



Research Paper

Mercury drives microbial community assembly and ecosystem multifunctionality across a Hg contamination gradient in rice paddies

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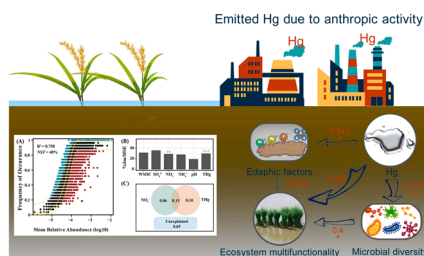
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HIGHLIGHTS

- Deterministic processes shaped community assembly in rice paddies across Hg gradient.
- Hg level was the most important predictor of microbial profiles.
- Hg contamination directly and indirectly affects ecosystem multifunctionality.
- Hg-induced reductions in soil biodiversity leads to reduced ecosystem services.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil microbial communities are critical for maintaining terrestrial ecosystems and fundamental ecological processes. Mercury (Hg) is a heavy metal that is toxic to microorganisms, but its effects on microbial community assembly and ecosystem multifunctionality in rice paddy ecosystems remain largely unknown. In the current study, we analyzed the microbial community structure and ecosystem multifunctionality of paddy soils across a Hg contamination gradient. The results demonstrated that Hg contamination significantly altered the microbial community structure. The microbial communities were predominantly driven by deterministic selection rather than stochastic processes. The random forest model and variation partition analysis demonstrated that the Hg level was the most important predictor of microbial profiles. Ecosystem multifunctionality decreased across the Hg concentration gradient, and multifunctionality was significantly correlated with soil biodiversity, suggesting that Hg-induced reductions in soil biodiversity led to reduced ecosystem services. A structural equation model showed that Hg contamination directly and indirectly affected ecosystem multifunctionality. The present work broadens our knowledge of the assembly of the microbiome in rice paddies across a Hg contamination gradient and highlights the significance of soil biodiversity in regulating ecosystem functions, especially in Hg-polluted rice paddies.

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1. Introduction

Microorganisms are widely distributed in nature. It is estimated that soil contains > 1000 kg of microbial biomass carbon per hectare, comparable to plant or animal aboveground biomass (Fierer et al., 2009; Serna-Chavez et al., 2013; Fierer, 2017). Over billions of years, microorganisms have played crucial roles in maintaining various ecosystem functions essential to nutrient cycling, plant production, and soil fertility maintenance (Falkowski et al., 2008; Reef et al., 2010; Bardgett and van der Putten, 2014; Wagg et al., 2014; Fierer, 2017). Anthropogenic factors contributing to global change, including greenhouse gas emission, fertilizer application, and landscape alteration, have exacerbated changes in microbial communities. This in turn has affected ecosystem multifunctionality, posing an immediate danger to food security and humankind (Sala et al., 2000; Hooper et al., 2012; Delgado-Baquerizo et al., 2016, 2020; Schuldt et al., 2018; Zhou et al., 2020b). Heavy metal pollution is also a global issue as it adversely affects the growth, morphology, and metabolism of soil microorganisms, thereby altering the diversity, composition, and function of microbial communities in terrestrial ecosystem (Harrison et al., 2007; Li et al., 2020; Xie et al., 2016).

Since the beginning of the Industrial Revolution in the mid-18th century, environmental pollution has become a global problem that affects atmospheric, aquatic, and terrestrial environments, causing a variety of adverse outcomes to humans (Hochella et al., 2019; Arrigo et al., 2020). Environmental heavy metal contamination strongly influences soil microbiota; previous studies have confirmed that heavy metal pollution reduces microbial diversity in soil (Singh et al., 2014; Abdu et al., 2017). For instance, cadmium, copper, and zinc have been found to decrease the species diversity and activity of microbial communities in forest and arable soils (Frostegard et al., 1993). The processes of microbial community assembly influence the composition of the microbial community and thus determine their functional traits (Liu et al., 2020). The assembly of microbial communities includes deterministic (driven by environmental determinants and species interaction) and stochastic processes (controlled by unpredictable disturbances) (Stegen et al., 2012). However, the processes of microbial community assembly in metal-polluted soils remain unclear, which impedes our ability to assess the ecosystem functions of soil microorganisms in metal-polluted soil. Therefore, it is imperative to comprehend the consequences of heavy metals on soil microbial community assembly and ecosystem multifunctionality.

Mercury (Hg) is a toxic heavy metal, and its organic form, methylmercury (MeHg), can bioaccumulate through the food chain, posing a potential threat to human health (Selin, 2009; Chen et al., 2019a; Perng et al., 2019; Schartup et al., 2019; Wu et al., 2020a). In recognition of its harmful effects, the United Nations Environment Programme developed an international treaty, the Minamata Convention on Mercury (<http://www.mercuryconvention.org>), with the aim of protecting people and the environment against anthropogenic Hg and Hg compounds emissions. It is estimated that a cumulative total of 1540 t of Hg has been released into the environment through human activities up until 2010 (Streets et al., 2017), resulting in serious global Hg contamination of soils (Gworek et al., 2020; Liu et al., 2021a; Xing et al., 2020). Because high levels of Hg in soil exert strong environmental stresses on microorganisms (Frossard et al., 2018; Liu et al., 2018; Hu et al., 2019; Wang et al., 2020), we hypothesized that Hg contamination could potentially affect microbial community assembly. Furthermore, numerous studies have demonstrated that microbial biodiversity plays a central role in ecosystem multifunctionality in natural ecosystems and agroecosystems (Delgado-Baquerizo et al., 2017; Chen et al., 2020a). However, the effect of Hg on soil multifunctionality is still unknown. Such knowledge is crucial for developing a management framework to maintain ecosystem functions and alleviate the effects of Hg contamination on terrestrial ecosystems.

