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Two microbes assisting Miscanthus floridulus in remediating multi-metal(loid)s contaminated soil

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Abstract

Miscanthus has good tolerance to muti-metal(loid)s and has received increasing attention in remediated studies of metal(loid)s-contaminated soil. In this study, we explored the synergic effects of *Miscanthus floridulus* (Lab.) and two plant growth-promoting bacteria (PGPB), TS8 and MR2, affiliated to Enterobacteriaceae on remediation of muti-metal(loid)s contaminated soil. The results exhibited a decrease of metal(loid)s except for copper contents in the soil in bacterial inoculation groups, indicating that MR2 and TS8 could enhance the remediation of metal(loid)s. Moreover, increased fresh/dry weight and height indicated that inoculated bacteria could promote Miscanthus growth. Although the activities of antioxidant enzymes and the content of chlorophyll in the overground tissues showed no significant increase or even decrease, the activities of antioxidant enzymes in the underground tissues and soil, as well as the contents of soil nutrients (available K and P), were significantly ($p < 0.05$) increased to some certain. Bacterial inoculants could also decrease the soil pH. High-throughput sequencing analysis showed that the bacterial inoculant affected the rhizosphere bacterial community and reduced community diversity, but the relative abundance of some PGPB were found to increase. Phylogenetic molecular ecological networks indicated that bacterial inoculants reduced interactions between rhizosphere bacteria and thereby led to a simpler network structure but increased the proportion of positive-correlation links and enhanced the metabiosis and symbiosis of those bacteria. Spearman's test showed that OTUs affiliated with *Enterobacteriaceae* and soil nutrients were critical for metal(loid) remediation and Miscanthus growth. The results of this study provide a basis for the synergic remediation of muti-metal(loid)s-contaminated soils by Miscanthus and PGPB and provide a reference for the subsequent regulation of Miscanthus remediation efficiency by the other PGPB or critical bacteria.

Key Points

Rhizosphere bacterial communities of *Miscanthus floridulus* were compared.

The mechanisms by bacteria assist *Miscanthus floridulus* absorb heavy metals.

Provide a reference for the subsequent regulation of Miscanthus remediation.

Introduction

The remediation of agricultural soil metal(loid)s pollution is urgently needed worldwide. The techniques of multip-metal(loid)s contaminated soil are challenging. Sustainable remediation approaches are preferred to ensure that the soil meets the needs of agricultural production. Physical and chemical approaches will change and destroy soil structure and usually come with high costs (Liu et al. 2020). Phytoremediation is a promising soil remediation technology, and plants are used to remove organic and inorganic pollutants from the soil (Chaney. et al. 1997). At the same time, it reduces remediation costs and sustainable use of the resource by selling plant biomass. Phytoremediation has the advantages of low cost and sustainable development. The factors that affect phytoremediation are the bioavailability of

soil metal(loid)s, soil properties, and climate et al. (Zhao et al. 2019). It is necessary to select specific implementation strategies according to the site and plant species.

In recent years researches, The common plants used for cadmium(Cd) remediation included *Medicago sativa* L. (Wang et al. 2018a), *Agave americana* L. (Ramana et al. 2021), *Chrysopogon zizaniodes* (Wasino et al. 2019), *Triticum aestivum* L. (Wang et al. 2018b), *Oryza sativa* L. (Liu et al. 2018a), *Brassica rapa* (Navarro-Leon et al. 2020), *Festuca arundinacea* (Dong et al. 2019), et al.. The common plants used for plumbum (Pb) remediation included *Bidens maximowicziana* (Wang et al. 2007), *Houttuynia cordata* Thunb (Liu et al. 2018b), *Solanum nigrum* L. (Sun et al. 2017), *Brassica napus* L. (Shakoor et al. 2014), *Panicum virgatum* L. (Guo et al. 2019), et al.. Some common plants for Zinc (Zn) remediation, such as *Betula pendula* Roth (Dmuchowski et al. 2014), *Fagopyrum esculentum* Moench (Kaplan &Akay 2018), *Hibiscus cannabinus* (An et al. 2020), et al.. *Sorghum sudanense* (Piper) Stapf. (Liu et al. 2020), *Zea mays* L. (Korzeniowska &Stanislawska-Glubiak 2018), *Ricinus communis* L. et al. are common plants that remediated nickel(Ni) and copper(Cu), et al. In addition, some plants have also been shown to have a capability of multip-metal(loid)s (Cd, Cu, Ni, Pb and Zn) remediation, such as energy plant *Elymus elongatus subsp ponticus* cv. Szarvasi-1(Andreazza et al. 2013, Sipos et al. 2013), *Miscanthus x giganteus* (Laval-Gilly et al. 2017), *Linum usitatissimum* L. (Šyc. et al. 2011), *Betula platyphylla* Suk. and *Picea asperata* Mast. (Reimann et al. 2008) et al.. The use of energy plants for phytoremediation can bring environmental benefits and reduce the economic expenditure of remediation, such as high biomass energy as renewable fuels and waste wood ash as fertilizer (Brosse et al. 2012, Reimann et al. 2008). However, because of the long growth cycle, phytoremediation-site remediation work is restricted.

Recently, phytoremediation combined with plant growth-promoting bacteria (PGPB) has been widely accepted and applied for the technology of site remediation engineering. Combined remediation technology promotes plant growth by inoculating PGPB with the production of indole-3-acetic acid (IAA), siderophore, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, nitrogen (N_2) fixation, phosphorus (P) and potassium (K) solubilization, and changes the bioavailability of metals by the production of acid, to enhance the efficiency of phytoremediation(Afegbua &Batty 2019, Chen et al. 2017, El-Meihy et al. 2019). However, research on the combined remediation of soil multi-metal(loid)s pollution by the combination of energy crops and PGPB is still limited, and there is more room to find other technologies that can be used for site remediation engineering.

Miscanthus spp. has a C4 photosynthetic pathway with a long growing season, and it was defined as an excellent energy crop combined with high non-edible biomass production, containing a lot of cellulose, hemicellulose and lignin (Hechelski et al. 2020). Biomass of *Miscanthus* spp. has become a vital fossil fuel substitute in Ukraine. According to the report, the biomass yield of *Miscanthus* spp. was 8–32 $t{\cdot}ha^{-1}$, energy production and lower heat combustion were 143–560 $GJ{\cdot}ha^{-1}$ and 17.5 $MJ{\cdot}kg^{-1}$ DM (Matys Grygar 2020, Pidlisnyuk et al. 2021). Meanwhile, this plant has a well-developed root to absorb deeper moisture and nutrients, making it more capable of surviving drought, heat, cold, and salt stresses (Wang et al. 2020). In addition, due to its excellent resistance and sustainable economy, *Miscanthus* spp. is also gradually used in the research of remediated metal(loid)s contaminated soil. It can tolerate, absorb and

stabilize metal(loid)s, promote carbon deposition, and improve soil physicochemical properties (Wang et al. 2020). In the study by Bang et al., the maximum removal of *Miscanthus* sp. Goedae-Uksae 1 for As, Cu, Pb, Ni, Cd and Zn was 97.7%, 86.4%, 77.5%, 61.0%, 56.2% and 42.9%, respectively (Bang et al. 2015).

Our previous studies have shown that PGPB combined with *M. floridulus* (Lab.) is a feasible remediation method (Liu et al. 2021). Based on pot experiments, this study aimed to explore the effects of PGPB under the multi-metal(loid)s contamination on the following parameters: (i) the growth index, physiological-biochemical characteristics and metal(loid)s accumulation capacity of *M. floridulus* (Lab.); (ii) the rhizosphere soil environment including soil physicochemical properties, metal(loid)s speciation and distribution, soil antioxidant enzyme activity and bacterial community structure; (iii) the co-occurrence networks of the soil bacterial community; (iv) the relationships among metals uptake, soil environment and bacterial community.

Materials and methods

Soil sampling and analysis

The experimental pot soil used in the study was collected from surface layer soil (5 – 30 cm) in Limei Village lead-zinc mining area, Huayuan County, Hunan Province (Lat. 28°30'46.47"N, Long. 109°21'41.12"E). The physicochemical properties of soil were as follows: pH value 5.54 (1:2.5 w/v water), available P 9.30 g/kg, available K 10.81 g/kg, total N 0.88 g/kg, organic matter 13.34 g/kg. The digestion method of total soil metal(loid)s consistent with our previous research method (Liu et al. 2021). The metal(loid)s concentrations were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (iCAP7200 HS Duo, Thermo Fisher Scientific Inc., China). Soil metal(loid)s including arsenic (As) 42.88 mg/kg, cadmium (Cd) 5.57 mg/kg, copper (Cu) 99.62 mg/kg, lead (Pb) 2973.33 mg/kg, zinc (Zn) 1234.39 mg/kg.

Experiment design

M. floridulus (Lab.) with 30–40 cm plant height used in this study were purchased from Xiaoshan Yishang Horticulture Farm, Hangzhou City, Zhejiang Province in China. The residual soil at the roots of *M. floridulus* (Lab.) was rinsed in tap water and surface-sterilized with 2% sodium hypochlorite solution for 10 min. Then sodium hypochlorite on the roots was rinsed three times with ultrapure water to clean. *M. floridulus* (Lab.) were planted into a pot (15 cm × 9 cm × 11.5 cm) containing 2 kg soil that passed through a 0.85 mm sieve. Each pot was planted with three *M. floridulus* (Lab.), and each treatment was repeated three times.

