#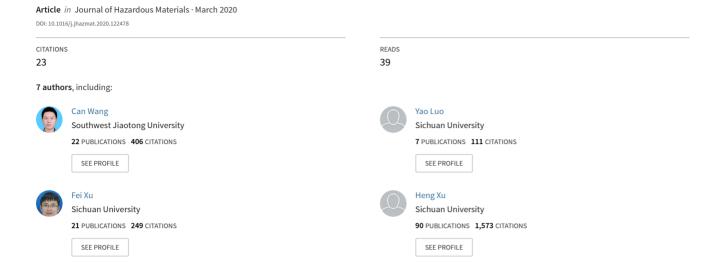 Ecological responses of soil microbial abundance and diversity to cadmium and soil properties in farmland around an enterprise-intensive region

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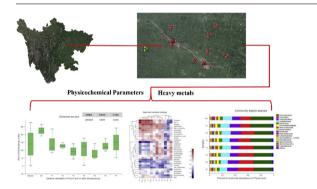**90** PUBLICATIONS **1,573** CITATIONS

Ecological responses of soil microbial abundance and diversity to cadmium and soil properties in farmland around an enterprise-intensive region

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GRAPHICAL ABSTRACT

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ABSTRACT

Microorganisms play a vital role in soil biochemical process in contaminated managed ecosystems. In the present study, a field investigation was conducted in farmland around an industrial intensive region contaminated with cadmium, and the changes of microbial assemblages in contaminated soils were assessed by 16S rRNA sequencing and the further statistical analysis. The results revealed obvious variations in microbial richness between referenced and contaminated soils, with *Proteobacteri*, *Chloroflexi*, *Actinobacteria*, *Acidobacteria* and *Nitrospirae* dominating the studied communities around the industrial intensive region. Redundancy analysis and Spearman correlation heatmap revealed that about 68.95 % of overall variation in microbial community composition was explained by soil physiochemical properties and Cd existence, among which pH, soil total phosphorus, total nitrogen, organic carbon (OC) and available Cd were identified as dominant factors. No significant difference was found in the similarities and Beta-diversity analysis among different groups. In conclusion, this study revealed the ecological effects of physiochemical parameters and Cd stress on the diversity and abundance of microbial communities, and these findings provided the detailed and integrated correlation between the main factors and microbial indexes in Cd contaminated farmland around the industrial intensive region.

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1. Introduction

For decades, excessive and unreasonable producing activities have caused serious heavy metals pollution problems, which has posed significant threat to biodiversity and functioning of soil microorganisms, food safety and public health (Bhakta et al., 2018). Soil microorganisms are crucial role in natural and managed soil ecosystems (Fierer, 2017). In farmland soil, as the core force of soil fertility, microorganisms can notably promote the cycling of nutrient elements, maintain the soil fertility and improve the health of crops (Fierer, 2017). It is known that soil bacteria are sensitive to pollutions, namely heavy metals and organic pollutants (e.g. PAHs, PAEs). Although the bulk of studies on mining sites or e-waste recycling sites have investigated and illustrated the obvious impacts of heavy metals on soil environmental quality (Guo et al., 2017; Jun et al., 2015), it is still unclear how the soil microbial community structure changes across cadmium gradient in agricultural soil (Chen et al., 2020). Therefore, a deeper insight into the environmental ecological effects of contaminated agricultural soil recently draws more attentions, which might be useful for land management in agricultural field around enterprise-intensive region.

It is also essential to understand how information about the structure of microbial community can help to evaluate the range of effects that bacterial may have on heavy metal contaminated soil ecosystems. The underground microbial taxa could directly drive the soil processes, to name a few, changing the soil acidity, promoting the cycling of nutrients (Organic carbon, nitrogen, phosphorus and other trace elements) and mediating soil carbon dynamics (Chen et al., 2020; Fierer, 2017; Hu et al., 2011). Many biogeochemical processes are also mediated by soil bacterial communities, for example, chelation, co-precipitation and detoxification of heavy metals (Rajkumar et al., 2012). The excess metals (cadmium, chrome, mercury) inputted into agricultural ecosystems could have obvious effects on crops quality and bacterial community diversity. Heavy metals could significantly affect the bacterial growth, metabolism, morphology and cellular functions (Kandeler et al., 2000; Walker et al., 2000). Previous studies have shown that elevated heavy metals have adverse effects on soil microorganisms abundance and enzymatic activities in contaminated soils (Bo Jiang et al., 2019; Kandeler et al., 2000). Additionally, exceed heavy metals might screen out some metal-tolerant bacterial species (*e.g. Serratia, Pseudomonas, Acinetobacter*) (Li et al., 2016). Soil microbial community composition have direct or indirect influence on such ecosystem-level processes, then how to identify dominant taxonomic communities (to the highest abundance possible) that are dominant in specific processes is important.