Rice paddies account for ~9% of the world's cropland area and are

widely distributed on all continents, especially in Asia (Liu et al., 2021b). As an intermittent wetland ecosystem, rice paddies are an ideal habitat for active microorganisms and their metabolic activities (Ishii et al., 2011; McGivern et al., 2021; Sun et al., 2021b). Anaerobic conditions induced by flooding promote methane emission and MeHg production in rice paddies (Rothenberg and Feng, 2012; Cai et al., 2016; Liu et al., 2019; Zhang et al., 2020). Furthermore, previous studies indicated that the variation of redox potential was one of the most important factors in determining the bioavailability and methylation of Hg in soils (Beckers et al., 2019; Frohne et al., 2012; Wang et al., 2021). Therefore, rice paddies are one of the most important ecosystems on earth, and healthy rice paddies are the key to maintaining soil sustainability and ensuring food security. In this study, rice paddy soil samples were collected from three rice paddy areas with different Hg sources and contents during the rice harvest season (Meng et al., 2010; Zhao et al., 2016). Then, the soil microbial communities and biogeochemical indicators were analyzed to explore the assembly of microbial communities and soil ecosystem multifunctionality along a Hg-contaminated gradient in paddy soils. This study aimed to (1) explore the effect of Hg on microbial community assembly and (2) assess ecosystem multifunctionality and its interaction with the soil microbial communities in paddy soils throughout a Hg gradient.

2. Materials and methods

2.1. Site description and sample collection

Tongren city in Guizhou is known as the "Hg capital" of China. It has a subtropical humid monsoon climate with an annual mean temperature of 17 °C and 1250 mm of precipitation. The Wanshan district of Tongren city has a long history of Hg mining and smelting, leading to various extents of Hg contamination in the local region. Previous studies have demonstrated that Hg concentrations in Hg mining and smelting areas are 3–5 orders of magnitude higher than those in non-Hg polluted control areas (Wu et al., 2020b; Liu et al., 2022). Therefore, in this study, paddy soil samples from varied Hg content locations were collected in September 2020, from sampling areas that included an artisanal Hg smelting area (Gouxi, GX) (27°33'50" N, 109°11'30" E) and an abandoned Hg mining area (Sikeng, SK) (27°31'02" N, 109°12'38" E). Meanwhile, another rice paddy in the southwest of Guiyang city (Huaxi, HX) was selected as the non-Hg polluted control (26°25'20" N, 106°31'28" E). The three selected areas exhibited similar climates and the soil types from the corresponding fields were classified as Cambisol (gleyic) (IUSS Working Group WRB, 2006).

Within each area, nine rice paddy fields were chosen; each paddy soil comprised a composite of three subsoil samples from the same rice paddy field. A total of 27 rice paddy soils from the root zone (10–20 cm depth) were collected and placed in sterilized polypropylene (PP) bottles. All PP bottles containing samples were sealed with Parafilm® and separately double-bagged with plastic bags to avoid cross-contamination. The soil samples were immediately transported to the lab in a cooler with ice packs and kept at 4 °C in the dark until analysis. The detailed information of the soil preparation, physicochemical properties and other selected heavy metals measurements are described in [Supplementary Material](#) (Text 1).

2.2. Mercury analysis

For total Hg (THg) analysis, 0.1–0.2 g soil was digested in 5 mL of freshly prepared aqua regia (HCl:HNO₃ = 3:1 v/v) with 5 mL of ultrapure deionized water in a water bath for 10 mins at 95 °C. Then, 0.5 mL of bromine chloride (BrCl, 25%) was added, followed by digestion for an additional 45 mins. After digestion, the samples were cooled down and made up to 25 mL with ultrapure deionized water. The supernatant was collected after 24 h, and the total Hg was determined using cold vapor atomic fluorescence spectrometry (AFS) (Brooks Rand Model III, Brooks

Rand Laboratories USA).

2.3. DNA extraction and characterization of microbial communities

Total microbial DNA was extracted from 0.5 g of soil using a FastDNA® Spin Kit (MP Biomedicals, Irvine, CA) according to the manufacturer's protocol. A NanoDrop spectrophotometer was used to determine the concentration and quality of DNA (ND-2000, NanoDrop Technologies, USA).

To assess the microbial communities, the V4 region of the microbial 16 S rRNA gene was amplified with the primer set F515: GTGY-CAGCMGCCGCGGTAA and R806: GGACTACNVGGGTWTCTAAT (Sun et al., 2021a). The PCR set up was as follows: 25 µl of 2 × premix (Ex Taq, TaKaRa, Japan), 1 µl of forward primer (10 µM), 1 µl of reverse primer (10 µM), 1 µl of DNA template, and 22 µl of sterilized DDW (Double Distilled Water). Each DNA sample was PCR amplified in duplicates under the following thermal cycles: 95 °C for 3 mins, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 mins. A no-template control was used to detect any contamination during the amplification process. The PCR products were purified using a purification kit (DP204, TIANGEN Biotech, China). The purified PCR products were quantified, pooled, and sequenced on the Illumina MiSeq platform (Majorbio, Shanghai, China).

After removing low-quality sequences with ambiguous bases (N), low-quality scores (< 25), and short lengths (< 100 bp), the remaining high-quality sequences were analyzed using the QIIME pipeline (version 1.9.1) (Caporaso et al., 2010; Chen et al., 2020b). Operational taxonomic units (OTUs) were identified using UPARSE (version 7.0.1090) with 97% sequence similarity (Edgar, 2013). Taxonomy classification was performed against the SILVA database (version 138) (Pruesse et al., 2007). In addition, sequences that matched the host chloroplast and mitochondria were discarded from the final OTU data set. To address differences in the sequencing depth of different samples, the sequences were rarefied to an equal sampling depth (sequence depth of 28,305). The InvSimpson index was used to calculate the alpha diversity of different soils. All sequences from this present work were deposited in the National Center for Biotechnology Information Sequence Read Archive under the accession number PRJNA800660.

