

1 **Title:** Assessment of ecological recovery potential of various plants in soil contaminated by multiple
2 metal(loid)s at various sites near XiKuangShan mine

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17 **Figures:** S7

18 **Supplementary materials:** 2

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1. Results

1.1 Contamination degree of metal(loid)s in TM, TS, MF and PF

The limit for soil Sb concentration was not specified in the Chinese Soil environmental quality standard (GB 15618-2018). The Sb concentrations in TM, TS, MF and PF all exceeded its soil background value (2.98 mg kg⁻¹) in Hunan Province, China by 219–1784 times (655.1 to 5316 mg kg⁻¹, on average, data not shown). The Sb concentration in TS (5316 mg kg⁻¹, on average) was highest (Fig. 2a), followed by TM (3989 mg kg⁻¹), PM (670.3 mg kg⁻¹) and MF (655.1 mg kg⁻¹). According to the above soil quality standard, the As and Cd concentrations (on average) in TM, TS, MF and PF were higher than their individual risk intervention values (Table S1). The As concentration (on average) in TS was highest (802.5 mg kg⁻¹, 8.03 times higher than its risk intervention value), followed by TM (437.3 mg kg⁻¹, 4.37 times), MF (182.0 mg kg⁻¹, 1.82 times) and PF (138.0 mg kg⁻¹, 1.38 times) (Fig. 2b, data not shown). The Cd concentration (on average) in TS was highest (67.56 mg kg⁻¹, 16.89 times higher than its risk intervention value), followed by TM (24.26 mg kg⁻¹, 6.07 times), PF (12.36 mg kg⁻¹, 3.09 times) and MF (8.87 mg kg⁻¹, 2.22 times) (Fig. 2c, data not shown). The Cr concentrations (on average) in TM, TS, MF and PF fell into the ranges of 41.85 mg kg⁻¹ (TM) to 124.3 mg kg⁻¹ (MF), which were all lower than its risk controlling value (250 mg kg⁻¹) and risk intervention concentration (1300 mg kg⁻¹) (Fig. 2d, data not shown). Similarly, the Pb (4.86 to 120.9 mg kg⁻¹) and Cu (51.06 to 56.61 mg kg⁻¹) concentrations (on average) in TM, TS, MF and PF were all lower than their risk controlling value (170 mg kg⁻¹ for Pb and 100 mg kg⁻¹ for Cu) and risk intervention concentration (1000 mg kg⁻¹ for Pb and risk intervention concentration of Cu was not specified) (Fig. 2ef, data not shown).

The index of I_{geo} can comprehensively reflect the impacts of natural geological processes and human activities on the accumulation of metal(loid)s in soils, and $I_{geo} > 0$ indicates that the soil is contaminated by a metal(loid) (Muller, 1969). The standards for contamination degrees were shown in Table S2. Overall, the I_{geo} values (on average and the same below) of Sb, As, Cd, Pb and Cu in TM, TS, MF and PF were all greater than 0 (Fig. S1a–f). The I_{geo} values of Sb (TS > TM > PF ≈ MF) and Cd (TS > TM > PF > MF) were all greater than 5 (Fig. S1a and S1e), indicating extreme pollution levels for Sb and Cd in these four soils. The I_{geo} values of As for MF and PF were within the range of 2–3 (Fig. S1b), indicating moderately severely polluted levels. The I_{geo} value of As for TM was 4.21, indicating moderately severely polluted levels.

Although the concentrations of Pb and Cu in these four soils did not exceed their individually risk intervention values (see the results above), the I_{geo} value of Pb was highest for TS (1.44, data not shown), followed by TM (1.17), MF (0.85) and PF (0.30) (Fig. S1e), indicating slight pollutions of Pb in MF and PF but moderate pollutions of Pb in TM and TS. The I_{geo} values of Cu were all lower than 1 (Fig. S1f), indicating slight pollutions of Cu in these four soils. In terms of the I_{geo} value for Cr, only the related value for MF was 0.21 and the I_{geo} values for TM, TS and PF were below 0 (Fig. S1d), indicating there were no pollution of Cr in TM, TS and PF but slight Cr pollution in MF.

