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Responses of microbial activity and diversity to cadmium in the rhizosphere of winter wheat seedlings

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ABSTRACT

Pot experiments were conducted to investigate the effect of cadmium on the rhizosphere microbial community and activity of winter wheat seedlings. The addition of Cd resulted in increases of the SMBC (soil microbial biomass carbon), SBR (soil microbial respiration), and MQ (microbial quotient) by 6.31 - 48.60%, 9.35 - 113.67%, and 8.29 - 76.24%, respectively, at the third week. However, the SMBC decreased at the seventh and twelfth weeks by 0.48-36.91% and 0.46-18.89%, respectively. The TOC (soil total organic carbon) decreased significantly ($p<0.01$) at the third and seventh weeks, except for the 5 and 10 mg/kg Cd treatments. The MMQ (metabolic quotient) increased at the third and seventh weeks by 8.490-44.739% and 2.79 - 46.48%, respectively, but significantly decreased by 1.72 -26.52% at the twelfth week. Furthermore, the microbial functional diversity decreased, and the ability of the rhizosphere microbes to utilize carbon resources was noticeably affected by Cd pollution. Cd resulted in the increase of phenolic acid utilization and the decrease of carbohydrate, carboxylic acid, and amino acid utilization. Moreover, the MQ was also found to be a sensitive ecophysiological parameter, indicating an environmental pressure. The responses to Cd of all of the microbial parameters determined did not display obvious time and dose dependences. The SMBC, MMQ, and MQ would be sensitive and precise indicators of the rhizosphere soil health under the stress of Cd.

KEYWORDS

Cadmium pollution;
Winter wheat seedlings;
Rhizosphere;
Microbial activity;
Microbial functional diversity.

INTRODUCTION

Cadmium (Cd) is a non-essential element for humans and an agricultural soil contaminant that accumulates in the soil through both natural and anthropogenic processes[1]. It is proven that the uptake of excess Cd by plants and its subsequent accumulation along the food chain is a potential risk to human health. Moreover, cadmium accumulates in plants more readily than most other heavy metals and can be transferred into the edible parts before any signs of phytotoxicity[1-3]. The mobility of Cd in the soil depends on physical, chemical, and biological processes that mainly include rhizosphere microbial activities and plant root growth behavior. Among the known biological processes, the rhizosphere microbes plays crucial roles in energy flow,

element cycling, and organic matter turnover in ecosystems, and a well-functioning microbial community is a prerequisite for soil fertility and the resilience to external factors[4]. Moreover, microbial communities are in close contact with soil microenvironments and, therefore, are easily subjected to change following an alteration of the soil chemical properties[5]. It is well known that microorganisms are sensitive indicators for environmental monitoring at contaminated sites (e.g., heavy metal contamination) because they are the most sensitive part of the soil ecosystem[6]. An increasing body of evidence also suggests that microorganisms are far more sensitive to the stress from heavy metals, e.g., Cd, than animals or plants in the same soils. Therefore, it is fundamental to understand how Cd influences soil microorganisms for environmental monitoring and sustainable management.

The rhizosphere is a zone of enhanced microbial activities due to the root exudates available to the microbial community[7], and the rhizosphere microbial properties influenced by plants are important factors in determining the survival and sustainable growth of those plant. Plant roots release a wide variety of compounds into the rhizosphere that create unique microenvironments for soil microorganisms[8]. It is commonly recognized that root exudates differ according to the plant species, cultivar, plant growth stage, and soil microenvironment[9-11]. The chronic exposures to Cd might influence the quantity and variety of plant exudates by affecting the soil microenvironment and plant root accumulation and would further affect the diversity and the activity of the rhizosphere microbial communities. Although there are many studies on the effect of Cd on soil microorganisms[12-15], due to the lack of relevant studies to date, it remains unclear the effects of Cd on microbial activity and diversity in the rhizosphere of winter wheat seedlings. Therefore, detailed research on the effects of Cd on the microbial activity and diversity in the rhizosphere of winter wheat seedlings is urgently needed.

The aim of this study was to evaluate the responses of microbial activity and diversity to Cd in the rhizosphere of winter wheat seedlings. To understand the effect of Cd on the microbial communities, information on the functional diversity, which is represented by the catabolic potential of the community, is essential. The BIOLOG system, which quickly, economically, and effectively determines diversity profiles, was used to assess the qualitative physiological fingerprinting of the potential functions of the microbial community. Soil microbial parameters, such as the SBMC (soil microbial biomass carbon), TOC (soil total organic carbon), SBR (soil microbial respiration), MQ (microbial quotient), and MMQ (metabolic quotient), were used to evaluate the responses of microbial activities to Cd in detail.

