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Changes in soil available cadmium and bacterial communities after fallowing depend on contamination levels

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Abstract

Purpose Fallowing is an improvement process aimed at promoting the soil health and the sustainability of cultivated land. How it affects the soil health-related abiotic properties and microbial community composition under different levels of cadmium (Cd) contamination is not well known.

Materials and methods This study involved applying consistent fallowing practices to paddy soils of low and high Cd content, in order to examine changes in the soil Cd, nutrient, and microbial community using a high-throughput sequencing method.

Results and discussion Fallowing significantly decreased available Cd and phosphorus (P) content, but increased ammonium nitrogen (N) on lightly contaminated plots, whereas only P variables changed significantly for heavily contaminated plots. Furthermore, fallowing significantly decreased bacterial Shannon diversity on lightly contaminated plots and altered bacterial community composition on heavily contaminated plots, but it had no impact on archaeal or fungal communities, indicating that bacteria are more sensitive to fallowing than archaea and fungi. Specifically, fallowing significantly promoted some copiotrophic bacteria (*Alphaproteobacteria* and *Betaproteobacteria*) but suppressed some oligotrophic taxa (*Chloroflexi* phylum and OTU5837 belonging to the phylum *Acidobacteria*) on highly contaminated plots. Interestingly, the soil microbial community in the lightly contaminated plots was mainly affected by soil pH, C, and N properties, whereas on heavily contaminated plots, it was largely influenced by soil Cd and P variables.

Conclusions Overall, these findings showed that fallowing significantly improved soil N availability but decreased Cd availability in lightly contaminated conditions, while shifts in microbial community composition under heavily contaminated conditions may indirectly enhance soil nutrient availability and reduce available Cd. These findings highlight the significance of fallowing to promote the health and the sustainability of Cd-contaminated cultivated land.

Keywords Fallowing cultivated land · Available cadmium · Microbial community · Life strategy · Soil health

1 Introduction

Healthy soil is the key to maintain the sustainability of agricultural ecosystem services, and ensure food security (Lehmann et al. 2020; Lu et al. 2020; Williams et al. 2020). With rapid growth in grain production, cultivated land is facing challenges to sustainable utilization, such as heavy metal (i.e., cadmium (Cd), lead, and arsenic) contamination and desertification (Liao et al. 2019, 2020; Qu et al. 2016; Shi et al. 2019; Zhao et al. 2015). Cultivated land in China is less than the world average, so protecting it from heavy metal contamination and degradation attracted significant attention in the government's agenda (Zhao et al. 2015). China has been conducting an experimental fallow policy, stop farming for a while (Gilley et al. 2001), to reduce problems on agricultural ecosystems in five pilot provinces since 2016 (Shi et al. 2019). Both of the abiotic factors (i.e., available heavy metal and nutrients content) and biotic factors (i.e., microbial diversity and community) are good indicators of soil sustainability and health because they mediate soil functions related to biogeochemical processes (Aponte et al. 2020; Lu et al. 2020;

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Wang et al. 2020). Most previous studies associated with fallowing have focused on management evaluation, operational analysis, and mapping of fallow land (Shi et al. 2019). However, the potential effects and underlying mechanisms of fallowing practices on soil abiotic and biotic factors related to soil health in Cd-contaminated agricultural systems have rarely been investigated.

A fallowing policy has been implemented by stopping rice farming, planting a green manure (typically *Astragalus sinicus* L.), and reducing Cd availability by the application of lime to improve the quality of cultivated land in Hunan province, China. These fallowing practices may directly enhance soil quality and health. For example, liming, by increasing soil pH, could decrease the mobility of Cd (precipitation of Cd) and thus reduce the toxicity of Cd to crop plants (Hong et al. 2010). Furthermore, planting *Astragalus*, which is a nitrogen (N)-fixing plant, may increase the N for plants and labile substrates for microorganisms, so improving soil quality (Yang et al. 2019). It has been shown that fertilizers are the sources of heavy metals (Zhao et al. 2015; Marini et al. 2020; Pizzol et al. 2014). For example, some imported phosphorus (P) fertilizer used to make compound fertilizers can contain considerable amounts of Cd (Luo et al. 2009; Marini et al. 2020; Pizzol et al. 2014). Therefore, the input of Cd and P may together be reduced by stopping farming and fertilizer applications. A report on the current status of soil Cd contamination in China showed that 13.5% of the soil samples analyzed were lightly contaminated (1~3 times of the standard (0.3 mg kg^{-1}) in GB15618-1995) and 0.5% were heavily contaminated (> 5 times the standard) (Ministry of Environmental Protection of the People's Republic of China (MEP) 1995, Ministry of Land and Resources of China (MLR) 2014). Therefore, it is necessary to determine whether fallow practices had similar effects on abiotic factors, such as soil Cd and nutrient contents at different contamination levels, and thus provide more useful information for the health management of Cd-contaminated cultivation land.

Soil microorganisms (bacteria, archaea, and fungi) play crucial roles in soil biogeochemical cycling (Liang et al. 2020; Luo et al. 2019; Torsvik and Øvreås 2002; Wang et al. 2018; Wei et al. 2020) and bioremoval of heavy metals in water and soils (Liao et al. 2019, 2020; Yang et al. 2018a, 2018b). Detection of changes in microorganisms is considered a more practical form of assessment than measuring abiotic properties for early identification of soil degradation or improvement (Garau et al. 2007) and soil health (Chaparro et al. 2012). Fallowing may alter microbial diversity by changes in available Cd because high Cd level leads to lower bacterial diversity indices (Lin et al. 2019; Lu et al. 2020; Luo et al. 2019). *Acidobacteria* and *Chloroflexi* are associated with low-quality resources, high tolerance (to environmental stress, such as heavy metal stress), and have been typically classified as oligotrophic, whereas *Proteobacteria* (in particular the *Alpha*- and *Betaproteobacteria* classes) and *Actinobacteria* are related to decomposition in resource-rich environments and are generally classified as copiotrophic (Fierer et al. 2007; Ho et al. 2017; Yao et al. 2017). Fallowing practices could influence microbial community composition and diversity by altering Cd and substrate availabilities (Cui et al. 2018; Vig et al. 2003; Wang et al. 2018; Xiao et al. 2020). For example, the relative abundance of some copiotrophic microbes in fallow soils may be higher than in paddy soils because the competition for nutrients between plants and microbes and Cd stress in the former may be lower than in the latter. However, the opposite result could also be true, since the quality and quantity of substrate from fertilization and rice roots may be less in fallow soils than in paddy soils. Furthermore, it has been shown that total and available Cd concentration were the major contributing factors influencing bacterial community in highly Cd-contaminated soils, while pH was the major factor in soils with lower Cd concentrations (Luo et al. 2019). However, the extent to which the microbial communities and their influencing factors are different between Cd contamination levels after fallowing is unclear. Figuring out the net effects of fallowing on the microbial community and the underlying drivers in different Cd-contaminated levels is of great significance to provide novel insight for soil health management.

