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# Heavy metal effects on multitrophic level microbial communities and insights for ecological restoration of an abandoned electroplating factory site<sup>☆</sup>

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## ABSTRACT

The response of soil microbes to heavy metal pollution provides a metric to evaluate the soil health and ecological risks associated with heavy metal contamination. However, a multitrophic level perspective of how soil microbial communities and their functions respond to long-term exposure of multiple heavy metals remains unclear. Herein, we examined variations in soil microbial (including protists and bacteria) diversity, functional guilds and interactions along a pronounced metal pollution gradient in a field surrounding an abandoned electroplating factory. Given the stressful soil environment resulting from extremely high heavy metal concentrations and low nutrients, beta diversity of protist increased, but that of bacteria decreased, at high versus low pollution sites. Additionally, the bacteria community showed low functional diversity and redundancy at the highly polluted sites. We further identified indicative genus and “generalists” in response to heavy metal pollution. Predatory protists in *Cercozoa* were the most sensitive protist taxa with respect to heavy metal pollution, whereas photosynthetic protists showed a tolerance for metal pollution and nutrient deficiency. The complexity of ecological networks increased, but the communication among the modules disappeared with increasing metal pollution levels. Subnetworks of tolerant bacteria displaying functional versatility (*Blastococcus*, *Agromyces* and *Opiritatus*) and photosynthetic protists (microalgae) became more complex with increasing metal pollution levels, indicating their potential for use in bioremediation and restoration of abandoned industrial sites contaminated by heavy metals.

## 1. Introduction

With rapid industrialization and urbanization, soil heavy metal pollution has become a prominent global issue (Mielke et al., 2019). After deposition to soil, these hazardous metals can persist for decades as they do not degrade, thereafter accumulate in the edible plants and amplify along food chains, thereby triggering a serious threat to the environment and human health (Chen et al., 2015; Sevik et al., 2020b). The majority of previous studies have concentrated on metal polluted soils in farmland and mine tailings (Sun et al., 2018; Wang et al., 2023).

There has been far less attention focused on metal pollution risks associated with industrial waste contaminated sites. Abandoned industrial waste complexes often contain extremely hostile habitats for soil organisms because of the combination of very high concentrations of toxic metals and very low nutrient levels in contrast to other metal polluted ecosystems (Xu et al., 2022). To fully assess soil organism toxicity, field studies are needed as heavy metal pollution in soils often involves a combination of heavy metals that may interact with other soil pollutants and natural soil constituents (e.g., soil organic matter and inorganic soil colloids) (Liu et al., 2015). Presently, there is a paucity of information

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concerning the impacts of long-term exposure of indigenous microorganisms to combined heavy metal pollution at highly contaminated industrial waste sites.

Soil bacteria display great diversity and drive biogeochemical cycles via various metabolic activities (Sokol et al., 2022). For instance, bacteria are actively involved in decomposition of organic carbon accompanied by respiration and nutrient mineralization, thereby improving plant growth (Edwards et al., 2015). Further, microbes have the potential to remediate heavy metal pollution by decreasing the toxicity and mobility of some heavy metals through biotransformation (Fakhar et al., 2022). Protists, a taxonomically diverse and functionally versatile part of the soil food web, represent an enormous seedbank of dormant bodies ranging from  $10^4$  to  $10^7$  individuals per gram of soil and play a disproportionate role in soil ecosystem functions (Adl and Coleman, 2005; Adl et al., 2019). Notably, the role of protists is often overlooked in soil ecological processes although protists are found across multiple trophic levels of the soil food web (Geisen et al., 2018; Wu et al., 2022). Phagotrophic protists can stimulate bacterial activity by eliminating aging individuals and releasing nutrients from microbial necromass into the soil environment, thereby contributing to plant growth and biogeochemical cycling of elements (Geisen et al., 2021; Sokol et al., 2022). Photosynthetic protists (traditionally termed microalgae) are essential primary producers contributing carbon to the soil organic matter pool to support heterotrophic microorganisms, which is especially important in early successional ecosystems (Geisen et al., 2018). In spite of the importance of protists in soil food webs, the majority of previous studies examining the effects of xenobiotic substances have focused exclusively on soil bacteria. Herein, we highlight the need for a multitrophic perspective to disentangle the ecological risks of metal pollution on soil biotic communities and their functions.

Useful bioindicators can be used to monitor human disturbance and environmental quality (Certin et al., 2020). Previous studies employed the tree-rings and the tissues of certain types of plant (i.e., cedar tree, rosemary and blue spruce) as bioindicators to monitor the heavy metals in air (Certin et al., 2020; Sert et al., 2019; Sevik et al., 2020a). In soil, both bacteria and protist have considerable potential as bioindicators for assessment of soil ecosystem health and ecological risks under anthropogenic environmental disturbances due to their high sensitivity to stressors and short generation times (Guo et al., 2021; Wilhelm et al., 2023). Metal pollution can exert a strong selective force on belowground microbial communities affecting their ecosystem functions (Liu et al., 2018; Xu et al., 2019). Microorganisms that are susceptible to heavy metals preferentially perish at higher heavy metal concentrations because of oxidative damage, and are thereby classified as “sensitive” taxon (Chen et al., 2020). In contrast, some microorganisms adapt to high concentrations of toxic metals leading to a tolerance to metal pollution, and resulting in their classification as “resistant” taxon (Chen et al., 2020). The functional roles of the specific microorganisms enriched at different metal pollution levels may be different and are largely unknown (Chen et al., 2020). Finally, some microorganisms have the ability to colonize across a wide range of pollution levels and are classified as “generalists”, which often display functional versatility (Geng et al., 2022).

Previous isolation of tolerant and abundant species from polluted soils identified divergent functions, such as enhancing phytoremediation efficiency by fixing nitrogen, promoting root growth, and alleviating metal toxicity for plants (Fakhar et al., 2022; Hao et al., 2015). Thus, it is warranted to explore the indicative microorganisms in combination with their functions under harsh environmental conditions, such as heavy metal polluted soils. Targeting the indicative taxa whose manipulation might have especially broad impacts on other important taxa allows practitioners to assess soil health surveillance and exploit bioremediation of polluted soil environments (Barnes et al., 2017).

The current study examines soils in an abandoned electroplating factory, which was polluted by a combination of heavy metals. To better evaluate heavy metal pollution levels, Nemerow index was adopted,

which can effectively provide a comprehensive measure of combined metal pollution and has been widely used in the previous studies (Chen et al., 2015; Ma et al., 2018; Qi et al., 2022). Amplicon sequencing provides an unprecedented approach to acquire a myriad of information on microbial community in soil “black box” (Ramirez et al., 2018). By way of amplicon sequencing, we determined the microbial community and filtered out the species adapting to different heavy metal niches along the combined metal pollution gradient. Herein, we addressed a set of questions fundamental for advancing our understanding of soil microbial communities at heavy metal contaminated industrial sites: 1) how do bacterial and protist diversity (including alpha and beta diversity) respond to compound heavy metal pollution? 2) how do bacterial and protist functional guilds vary among different pollution levels? and 3) what roles do indicative taxa and “generalists” play in network interactions under different pollution levels? We hypothesize that: 1) the protist community would be more sensitive to metal pollution than the bacterial community because protists have a wider spectrum of feeding habits across multiple trophic levels; 2) the functional redundancy of microorganisms would be attenuated under metal pollution; and 3) the complexity of co-occurrence networks would increase with an increase in metal pollution due to the tolerant taxa and “generalists”. Understanding how microbial diversity, community structure, function and species interactions are subject to heavy metal pollution improves our ability to predict and manage microbiome functions for soil ecosystem services and human/environmental health. Results of this study provide information to enhance ecosystem biomonitoring and provide a theoretical basis for ecological restoration of abandoned industrial sites with severe heavy metal contamination.

## 2. Materials and methods

### 2.1. Study site and soil sampling

Soils were collected in April 2019 from a field at an electroplating factory abandoned (operational from 1980s to 2004) in Jingjiang City ( $120^{\circ}23'E$ ,  $32^{\circ}01'N$ ), Jiangsu Province, China. This site has a subtropical monsoon climate with a mean annual temperature of  $18^{\circ}C$  and precipitation of 1079 mm (<http://www.nmc.cn/>). Dominant plants on the abandoned site were *Erigeron multiradiatus*, *Taraxacum mongolicum* and *Phragmites australis*. Twenty-six sampling points were evenly distributed across the 0.3 ha study site and at each sampling point, five soil cores from the 0–20 cm depth were mixed to obtain a composite sample. The soil is developed on silica-aluminum parent material, has a silt loam texture and is classified as Oxisols according to USDA Soil Taxonomy. Soil samples were stored in sealed polyethylene bags and transported on ice to the laboratory for immediate processing. Each fresh sample was homogenized by passing through a 2-mm sieve to remove any plant debris and coarse fragments. Samples were then allocated for two sets of analyses: one was stored at  $4^{\circ}C$  for determination of physicochemical properties and the second was stored at  $-80^{\circ}C$  for DNA extraction/analysis.