MATERIALS AND METHODS

Soil characterization and experimental design

We selected five levels of Cd (5, 10, 20, 50, and 70 mg/kg dry weight soil) according to the soil environment quality standard (GB 15168-1995) in China. A pot (H 46 cm × D 36 cm) experiment was used to culture the winter wheat seedlings. The soil for the pot experiment was collected from 8 locations of the top 20 cm layer in the same cornfield in Central Shaanxi, China (108°54' E, 34°16' N). Some properties of the soil are as follows: organic matter content, 17.17 g/kg; pH, 8.45; total nitrogen, 1.12 g/kg; available P, 71.17 mg/kg; exchangeable K, 575.00 mg/kg; soluble salt 0.74 g/kg and total Cd, 0.22 mg/kg. The soil type is brown soil.

After the soil was air-dried at ambient temperature, and the fine roots and other debris were removed, the soil was sieved (2 mm), mixed, and homogenized for the pollution treatment with Cd. The soil was artificially contaminated with $CdCl_2$: the plastic pots were filled with 15 kg of contaminated soil after it was incubated for 30 days, and three replicates for each treatment were prepared. The control pots (0 mg/kg dry weight soil) were filled with uncontaminated soil. The pot-soil moisture was brought to 60% field capacity, and the pots were placed in the open air. The seeds of winter wheat (*Triticum aestivum L.*) were planted in all of the pots on October 1st 2010, with field one hundred seedlings per pot after emergence. The pots were maintained at 60% field capacity (using constant weight) by watering during the seedling growth stage.

Rhizosphere soil sampling

The soil strongly adhering to the roots and within the space explored by the roots was considered the

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rhizosphere soil[16]. Rhizosphere soil samples were collected when the winter wheat seedlings had grown for three weeks, seven weeks, and twelve weeks. The roots were retrieved from five areas in each pot, and the rhizosphere soil was carefully collected, mixed, and homogenized to obtain 0.3 - 0.5 kg. Each sample was then divided into three subsamples and analyzed for the microbial community diversity, microbial activity, and soil properties.

Microbial activity analysis

The soil total organic carbon (TOC) content was analyzed using the $K_2Cr_2O_7$-H_2SO_4 calefaction method[17].

The soil microbial biomass carbon (SMBC) was determined using the fumigation-extraction method[18]. Subsamples of sieved soil (25 g dry weight) were fumigated with ethanol-free $CHCl_3$ for 24 h and immediately extracted with 0.5 M K_2SO_4 solution; unfumigated soils were extracted in the same way. The K_2SO_4 soil extract was analyzed for the total dissolved organic carbon (DOC). The SMBC was calculated as follows: SMBC = EC/K_{EC}, where EC = (the organic C extracted from the fumigated soils) – (the organic C extracted from the unfumigated soils) and K_{EC} = 0.45[19].

The soil microbial respiration (SBR) was estimated via the evolution of CO_2 at 25°C in samples incubated for 24 h; any CO_2 respired was trapped in NaOH, and the residual NaOH was titrated with HCl. The metabolic quotient (MMQ) was calculated as the SBR per unit of MBC (SBR/MBC). The microbial quotient (MQ) was also calculated by MBC/TOC[7].

Substrate utilization patterns

The BIOLOG EcoPlate system (Biolog Inc., USA) was used to discriminate the metabolic diversity of the microbial community. Rhizosphere soil samples were homogenized with sterile saline 0.85% NaCl into slurries having a final soil concentration of 1.000 g dry weight/L. The carbon source utilization patterns of the microbial communities were assessed using BIOLOG Eco-Micro-plates that contained three replicate wells of 31 carbon sources and a water blank[20]. A 150 µL aliquot of soil suspension was added to each well of the microplate, which was then incubated in the dark at 28°C for 240 h until no further color development occurred. The color development in the wells at 590 nm was measured every 12 h.

Data analysis

Before the data were further processed, the mean value was calculated from the three replicates included in each BIOLOG EcoPlate. The OD_{590} at time zero was subtracted from the latter reading, yielding the net OD_{590}, and the data were further adjusted by subtracting the OD_{590} value from the blank (A1 well). The average well color development (AWCD) was calculated for each microplate as AWCD = •(C-R)/N, where C is the raw absorbance of in each well, R is the absorbance in the control well (A1 well), and N is the number of substrates in the plate. The functional diversity index of the rhizospheric microbial flora was calculated according to a described method[21,22].

A principal component analysis (PCA) was used to normalize the Biolog absorbance values. The normalization of the Biolog values was performed by dividing the absorbance values for individual wells by the AWCD for the whole plate to account for differences in the inoculum density, as suggested by Garland and Mills[23]. All of the statistical analyses were all conducted using SPSS 15.0 software.