In the present study, typical Cd contaminated regions were selected in Mianyang City, China, where farmland around the industrial intensive area were sampled and investigated. We investigated changes in microbial communities and estimated underlying functional influences across Cd pollution gradient in the contaminated soil by analyzing the relationship between environmental factors (soil properties, heavy metal speciation and concentration) and soil microbial communities. This survey will fully reveal the ecological responses of soil microbial community to Cd distribution and soil properties variation.

2. Materials and methods

2.1. Sites description

The study area is located in Anzhou district, Mianyang, China (Fig. 1), which is farmland around an enterprise-intensive region (N 31°26′-31°29′ E 104°16′-104°23′). Long-term disorderly phosphorus chemical production has caused severe Cd pollution in this research area. The region has a typical central subtropical monsoon climate with annual temperature and rainfall of 21℃ and 1000 mm, respectively. The main wind direction in this region is influenced by monsoon circulation and topography.

2.2. Samples collection and physiochemical properties analysis

The Cd contaminated soils were sampled from 7 sites in the study area (T1-T7), and those sites, far away (about 6.10 km) from polluted area, were set as the reference point (CK). The geographic locations of the sampling sites were described in Fig. 1 and Tab. S1. Meanwhile, three soil sampling points were collected for each site at a depth of $0-20$ cm. All samples (8 sites $\times$ 3 sampling points) were transported to the laboratory with cooler boxes. Each sample (200 g) was homogenized and sieved through a 0.45 mm sieve, then divided to two parts for the further research. One part was air-dried and stored at 4 ℃ for physiochemical properties analyses. And another was collected at -80 ℃ for DNA extraction (within 48 h) and microbial community analyses.

The soil pH was measured by the pH meter (METTLER-S220) with soil/water slurry at 1:20 (w/v) ratio after shaking 30 min. Cation exchange capacity (CEC) was determined by modified method (Skjemstad et al., 2008). Soil organic carbon (SOC) was calculated by subtracting the inorganic carbon from total carbon measured by monitoring the content of CO_2 during dry combustion (Bo Jiang et al., 2019; Nelson et al., 1982). Soil total phosphorus (TP) was assayed through Olsen's method (Rajan et al., 1991). Available phosphorus (AP) was measured by spectrophotometry at 700 nm (NY/T 1121.7–2014) (Huakang et al., 2018). Kelvin digestion-constant distillation-titration method was used to determine total nitrogen (TN) in soil (Wang et al., 2018b). Soil alkali hydrolysable nitrogen (AN) was measured by the modified method of Page et al. (Page, 1982). Total potassium was measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) after being digested with the mixed solution of HNO_3, $HClO_4$ and HF (5:4:3, v/v) (Liu et al., 2013). Soil available potassium (AK) could be extracted with 2 mol L^{-1} HNO_3 (v:w 20:1) and determined with ICP-MS (Lean and Watson, 1985).

2.3. Heavy metal fractions analysis

The total and available heavy metals in contaminated soil were measured by ICP-MS. The contaminated soil 1.0 g was digested with a mixture of 6 mL HNO_3, 5 mL $HClO_4$ and 4 mL HF for measuring the concentration of total heavy metals (Huakang et al., 2018). For available heavy metals, Diethylene-triamine pentacetic acid (DTPA) extraction was utilized as an effective method for obtaining available (DTPA-extractable) heavy metals from soils, which are highly related with bio-accumulating in crops (Zahedifar et al., 2016). Air-dried soil (5 g) was mixed and shaken for 2 h with 25 mL mixture of 0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$, then centrifuged at 3000 rpm for 5 min and the supernatant was detected for DTPA-extractable heavy metals (Bo Jiang et al., 2019). Contamination index (*Ci*) is defined as the ratio of the total Cd (T-Cd) concentration to ecology-soil screening limit values (Eco-SSLs), as shown in Eq. (1). Eco-SSLs were referred to Soil environmental quality – Risk control standard for soil contamination of agricultural land of China (GB 15618-2018) (Xiaoxia et al., 2018), which was described in Tab. S2.

$$Ci = \frac{[Heavy\ metal\ in\ sample\ soils]}{[Eco-SSLs\ in\ different\ reference\ soils]} \tag{1}$$