In our previous study, *Lelliottia jeotgali* MR2 and *Klebsiella michiganensis* TS8 were plant growth-promoting bacteria (PGPB), both of them had better PGP traits and Cd resistance (Liu et al. 2021). The experiments design was as follows: (1) CK: inoculation without PGPB; (2) MR: inoculation with *L. jeotgali* MR2; (3) TS: inoculation with *K. michiganensis* TS8; (4) MT: inoculation with a mixed bacteria liquid composed by *L. jeotgali* MR2 and *K. michiganensis* TS8 (1:1, c/c). *L. jeotgali* MR2, *K. michiganensis* TS8

and the liquid of the mixed bacteria were suspended in sterile ultrapure water, and the concentration of the bacterial suspension was 1×10^{11} cfu·mL^{-1}. 10 ml of three different bacterial suspensions were added to treatment MR (*L. jeotgali* MR2), TS (*K. michiganensis* TS8) and MT (*L. jeotgali* MR2 and *K. michiganensis* TS8), and the same volume of ultrapure water was added to CK. Bacteria liquids were added once a week for five weeks. Each pot was watered with 100ml of water every two days and left in the open air for nine weeks on October 1, 2020. Climatic conditions during the planting period are 25–35°C daytime, 15–25°C nighttime and humidity around 50%.

Samples collection and physicochemical analysis

After 9 weeks, the plant samples were taken out of the pots and used to determine plant height. Then it was divided into the overground and underground parts to determine biomass, antioxidant enzyme activity (SOD, POD, CAT), chlorophyll content and metal(loid)s concentration. The antioxidant enzyme activity was determined using CheKine™ Superoxide Dismutases (SOD) Assay Kit (KTB1030), CheKine™ Peroxidase (POD) Activity Colorimetric Assay Kit (KTB1150) and CheKine™ Catalase (CAT) Activity Assay Kit (KTB1040) (Abbkine Scientific Co., Ltd, Wuhan, China), respectively. The determining method of chlorophyll content in the overground was as follows: (1) 10 mL ddH_2O and 0.1000 g *M. floridulus* (Lab.) leaves were fully ground with quartz sand and $CaCO_3$, and the slurry was transferred to a test tube containing 10 ml of water.; (2) the slurry was added to 10 mL by extracting solution, the test tube was kept in the dark place for 5 hours until the bottom sample turns white. The extracting solution is made of absolute ethanol and acetone (1:2, v/v); (3) 200 μL supernatant was measured at 663 nm and 645 nm, the equal volume of extracting solution as a control; (4) The concentrations of chlorophyll were calculated using the following equations:

Chlorophyll a = 0.01 × (12.70 × OD663–2.69 × OD645) × dilution ratio / sample quality;

Chlorophyll b = 0.01 × (22.90 × OD663–4.68 × OD645) × dilution ratio / sample quality.

The metal(loid)s concentration in the overground and underground was consistent with the previous method (Liu et al. 2021). Then, the bioconcentration factor (BCF) and the translocation factor (TF) were calculated according to the following formulae:

BCF = the metal(loid)s concentration$_{(plant)}$ / the metal(loid)s concentration$_{(soil)}$;

TF = the metal(loid)s concentration$_{(overground)}$ / the metal(loid)s concentration$_{(underground)}$.

As part of the quality control of the method, the standard reference of plant material (GBW07603 GSV-2) was used, and the recovery of metal(loid)s was 95.2% − 101.5%.

Rhizosphere soil samples were divided into two parts. One part was dried to constant weight before a 0.15 mm sieve. Then, soil physicochemical properties and enzyme activities, including organic matter (OM), total N (TN), available P (AP), available K (AK), soil POD (S-POD), soil SOD (S-SOD), soil CAT (S-CAT) and soil metal(loid)s morphology were determined. The determination method of OM and TN was

according to our previous study (Liu et al. 2021). The concentration of AP, AK, S-POD, S-SOD and S-CAT were determined according to instructions of the Acid soil available phosphorus Assay box (ZC-S0841), Soil available potassium Assay box (ZC-S0850), Soil peroxidase (S-POD) Assay kit (ZC-S0837), Soil catalase (S-CAT) Assay kit (ZC-S0830), Soil superoxide dismutase (S-SOD) Assay kit (ZC-S0350), all of these kits were bought from ZCIBIO Technology Co., Ltd, Shanghai, China. According to BCR sequential extraction, soil metal(loid)s morphology was defined as acid-soluble phase, reducible phase, oxidizable phase and residual phase (Ure et al. 1993). The metal(loid)s morphology was determined as reported by Chen et al. (Chen et al. 2010). The standard reference of plant material (GBW07437) was used to control the quality of the method, and the recovery of metal(loid)s was 96.4% − 103.2%. The other part was stored at -80℃ for microbial community analysis.

DNA extraction and Illumina sequencing

Microbial DNA extraction was performed according to the E.Z.N.A.® Soil Kit (Omega Bio-tek, Norcross, GA, U.S.) manufacturer's protocols from rhizosphere soil samples. The NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA) was used to determine final DNA concentration and purification, and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 region of the bacterial 16S rRNA gene was amplified using the primer pair 515F (5’-GTGCCAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’). PCR reactions were performed using the following programme: 1 min of denaturation at 98°C, 30 cycles of 10 s at 98°C, 30 s for annealing at 50°C, and 30 s for elongation at 72°C, and a final extension at 72°C for 10 min. The PCR products were extracted from a 2% agarose gel. Further purification was performed using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer’s protocol. The raw read data were submitted to the NCBI Sequence Read Archive (SRA) database (PRJNA950185).

Molecular ecological network construction and characterization

Approaches to network construction based on Random Matrix Theory (RMT) (Francois et al. 2021), The Hub and Connector OTUs were identified and the topological property determined using a similar threshold (0.94). To ensure the reliability of the data, OTUs presented in 6 out of 6 replicates were used for network analysis. Several network properties were measured, including average degree, average path distance, average clustering coefficient, and modularity index. Rapid greedy modularity optimisation was used to generate the network modules. The experimental data based on 16S rRNA gene sequences were used to construct phylogenetic molecular ecological networks (pMENs), and the network graph visualisation software was Gephi 0.9.2. The pMENs were constructed separately based on sequencing data of 4 treatments to reveal the effect of bacterial inoculant on microbial network interactions.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 25.0 was used for all statistical analyses. All data were presented as means ± standard deviation (SD) of data collected from at least three replicates. One-way analysis of variance (ANOVA) was used to determine significant differences at the 5% level for differences in parameters based on Tukey's test. The different lower-case alphabets above the bars indicate a significant difference. Details for bioinformatics analysis were described in our previous study (Xiao et al. 2022). The index of species richness and diversity, including the Shannon diversity, the Pielou evenness and the OTUs of all the samples, had been evaluated by the Mothur software. Mantel and Spearman tests and non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity were performed using the R package 'vegan' to reveal the association of microbial communities with environmental factors (De Silva et al. 2021, Yang et al. 2020).

Result

Growth indices of M. floridulus (Lab.)

In general, the addition of *L. jeotgali* MR2 and *K. michiganensis* TS8 significantly ($p < 0.05$) increased plant height, fresh weight and dry weight of *M. floridulus* (Lab.) after 9 weeks of planting. (Fig. S1). Compared with the CK group (33.07 cm), the plant height was increased by 41.22%, 59.81% and 20.40%, respectively, in the MR (46.70 cm), TS (52.85 cm) and MT (39.82 cm) groups (Fig. 1a). In addition, fresh plant weight (Fig. 1b) and dry weight (Fig. 1c) were also increased after inoculating this two PGPB: Compared with the CK group (3.06 g; 1.18 g), the fresh weight in the underground and overground was increased by 0.35–1.01 and 1.28–3.05 times, respectively in the other groups (4.14–6.16 g; 2.68–4.77 g); compared with the CK group (0.84 g; 0.29 g), the dry weight in the underground and overground parts was increased by about 2.5 and 1.3 times, respectively in the other groups (2.99–3.10 g; 0.67–0.71 g). Overall, *K. michiganensis* TS8 showed the highest growth-promoting efficiency.

Antioxidant enzyme activities and chlorophyll content in M. floridulus (Lab.)

To some extent, the activities of SOD, POD and CAT in plants were changed by the addition of MR2 and TS8 (Fig. 2). Compared with the CK group (SOD, 1164.14 $U \cdot g^{-1}$; POD, 373.02 $U \cdot g^{-1}$; CAT, 34.54 $nmol \cdot min^{-1} \cdot ml^{-1}$) in the underground parts, MR2 inoculants could significantly increase the activities of POD (1682.49 $U \cdot g^{-1}$) and CAT (74.63 $nmol \cdot min^{-1} \cdot ml^{-1}$); TS8 inoculants could significantly increase the POD activity (857.87 $U \cdot g^{-1}$); and the mixed strains promoted the activities of these three enzymes (SOD, 1939.97 $U \cdot g^{-1}$; POD, 2715.59 $U \cdot g^{-1}$; CAT, 83.50 $nmol \cdot min^{-1} \cdot ml^{-1}$). Compared with the CK group in the aboveground parts, only the CAT activity was significantly increased by the TS8 inoculant, while the additions of MR2 and mixed strains decreased it. In addition, in the MR group (077 $mg \cdot g^{-1}$; 0.26 $mg \cdot g^{-1}$), compared to the CK group (1.27 $mg \cdot g^{-1}$; 0.42 $mg \cdot g^{-1}$), the chlorophyll a and b content in the leaves was only significantly reduced by 37.16–39.05%.

Metal(loid)s contents in M. floridulus (Lab.)