In order to assess the ecological risks of metal(loid) pollution, we calculated the indexes of E_r^i and RI . The explanations for the relationships between comprehensive pollution levels and values of E_r^i and RI were shown in Table S2. Regarding to E_r^i values of Sb, As and Cd, their values were all highest in TS, followed by TM (except for E_r^i value of Cu) (Fig. 3abc). The E_r^i values of Sb and Cd (Fig. 3ac) in TS, TM, MF and PF were all higher than 320, suggesting very high pollution levels for Sb and Cd in these four types of soils. The E_r^i value of As (Fig. 3b) in TS, TM, MF and PF was 512.2, 278.5, 115.9, 87.9 (data not shown), suggesting a very high pollution level in TS, a high pollution level in TM, a considerable pollution level in MF and a moderate pollution level in PF, respectively. The E_r^i values of Cr (Fig. 3d), Pb (Fig. 3e) and Cu (Fig. 3f) in TS, TM, MF and PF were all lower than 40, indicating low pollution levels for Cr, Pb and Cu. The RI values of TM, TS, MF and PF were all higher than 600 (Fig. 3g), suggesting serious pollution levels in these four soils.

1.2 Basically physicochemical properties of TM, TS, MF and PF

The soil pH values (on average) of TM, TS, MF and PF were all higher than 7.8, and the pH value of TS (8.68) was highest (Fig. 4a), followed by MF (8.46), TM (8.41) and PF (7.89). The pH values of TM and PF were significantly lower than that of TS, and pH value of PF was significantly lower than that of TM (Fig. 4a). The EC values of TS, PF and MF were significantly lower than that of TM, and there were no significant differences in the EC values between TS, PF and MF (Fig. 4b). The OM concentrations in PF and MF were higher than that in TS and TM, and there were no significant differences in OM concentration between PF and MF, and between TS and TM (Fig. 4c). There were no significant differences in the total N concentration in TS, MF and TM, which were all significantly higher than that in PF (Fig.

4d). The total P concentration in MF was significantly higher than that in TS and TM, and there were no significant differences in the total P concentration between TS, PF and TM (Fig. 4e). The available P concentration in PF and MF were both significantly higher than that in TS and TM, but there were no significant differences in available P concentration between TS and TM, and between PF and MF (Fig. 4f). The available K concentrations in TS and PF were both significantly higher than that in MF and TM (Fig. 4g). The available K concentration in TS and PF did not differ significantly, and the available K concentration in TM was lowest among TM, TS, PF and MF.

1.3 Basically physicochemical properties and metal(loid) concentrations in rhizosphere soils of 8 crops

The rhizosphere soil pH values decreased significantly in the following order: ZM, SM > AF, VU > LAM > CMD, CS > CA (Fig. S2a). The pH values of rhizosphere soils of 8 crops were in the range of 7.00 (pepper) to 8.13 (maize) (Fig. S2a), but the pH values of non-rhizosphere soils of pepper (PF) and maize (MF) was 7.97 and 8.44 (Fig. 4a). The above results suggested that cultivation of crops can reduce soil pH in alkaline soils.

The EC values followed the order: CA > AF > LAM > CS > VU > ZM > CMD > SM (Fig. S2b). The EC values of rhizosphere soils of 8 crops fell into the range of 90.00 $\mu\text{S cm}^{-1}$ (SM) to 207.0 $\mu\text{S cm}^{-1}$ (pepper, CA), and that of ZM was 105.2 $\mu\text{S cm}^{-1}$ (Fig. S2b). However, the EC values of non-rhizosphere soils of CA (PF) and ZM (MF) was 90.97 $\mu\text{S cm}^{-1}$ and 102.7 $\mu\text{S cm}^{-1}$, respectively (Fig. 4b).

The OM concentrations followed the order: CMD > AF, CA > LAM, SM, VU and ZM, and there were no significant differences in the rhizosphere soil OM concentrations between CMD and CS, and among CS, AF and CA (Fig. S2c). The OM concentrations of rhizosphere soils of 8 crops fell into the ranges of 30.78 g kg^{-1} (LAM) to 52.14 g kg^{-1} (CMD), and that of CA and ZM was 44.95 g kg^{-1} and 33.50 g kg^{-1} , respectively (Fig. S2c). The OM concentration in PF and MF was 30.69 and 33.44 g kg^{-1} , respectively (Fig. 4c).