2.4. Microbial community structure and diversity analysis

A soil DNA kit (Omega Bio-tek Inc, Norcross, GA, USA) was utilized to isolate the soil microbial DNA from 5 g soil samples. V4 hypervariable region of bacterial and actionbacterial genes were amplified by polymerase chain reaction (PCR) using a pair of primers 530 F (5′ GTG CCA GCM GCN GCG G-3′) and 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′) (Song et al., 2015). Sequencing was carried out with Illumina Hiseq4000 after PCR processing finished. The raw sequence results were processed and analyzed with Quantitative Insights Into Microbial
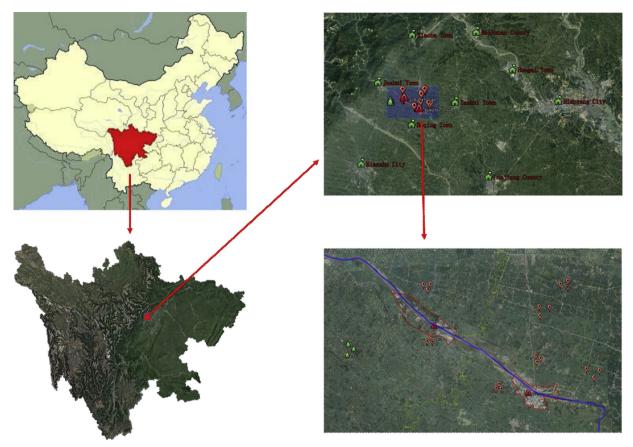
Fig. 1. Map of enterprise-intensive area and locations of sampling sites contaminated with cadmium.

Table 1
Physicochemical properties in agricultural land around the enterprise-intensive region (mean $\pm$ S.E., n = 3).

Samples	pH	CEC(cmol kg^{-1})	OC (g kg^{-1})	TN(g kg^{-1})	AN(mg kg^{-1})	TP(g kg^{-1})	AP(mg kg^{-1})	TK(g kg^{-1})	AK(mg kg^{-1})
CK	6.20 ± 0.62[ab]	9.57 ± 0.45[a]	16.31 ± 1.50[a]	1.45 ± 0.20[a]	144.78 ± 9.11[a]	0.71 ± 0.06[a]	20.70 ± 2.72[b]	15.83 ± 0.39[ab]	122.64 ± 3.05[ab]
T1	5.04 ± 0.08[a]	12.41 ± 0.48[b]	18.84 ± 0.82[ab]	1.71 ± 0.07[b]	169.76 ± 5.34[ab]	1.08 ± 0.03[b]	6.44 ± 0.88[a]	15.65 ± 0.35[a]	84.20 ± 5.17[ab]
T2	5.23 ± 0.28[a]	14.89 ± 0.70[cd]	21.37 ± 0.40[bc]	1.90 ± 0.02[bc]	180.63 ± 0.33[b]	1.55 ± 0.13[c]	7.62 ± 0.70[a]	15.03 ± 0.26[a]	109.60 ± 3.31[ab]
T3	5.87 ± 0.58[ab]	14.12 ± 0.09[bcd]	21.38 ± 0.26[bc]	1.90 ± 0.09[bc]	174.04 ± 5.13[b]	1.81 ± 0.09[c]	5.16 ± 0.11[a]	16.18 ± 0.17[abc]	109.91 ± 9.46[ab]
T4	5.43 ± 0.36[a]	13.43 ± 0.51[bc]	22.53 ± 0.21[c]	2.05 ± 0.02[c]	191.26 ± 0.94[b]	1.14 ± 0.13[b]	10.54 ± 1.57[a]	15.40 ± 0.78[a]	111.35 ± 5.00[ab]
T5	5.09 ± 0.22[a]	14.13 ± 0.32[bcd]	20.70 ± 0.46[bc]	1.87 ± 0.03[bc]	175.33 ± 3.93[b]	1.58 ± 0.02[c]	5.01 ± 0.53[a]	17.36 ± 0.29[c]	109.48 ± 6.04[ab]
T6	5.34 ± 0.12[a]	16.01 ± 0.21[d]	21.40 ± 0.57[bc]	1.99 ± 0.03[bc]	179.81 ± 2.64[b]	2.25 ± 0.08[d]	5.70 ± 0.21[a]	17.09 ± 0.34[bc]	148.28 ± 4.62[b]
T7	6.99 ± 0.40[b]	14.15 ± 0.22[bcd]	20.58 ± 0.21[bc]	1.87 ± 0.01[bc]	164.31 ± 0.96[ab]	2.51 ± 0.04[d]	7.78 ± 1.01[a]	16.19 ± 0.41[abc]	64.37 ± 3.31[a]

Abbreviations: Cation exchange capacity (CEC); organic carbon (OC); total nitrogen (TN); alkali hydrolysable nitrogen (AN); total phosphorus (TP); available phosphorus (AP); total potassium (TK); available potassium (AK); Standard errors (S.E.). Different letters in the same column represent significant differences at $p <$ 0.05.

Ecology (QIIME) and Mothur (Bo Jiang et al., 2019). Next, making all reads pass the quality filtering, then obtaining the effective sequences by the barcodes (5′ and 3′) and primer sequence distinguished with FLASH and Trimmomatic (Guo et al., 2017). Representative sequences were clustered at a similarity level of 97 % of operational taxonomic units (OTUs). In order to obtain the corresponding species classification information for each OTU, the RDP classifier Bayesian algorithm was used to classify and analyze representative sequences at a similarity level of 97 %. The relative abundance of each OTU and taxon was calculated via its number of total sequences.