Compared with the CK group (5.49 mg/kg), the three different treatments significantly reduced As and Cu accumulation in the underground part of *M. floridulus* (Lab.) by 47.91%-77.60% and 29.06%-37.31%, respectively (Table 1). However, Cd accumulation in the subterranean part of M. floridulus (Lab.) was significantly increased by 64.71% and 76.47% in MR and MT groups; Pb accumulation was enhanced considerably by 40.41% in the TS group. Compared with the CK group in the overground part, Cd accumulation was significantly increased by 4.54 and 3.24 folds in the MR and TS groups, and Pb accumulation was significantly enhanced by 61.62% and 102.22% in the TS and MT groups. Besides, the concentrations of Zn showed no significant differences among all groups.

Table 1
Different metal(loid)s concentrations in *M. floridulus* (L.) underground and overground parts under the different treatments. (Unit: $mg \cdot kg^{-1}$)

	Underground				Overground			
	CK	MR	TS	MT	CK	MR	TS	MT
As	5.49 ± 0.31^{a}	1.23 ± 0.04^{c}	1.76 ± 0.28^{c}	2.86 ± 0.24^{b}	0.94 ± 0.08^{ab}	1.50 ± 0.19^{ab}	2.50 ± 1.29^{a}	0.71 ± 0.03^{b}
Cd	0.34 ± 0.04^{b}	0.53 ± 0.15^{a}	0.43 ± 0.06^{ab}	0.56 ± 0.05^{a}	0.33 ± 0.11^{b}	1.83 ± 0.44^{a}	1.40 ± 0.27^{a}	0.43 ± 0.24^{b}
Cu	26.43 ± 4.43^{a}	18.52 ± 1.62^{b}	16.57 ± 1.26^{b}	18.75 ± 0.72^{b}	23.32 ± 2.92^{b}	17.29 ± 1.10 ^{c}	22.53 ± 1.65^{b}	35.76 ± 7.91^{a}
Pb	17.84 ± 0.71^{b}	9.04 ± 1.40^{c}	25.05 ± 0.67^{a}	13.65 ± 3.03^{bc}	9.90 ± 2.33^{b}	9.43 ± 0.21^{b}	16.00 ± 3.81^{a}	20.02 ± 0.51^{a}
Zn	58.92 ± 5.57^{a}	46.87 ± 12.11^{a}	43.99 ± 0.93^{a}	56.26 ± 4.37^{a}	65.39 ± 5.74^{a}	55.15 ± 8.02^{a}	54.44 ± 6.83^{a}	51.83 ± 10.10^{a}

Soil physicochemical properties and enzymes activities

Soil enzyme activities including SOD, POD and CAT, were shown in Fig. 3a-d. Compared with the CK group, the activities of three enzymes were significantly increased in the MR and MT groups by 48.95-354.17%. TS8 showed an insignificant effect on the enzyme activities. Besides, the pH values were significantly lower in the three experimental groups (MR: 6.67; TS: 6.61; MT: 6.54) than in the CK group (6.84). MR2 and mixed strains significantly improved the contents of available phosphorus (19.07% and 23.02%) and potassium (15.34% and 17.79%) in soil compared with the CK group (Fig. 3e-h). In addition, TS8 also significantly improved the available potassium content by 10.99%. Still, it significantly reduced the content of soil organic matter (41.80 ± 6.63 $g \cdot kg^{-1}$) (Fig. 3c). The content of TN in the MR, TS and MT groups showed no significant difference compared with the CK group (Fig. 3d).

Metal(loid)s morphology and contents in soil

The PGPB inoculants showed some changes in the metal(loid)s content of the soil (Table 2). The concentrations of As, Pb and Zn in the MR (23.91 mg/kg; 2768.66 mg/kg; 818.61 mg/kg), TS (25.11 mg/kg; 2567.52 mg/kg; 851.56 mg/kg) and MT (25.81 mg/kg; 2750.17 mg/kg; 740.61 mg/kg) groups were significantly decreased by 15.27–21.50% and 14.59%-25.72% compared with the CK group (30.46 mg/kg; 2942.89 mg/kg; 977.04 mg/kg). The concentrations of Cd were also significantly decreased by 8.64–15.52% in the MR and TS groups. However, Cu concentrations were not significantly different between the CK group and the PGPB inoculation groups. Figure 3i showed that PGPB could significantly enhance the remediation efficiencies of metal(loid)s, including As, Cd, Pb and Zn. However, the best treatment for removing different metal(loid) was different, such that MR2 showed the best effect on remediating Cd and As, TS8 on remediating Pb, and the mixed strains on remediating Zn.

We further explored the speciation distributions of metal(loid) using the method of BCR sequential extraction (Table S1 and Figure S2). The residual fraction was the dominant fraction of soil As (86.95%-90.23%), Cu (77.67%-79.35%), and Zn (81.50%-82.77%), the reducible fraction occupied the most proportion (80.20%-80.88%) of Pb, while four fractions of Cd were relatively uniformly distributed (Figure S2). It showed that PGPB significantly ($p < 0.05$) decreased the fraction concentrations of metals (except Cu) to some extent, which suggested PGPB could change the transformation of speciations (Table S1). For example, compared with the CK group (0.85 mg/kg; 1.43 mg/kg), MR2 (0.61 mg/kg; 1.17 mg/kg) significantly reduced the oxidisable and residual fractions.

Table 2
Total concentration of different metal(loid)s under the different treatments. (Unit: $mg \cdot kg^{-1}$)

	CK	MR	TS	MT
As	30.46 ± 0.49^{a}	23.91 ± 1.05^{b}	25.11 ± 2.01^{b}	25.81 ± 1.57^{b}
Cd	5.09 ± 0.17^{a}	4.30 ± 0.24^{c}	4.65 ± 0.06^{b}	4.80 ± 0.21^{a}
Cu	78.61 ± 9.6^{a}	79.42 ± 1.95^{a}	86.13 ± 3.25^{a}	79.95 ± 2.45^{a}
Pb	2942.89 ± 30.44^{a}	2768.66 ± 125.12^{b}	2567.52 ± 37.57^{c}	2750.17 ± 78.4^{b}
Zn	977.04 ± 49.01^{a}	818.61 ± 25.83^{bc}	851.56 ± 37.92^{b}	740.64 ± 15.93^{c}

Factor of bioconcentration and translocation of M. floridulus (Lab.) for metal(loid)s

In comparison with the control (0.07; 0.0054), MR2 (0.18) showed significantly ($p < 0.05$) enhanced the BCF of *M. floridulus* (Lab.) for Cd by 157.14% and TS8 (0.0091) significantly ($p < 0.05$) enhanced the BCF of *M. floridulus* (Lab.) for Pb by 68.52% (Table 3). For the TF of *M. floridulus* (Lab.) for Metal(loid)s (except Zn), significant differences were detected between the CK group and bacterial-inoculated groups (Table 3). MR2 significantly ($p < 0.05$) enhanced As (1.22), Cd (1.22) and Pb (1.22) translocation by 6.18, 2.56, 0.89 folds, respectively; TS8 significantly ($p < 0.05$) enhanced As (1.42), Cd (3.26) and Cu (1.36)

translocation by 7.35, 2.36, 0.55 folds, respectively; MT mixed culture significantly ($p < 0.05$) enhanced As (0.25), Cu (1.91) and Pb (1.47) translocation by 0.47, 1.17, 1.67 folds, respectively.

Table 3
Bioconcentration factors and translocation factors of *M. floridulus* (L.) in the different metal(loid)s

	Bioconcentration factors				Translocation factors			
	CK	MR	TS	MT	CK	MR	TS	MT
As	0.14 ± 0.01^{a}	0.05 ± 0.01^{b}	0.08 ± 0.03^{b}	0.08 ± 0.02^{b}	0.17 ± 0.02^{c}	1.22 ± 0.19^{a}	1.42 ± 0.69^{a}	0.25 ± 0.02^{b}
Cd	0.07 ± 0.03^{b}	0.18 ± 0.04^{a}	0.13 ± 0.03^{ab}	0.11 ± 0.02^{b}	0.97 ± 0.23^{b}	3.45 ± 0.18^{a}	3.26 ± 0.20^{a}	0.77 ± 0.35^{b}
Cu	0.33 ± 0.05^{a}	0.23 ± 0.02^{b}	0.21 ± 0.04^{b}	0.30 ± 0.03^{a}	0.88 ± 0.28^{c}	0.93 ± 0.13^{c}	1.36 ± 0.05^{b}	1.91 ± 0.41^{a}
Pb	0.0054 ± 0.0011^{b}	0.0033 ± 0.0005^{c}	0.0091 ± 0.0022^{a}	0.0057 ± 0.0006^{b}	0.55 ± 0.11^{b}	1.04 ± 0.18^{a}	0.64 ± 0.14^{b}	1.47 ± 0.39^{a}
Zn	0.062 ± 0.012^{a}	0.059 ± 0.009^{a}	0.054 ± 0.010^{a}	0.74 ± 0.014^{a}	1.11 ± 0.10^{a}	1.18 ± 0.15^{a}	1.24 ± 0.17^{a}	0.92 ± 0.12^{a}

Structure, diversity, and compositions of soil bacterial communities

We tested the bacterial community using 16S rRNA sequencing method, and the number of sequenced reads in each sample was more than 30,000. NMDS analysis showed clustering of the samples among different groups (Fig. 4a). Dissimilarities tests also showed significant differences in bacterial communities' structure among different groups (Table S2). Venn diagram (Fig. 4b) showed the numble of common OTUs was 1039, and the unique OTUs were 459, 350, 271 and 205, respectively, in CK, MR, TS and MT groups. There was a lower diversity of bacterial species in the MR, TS and MT groups than in the CK group (Fig. 4c). It suggested that inoculations of functional bacteria significantly affected the structure and diversity of the bacterial community.