Taken together, the comprehensive effects of fallow on soil health-related abiotic (available Cd, nutrient content) and biotic factors (the microbial community composition and diversity) factors under different Cd contamination levels are poorly understood and deserve further exploration (Luo et al. 2019). Our work was conducted to examine the fallow-induced changes in soil abiotic and biotic factors, by studying situations with similar fallow practices on paddy soils of low and high Cd content. Specifically, we addressed the following questions: (1) How do fallow measures affect the soil Cd content and nutrient status? (2) How do fallow practices influence the soil microbial community composition? (3) Do changes in soil microbial communities under different Cd contamination levels have different influencing factors?

2 Materials and methods

2.1 Experiment design

The experiment was conducted in Zhuzhou, Hunan province, China. This region has some emission sources of Cd, such as mining and smelting industries. Atmospheric deposition is the major contamination source for agricultural soils. In November 2019, two representative agricultural sites with different degrees of Cd contamination were selected: Chaling county (CL, 26.82°N, 113.53°E) and Zhuzhou county (ZZ, 27.67°N, 113.13°E), designated as light (~ 0.8 mg kg^{-1}) and heavy (~2.5 mg kg^{-1}) contamination level, respectively. At each location, a nearby 3-year fallow site was selected that had similar soil type, texture, and drainage. Five 20 m × 20 m replicates of paddy and fallow soils were established in both the low and high Cd contamination sites. The specific fallow measures were all carried out in the first year and were as follows: firstly, deep plowing up to 15–20 cm; secondly, application of quicklime for 1.2 t hm^{-2}; and thirdly, planting of green manure (*Astragalus sinicus* L.).

2.2 Soil sampling and chemical analysis

Soil from 0 to 15 cm was sampled. Eight soil cores were randomly collected from each replicate plot and were mixed thoroughly to form a composite sample. Soil samples were sieved (through a 2 mm mesh screen) to remove stones, plant residues, and other impurities. They were then separated into two parts, one stored at 4 °C and one at − 80 °C for physicochemical analysis and DNA extraction, respectively.

Soil Cd was measured in terms of total and available fractions. For total Cd, the soil was treated with aqua regia digestion using HNO_3 and HCl, and available Cd was extracted by EDTA. Soil pH was measured using a pH meter (Mettler-Toledo FE28, Switzerland) in a soil:water mix of 1:2.5 (weight:volume ratio). Soil organic carbon (C) was measured using the dichromate oxidation method (Walkley and Black 1934). Soil N and P properties were measured according to Olsen et al. (1954) and Page et al. (1982). Specifically, soil ammonium (NH_4^+) and nitrate (NO_3^-) were extracted with KCl. Soil available phosphorus (P) was extracted by $NaHCO_3$. Soil total N and P were extracted by digesting the soil sample with H_2SO_4. All the extracted solutions were analyzed for the N and P concentrations with an Auto Analyzer (BRAN + LUEBBE-AA3, Germany).

2.3 DNA extraction and high-throughput sequencing

The FastDNA Spin Kit for soil was used to extract soil DNA (MP bio, CA, USA). NanoDrop (ND-1000, USA) was used to analyze DNA concentration and OD260/OD280 ratio. After being amplified by PCR, the products were sequenced targeting the V3-V4 hypervariable regions of the 16S rRNA gene and ITS1 region of the fungal ITS gene at the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). For bacteria, the 16S rRNA genes were amplified with the primer 338F_806R. For archaea, the 16S rRNA genes were amplified with the primer 524F10extF_Arch958RmodR. For fungi, the ITS1 regions were amplified with the primer ITS1F_ITS2R. Refer to SI (Table S1) for the details of PCR system and primers.