RESULTS

Effects of Cd on rhizosphere microbial activities

Cd significantly stimulated the SMBC, SBR, MQ, and MMQ at the third week of winter wheat growth by 6.31 - 48.60%, 9.35 - 113.67%, 8.29 - 76.24%, and 2.79 - 46.48%, respectively (TABLE 1). However, the MBC under the stress of Cd decreased significantly by 0.48 - 36.91% at the seventh week and by 0.46 - 18.89% at the twelfth week compared to the control. The TOC under different Cd treatments decreased by 0.69 - 10.08% at the twelfth week and also decreased at the third and seventh weeks, except for T1 and T2. With the addition of Cd, the SBR significantly ($p<0.01$) decreased by 20.00 - 30.56% at the twelfth week and by 0.78-11.72% at the seventh week, except for T1 and T2. The MQ significantly ($p<0.01$) decreased by 4.29 - 51.11% with the addition of Cd at the seventh week and increased by 0.67 - 1.72% at the twelfth week, except for T4 and T5. Simultaneously, the MMQ

with added Cd increased significantly by 8.49 - 44.74% at the seventh week but decreased significantly by 1.72 - 26.52% at the twelfth week. However, the responses of all of the parameters determined with the addition Cd did not exhibit obvious time and dose dependences. In addition, a significant ($p<0.05$) and negative correlation between the TOC and Cd was observed at the third week. There were also significant ($p<0.05$) and negative correlations between the MQ and Cd at the seventh and twelfth weeks, and the significant ($p<0.01$) and a negative correlation between the MBC and Cd concentration was also observed at the third week (TABLE 2). Furthermore, the correlation between the MMQ, SMBC, and MQ and Cd intensified with time. In contrast, the correlation between the TOC and Cd decreased with time (TABLE 2).

TABLE 1 : Soil microbial biomass carbon (SMBC), total organic carbon (TOC), soil basic respiration (SBR), microbial metabolic quotient (MMQ), and microbial quotient (MQ) with the addition of Cd

Growth stage	Iterms	Treatments					
		The control	T1	T2	T3	T4	T5
Three weeks	SMBC mg/kg	49.66(4.95)^{Aa}	65.69(2.65)^{Bb}	87.96(3.46)^{C}	73.79(3.21)^{Bc}	56.84(5.29)^{Ad}	52.79(3.24)^{A}
	TOC mg/g	9.66(0.02)^{A}	9.98(0.03)^{B}	9.70(0.04)^{A}	9.71(0.01)^{A}	9.51(0.07)^{C}	9.48(0.07)^{C}
	SBR mg/g	0.14(0.01)^{Aa}	0.27(0.06)^{B}	0.30(0.07)^{Bb}	0.22(0.01)^{c}	0.17(0.01)^{Aa}	0.15(0.01)^{A}
	MQ 10^{-3}	5.14(0.29)^{Aa}	6.72(0.08)^{Bb}	9.07(0.20)^{C}	7.60(0.19)^{Bc}	5.98(0.29)^{ABd}	5.57(0.17)^{A}
	MMQ	2.80(0.03)^{A}	4.17(0.48)^{B}	3.38(0.36)^{B}	2.95(0.04)^{A}	2.91(0.06)^{A}	2.88(0.03)^{A}
Seven weeks	SMBC mg/kg	23.54(2.29)^{A}	17.54(0.12)^{Ba}	23.43(1.57)^{A}	21.71(1.44)^{Ab}	17.11(1.41)^{B}	14.85(2.43)^{B}
	TOC mg/g	9.06(0.10)^{A}	9.37(0.02)^{B}	9.29(0.03)^{B}	8.99(0.03)^{A}	8.99(0.05)^{A}	9.03(0.05)^{B}
	SBR mg/g	0.13(0.03)^{A}	0.14(0.01)^{a}	0.18(0.01)^{Bb}	0.13(0.01)^{A}	0.11(0.03)^{A}	0.11(0.01)^{A}
	MQ 10^{-3}	2.60(0.13)^{A}	1.87(0.01)^{B}	2.49(0.09)^{A}	2.41(0.07)^{A}	1.90(0.04)^{B}	1.65(0.01)^{C}
	MMQ	5.44(0.36)^{Aa}	7.81(0.03)^{Bb}	6.94(0.15^{B}	5.85(0.05)^{A}	6.61(0.63)^{c}	7.68(0.43)^{Bb}
Twelve weeks	SMBC mg/kg	23.77(1.91)^{A}	23.62(0.81)^{Aa}	22.51(1.31)^{a}	22.38(0.51)^{a}	20.05(2.10)^{B}	19.28(1.19)^{Bb}
	TOC mg/g	10.18(0.07)^{A}	9.96(0.02)^{B}	9.58(0.01)^{C}	9.15(0.04)^{D}	9.54(0.08)^{C}	10.11(0.09)^{AB}
	SBR mg/g	0.18(0.00)^{A}	0.15(0.00)^{B}	0.15(0.01)^{B}	0.13(0.03)^{C}	0.14(0.03)^{B}	0.15(0.01)^{B}
	MQ 10^{-3}	2.34(0.09)^{A}	2.38(0.04)^{Aa}	2.35(0.08)^{Aa}	2.45(0.03)^{A}	2.10(0.11)^{Ab}	1.93(0.05)^{B}
	MMQ	7.57(0.30)^{A}	6.29(0.05)^{Ba}	6.57(0.11)^{Ba}	5.59(0.04)^{Bc}	7.18(0.35)^{Ab}	7.73(0.03)^{A}