### 2.2. Soil physicochemical properties

Physicochemical properties were determined on air-dried soils. Soil pH was measured in a 1:2.5 soil/water suspension with a glass electrode after a 30 minutes equilibration (Mettler, S220-Bio-CN, Giessen, German). The soil in this electroplating factory was alkaline because the most electroplating factories apply alkaline precipitation to deal with the waste water and thus produce electroplating sludge, which can seep into ground water and result in alkaline soil (Xu et al., 2015; Peng and Tian, 2010). Soil total carbon (TC) and total nitrogen (TN) were analyzed on an elemental analyzer (Elementar Vario EL Cube, Germany). The C:N mass ratio was calculated from TC and TN. A subsample of soil was passed through a 0.15-mm sieve and digested with a perchloric-nitric-hydrochloric acid mixture using microwave digestion

to quantify total concentrations of seven heavy metals: zinc, nickel, lead, cadmium, chromium, copper and arsenic (Yang et al., 2018). Available heavy metals (Zn, Ni, Pb, Cd, Cr and Cu) were determined by 0.01 M CaCl<sub>2</sub> (1:10 soil:water) extraction (Song et al., 2018), whereas available As was extracted by 0.5 M NaH<sub>2</sub>PO<sub>4</sub> (1:15 soil:water) (Huang et al., 2006). Heavy metal concentrations in acid digests and extracts were measured by ICP-MS (PerkinElmer Nexlon300X, USA).

To overcome the challenges of characterizing the combined pollution level of multiple heavy metals that co-varying across the landscape, the integrated Nemerow pollution index (PI<sub>N</sub>) was used to quantify the overall heavy metal pollution risk (Chen et al., 2015; Ma et al., 2018). The integrated Nemerow pollution index was calculated as:  $PI_N = \sqrt{\frac{MaxPI_i^2 + AvePI_i^2}{2}}$ , wherein the single pollution index (PI<sub>i</sub>) is defined as the ratio of the detected concentration of each heavy metal and its background value (Ma et al., 2018). The background concentrations of heavy metals in Jiangsu Province soils were originated from Yang et al. (2018) and summarized in Table S1.

### 2.3. High-throughput DNA sequencing and bioinformatics analysis

Genomic DNA was extracted from 0.4 g of soil using a MoBio PowerSoil DNA Isolation kit (MoBio, Carlsbad, CA, USA) following manufacturer instructions. DNA quantity and quality were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Germany). Universal primers FW-TAREuk454FWD1 (5'-CCAGCASCYCGCGTAATCC-3') and REV-TAREukREV3 (5'-ACTTTCGTTCTTGATYRA-3') were used to amplify ca. 418 bp fragments covering the V4 hypervariable region of the protist 18S rRNA gene (Stoeck et al., 2010). Primer sets 515F (5'-GTGCCAGMCGCCGGTAA-3') and 806R (5'-GGACTACCCGGTATCTAAT-3') were applied to cover the V4 hypervariable region to target the bacterial 16S rRNA gene (Bates et al., 2011). Paired-end sequencing was conducted on an Illumina HiSeq 2500 platform (Illumina, USA).

Sample sequences were de-multiplexed based on their barcode sequences. Then, paired-end reads were aligned using a maximum mismatch of 10 bases and a minimum alignment ratio of 80%. Aligned sequences were further filtered to discard reads with expected errors >0.5. VSEARCH was applied to dereplicate the remaining sequences. The resulting sequences were clustered into OTUs using the UPARSE algorithm at 97% similarity and chimeras were discarded with UCHIME (Edgar, 2013). Representative sequences for each OTU were determined and an OTU abundance table was generated by mapping reads to OTUs. Each record in the OTU table was rarefied to the level representing the lowest number of sequences per library across all samples. Taxonomic assignments for protist OTUs were obtained using SINTAX classifier against the PR2 database (Protist Ribosomal Reference database) (Guillou et al., 2013). Bacteria taxonomy was assigned against the RDP database in the same way (Cole et al., 2013). OTUs affiliated with *Fungi*, *Metazoa*, *Opisthokonta*, *Rhodophyta*, *Streptophyta* and other unassigned species in the protist OTU table were filtered prior to alpha diversity analysis (Zhao et al., 2019). The raw sequence data reported in this paper were deposited in the Genome Sequence Archive (GSA) database (accession number: PRJNA907876).

### 2.4. Statistical analyses

All statistical analyses were performed with R statistical software. Inverse Distance Weighting (IDW) interpolation in arcGIS (10.2) was applied to depicted the spatial distribution of various heavy metals across the study area. LOESS non-parametric regression was applied to assess the relationship between bacteria and protist diversity with the integrated Nemerow index. Based on the fitted curve, we identified two turning points at Nemerow index values of 100 and 200. Using the integrated Nemerow indices values of 100 and 200 as cut-off points, we divided the 26 soil samples into three heavy metal pollution levels,

designated as low, moderate and high.

Relationships between various soil factors were analyzed with Pearson's correlations. The Mantel test was applied to evaluate relationships between soil factors and the two organism communities (bacteria and protist). These relationships were presented in a Mantel test plot using the *ggcor* package (Huang H, 2020). As shown in Table S2, functional guilds of protists, which are often conserved within the same genus, were identified at the genus level based on Adl et al. (2019). Functional profiles and functional redundancy of the bacteria community were predicted using FAPROTAX (Louca et al., 2016) and R package "Tax4Fun2" (Wemheuer et al., 2020). The function annotation results are provided in Table S3. Levins' niche breadth index was applied to evaluate the niche breadth of soil species with "spaa" package (Pandit et al., 2009).

To identify the taxa responding to heavy metals (i.e., bioindicators), linear discriminant analysis effect size (LEfSe) was utilized to identify significantly different taxon at different heavy metal levels using default settings (Alpha = 0.05, LDA >2.0, All-against-all or strict comparison) (Segata et al., 2011). The LEfSe results were calculated online (<http://huttenhower.sph.harvard.edu/galaxy>). The most abundant 948 OTUs present in 80% of all replicates within each pollution level were applied to network construction. Only significant correlations ( $P_{adj} < 0.05$ ,  $P$  values adjusted by Bonferroni-Holm's correction) with Spearman's rank correlations  $\rho > 0.6$  were conserved in the networks. The co-occurrence network containing protists and bacteria was depicted in *gephi*. Differences in soil properties, alpha diversity and abundance of soil organisms among the three metal contamination levels were assessed by one-way ANOVA and Tukey's HSD post-hoc test after normality and homogeneity of variance test. Differences in microbial community composition were assessed by PERMANOVA.

## 3. Results

### 3.1. Heavy metal pollution status

The spatial heterogeneity and the maximum concentrations of heavy metals at the abandoned electroplating site was extremely high (Fig. S1 and Fig. 1). Notably, the heterogeneity increased along the pollution gradients as shown by the increasing standard deviation in Table S4, whereas the concentrations and heterogeneity of the nutrient elements (C and N) were very low (Table S4). While heavy metal hotspots were common, most of the sampling points possessed lower heavy metal concentrations (Fig. 1). The distribution patterns of Zn and the Nemerow index were similar, with Zn being the dominant toxic metal reaching concentrations up to 38700 nullmg nullkg<sup>-1</sup> (Fig. 1 and Fig. S1).

As the spatial distribution of the seven heavy metals was often contrasting, we assessed the overall metal pollution level by calculating the Nemerow index, which takes into account all the heavy metals. To assess the impact of combined heavy metals on soil organism diversity and group sampling points, we considered the Nemerow index as the independent variable and the Shannon diversity indices for protists and bacteria as dependent variables. The variation of microbial diversity with increasing Nemerow pollution index was fitted using a LOESS non-parametric regression (Fig. S2). There were two turning points (shown as black arrows) in both protist and bacteria trends at Nemerow index values of approximately 100 and 200 (Fig. S2). Thus, we divided the soil samples into three metal pollution levels based on the two turning points: low (<100, n = 15), moderate (100–200, n = 6) and high (>200, n = 5). There were almost no strong differences in TC, TN, C:N ratio and pH among the three metal pollution levels (Table S4).

### 3.2. Effects of heavy metal pollution on bacteria and protist diversity

There were no significant correlations between individual heavy metals and bacteria alpha diversity indices (Fig. S3). In contrast, there were significant negative relationships between several metal pollution