2.4 Statistical analysis

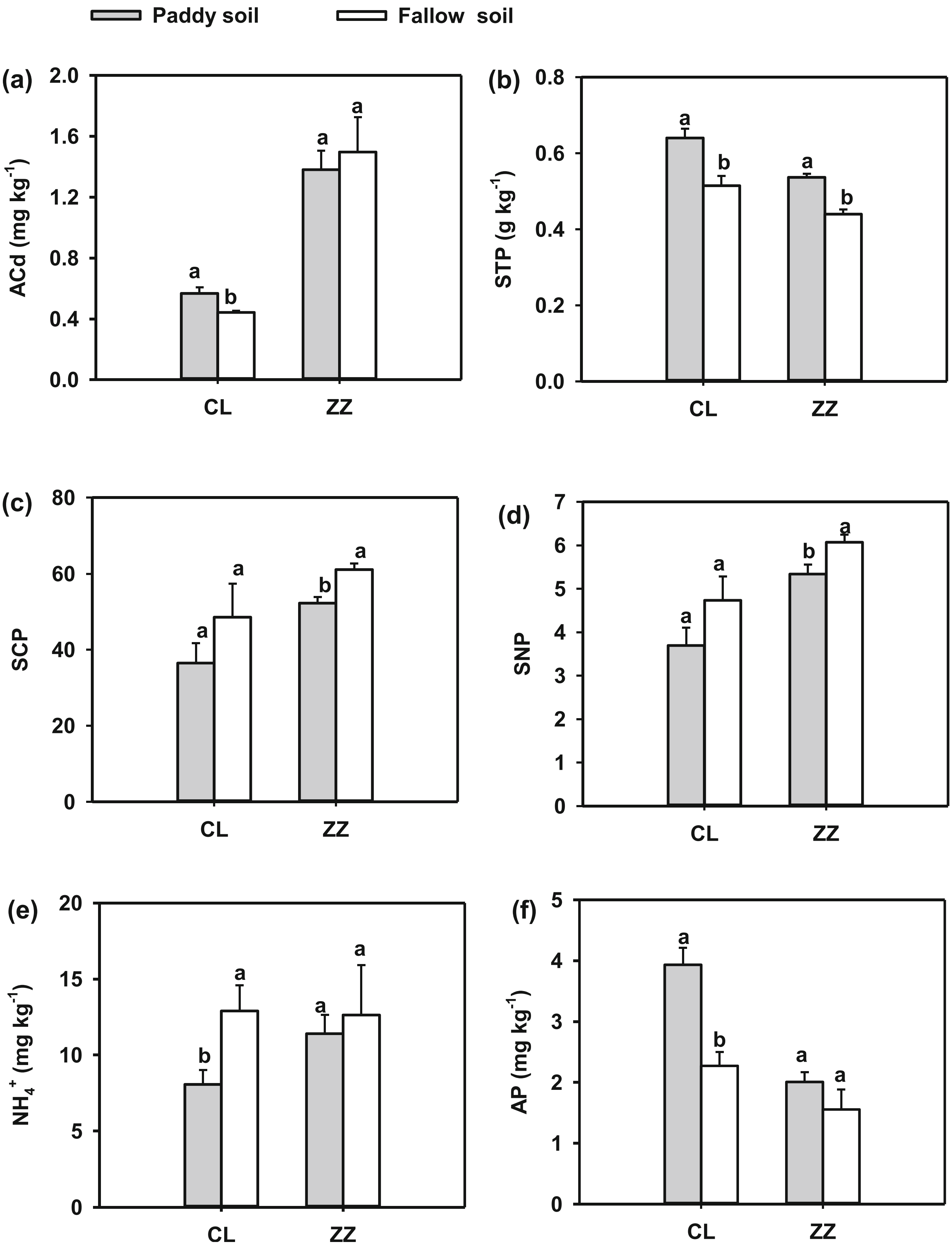
The significance of differences in soil properties between paddy and fallow soils was detected with the *t* test performed in SPSS 22.0 ($\alpha = 0.05$). A linear regression procedure was applied to examine relationships between soil abiotic factors. The difference of relative abundance of the microbial community between paddy and fallow soils was examined by statistical analysis of taxonomic and functional profiles (STAMP). Principal coordinates analysis (PCoA) and analysis of similarities (ANOSIM) were used to visualize and determine community differences ($\alpha = 0.05$). The sequences of the corresponding OTUs were determined by doing a BLAST from National Center for Biotechnology Information (NCBI) at 97% similarity level. The Mega 5.0 software was used to set up the phylogenetic tree. Spearman correlation analysis and redundancy analysis (RDA) were carried out between microbial parameters (Chao, Shannon, dominant genera of bacteria, archaea, and fungi) and the physicochemical properties.

3 Results

3.1 Comparisons and relationships of soil biotic properties between paddy and fallow soils

Some of the selected soil abiotic properties are significantly different between paddy and fallow soils (Fig. 1). For the soil Cd concentration, the mean value of available Cd in the paddy soil is significantly higher (22.0%) than in the fallow soil on lightly contaminated plots ($P < 0.05$), whereas this difference under heavily contaminated plots is not significant ($P > 0.05$; Fig. 1a). For the nutrient factors, soil total P content of the paddy soil is 19.7% and 17.9% greater than that of fallow soil under light and heavy contamination, respectively ($P < 0.05$; Fig. 1b). The soil C:P and N:P ratios of the paddy soil are significantly lower than those in the fallow soil in the heavily contaminated plots ($P < 0.05$; Fig. 1c and d). Additionally, there is 59.7% higher soil NH_4^+ in fallow soil than in paddy soil ($P < 0.05$; Fig. 1e), while the available P content of the paddy soil is significantly greater than that of the fallow soil in the lightly contaminated plots ($P < 0.05$; Fig. 1f). However, the total Cd concentration, soil pH, and other soil abiotic factors (soil organic C, dissolved organic C, total soil N, NO- 3) did not differ significantly between paddy and fallow soils (Fig. S1).

The available Cd in the lightly contaminated plots is significantly and only positively correlated with available P (Fig. 2a), whereas that in the heavily contaminated plots is positively related to NH_4^+ (Fig. 2b). Furthermore, the total Cd in the heavily contaminated plots also had positive relationships with soil organic C, total N, NH_4^+, and NO_3^- (Fig. 2c–f).


Paddy soil
Fallow soil
(a)
ACd (mg kg^{-1})
2.0
1.6
1.2
0.8
0.4
0.0
a
b
a
a
CL
ZZ
(b)
STP (g kg^{-1})
0.8
0.6
0.4
0.2
0.0
a
b
a
b
CL
ZZ
(c)
SCP
80
60
40
20
0
a
a
b
a
CL
ZZ
(d)
SNP
7
6
5
4
3
2
1
0
a
a
b
a
CL
ZZ
(e)
NH$_4^+$ (mg kg^{-1})
20
15
10
5
0
b
a
a
a
CL
ZZ
(f)
AP (mg kg^{-1})
5
4
3
2
1
0
a
b
a
a
CL
ZZ

◀ **Fig. 1** Soil abiotic properties between paddy and fallow plots (mean ± SE, $N = 5$). ACd = available Cd; STP = soil total P; SCP = soil C:P ratio; SNP = soil N:P ratio; AP = available P. CL = the lightly contaminated plots; ZZ = the heavily contaminated plots. Different lowercase letters indicate a significant difference at 0.05 level

3.2 Comparisons of α-diversity of soil microbial community between paddy and fallow soils

By clustering at the 97% similarity level, 2240 bacterial operational taxonomic units (OTUs), 425 archaeal OTUs, and 917 fungal OTUs were obtained from 20,946, 27,082 16S rRNA gene sequences, and 28,628 ITS gene sequences, respectively.

With regard to lightly contaminated plots, the Shannon diversity of the bacterial community for the paddy soil is significantly more diverse than that for the fallow soil, but differences between Chao diversity are not distinct (Fig. 3a). For heavily contaminated plots, there is no significant difference between either the Chao or Shannon diversity (Fig. 3b). Additionally, the Chao and Shannon differences of archaeal and fungal on lightly and heavily contaminated plots are not significant (Fig. S2).