There were 22 phyla, 130 families, 156 genera and 3303 OTUs. The community composition shifted with bacterial inoculants. At the phylum level (Fig. 4d), the dominant phyla (relative abundance > 1%) were Proteobacteria (37.46–52.10%), Actinobacteria (26.90-34.82%), Acidobacteria (8.91–11.10%), Gemmatimonadetes (4.87–7.54%), Chloroflexi (2.17–3.19%) and Nitrospirae (2.33–3.26%). Compared with the CK group, the groups with bacterial inoculants showed increases in the relative species abundance in the phylum Proteobacteria and showed decreases in the phylum *Acidobacteria*, *Actinobacteria, Chloroflexi*, *Gemmatimonadetes* and *Nitrospirae* (Fig. 4e).

At the family level, the dominant families (relative abundances > 1%) were *Xanthomonadaceae*, *Nocardioidaceae*, *Gaiellaceae*, *Enterobacteriaceae*, *Hyphomicrobiaceae*, *Syntrophobacteraceae*,

Micromonosporaceae, *Sphingomonadaceae*, *Micrococcaceae*, *Intrasporangiaceae*, *Oxalobacteraceae*, etc.. The *Enterobacteriaceae*, *Pseudomonadaceae*, *Comamonadaceae*, *Rhizobiaceae*, *Cytophagaceae*, *Rhodocyclaceae* and *Ellin517* families showed significant increases in relative abundance, and significantly decreased in the family *Hyphomicrobiaceae*, *Syntrophobacteraceae*, *Micromonosporaceae*, *Nocardioidaceae* and *Gaiellaceae* in the bacterial inoculant groups (Table S3). At the genus level (Table S4), the relative abundances of *Serratia* and *Enterobacter*, the same as MR2 and TS8 affiliated with the family *Enterobacteriaceae*, significantly increased in bacterial inoculant groups (Fig. 4f).

Network interactions of bacterial communities shifted under the bacterial inoculant

To discern possible microbial interactions in response to bacterial inoculants, RMT-based network approaches were used to construct molecular ecological networks (MENs) using 16S rRNA sequencing data. Table S5 shows the main topological properties of the empirical MENs of the microbial communities in the four groups. Using the same threshold (0.940), their correlations were greater than 0.722. This indicates that the degree distributions in the two constructed molecular ecological networks fit the power-law model well. There were more nodes and links in the CK group (475 nodes and 846 links) than those in the MR (392 and 765), TS (365 and 556) and MT (340 and 653) groups (Fig. 5a-d). It showed interactions of bacterial communities could be disrupted by bacterial inoculants, especially strain TS8. However, the proportion of positive links was increased in the bacterial inoculant groups (Table S5).

There were 3 module hubs and 3 connectors in the CK group, whereas only 1 connector in the TS group, and it was interesting that 8 (2 module hubs and 6 connectors) and 7 (1 module hub and 5 connectors) key nodes in the MR and MT groups, respectively (Fig. 5e). The key nodes were mainly affiliated to the phylum Proteobacteria, Actinobacteria and Chloroflexi (Fig. 5f).

Linkage of parameters in soil/plant and bacterial communities

To explore the interactions between soil/plant parameters and bacterial communities, all soil/plant parameters, e.g. antioxidant enzymes, nutrients, pH, chlorophyll, were considered and the Mantel test was performed (Table 4). The results showed that the parameters in soil ($r = 0.363$, $p = 0.001$) and plant ($r = 0.397$, $p = 0.001$) had a significant correlation with bacterial communities. Further analysis found that SOD/pH/available phosphorus in the soil, SOD/POD in underground tissues, and SOD/CAT/chlorophyll in overground tissues were significantly ($p < 0.05$) correlated with the structure of bacterial communities. In addition, we found soil bacterial communities showed more close relationships with the antioxidant enzymes in soil than in plant tissues, especially in the overground tissues.

To explore the relationships between different phyla/genera/typical OTUs and soil and plant parameters, Mantel tests were also performed. Similar results were found that fewer bacterial groups showed significant relationships with the parameters in the overground tissues. Phylum Proteobacteria and

Bacteroidetes were significantly ($p < 0.05$) and positively correlated with AK, AP, CAT, and SOD in soil and SOD and POD in the underground tissues (Figure S3). Some genera, e.g., *Enterobacter*, *Serratia*, *Sphingomonas*, *Pseudomonas*, *Ramlibacter*, and *Janthinobacterium*, had significant positive relationships with the parameters in soil and underground tissues while showing a significantly negative relationship with soil pH (Figure S4). The typical OTUs, including 21 key nodes in the co-occurrence network (hubs and connectors) and 11 OTUs affiliated to *Enterobacteriaceae* same as inoculated strains MR2 and TS8 (Figure S5). It was interesting that the OTUs (OTU_4, OTU_3357, OTU_282, OTU_2645 and OTU_17) affiliated to *Enterobacteriaceae* were significantly and positively correlated to the soil nutrient and antioxidant enzymes in soil and root of *Miscanthus*, while the key nodes (OTU_6, OTU_264 and OTU_223) in the ecological network was on the contrary.

Table 4
The interactions between parameters in soil, plant and bacterial communities

Parameters			r	p
Soil parameters	whole		**0.363**	**0.001**
	Antioxidant enzyme in soil	SOD in soil	**0.644**	**0.001**
		POD in soil	0.191	0.103
		CAT in soil	0.026	0.345
	Nutrient	AP	**0.379**	**0.001**
		AK	-0.028	0.500
		OM	-0.080	0.618
		TN	0.160	0.056
	-	pH	**0.293**	**0.008**
Plant parameters	whole		**0.397**	**0.001**
	Antioxidant enzyme in underground tissues	SOD in underground tissues	**0.347**	**0.001**
		POD in underground tissues	**0.421**	**0.001**
		CAT in underground tissues	-0.066	0.714
	Antioxidant enzyme in overground tissues	SOD in overground tissues	**0.142**	**0.026**
		POD in overground tissues	-0.092	0.721
		CAT in overground tissues	**0.145**	**0.040**
	Chlorophyll	Chlorophyll a	**0.345**	**0.003**
		Chlorophyll b	**0.498**	**0.001**
Antioxidant enzyme		Antioxidant enzyme in soil	**0.492**	**0.001**
		Antioxidant enzyme in underground tissues	**0.305**	**0.001**
		Antioxidant enzyme in overground tissues	0.055	0.234

Key factors affected the growth and metal uptake of Miscanthus

Spearman's tests were conducted to explore the key factors that affected the growth and metal uptake of *Miscanthus* (Fig. 6). We found the height, fresh and dry weight had significantly ($p < 0.05$) positive relationships with the antioxidant enzymes in soil/underground tissues and soil nutrients, except for total nitrogen. Different metals showed different responses to environmental parameters. The higher contents

of AK, AP, SOD, POD and CAT in soil and underground tissues might enhance the translocation factor of Pb/Cu and the remediation efficiency of Zn/Pb/As. AK and organic matter could affect the BCF of Cd, AP and SOD in underground tissues could affect the BCF of Zn, total nitrogen could affect the BCF of Zn and Cu, and chlorophyll could affect the BCF of metals except for Cu. In addition, low pH benefited Miscanthus for metals uptake.

Then, we explored the relationships between the key microbial population (genera/OTUs) and the growth/metal uptake of *M. floridulus* (Lab.). We found that some genera played their roles in affecting both growth and metal uptake (Figure S6), including *Bradyrhizobium*, *Catellatospora*, *Candidatus_Solibacter*, *DA101*, *Massilia*, *Mycobacterium* and *Pilimelia*, and some genera affected only growth or metal uptake, e.g., *Enterobacter*, *Bacillus*, *Pseudomonas* and *Sphingomonas*. The results indicated that the growth of *Miscanthus* showed significantly positive relationships with the OTUs (OTU_4, OTU_3357, OTU_282, OTU_2645 and OTU_17) affiliated with Enterobacteriaceae while showing negative relationships with the key nodes (OTU_6, OTU_489 and OTU_223) in the ecological network (Figure S7). The correlation analysis showed the remediation efficiency of all metals showed few correlations with the selected OTUs. In contrast, the BCF and TF of metals showed different responses to the variations of the bacterial community. Among these, the BCF of Cu and Pb and the TF of Cu, Pb and As were closely correlated to some OTUs. The correlation coefficient was the opposition between the BCF of Cu and Pb. For example, OTUs affiliated with *Enterobacteriaceae* showed positive relationships with the BCF of Pb while negatively correlated with the BCF of Cu.

The above 47 parameters were classified into plant chlorophyll, antioxidant enzymes in overground tissues, antioxidant enzymes in underground tissues, antioxidant enzymes in soil, soil nutrition, soil pH, bacterial OTUs affiliated to *Enterobacteriaceae* and bacterial OTUs in the ecological network, and further correlation analysis was conducted (Fig. 6c). It was found that the roles of antioxidant enzymes in overground tissues and bacterial OTUs in the ecological network were relatively weaker than other parameters in the metal uptake, and antioxidant enzymes in overground tissues and chlorophyll play weaker roles in miscanthus growth. Chlorophyll might be the most critical factor in Cd uptake; soil pH might be in As uptake; OTUs affiliated to Enterobacteriaceae and antioxidant enzymes in underground tissues might be in Cu uptake; Chlorophyll and antioxidant enzymes in underground tissues might be in Pb uptake; and antioxidant enzymes in underground tissues might be in Zn uptake. Besides, *Miscanthus* trait indices were significantly correlated with As, Pb and Zn remediation efficiencies. There was a significant positive correlation between the BCF or TF of cadmium and its removal efficiency, TF of As and Pb showed a significant and positive correlation with removal efficiencies. However, it was not guaranteed that the higher BCF or TF enhanced the remediation efficiency for metals, e.g., Zn.