The total N concentrations followed the order: CA, VU, AF > CMD, LAM, CS and SM, and there were no significant differences in total N concentrations among AF, VU, CA and ZM, and among CMD, LAM, CS and SM (Fig. S2d). The total N concentrations of rhizosphere soils of 8 crops fell into the ranges of 0.02 g kg^{-1} (SM) to 0.46 g kg^{-1} (VU), and that of CA and ZM was 0.45 g kg^{-1} and 0.25 g kg^{-1} , respectively (Fig.

S2d). The total N concentrations in PF and MF was 0.02 g kg⁻¹ and 0.39 g kg⁻¹, respectively (Fig. 4d).

The total P concentration in the rhizosphere soil of CA was highest, which did not significantly differ with CMD, CS and AF. The total P concentration in the rhizosphere soil of CMD was significantly higher than LAM and SM, but there were no significant differences among LAM, SM, VU and ZM, and among CMD, CS, AF, VU and ZM (Fig. S2e). The total P concentrations of rhizosphere soils of 8 crops fell into the ranges of 0.19 g kg⁻¹ (SM) to 0.50 g kg⁻¹ (CA), and that of ZM was 0.34 g kg⁻¹ (Fig. S2e). The total P concentrations in PF and MF was 0.26 g kg⁻¹ and 0.36 g kg⁻¹, respectively (Fig. 4e).

The available P concentrations followed the order: AF > CMD > LAM, CS, SM, VU, ZM, but there were no significant differences among CA, LAM, CS, SM and ZM (Fig. S2f). The AP concentrations of rhizosphere soils of 8 crops fell into the ranges of 0.007 g kg⁻¹ (VU) to 0.049 g kg⁻¹ (AF), and that of CA and ZM was 0.021 g kg⁻¹ and 0.01 g kg⁻¹ (Fig. S2f). The AP concentrations in PF and MF was 0.01 g kg⁻¹ and 0.01 g kg⁻¹ (Fig. 4f).

The available K concentrations followed the order: CMD > CA, AF > CS > SM, VU > LAM, but there were no significant differences between CS and ZM, and among SM, VU and ZM (Fig. S2g). The AK concentrations of rhizosphere soils of 8 crops fell into the ranges of 49.96 mg kg⁻¹ (LAM) to 206.7 mg kg⁻¹ (CMD), and that of CA and ZM was 183.3 mg kg⁻¹ and 94.87 mg kg⁻¹ (Fig. S2g). The AK concentrations in PF and MF was 77.89 mg kg⁻¹ and 61.23 mg kg⁻¹ (Fig. 4g).

The As concentration in the rhizosphere soil of all crops was highest (18046 to 23111 mg kg⁻¹) among all tested metal(loid)s, followed by Cr (163.7 to 591.6 mg kg⁻¹), Sb (144.7 to 541.9 mg kg⁻¹), Pb (17.67 to 20.33 mg kg⁻¹), Cu (8.49 to 11.87 mg kg⁻¹) and Cd (3.89 to 4.21 mg kg⁻¹) (Table S3). The rhizosphere soil As concentration of maize (ZM) was highest among all crops, and rhizosphere soil concentrations of Cr (419.2 mg kg⁻¹), Cu (9.69 mg kg⁻¹), Cd (4.04 mg kg⁻¹), Sb (423.2 mg kg⁻¹) and Pb (19.35 mg kg⁻¹) were also relatively high among all crops (Table S3). In the rhizosphere soils of pepper (CA), the concentrations of Sb, As, Cd, Cr, Cu, and Pb reached up to 464.7 mg kg⁻¹, 18046 mg kg⁻¹, 4.10 mg kg⁻¹, 445.3 mg kg⁻¹, 11.87 mg kg⁻¹ and 17.67 mg kg⁻¹, respectively (Table S3). The highest concentration of Cr, Cu, Cd, Sb and Pb occurred in the rhizosphere soil of SM, CA, CS, SM and CS, respectively; and the lowest concentration of Cr, Cu, As, Cd, Sb and Pb occurred in the rhizosphere soil of CS, SM, CA, CMD, AF and CA, respectively (Table S3).

