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ENVIRONMENTAL BIOTECHNOLOGY

Response of rhizosphere microbial community structure and diversity to heavy metal co-pollution in arable soil

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Abstract Due to the emerging environmental issues related to heavy metals, concern about the soil quality of farming lands near manufacturing district is increasing. Investigating the function of soil microorganisms exposed to long-term heavy metal contamination is meaningful and important for agricultural soil utilization. This article studied the potential influence of several heavy metals on microbial biomass, activity, abundance, and community composition in arable soil near industrial estate in Zhuzhou, Hunan province, China. The results showed that soil organic contents (SOC) were significantly positive correlated with heavy metals, whereas dehydrogenase activity (DHA) was greatly depressed by the heavy metal stress. Negative correlation was found between heavy metals and basal soil respiration (BSR), and no correlation was found between heavy metals and microbial biomass content (MBC). The quantitative PCR (QPCR) and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis could suggest that heavy metal pollution has significantly decreased abundance of bacteria and fungi and also changed their community structure. The results could contribute to evaluate heavy metal pollution level in soil. By combining different environmental parameters, it would promote the better understanding of heavy metal effect on the size, structure, and activity of microbial community in arable soil.

Keywords Soil pollution · Heavy metals · Rhizosphere · PCR-DGGE · Microbial community

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Introduction

Heavy metal pollutions can retain a long time in soil, even migrate to crops, and may enter into food chain in the end so that it can endanger people's health. Rice as the main planted crop played a huge impact on the migration of heavy metal to the food chain. Heavy metal pollution has been widely reported in the arable fields of South China in recent years due to the rapid development of mining activities, metallurgical industry, sewage irrigation, and the application of pesticides and fertilizers (Chen et al. 2014; Gong et al. 2009; Zeng et al. 2013a). Heavy metal pollution in soil has been paid extensively attention worldwide, especially the rapid industrialization and urbanization countries. It was noticed that human induced metals like Pb, Hg, and Cu had been detected in both Greenland and Antarctica snow samples where were remote from human beings (Lobinski et al. 1994; Van de Velde et al. 2005). The most common heavy metals mainly include mercury, cadmium, lead, chromium, arsenic, zinc, copper, nickel, cobalt, etc., which can cause toxicities and serious side effects toward human health (Tang et al. 2014; Xu et al. 2012). Soil as the basis of human production and living was not only basic components of our environment as they provided nutrients for living organisms but also served as reservoirs for poisonous chemical substances which could cause a negative effect on

microbial system and human health (Akcay et al. 2003; Khan et al. 2010; Wang et al. 2007).

In topsoil ecosystems, bacteria and fungi generally constituted more than 90 % of the total soil microbial biomass and were the key regulators of soil organic matter dynamics and nutrient availability (Chen et al. 2014). Soil microorganisms played an important role in energy flow, nutrient cycling, and organic matter turnover in terrestrial ecosystems (Eguchi et al. 2001; Frische and Hoper 2003; Sabater et al. 2003; Tonkovic 1998). Soil offered survival environment for bacteria and fungi, which in turn were more sensitive to harmful substance, then they can produce different reaction mechanism leading to different biological structure.

Zhuzhou is the second largest city in Hunan Province, and it is an industrial city with four key industries (metallurgy, machine manufacture, chemicals, and building materials). Due to industrial structure and historical reasons, environmental pollution of Qingshuitang District was very severe, making it one of the most serious areas of national environmental issues (Jiang et al. 2013). Industries' wastewater was discharged into Xiawangang River, which was used for agricultural irrigation of surrounding farmlands; thus, it would lead to pollutant entering into soil and crops. For examples, 36 % of rice grown in Hunan province was found to have cadmium levels above those specified by China's food standards regulation (Zeng et al. 2013b). Rice and rapeseed are the main production crops of Zhuzhou city in Hunan province due to geography and climate conditions, so it is very necessary to study the relationship between microbial and heavy metal in rape field (RF) and paddy field (PF). Many previous studies have carried out researches about the relation of heavy metals with microbial community structure and diversity (Gremion et al. 2004; Solís-Domínguez et al. 2011), but few studies have researched relation of heavy metal with rhizosphere microbial community in arable soil.

In this article, the objective was to examine the effect of heavy metal pollution on the shift in microbial community structure in RF and PF using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) to determine the community dynamics in response to environmental variations. It revealed the relationship of rhizosphere microbial community and their environmental parameters including the joint action of a variety of heavy metals (Cd, Pb, Zn, Cu, and Cr). This study would arouse people's attention to heavy metal pollution and further promote the understanding of the heavy metal toxicity to soil microorganism near manufacturing districts in China.

Materials and methods

Sampling locations

Agricultural land activities, such as cultivation, fertilization, and irrigation, were the important impetus of microbial community dynamics and physiochemical phenomenon (Gupta and Germida 1988; Ronnenberg and Wesche 2011). On the basis of the influence of those practices, two completely different types of farming land (RF and PF) were selected in field condition. These sites were located in area of Zhuzhou, Hunan province, China. The samples were collected on 26 April 2014, and sampling locations were shown in Fig. 1. In order to get the comprehensive analysis of the effect of heavy metals on rhizosphere microorganism, seven sites were selected in RF and PF. RF1, RF2, PF1, and PF2 were in the upstream of the Xiang River in Dajing Scenic Area. RF3 was near a cement plant. RF5 and PF5 were the control in downstream of the Xiang River. RF6 was close to Smelting of Zhuzhou. RF7 and PF7 were located in Huaxin Concrete Plant. All samples were collected around the rhizosphere of rape and paddy in triplicate (approximately apart 15 cm of two duplicates in a line) in every sampling location, and they were free of roots and homogeneous with depth (15 cm of top soil), then mixed thoroughly to obtain a uniform sample for each site to reduce the error. The collected samples were kept in polyethylene sealed plastic bags and carried back to the lab in iced box. After getting rid of plant residues and gravels, all of the samples were in duplicate, one was air-dried at ambient temperature for chemical analysis, and the other was stored at −20 °C to extract soil DNA.