Analysis of predictive functional enzyme and genes

The relative abundances of the enzyme about ROS, such as glutathione peroxidase, superoxide dismutase, and glutathione synthase, were the most abundant in TS (Table S6). Genes function prediction results showed that the genes related to metal transport about Mn, Zn, Cd, Cu et al. (Table S7)

were ABC.ZM.A, corA, corC, zntB, zipB, ycnJ, mtsB. Meanwhile, the relative abundances of the genes ABC.ZM.A, corC, and zntB were the most in MT.

Discussion

M. floridulus has become rooted and developed a large biomass, in their preliminary vegetation survey, reported that *M. floridulus* had high coverage and a strong phytoremediation ability in metal(loid)s contamination (Wu et al. 2021). Soil bacterial communities play lots of roles in the ecosystem (Strickland &Rousk 2010). Recently, a large number of studies have shown that PGPB is an excellent choice for improving the efficiency of phytoremediation of metal(loid)s (Xiao et al. 2019b). In our previous study, TS8 and MR2, affiliated with Enterobacteriaceae, were found to be PGPB and to enhance the phytoremediation efficiency of Cd (Liu et al. 2021). This study showed that these two strains also enhanced the phytoremediation efficiency of multi-metal(loid)s, including Cd, As, Pb and Zn.

Enterobacteriaceae potential to promote the growth and metal(loid)s uptake of Miscanthus in soil contaminated with multiple metals(loids)

Enterobacteriaceae, affiliated with Enterobacteriales, γ-proteobacteria, and Proteobacteria, now includes dozens of genera (Adeolu et al. 2016), e.g., *Klebsiella*, *Lelliottia*, *Shigella*, *Enterobacter*, *Escherichia*, *Pantoea*, *Salmonella* and *Serratia*. Species such as humans have been found in a wide range of environmental niches belonging to this family, infections, food, insects, soil, plants, and even in many extreme or hostile environments, e.g., saline-alkali land and diesel fuel-contaminated sites. Due to their pathogenic effects on agriculture, livestock and food safety, this family plays an essential role in the economy. Inversely, some members of this family, such as *Mangrovibacter spp.* (Behera et al. 2016), *Jejubacter spp.* (Jiang et al. 2020), *Pantoea spp.* (Gkorezis et al. 2016), *Serratia spp.* (Neupane et al. 2012), *Enterobacter spp.* (Ali et al. 2022, Francois et al. 2021) and *Klebsiella spp.* (Aloo et al. 2020, Gang et al. 2018) were found to have beneficial effects, such as improving plant growth by inhibiting plant pathogens, auxin production or nitrogen fixation.

In this study, *L. jeotgali* MR2 and *K. michiganensis* TS8 were found to enhance Miscanthus growth under multi-metal(loid)s toxic stress (Fig. 1), mainly because MR2 and TS8 had multi-metal(loid)s tolerance and plant growth-promoting (PGP) traits, including production of IAA and siderophore, nitrogen fixation, solubilization of P/K, which was shown in our previous study (Liu et al. 2021). And this study also showed that the soil nutrients (e.g., AP and AK) were improved with the bacterial inoculant (Fig. 3). It was not first found the PGP ability of *K. michiganensis*. Mitra et al. also found *K. michiganensis* MCC3089 had PGP traits and could enhance rice seedling growth (Mitra et al. 2018). *Lelliottia* was a relatively newly characterized genus within the Enterobacteriaceae family in 2013. *Lelliottia jeotgali* sp. nov. was found in fermented clam but did not detect the PGP traits (Yuk et al. 2018). The production of the IAA by some PGPR is a direct mechanism by which microorganisms can modulate plant growth. Therefore, it was predictable and reliable to the growth-promoting effects of *L. jeotgali* and *K. michiganensis* TS8.

Soil metal(loid)s can cause the increase of reactive oxygen species (ROS) in the plant body, such as superoxide anion (O^{-}), hydroxyl radical (OH^{-}), and hydrogen peroxide (H_2O_2), and ultimately produce oxidative stress (Xiao et al. 2019a). Mitra et al. reported that *K. michiganensis* MCC3089 reduced the Cd content within rice tissues (Mitra et al. 2019). However, through its ROS-dependent MAP kinase pathway, metal(loid)s-induced phytotoxicity can disrupt the phytohormone synthesising machinery, leading to senescence and cell death (Mitra et al. 2018). Under these conditions, the phytohormones produced by the PGPB come to the rescue to mitigate this damage and promote the growth of the seedling, as has been seen in rice and barley (Chang et al. 2014, Fernandez-Llamosas et al. 2020). In contaminated agricultural fields near the industrial belt, the strain's ability to bioaccumulate Cd could effectively eliminate Cd-induced phytotoxicity. Intracellular accumulations of Cd by metal-resistant bacteria have been previously reported in *Enterobacter* EG16 by Chen et al. Over the last decades, studies have indicated high concentrations of metal(loid)s can disrupt the function and metabolism of the cell and induce cell death (Xu et al. 2012) and denature enzymes and proteins (Anjum et al. 2016). To alleviate the damage of oxidative stress, plants removed excess ROS and suppressed lipid peroxidation by antioxidative enzymes system, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase (GPX) et al. (Xu et al. 2012). The high SOD, CAT, POD and GPX activities of *Zea mays* L. (Dong Dan 80) reduced the concentration of ROS and increased the accumulation of Cd and As in the 100 μM Cd and 200 μM As soil (Anjum et al. 2016).

Besides, the genome of Homologues of genes involved in plant growth-promoting traits such as inorganic phosphorus solubilization, nitrogen fixation, and siderophore production have been identified in *Pantoea ananatis* GB1. Although 16S rRNA gene sequence data and phenotypic profiling indicated that GB1 was closely related to the rice pathogen *P. ananatis* PA13, it was able to promote the growth of hybrid poplar cuttings and Lupinus luteus and Cytisus striatus. (Choi et al. 2012).

Other potential and benefit bacteria in Miscanthus rhizosphere

Microbial inoculants can affect native soil microbial communities in the short or long term. In this study, MR, TS and MT inoculation significantly changed microbial community composition and structure. In line with other similar studies, Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes and Nitrospirae were the most abundant at the phylum level in the *M. floridulus* rhizosphere soil (Fig. 4e). Pham et al. recently demonstrated that the rhizosphere bacteria of *Miscanthus giganteu*s were predominantly composed of Proteobacteria, Acidobacteria, and Gemmatimonadetes (Pham et al. 2018). Specifically, Proteobacteria was identified as the most abundant phylum, consistent with previous reports indicating that this taxon dominates in long-term contaminated soils.

Moreover, Proteobacteria is known to harbor many facultative anaerobic members capable of thriving in extreme pH conditions and has been reported to exhibit exceptional tolerance to metal(loid)s. Additional research conducted in the Hatu mining region also found that Actinobacteriota and Proteobacteria were

the dominant phyla (Qi et al. 2022). These findings underscore the importance of understanding the ecological significance of microbial communities in polluted environments and highlight the potential role of Proteobacteria as a critical contributor to soil remediation efforts. The findings above suggest that the application of MR, TS, and MT bacterial strains leads to a regulation of the microbial community's composition in soil. Notably, certain Proteobacteria are recognized for their positive impact on plant development, as they can facilitate nitrogen fixation, participate in carbon cycling, and enhance nutrient availability for plant growth. Both TS8 and MR2 strains fall into this category of plant growth-promoting bacteria, thereby exhibiting potential significance in enhancing soil quality and agricultural productivity. Taken together, these results help to understand the ecological implications of bacterial inoculation and highlighted the importance of exploring microbial-based strategies for sustainable agriculture. The study conducted by Yu et al. revealed the abundance of Proteobacteria had a significant positive correlation with the content of soil organic matter and total nitrogen (Yu et al. 2021).

A majority of the bacterial species belonging to the phylum Proteobacteria are categorized as anaerobic microorganisms, making them highly valuable in the process of pollutant degradation (Ma et al. 2022), and Proteobacteria exhibit remarkable tolerance towards multiple metal(loid)s (Ma et al. 2023). The potential of using plant growth promoting bacteria (PGPB) such as *Bacillus*, *Gemmatimonas* and *Sphingomonas* for the co-remediation of soils contaminated with multiple metals has been demonstrated in several previous studies (Chen et al. 2021). These bacteria have been shown to be effective enhancers of plant growth and tolerance to heavy metals and metal(loid)s, thus improving the phytoremediation of contaminated soils.

Actinobacteria are the second most abundant taxon, according to the results of this study. Actinobacteria show a strong response to changes in the physico-chemical properties of the soil in the rhizosphere (Yu et al. 2020). It was consistent with the previous research demonstrating correlations between Actinobacteria and soil physical properties (Jiang et al. 2017). Actinobacteria's ability to promote plant growth through biological nitrogen fixation, solubilisation of phosphorus, potassium and zinc, production of plant growth hormones and production of antagonists has also been recognised as a potential biological inoculant in agriculture (Igalavithana et al. 2019, Yadav et al. 2018). Overall, these findings provided a deeper understanding of the ecological role and potential utility of Actinobacteria in the environmental remediation systems.