2.4. Assessing ecosystem multifunctionality

The ability of an ecosystem to provide multiple functions is referred to as ecosystem multifunctionality (Chen et al., 2020a). In this study, 15 parameters involved in nutrient cycling were employed to assess multifunctionality, including (1) soil pH, (2) dissolve organic carbon (DOC), (3) SO_4^{2-} , (4) NO_3^- -N, (5) NH_4^+ -N, (6) β -glucosidase, (7) urease, (8) acid phosphatase, (9) cellulase, (10) nitrate reductase, (11) sucrose, (12) carbon cycling genes, (13) nitrogen cycling genes, (14) phosphorus cycling genes, and (15) sulfur cycling genes. DOC and SO_4^{2-} were extracted with ultrapure deionized water (soil: water = 1:10 w/v), filtered using polyethersulfone (PES) membranes (0.45 µm, Bizcomr, China), and analyzed with a total organic carbon analyzer (Vario TOC cube, Elementar, Germany) and a UV-Vis spectrophotometer (UV-5100B, METASH, China), respectively. NO_3^- -N and NH_4^+ -N were extracted with 2 M KCl, filtered using PES membranes (0.45 µm, Bizcomr, China), and measured by UV spectrophotometry (UV-1200, Macy Analysis Instrument Co. Ltd., China) and the indophenol blue method, respectively. Soil enzyme activities (β -glucosidase, urease, acid phosphatase, cellulase, nitrate reductase, and sucrose) were measured using a biochemical assay kit (Solarbio, China) according to the manufacturer's recommended protocols. The functional genes were quantified according to previously published methods in a 25 µl PCR mixture using real-time quantitative PCR (LightCycle 480 II, USA) (Henry et al., 2006; Kandeler et al., 2006; Meyer et al., 2007; Blazejak and Schippers, 2011; Fraser et al., 2017; Zheng et al., 2017; Zhou et al., 2020a; Hussain et al., 2021). Details of functional genes and the primer information are shown

in Table S1.

2.5. Statistical analysis and QA/QC

Averages and standard deviations of all data were analyzed using Microsoft Excel 2016 (Microsoft, USA). The Mann–Whitney U test was performed using SPSS 20.0 software (SPSS, Chicago, IL). All statistical tests were considered significant at p value < 0.05; significance levels of 99% and 99.9% were considered more significant. Principal coordinates analysis (PCoA) of unweighted UniFrac distances, the Adonis test, and microbial alpha diversity were calculated and plotted using the R platform (R Core Team, 2016) with the “vegan” package (Oksanen et al., 2017). In the present study, three metrics of multifunctionality were evaluated: the averaging, single threshold, and multiple threshold approaches were adopted using the “multifunc” package (Byrnes et al., 2014). A neutral community model was constructed in R with the “minpack.lm” package and the “Hmisc” package to assess the importance of stochastic processes in community assembly (Sloan et al., 2006; Elzhov et al., 2013; Harrell, 2014). The normalized stochasticity ratio (NST) index was further used to estimate ecological stochasticity, i.e., the relative importance of deterministic and stochastic processes in community assembly with 50% as the boundary point between deterministic dominance (NST < 50%) and stochastic dominance (NST > 50%) (Ning et al., 2019). To identify the significant drivers of microbial community assembly, a random forest model was constructed using the “randomForest” package (Liaw and Wiener, 2002). To calculate the contributions of multiple drivers to the microbial profiles, variation partition analysis was performed in R with the “vegan” package (Oksanen et al., 2017). Pearson correlation analysis was used to determine the correlations between biodiversity and various soil attributes and examine the relationships between biodiversity and multifunctionality; correlations were computed and visualized with the “ggpubr” and “ggplot2” packages (Wickham, 2009). Structural equation modeling (SEM) is useful for investigating the causal links between multiple variables (Grace, 2006). SEM was used in this study to evaluate the effects of Hg concentration, edaphic factors, and soil microbial diversity on ecosystem multifunctionality. SEM was performed using AMOS 25 (SPSS Inc., Chicago, USA). The data were standardized using Z scores; Z scores can be used to standardize scores on the same scale and are calculated by dividing a score's deviation by the standard deviation of the data set (Chen et al., 2020b).

Quality control for the total Hg analysis was ensured by analyzing method blanks and soil standard reference materials: GSS-5 (total Hg: 290 ± 30 ng/g) and Montana (total Hg: 7420 ± 180 ng/g). The typical recoveries of total Hg from GSS-5 and Montana were $97.0\% \pm 8\%$ ($n = 6$) and $98.2\% \pm 9\%$ ($n = 6$), respectively. For the analysis of triplicate samples, the relative standard deviation (RSD%) was less than 5.2%.

3. Results

3.1. Mercury concentrations in soils across a Hg concentration gradient

The soil physicochemical properties and selected heavy metals (e.g., Cd, Cr, Cu, As, and Pb) concentrations in soils are shown in Table S2. There were slight but insignificant differences in the basic properties of the soils obtained from the different rice paddies. The soil pH ranged from 7.45 to 7.54, and the TC and TN varied from 0.21% to 0.48% and 3.13–7.99%, respectively (Table S2). Furthermore, no discernable difference in the concentrations of other selected heavy metals (e.g., Cd, Cr, Cu, As, and Pb) in paddy soils was observed among HX, GX, and SK ($p > 0.05$) (Table S2). The THg concentrations increased along the contaminated gradient, from HX to SK, with average contents of 0.27 ± 0.05 , 22.15 ± 2.39 , and 588 ± 44.81 µg/g, respectively (Fig. 1). Hg concentrations in paddy soils at GX and SK surpassed the limits of the soil environmental quality-risk control standard for agricultural land soil contamination in China (GB15618–2018), but the paddy soils at HX