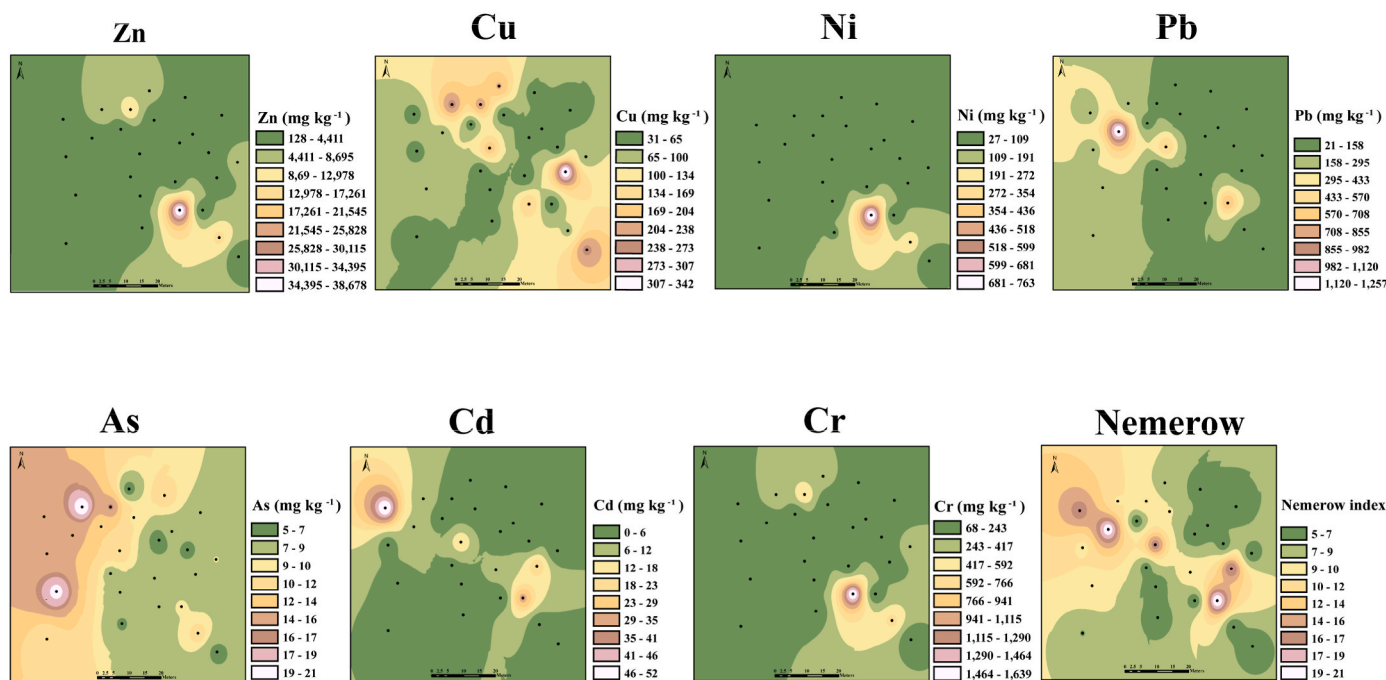


Fig. 1. Spatial distribution of total heavy metal concentrations (Zn, Cu, Ni, Pb, As, Cd and Cr) and Nemerow index as depicted by Inverse Distance Weighting (IDW) interpolation. Points in black indicate the sampling points.

factors (Nemerow index, total/available Zn and Pb) and protist alpha diversity (Fig. S3). Notably, the niche breadth of the protist genus was narrower than bacteria (Fig. S4). Overall, there were stronger negative correlations between the protist community and heavy metals (especially Zn, Pb and Nemerow index) than between bacteria and heavy

metals.

Mantel tests based on Pearson correlations indicated that the protist community was more closely correlated to heavy metal pollution, such as total and available concentrations of Zn, Cd and Pb, as well as Nemerow index (Fig. S5). There were significant differences within the

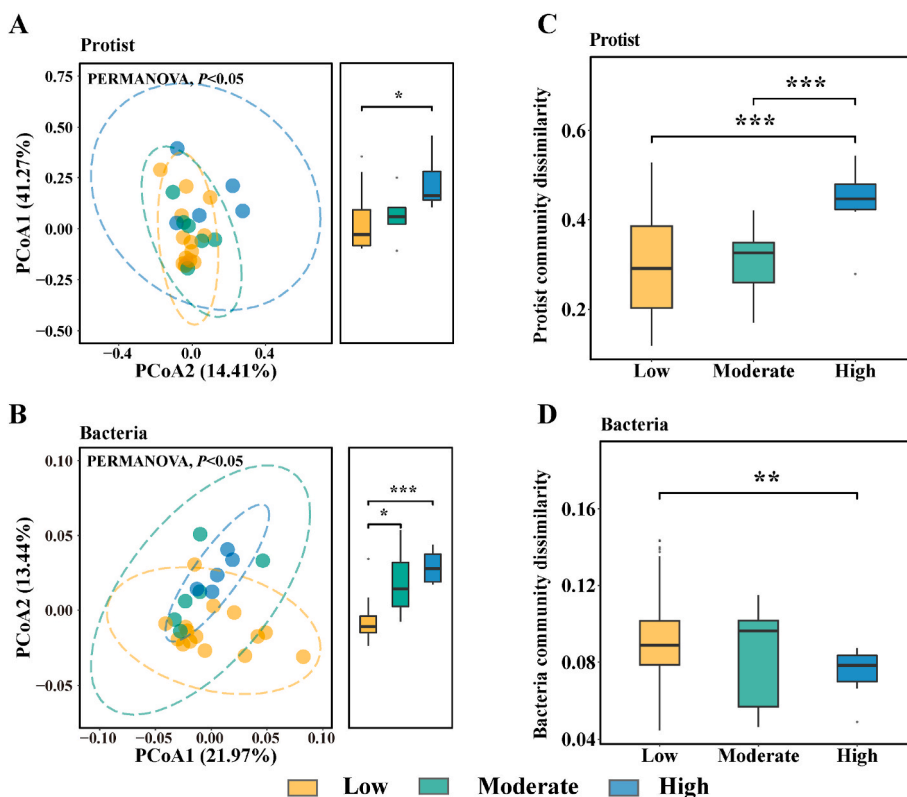


Fig. 2. Protist (A) and bacteria (B) community composition and protist (C) and bacterial (D) community dissimilarity in low, moderate and high pollution levels based on weighted UniFrac distance. Significant differences from Tukey's HSD post-hoc test are depicted with asterisks (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ).

two microbial communities among the three pollution levels ( $P < 0.05$ , PERMANOVA) (Fig. 2). Points for the three pollution levels spread out along PCoA1 axis for the protist community, whereas the points for the bacteria community dispersed along the PCoA2 axis (Fig. 2A and B). In both cases, the bacterial and protist communities at low pollution sites were distinguishable from high pollution sites, but were indistinguishable from moderate pollution sites (Fig. 2C and D). Greater community dissimilarity for protist was found under the high pollution versus the low and moderate pollution levels (Fig. 2C). In contrast, the bacterial community dissimilarity in low pollution sites was lower than found in high pollution sites (Fig. 2D).

### 3.3. Effects of heavy metal pollution on functional guilds of bacteria and protist

A functional annotation of prokaryotic taxa (FAPROTAX) was performed to assess the influence of combined heavy metal pollution on soil bacterial ecological functions. The bacteria community in the highly pollution sites showed low functional diversity and redundancy. The functional group with the highest species number was aerobic chemoheterotrophic bacteria followed by parasitic bacteria (Fig. 3A). Species number and relative abundance of bacterial functional guilds, except for aerobic chemoheterotrophic bacteria, were extremely low (Fig. 3A). The relative abundance of aerobic chemoheterotrophic bacteria at moderate and high pollution levels was significantly higher than for low pollution (Fig. 3B). Moreover, the relative abundance of anaerobic chemoheterotrophic bacteria and bacteria related to ureolysis was significantly higher in the high versus low pollution level (Fig. 3B). Tax4Fun2 was applied to predict habitat-specific functional profiles based on 16S rRNA gene sequences (Wemheuer et al., 2020). Half of the genes performing degradation (mainly degradation of aromatic compounds) had

significant positive correlations with at least one heavy metal (Fig. S6).

Based on a summary of protist feeding habits (Adl et al., 2019), protists were classified into five functional guilds: bacterivores, phototrophs, omnivores, parasites and fungivores (Table S2). Although there were no evident differences for the five protist functional guilds among the three pollution levels (Fig. S7), the relative abundance of phototrophic protists was positively correlated to total and available Cd (Fig. S8). Most genera belonging to photosynthetic protist were positively correlated with heavy metals, such as *Pseudomuriella*, *Bracteacoccus*, *Leptosira*, *Myrmecia*, *Borodinellopsis*, *Chlorococcum* and *Sphaeropleales\_XX* in *Chlorophyta*, *Chrysophyceae\_Clade-C\_X* and *Heterococcus* in *Ochrophyta* (Fig. S9).

### 3.4. Bioindicators for heavy metal pollution and “generalists” across metal polluted sites

Microorganisms enriched in the low, moderate and high pollution groups were distinguished based on LEfSe analysis, resulting in 49 and 22 indicative bacterial genera and protist OTUs, respectively (Fig. S10). We particularly concentrated on the sensitive and tolerant species (i.e., species enriched at low and high pollution levels). In terms of the number of indicative species, half of the indicative bacterial species were enriched in the moderate and high pollution groups (27 of 49 species) (Fig. S10A), whereas half of the indicative protist species were enriched in the low pollution group (9 of 22 species) (Fig. S10B). All genera assigned to *Bacteroidetes* showed resistance to heavy metals and were enriched in the high pollution group (Fig. S10A). Predominantly, members of the recalcitrant phylum *Actinobacteria*, such as *Gordonia*, *Agromyces*, *Blastococcus* and *Iamia*, were enriched in the moderate and high pollution groups (Fig. S10A). All taxa under *Cercozoa*, a small protist consumer, were enriched at low pollution level (Fig. S10B).