Chemical analyses

The pH, soil moisture content (SMC) and organic content (SOC) of samples were measured according to Jiang et al. (2013). The total concentrations of Cd, Pb, Zn, Cu, and Cr in samples were measured after wet digestion in concentrated HNO_3-HCl-$HClO_4$-HF with a gradual temperature increasing from 80 to 130 °C using flame atomic absorption spectrometry

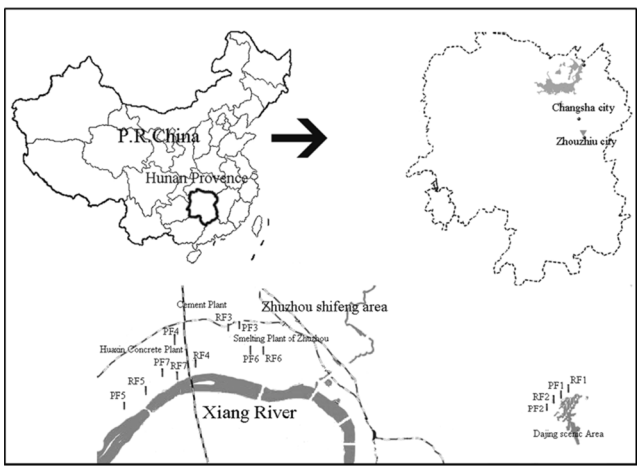

Fig. 1 Sketch of study area and geographical location of sample (RF and PF)

(AAnalyst700, Perkin-Elmer Inc, USA). The selected chemical properties were shown in Table 1.

Microbial biomass, microbial quotient, enzyme activity, and basal soil respiration

The microbial biomass contents (MBC) were measured using a fumigation-extraction method (Vance et al. 1987). Carbon in the extracts was determined by an automated TOC Analyzer (Shimazu, TOC-5000, Japan) and used an extraction efficiency coefficient of 0.45 to convert the measured C to MBC. The microbial quotient was the proportion of MBC to SOC. Dehydrogenase activity (DHA) was measured following the protocol described by Serra-Wittling et al. (1995). The absorbance was assayed at 485 nm using a spectrophotometer (Shimazu, UV-2550, Japan). Basal soil respiration (BSR) was determined according to Anderson and Domsch (1973). The released CO_2 was analyzed by gas chromatography (Agilent 6890D). The metabolic quotient (the respiration per unit biomass) represented one of indicators about C utilization efficiency in the soil. The selected biological properties were shown in Table 1.

QPCR

Genomic DNA of soil samples was extracted using E.Z.N.A.™ Soil DNA Kit (Omega Biotek, USA) according to the instruction. The crude DNA was purified using a kit (Bioteke, Beijing, China). Ultimately, DNA was dissolved in the 100 μL TE buffer and stored in −20 °C before used.

Quantitative PCR (QPCR) was operated in a Cycler iQ5 thermocycler (Bio-Rad, USA). The primer pairs of 338 F/518R (Ovreås et al. 1997) and Fung/NS1 (Mitchell and Zuccaro 2006) were used to quantify bacterial 16S rRNA and fungal 18S rRNA coding genes. The protocol was as follows: 2 min at 94 °C; 35 cycles consisting of 10 s at 94 °C, 30 s at 55 °C for bacteria, or 45 s at 55 °C for fungi; a final extension of 7 min at 72 °C; and ending at 4 °C. A negative control without the corresponding template DNA was included in every QPCR assay for each primer. Melting curve analysis was manifested to prove the specificity of amplification. All experiments were done in triplicate. Serial 10-fold plasmid dilutions of plasmids DNA from 1.0×10^4 to 1.0×10^{11} copies were acted as templates for standard curve generation (Lu et al. 2014).

Microbial community fingerprinting by PCR-DGGE analysis

Universal bacterial 16S rRNA coding gene primers GC-338 F/518R (Ovreås et al. 1997) and fungal 18S rRNA coding gene primers GC-Fung/NS1 (Mitchell and Zuccaro 2006)

Table 1 The physical and biochemical properties in rape fields (RF) and paddy fields (PF)

Samples	SMC[a]	pH	SOC[b]	MBC[c]	Microbial quotient[a]	BSR[d]	Metabolic quotient[e]	DHA[f]
RF1	23.47	6.00	57.90	388.16	0.67	56.32	0.81	28.03
PF1	45.83	6.74	54.22	148.42	0.27	18.85	0.71	22.85
RF2	22.21	6.70	65.51	406.54	0.62	60.25	0.82	26.97
PF2	35.71	6.21	55.51	791.55	1.43	92.36	0.65	25.64
RF3	24.35	7.36	84.21	440.07	0.52	41.23	0.52	12.63
PF3	37.84	6.30	53.38	742.71	1.39	120.63	0.90	21.32
RF4	19.66	6.81	51.53	478.15	0.93	100.65	1.17	20.52
PF4	42.14	6.93	54.54	349.76	0.64	64.52	1.02	17.3
RF5	25.84	6.43	40.85	161.78	0.40	35.35	1.21	34.25
PF5	20.02	7.28	40.61	20.58	0.05	14.91	4.02	28.31
RF6	19.52	6.22	51.17	381.18	0.74	60.35	0.88	16.33
PF6	35.31	7.51	35.83	19.33	0.05	15.36	4.41	27.65
RF7	23.21	7.39	49.35	331.92	0.67	65.39	1.09	22.31
PF7	49.41	6.42	41.59	147.11	0.35	38.47	1.45	25.98