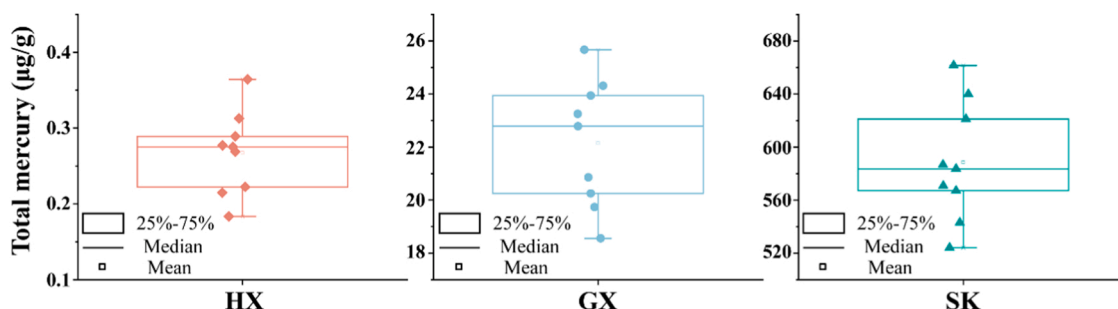


Fig. 1. Total Hg concentrations in soils in different rice paddy areas. The data shown in the figure are from nine replicates (n = 9). Abbreviations: HX (Huaxi, a control area), GX (Gouxi, an artisanal Hg smelting area), and SK (Sikeng, an abandoned Hg mining area).

did not exceed this standard. Moreover, the Hg concentrations of paddy soils at GX and SK were 2 and 3 orders of magnitude higher, respectively, than that of the paddy soils at HX, indicating that HX represents a background area, while GX and SK represent Hg-contaminated areas.

3.2. Soil microbial communities in paddy soils across a Hg concentration gradient

After quality filtering and assembly, a total of 1977,207 high-quality sequences were obtained; these sequences were clustered into 13,256 OTUs. *Proteobacteria* (~ 21%), *Chloroflexi* (~ 19%), and *Acidobacteria* (~ 10%) were the three most abundant phyla (Fig. 2). The microbial community compositions differed significantly ($p < 0.05$) in the paddy soils across the Hg concentration gradient at the phylum level. Compared to the non-polluted control, the relative abundance of *Planctomycetes* and *Actinobacteria* was significantly decreased in Hg-

contaminated paddy soils. Moreover, the relative abundance of *Actinobacteria* decreased with increasing Hg concentration (Fig. 2).

Hg pollution induced significant changes ($p < 0.01$) in the microbial community diversity (based on the InvSimpson index); the highest microbial diversity was found in HX (Fig. S1). Unweighted UniFrac distance-based principal coordinate analysis (PCoA) of microbial community structure showed that microbial communities in HX were clearly distinct from GX and SK. It suggests that the microbial community structure of paddy soil was altered across the Hg concentration gradient (Adonis test, $p < 0.01$) (Fig. S2).

3.3. Microbial community assembly processes in paddy soils across a Hg concentration gradient

A neutral community model was used in this study to anticipate the possible impact of stochastic processes in community assembly in paddy

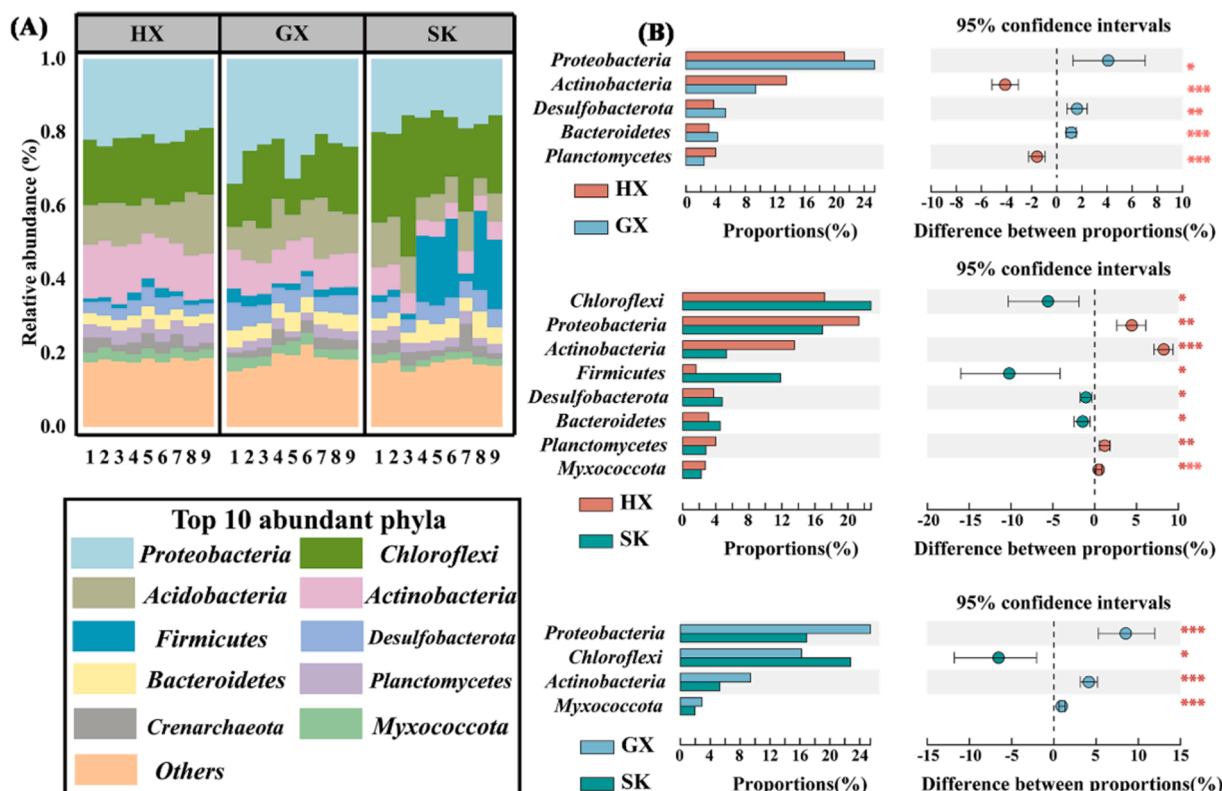


Fig. 2. Taxonomic profiles of microbial communities in soils in different rice paddy areas. (A) The relative abundance of microbial phyla presents in paddy soils. The numbers shown in the figure represent replicates (n = 9). (B) Extended error bar plots showing the ten most abundant phyla that had significant differences between different paddy soils. Data are shown as the relative abundance (%) of phyla in each group. Abbreviations: HX (Huaxi, a control area), GX (Gouxi, an artisanal Hg smelting area), and SK (Sikeng, an abandoned Hg mining area). Statistical analysis was performed by the Mann-Whitney U test. Asterisks indicate a significant difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

soils across a Hg contamination gradient. The results showed that the microbial communities fitted to a neutral community model (NCM) (Fig. 3A). Then, the NST was further analyzed to estimate the relative importance of deterministic and stochastic processes in microbial community assembly. The NST value was lower than the 50% boundary point for microbial communities, suggesting that deterministic processes played a more critical role than stochastic processes in microbial community assembly in paddy soils throughout the Hg contamination gradient.