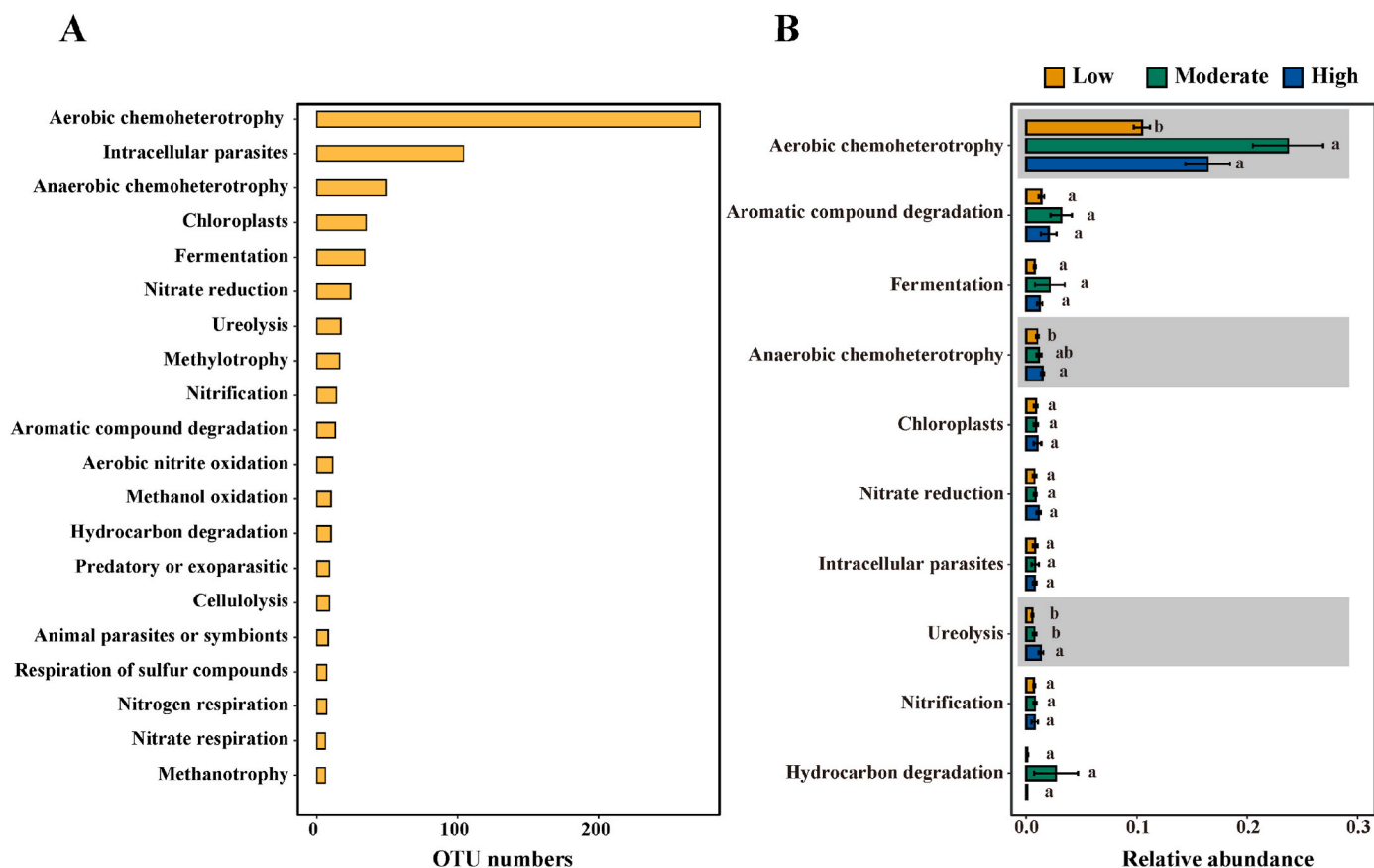


Fig. 3. OTU numbers for the top-20 bacterial functional guilds (A) and relative abundance (mean ± std dev) of the top-10 bacterial functional guilds (B). Different letters in right panel indicate significant differences ( $P < 0.05$ ) between different pollution levels based on a Tukey HSD post-hoc test.

Similarly, OTUs in *Apusomonadidae* (a gliding biflagellated bacteria-eating protist) were enriched in the low pollution group (Fig. S10B). In contrast, two phototrophic protists (*Chlorococcum* and *Chlorogonium*) belonging to *Chlorophyta* and *Ochrophyta*, showed resistance to combined heavy metal pollution (Fig. S10B).

We defined the species with the highest relative abundance and present in all samples as “generalists”. Relationships between relative abundance of top-20 ubiquitous genera and heavy metal pollution were evaluated by Spearman correlation. Most of the correlations between “generalists” and heavy metal pollution were positive and three heavy metals (Zn, Cd and Pb) formed the most correlations with “generalists” (Fig. 4). For instance, genera in *Actinobacteria* (e.g., *Agromyces*, *Blastococcus*, *Gaiella* and *Nocardioides*) were positively correlated with at least one heavy metal (Fig. 4). Of note, *Massilia* in *Proteobacteria*, *Opiritutus* in *Verrucomicrobia* and *Ohtaekwangia* in *Bacteroidetes* formed strong positive correlations with Zn, Cd, Pb and the Nemerow index (Fig. 4A). As for protist “generalists”, we observed positive correlations between the phototrophic protist *Bracteacoccus* with Zn, Cd, Pb and the Nemerow index (Fig. 4B). Consistent with the results revealed by LEfSe, pervasive predatory protists (*Glissomonadida Clades-Y\_X*, *Sandona*, *Neoheteromita* and *Eocercomonas*) were negatively correlated with Ni, Zn, Cd, Pb and the Nemerow index (Fig. 4B). Similarly, there were intersections between the bioindicators revealed by LEfSe and “generalists”, such as *Blastococcus*, *Agromyces* and *Opiritutus*, assigned to aerobic chemoheterotrophic bacteria, as well as protist consumers, such as *Eocercomonas* and *Sandona*. Notably, *Opiritutus* performs other functions, such as nitrate reduction and fermentation according to the annotation of FAPROTAX (Table S3).

### 3.5. Effects of heavy metal pollution on the co-occurrence networks of bacteria and protists

Co-occurrence networks were constructed at the OTU level. The complexity of bacteria and protist co-occurrence networks (as indicated by nodes and edges numbers) increased strikingly with increasing combined heavy metal levels, and the modules were increasingly isolated from each other as metal concentrations increased (Fig. 5a). In moderate and high pollution networks, the modules were like “islands” (Fig. 5a). We extracted the modules containing photosynthetic protists and found that the complexity of these subnetworks also increased along the pollution gradient (Fig. 5b). The modules containing *Blastococcus*, *Agromyces* and *Opiritutus*, which showed resistance to combined heavy metal pollution, were prevalent in all soil samples. These modules of photosynthetic protists and the three bacteria (*Blastococcus*, *Agromyces* and *Opiritutus*) displayed consistent patterns with the whole networks and the modules (Fig. S11).

## 4. Discussion

The majority of previous research on heavy metal polluted soils has examined farmlands, compost and sediments (Wang et al., 2019; Chen et al., 2020; Li et al., 2020a). In contrast, research on heavy metal polluted industrial sites which is characterized by extremely high concentrations of toxic metals and limited nutrient availability (Sun et al., 2018), has received considerably less attention and has been focused primarily on mining sites (Beattie et al., 2018). In our study, total Zn concentrations reached up to 38700 nullmg nullkg<sup>-1</sup>, ~200-fold higher than typically found in metal polluted farmlands (Song et al., 2018). However, the content of nitrogen, an essential element of all living organisms, was only 2% of that of other heavy metal polluted soils (Song et al., 2018; Zhao et al., 2021). Heavy metal concentrations at industrial sites are highly heterogeneous because of the spatial distribution of various industrial processes emitting metals to the environment, resulting in hot-spots with niche differentiation of the microbial community and thus an increase of beta diversity (Huber et al., 2020). Thus, metal polluted industrial sites represent an extremely hostile

environment and exert a strong stress and selective force on soil biota (Xu et al., 2022).

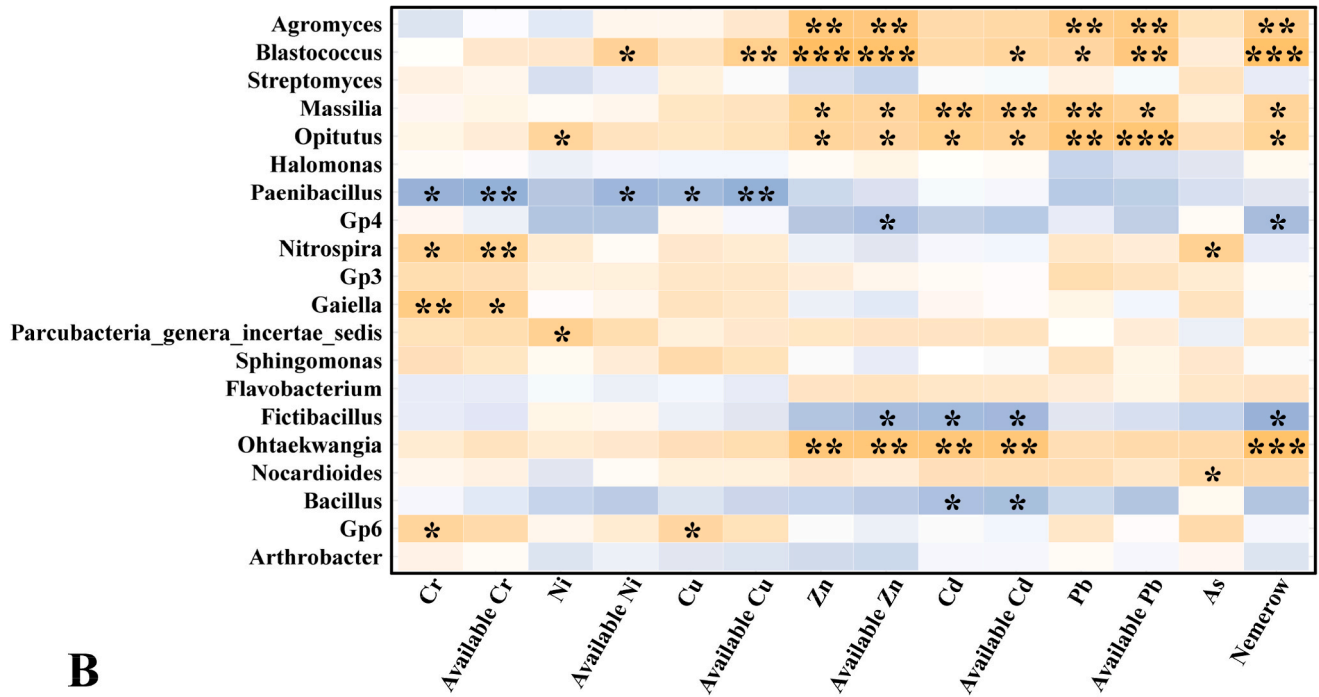
The protist community exhibited greater sensitivity to heavy metal pollution than the bacterial community, as reflected by the stronger negative relationships between protist alpha diversity and heavy metal pollution along with a narrower niche breadth for the protist genus. These findings are in agreement with previous research that found the protist community was more sensitive to environmental perturbances than bacterial and fungal communities (Zhao et al., 2019), since heavy metals can be directly toxic to protists and also poison their food source or host (Nguyen et al., 2020). Notably, bacteria displayed a decreasing community dissimilarity while protist displayed an increasing one along the pollution gradients (Fig. 2), which was ascribed to the divergence in niche breadth between bacteria and protist (Fig. S4). Specifically, as shown by the increasing standard deviation in Table S4, the heterogeneity of heavy metals increased along the pollution gradients. Bacteria have a wider niche breadth and can tolerate a wider range of heavy metals, resulting in a lower community dissimilarity at higher pollution levels. On the contrary, due to lower niche breadth of protist, the increasing heavy metal heterogeneity triggered niche differentiation among protists, contributing to a greater protist community dissimilarity. This is consistent with the previous study which has found that species with narrower niche breadth tend to have greater community dissimilarity (Li et al., 2020b). The higher sensitivity in higher trophic level highlighted the need for a multitrophic perspective to reveal the impacts of heavy metal pollution and potential concerns for the ecological risks of heavy metal pollution on soil food web. (Barnes et al., 2017).