2.5. Data analysis

All bacterial sequences statistical analyses were performed using various packages in R version 3.1.3 (Vermeulen et al., 2013). The microbial community relative abundance and diversity were reflected by the Alpha-diversity (indices of Chao 1, Ace, Shannon and Simpson) and

unweighted pair group method with arithmetic mean (UPGMA) cluster analysis, which were calculated by QIIME (v 1.80). Principal co-ordinates analysis (PCoA), Redundancy analysis (RDA), Heatmap and other correlation analyses of environmental factors and microbial community were carried out with Majorbio Cloud Platform (www.majorbio.com). All Statistical analyses were performed using SPSS 20, and mean values of groups were deemed to be different when $P < 0.05$ using least significant difference (LSD). A part of statistics were performed using the Origin 9.0 (USA).

3. Results

3.1. Soil physicochemical properties

Physicochemical properties and Cd contamination characteristics of surface soils (0 − 20 cm) were listed in Table 1 and Fig. S1, respectively. The pH of samples was strongly acidic to acidic in T1-T6

(5.04 ± 0.08–5.87 ± 0.58), the neutral in T7 (6.99 ± 0.40), and slightly acidic in CK (6.20 ± 0.62). The results showed that OC, CEC, TN, AN, TP in T1-T7 were significantly higher than those in CK about 15.51–38.14 %, 29.68–67.29 %, 17.93–41.38 %, 13.49–32.10 %, 52.11–253.52 %, respectively. Specially, the concentration of AP (5.01 ± 0.53–10.54 ± 1.57 mg kg^{-1}) in T1-T7 was significantly lower than CK (Table 1). Except T5, there were no obvious difference about TK and AK in contaminated samples compared with CK (Table 1).

3.2. Heavy metal contamination level

Soil physicochemical properties varied in different samples, which would significantly impact Cd speciation. So it was necessary to further detect the total and available content of Cd in soil (Bo Jiang et al., 2019; Jun et al., 2015). otal and DTPA-extractable Cd (A-Cd) in the studied region were far beyond Eco-SSLs (Fig. S1 and Tab. S2). The lowest Cd concentration (0.32 ± 0.03 mg kg^{-1}) was showed in CK, while the contaminated sites ranged from 0.89 ± 0.12–1.93 ± 0.10 mg kg^{-1}. The results indicated that A-Cd content increased with the increase of total Cd (Fig. S1). Although the highest rate of available Cd to total Cd was found in T6 (54.88 % ± 0.48), it varied in different samples. Ci can evaluate the holistic level of soil pollution. The values of Ci (Fig. S1) showed that all samples (T1-T7) in the study area were polluted with the mean values of Ci as T6 (6.42 ± 0.31) > T7 (5.89 ± 0.21) > T5 (5.78 ± 0.18) > T2 (5.20 ± 0.22) > T3 (4.58 ± 0.21) > T1 (3.13 ± 0.11) > T4 (2.97 ± 0.08). It was noteworthy that the sites of T2, T5, T6 and T7 have been contaminated heavily according to GB 15618-2018 (Xiaoxia et al., 2018).

3.3. Change of bacterial community in different sampling sites

The 16S rRNA genes in 24 samples were detected by Illumina Hiseq4000 and generated a total of 813,187 qualified sequences, with a range of 30532–36541 sequences in each sample (Tab. S3). In the aggregate, there were 8668 OTUs identified in all samples. Rarefaction curves analysis suggested that the amount of sequencing data was large enough to reflect the vast majority of microbial diversity information in each contaminated and reference soil (Fig. S2).

Effect of different Cd contaminated levels on relative abundance and diversity of bacterial communities in study area were incarnated by Alpha diversity (indexes of Chao, Ace, Shannon and Simpson), β-diversity and UPGMA cluster analysis, etc.. From Tab. S3, T3 (Ci 4.58 ± 0.21, A-Cd 0.71 ± 0.03 mg kg^{-1}) reached the highest relative abundance, and the lowest relative abundance was observed in CK (Ci 1.07 ± 0.13, A-Cd 0.13 ± 0.04 mg kg^{-1}). The results of Ace and Chao indexes in Tab. S3 suggested that T2, T3 and T6 were also significantly higher than CK on species richness. However, the indexes of Shannon and Simpson (Tab. S3) indicated that there were no significant differences with bacterial diversity and homogeneity in all sampling sites, which could also be proved by the result of significance test of difference among groups on phyla and genus level (Fig. S3A and S3B). Moreover, the result of Analysis of Similarities (ANOSIM) (Fig. S4) revealed that there was no significant difference (R: 0.0589, p-value: 0.2160) between and within groups of Cd-contaminated and reference soils, which was also represented in analysis of samples distances heatmap on phylum level by Bray-Curtis distance algorithm (Fig. S6).