SMC soil moisture content, *SOC* soil organic carbon, *MBC* microbial biomass carbon, *BSR* basal soil respiration, *DHA* dehydrogenase activity

[a] %

[b] mg/g

[c] mg/kg

[d] mg CO_2-C kg^{-1} soil

[e] mg CO_2-C g^{-1} MBC h^{-1}

[f] μg TPF g^{-1} 24 h^{-1}

were used for the PCR amplification. The PCR was performed under protocol by Zhang et al. (2011). The PCR amplification was run on a MyCycler thermal cycle (Bio-Rad, Hercules, CA, USA). Then, PCR products (30 μL) were loaded onto the 1-mm-thick 8 % (*w*/*v*) polyacrylamide gels with a denaturing gradient of 35–70 % for bacteria and 20–50 % for fungi. Electrophoresis were operated in 1×TAE buffer at 60 °C, 80 V for 14 h. DGGE was carried out using a DCode™ Universal Detection System (Bio-Rad, USA). After staining with SYBR solution (TianGen, Beijing, China), for 30 min, gels were visualized with the Gel Doc XR System (Bio-Rad, USA).

Statistical analyses

The pollution level was characterized by the toxicity index (TI) as following equation (Stefanowicz et al. 2008):

$$\mathrm{TI} = \sum C_i / \mathrm{EC}_{50i} \quad (1)$$

where C_i was the concentration of heavy metal i in soil and EC_{50i} was the concentration of that heavy metal causing 50 % reduction in DHA (Welp 1999). All results were tested for normality and homogeneity of variance using SPSS 16.0 (SPSS Inc, Chicago, Illinois, USA).

Prior to further analysis, DGGE bands of microbial community were detected and digitized after average background subtraction for the entire lane (Quantity One 4.62, Bio-Rad, USA). Then, band intensity for each lane was normalized by SPSS 16.0 to eliminate the difference generated by the volume of PCR products added to the DGGE system. Shannon-Wiener diversity index (H) for rhizosphere bacterial and fungal DGGE community fingerprinting calculated as follows:

$$H = \sum \left(\frac{N_i}{N}\right) \ln\left(\frac{N_i}{N}\right) \quad (2)$$

where N_i was the height of a peak of each band i, i was the number of bands in each DGGE profile, and N was the sum of all peak heights in a given DGGE profile.

CANOCO software V4.5 (Biometris, Wageningen, The Netherlands) was used for determination of multivariate relationship between bacterial and fungal community composition and physicochemical parameters. Redundancy analysis (RDA) was performed to ordinate the spatial and temporal compositions of the bacterial and fungal community to the measured environmental parameters (Zhang et al. 2011).

Results

Distribution of heavy metals

The heavy metal contents and TI_{tot} were shown in Table 2. A relatively high content of heavy metals was found in RF3 and RF6, especially for Zn and Cd. The reasons that RF3 and RF6 had high levels of heavy metals might be that they were closed to a cement plant and smelting plant. Expressing the toxicity of several metals (Cr, Cd, Pb, Zn, and Cu) by TI_{tot} calculated on the basis of EC_{50} was satisfactory. We checked by comparing the results of TI_{tot} values and the sums of five heavy metal concentrations for each site. TI_{tot} was higher in RF3 and RF6

Table 2 Total concentration of several kinds of heavy metals in all the samples

Samples	Cr (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	TI_{tot}
RF1	83.22bc	3.98a	64.38a	574.06a	53.75b	7.84bc
PF1	85.28bc	4.34a	74.30ab	791.15a	65.99bcd	10.13de
RF2	133.07cd	5.68a	56.3abc	595.48a	79.73de	9.48cde
PF2	87.17bc	3.87a	53.20a	724.36a	81.84de	9.99cde
RF3	154.47d	62.43e	558.09d	4020.47c	191.75h	44.16j
PF3	105.14cd	5.05a	78.21ab	1069.70a	75.82cde	13.12f
RF4	109.33cd	15.60c	185.15bc	1498.70a	140.87g	19.05g
PF4	124.84cd	13.60bc	205.01c	2179.85ab	100.56f	24.05h
RF5	10.50a	3.33a	3.82a	560.35a	31.32a	5.96ab
PF5	12.90a	4.10a	6.19a	491.37a	29.62a	5.36a
RF6	113.43cd	46.10d	96.96abc	3455.02bc	34.22a	33.28i
PF6	22.51a	4.72a	99.12abc	758.78a	60.20bc	8.84cd
RF7	13.90a	4.37a	71.50ab	895.41a	90.12ef	10.72ef
PF7	25.30a	3.52a	21.94a	857.36a	70.38cde	9.90de

Values in each column followed with different letters indicated statistically different (ANOVA; Tukey's test, $P<0.05$)