1.4 Concentrations of metal(loid)s in different tissues of 6 plant species gathered in the wasteland

The highest concentrations of Cr (2.48 mg kg⁻¹), Cu (12.10 mg kg⁻¹), As (46.10 mg kg⁻¹) and Pb (3.64 mg kg⁻¹) all occurred in the roots of PM, but the roots of ECL accumulated the highest Cd concentration (1.25 mg kg⁻¹) and CT accumulated the highest Sb concentration (571.7 mg kg⁻¹) (Table S3). The roots of PM also accumulated a relatively high Sb concentration up to (548.6 mg kg⁻¹). The lowest concentration of Cr (1.06 mg kg⁻¹), Cu (3.25 mg kg⁻¹), As (4.79 mg kg⁻¹), Cd (0.18 mg kg⁻¹), Sb (25.15 mg kg⁻¹) and Pb (0.57 mg kg⁻¹) occurred in the roots of ECL, BP, AC, BP, AC and BP (Table S3).

The highest concentration of Cr (1.66 mg kg⁻¹), Cu (9.03 mg kg⁻¹), As (4.76 mg kg⁻¹), Cd (1.85 mg kg⁻¹), Sb (86.87 mg kg⁻¹) and Pb (2.28 mg kg⁻¹) occurred in the stems of IC, PM, BP, PM, AC and BP, respectively (Table S3). The lowest concentration of Cr (0.13 mg kg⁻¹), Cu (1.60 mg kg⁻¹), As (1.03 mg kg⁻¹), Cd (0.41 mg kg⁻¹), Sb (2.32 mg kg⁻¹) and Pb (0.12 mg kg⁻¹) occurred in the stems of AC, IC, IC, BP, IC and IC (Table S3).

The highest concentration of Cr (0.74 mg kg⁻¹), Cu (19.68 mg kg⁻¹), As (30.44 mg kg⁻¹), Cd (1.44 mg kg⁻¹), Sb (275.7 mg kg⁻¹) and Pb (4.96 mg kg⁻¹) occurred in the leaves of IC, AC, AC, AC, PM and PM, respectively (Table S3). The lowest concentration of Cr (0.30 mg kg⁻¹), Cu (2.18 mg kg⁻¹), As (8.16 mg kg⁻¹), Cd (0.20 mg kg⁻¹), Sb (14.43 mg kg⁻¹) and Pb (1.66 mg kg⁻¹) occurred in the leaves of AC, IC, CT, IC, IC and IC (Table S3).

1.5 Concentrations of metal(loid)s in different tissues of 8 crops

Roots. The highest concentration of Cr (7.68 mg kg⁻¹), Cu (17.14 mg kg⁻¹), As (4.45 mg kg⁻¹), Cd (2.11 mg kg⁻¹), Sb (7.18 mg kg⁻¹) and Pb (1.91 mg kg⁻¹) occurred in the roots of SM, AF, AF, SM, CMD and CMD, respectively (Table S3). The lowest concentration of Cr (2.13 mg kg⁻¹), Cu (6.90 mg kg⁻¹), As (1.33 mg kg⁻¹), Cd (0.42 mg kg⁻¹), Sb (1.35 mg kg⁻¹) and Pb (0.41 mg kg⁻¹) occurred in the roots of AF, ZM, ZM, ZM, LAM and VU (Table S3).

Stems. The highest concentration of Cr (3.52 mg kg⁻¹), Cu (9.84 mg kg⁻¹), As (7.41 mg kg⁻¹), Cd (1.30 mg kg⁻¹), Sb (9.79 mg kg⁻¹) and Pb (1.05 mg kg⁻¹) occurred in the stems of LAM, CA, CS, CA, CS and CS, respectively (Table S3). The lowest concentration of Cr (0.38 mg kg⁻¹), Cu (2.05 mg kg⁻¹), As (0.44 mg kg⁻¹), Cd (0.32 mg kg⁻¹), Sb (0.87 mg kg⁻¹) and Pb (0.09 mg kg⁻¹) occurred in the stems of CS, ZM, SM, CMD, SM and SM (Table S3).

Leaves. The highest concentration of Cr (1.78 mg kg⁻¹), Cu (17.95 mg kg⁻¹), As (8.64 mg kg⁻¹), Cd (1.82 mg kg⁻¹), Sb (18.17 mg kg⁻¹) and Pb (3.65 mg kg⁻¹) occurred in the leaves of ZM, LAM, SM, SM, CA and LAM, respectively (Table S3). The lowest concentration of Cr (0.21 mg kg⁻¹), Cu (4.28 mg kg⁻¹), As (0.42 mg kg⁻¹), Cd (0.16 mg kg⁻¹), Sb (1.23 mg kg⁻¹) and Pb (0.12 mg kg⁻¹) occurred in the leaves of AF, AF, AF, VU, AF and AF, respectively (Table S3).