3.3 Comparisons of β-diversity and relative abundance of soil microbial community between paddy and fallow soils

Principal coordinates analysis (PCoA) and analysis of similarities (ANOSIM; Table S2) at genus level show that there was a significant difference in the β-diversity of bacterial community between paddy and fallow soil in heavily contaminated plots ($P < 0.05$), while those differences in lightly contaminated plots are not significant ($P > 0.05$; Fig. 4a). However, the archaeal and fungal communities at the phylum or genus level are not clustered distinctly between paddy and fallow soils in either light or heavy contamination conditions (Fig. S3 and Table S2).

The main phyla in the bacterial community of paddy and fallow soils are largely the same: *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, and *Acidobacteria* dominated and accounted for about 30.6~42.2%, 11.9~19.2%, 8.4~16.7%, and 13.1 ~ 16.2% of all the sequences, respectively (Fig. S4). There are two genera (*norank_c__Acidobacteria* and *norank_f__Nitrosomonadaceae*) significantly different between paddy and fallow soils in the heavily contaminated plots (Fig.

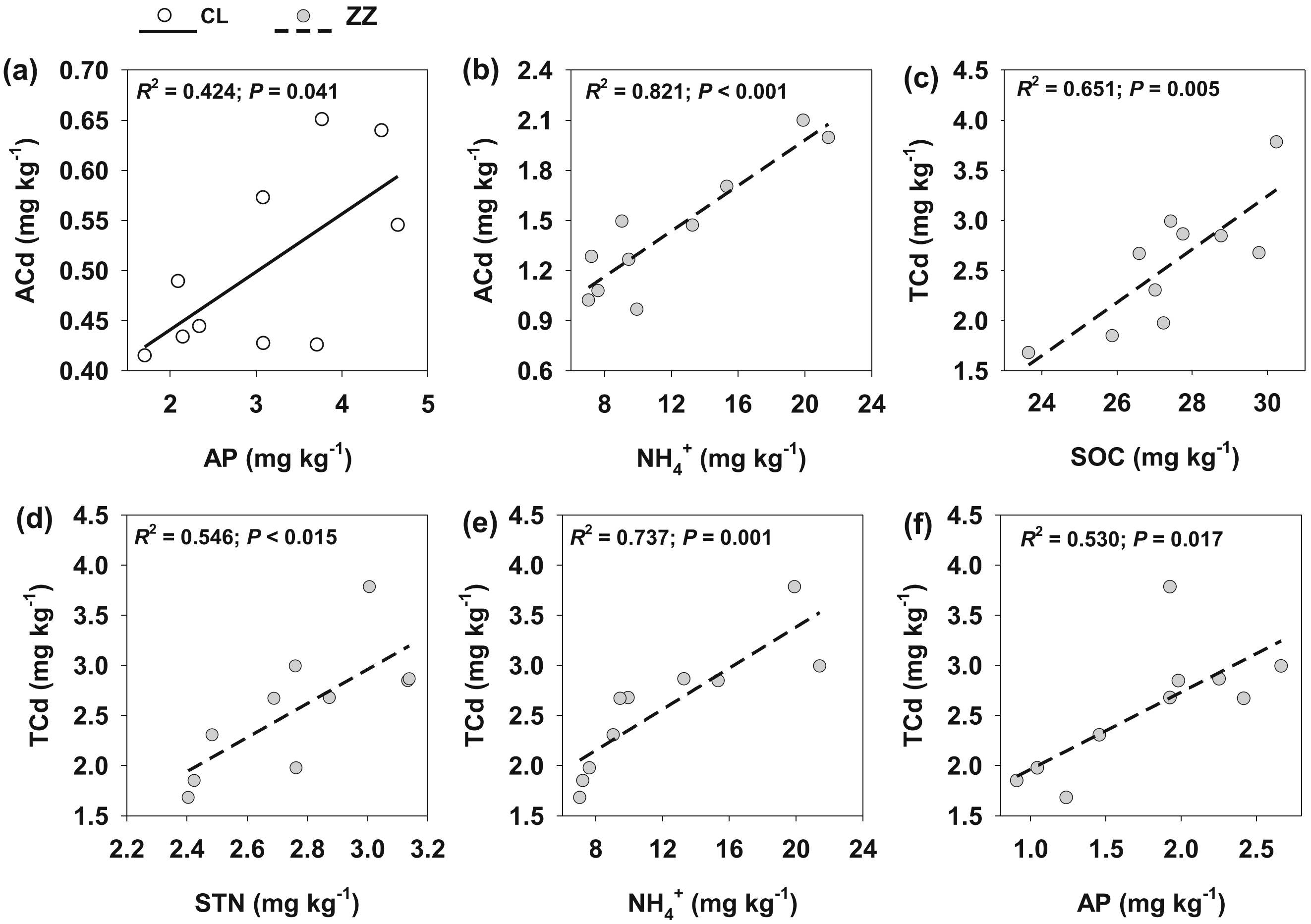


Fig. 2 Relationships between cadmium and nutrient variables. CL = the lightly contaminated plots; ZZ = the heavily contaminated plots. ACd = available Cd, TCd = total Cd; AP = available P; SOC = soil organic C; STN = soil total N