Industrial heavy metal pollution caused the loss of functional diversity within soil ecosystems, as suggested by low functional redundancy. There were very few species (as far as species number and abundance) involved in the same functions, besides aerobic chemoheterotrophs. This contrasts with microbial functional assemblages in non-polluted systems that contain an abundance of OTUs participating in carbon, nitrogen and sulfur cycles (Yang et al., 2022). Low redundancy may result in a permanent loss of ecological function due to the non-random loss of species (Schuldt et al., 2018). Thus, low functional redundancy could hinder ecological restoration of abandoned metal contaminated sites, thereby limiting land rehabilitation for future uses.

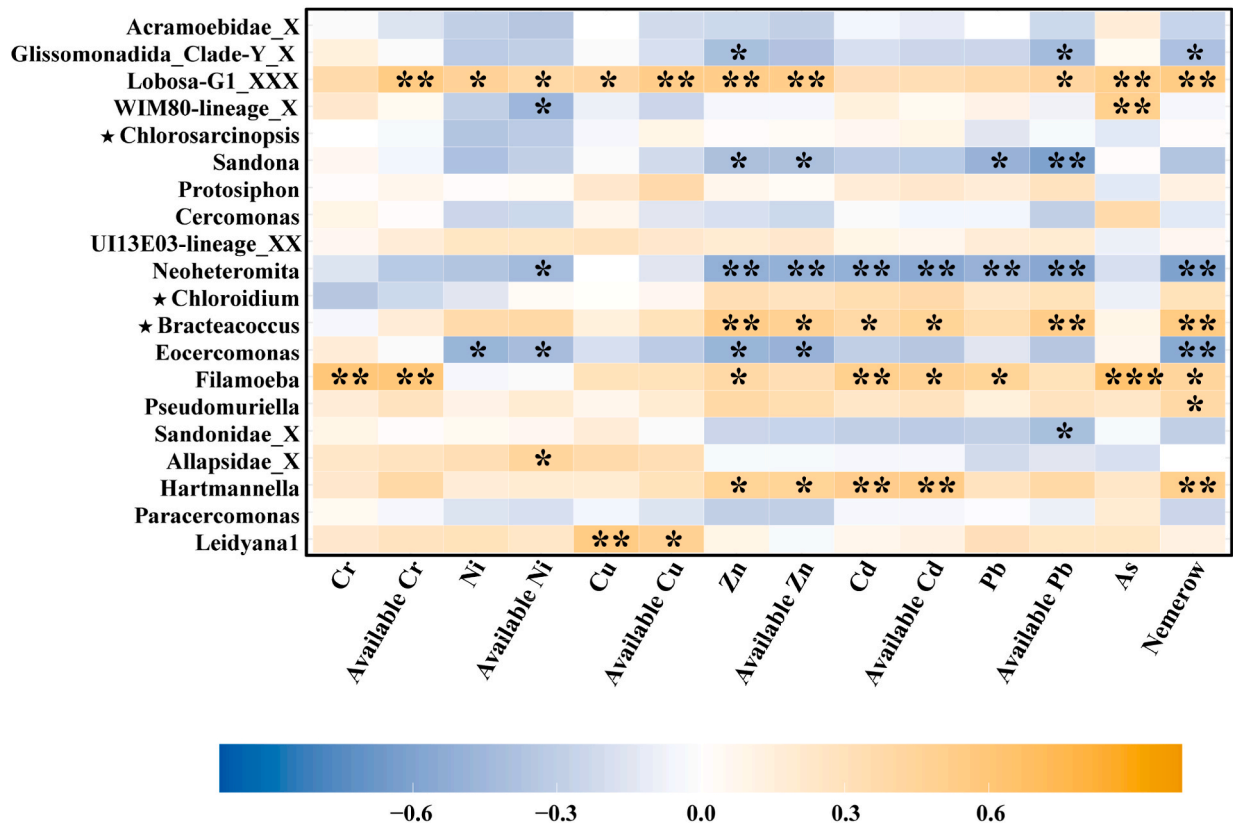
Previous studies reported that heterotrophic protists were very sensitive to environmental disturbances making them a responsive bioindicator for monitoring soil health status (Wu et al., 2022). *Cercozoa*, a major protistan lineage in soil, comprises a vast array of predatory protists, feeding on bacteria, algae, fungi and other eukaryotes (Fior-Donno et al., 2019). In our study, indicative protist genus assigned to *Cercozoa* were only enriched in the low pollution soils, thereby showing a high sensitivity to heavy metal pollution. Previous studies suggest that predatory protists are more sensitive to environmental stress than the other feeding guilds because they receive extra influences from their food sources (Nguyen et al., 2020). Some protist consumers, like amoebae and ciliates, have similar metabolic traits with human cells, and thus have been applied as suitable models in ecotoxicological research (Johansen et al., 2018; Cosson and Lima, 2014). Microbes responding sensitively to toxicants have been previously implanted into portable biosensors to monitor environmental pollution including water, sewage and soil systems (Saltepe et al., 2022). Notably, these sensitive taxa have the potential to be utilized for in vitro detection of heavy metal pollutant status without requiring complex devices and operations.

We also focused on the indicative species tolerant to heavy metals and dominant “generalists”, which thrive under different pollution status. These species constitute a potential resource as bioremediation agents, in particular to enhance phytoremediation (Fakhar et al., 2022). Relative abundance of chemoheterotrophic bacteria increased considerably in the moderate and high pollution soils. Under metal pollution stress, bacteria expend more energy to defend themselves against metal

**A**



**B**



**Fig. 4.** Correlations among the top-20 “generalists” bacteria (A) and protists (B) with heavy metal (total and available metal) concentrations and Nemerow values. Genera with stars were phototrophic protists. Significant relationships identified by Spearman correlation are depicted with asterisks (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ).