***The T1, T2, T3, T4, and T5 treatments were 5, 10, 20, 50, and 70 mg/kg dry weight soil, respectively, the same below; The data are presented as the mean values (n = 9), with the standard errors in parentheses; The different capital and lowercase letters in the same row at the same growth stage indicate significant differences at $p<0.01$ and $p<0.05$(LSD test), respectively.**

Biolog analysis on rhizosphere microbial functional diversities

Temporal changes in the average well color development (AWCD)

The rhizosphere microbial community at the different doses of Cd showed different AWCD values, and the AWCD curves declined significantly at the twelfth week (Figure 1). However, as based on the AWCD values, there was evident stimulation by 10 mg/kg dry weight soil Cd on the microbial activity at the third week and 10 and 70 mg/kg dry weight soil Cd at the seventh week (Figure 1). The AWCD values under all of the treatments increased rapidly in the first 120 h and reached a maximum after 192 h in three growth stages. Further analysis on the average color differences among the 31 single-carbon sources showed that the differences of the AWCD values among the different treatments at all of the incubation time intervals was significant; the effects of Cd on the AWCD values were evident.

TABLE 2 : Pearson correlation coefficient between the Cd concentration and microbial activities

Iterms	Growth stages		
	Three weeks	Seven weeks	Twelve weeks
MBC	-0.38	-0.77	-0.99**
TOC	-0.90*	-0.78	-0.07
SBR	-0.53	-0.60	-0.31
MQ	-0.34	-0.81*	-0.91*
MMQ	0.02	0.40	0.45

***p<0.05, **p<0.01**

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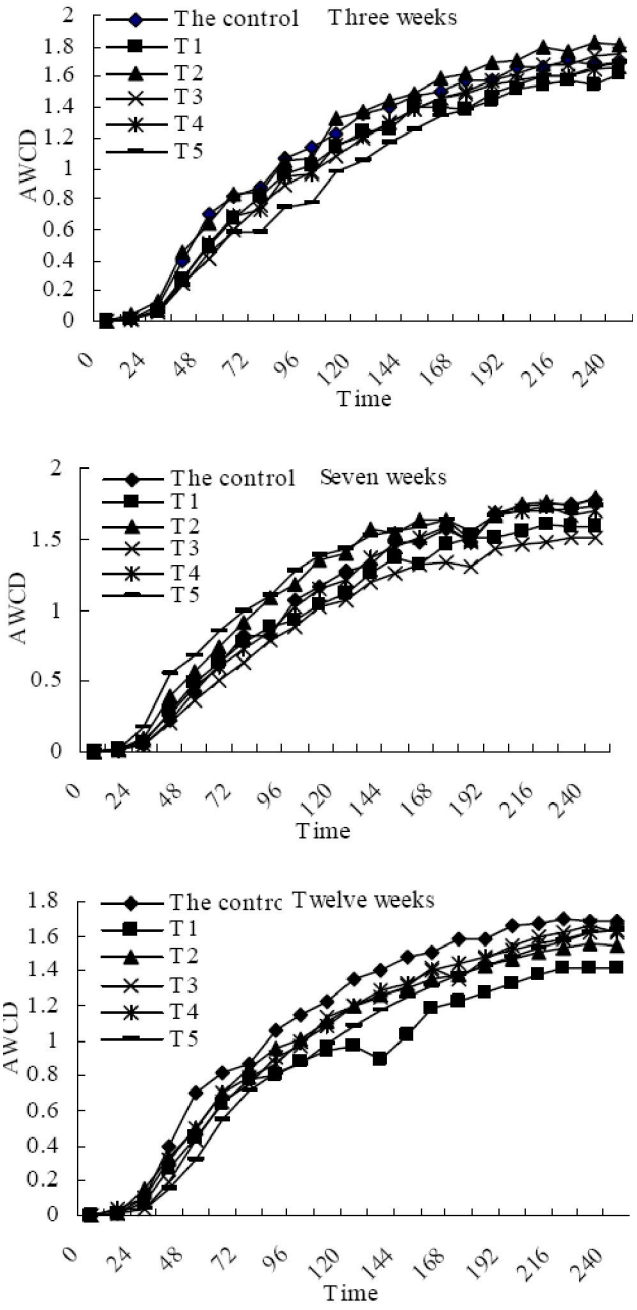

Figure 1 : AWCD values of the rhizosphere microorganisms under cadmium

The utilization rate of six carbon source categories by the rhizosphere microorganisms