Enterobacteriaceae inoculant shifted the soil microecological environment

The rhizosphere soil environment plays an essential role in phytoremediation and is the reaction site of complex biological and ecological processes, especially when it connects plant roots and soil biota (Tian et al. 2020). Soil microorganisms play an essential role in plant growth and regulating available nutrients, such as participation in energy flow, organic matter conversion, and the biochemical decomposition of other substances (Tong et al. 2017), depending on the structure and diversity of the soil microbial community. In this study, the relative abundances of *Serratia* and *Enterobacter* were significantly

increased in bacterial inoculant groups (Fig. 4f). Antioxidant enzymes are known to play a crucial role in promoting plant growth and maintaining the health of soil microbial communities by reducing lipid peroxidation levels (Parvin et al. 2020). These studies have highlighted the potential of MR2, TS8 and MT to enhance soil antioxidant enzyme activity and mitigate microbial oxidative stress (Fig. 3).

Soil metal(loid)s play a significant role in shaping the inter-rhizosphere soil bacterial community. In addition to metal(loid)s, soil nutrients, including available phosphorus (AP), available potassium (AK), and total nitrogen (TN), also greatly impact bacterial diversity and distribution (Ma et al. 2020). However, after *Miscanthus floridulus* (Lab.) was inoculated with PGPB, the interaction of the soil microbial community was not clearly understood. Revealing the relationship helps evaluate the effectiveness of the combined remediation of *M. floridulus* (Lab.) and microorganisms. In our study, *Bradyrhizobium*, *Catellatospora*, *Massilia* could affect the growth and sorption of multi-metal(loid)s by *Miscanthus* (Figure S6). This study demonstrates significantly positive relationships between soil parameters and the presence of *Enterobacter*, *Serratia*, *Sphingomonas*, *Pseudomonas.* These bacteria have been identified as producers of phytohormones (Abreo et al. 2021, Pan et al. 2017, Srisuk et al. 2018), such as *Enterobacter cloacae* CTWI-06, which has been shown to increase seed germination by synthesizing growth hormones. (Pattnaik et al. 2020). Additionally, PGPB protects plants from harmful metal(loid) poisoning and enhances growth and productivity by producing indole-3-acetic acid, ammonia, potassium, phosphate solubility and antifungal activity (Ndeddy Aka &Babalola 2016). Furthermore, Guo et al. observed that catalase activity was enhanced and the accumulation of reactive oxygen species in plants was reduced by inoculating *Suaeda salsa* with *Sphingomonas prati*. (Guo et al. 2021). The introduction of mixed bacterial agents, such as MR, TS, and MT, into the inter-rhizosphere soil could enrich other beneficial bacteria and, in turn, enhance plant growth under metal stress. These findings suggest the potential application of PGPB and mixed bacterial agents as effective bioremediation strategies in metal-contaminated soil.

The utilization of co-occurrence networks for the characterization of soil microbial communities and their response to environmental changes is gaining momentum in microbial ecology. A study by Hou et al. (Hou et al. 2019) demonstrated that the intensity and activity of microbial interactions are critical determinants of the complexity of co-occurrence networks. The identification and characterization of cooperative and competitive microbial interactions, as well as the determination of network modularity, are crucial for understanding the stability of microbial communities in response to various environmental factors (Xu et al. 2023). The results obtained through network analysis indicate that microbial networks processed with MR, TS, and MT displayed lower levels of nodes and links when compared to CK (Fig. 5). Nevertheless, a notable increase in the proportion of positive links was observed in bacterial inoculant groups, the observed elevation in positive linkages implies a reduction in competitive forces and a simultaneous strengthening of synergistic interactions among microorganisms, underscoring the efficacy of the intervention in shaping microbial networks. This phenomenon can be attributed to the enhancement of soil microbial functions and interactions through the inoculation of PGPB (Xu et al. 2023). The intricate microbial network becomes less susceptible to external stresses and enlists the aid of highly efficacious PGPB to safeguard the inter-root microecological environment of plants, thereby

bolstering their resilience against external stressors and promoting natural growth (Tao et al. 2020). These findings hold significant implications for research and development in microbiology and highlight the crucial role of bacterial inoculants in modulating microbial communities and promoting plant growth.

Conclusion

This research article delves into the possibility of efficacious synergistic remediation of multi-metal(loid)s contaminated soil through the inoculation of *Miscanthus floridulus* (Lab.) with *Lelliottia* MR2 and *Klebsiella michiganensis* TS8. The results indicate that the use of plant-promoting bacteria facilitated the growth of *M. floridulus* (Lab.), particularly with the application of *K. michiganensis* TS8.

After implementing a synergistic remediation approach, the concentrations of As, Cd, Pb, and Zn in the soil were observed to decrease at varying degrees, the activities of antioxidant enzymes in the underground tissues and in the soil were observed to increase, while the contents of available K and P also increased. While biodiversity was diminished and interactions among rhizosphere bacteria were curtailed, the inoculation bolstered the prevalence of bacteria with plant growth-promoting attributes and augmented the metabiosis and symbiosis of rhizosphere bacteria. In addition, the OTUs associated with Enterobacteriaceae, as well as soil nutrients, have been identified as crucial factors for the remediation of metal(loid) pollutants and the growth of *M. floridulus* (Lab.). The results of this study indicate that the introduction of *Lelliottia* MR2 and *Klebsiella michiganensis* TS8 can serve as effective measures to enhance the phytoremediation of multi-metal(loid)s-contaminated soils. Overall, these findings hold significant implications for improving environmental sustainability and promoting the remediation of contaminated soil.

Declarations

Ethical Approval This article does not contain any studies with human participants performed by any of the authors.

Consent to Participate This manuscript is approved by all authors for participation.

Consent to Publish This manuscript is approved by all authors for publication.

Author contribution YHX and JF contributed to the study's conception and design. Research, material preparation, data collection and analysis were performed by JJM, LC, RC, and SML. The first draft of the manuscript was written by JJM and SML. YHX and BY commented on previous versions of the manuscript. All aforementioned authors read and approved the final manuscript.

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Data availability The 16S rRNA sequencing data were deposited to the sequence read archive (SRA) of the National Center for Biotechnology Information (NCBI) under accession number PRJNA950185.

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Figures

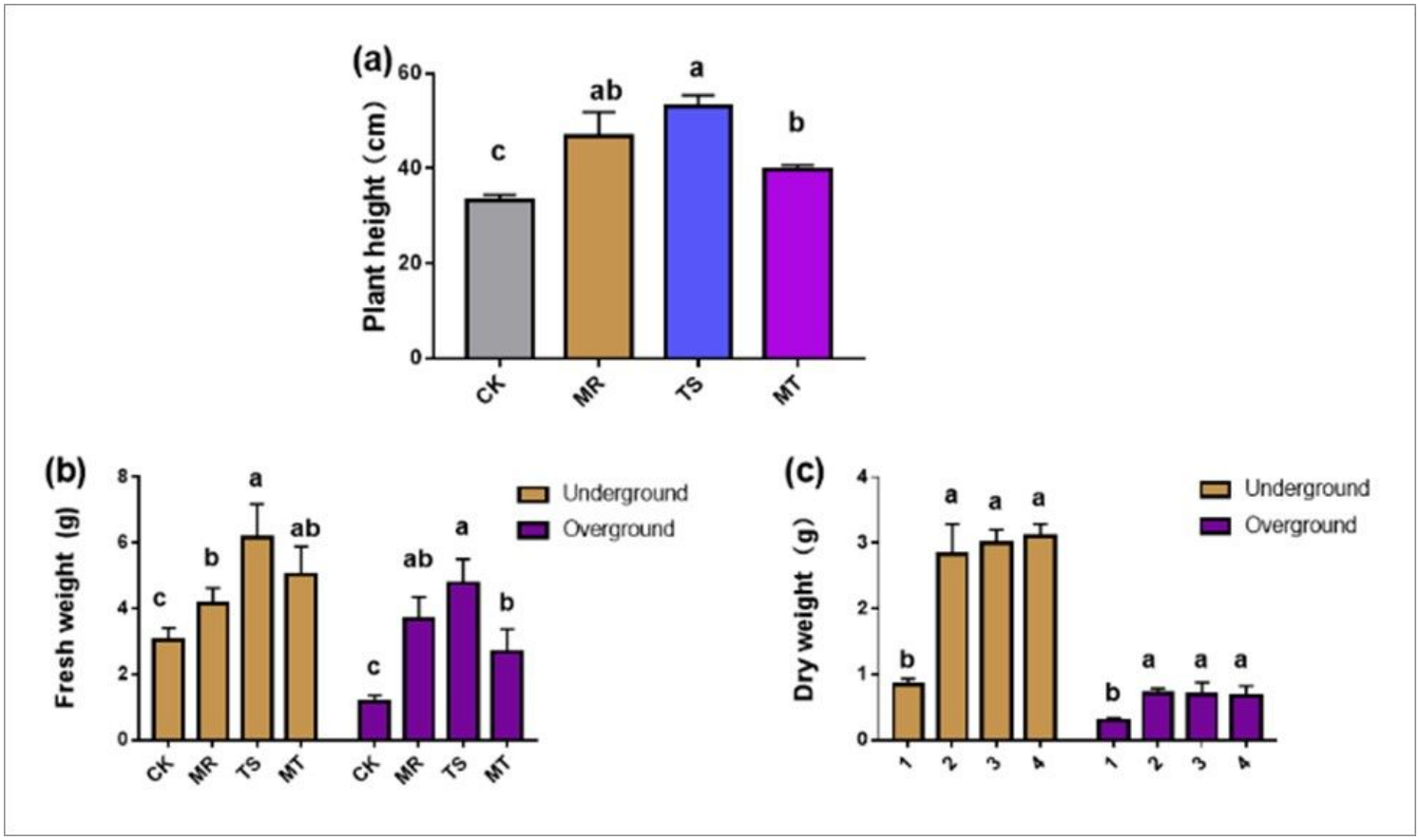


Figure 1

Growth parameters of *M. floridulus* (Lab.). **(a)** Plant height; **(b)**Fresh weight; **(c)** Dry weight.