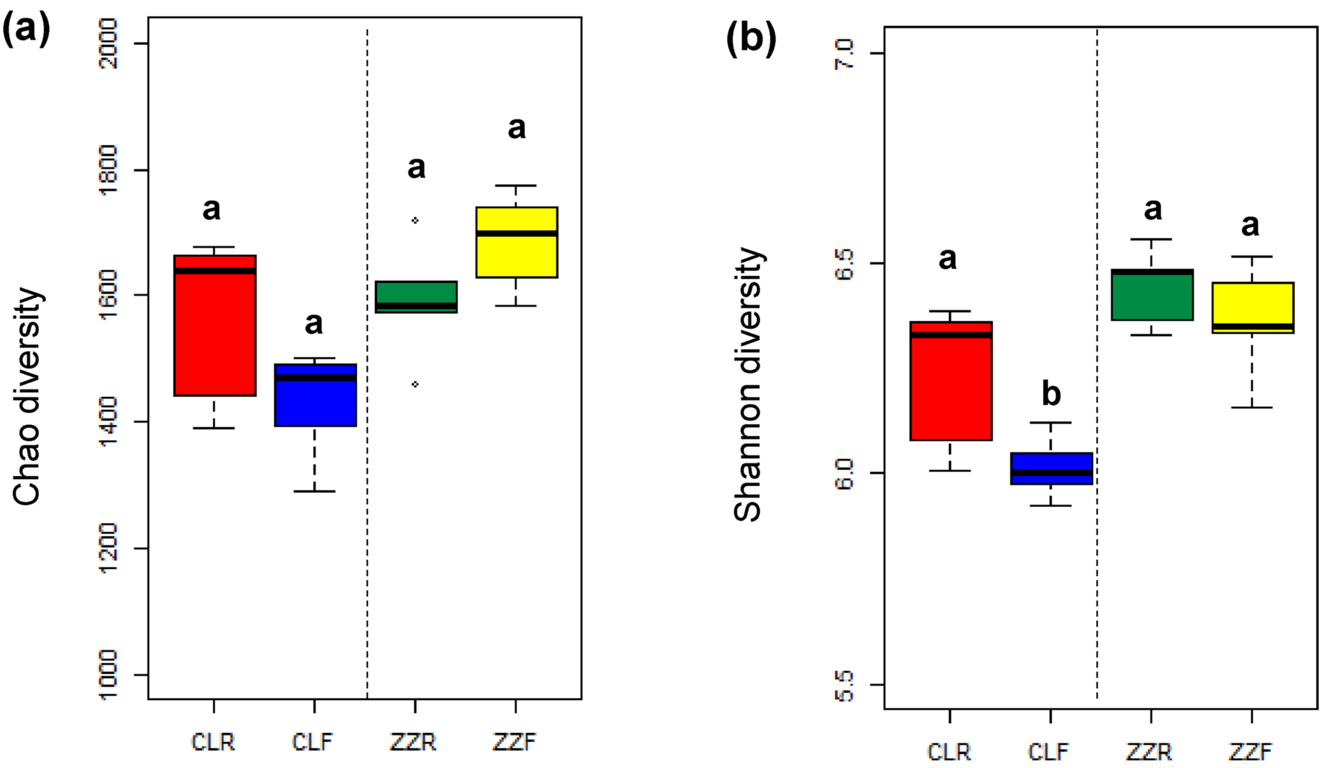


Fig. 3 Comparisons of the estimated value of bacterial Chao and Shannon diversity index between paddy and fallow soil. $N = 5$. CLR = lightly contaminated paddy soil; CLF = lightly contaminated fallow soil; ZZR = heavily contaminated paddy soil; ZZF = heavily contaminated fallow soil. Different lowercase letters indicate a significant difference at 0.05 level

4b). Furthermore, *Chloroflexi* and *norank_c__Acidobacteria* are 26.5% and 35.6% more abundant at the phylum/genus level in the paddy soil than in the fallow soil, respectively (Fig. 4c). However, *Alphaproteobacteria*, *Betaproteobacteria*, and *Syntrophobacterales* are 72.9%, 35.9%, and 47.6% less abundant at the class/order level in the paddy soil than in the fallow

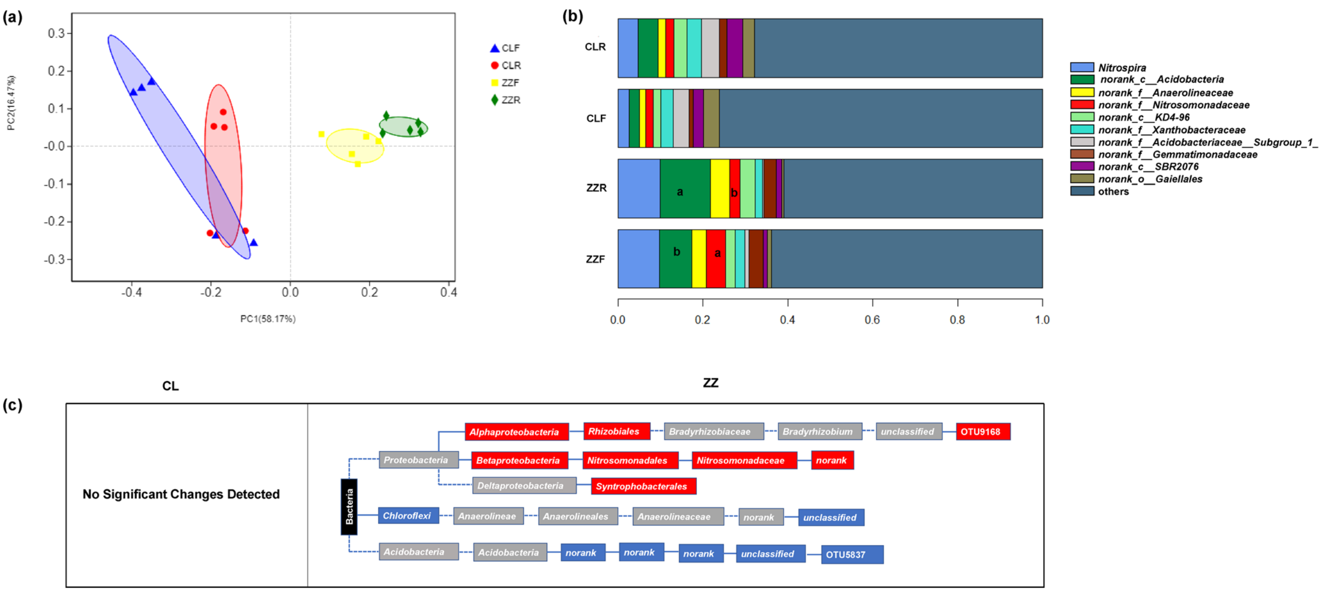


Fig. 4 Comparisons of the soil bacterial community between paddy and fallow soil. PCoA of the bacterial community at the genus level (**a**). The relative abundance of the bacteria community at genus level (different lowercase letters indicate a significant difference at 0.05 level; **b**). Significant differences in taxa from STAMP differential abundance testing (taxa in red indicates lower abundance in the paddy soil relative to the fallow soil, while taxa in blue indicates higher relative abundance in the former than the latter; **c**). CLR = lightly contaminated paddy soil; CLF = lightly contaminated fallow soil; ZZR = heavily contaminated paddy soil; ZZF = heavily contaminated fallow soil. CL = the lightly contaminated plots; ZZ = the heavily contaminated plots

soil in the heavily contaminated plots (Fig. 4c). To further characterize the differences in bacterial community between paddy and fallow soils, OTUs are selected and analyzed by STAMP (Fig. S5). The fallow soil had a 3.5× higher relative abundance of OTU9168, which had a high degree of similarity with *Bradyrhizobium* sp. Conversely, the relative abundance of OTU5837 in the paddy is significantly higher (2.7×) than in the fallow soil, and it is similar to *Acidobacteria bacterium* (Fig. S5 and Fig. 4c). However, there is no significant difference in relative abundance of the bacterial community on lightly contaminated plots (Fig. 4c).