3.4. Multiple drivers accounting for microbial community assembly in paddy soils across a Hg concentration gradient

The most critical predictors of microbial community assembly in paddy soils throughout the Hg contamination gradient were identified using random forest modeling. The random forest model (ntree = 5000) explained 77.56% of the microbial community assembly variance (Fig. 3B). Soil NO_3^- ($p < 0.01$) exhibited a significant effect on the microbial community composition of paddy soils across the Hg contamination gradient. THg ($p < 0.001$) also significantly influenced the microbial composition. Among all the factors, THg was the most crucial driver of the microbial community composition of paddy soils across the Hg contamination gradient. Variation partitioning analysis further indicated that Hg contamination was the major factor shaping microbial communities (Fig. 3C). Nevertheless, it should be noted that 69% of the variation remained unexplained when exploring the contributions of NO_3^- and THg on microbial communities in paddy soils across the Hg contamination gradient.

3.5. Impacts of Hg on paddy soil ecosystem multifunctionality and its relationship with microbial diversity

A significant decrease in the ecosystem multifunctionality index (Z score) was observed with increasing Hg concentration, with average multifunctionality indices of 0.64 ± 0.26 , 0.22 ± 0.17 , and -0.86 ± 0.38 in paddy soils at HX, GX, and SK, respectively (Fig. 4A). In addition, microbial diversity was significantly related to most of the individual functions of soils in different rice paddy areas (Fig. S3). Moreover, microbial diversity was positively correlated with acid phosphatase ($R = 0.49$, $p < 0.01$), urease ($R = 0.55$, $p < 0.01$), and nitrogen cycling-related genes ($R = 0.45$, $p < 0.05$).

The relationship between microbial diversity (InvSimpson index) and ecosystem functions was investigated in this study utilizing different multifunctionality metrics: averaging, single threshold, and multiple threshold approaches. By standardizing each function to a common

scale, the averaging approach was employed to determine the average level of multiple functions. The results revealed a positive linear correlation between soil microbial diversity and ecosystem multifunctionality ($R = 0.55$, $p < 0.01$) (Fig. 4B). When analyzed with the single threshold approach to determine the overall level of multiple functions under a certain threshold, the results exhibited that microbial diversity was significantly ($p < 0.05$) correlated with ecosystem multifunctionality at thresholds of 20%, 40%, and 80% (Fig. S4). Given that the multiple threshold approach does not require setting a threshold value, a multiple threshold approach was employed to investigate the relationship between the diversity of the microbial community and ecosystem multifunctionality with a continuous gradient of thresholds (Fig. 5). Microbial diversity boosted ecosystem multifunctionality across a greater range of thresholds (beginning at a threshold of 16%), and the Threshold of Maximum Diversity Effect (Tmde) was 81%, with a Realized Maximum Effect of Diversity (Rmde) of 0.042. It suggests that adding one microbial species increased ecosystem functions by 0.042.

SEM was further performed to assess the direct and indirect relationships between Hg levels, microbial diversity, edaphic factors, and ecosystem functions. As illustrated in Fig. 6, the model explained 88.9% of the variation in ecosystem multifunctionality associated with Hg contamination and was a good fit according to the χ^2 test, RMSEA, and GFI. The results suggested that Hg pollution directly affected ecosystem multifunctionality ($\lambda = -0.909$, $p < 0.001$) and indirectly affected ecosystem multifunctionality by influencing microbial diversity ($\lambda = -0.547$, $p < 0.001$). In addition, the model provided evidence that microbial diversity was strongly associated with ecosystem multifunctionality even when considering edaphic factors.

4. Discussion

4.1. Hg-based deterministic selection dominates microbial community assembly in paddy soils across a Hg concentration gradient

To date, little attention has been focused on microbial community assembly processes in paddy soils across Hg contamination gradient. The current work provides a deeper understanding of how Hg concentration gradients affect microbial community assembly and highlights the underlying processes and mechanisms. Previous studies have demonstrated that Hg at low concentrations represented a major hazard to microorganisms, and organomercury compounds can exert the same effect at a much lower concentration than inorganic mercury (Boening, 2000; Liu et al., 2018; Mahbub et al., 2017). Hg can inhibit protein synthesis and disrupt enzymatic and cellular functions (Bruins et al., 2000; Kuznetsov et al., 1986). Inorganic Hg and organomercury