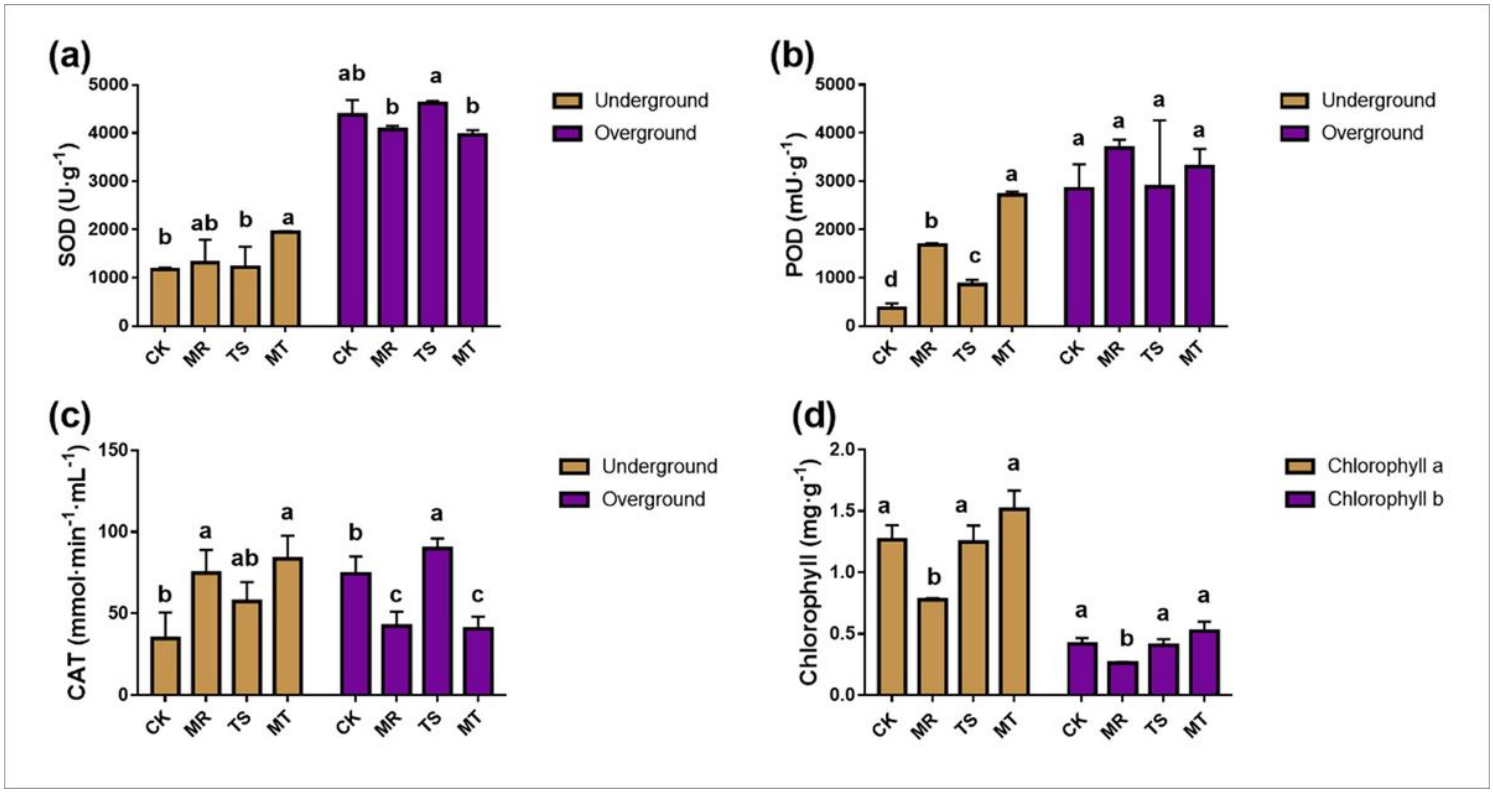


Figure 2

Enzymes activities and leaves chlorophyll content of *M. floridulus* (Lab.). **(a)** SOD; **(b)**POD; **(c)** CAT; **(d)** chlorophyll.

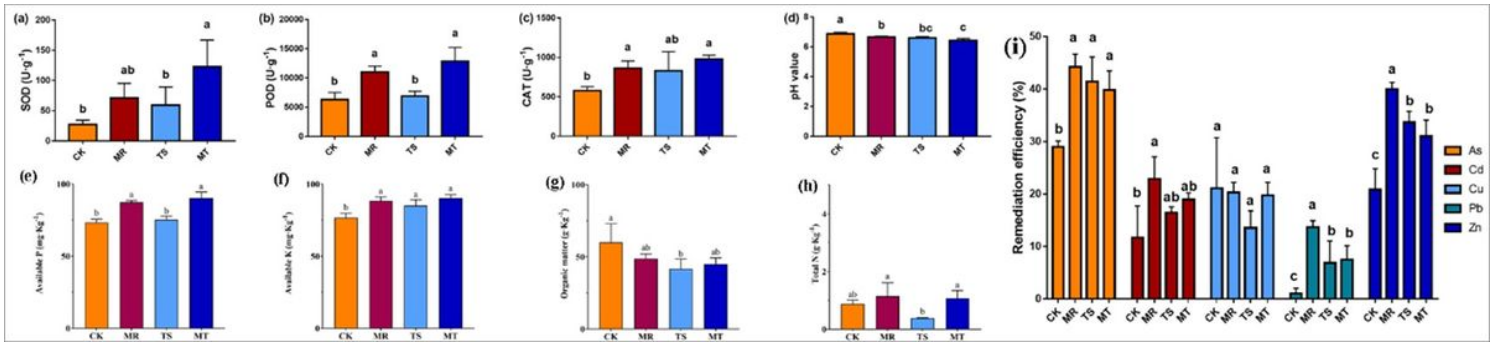


Figure 3

Soil enzyme activities and nutrients. **(a)** SOD; (b) POD; **(c)** CAT; **(d)** pH value; **(e)** Available P; **(f)** Available K; **(g)** Organic matter; **(h)**Total N; **(i)** Remediation efficiency of As, Cd, Cu, Pb, Zn

