere. In addition, it has been reported that heavy metals can impact microbial biomass, specific microbial groups, also C_{mic}-to-C_{org}, and FDA (Shen et al., 2005; D'Aascoli et al., 2006; Gomes et al., 2010). Our results also showed that fungi were more sensitive than bacteria and actinomycetes to Cd and Pb (Fig. 2), consistently with previous studies (Pan and Yu, 2011). Heavy metals can denature enzyme proteins and can interact with both enzyme active sites and substrate complexes (Vig et al., 2003). The decreased urease, dehydrogenase, invertase, and β-glucosidase activities was also consistent with previous studies (Tripathy et al., 2014; Ma et al., 2015), confirming that heavy metals can impact C and N cycling in the rhizosphere. Increased concentrations of soluble sugars and organic acids could increase availability of heavy metals (Lu et al., 2007; Wang et al., 2009), which in turn lead to a decrease in enzyme activity in the rhizosphere. However, the response of l-asparaginase to Cd differed from the response to Pb (Fig. 3), indicating that two heavy metals can have different effects on a specific enzyme activity.

4.3. Interactions between temperature and heavy metals

The increase in organic compounds (except for phenolic acids) in rhizosphere soils under elevated temperature in combination with heavy metals supported our working hypothesis. Consistent with our observations for black locust, increased concentrations of organic compounds were also observed in rhizosphere soils of wheat seedlings exposed to Cd and slightly elevated temperature (Jia et al., 2015).

Slightly elevated temperature and heavy metals had contrasting effects on microbial activity. Increased soluble sugars, amino acids, and organic acids in the rhizosphere under elevated temperature plus Pb and Cd had a stimulatory effect on soil microorganisms through a greater substrate abailabity (Fig. 1). An increase in organic compounds in the rhizosphere might also stimulate the bioavailability of Cd and Pb, which would have adverse effects on microbial activity. However, the stimulatory effect of elevated temperature might be greater than the inhibitiory of Cd and Pb, which would help to explain the increased microbial activity under elevated temperature plus the combined metals, which was contrary to our second hypothesis. The effect of elevated temperature in combination with Pb and Cd on microbial dynamics in the black locust rhizosphere was similar to that observed in wheat seedlings (Jia et al., 2015), suggesting that plant species might not have a strong effect on the response of soil micro-organisms to these environmental changes.

The increased activity of most enzymes in the elevated temperature + heavy metal treatments indicated that a slight