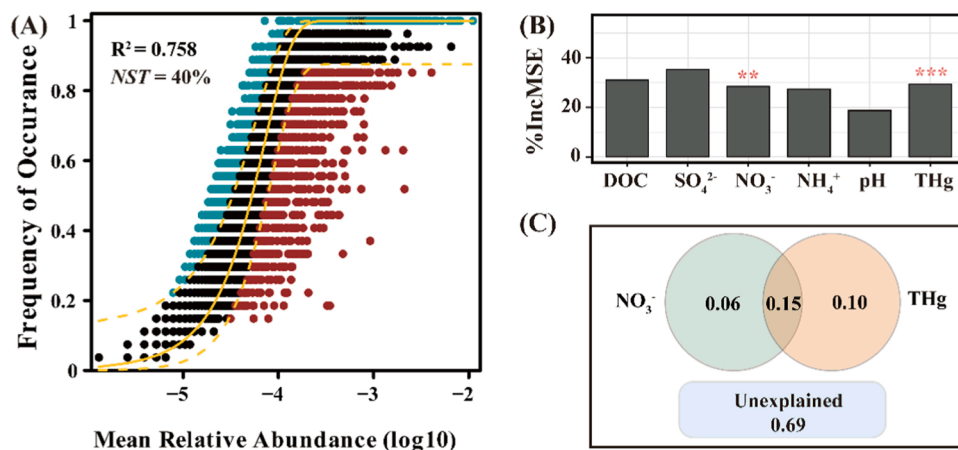


Fig. 3. Microbial community assembly in paddy soils across a Hg contamination gradient. (A) Fit of the neutral community model (NCM) of community assembly. The solid yellow line indicates the best fit to Sloan's neutral model, and the dashed yellow lines represent 95% confidence intervals around the model prediction. OTUs with frequencies higher or lower than the predicted occurrence frequency by NCM are shown in different colors. R^2 indicates the fit to NCM. (B) Random forest modeling indicating the importance of different predictors for microbial profiles. The accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). %IncMSE was used to estimate the importance of these predictors. Asterisks indicate a significant difference. ** $p < 0.01$, *** $p < 0.001$. (C) Variation partitioning analysis (VPA) differentiating effects of NO_3^- and THg on microbial profiles.

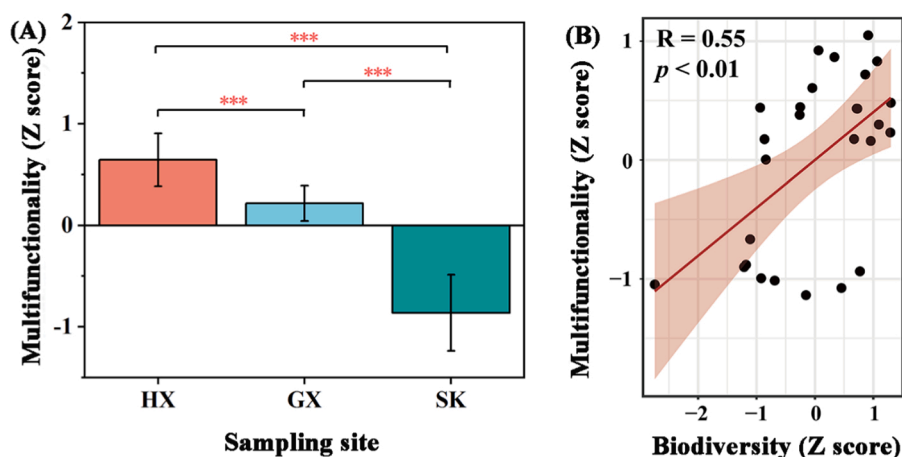


Fig. 4. Ecosystem multifunctionality in response to mercury pollution. (A) Average multifunctionality index in soils in different rice paddy areas. The multifunctionality index was calculated using the data from soil chemical properties, enzyme activities, and functional genes involved in nutrient cycling. Abbreviations: HX (Huaxi, a control area), GX (Gouxi, an artisanal Hg smelting area), and SK (Sikeng, an abandoned Hg mining area). The data shown in the figure are from nine replicates ($n = 9$) and are represented as the means \pm SD. Statistical analysis was performed by the Mann-Whitney U test. Asterisks indicate a significant difference. *** $p < 0.001$. (B) Relationship between the multifunctionality index and microbial diversity. The solid red line represents the smooth curve that fits between variables, and the black dots represent paddy soil samples ($n = 27$).

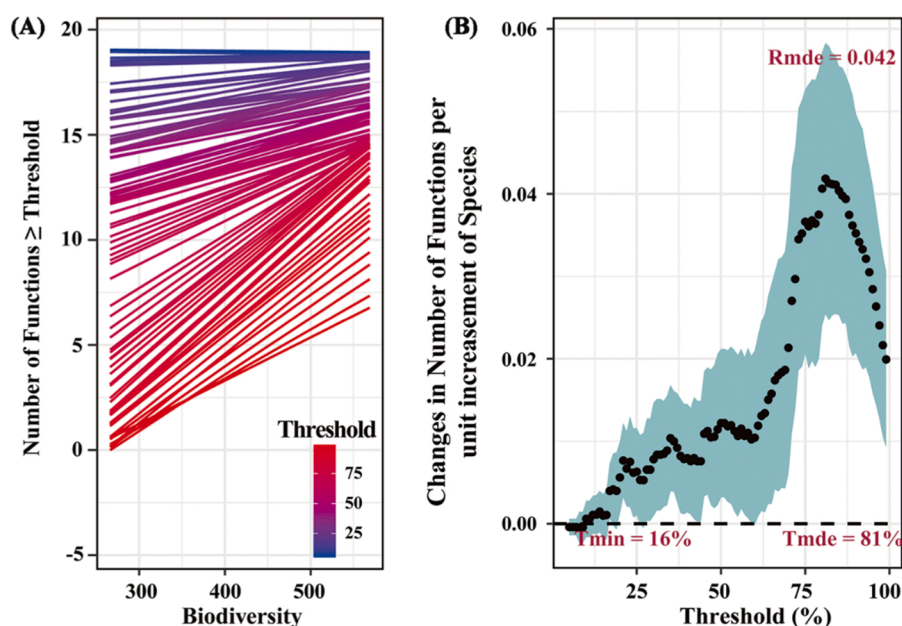


Fig. 5. The shift of ecosystem functions with a continuous gradient of thresholds. (A) Effects of microbial diversity on the number of functions above thresholds. Lines represent the slope between microbial diversity and the number of functions greater than or equal to a threshold value. (B) The dotted curves indicate the changes in the number of functions per unit increment of microbial diversity. The 95% confidence intervals (cadet blue) around the estimated slopes (black dot) indicate whether the intervals contain zero, giving a test of the threshold values at which diversity has an effect on multifunctionality. T_{min} is the slope with the lowest threshold that is different from zero; T_{mde} is the threshold with the steepest slope; and R_{mde} shows the maximum slope estimated at T_{mde} .