Fruits. The highest concentration of Cr (0.31 mg kg⁻¹), Cu (13.92 mg kg⁻¹), As (0.93 mg kg⁻¹), Cd (1.99 mg kg⁻¹), Sb (2.38 mg kg⁻¹) and Pb (0.13 mg kg⁻¹) occurred in the fruits of CA, LAM, CS, SM, CA and CA, respectively (Table S3). The lowest concentration of Cr (0.08 mg kg⁻¹), Cu (3.35 mg kg⁻¹), As (0.07 mg kg⁻¹), Cd (0.25 mg kg⁻¹), Sb (0.22 mg kg⁻¹) and Pb (0.02 mg kg⁻¹) occurred in the fruits of ZM, ZM, ZM, ZM, ZM and VU, respectively (Table S3).

According to the National food safety standards–limit of pollutants in foods (Table S1, GB 2762–2022), the limited fruit concentration of Pb, Cd and Cr is 0.1, 0.05 and 0.5 mg kg⁻¹, respectively. The limited leaf concentration of above elements in vegetables is 0.3, 0.2 and 0.5 mg kg⁻¹, respectively. The grain limited concentration of above elements is 0.2, 0.1 and 1.0 mg kg⁻¹, respectively. The limited concentration of above elements in legume vegetables is 0.2, 0.1 and 0.5 mg kg⁻¹. The concentration of As in fresh vegetables is 0.5 mg kg⁻¹.

1.6 Diversity of soil bacterial communities in TM, TS, MF and PF

After the 16 samples (each of TM, TS, MF and PF had 4 samples) were sequenced, and identified via Barcode, 102596 CCS sequences in total were obtained (data not shown). Each sample generated at least 4082 CCS sequences with an average of 6412 CCS sequences (data not shown).

In this study, the sequencing coverage of all samples were 86.12% –97.69% (data not shown), suggesting that the sequencing depths was enough and can be used to assess the microbial diversity. Rarefaction Curve and Rank Abundance Curve, as well as the results of NMDS analysis and PCoA analysis were shown in Fig. S3a–d. The number of OUTs in TM, TS, MF, and PF was (on average) 652, 777, 794, and 742, respectively, with MF having the highest OUTs (Fig. S4a). A total of 1238 OUTs were acquired from 16 samples (Fig. S4a). The above results showed that microbial relative abundance in samples followed the order of MF > PF > TS > TM (Fig. S3ab and Fig. S4a). The soil bacterial diversity of

PF and MF was similar (Fig. S4bc). Soil bacterial diversity of MF was significantly higher than that of TM and TS, and soil bacterial diversity of PF was only significantly higher than that of TM (Fig. S4bc).

1.7 Analysis of soil bacterial community structure

The bacteria in the 16 soil samples were assigned to 19 phyla and 206 genera (Table S4). There were significant differences among TM, TS, MF and PF in the relative abundances of only eight phyla, including Acidobacteria, Actinobacteria, Chloroflexi, Epsilonbacteraeota, Gemmatimonadetes, Nitrospirae, Proteobacteria and Verrucomicrobia (Fig. S5a–h). At the genus level, we selected the TOP 10 genera to perform further analysis, including *Gemmatimonas*, *MND1*, *Zavarzinella*, *Terrimonas*, *Tepidisphaera*, *Vicinamibacter*, *RB41*, *Nitrospira*, *Thiobacillus*, and *Sphingomonas* (Fig. 5a–j).

At the phylum level, MF had the highest relative abundances (on average) of Acidobacteria (Fig. S5a), Chloroflexi (Fig. S5c) and Verrucomicrobia (Fig. S5h) among TM, TS, MF and PF. The relative abundance of Acidobacteria in MF was significantly higher than that in TM and TS, which in PF was significantly higher than that in TM (Fig. S5a). The relative abundance of Actinobacteria in TS was significantly higher than that in MF, PF and TM, which did not significantly differ among MF, PF and TM (Fig. S5b). The relative abundance of Chloroflexi in MF was significantly higher than that in PF and TM, which was significantly lower in TM than that in TS (Fig. S5c). The relative abundance of Epsilonbacteraeota in TM was significantly higher than that in MF, PF and TS, which did not significantly differ among MF, PF and TS (Fig. S5d). There were significant differences in the relative abundance of Epsilonbacteraeota among MF, PF, TM, and TS with the following order of TM < PF < MF < TS (Fig. S5e). The relative abundance of Nitrospirae in TM was significantly lower than that in MF and PF (Fig. S5f). The relative abundance of Proteobacteria in TM was significantly higher than that in MF and PF, which was significantly higher in TS than that in MF (Fig. S5g). The relative abundance of Verrucomicrobia in MF and PF was both significantly higher than that in TM and TS, which did not significantly differ between MF and PF, and between TM and TS (Fig. S5h).