TABLE 3 shows that the utilization rate of six carbon sources by the rhizosphere microorganism under the different treatments. The rhizosphere microbial communities under the different treatments varied tremendously in their utilization of carbohydrate, carboxylic acid, amino acid, polymer, phenolic acid, and propylamine substances in the three growth stages. The metabolism of carboxylic acids was the fastest for all of the treatments in the three growth stages. Moreover, the difference between the metabolism of carbohydrates and carboxylic acids under the different treatments was significant ($p<0.05$). The utilization rates of carbohydrates, carboxylic acids, and propylamine by the rhizosphere microbes in the control were higher than the others at the third week. The sequence of the metabolism of amino acids, polymers, and phenolic acids was different between the Cd treatments and the control, and the sequence of the utilization of the six carbon sources exhibited marked changes among the different treatments. Furthermore, the sequence of the utilization of the same carbon source among the treatments was also significantly different. In contrast, the utilization of phenolic acids increased with added Cd in the three growth stages. The addition of Cd also resulted in a significant decrease in carbohydrate, carboxylic acid and propylamine utilization at the third and twelfth weeks and a decrease of the utilization of amino acids at the twelfth week.

Analysis of the major components

The first two principal factors accounted for 88.94, 88.19, 86.60, 91.74, 88.45, and 86.06%, and the first principal component axis (PC1) explained 81.46, and the first principal component axis (PC1) explained 81.46, 81.40, 79.21, 81.768, 81.90, and 79.50% for the control, T1, T2, T3, T4, and T5, respectively. A component plot in rotated space (Figure 2) showed that the microbial functional diversity under the stress of Cd decreased markedly, except for T3. The number of substrates utilized under the T3 treatment was the same as in the control. The load values exceeding 0.90 under the T3 treatment were D-mannitol, D-galactonic acid-γ-lactone, D, L-α- glycerophosphate, D-glucosaminic acid, L-phenylalanine, and α-cyclodextrin. However, the rhizosphere microbes in the control were more likely to use D-cellobiose, D-mannitol, and Tween 80 for which the load value was greater than 0.900. In addition, the type of substrates utilized preferentially in Cd was evidently different from that in the control. The results indicated that, between the control and Cd treatments, some significant changes occurred in the responses of the rhizosphere microbial communities to the carbon sources provided.

TABLE 3 : The utilization ratio of six carbon sources by rhizosphere microorganisms (absorbance)

Growth stage	Treatm-ents	Carbohydrate	Carboxylic acids	Amino acid	Polymer	Phenolic acid	Propylamine
Three weeks	Control	1.29(0.07)^{A}	1.45(0.05)^{A}	1.00(0.08)^{Aa}	0.93(0.04)^{A}	0.80(0.03)^{A}	1.21(0.03)^{A}
	T1	1.17(0.02)^{Ba}	1.24(0.02)^{B}	0.94(0.02)^{Ab}	1.04(0.02)^{B}	0.89(0.04)^{B}	1.05(0.02)^{Ba}
	T2	1.43(0.01)^{C}	1.41(0.03)^{AC}	1.10(0.01)^{Bc}	1.13(0.03)^{C}	1.07(0.02)^{C}	1.10(0.02)^{BCb}
	T3	1.24(0.02)^{ABb}	1.31(0.03)^{Ca}	0.83(0.02)^{C}	0.92(0.04)^{A}	0.90(0.01)^{B}	1.03(0.01)^{BD}
	T4	1.15(0.01)^{B}	1.37(0.03)^{Cb}	1.01(0.01)^{ABa}	0.91(0.02)^{A}	0.92(0.02)^{B}	1.11(0.02)^{c}
	T5	1.17(0.02)^{Bc}	1.28(0.01)^{BC}	0.83(0.01)^{C}	0.80(0.02)^{D}	0.88(0.04)^{B}	1.07(0.01)^{Ba}
Seven weeks	Control	1.28(0.04)^{Aa}	1.34(0.03)^{Aa}	0.84(0.09)^{A}	0.7(0.07)^{Aa}	0.84(0.04)^{A}	1.30(0.16)^{Aa}
	T1	1.18(0.07)^{ABb}	1.33(0.02)^{ABa}	0.80(0.01)^{ABa}	0.91(0.01)^{Bb}	0.94(0.01)^{B}	1.04(0.02)^{B}
	T2	1.38(0.08)^{AC}	1.50(0.07)^{C}	0.98(0.01)^{C}	0.92(0.02)^{BC}	1.05(0.01)^{C}	1.54(0.03)^{C}
	T3	1.15 ± 0.01^{B}	1.23(0.03)^{Bb}	0.70(0.02)^{Bb}	0.87(0.02)^{b}	0.92(0.03)^{B}	0.81(0.02)^{D}
	T4	1.29(0.03)^{Aa}	1.45(0.05)^{ACc}	0.86(0.01)^{A}	0.83(0.02)^{ABc}	1.01(0.01)^{C}	1.14(0.01)^{B}
	T5	1.40(0.03)^{ACc}	1.52(0.05)^{C}	1.01(0.01)^{C}	1.10(0.02)^{D}	1.14(0.02)^{D}	1.45(0.03)^{ACb}
Twelve weeks	Control	1.16(0.02)^{Aa}	1.46(0.01)^{A}	0.94(0.01)^{A}	1.04(0.02)^{A}	0.87(0.02)^{A}	1.20(0.03)^{A}
	T1	1.04(0.02)^{B}	1.04(0.01)^{B}	0.79(0.01)^{B}	0.91(0.02)^{B}	0.89(0.01)^{A}	1.04(0.01)^{B}
	T2	1.18(0.01)^{AC}	1.11(0.02)^{C}	0.88(0.03)^{C}	1.00(0.02)^{Aa}	1.28(0.03)^{B}	1.16(0.01)^{A}
	T3	1.14(0.02)^{AD}	1.34(0.03)^{D}	0.81(0.02)^{B}	1.07(0.02)^{A}	1.19(0.02)^{C}	1.13(0.03)^{CAa}
	T4	1.13(0.01)^{ADb}	1.32(0.01)^{D}	0.78(0.01)^{B}	0.93(0.01)^{B}	1.02(0.02)^{D}	1.24(0.04)^{Bb}
	T5	1.21(0.02)^{C}	1.25(0.02)^{E}	0.57(0.01)^{D}	0.96(0.02)^{Cb}	0.85(0.01)^{A}	1.18(0.02)^{c}