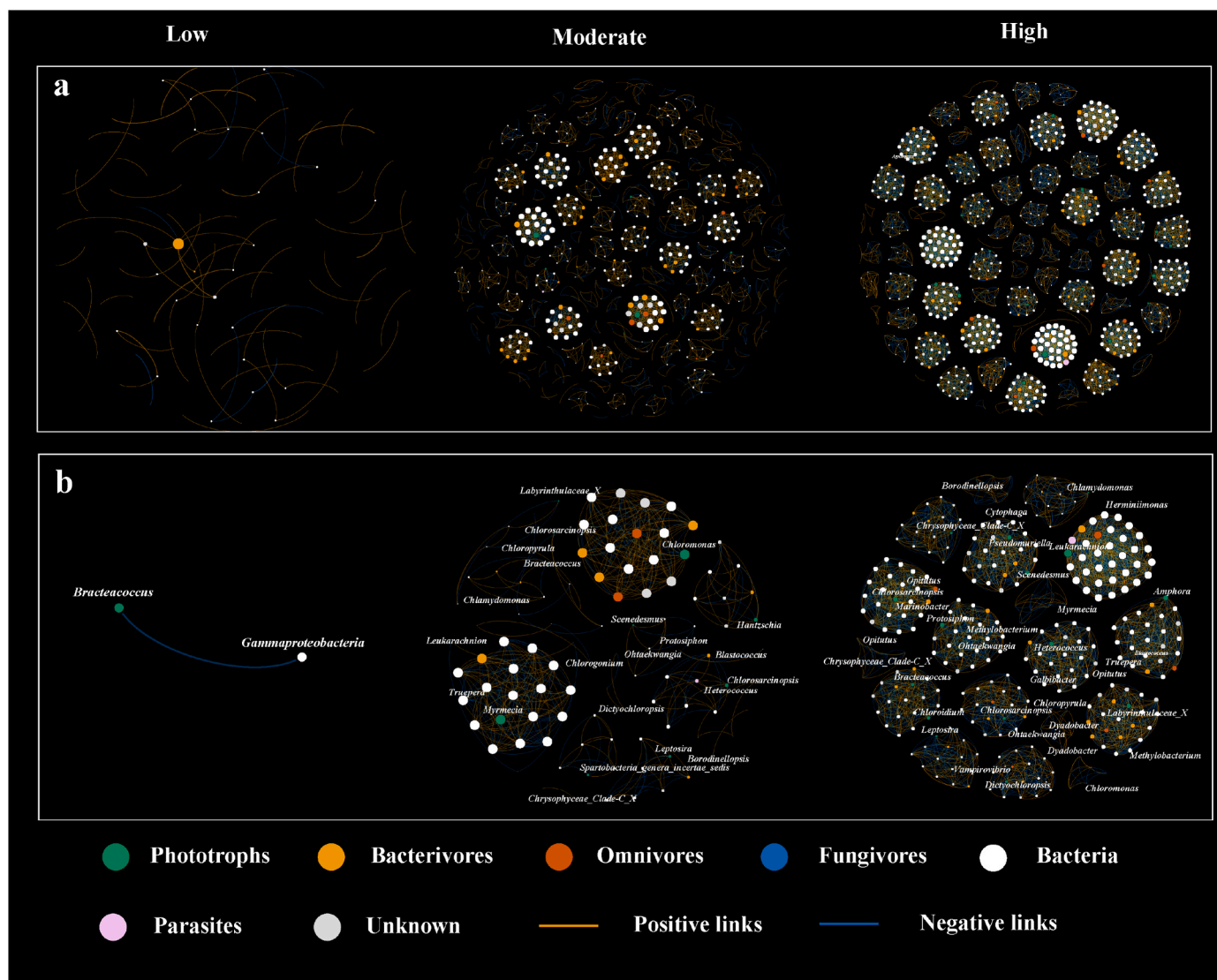


Fig. 5. Co-occurrence networks for bacteria and protists (a) and subnetworks of photosynthetic protist (b) under low, moderate and high pollution levels. The node size is proportional to the node degrees that show the connections of one taxon with other taxa.

toxicity (Zhao et al., 2021). There were three dominant aerobic chemoheterotrophic bacterial genera found at the intersection of tolerant and generalist species: *Opitutus*, *Blastococcus* and *Agromyces*. The metabolic versatility of *Opitutus* with respect to nitrate reduction and fermentation (Chin and Janssen, 2002; Espenberg et al., 2018) makes them highly successful bacterial forager of soil carbon (Hünninghaus et al., 2019), which has led to their use in bioremediation, especially for the biodegradation of organic pollutants (Sun et al., 2015; Ma et al., 2013). Due to the dominance of *Opitutus* and the co-occurrence of heavy metals and organic pollutants in most contaminated sites (Tufail et al., 2022), *Opitutus* can be a good candidate for organic pollution remediation in sites also experiencing heavy metal contamination. The species tolerant to heavy metals showed functional versatility, which provide insights on the bioremediation potential of these species.

In our study, predatory protists were repressed by heavy metal pollution, while phototrophic protists (i.e., microalgae) generally show more tolerance to toxic substances than heterotrophic protists. Previous study reported that microalgae had a higher EC50 value than amoebae, ciliates and flagellates (Wu et al., 2022), which was ascribed to algae having thick cell walls, whereas other protozoa lacking cell walls. Further, some heavy metals serve as micro-nutrients that are necessary for maintaining certain physiological processes in microalgae (Chen

et al., 2016). For example, Zn and Cu are considered essential heavy metals that play crucial roles in microalgae growth by participating in several physiological processes, such as photosynthesis, respiration, nutrient assimilation and electron transport (Tripathi and Poluri, 2021). Thus, microalgae are equipped with a larger biologically detoxified metal “pool” to accommodate higher levels of heavy metals than the other protists. As such, unicellular algae could be utilized as a pioneer community for colonizing abandoned factory sites contaminated by heavy metals, which promotes secondary succession by fixing carbon to accelerate the succession and restoration of abandoned polluted ecosystems (Geisen et al., 2018).

Although the complexity of networks increased as indicated by the numbers of nodes and edges, the modules in the high pollution network became more isolated from the other modules when compared to the lower pollution network. There were few edges spanning different modules resulting in each module occurring as an isolated patch. According to niche-based theory, environmental heterogeneity can contribute to the differentiation of the niche space across the species within a community (Dumbrell et al., 2010). In our study, the increasing heterogeneity of heavy metals along the pollution gradients can decrease niche overlap of soil biotas (Huber et al., 2020). The species sharing the similar niche or ecological traits (e.g., ability to tolerate

certain heavy metal) tend to form independent modules and the connectivity between these modules is low under high environmental heterogeneity (Yin et al., 2022). Therefore, high heterogeneity of heavy metals displayed strong selective pressures and crushed the soil biota communities into fragments, thus increasing beta diversity (Huber et al., 2020). Communications between modules are attenuated due to the high environmental heterogeneity and may further contribute to the loss of functionality, such as functions depending on cross-feeding (Schuldt et al., 2018).

Three bacteria genera (*Opitutus*, *Blastococcus* and *Agromyces*) were identified as keystones in the networks and were highly connected with other species. These interactions increased as the heavy metal pollution level increased. The roles of phototrophic protists (e.g., *Chrysothrix* *Clade-C\_X*, *Amphora* and *Heterococcus* in *Ochrophyta*, *Protosiphon* and *Chlorosarcinopsis* in *Chlorophyta*) in subnetworks of *Opitutus*, *Blastococcus* and *Agromyces* were strengthened with increasing pollution levels. Moreover, the whole networks followed the same patterns as the subnetworks for photosynthetic protists, which became more complex with increasing pollution levels. The same phenomenon was observed at an abandoned copper smelting plant where the complexity of co-occurrence networks increased with increasing pollution level and the generalists played a crucial role in the networks (Qi et al., 2022). The existence of photosynthetic protists may alleviate nutrient deficiency stress of other heterotrophic microbes in nutrient-deficient sites (Bielewicz et al., 2011). Previous studies indicated that metal-resistant species can secrete metal detoxification substances into the soil environment, thereby favoring the growth of some metal-sensitive species under high metal pollution stress and enhancing the performance of these functional taxa (Lin et al., 2021). For example, algae synthesize extracellular dissolved organic compounds that support bacterial growth and bacteria can supply organic cofactors and siderophores for algae in return, thus algae and bacteria formed a mutually beneficial and symbiotic relationship, i.e., algae-bacteria consortia (Li et al., 2022). It has been reported that algae-bacteria consortia showed an outstanding performance in coping with soil contaminants, which confirmed that algae-bacteria consortia treatment is a feasible way in soil remediation (Sheng et al., 2022; Wu et al., 2018; Yang et al., 2016). Although many efforts have been devoted to apply algae-bacteria consortia in aqueous remediation (Li et al., 2022), algae-bacteria consortia treatment has been considered as a new biotechnology of great prospects (Subashchandrabose et al., 2019). Soil and terrestrial ecosystem restoration following severe contamination by abandoned factory sites is a grand challenge in rapidly industrializing and urbanizing areas (Wong, 2003). Utilizing a remediation system containing microalgae (photosynthetic protists) and bacterium adapted to severe heavy metal pollution poses a promising approach to promote the ecological recovery of heavy metal polluted sites with low soil nutrient level. In our study, the tolerant and generalist species (*Opitutus*, *Blastococcus* and *Agromyces*) formed close relationships with microalgae, provides a theoretical basis for optimizing this approach to the range of site-specific conditions associated with abandoned industrial sites.

## 5. Conclusion

The results obtained in this study provide new insights into the multitrophic response of bacteria and protist communities to heavy metal pollution in an abandoned industrial site. The land surrounding the abandoned electroplating factory contained very high heavy metal levels and low C and N concentrations. Under this harsh environmental stress, the protist community responded to heavy metal pollution more sensitively than the bacteria community. The bacteria community was characterized by low functional diversity and redundancy. Communications among the modules in the co-occurrence networks of bacteria and protist decreased or even disappeared with increasing heavy metal pollution. These results highlight the potential ecological challenges for ecological restoration of abandoned factory sites. With increasing heavy

metal pollution levels, the connections between some metal-tolerant species (functionally versatile bacteria and photosynthetic protists) became tighter, forming potential algae-bacteria consortia, which holds great promise for the remediation and restoration of severely polluted abandoned lands degraded by industrial activities. This calls for a future attempt to isolate the resistant species from soil and translate this bioremediation materials to the field to demonstrate its efficacy under real-world environmental conditions.

## Author contribution

**Xuehua Wang:** Investigation, Formal analysis, Writing – original draft, Visualization. **Zhongmin Dai:** Formal analysis. **Haochun Zhao:** Formal analysis. **Lingfei Hu:** Conceptualization, Supervision, Writing – review & editing. **Randy A. Dahlgren:** Writing – review & editing. **Jianming Xu:** Conceptualization, Resources, Funding acquisition, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2023.121548>.