At the genus level, the relative abundance of *Gemmatimonas* in TS was significantly higher than that in other samples, which was significantly higher in MF than that in TM (Fig. 5a; Table S5). The relative abundance of *MND1* in TM was significantly lower than that in MF and TS (Fig. 5b; Table S5). The relative

abundance of *Zavarzinella* in TM was significantly higher than that in other samples, which did not significantly differ among MF, PF and TS (Fig. 5c; Table S5). The relative abundances of *Terrimonas* in MF and PF were significantly higher than that in TM and TS, but there were no significant differences between MF and PF, and between TM and TS (Fig. 5d; Table S5). The relative abundance of *Tepidisphaera* in MF was significantly higher than that in TM and TS, which was significantly higher in PF than that in TM (Fig. 5e; Table S5). The relative abundance of *Vicinamibacter* significantly differed among MF, PF, TM and TS except for the non-significant differences between PF and TS (Fig. 5f; Table S5). The relative abundances of *RB41* in MF and PF were significantly higher than that in TM and TS, which did not significantly differ between MF and PF, and between TM and TS (Fig. 5g; Table S5). The relative abundance of *Nitrospira* in PF was significantly higher than that in TM and TS, which was significantly higher in MF than that in TM (Fig. 5h; Table S5). The relative abundances of *Thiobacillus* in TM was significantly higher than that in MF and PF (Fig. 5i; Table S5). The relative abundance of *Sphingomonas* in TM was significantly lower than that in MF, PF and TS (Fig. 5j; Table S5).

1.8 Correlation analysis between microbial communities and environmental factors

The soil environmental factors driving the relative abundance changes of soil microbial communities at genus level (TOP10, Fig. 6) was assessed through Spearman correlation analysis. There were no significant correlations between soil Cu (Pb) concentration and the selected microbial communities (Fig. 6). AP was significantly positively correlated with *RB41*, *Terrimonas*, *Tepidisphaera* and *Vicinamibacter* but negatively correlated with *Thiobacillus*. TP was significantly positively correlated with *Tepidisphaera*, *Vicinamibacter*, and *Sphingomonas*, but negatively correlated with *Zavarzinella*. AK was significantly positively correlated with *RB41*, *Terrimonas*, *Tepidisphaera*, *Vicinamibacter*, *Gemmatimonas*, *MND1*, and *Sphingomonas*. Cr was significantly positively correlated with *RB41*, *Terrimonas*, *Nitrospira*, *Tepidisphaera*, *Vicinamibacter*, *MND1*, and *Sphingomonas*, but negatively correlated with *Thiobacillus* and *Zavarzinella*. SOM was beneficial for increasing the abundance of *RB41*, *Terrimonas*, *Nitrospira*, *Tepidisphaera*, and *Vicinamibacter*, but negatively correlated with *Thiobacillus*. EC was significantly positively correlated with *Thiobacillus* and *Zavarzinella*, but negatively correlated with *RB41*, *Terrimonas*, *Nitrospira*, *Tepidisphaera*, and *Vicinamibacter*. TN was only significantly positively correlated with *Thiobacillus*. Both Sb and As were

significantly negatively correlated with *RB41*, *Terrimonas*, *Nitrospira* and *Tepidisphaera*, but positively correlated with *Thiobacillus*. However, Sb alone was significantly negatively correlated with *Vicinamibacterium*. Cd was significantly negatively correlated with *RB41*. PH was significantly negatively correlated with *Terrimonas*, but positively correlated with *Gemmatimonas* (Fig. 6).