The different capital and lowercase letters in the same column at the same growth stage indicate significant differences $p<0.05$ and $p<0.01$(LSD test), respectively.; The data are presented as the mean values (n = 9), with the standard errors in parentheses.

DISCUSSION

Effects of cadmium on rhizosphere microbial activities

The SMBC can respond more rapidly to changes in the ecological environment of the soil, e.g., heavy metal contamination, and to continuous changes in plant root exudates[7,24,25]. However, the colonization and establishment of microorganisms in rhizosphere soil is affected by many factors, including the quantity and quality of root exudates secreted by a particular species, soil physicochemical properties, and climate conditions. This suggests that the rhizosphere microenvironment of a plant will be continuously changing as a result of these effects, giving an opportunity for the development of a specialized rhizoflora[7]. Furthermore, several studies have also demonstrated that root exudation is a major factor controlling the rhizosphere microbial activity and community structure[26-29]. Therefore, changes in the content and the species of carbohydrates, organic acids, amino acids, and vitamins secreted by winter wheat roots under Cd treatment would have a direct effect on the rhizosphere microbial populations[30,31], which would further influence the microbial biomass due to the positive correlation between microbial biomass and microbial populations[32]. The increase in the SMBC with added Cd at the third week (TABLE 1) could be explained by the fact that the release of exudates might be more favorable for a microbial population. However, the decrease of the SMBC under Cd contamination at the seventh and twelfth weeks (TABLE 1) suggested that the exudates might act on the rhizosphere microbes as allelochemicals, e.g., phenolic acids, with an extension of the growth stage of the seedlings. Additionally, the direct effect of Cd was not negligible. Furthermore, the decrease in the SMBC with added Cd was also likely because the microorganisms needed more energy to survive for long time under unfavorable conditions and a reduction in the conversion of substrates into new microbial biomass and other metabolic processes[33]. Therefore, a higher percentage of energy is lost and less C is incorporated into organic components[34]. This assumption is also supported by the higher metabolic quotient with added Cd at the seventh week (TABLE 1). Moreover, the

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fact that the SMBC was more sensitive to Cd than the TOC was supported by the evident correlation between the SMBC and the concentration of Cd (TABLE 2). However, an obvious time and dose dependence was not found in the response of the SMBC to Cd.

The significant ($p<0.05$) and negative correlation between the MQ and the concentration of Cd at the seventh and twelfth weeks (TABLE 2) showed that the MQ was a more sensitive ecophysiological parameter than the others, indicating an environmental pressure that suppressed the rhizosphere microbial biomass, as was indicated by Anderson and Domsch[35]. Moreover,