compounds, therefore, are both toxic to most organisms, which could significantly alter microbial communities. Current study revealed that the relative abundance of *Planctomycetes* and *Actinobacteria* decreased with increasing Hg concentration; these phyla play vital roles in maintaining ecosystem functions (Kalam et al., 2020; Tran et al., 2021). Furthermore, significant shifts in microbial community structure and decreased microbial community diversity were observed in soils with higher Hg concentration, which is consistent with observations in other heavy metal-contaminated soils (Gough and Stahl, 2011; Kuang et al., 2016). These results indicated that Hg alters taxonomic composition and diversity of soil microbial communities. In this case, shifts in microbial communities in paddy soils through a Hg contamination gradient may be related to ecological selection, i.e., deterministic processes (Dini-Andreote et al., 2015). Based on the neutral community model, the fitness value (Fig. 3, $R^2 = 0.758$) observed in this study was lower than that of previous studies, in which stochastic processes were found to be more important than deterministic processes in the microbial community assembly of microeukaryotic plankton ($R^2 = 0.899$ for wet season and $R^2 = 0.885$ for dry season) and zebrafish gut ($R^2 = 0.81$ for four days post-fertilization) (Burns et al., 2016; Chen et al., 2019b). Thus, the current results demonstrate that deterministic processes might play an essential role in microbial community assembly in paddy soils

throughout a Hg contamination gradient. A general mathematical framework was further used to quantitatively assess the relative importance of deterministic and stochastic assembly processes in microbial communities (Ning et al., 2019). The NST value was lower than the 50% boundary point for microbial communities, indicating that deterministic processes are more critical than stochastic processes in influencing the microbial communities in paddy soils over a Hg contamination gradient.

The present study further investigated the soil microbial characteristics and the factors that influence the microbes in paddy soils across a Hg contamination gradient. We found that soil NO_3^- and THg significantly affected microbial community composition among all parameters. NO_3^- content was significantly correlated with expression of genes involved in the NO_3^- reduction process (narG: Pearson's $r = 0.713$, $p < 0.001$; nirS: Pearson's $r = 0.706$, $p < 0.001$), suggesting that NO_3^- significantly altered abundance and diversity of denitrifying bacteria. However, THg significantly altered microbial community structure and diversity, suggesting that THg may be the most important driver that shapes microbial profiles, which was also supported by random forest and variation partitioning analyses. A major limitation of this study, however, is that an insufficient number of physicochemical properties were examined, as previous studies have confirmed that microbial

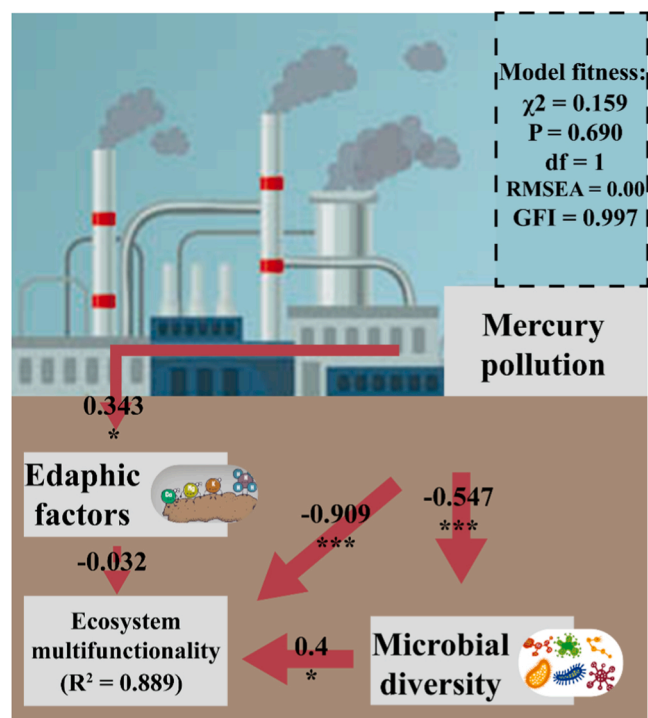


Fig. 6. Structural equation models showing the effects of mercury, microbial diversity (InvSimpson index), and edaphic factors (soil chemical properties and enzyme activities) on ecosystem multifunctionality. Value adjects to arrows are path coefficients and indicative of the effect size of the relationship. R^2 denotes the proportion of variance explained. Significance levels are as follows: * $p < 0.05$, *** $p < 0.001$. χ^2 , P , df , $RMSEA$, GFI present in figure are model fitness details.

profiles are affected by multiple factors (Wang et al., 2017; Bahram et al., 2018; Jiao et al., 2020; Chen et al., 2021). Along with unidentified environmental variables, this limitation could explain why 69% of the variation in these microbial communities could not be explained. It is currently unfeasible to study the impact of all abiotic variables on microbial communities, so variables involved in nutrient cycling were selected. The findings highlighted that Hg is the dominant factor affecting microbial communities, compared to the contributions of the other selected variables. Therefore, these results indicate that Hg contamination should be carefully considered when exploring patterns and processes of microbial community assembly across these ecosystems.