Taxonomic classification of whole qualify OTUs identified 51 different phyla, 917 different genus and 2068 different species. The sites' specific physicochemical parameters and Cd concentration would influence bacterial population. In addition, taxonomic analysis showed slight differences in bacterial community structure across all samples. The highest richness of bacterial phylum was *Proteobacteria* (27.19 %–34.15 %), followed by *Chloroflexi* (16.11 %–25.67 %), *Actinobacteria* (12.80 %–17.97 %), *Acidobacteria* (11.13 %–14.63 %), *Nitrospirae* (3.07 %–5.26 %), *Gemmatimonadetes* (2.34 %–3.43 %), *Verrucomicrobia* (1.17 %–3.43 %), as shown in Fig. 2. Besides, *Proteobacteria, Actinobacteria,*

Acidobacteria and *Chloroflexi* were more abundant in T2, T6 and T5, in which the soil was heavily contaminated (Ci > 5). Community heatmap analysis (n = 3) also generally reflected analogical patterns of relative richness distribution of main bacterial phyla in Cd contaminated and reference soils (Fig. S5). The species of *Proteobacteria* (Kevin et al., 2003), *Nitrospirae* (Wu et al., 2017) and *Acidobacteria* (Li et al., 2017) in phyla could resist toxicity of Cd, Cu, Pb, etc., which were also detected in this study (Tab. S4 and S6).

For visually expressed and thoroughly investigated the microbial composition, PCA was calculated by Weighted Unifrac distance algorithm (Wang et al., 2019), which revealed synergistic effect of soil physicochemical parameters and Cd contamination level on β-diversity of bacterial community structure (Fig. 3). Approximately 73.20 % of the bacterial community diversity variation was interpreted (the first two dimensions, PC1 and PC2 were 53.80 % and 19.40 %, respectively), implying that environmental parameters (pH, CEC, OC, N, P, K, Cd, etc.) had a significant effect on the bacterial community structure. PCA results also suggested that the samples of CK, T1 (Ci 3.13, medium contamination), T4 (Ci 2.97 slight contamination) were more scattered than the other samples, and T2, T5, T6 and T7 were rather more affected by physicochemical properties.

3.4. Relative influences of soil physicochemical parameters and Cd contamination on bacterial community

To amply evaluate the relative effect of physicochemical variables (pH, CEC, OC, TN, TP, TK, AN, AP, AK) and Cd concentration on the abundance and diversity of bacterial community in Cd contaminated farmland, redundancy analysis (RDA) was conducted (Fig. 4). The first two coordinate axes of the RDA could explain 25.37 % and 12.51 % of the holistic variation, respectively. The results of RDA further showed that the pH, TP, TN, A-Cd, T-Cd and CEC played a vital role in structuring the bacterial communities (Fig. 4). Meanwhile, subsequent analysis revealed that soil physiochemical properties and heavy metals explained for 68.95 % of overall variation in microbial community composition, leaving 31.05 % of the variation unexplained. Furthermore, the results of significance test of difference (Fig. 4) showed that environmental factors had a significant influence on microbial community (p < 0.05), excepted AN, AP and TK. The spearman correlation heatmap analysis was used to investigate the relationship among the soil physicochemical parameters, Cd concentration and relatively microbial abundance of dominated bacterial phyla and genera (Fig. 5, Tab. S4 and Tab. S5). The abundance of the phyla *Latescibacteria, Chloroflexi, GAL15* and *Nitrospinae* were positively correlated with CEC (p < 0.05). In addition, AN was positively correlated with the abundance of *Latescibacteria, Armatimonadetes, Verrucomicrobia, Planctomycetes* and *Elusimicrobia*, and with the abundance of the genera *Acidobacteriaceae, Latescibacteria, DA101* and *OPB35* (all p < 0.05). Interestingly, the results of Fig. 5 showed that T-Cd, A-Cd and Ci were significantly and positively correlated with *Chloroflexi, GAL15* and *Nitrospinae* (p < 0.05), and positively correlated with *Latescibacteria, Actinobacteria, Aminicenantes, RBG-1-Zixibacteria* (not significant). However, Cd concentration was negatively correlated with the abundance of phylum *FCPU426* and genus *Rhodobiaceae* (all p < 0.05). Pearson correlation analysis also indicated that microbial abundance (Chao and Ace) had a positive correlation with CEC, OC, TN, AN, TP, T-Cd, A-Cd and Ci, but negatively correlated with pH and AP (Tab. S6).