TI_{tot} toxicity index

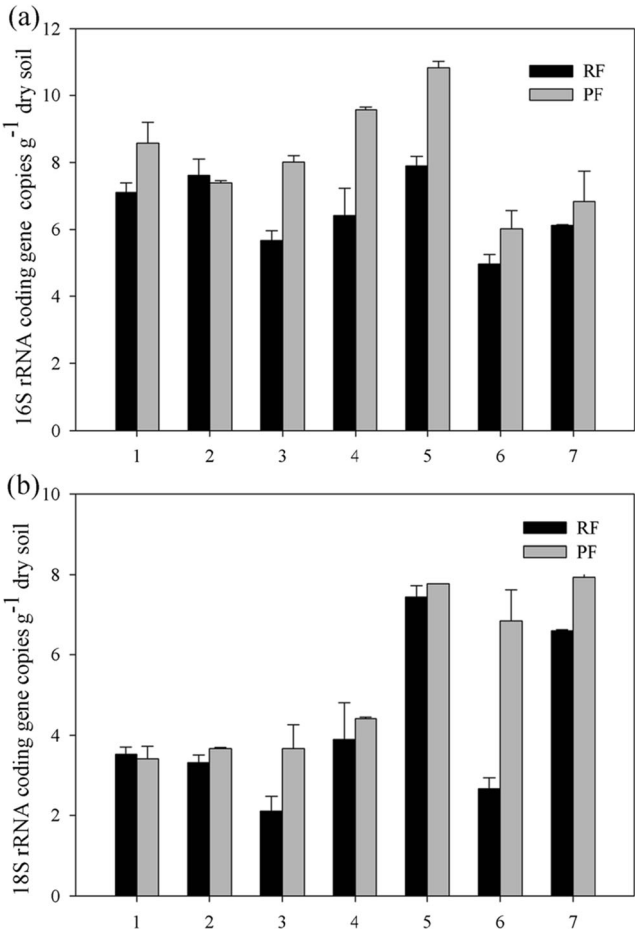

Fig. 2 Lg-transformed gene copies of total bacteria (**a**) and total fungi (**b**) according to QPCR analysis in rape fields (RF, *black bar*) and paddy field (PF, *gray bar*) (n=3; *error bars* are +SD). (copies g^{-1} dry soil)

than other studied samples, and the tendency was consistent with those of heavy metal content despite the fact that the EC_{50} reported by Welp (1999) was estimated for dehydrogenase activity in a single metal-spiked soil. Heavy metals in different places showed an obvious difference, especially for Pb (3.82 to 5580.9 mg/kg). A trend of relatively higher levels of heavy metals was found in rape field than those in paddy field. It was supposed that heavy metals might transfer from soil to paddy with the joint action of rhizosphere microorganisms and other environmental factors. Previous study surveyed the relationship of rapeseed residue into the metal contaminated rice paddy soils, indicating that the incorporation of them could decrease the heavy metal phytoavailability (Ok et al. 2011). The results supposed that heavy metal contents in rape were higher than those in paddy.

Bacterial 16S rRNA and fungal 18S rRNA coding genes

The rhizosphere bacterial and fungal gene abundance in RF and PF was shown in Fig. 2. The highest bacterial gene abundance was observed in PF5, while the lowest were in RF6. Abundance of bacterial 16S rRNA coding genes showed significant difference in various sites. Obvious differences of bacteria population size were observed among rice paddies, while few differences were detected in rape fields (Fig. 2a). For fungi, the biggest difference appeared between RF6 and PF6. The samples with higher heavy metal contents revealed lower lever of fungal abundance, which was consisted with that of bacteria (Fig. 2b).

Pearson's correlations

The Pearson correlation analysis of heavy metals, physical and biochemical properties were shown in Table 3. Negative correlation was found between heavy metals and SMC. No correlation was found between heavy metals and pH; SOC were significantly positive correlated with heavy metals, especially for Cr and Pb, which indicated that heavy metal pollution had a marked impact on chemical properties of arable soils. DHA and metabolic quotient were significant negative correlated with heavy metals, whereas no correlation was found between heavy metals and MBC or microbial quotient or bacteria diversity. Fungal abundance was significant negative correlation with Cr, but had unobvious negative correlation with other types of heavy metals, while abundance of bacteria was positive correlated with heavy metals. Diversity

Table 3 Correlation coefficients of heavy metals and physical and biochemical properties

	SMC	pH	SOC	MBC	Microbial quotient	BSR	Metabolic quotient	DHA	Bacteria abundance	Fungal abundance	*H* of bacteria	*H* of fungi
Cr	−0.272	−0.235	0.719[b]	0.501	0.385	0.353	−0.576[a]	−0.441	0.27	−0.604[a]	0.247	−0.07
Cd	−0.347	0.15	0.619[a]	0.15	0.016	0.02	−0.273	−0.745[b]	0.006	−0.23	0.119	−0.105
Pb	−0.145	0.338	0.735[b]	0.181	0.008	0.002	−0.237	−0.674[b]	0.188	−0.333	−0.114	−0.236
Zn	−0.223	0.14	0.558[a]	0.177	0.07	0.056	−0.299	−0.833[b]	0.218	−0.242	0.16	0.016
Cu	−0.213	0.475	0.591[a]	0.185	0.054	0.115	−0.179	−0.45	0.079	−0.13	−0.18	−0.034
EI	−0.188	0.154	0.655[a]	0.266	0.141	0.135	−0.362	−0.890[b]	0.227	−0.321	0.096	−0.023

[a] Correlation is significant at the 0.05 level

[b] Correlation is significant at the 0.01 level

of fungi was negative correlation with heavy metals, except Zn.