4.2. Decreased soil ecosystem multifunctionality and its association with microbial diversity in paddy soils across a Hg concentration gradient

A large number of studies have claimed that ecosystem functions are affected by anthropic activity (Sala et al., 2000; Foley et al., 2005; Hooper et al., 2012). However, human-induced global change is multifactorial and leads to significant uncertainty in the prediction of ecosystem functions (Lehmann and Rillig, 2014; Rillig et al., 2019). For example, anthropogenic activities release large amounts of Hg into the environment, but the effects of Hg on ecosystem multifunctionality in paddy soils are poorly understood due to the fact that Hg content can be highly variable in soils (Beckers and Rinklebe, 2017). Given that each approach has advantages and limitations (Byrnes et al., 2014), ecosystem multifunctionality was calculated in this study using different metrics of multifunctionality, namely, the averaging, single threshold, and multiple threshold approaches (Maestre et al., 2012; Byrnes et al., 2014; Perkins et al., 2015; Valencia et al., 2015; Delgado-Baquerizo et al., 2016; Manning et al., 2018). When the results of three different

metrics were examined, it was clear that biodiversity significantly affected ecosystem multifunctionality. Given that Hg is a significant factor influencing microbial communities and that Hg, directly and indirectly, affects ecosystem multifunctionality, this study implies that Hg plays a vital role in ecosystem functions. Pollutants, such as heavy metals (including Cu, Ni, Cr, and Pb) and microplastics have a significant effect on the ecological functions of aquatic and terrestrial systems (Rillig and Lehmann, 2020; Nava and Leoni, 2021; Zhang et al., 2021). However, this study is the first insightful exploration into the effects of Hg on ecosystem multifunctionality in paddy soils through a Hg contamination gradient. Previous reports indicate that agricultural practices can positively or negatively affect ecosystem multifunctionality, and microbial function redundancy is widespread in microbial communities (Bender et al., 2016; Chen et al., 2020a; Tsiafouli et al., 2015; Wertz et al., 2007). The results of the current study demonstrated that Hg contamination has especially strong negative consequences for ecosystem functions, suggesting that the loss of soil diversity will inevitably lead to a decline in ecosystem function. In this regard, increasing one species of microorganism can improve ecosystem function, supported by the study showing that soil microbial diversity is essential for maintaining soil ecosystem function (Chen et al., 2020b). Therefore, it is important to employ environmental management and conservation strategies to mitigate the adverse effects of Hg on soil diversity and soil provisioning ecosystem services in terrestrial ecosystems.

An increasing body of evidence has now provided sufficient insight into the roles of microbial communities in the multifunctionality of belowground soil ecosystems (Delgado-Baquerizo et al., 2016; Meyer et al., 2018; Chen et al., 2020a). In this respect, decreased ecosystem multifunctionality could be attributed to a shift in microbial communities. This is indicated by the observed positive correlation between the diversity of the microbial community and ecosystem multifunctionality in paddy soils across the Hg gradient in this study. Therefore, Hg-induced changes in microbial diversity have an even more significant impact on paddy soil's multifunctionality; indeed, many studies have identified a positive correlation between microbial community diversity and multifunctionality in agroecological (Chen et al., 2020a) and natural systems (Jing et al., 2015; Delgado-Baquerizo et al., 2017; Gross et al., 2017).

Positive correlations were also observed in our study between microbial community diversity and the majority of individual characteristics in different rice paddy areas, such as pH, DOC, SO_4^{2-} , NO_3^- , NH_4^+ , acid phosphatase, β -glucosidase, sucrase, urease, and nitrogen- and phosphorous-related functional genes. However, weak correlations were observed with cellulase, nitrate reductase, and carbon- and sulfur-related functional genes related. It is hypothesized that these results can be attributed to the fact that Hg reduces soil microbial diversity, which, in turn, adversely affects nutrient cycling. A previous study confirmed that the relationships between biodiversity and ecosystem functions might be compromised under adverse soil conditions (Domeignoz-Horta et al., 2020). In this study, Hg played a more critical role in shaping microbial profiles than other nutrient factors; therefore, the Hg contamination gradient altered microorganism interactions and, ultimately, ecosystem multifunctionality (Reich et al., 2012; Louca et al., 2018). However, it should be kept in mind that the effect of biodiversity on multifunctionality depends on the number of functions (Hector and Bagchi, 2007; Gamfeldt and Roger, 2017; Meyer et al., 2018). Therefore, this study, which provides evidence on biodiversity-multifunctionality relationships in paddy soils throughout a Hg concentration gradient, is limited. For example, this study assessed 15 ecosystem functions that represent nutrient cycling but did not include variables related to climate change, land-use change, etc. While nutrient cycling could be considered an indicator of ecosystem functions that are essential to the elemental biogeochemical cycle, the inclusion of factors involved in climate change, land-use change, etc. would better reflect the current status of ecosystem multifunctionality (Sala et al.,

2000; Foley et al., 2005). Overall, based on the findings of the three study areas examined in this study, Hg contamination is the major factor affecting ecosystem multifunctionality. This study highlights the importance of assessing Hg levels before evaluating ecosystem services in paddy soils.

5. Conclusions and implications

In summary, this study demonstrates that paddy soil microbial communities are predominantly driven by Hg-based deterministic processes rather than stochastic processes. Ecosystem multifunctionality decreased across the Hg concentration gradient, and significant correlations were observed between the microbial community diversity and ecosystem multifunctionality in paddy soils across the Hg contamination gradient. Altogether, our findings indicate that Hg affects microbial community assembly processes and significantly reduces the ecosystem multifunctionality, suggesting that Hg-induced shifts in microbial diversity are the major drivers shaping paddy soil functions. However, soil function can be affected by various biological, chemical, and physical factors, along with complex interactions between these factors. Therefore, more environmental factors and Hg chemical speciation need to be considered in future studies to more comprehensively determine the effects of Hg on microbial community assembly and ecosystem multifunctionality. The findings of this study also call for microbial diversity conservation strategies to alleviate the negative effects of Hg pollution on ecosystem functions. Consequently, further work is required to uncover the effect of a gradient of microbial diversity in Hg-contaminated paddy soils on soil functions.

CRedit authorship contribution statement

Qiang Pu: Data curation, Formal analysis; Visualization, Writing – original draft. **Kun Zhang:** Data curation, Formal analysis, Writing – review & editing. **Alexandre J. Poulain:** Writing – review & editing, Methodology. **Jiang Liu:** Formal analysis, Writing – review & editing. **Rui Zhang:** Data curation, Writing – review & editing. **Mahmoud A. Abdelhafiz:** Formal analysis, Writing – review & editing. **Bo Meng:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Xinbin Feng:** Funding acquisition, Resources, 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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2022.129055](https://doi.org/10.1016/j.jhazmat.2022.129055).

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