4. Discussion

Heavy metal pollution is always found around mining, smelting, e-waste recycle and some manufacturing industry sites, as they are widely existing in the raw materials and products of these industries, e.g. Cd in computer batteries, Cd and Zinc in phosphogypsum (Bo Jiang et al., 2019; Mohammad and Omar, 2015). Long-term phosphate fertilizer producing activities have caused seriously Cd contamination in studied
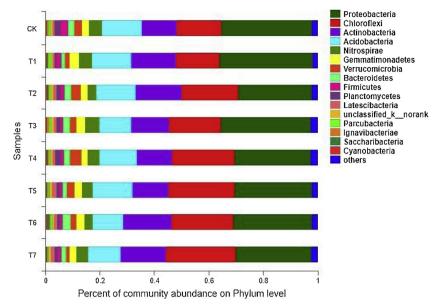
Fig. 2. Relative abundance (n = 3) of staple microbial taxa on phylum level across all samples (CK, T1-T7) detected by 16S rRNA gene sequencing. Phyla with less than 0.5 % relative abundance in all samples are classified as others.
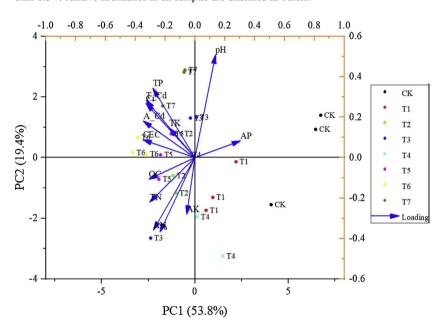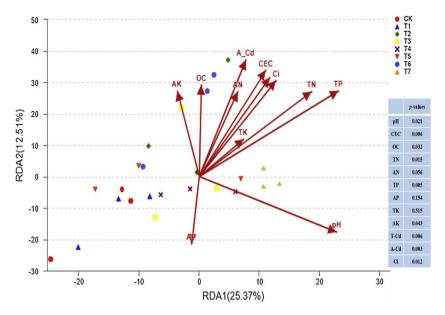

Fig. 3. Principal component analysis (PCA) of sampling sites and related environmental parameters in the studied area. Note: cation exchange capacity (CEC), organic carbon (OC), total phosphorus (TP), available phosphorus (AP), total nitrogen (TN), alkali hydrolysable nitrogen (AN), Total potassium (TK), available potassium (AK), total Cd (T-Cd), available Cd (A-Cd), contamination index (*Ci*).

areas through various diffusing pathways (atmospheric deposition, sewage irrigation, etc.) (Bo Jiang et al., 2019; Jun et al., 2015). The poor homogeneity of soil and the main wind direction might explain the different Cd concentrations of samples around the enterprise-intensive region. Total Cd concentrations couldn't holistically reflect Cd toxicity, so it was necessary to estimate the ecological effect of A-Cd and soil physicochemical parameters on soil indigenous bacterial abundance and diversity (Bo Jiang et al., 2019; Mohammad and Omar, 2015; Shahid et al., 2016). Previous studies have shown that soil pH was one of the most important factors impacting Cd fractions in studied soil, which was negatively correlated with available Cd concentration through enhancing competition for negative ions surfaces between Cd^{2+} and H^+ (Ardestani and van Gestel, 2013; Shahid et al., 2016). The relationship between pH and Cd was also represented in Tab. S6. Traditional agronomic practices (straw application), excessive chemical fertilizer application and dry deposition of phosphogypsum could bring about soil acidification and Cd accumulation (Gao et al., 2018; RUT-HERFORD et al., 1994), which might be the reason for the stronger

acidity of heavily polluted samples in studied region. Previous studies showed that organic materials also could render pH decrease during hatching process (Ingelmo et al., 2012), The released low molecular weight organic acids could be combined with heavy metals to enhance metals' solubility (Ingelmo et al., 2012; Rojjanateeranaj et al., 2017). Similarly, Pearson correlation analysis (Tab. S6) indicated that OC was negatively correlated with pH and positively correlated with A-Cd in this study. This conclusions would provide basic information for improving agricultural safety production in the contaminated farmland. The variation of percentage of available Cd also suggested that the chemical fractions of Cd in contaminated soil was also governed by CEC, redox conditions, organic and inorganic substances, biological and bacterial activities, etc. (Fierer, 2017; Shahid et al., 2016).

Soil bacterial communities are obviously an essential component for both natural and human-managed ecosystems, no matter whether contaminated or not (Fierer, 2017). There is a long-range study that describes the microbial inhabitation in soil, their effect on soil fertility and their influence on heavy metals toxicity. But, the questions of how

Fig. 4. Redundancy analysis (RDA) of environmental parameters with the microbial community abundance at the contaminated and referenced soil. Arrows present the magnitude and direction of environmental factors associated with bacterial community structure. "p-values" indicate the results of significance test of difference among environmental factors and soil microbial community.

	p-values
pH	0.021
CEC	0.006
OC	0.032
TN	0.015
AN	0.056
TP	0.005
AP	0.154
TK	0.515
AK	0.043
T-Cd	0.006
A-Cd	0.003
Ci	0.012