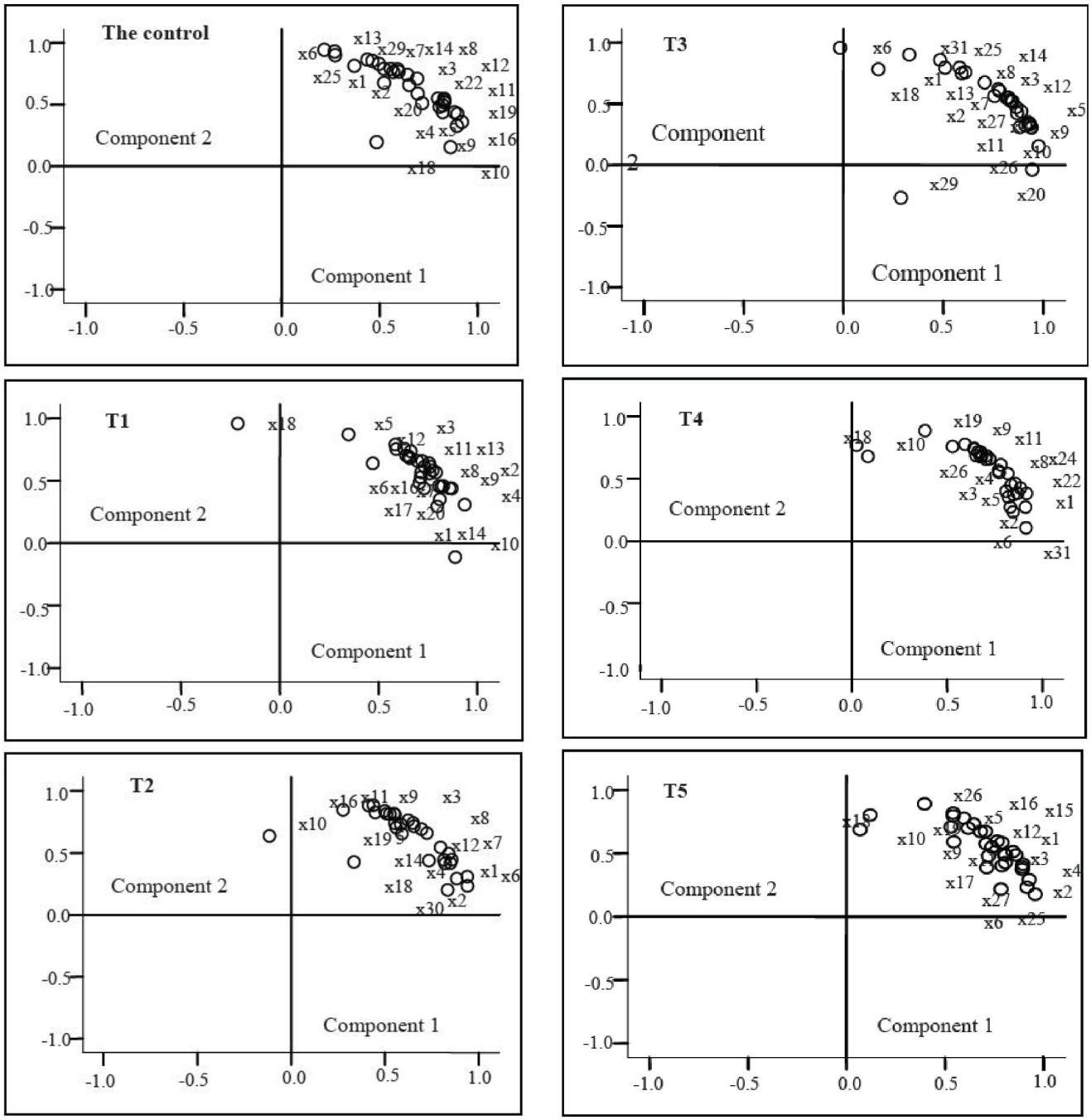

Figure 2 : Component plot in rotated space. X_1-X_{31} is D-cellobiose, α-D-lactose, β-methyl-D-glucoside, D-xylose, I-erythritol, D-mannitol, N-acetyl-D-glucosamine, glucose-1-phosphate, D-galactonic acid-γ-lactone, D, L-α- glycerophosphat, D-glucosaminic acid, pyruvic acid methyl ester, D-galacturonic acid, γ-hydroxybutyric acid, itaconic, α-ketobutyric acid, D-malic acid, L-arginine, L-asparagine, L-phenylalanine, L-serine, L-threonine, glycyl-L-glutamic acid, tween 40, tween 80, α-cyclodextrin, glycogen, 2-hydroxy benzoic acid, 4-hydroxy benzoic acid, phenylethylamine, and putrecine, respectively.

the SBR has been largely used to estimate heavy metal toxicity[25]. The high SBR under the stress of Cd at the third week (TABLE 1) was possible because the microorganisms needed more energy to survive under the unfavorable conditions; however, the microorganisms in the control used a higher percentage of consumed carbon for assimilation and, thus, a smaller percentage was released as CO_2 in dissimilation processes[7]. The decrease of the bioavailability of Cd, which was due to the increase of Cd complexation by accumulating exudates, with the extension of the growth stage was a probable reason for the decrease of the SBR in the contaminated soils at the twelfth week (TABLE 1)[36]. Moreover, in general, lower TOC and SMBC values in polluted soils might well explain the lower SBR value under the stress of Cd at the twelfth week (TABLE 1); the negative correlation between the SBR and the concentration of Cd also confirmed this point.