1.8 Bugbase phenotypic prediction of functional gene

We performed bugbase phenotypic prediction, and the results were shown in Fig. 7 (according to relative abundance) and Fig. 8 (at phylum level or genus level). BugBase is a tool for high-level phenotype classification of microbiome, which can classify microbial communities to seven phenotypes: Gram Positive, Gram Negative, Biofilm Forming, Pathogenic, Mobile Element Containing, Oxygen Utilization (Aerobic, Anaerobic and Facultatively Anaerobic), and Oxidative Stress Tolerant.

1.8.1 Distribution of relative abundance of microbial communities belonging to different phenotypes

Relative abundance percentages of microbial communities classified into different phenotypes. Relative abundances of microbial communities classified into Stress_tolerant phenotype were highest in TM (20.69%), followed by TS (18.05%), PF (15.92%) and MF (15.80%) (Fig. 7a). Similarly, microbial communities classified into Potentially_pathogenic phenotype were highest in TM (9.97%), TS (7.78%), PF (5.38%) and MF (4.57%). TS had the most Gram_positive microbial communities (1.45%), followed by MF (0.46%), PF (0.35%) and TM (0.14%). Microbial communities classified into Grm_negative phenotype were highest in PF (24.65%), MF (24.58%), TM (22.24%) and TS (21.29%). Microbial communities classified into Forms_biofilms phenotype were highest in PF (20.72%), MF (20.52%), TS (17.66%) and TM (12.30%). Microbial communities classified into Facultatively_anaerobic phenotype were highest in TM (5.83%), TS (2.39%), PF (2.14%) and MF (1.51%). Microbial communities classified into Contains_mobile_elements phenotype were highest in TM (21.12%), TS (18.85%), MF (17.42%) and PF (16.83%). Microbial communities classified into Anaerobic phenotype were highest in MF (6.51%), PF (4.72%), TS (3.24%) and TM (1.69%). Microbial communities classified into Aerobic phenotype were highest in PF (9.31%), TS (9.30%), MF (8.63%) and TM (6.02%).

Differences in relative abundances of microbial communities classified into different phenotypes

among MF, PF, TM and TS. There were no significant differences in the relative abundances of microbial communities belonging to aerobic phenotype among MF, PF, TM and TS (Fig. 7b). On average, MF had the highest relative abundance of microbial communities classified into Anaerobic_phenotype, followed by PF, TS and PF (Fig. 7c). The relative abundance of microbial communities classified into Facultatively_anaerobic phenotype in TM was significantly higher than that in MF, PF and TS, and was significantly higher in TS than that in MF (Fig. 7d). The relative abundance of microbial communities classified into Gram_positive phenotype was significantly higher in TS than that in MF, PF and TM, and was significantly higher in MF than that in MF (Fig. 7e). The relative abundance of microbial communities classified into Gram_negative phenotype was significantly lower in TS than that in MF, PF and TM, and was significantly higher in TM than that in MF (Fig. 7f). The relative abundance of microbial communities classified into Contains_mobile_elements phenotype was significantly higher in TM or in TS than that in MF or in PF (Fig. 7g). The relative abundance of microbial communities classified into Stress_tolerant phenotype was significantly higher in TM than that in MF, PF and TS, and was significantly higher in TS than that in MF and PF (Fig. 7h). The relative abundance of microbial communities classified into Potentially_pathogenic phenotype was significantly higher in TM than that in MF and PF, and was significantly higher in TS than that in MF (Fig. 7i). The relative abundance of microbial communities classified into Forms_Biofilms phenotype was significantly lower in TM than that in MF, PF and TS (Fig. 7j).

1.8.2 Distribution of important phenotypes at phylum levels

In this study, we only selected three important phenotypes including Contains_Mobile_Elements, Stress_Tolerant, and Potentially_Pathogenic.

Contains_Mobile_Elements. Microbial communities classified into the phenotype of Contains_mobile_elements were mainly found in the phyla Bacteroidetes, Planctomycetes and Proteobacteria in MF, PF and TS (Fig. 8a and Table S6). Among MF, PF, TM and TS, TS had characteristically microbial communities belonged to Gemmatimonadetes; and MF had characterized microbial communities belonged to Verrucomicrobia. TM had characteristically microbial communities belonged to Cyanobacteria, but it did not have the microbial communities belonged to Bacteroidetes and

Planctomycetes. Microbial communities of Contains_mobile_elements phenotype belonged to Proteobacteria phylum were highest in TM, followed by TS and PF/MF.