Bacterial and fungal community analysis

Analyzing of the DGGE profile about 16S rRNA and 18S rRNA suggested that rhizosphere bacterial and fungal community changed under heavy metal pollution compared with the controls (Figs. 3 and 4). Generally, there were significant shifts in composition of total rhizosphere bacterial community at different sites. Some of flush obvious stripes exhibited similar intensities across all of the samples, regardless of pollution, indicating that all of the fractions presented a similar predominant bacterial community. Samples of control soils (RF5 and PF5) showed very stable profiles, indicating that few changes occurred in the molecular structure of soil bacterial community due to low toxicity index and the co-abundance of different organisms in soil. For fungi, the number and definition of bands were significantly decreasing contrasting to bacteria (Fig. 4). The large difference of each sample was recorded, and the shifts in community composition occurred in different patterns. It was not only because the species of fungi were relative less, but also fungi were more sensitive corresponding to co-pollution of heavy metals (Chen et al. 2014; Xu et al. 2015). This result suggested that the difference between the bands at the different sampling sites (some bands become obvious or faint, sometimes difficult to distinguish from the background and new bands were formed) might have been due to combined effect of different heavy metal co-pollution and environmental parameters, such as pH can affect the mobility of heavy metals. Furthermore, heavy metal co-pollution looked like to have a much forceful impact on the fungal community structure, leading to less diverse banding patterns with lower intensities and decreasing number of bands observed in the DGGE patterns comparing with bacteria.

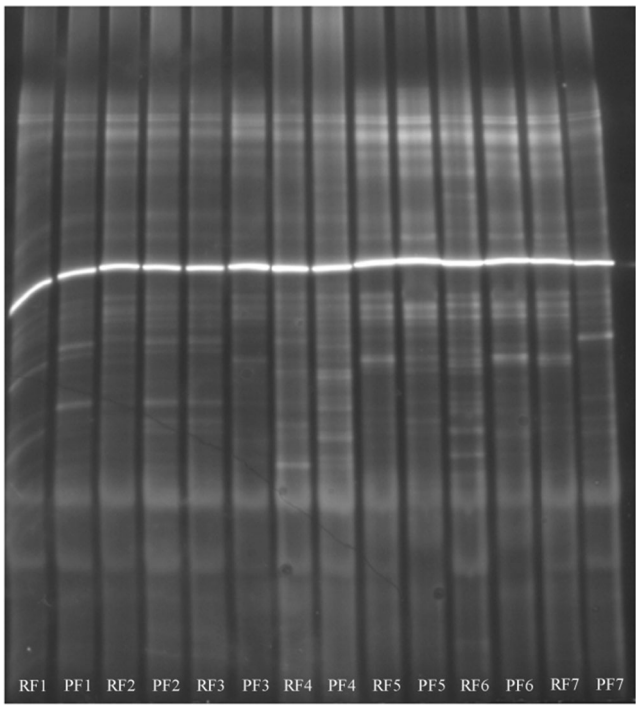

Fig. 3 DGGE profiles of rhizosphere bacterial communities in rape fields (RF) and paddy fields (PF)

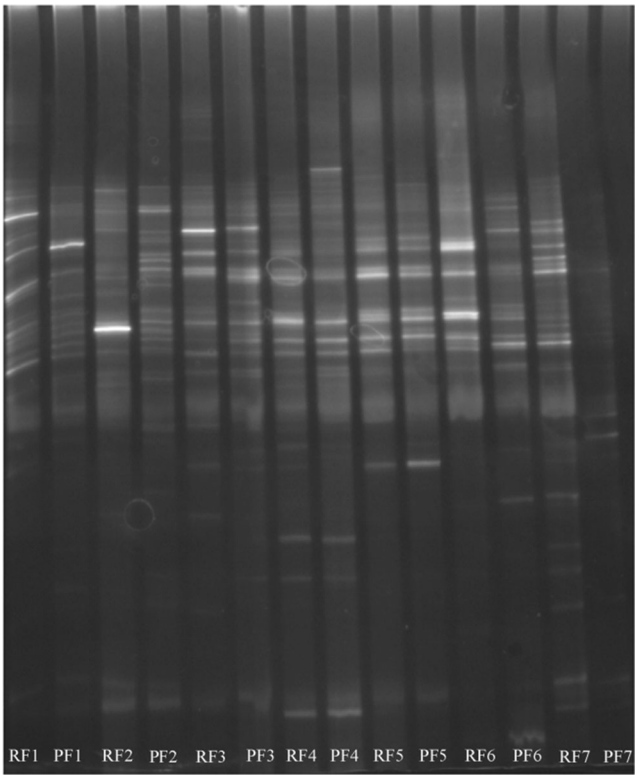

Fig. 4 DGGE profiles of rhizosphere fungal communities in rape fields (RF) and paddy fields (PF)

The DGGE gel profiles were further visualized by the Shannon-Wiener diversity index (*H*), which provided a direct indication of the apparent diversity of a microbial community (Fig. 5). The diversity index of bacteria was basically at the same level; however, diversity of fungi showed significant difference. Under co-pollution of heavy metals, tolerance of bacteria was more than fungi, resulting in more abundance and diversity of bacteria as the spatial and temporal change.