Fig. 5. Heatmap of Spearman correlation on phyla combined with UPGMA cluster analysis between the relative abundance of series of bacteria phyla and samples physicochemical parameters, including the relative richness (n = 3) of the top 30 microbial phyla. Note: Horizontal ordinate represent soil physicochemical properties and Cd information, vertical ordinate represent bacterial community abundance information. Negative correlation and positive correlation were represented by blue color and red color. * indicates $0.01 < p \leq 0.05$, ** indicates $0.001 < p \leq 0.01$, *** indicates $p \leq 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

to manage the soil microorganisms to enhance soil fertility and quantity, and how bacterial communities will respond to heavy metal contaminated soil ecosystem remain unclear (Fierer, 2017). Recent methodological development provided opportunities to chart complex microbial community structure and potential mechanisms for the full variation with dominant environmental parameters (Wu et al., 2017). 16S rRNA-based high-throughput sequencing is now relatively common tool for in-depth exploring soil microbial community composition and abundance (Bhakta et al., 2018; Fierer, 2017). Toxic stress of heavy metal(loid)s would adversely influence the diversity and abundance of soil sensitive microorganisms, while resistant bacteria could readily adapt and increase in abundance (Xu et al., 2018; Zhao et al., 2019), thereby structuring microbial community (Guo et al., 2017). A suite of studies have shown that heavy metals (Cd, Pb and Hg) were negatively correlated with microbial abundance in farmland soil (Bo Jiang et al., 2019; Huang et al., 2017; Wu et al., 2017). Yin et al. (2015) also observed that the diversity and abundance of microbial communities in studied soil were decreasing with long-term contamination. However, according to the sequence data obtained by Alpha-diversity (Tab. S3) and correlation analysis between Cd and abundance of bacterial community (Tab. S6). T-Cd, A-Cd and Ci were positively correlated with microbial abundance at various contaminated levels ($1.07 < Ci < 6.42$). Rarefaction curves (Fig. S2) also showed a higher abundance in the contaminated soils than reference soil. The dominating bacterial phyla detected in studied region, in the present research (Tab. S4 and S6), were analogous to other studies in heavy metal contaminated farmland or e-waste sites, in which *Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Nitrospirae, Gemmatimonadetes* and *Verrucomicrobia* were regularly observed (Bo Jiang et al., 2019; Guo et al., 2017; Jun et al., 2015; Kandeler et al., 2000; Wu et al., 2017). Some of those bacterial phyla, including *Proteobacteria* (Kevin et al., 2003), *Nitrospirae* (Wu et al., 2017) and *Acidobacteria* (Li et al., 2017), could resist metal(loid) toxicity depending on their complexation and adsorption capacities. A range of physiochemical and biotic factors, including the amount of organic carbon, pH, CEC, available N, P, K, etc. and the richness of microbial predators, could also affect the amount of microbial biomass (Fierer, 2017). The above conclusions might provide a new perspective for the screening and application of indigenous resistant bacteria. Wu et al. (2017) has summarized that TP, moisture content, TOC were identified as more important factors to structure the microbial communities than Cu, Pb in e-waste recycle region. These might be the reason why the microbial abundance of contaminated soil is higher than that of reference soil in this study. Taxonomic analysis (Fig. 2) and Kruskal-Wallis H test (Fig. S3) among different groups indicated that there were no significant differences in bacterial communities at the phyla level with Cd contamination level, and alpha-diversity analysis (Shannon and Simpson) also showed the same conclusion. Similarly, Wu et al. (2017) indicated that microbial diversity had a weak correlation with heavy metal contamination. Some research have demonstrated the scientific consensus that some metals (Fe, Ni and Cd) could accelerate microbial metabolism process and their toxicity was dose-dependent (Oladipo et al., 2018). Meanwhile, most microorganisms could tolerate varying degree of Cd toxicity through extracellular precipitation, cell wall adsorption, enzymatic oxidation and intracellular complexation (Dong et al., 2007). Taken together, these proved conclusions might be the reason why soil microbial community composition was not significantly influenced by Cd pollution ($1.07 < Ci \leq 6.42$) in farmland, although that heavily polluted soil (T2, T5, T6, T7) seriously affected the quality and security of agricultural products (Kim et al., 2017).

However, the data of Analysis of Similarities (ANOSIM) (Fig. S4), PCA and RDA illustrated that physiochemical factors and Cd were important factors associated with microbial diversity in the studied region. In this study, the Cd contaminated index of contaminated samples were 2.97 ± 0.08–6.42 ± 0.31. Unlike essential elements (Zn and Fe), Cd has not proved biological effects on metabolism and biosynthesis