The dominant archaeal phyla (*Bathyarchaeota*, *Euryarchaeota*, and *Thaumarchaeota*) and genera show no significant difference between paddy and fallow soils; the dominant fungal phyla (*Ascomycota* and *Basidiomycota*) and genera also did not significantly differ between paddy and fallow soils (Fig. S4), further supporting the evidence that fallowing did not significantly alter the communities of archaea and fungi.

3.4 Relationships between the bacterial community and abiotic properties

To further figure out the influencing factors on changes in bacterial community to fallowing at light and heavy contamination levels, relationships between the bacterial community and abiotic properties were conducted. Since the differences in archaeal and fungal community between paddy and fallow soils were not significant, their potential influencing factors were not analyzed.

The RDA results indicated that soil pH, C, and N variables (i.e., the stoichiometry of C:N:P, dissolved organic C, total N content) are the major influencing factors for the microbial community on lightly contaminated plots (Fig. 5a), while available Cd, NH_4^+, and soil C:P are the most important influencing factors on heavily contaminated plots (Fig. 5b).

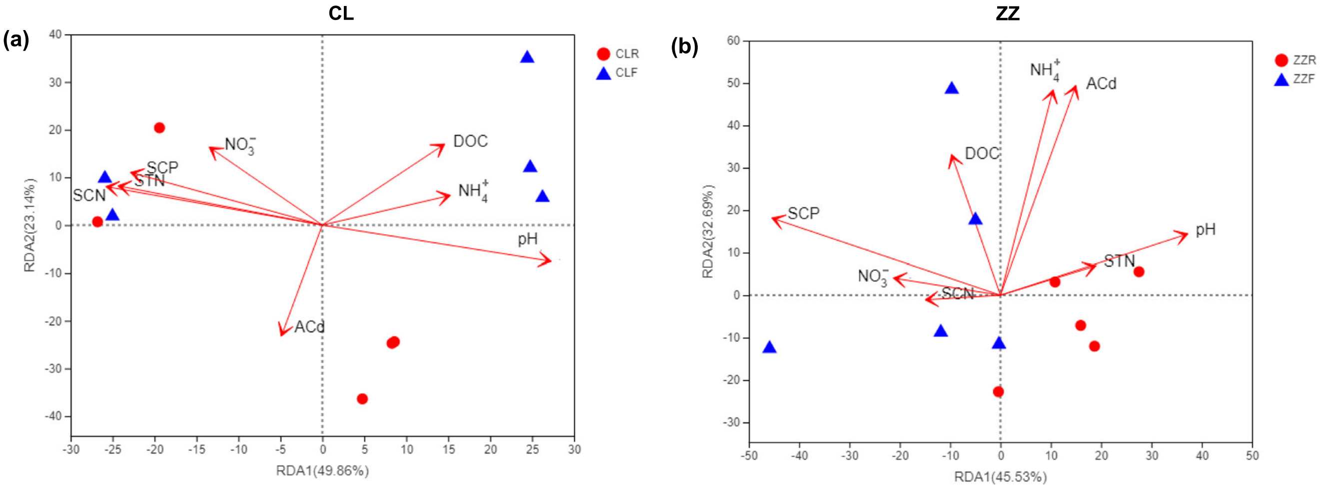
The factors contributing significantly to the α-diversity and the dominant bacteria vary with the contamination levels (Fig. 6). On lightly contaminated plots, the α-diversity of bacteria is widely and significantly correlated with soil variables (Fig. 6). Specifically, the bacterial Chao index increases with total soil P content and decreases with soil C:P ratio, N:P ratio, and NO_3^--N content, while the bacterial Shannon index increases with available Cd content on lightly contaminated plots (Fig. 6). With regard to the heavily contaminated plots, only the Chao of bacteria positively correlated with soil C:N, while the other correlations were not significant. Furthermore, nearly a quarter of the top 20 bacterial genera significantly related to soil C, total N content, and soil C:N ratio, while only two bacterial genera significantly correlated with total P content on lightly contaminated plots. With regard to the heavily contaminated plots, nearly half of the top 20 bacterial genera show significant relationships with soil P variables (soil total P, soil C:P, available P), whereas these genera had fewer correlations with soil organic C or total N content (Fig. 6).

4 Discussion

4.1 Fallowing directly reduced available Cd and increased available N at lightly contaminated plots but not at heavily contaminated plots

Our results show that fallowing reduced available Cd in the lightly contaminated plots (Fig. 1a), indicating that fallow practices may effectively reduce Cd toxicity for crop plants when contamination is light. The application of lime was supposed to increase soil pH and reduce available Cd (Hong et al. 2010), but the soil pH was not significantly influenced by liming in this study. This result could be partly attributed to the acidic nature of the soil and acidic rain in this region (Zhao et al. 2015) counteracting the influence of the lime. We found a positive relationship between available Cd and available P (Fig. 2a), indicating that P fertilizer might lead to some inputs of Cd on lightly contaminated plots (Marini et al. 2020; Pizzol et al. 2014). Another fallowing practice, planting *Astragalus*, is supposed to increase soil nutrients. We did find that there was higher soil NH_4^+ in the fallow soil than in the paddy soil on lightly contaminated plots.