The principal component analysis (PCA) of the bacterial DGGE profiles yielded a relatively goodish separation in different positions, which can be exhibited in Fig. 6a. Principal components 1 and 2 accounted for 66.2 % of the total sample variability (52.9 and 13.3 % for PC1 and PC2, respectively). The bacterial community structure in polluted fields (RF6, RF7, and RF6) and controls (RF5 and PF5) formed a bunch that was separated from other co-polluted points in PCA co-ordinates, which can be interpreted by their similar primary species composition of the sampling point. For fungal colony, the PCA showed the genetic diversity in rape and paddy fields, which was consisted with the results of Shannon index

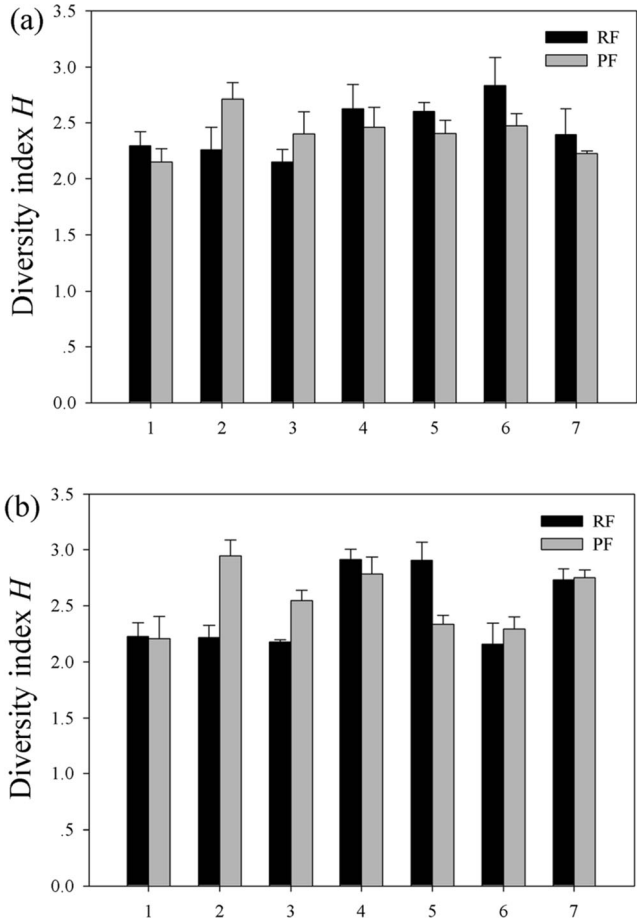

Fig. 5 The diversity index *H* rooted in DGGE profiles of amplified rhizosphere bacterial 16S rRNA genes (**a**) and fungal 18S rRNA genes (**b**) from arable rape fields (RF, *black bar*) and paddy fields (PF, *gray bar*)

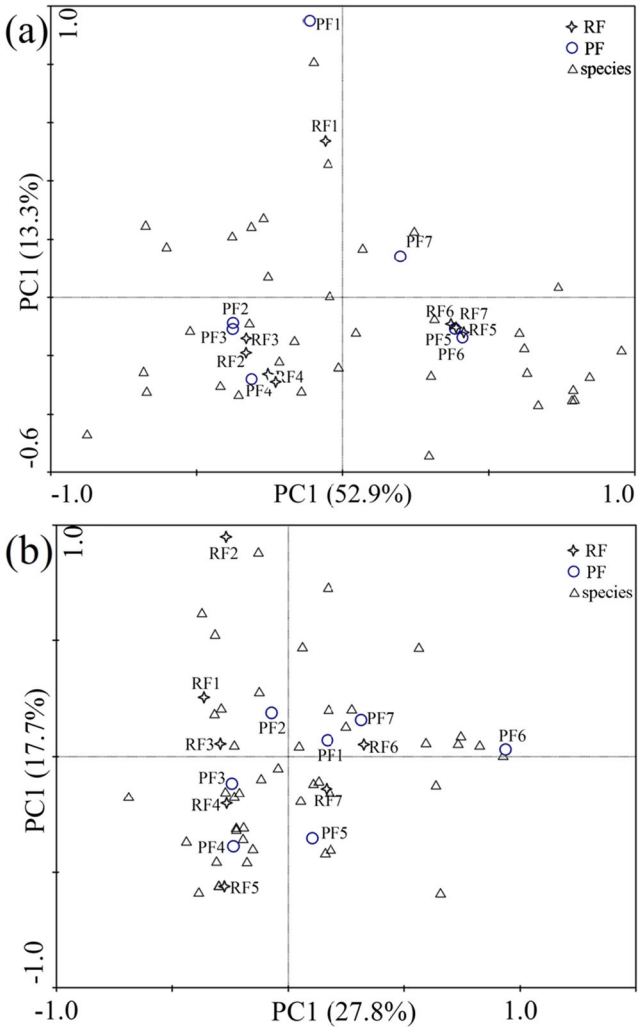

Fig. 6 Principal component analysis (PCA) of amplified 16S rRNA gene fragments from rhizosphere bacterial community (**a**) and 18S rRNA gene fragments from rhizosphere fungal community (**b**) based on DGGE profiles in rape fields (RF) and paddy fields (PF)

H. The first two components accounted for 45.5 % of total variation (Fig. 6b). In spite of PC2 explained only 17.7 % of total variability, all fractions suffered from pollution were discrete from those controls.