processing (Mahbub et al., 2017). Recent studies have verified that dose-dependent toxicity of Cd and bacterial sensitivity difference might cause the different microbial communities (Bo Jiang et al., 2019; Mahbub et al., 2017; Wu et al., 2017). For instance, previous studies have reported that bacterial abundance significantly decreased with the high concentration Hg ($32\,mg\,kg^{-1}$) in soils, whereas it enhanced at $3.2\,mg\,kg^{-1}$ (Wu et al., 2017). Moreover, Guo et al. (2017) has shown that *Ralstonia* and *Gemmatimonas* were negatively correlated with available Cd, Hg, Pb and pH ($p < 0.05$), but there was a positive correlation between *Unidentified-Nitrospiraceae, Blastocatella, Acidobacteria* and Pb, Cd, Zn and OC ($p < 0.01$). The hypothesis of Connell (1978) that heavy metal (Zn, Cu, Cd, Hg) might enhance microbial abundance at low concentrations was supported by these studies. In the present research, the Cd dosage in studied areas was not high enough to inhibit the bacterial activity, and the index of Chao in Alpha-diversity was positively related with soil T-Cd, A-Cd (Tab. S6). Moreover, the main abundant bacterial phyla (*Chloroflexi, Actinobacteria, Nitrospinae*) in contaminated soils (0.89 ± 0.12–$1.93 \pm 0.10\,mg\,Cd\,kg^{-1}$) were positively and significantly correlated with A-Cd, which was well compared with previous studies. However, the highest abundance of bacterial community was detected in T3 ($1.37 \pm 0.09\,mg\,Cd\,kg^{-1}$). So it could be concluded that the synergistic effect of bio-stimulating with OC, TP, TN, TK and Cd stress will structure the microbial community (Kevin et al., 2003; Rojjanateeranaj et al., 2017).

OC could significantly structure the bacterial community in studied farmland. Guo et al. (2017) and Huang et al. (2017) also have proven that OC played a vital role in bio-stimulating and governing metals (Cd, Pb, Cr) fractions in contaminated soil. In present study (Fig. 5 and Tab. S6), OC was significantly and positively correlated with Chao and Ace indexes and the amount of certain microorganisms, such as *Nitrospinae, Chloroflexi, Latescibacteria*, etc. on phyla level and *Anaerolineaceae, norank-OPB35* on genera level. Therefore, the changes in microbial communities structure might be caused by the effects of bio-stimulating of carbon source and the reduction of Cd bioavailability by Cd^{2+} binding with OC. Wu et al. (2017) have documented that bacterial communities could be driven by multiple factors. Fig. 4 and 5 indicated TP as an important factor in shaping soil bacterial community (closely followed by pH). Furthermore, TN and AN also were known as dominated factors in structuring microbial community because their crucial functions in cellular metabolism processes, such as energy metabolism, protein synthesis, cell division, etc. (Rajan et al., 1991; Shahid et al., 2016). Therefore, TP, TN and AN have positive correlation with abundance of bacterial communities (Tab. S6). Several physicochemical and biochemical parameters (adsorbing, pH, microbial activity, etc.) would significantly affect the content of available phosphorus in realistic farmland (Zhang et al., 2013). The application of phosphorus fertilizer and its aging effect are the main factors that determine the fate of phosphorus in farmland soil. Long-term anthropogenic phosphate fertilizer additions caused the highest AP content in reference soil compared with contaminated samples (Wang et al., 2018a; Xin et al., 1997). However, the lowest relative abundance was observed in CK. In this case, AP was negatively correlated with microbial abundance and diversity in studied soils with statistical analysis.

5. Conclusion

The present study provided an original insight into the abundance and composition of bacterial communities based on 16 rRNA sequencing in Cd contaminated farmland around an enterprise-intensive region. Considerable differences in soil microbial relative abundance were found with different Cd contaminated levels ($1.07 \pm 0.13 < Ci \leq 6.42 \pm 0.31$). But there was no significant difference between reference soils with contaminated soils at genus and phyla level. Subsequent further analysis (PCA, RDA, UPGMA cluster analysis, ANOSIM, etc.) revealed that bacterial communities were shaped by soil physiochemical parameters and Cd pollution, and pH, TP, TN, A-Cd and

CEC were verified as the five vital parameters. We also found that *Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria* and *Nitrospirae* were the most dominant phyla in contaminated soils. And the bacteria of *Actinobacteria, Chloroflexi, Nitrospirae* on phyla level and *Anaerolineaceae, KD4-96, norank-SJA-15* on genus level were positively and significantly correlated with A-Cd in studied area. These findings might provide a new perspective for the ecological assessment of Cd contaminated farmland in natural environment. The further research will focus on how to establish the most suitable living ecosystem for exogenous tolerant bacteria, and understand how information were summarized in this study could assist researchers to evaluate the various effects that bacterial communities might have on Cd contaminated farmland soils.

Declaration of competing interest

We declare that we have **NO** financial and personal relationships with other people or organizations that can inappropriately influence our work, there is **NO** professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "Ecological responses of soil microbial abundance and diversity to cadmium and soil properties in farmland around an enterprise-intensive region".

CRediT authorship contribution statement

Huakang Liu: Conceptualization, Investigation, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Can Wang:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Yanluo Xie:** Methodology, Data curation. **Yao Luo:** Investigation, Methodology. **Mingping Sheng:** Investigation, Data curation. **Fei Xu:** Software, Investigation, Visualization. **Heng Xu:** Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.122478.

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