For heavily contaminated plots, the available Cd is not affected by fallowing (Fig. 1a), suggesting that the reduction of available Cd at high contamination levels is a slow process. The positive relationships between Cd and soil organic matter (soil organic C and total N) at the heavily contaminated plots indicated that the inputs of Cd might be mainly derived from the organic fertilization during farming, such as applications of manure from livestock and poultry (Pizzol et al. 2014). The reduction of Cd in heavily contaminated soil may be significant if the fallowing is implemented for a longer time. Overall, these relationships between Cd and soil nutrient factors indicate that the changes in Cd content after fallowing are largely due to the absence of fertilization (Marini et al. 2020; Pizzol et al. 2014). The fertilizers previous applied in this region should be substituted by fertilizers with a low level of Cd (Zhao et al. 2015). Additionally, to effectively immobilize available Cd, soil amendments containing more sorption sites, such as biochar, could be applied to the paddy soils when fallowing (Luo et al. 2020; Palansooriya et al. 2020). Collectively, the fallowing practices could directly improve the soil abiotic factors that related to soil health (decreased Cd availability but increased N availability) when Cd-contaminated level was relatively low, whereas the direct effect of fallowing on the abiotic factors for the heavily contaminated conditions might be significant after longer fallowing time.



	R^2	*P*
pH	0.924	0.002
SCN	0.840	0.005
STN	0.759	0.009
SCP	0.738	0.014
DOC	0.566	0.032
NO_3^-	0.504	0.049
ACd	0.629	0.051
NH_4^+	0.305	0.281

	R^2	*P*
ACd	0.789	0.003
NH_4^+	0.724	0.013
SCP	0.699	0.014
pH	0.465	0.106
DOC	0.344	0.234
NO_3^-	0.131	0.607
STN	0.114	0.677
SCN	0.061	0.818

Fig. 5 Redundancy analysis (RDA) of bacterial community and soil biotic properties. After removal of the redundant variables, eight soil characteristics were chosen for redundancy analysis. ACd = available Cd; STN = soil total N; SCN = soil C:N ratio; SCP = soil C:P ratio; DOC = dissolved organic C

4.2 Distinct responses of microbial diversity and community to fallowing at different Cd contamination levels

Our results show that fallowing significantly altered the diversity and community structure of bacteria, but had no significant effect on archaeal or fungal communities (Figs. 3 and 4 and Fig. S2–4). The different responses of these three domains may be due to the fact that archaea generally have higher tolerance to heavy metal stress, and fungal cells, being usually larger than bacteria (Mueller et al. 2015), are supposed to be less easily influenced by the soil microenvironment.

Microbial community and diversity are crucial indicators related to soil health for evaluating ecosystem function (Lehmann et al. 2020; Lu et al. 2020; Torsvik and Øvreås 2002; Williams et al. 2020). The difference in bacterial

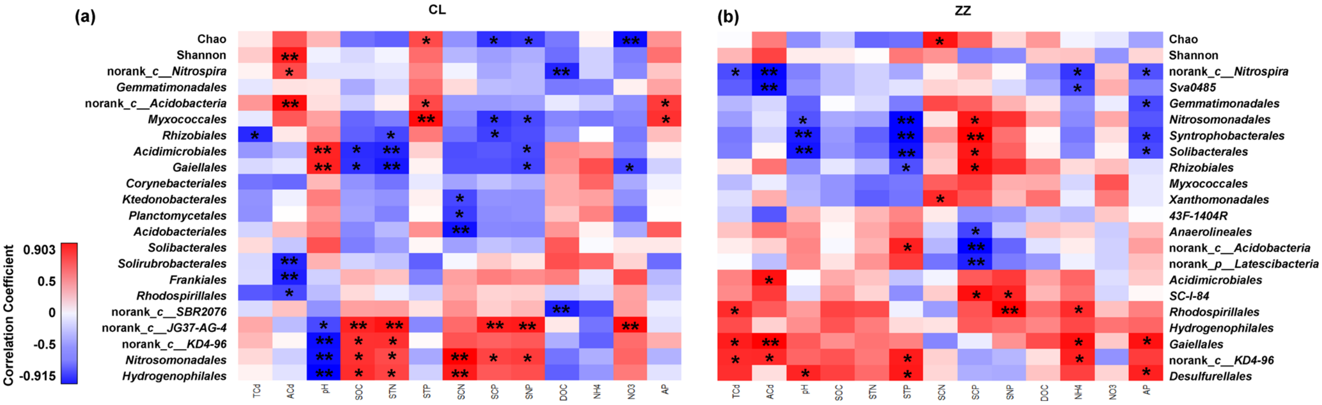


Fig. 6 Relationships between the microbial diversity, dominant genera, and soil properties. The red and blue colors indicate positive and negative relationships, respectively. The * and ** indicate a significant correlation for $P < 0.05$ and $P < 0.01$ levels, respectively. CL = the lightly contaminated plots; ZZ = the heavily contaminated plots; TCd = total Cd; ACd = available Cd; SOC = soil organic C; STN = soil total N; STP = soil total P; SCN = soil C:N ratio; SCP = soil C:P ratio; SNP = soil N:P ratio; DOC = dissolved organic C; AP = available P

diversity on lightly contaminated plots is distinct (Fig. 2), while the difference in richness between paddy and fallow soils is not significant, whether on lightly or heavily contaminated plots. These results indicate that the response of microbial richness to 3 years of fallowing in Cd-contaminated plots is also a slow process. More and more evidence has suggested that heavy metal contamination could decrease soil microbial diversity (Luo et al. 2019; Wang et al. 2019). Although the available Cd was higher for paddy soil than for fallow soil on our lightly contaminated plots, the Shannon diversity for the former is greater than for the latter (Fig. 2). Yan et al. (2019) also found that a lower concentration of Cd (< 3 mg kg^{-1}) was able to enhance microbial diversity. This is likely due to the Cd dosage in the studied areas not being high enough to inhibit bacterial diversity (Liu et al. 2020), and lower doses of Cd could promote some microorganisms (Liao et al. 2010; Oladipo et al. 2018). Furthermore, the higher diversity in the paddy soil may result from agricultural practices associated with a form of cultivation that provides more microbial niches, such as fertilizer application or the higher organic C turnover derived from rice production and litter inputs (Mackelprang et al. 2018).