## References

- Adl, S.M., Bass, D., Lane, C.E., et al., 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. *J. Eukaryot. Microbiol.* 66, 4–119. <https://doi.org/10.1111/jeu.12691>.
- Adl, S.M., Coleman, D.C., 2005. Dynamics of soil protozoa using a direct count method. *Biol. Fertil. Soils* 42, 168–171. <https://doi.org/10.1007/s00374-005-0009-x>.
- Barnes, A.D., Allen, K., Kreft, H., et al., 2017. Direct and cascading impacts of tropical land-use change on multi-trophic biodiversity. *Nat. Ecol. Evol.* 1, 1511–1519. <https://doi.org/10.1038/s41559-017-0275-7>.
- Bates, S.T., Berg-Lyons, D., Caporaso, J.G., et al., 2011. Examining the global distribution of dominant archaeal populations in soil. *ISME J.* 5, 908–917. <https://doi.org/10.1038/ismej.2010.171>.
- Beattie, R.E., Henke, W., Campa, M.F., et al., 2018. Variation in microbial community structure correlates with heavy-metal contamination in soils decades after mining ceased. *Soil Biol. Biochem.* 126, 57–63. <https://doi.org/10.1016/j.soilbio.2018.08.011>.
- Bielewicz, S., Bell, E., Kong, W., et al., 2011. Protist diversity in a permanently ice-covered Antarctic Lake during the polar night transition. *ISME J.* 5, 1559–1564. <https://doi.org/10.1038/ismej.2011.23>.
- Certin, M., Sevik, H., Cobanoglu, O., 2020. Ca, Cu, and Li in washed and unwashed specimens of needles, bark, and branches of the blue spruce (*Picea pungens*) in the city of Ankara. *Environ. Sci. Pollut. Res.* 27, 21816–21825. <https://doi.org/10.1007/s11356-020-08687-3>.
- Chen, H., Teng, Y., Lu, S., et al., 2015. Contamination features and health risk of soil heavy metals in China. *Sci. Total Environ.* 512–513, 143–153. <https://doi.org/10.1016/j.scitotenv.2015.01.025>.
- Chen, X., Zhao, Y., Zhao, X., et al., 2020. Selective pressures of heavy metals on microbial community determine microbial functional roles during comp-osting: sensitive, resistant and actor. *J. Hazard Mater.* 398, 122858 <https://doi.org/10.1016/j.jhazmat.2020.122858>.

- Chen, Z., Song, S., Wen, Y., et al., 2016. Toxicity of Cu (II) to the green alga *Chlorella vulgaris*: a perspective of photosynthesis and oxidant stress. *Environ. Sci. Pollut. Res.* 23, 17910–17918. <https://doi.org/10.1007/s11356-016-6997-2>.
- Chin, K.J., Janssen, P.H., 2002. Propionate formation by *Opirititus terrae* in pure culture and in mixed culture with a hydrogenotrophic methanogen and implications for carbon fluxes in anoxic rice paddy soil. *Appl. Environ. Microbiol.* 68, 2089–2092. <https://doi.org/10.1128/AEM.68.4.2089-2092.2002>.
- Cole, J.R., Wang, Q., Fish, J.A., et al., 2013. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* 42, D633–D642. <https://doi.org/10.1093/nar/gkt1244>.
- Cosson, P., Lima, W.C., 2014. Intracellular killing of bacteria: is *Dicystostelium* a model macrophage or an alien? *Cell Microbiol.* 16, 816–823. <https://doi.org/10.1111/cmi.12291>.
- Dumbrell, A.J., Nelson, M., Helgason, T., et al., 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME J.* 4, 337–345. <https://doi.org/10.1038/ismej.2009.122>.
- Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998. <https://doi.org/10.1038/nmeth.2604>.
- Edwards, J., Johnson, C., Santos-Medellín, C., et al., 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci. U.S.A.* 112, E911–E920. <https://doi.org/10.1073/pnas.1414592112>.
- Espenberg, M., Truu, M., Mander, Ü., et al., 2018. Differences in microbial community structure and nitrogen cycling in natural and drained tropical peatland soils. *Sci. Rep.* 8, 4742. <https://doi.org/10.1038/s41598-018-23032-y>.
- Fakhar, A., Gul, B., Gurmani, A.R., et al., 2022. Heavy metal remediation and resistance mechanism of *Aeromonas*, *Bacillus*, and *Pseudomonas*: a review. *Crit. Rev. Environ. Sci. Technol.* 52, 1868–1914. <https://doi.org/10.1080/10643389.2020.1863112>.
- Fiore-Donno, A.M., Richter-Heitmann, T., Degruene, F., et al., 2019. Functional traits and spatio-temporal structure of a major group of soil protists (*Rhiza-ria: Cercozoa*) in a temperate grassland. *Front. Microbiol.* 10, 1332. <https://doi.org/10.3389/fmicb.2019.01332>.
- Geisen, S., Hu, S., dela Cruz, T.E.E., et al., 2021. Protists as catalyzers of microbial litter breakdown and carbon cycling at different temperature regimes. *ISME J.* 15, 618–621. <https://doi.org/10.1038/s41396-020-00792-y>.
- Geisen, S., Mitchell, E.A.D., Adl, S., et al., 2018. Soil protists: a fertile frontier in soil biology research. *FEMS Microbiol. Rev.* 42, 293–323. <https://doi.org/10.1093/femsre/fuy006>.
- Geng, M., Zhang, W., Hu, T., et al., 2022. Eutrophication causes microbial community homogenization via modulating generalist species. *Water Res.* 210, 118003. <https://doi.org/10.1016/j.watres.2021.118003>.
- Guillou, L., Bachar, D., Audic, S., et al., 2013. The Protist Ribosomal Reference database (PR<sup>2</sup>): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.* 41, D597–D604. <https://doi.org/10.1093/nar/gks1160>.
- Guo, S., Xiong, W., Hang, X., et al., 2021. Protists as main indicators and determinants of plant performance. *Microbiome* 9, 64. <https://doi.org/10.1186/s40168-021-01025-w>.
- Hao, X., Xie, P., Zhu, Y., et al., 2015. Copper tolerance mechanisms of *Mesorhizobium amorphae* and its role in aiding phytostabilization by *Robinia pseudoacacia* in copper contaminated soil. *Environ. Sci. Technol.* 49, 2328–2340. <https://doi.org/10.1021/es504956a>.
- Huang, H., 2020. ggcor: extended tools for correlation analysis and visualization. <https://github.com/J-P-Zhang/ggcor>.
- Huang, R.Q., Gao, S., Wang, W., et al., 2006. Soil arsenic availability and the transfer of soil arsenic to crops in suburban areas in Fujian Province, southeast China. *Sci. Total Environ.* 368, 531–541. <https://doi.org/10.1016/j.scitotenv.2006.03.013>.
- Huber, P., Metz, S., Unrein, F., et al., 2020. Environmental heterogeneity determines the ecological processes that govern bacterial metacommunity assembly in a floodplain river system. *ISME J.* 14, 2951–2966. <https://doi.org/10.1038/s41396-020-0723-2>.
- Hünninghaus, M., Dibbern, D., Kramer, S., et al., 2019. Dismantling carbon flow across microbial kingdoms in the rhizosphere of maize. *Soil Biol. Biochem.* 134, 122–130. <https://doi.org/10.1016/j.soilbio.2019.03.007>.
- Johansen, J.L., Rønn, R., Ekelund, F., 2018. Toxicity of cadmium and zinc to small soil protists. *Environ. Pollut.* 242, 1510–1517. <https://doi.org/10.1016/j.envpol.2018.08.034>.
- Li, C., Quan, Q., Gan, Y., et al., 2020a. Effects of heavy metals on microbial communities in sediments and establishment of bioindicators based on microbial taxa and function for environmental monitoring and management. *Sci. Total Environ.* 749, 141555. <https://doi.org/10.1016/j.scitotenv.2020.141555>.
- Li, P., Liu, M., Ma, X., et al., 2020b. Responses of microbial communities to a gradient of pig manure amendment in red paddy soils. *Sci. Total Environ.* 705, 135884. <https://doi.org/10.1016/j.scitotenv.2019.135884>.
- Li, S., Zhang, C., Li, F., et al., 2022. Recent advances of algae-bacteria consortia in aquatic remediation. *Crit. Rev. Environ. Sci. Technol.* 53, 315–339. <https://doi.org/10.1080/10643389.2022.2052704>.
- Lin, Y., Wang, L., Xu, K., et al., 2021. Revealing taxon-specific heavy metal-resistance mechanisms in denitrifying phosphorus removal sludge using genome-centric metaproteomics. *Microbiome* 9, 67. <https://doi.org/10.1186/s40168-021-01016-x>.
- Liu, J., He, X., Lin, X., et al., 2015. Ecological effects of combined pollution associated with e-waste recycling on the composition and diversity of soil microbial communities. *Environ. Sci. Technol.* 49, 6438–6447. <https://doi.org/10.1021/es5049804>.
- Liu, Y., Delgado-Baquerizo, M., Bi, L., et al., 2018. Consistent responses of soil microbial taxonomic and functional attributes to mercury pollution across China. *Microbiome* 6, 183. <https://doi.org/10.1186/s40168-018-0572-7>.
- Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonom-y in the global ocean microbiome. *Science* 353, 1272–1277. <https://www.science.org/doi/10.1126/science.aaf4507>.
- Ma, J., Nossa, C.W., Xiu, Z., et al., 2013. Adaptive microbial population shifts in response to a continuous ethanol blend release increases biodegradation potential. *Environ. Pollut.* 178, 419–425. <https://doi.org/10.1016/j.envpol.2013.03.057>.
- Ma, W., Tai, L., Qiao, Z., et al., 2018. Contamination source apportionment and health risk assessment of heavy metals in soil around municipal solid waste incinerator: a case study in North China. *Sci. Total Environ.* 631–632, 348–357. <https://doi.org/10.1016/j.scitotenv.2018.03.011>.
- Mielke, H.W., Gonzales, C.R., Powell, E.T., et al., 2019. The concurrent decline of soil lead and children's blood lead in New Orleans. *Proc. Natl. Acad. Sci. U.S.A.* 116, 22058–22064. <https://doi.org/10.1073/pnas.1906092116>.
- Nguyen, B.A.T., Chen, Q., He, J., et al., 2020. Oxytetracycline and ciprofloxacin exposure altered the composition of protistan consumers in an agricultural soil. *Environ. Sci. Technol.* 54, 9556–9563. <https://doi.org/10.1021/acs.est.0c02531>.
- Pandit, S.N., Kolasa, J., Cottenie, K., 2009. Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. *Ecology* 90, 2253–2262. <https://doi.org/10.1890/08-0851.1>.
- Peng, G., Tian, G., 2010. Using electrode electrolytes to enhance electrokinetic removal of heavy metals from electroplating sludge. *Chem. Eng. J.* 165, 388–394. <https://doi.org/10.1016/j.cej.2010.10.006>.
- Qi, Q., Hu, C., Lin, J., et al., 2022. Contamination with multiple heavy metals decreases microbial diversity and favors generalists as the keystones in m-icrobial occurrence networks. *Environ. Pollut.* 306, 119406. <https://doi.org/10.1016/j.envpol.2022.119406>.
- Ramirez, K.S., Knight, C.G., de Hollander, M., et al., 2018. Detecting macroecological patterns in bacterial communities across independent studies of global soils. *Nat. Microbiol.* 3, 189–196. <https://doi.org/10.1038/s41564-017-0062-x>.
- Saltepe, B., Wang, L., Wang, B., 2022. Synthetic biology enables field-deployable biosensors for water contaminants. *TRAC, Trends Anal. Chem.* 146, 116507. <https://doi.org/10.1016/j.trac.2021.116507>.
- Schuld, A., Assmann, T., Brezzi, M., et al., 2018. Biodiversity across trophic levels drives multifunctionality in highly diverse forests. *Nat. Commun.* 9, 2989. <https://doi.org/10.1038/s41467-018-05421-z>.
- Segata, N., Izard, J., Waldron, L., et al., 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12, R60. <https://doi.org/10.1186/gb-2011-12-6-r60>.
- Sert, E.B., Turkmen, M., Cetin, M., 2019. Heavy metal accumulation in rosemary leaves and stems exposed to traffic-related pollution near Adana-İskenderun Highway (Hatay, Turkey). *Environ. Monit. Assess.* 191, 553. <https://doi.org/10.1007/s10661-019-7714-7>.
- Sevik, H., Cetin, M., Ozel, H.B., et al., 2020a. Analyzing of usability of tree-rings as biomonitors for monitoring heavy metal accumulation in the atmosphere in urban area: a case study of cedar tree (*Cedrus* sp.). *Environ. Monit. Assess.* 192, 23. <https://doi.org/10.1007/s10661-019-8010-2>.
- Sevik, H., Cetin, M., Ozel, H.B., et al., 2020b. Changes in heavy metal accumulation in some edible landscape plants depending on traffic density. *Environ. Monit. Assess.* 192, 78. <https://doi.org/10.1007/s10661-019-8041-8>.
- Sheng, Y., Benmati, M., Guendouzi, S., et al., 2022. Latest eco-friendly approaches for pesticides decontamination using microorganisms and consortia microalgae: a comprehensive insights, challenges, and perspectives. *Chemosphere* 308, 136183. <https://doi.org/10.1016/j.chemosphere.2022.136183>.
- Sokol, N.W., Slessarev, E., Marschmann, G.L., et al., 2022. Life and death in the soil microbiome: how ecological processes influence biogeochemistry. *Nat. Rev. Microbiol.* 20, 415–430. <https://doi.org/10.1038/s41579-022-00695-z>.
- Song, J., Shen, Q., Wang, L., et al., 2018. Effects of Cd, Cu, Zn and their combined action on microbial biomass and bacterial community structure. *Environ. Pollut.* 243, 510–518. <https://doi.org/10.1016/j.envpol.2018.09.011>.
- Stoeck, T., Bass, D., Nebel, M., et al., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol. Ecol.* 19, 21–31. <https://doi.org/10.1111/j.1365-294X.2009.04480.x>.
- Subashchandrabose, S.R., Venkateswarlu, K., Venkidasamy, K., et al., 2019. Bio-remediation of soil long-term contaminated with PAHs by algal-bacterial s-ynergy of *Chlorella* sp. MM3 and *Rhodococcus wratislaviensis* strain 9 in slurry phase. *Sci. Total Environ.* 659, 724–731. <https://doi.org/10.1016/j.scitotenv.2018.12.453>.
- Sun, W., Li, J., Jiang, L., et al., 2015. Profiling microbial community structures across six large oilfields in China and the potential role of dominant microorganisms in bioremediation. *Appl. Microbiol. Biotechnol.* 99, 8751–8764. <https://doi.org/10.1007/s00253-015-6748-1>.
- Sun, W., Xiao, E., Häggblom, M., et al., 2018. Bacterial survival strategies in an alkaline tailing site and the physiological mechanisms of dominant phylotypes as revealed by metagenomic analyses. *Environ. Sci. Technol.* 52, 13370–13380. <https://doi.org/10.1021/acs.est.8b03853>.
- Tripathi, S., Poluri, K.M., 2021. Heavy metal detoxification mechanisms by microalgae: insights from transcriptomics analysis. *Environ. Pollut.* 285, 117443. <https://doi.org/10.1016/j.envpol.2021.117443>.
- Tufail, M.A., Iltaf, J., Zaheer, T., et al., 2022. Recent advances in bioremediation of heavy metals and persistent organic pollutants: a review. *Sci. Total Environ.* 850, 157961. <https://doi.org/10.1016/j.scitotenv.2022.157961>.
- Wang, M., Chen, S., Chen, L., et al., 2019. Responses of soil microbial communities and their network interactions to saline-alkaline stress in Cd-contaminated soils. *Environ. Pollut.* 252, 1609–1621. <https://doi.org/10.1016/j.envpol.2019.06.082>.
- Wang, C., Zhang, Q., Kang, S., et al., 2023. Heavy metal(loid)s in agricultural soil from main grain production regions of China: bioaccessibility and health risks to humans. *Sci. Total Environ.* 858, 159819. <https://doi.org/10.1016/j.scitotenv.2022.159819>.