Relationship between environmental parameters and bacterial and fungal community structure

According to the results of DCA, RDA was used to assess the significant correlation between environmental parameters and rhizosphere bacteria and fungi in soil (Fig. 7). The first two axes of the RDA explained 50.1 and 10.4 % for bacteria, 27.4 and 15.9 % for fungi of the variance between environmental variables and species data. As it was shown in Fig. 7, it was found that pH was positively correlated with axis 1 both bacterial and fungal community structure. And SMC only had positive relationship with axis 1 for fungal community structure, while other environmental properties had inverse correlation with dynamic of both bacterial and fungal community structure. The majority of environmental variables in soils were located in the left part of RDA ordination diagram both bacteria and fungi, excepting for pH and SMC. According to the RDA profiles, SMC and SOC, TI_{tot}, the pH value, and MBC of the soils were the most important factors governing bacterial and fungal community structures.

Discussion

Physical and biochemical properties in arable soil under heavy metal co-pollution

The phenomenon of relative higher level heavy metals in rape fields than those in paddy fields might be due to the special properties of the paddy fields. The paddy microenvironment scale is often irrigated, which might be responsible for the lower levels of heavy metal contamination compared to rape soil. Study reported by Zheng and Zhang (2011) suggested

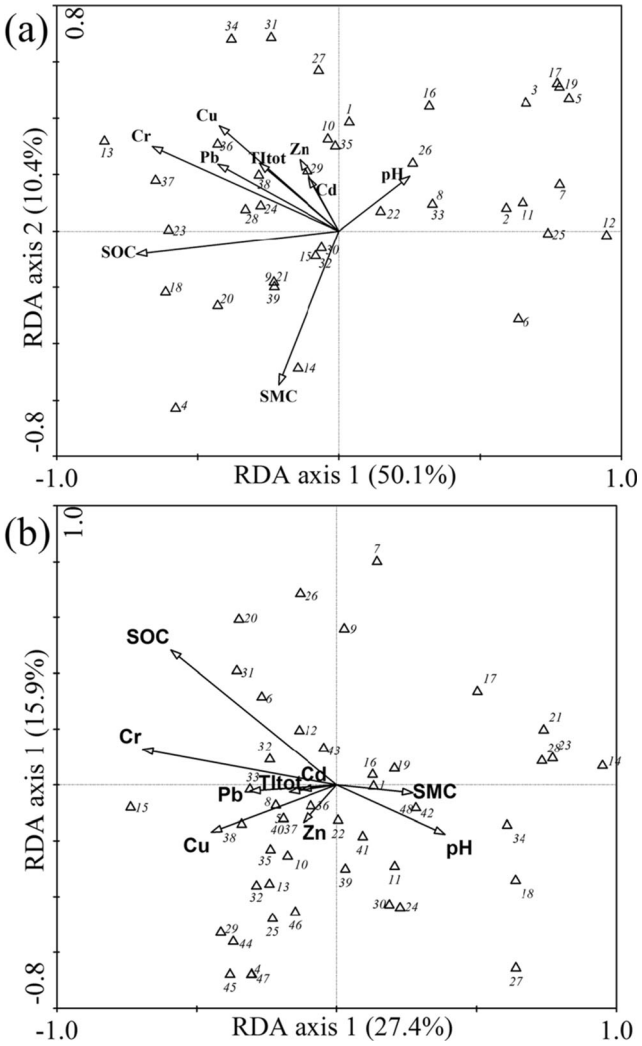

Fig. 7 Redundancy analysis (RDA) of rhizosphere bacterial species (**a**) and fungal species (**b**) in the rape fields and paddy fields with soil chemical variables. Environmental variables and bacterial species or fungal species were represented as *arrows* and *empty triangles*, respectively. The length of the arrows manifested the relative importance of that environmental factor in explaining the variation of bacteria and community structures, while the *angles* between the *arrows* reflected the degree of their correlations. The *percentages presented on the first and second axis* correspond to the percentages of variance of microbial sequence type data explained by the particular axis

that the paddy soil under moisture regime had higher metal reactivity compared with wetting-drying cycle regimes.

The pH value is an important factor affecting the availability of heavy metals. Wen et al. (2013) have clearly revealed that simulated acid rain can increase the environmental risk of heavy metals by increasing the soluble content of metals in soil solutions. Most of our samples exhibited faintly acid. Also, acidic pH could promote dissolution of secondary minerals and movement of Pb in transects (Sanderson et al. 2012). In our study, SOC have significant positive correlation with all studied heavy metals. The trend that the higher the soil organic content, the higher the metal concentration in the solid or water phase was well documented (Park et al. 2011; Wu et al. 2011).

Based on Pearson correlation analysis, there was negative correlation between stress of heavy metals and metabolic quotient. The similar results were reported in literatures (Bååth 1989; Chen et al. 2014). Cr was observed to be significant negative correlation with metabolic quotient at $P<0.05$. Basal soil respiration of soil microflora offered useful information on the physiological condition of the ecosystem. Joint toxicity of heavy metals on microbial biomass has been well documented (Barajas Aceves et al. 1999; Niklińska et al. 2006). There was no significant relationship between microbial biomass and heavy metals in this article. The difference of microbial biomass and activity under heavy metal co-pollution could probably stem from distinction of soil carbon content. In theory, this might be caused either by restricting bioavailability of the metals or by elevating resistance to pollution.

DHA, a kind of extracellular enzymes participating in oxidative phosphorylation in microorganism, was widely used to assessing the metabolic activity of arable soil microorganism. It has been reported that DHA was correlated with the availability of organic carbon (Serra-Wittling et al. 1995) and microbial respiratory processes (Insam 2001) in soils. Generally, significant negative relationship was observed between the total heavy metal contents and soil DHA (Kızılkaya et al. 2004; Leirós et al. 2000), which supported our results. Presence of Zn in soil significantly decreased DHA compared with controls (Kelly et al. 1999). At the same time, DHA was obviously negatively related to TI_{tot} at $P<0.01$.