Stress_Tolerant. Microbial communities classified into the phenotype of Stress_tolerant in MF, PF and TS mainly occurred in the phyla of Bacteroidetes, Planctomycetes and Proteobacteria (Fig. 8b and Table S6). Among MF, PF, TM and TS, MF had characterized microbial communities belonged to Verrucomicrobia; and TM had characteristically microbial communities belonged to Cyanobacteria, but it did not have the microbial communities belonged to Planctomycetes. MF and TS both had the microbial communities belonged to Gemmatimonadetes, but PF and TM both did not have. Microbial communities of Stress_tolerant phenotype belonged to Proteobacteria phylum were highest in TM, followed by TS and PF and MF. Microbial communities of Stress_tolerant phenotype belonged to Planctomycetes phylum were highest in MF, followed by PF, TS and TM.

Potentially_Pathogenic. Microbial communities classified into the Potentially_pathogenic phenotype in all samples mainly occurred in the phylum of Proteobacteria, which were highest in TM, followed by TS, PF and MF (Fig. 8c and Table S6).

1.8.3 Distribution of different phenotypes at genus levels

Contains_Mobile_Elements. Genera classified into the phenotype of Contains_Mobile_Elements mainly included *Chitinophagaceae* (MF > PF > TS > TM), *Comamonadaceae* (PF > TM), *Cytophagaceae* (only in TM), *Gaiellaceae* (only in TS), *Haliangiaceae* (only in TS), *Helicobacteraceae* (only in TM), *Pirellulaceae* (TS > PF > MF), *Rhodospirillaceae* (only in PF), *Saprospiraceae* (only in TS), *Sinobacteraceae* (TS > MF > PF), *Syntrophobacteraceae* (MF > PF), *Xanthomonadaceae* (PF > TS), *DA101* (only in PF), *Flaviumibacter* (only in TM), *Flavisolibacter* (only in PF), *Hydrogenophaga* (only in TM), *Kaistobacter* (TS > MF > PF), *Leptothrix* (TM > TS > MF), *Limnohabitans* (PF > TS > MF), *Methylibium* (PF > MF > TM > TS), *Pirellula* (MF > PF), *Planctomyces* (TS > PF), *Rhodobacter* (only in TM), *Rhodoferrax* (only in TM), *Rubrivivax* (only in TM), *Sulfuritalea* (only in TM), *Thermomonas* (only in PF), and *Thiobacillus* (TM > TS) (Fig. 8d and Table S6).

Stress_Tolerant. Genera classified into the phenotype of Stress_Tolerant mainly included *Chitinophagaceae* (MF > PF > TS > TM), *Comamonadaceae* (PF > TM), *Cytophagaceae* (only in TM), *Gaiellaceae* (only in TS), *Haliangiaceae* (only in TS), *Pirellulaceae* (TS > PF > MF), *Rhodospirillaceae* (only in PF),

Saprospiraceae (only in TS), *Sinobacteraceae* (TS > MF > PF), *Xanthomonadaceae* (TS > PF), *DA101* (only in PF), *Flaviumibacter* (only in TM), *Flavisolibacter* (only in PF), *Hydrogenophaga* (only in TM), *Leptothrix* (TM > TS > MF), *Limnohabitans* (PF > TS > MF), *Methylibium* (PF > MF > TM > TS), *Pirellula* (MF > PF), *Planctomyces* (TS > PF), *Rhodobacter* (only in TM), *Rhodoferrax* (only in TM), *Rubrivivax* (only in TM), *Sulfuritalea* (only in TM), *Thermomonas* (only in PF), and *Thiobacillus* (TM > TS) (Fig. 8e and Table S6).

Potentially_Pathogenic. Genera classified into the phenotype of Potentially_Pathogenic mainly included *Comamonadaceae* (PF > TM), *Sinobacteraceae* (TS > MF > PF), *Xanthomonadaceae* (TS > PF), *Hydrogenophaga* (only in TM), *Leptothrix* (TM > TS > MF), *Limnohabitans* (PF > TS > MF), *Methylibium* (PF > MF > TM > TS), *Rhodoferrax* (only in TM), *Rubrivivax* (only in TM), *Sulfuritalea* (only in TM), *Thermomonas* (only in PF), and *Thiobacillus* (TM > TS) (Fig. 8f and Table S6).

Reference

Muller, G., 1969. Index of geoaccumulation in sediments of the Rhine river. *GeoJournal*. 2(3), 109–118.