- Wemheuer, F., Taylor, J.A., Daniel, R., et al., 2020. Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. *Environ. Microbiome*. 15, 11. <https://doi.org/10.1186/s40793-020-00358-7>.
- Wilhelm, R.C., Amsili, J.P., Kurtz, K.S.M., et al., 2023. Ecological insights into soil health according to the genomic traits and environment-wide associations of bacteria in agricultural soils. *ISME COMMUN* 3, 1. <https://doi.org/10.1038/s43705-022-00209-1>.
- Wong, M., 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere* 50, 775–780. [https://doi.org/10.1016/S0045-6535\(02\)00232-1](https://doi.org/10.1016/S0045-6535(02)00232-1).
- Wu, C., Chao, Y., Shu, L., et al., 2022. Interactions between soil protists and pollutants: an unsolved puzzle. *J. Hazard Mater.* 429, 128297 <https://doi.org/10.1016/j.jhazmat.2022.128297>.
- Wu, Y., Liu, J., Rene, E.R., 2018. Periphytic biofilms: a promising nutrient utilization regulator in wetlands. *Bioresour. Technol.* 248, 44–48. <https://doi.org/10.1016/j.biortech.2017.07.081>.
- Xu, R., Zhang, M., Lin, H., et al., 2022. Response of soil protozoa to acid mine drainage in a contaminated terrace. *J. Hazard Mater.* 421, 126790 <https://doi.org/10.1016/j.jhazmat.2021.126790>.
- Xu, Y., Seshadri, B., Bolan, N., et al., 2019. Microbial functional diversity and carbon use feedback in soils as affected by heavy metals. *Environ. Int.* 125, 478–488. <https://doi.org/10.1016/j.envint.2019.01.071>.
- Xu, Z., Gao, G., Pan, B., et al., 2015. A new combined process for efficient removal of Cu (II) organic complexes from wastewater: Fe(III) displacement/UV degradation/alkaline precipitation. *Water Res.* 87, 378–384. <https://doi.org/10.1016/j.watres.2015.09.025>.
- Yang, J., Tang, C., Wang, F., et al., 2016. Co-contamination of Cu and Cd in paddy fields: using periphyton to entrap heavy metals. *J. Hazard Mater.* 304, 150–158. <https://doi.org/10.1016/j.jhazmat.2015.10.051>.
- Yang, Q., Li, Z., Lu, X., et al., 2018. A review of soil heavy metal pollution from industrial and agricultural regions in China: pollution and risk assessment. *Sci. Total Environ.* 642, 690–700. <https://doi.org/10.1016/j.scitotenv.2018.06.068>.
- Yang, Z., Peng, C., Cao, H., et al., 2022. Microbial functional assemblages predicted by the FAPROTAX analysis are impacted by physicochemical properties, but C, N and S cycling genes are not in mangrove soil in the Beibu Gulf, China. *Ecol. Indic.* 139, 10887 <https://doi.org/10.1016/j.ecolind.2022.108887>.
- Yin, C., Tan, C., Chen, H., et al., 2022. Insight into the role of competition in niche differentiation between ammonia-oxidizing archaea and bacteria in ammonium-rich alkaline soil: a network-based study. *Soil Biol. Biochem.* 168, 108638 <https://doi.org/10.1016/j.soilbio.2022.108638>.
- Zhao, H., Lin, J., Wang, X., et al., 2021. Dynamics of soil microbial N-cycling strategies in response to cadmium stress. *Environ. Sci. Technol.* 55, 14305–14315. <https://doi.org/10.1021/acs.est.1c04409>.
- Zhao, Z., He, J., Geisen, S., et al., 2019. Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. *Microbiome* 7, 33. <https://doi.org/10.1186/s40168-019-0647-0>.