Abundance of bacteria and fungi in arable soils under stress of heavy metals

To assay the distinction in soil microbial abundance under heavy metal stress, QPCR aiming at the bacterial 16S rRNA genes and fungal 18S rRNA genes was adopted. In our study, it uncovered decrease both in rhizosphere bacterial 16S rRNA and fungal 18S rRNA gene copy numbers. Previous research (Frey et al. 2003) has revealed that the amount of litter-derived C found in macroaggregate was positively correlated with litter-associated fungal biomass; however, in this article, the abundance of fungi in soil samples PF5, PF6, RF7, and PF7 with lower SOC was relatively higher in soil than the samples with higher SOC.

Literatures described abundance of soil bacteria and fungi under the effect of heavy metals varied from each other (Khan et al. 2010; Oliveira and Pampulha 2006). In tested conditions, pollutants (phenanthrene and arsenic) did not have a major effect on community abundance or taxonomic composition but rather had an impact on metabolic and functional bacterial properties (Cebron et al. 2014). Liu et al. (2012) revealed a consistent change in soil microbial community under heavy metal pollution of rice paddy across South China. These changes could be characterized by a decline in abundance of

overall microbial community and specifically for fungi which was consistent with our results.

In this study, it was observed that fungi abundance was mostly lower in all fields under heavy metal pollution compared with that of bacteria, indicating that heavy metal co-pollution may have more long-range effect on the fungal community. One explanation for these different results could be that bacteria could probably be well accommodated to chronic toxicity or stress in long-term heavy metals co-contaminated soils compared with fungi, due to their wide substrate using profile and high metabolic activity. Beyond that, bacteria dominated in arable fields may compete with fungi for nutrients, resulting in greater survive stress on the fungal community. The abundance of the extramatrical mycelium was shown to be important for heavy metals binding by the fungi (Galli et al. 1994). In this article, there was a negative correlation between fungi abundance and TI_{tot}, so it speculated that fungal hyphae could improve aggregate stability by combining metal ions with extracellular polysaccharides to form macroaggregates under stress of several heavy metals, resulting in lower abundance of fungi in soil with the high content of heavy metals compared with bacteria. Our results showed that fungi abundance was significantly related to Cr, suggesting that Cr might have an important impact on the fungal population (Joynt et al. 2006).

Community structure of bacteria and fungi in arable soils under pollution of heavy metals

Heavy metals not only have an effect on microbial abundance but also on microbial community structure and diversity. The changes in microbial populations or processes under field-contaminated differed from laboratory-spiked (Bååth 1989; Smolders et al. 2004), and those with short-term pollution (Frostegård et al. 1996; Rajapaksha et al. 2004) differed from with long-term pollution (Abaye et al. 2005; Ge and Zhang 2011). It was known that the high concentration of heavy metals could affect the microbiota directly through the transformation of the population size, diversity, and structure.

In the present study, significant difference of the number of bacterial specific DGGE bands and bacterial diversity index (Fig. 5) was observed between RF and PF because of heavy metal pollution, while diversity of bacteria was mainly invariable. One explanation for this finding was that bacteria as indigenous microbial in agricultural soil might adapt multiple microhabitats and utilize more labile SOC and form a favorable mechanism against heavy metal. The increased heavy metal tolerance of the microbial community could be due either to an acquired tolerance by adaptation, or to a shift in species composition, where organisms already tolerance became more competitive and thus more numerous (Bååth 1989). In the study done by Ying et al. (2009), resistance effect of resistant bacteria was better than that of resistant fungi in microorganism-plant bioremediation for heavy metal (Zn and Cr) contaminated soil.

Contrary to the variation of bacterial diversity observed under pollution, the fungal community structure showed a different DGGE pattern with a decreased diversity index being observed in fields compared to the controls. The reduced fungal community diversity shown by the DGGE profiles in the present study provided further evidence of fungi responding to long-term heavy metal stress. Hence, the appearance and disappearance of fragments in the DGGE profiles approximated shifts in the microbial community structure.

In conclusion, this study made advance toward providing insight into the population, distribution, and activity of the microbial community in arable soils. Our results illustrated that the microbial community responded to long-term Cd, Pb, Zn, Cu, and Cr contamination through changes of microbial community structure. Microorganism could be better adapted to the soil medium under stress. The general soil properties most closely correlated with the biochemical properties were the SOC and available nutrient contents, suggesting that the number and activity of soil microorganisms depended mainly on the quantity of mineralizable substrate and the availability of nutrients. It was not surprising that these parameters had a significant and complex influence on the microbial community. The heavy metal stress cannot only lead to adverse effects on the physicochemical property of agricultural soil but also result in size, composition, and activity changes of soil microbial community. A decrease of abundance was found in both bacteria and fungi. Fungi was more sensitive to toxicants of heavy metal co-pollution than bacteria, which varied in sensitivity to heavy metal toxicity, may be an important factor in explaining discrepancies among studies.

In this study, different monitoring indicators were used to investigate soil microbial community structure and activities to estimate soil quality indices. Because of the limitations of the DGGE technique, further monitoring using new fingerprinting methods (e.g., cloning sequencing and pyrosequencing) might be necessary to evaluate the changes precisely in the soil microbial population and its relationships with physicochemical dynamics.

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