The MMQ (microbial metabolic quotient) has been used as an ecophysiological index for soil microbes and reflects the bioenergetic status of the microbial biomass[8]. A high MMQ indicates that soil microbial activity is low in efficiency and that the soil microorganisms are living under environmental stress[37]. The change in the MMQ showed that the stress of Cd on the rhizosphere microorganisms was larger at the third and seventh weeks than at the twelfth week (TABLE 1), which was likely due to the direct stress of Cd and the indirect effect on the microorganisms by the root exudates. However, the direct effect would become weak because the Cd absorption and accumulation ability of the roots would intensify with the extension of the growth stage[36], which can also explain the significant decrease of the MMQ at the twelfth week (TABLE 1).

The functional diversity of the rhizosphere microbes

The BIOLOG data reflect the diversity of carbon-oxidation pathways (functional diversity) of the soil microbial community; moreover, the AWCD values can be used as indicators of the microbial activity and reflect the sole-carbon-source utilization (SCSU) ability of the soil microbial community[38,39]. The decrease of the AWCD values (Figure 1) suggested that the total microbial activity decreased, and the stress of Cd on the rhizosphere microbe communities was evident. However, the increase of the AWCD values in T2 at the third and seventh weeks (Figure 1) indicated that 10 mg/kg dry weight soil Cd stimulated the metabolic function of the microbial community by altering the soil microenvironment due to the shifts of the species and the quantity of the root exudates; these events can also be described by the changes in microbial parameters, such as respiratory capacities and microbial biomass. This stimulation weakened with time, such that the AWCD value in T2 was reduced at the twelfth week. However, this result was not consistent with the findings of Akmal et al. who studied the response of unplanted soil microbial functional activity to Cd (2005).

Amino acids are well-known wheat root exudates[40,41], and the addition of Cd resulted in a decrease in amino acid utilization at the twelfth week (TABLE 3). The paradox that amino acid utilization in the rhizosphere decreased with the addition of Cd when the root exudation of amino acids most likely increased may be explained by the nature of nitrogen cycling in the soil[42]. Under normal circumstances, microbes will take up organic N sources and retain them, but the mineralization of simple N compounds, such as NH_4^+ and NO_3^-, is stimulated when organic N concentrations increase[42,43]. In the rhizosphere, this could result in a reduced reliance on amino acids as N sources, as heterotrophs are more likely to utilize simpler N compounds. Furthermore, the increase in the utilization of amino acids in T2, T4, and T5 was mainly due to the high utilization rate of L-phenylalanine, L-serine, L-threonine, and glycyl-L-glutamic acids at the third and seventh weeks. Interestingly, these amino acids have been associated with wheat root exudates, and the quantity of exudates increase under the stress of Cd[30]. In our study, the T2, T4, and T5 treatments most likely stimulated the increase of amino acid exudation at the third week. However, the increase of the quantity of other exudates, e.g., phenolic acids and carboxylic acids, which inhibit microbial activities as allelochemicals most likely reduced the utilization rate of amino acids with time under Cd treatment[30], showing a decrease in the utilization of amino acids at the twelfth week. The increase in the utilization of phenolic acids (TABLE 3) was most likely related with the stimulation by Cd on the secretion of phenolic acids, which has been demonstrated previously[30]. The utilization of carbohydrates, carboxylic

acids, polymers, and propylamines further indicated the evident effects of Cd on the rhizosphere microbial functional diversity. In addition, the principal component analysis showed that the ability of the rhizosphere microbes to utilize different carbon resources was affected by Cd, and the characteristic C source utilization patterns were different between the Cd treatment and the control. The composition of microbial communities determines the biological quality of the soil, and the resilience of soil microbial communities to contamination events is related to a high degree of functional redundancies of the microbes[31,44]. It could be deduced that the winter wheat rhizosphere soil health was poor with added Cd due to the decline of the microbial functional diversity. Therefore, it is impossible that the input of Cd in the soil mainly induced physiological adaptations rather than microbial selection in the rhizosphere of winter wheat seedlings.

CONCLUSIONS

The SMBC and SBR under the Cd treatment increased at the third week and decreased at the seventh and twelfth weeks. The effect of Cd on the TOC was related to the growth period of the winter wheat. The MMQ increased significantly at the third and seventh weeks and decreased significantly at the twelfth week. Moreover, the SMBC, MMQ, and MQ represent possible sensitive and precise indicators of winter wheat rhizosphere soil health under the stress of Cd. The response of all of the rhizosphere microbial parameters determined with added Cd did not indicate obvious time and dose dependences. Moreover, the rhizosphere microbial functional diversity decreased under Cd contamination. The addition of Cd mainly resulted in the increase of phenolic acid utilization and the decrease in carbohydrate, carboxylic acid, and amino acid utilization. In conclusion, there were some evident differences in the responses of the microbial activity and functional diversity to Cd in the rhizosphere of winter wheat seedlings.

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