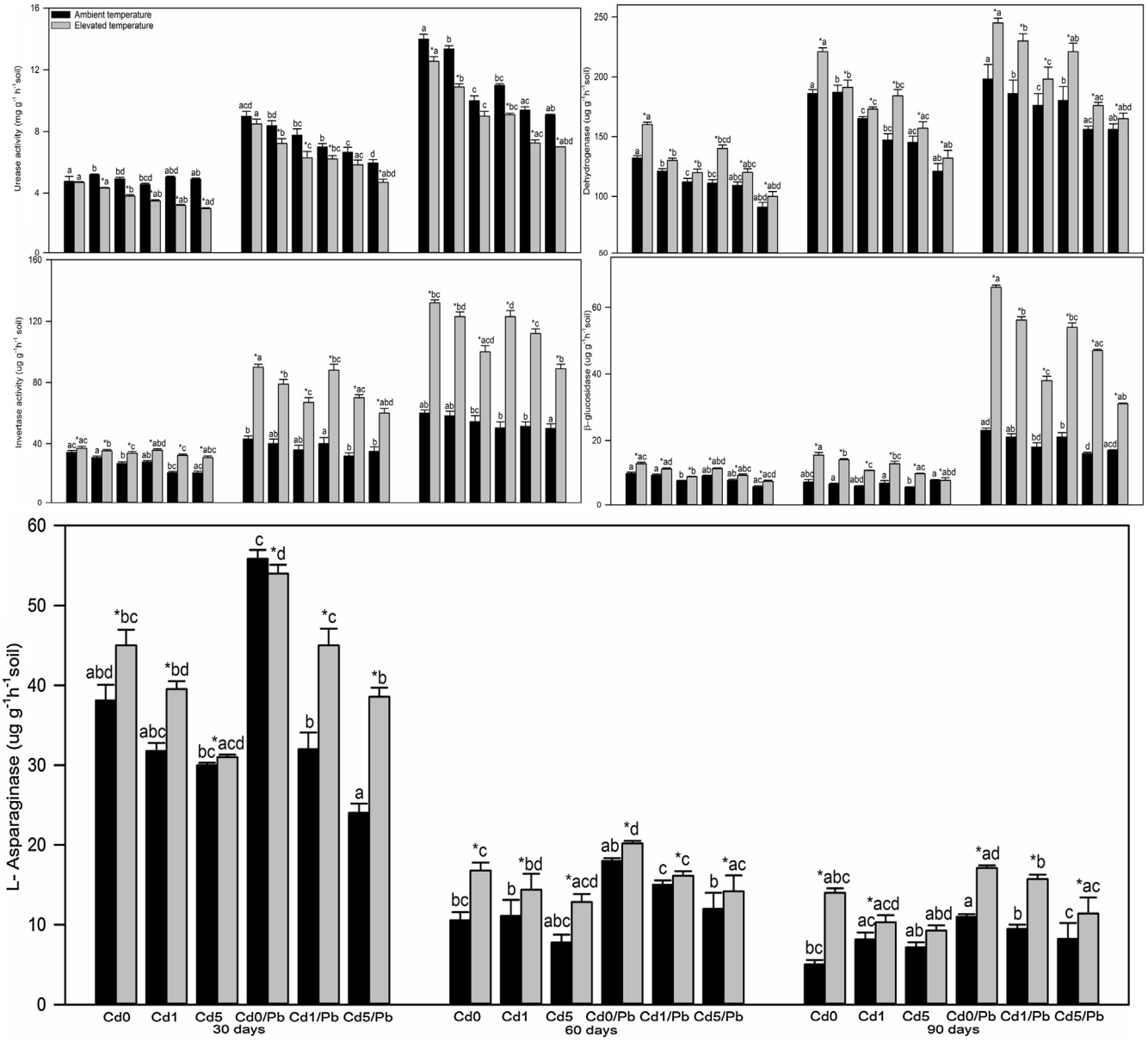

Fig. 3. The activity of urease, dehydrogenase, invertase, β-glucosidase, and L-asparaginase in rhizosphere soils of black locust under different treatments. (Date are means ± SE; n = 3).

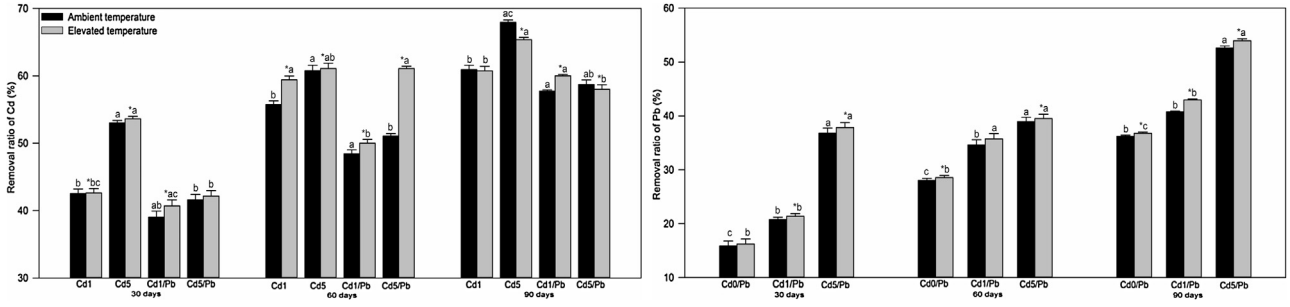

Fig. 4. Removal rate of Cd and Pb in rhizosphere soils of black locust under different treatments. (Date are means ± SE; n = 3).

increase in temperature could counteract the negative effects of Cd or Pb on enzyme activity in the rhizosphere. Furthermore, the increased microbial abundance and activity in these treatments could explain the increased enzyme activity in rhizosphere soil. Negative effects of Cd and Pb could explain the lower enzyme activity under elevated temperature in plants exposed to both metals relative to those exposed to individual metals.

5. Conclusions

Overall, the effects of elevated temperature in combination with soil Cd and Pb pollution on enzyme activity are complex. Elevated temperature increased biological activity in rhizosphere soil of black locust seedlings under Cd and Pb stress by stimulating microbial abundance, microbial activity, and enzyme activity. The increased removal of Cd and Pb under elevated temperature indicated that increased atmospheric temperature might improve the phytoremediation efficiency of black locust in Cd- and Pb-contaminated soils.

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