In this study, *Proteobacteria* was the most abundant bacterial phylum both in paddy and fallow soils for lightly contaminated soil, consistent with the previous studies (Liu et al. 2016; Luo et al. 2019; Wei et al. 2020). However, lightly contaminated plots show no significant differences in the relative abundance of bacterial taxa between paddy and fallow soils (Fig. 4a–c). Madrova et al. (2018) also proved that the bacterial community on a lightly contaminated plot had higher resistance to change than in a heavily contaminated plot. We can therefore conclude that while changes in the α-diversity of bacteria in lightly contaminated soils occurred in response to fallowing, there was no linked change in the relative abundance of bacteria (Jenkins et al. 2017).

Contrary to the lightly contaminated soil, the β-diversity of bacteria in heavily contaminated soil is significantly influenced by fallowing practices (Fig. 4a), and the relative abundance of some taxa significantly changes (Fig. 4b, c). *Gammaproteobacteria* has been considered a signal phylum that is very sensitive to heavy metal contamination (Cui et al. 2018). Although there was a significant decline of available Cd in fallowed soil on the lightly contaminated plots (Fig. 1), the relative abundance of *Gammaproteobacteria* is not significantly decreased (Fig. 4c). However, the relative abundance of *Alphaproteobacteria* and *Betaproteobacteria* in the fallow soils is significantly higher than those in the paddy soils on heavily contaminated plots (Fig. 4c). These classes are both associated with C cycling and generally classified as copiotrophic microbes (Li et al. 2019). *Alphaproteobacteria* are known to be able to metabolize arabinose, potentially indicating an enhanced role for them in the decomposition of soil organic matters (Verastegui et al. 2014) and thus may lead to an increase in nutrient availability. Additionally, fallowing may promote N fixation, since approximately 16.8% of the increase of *Alphaproteobacteria* was in the OTU 9168, which may belong to the genus of *Bradyrhizobium* (Fig. 4c and Fig. S5). This is reported to be a genus of root nodule bacteria fixing N (Bourebaba et al. 2016). *Betaproteobacteria* are involved in the assimilation of C derived from cellulose (Verastegui et al. 2014). Fallow soil shows a significantly higher abundance of the *Nitrosomonadaceae* families, which belong to the *Betaproteobacteria* class (Fig. 4c). These can oxidize nitrite to nitrate, thus increasing nitrification activity. The greater abundance of *Nitrosomonadaceae* may be a response to the higher N availability derived from the planting of *Astragalus sinicus* L., which is an N-fixing plant. Therefore, although fallowing practices did not directly alter soil available N content, it had the potential to mediate soil N cycling by changing soil health-related biotic factors-microbial community compositions. On the other hand, the relative abundances of *Chloroflexi* and OTU5837 in the fallow soil are significantly lower than those in the paddy soil (Fig. 4c). *Chloroflexi* are generally considered oligotrophic microbes (Ho et al. 2017), and are predicted to degrade plant compounds, such as cellulose, starch, and long-chain sugars (Hug et al. 2013). Additionally, OTU5837 may belong to the *Acidobacteria* phylum (Fig. S5), also typically classified as oligotrophic microbes (Verastegui et al. 2014; Yi et al. 2019). Taken together, these results indicate that fallowing improves the relative abundance of some copiotrophic microbes but decreases the relative abundance of some oligotrophs in heavily contaminated soils. These shifts in microbial life strategy can further enhance soil nutrient availability and contribute to soil health and the sustainability of agricultural ecosystems (Liang et al. 2020).

4.3 Potential links between bacteria and soil properties depend on Cd contamination level

Variations of soil microbial community and composition are thought to be related to a number of soil biotic and abiotic factors (Deng et al. 2017; Liu et al. 2014; Yi et al. 2019). The main influencing factors for the microbial community were different between the low and high contamination plots. Specifically, the microbial community of the lightly contaminated plots was mainly affected by the soil pH, C, and N properties, whereas the microbial community of heavily contaminated plots was largely influenced by Cd and P availability.

Recent studies have found that Cd contamination of soil can cause a change of microbial community composition (Cui et al. 2018; Liu et al. 2020; Luo et al. 2019). However, the available Cd had no significant impact on the bacterial β-diversity by RDA analysis (Fig. 5), and the total Cd content had fewer relationships with the main genera in the less contaminated plots (Fig. 6). On the other hand, the bacterial β-diversity and the main genera in the heavily contaminated plots are significantly influenced by soil Cd (Fig. 6). Luo et al. (2019) also found that total Cd concentration and available Cd concentration were the strongest influencing factors affecting communities in high concentration Cd soils but not in situations where it was present in medium or trace amounts. These results indicate that bacteria under higher Cd stress are more sensitive to changes in Cd concentration. Soil pH is the major factor determining soil microbial diversity (Jenkins et al. 2017; Luo et al. 2020). Interestingly, we found that the contributions of soil pH to microbes at different levels of Cd contamination are distinct (Fig. 5). Microbes in lightly contaminated plots had more relationships with soil pH, while microbes in heavily contaminated plots were less affected, which was consistent with previous work (Luo et al. 2019).

In addition to Cd content and soil pH, other factors, such as soil C, N, and P availability, were also significantly related to the microbial communities (Deng et al. 2017; Zhang et al. 2019). We note that the relative abundance of microbial taxa on heavily contaminated plots had more correlations with soil P variables, while on lightly contaminated plots, it is strongly related to soil C and N properties (Fig. 6). These inconsistent responses of microbial communities to soil nutrients between lightly and heavily contaminated plots are likely due to that the soil organic matter (soil organic C and N) in the lightly contaminated plots was lower than that in the heavily polluted plots, while the soil P content in the former is higher than that in the latter (Fig. S1), and thus, microorganisms had different nutrient limitation status.

5 Conclusion

In this study, fallowing was shown to directly promote soil health-related abiotic factors, that is, improved soil N availability but decreased Cd availability in the lightly contaminated soil, whereas these changes in heavily contaminated soil were not significant. However, fallowing had stronger impacts on microbial community composition in the heavily contaminated soil than that in the lightly ones. Specifically, some copiotrophic bacteria were promoted, and some oligotrophs were suppressed on the heavily contaminated plots, which resulted from the contributions of soil available Cd and P. Overall, our study found that changes in soil health-related abiotic and biotic factors after fallowing depend on Cd contamination levels. Therefore, the implemented time of the fallowing policy for the cultivated land with heavily Cd-contaminated level could be longer than that for the lightly ones to enhance more healthy soil status. These findings should provide useful information for Cd remediation and enhancing sustainability of Cd-contaminated agricultural system.

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