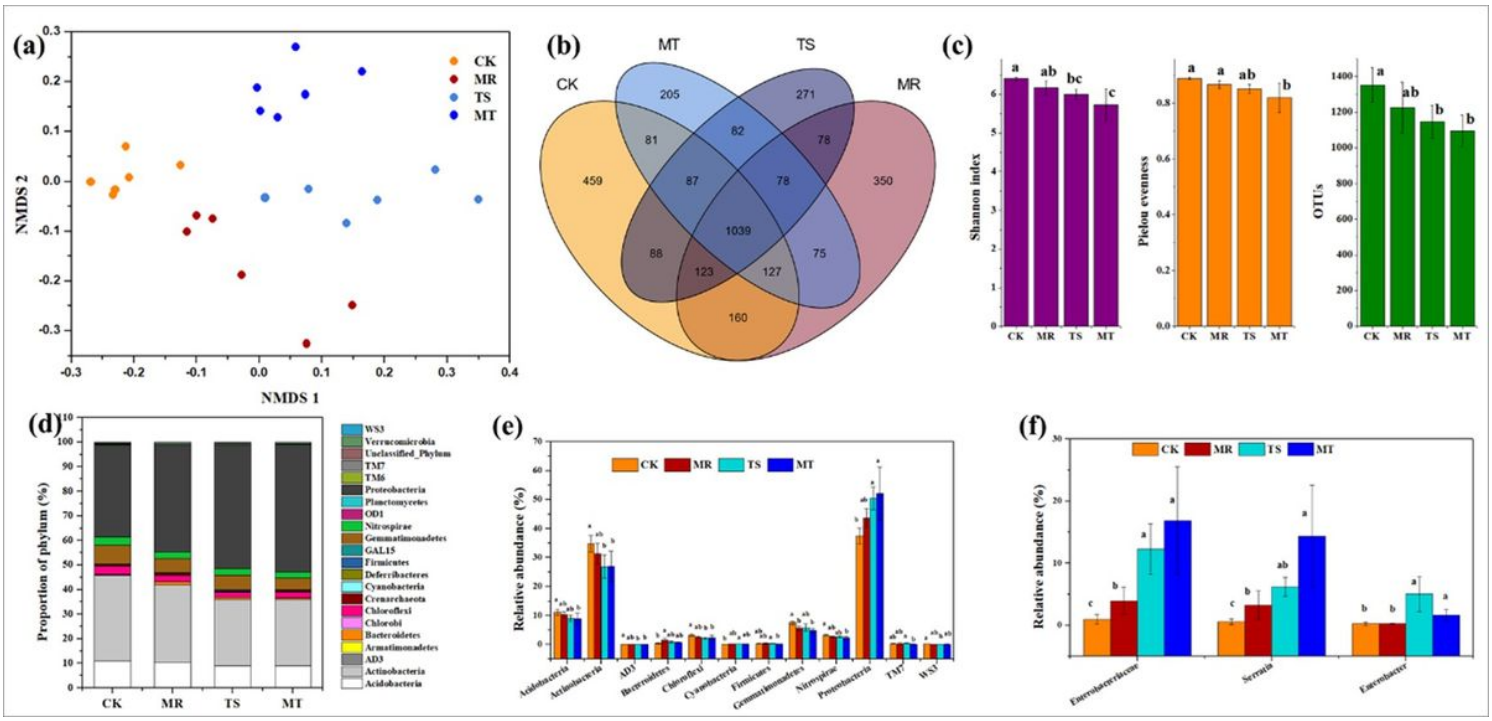
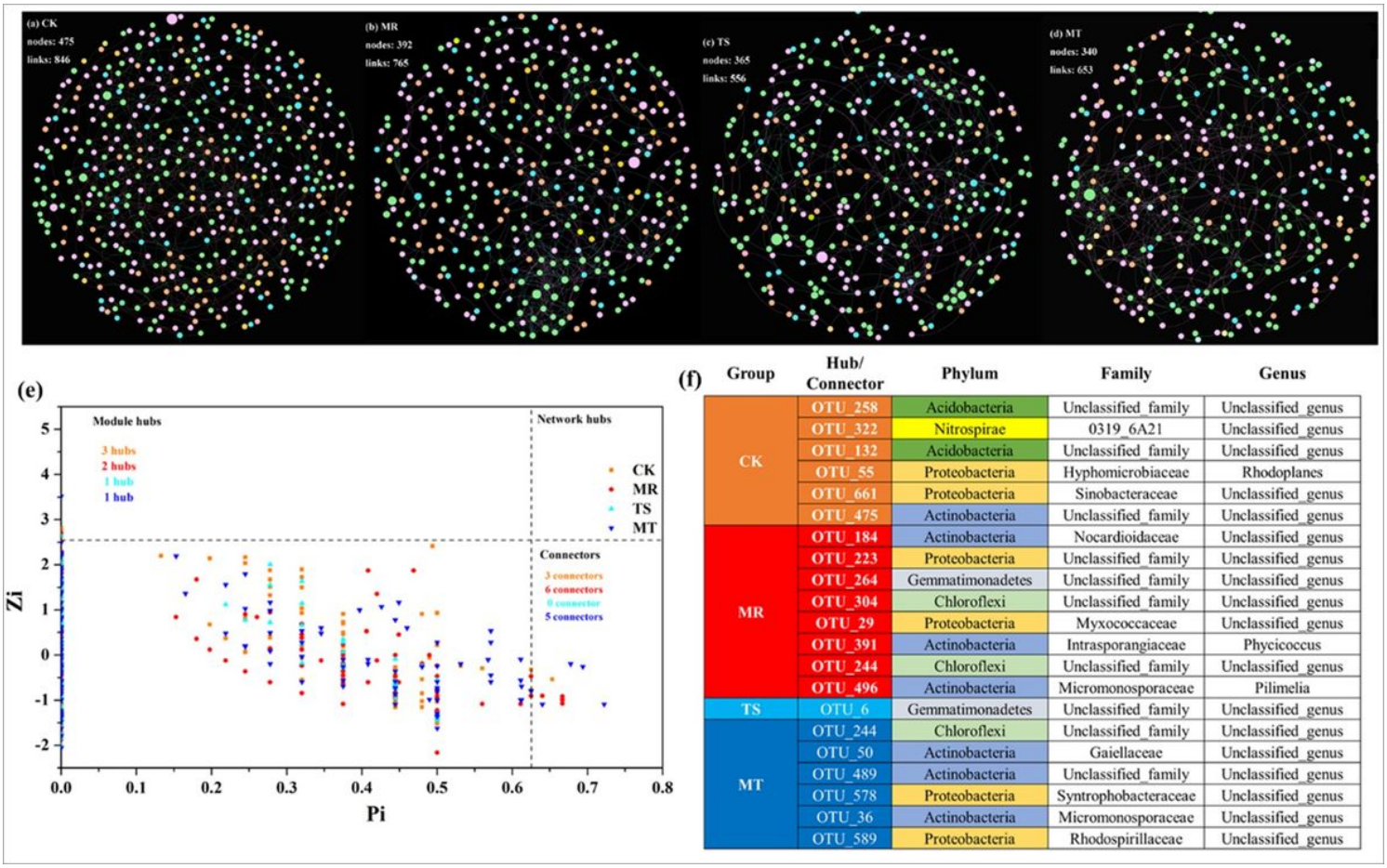


Figure 4

Structures, diversities and compositions of bacterial communities in different groups. **(a)** Plots of NMDS; **(b)** Venn plot; **(c)** Diversity indexes; **(d)**Compositions in phylum level; **(e)** Significant differences in phylum level; **(f)** Significant differences of Enterobacteriaceae and its genera



Group	Hub/ Connector	Phylum	Family	Genus
CK	OTU_258	Acidobacteria	Unclassified_family	Unclassified_genus
	OTU_322	Nitrospirae	0319_6A21	Unclassified_genus
	OTU_132	Acidobacteria	Unclassified_family	Unclassified_genus
	OTU_55	Proteobacteria	Hyphomicrobiaceae	Rhodoplanes
	OTU_661	Proteobacteria	Sinobacteraceae	Unclassified_genus
	OTU_475	Actinobacteria	Unclassified_family	Unclassified_genus
MR	OTU_184	Actinobacteria	Nocardioidaceae	Unclassified_genus
	OTU_223	Proteobacteria	Unclassified_family	Unclassified_genus
	OTU_264	Gemmatimonadetes	Unclassified_family	Unclassified_genus
	OTU_304	Chloroflexi	Unclassified_family	Unclassified_genus
	OTU_29	Proteobacteria	Myxococcaceae	Unclassified_genus
	OTU_391	Actinobacteria	Intrasporangiaceae	Phycicoccus
	OTU_244	Chloroflexi	Unclassified_family	Unclassified_genus
	OTU_496	Actinobacteria	Micromonosporaceae	Pilimelia
TS	OTU_6	Gemmatimonadetes	Unclassified_family	Unclassified_genus
MT	OTU_244	Chloroflexi	Unclassified_family	Unclassified_genus
	OTU_50	Actinobacteria	Gaiellaceae	Unclassified_genus
	OTU_489	Actinobacteria	Unclassified_family	Unclassified_genus
	OTU_578	Proteobacteria	Syntrophobacteraceae	Unclassified_genus
	OTU_36	Actinobacteria	Micromonosporaceae	Unclassified_genus
	OTU_589	Proteobacteria	Rhodospirillaceae	Unclassified_genus

Figure 5

Co-occurrence Networks analysis. **(a)** Ck; **(b)** MR; **(c)** TS; **(d)** MT; **(e)**ZP diagram; **(f)** connector and hubs

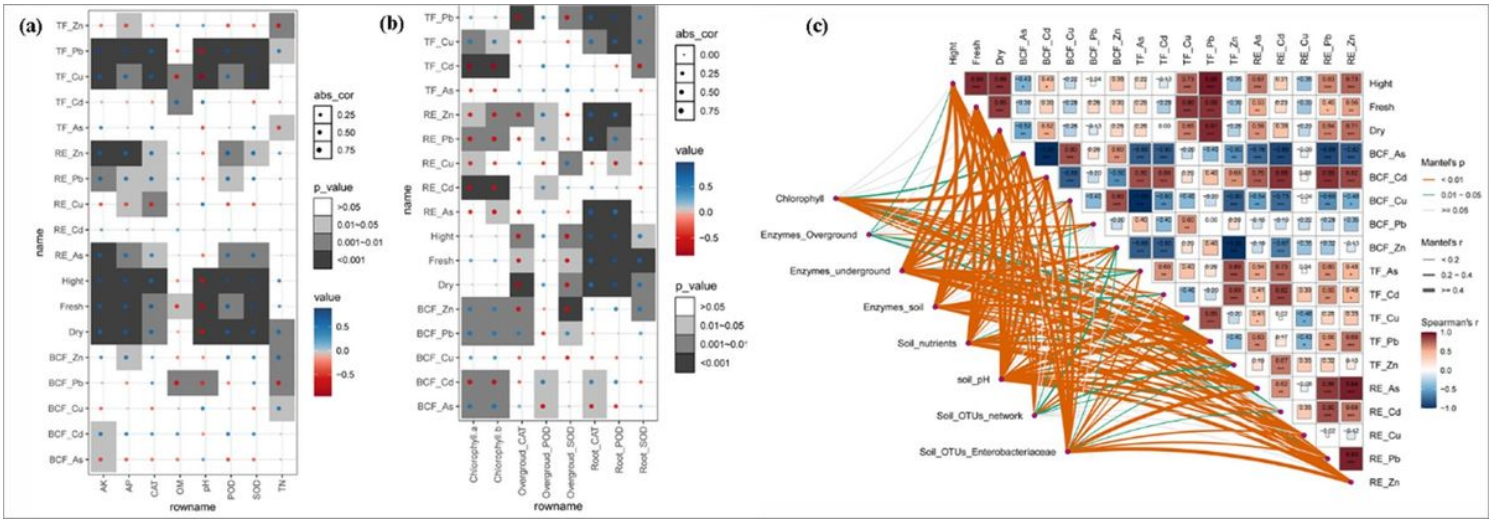


Figure 6

Analysis of the correlations between the bacterial communities, the TFs, Res and BCFs of heavy metals and the physico-chemical properties of the soil. **(a)** Spearman correlations of the plant growth, metal uptake and soil environmental factors; **(b)** Spearman correlations of plant growth, metal uptakes and

enzymes activity; **(c)** The relationships among the chlorophyll, enzymes in overground, enzymes in underground, enzymes in soil, soil nutrition, soil pH, soil OTUs network, soil bacterial OTUs affiliated to *Enterobacteriaceae* Mantel tests

Supplementary Files

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