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The mutual effects of water quality and microbial community at water and sediments polluted by acid mine drainage from a typical abandoned coal mines

Hong-Ji Zhao[1], Juan Chu[2], Qian Wang[3], Quan-Jia Wu[3], Xiang-Dong Li*[3]

[1]School of Environment Science and Spatial Informatics, China University of Mining and Technology, Jiangsu, China
2Ecological Environment Monitoring Centre of Qiandongnan, Guizhou, China
3School of Environment Science and Spatial Informatics, China University of Mining and Technology, Jiangsu, China

* Corresponding author's e-mail: 4549@cumt.edu.cn

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Abstract: The stochastic groundwater flow paths and hydrogeological complexity inherent to karst systems significantly exacerbate acid mine drainage (AMD) contamination risks in these regions. This study examines the impacts of AMD from abandoned coal mines on aquatic microbial communities and water quality parameters in both water and sedimentary environments of the karst-dominated Yudong River Basin. Water and sediment samples were collected from upstream, AMD discharge points, and downstream areas. Physicochemical parameters (pH, Fe, Mn, SO_4^{2-}, NH_3-N, COD) were analyzed, and microbial communities were characterized using high-throughput 16S rRNA sequencing. Taxonomic composition and correlations between environmental factors and microbial taxa were evaluated. The analysis demonstrated that AMD significantly reduced pH (from 7.63 to 3.22) and increased pollutant concentrations, with gradual downstream recovery. Microbial diversity declined near the discharge point, accompanied by shifts in dominant taxa: *Campylobacterota* (55.0 ± 1.5%) in water and *Firmicutes* (48.4 ± 5.6%) in sediments. Key genera exhibited strong correlations with pollutants ($r > 0.65$) and acidic conditions. Archaeal communities were dominated by *Thermoplasmatota* (water) and *Halobacterota* (sediments), reflecting habitat-specific adaptations. AMD drastically restructured microbial communities, selecting for acid- and metal-tolerant taxa involved in sulfur, iron, and nitrogen cycling. These findings highlight the ecological resilience of specific microbial groups and their potential for bioremediation in AMD-contaminated karst ecosystems.

Introduction

Acid mine drainage (AMD), a typical environmental byproduct of mining activities, has become a global environmental issue. In recent years, policy reforms in China have led to the closure of numerous coal mines due to resource depletion or outdated production capacity, yet the legacy of AMD contamination continues to threaten ecosystems. Untreated AMD from abandoned coal mines is characterized by low pH and elevated concentrations of heavy metals and sulfate (SO_4^{2-}) (Ma et al. 2024), causing severe pollution of surface water, groundwater, sediments, and soils (Naghoum et al. 2025). Notably, the transport paths of karst groundwater, which is typically mineral-rich, exhibit random orientations. The complexity of karst systems, both above and below the surface, in coal-mining regions significantly contributes to the likelihood of water contamination and the difficulty in its remediation. （Li et al. 2023）. Given the high sensitivity and fragility of karst ecosystems, the escalating impact of AMD on water quality jeopardizes regional aquatic ecological security and generates adverse societal consequences. Thus, elucidating the environmental influence of AMD in karst areas is urgently needed.

Surface water serves as a critical pathway for karst water pollution (Malá et al. 2022). Current research has primarily focused on the impacts of AMD discharge on surface water in karst regions. For instance, Bu et al. (2024) reported severe exceedances of iron and manganese concentrations in riverine surface waters within the karst areas of southwestern China, identifying these metals as dominant pollutants in river ecosystems. Similarly. Qin et al. (2024) demonstrated that AMD-derived heavy metal concentrations gradually declined downstream after tributaries from mining areas merged with main rivers, with metals predominantly accumulating in suspended particulate matter and sediments. However, existing studies have mainly addressed the geochemical processes of heavy metal pollutants in karst surface waters, while limited attention has been paid to AMD-induced shifts in microbial communities.

As fundamental components of ecosystems, microorganisms play pivotal roles in pollutant transformation within river water and sediments (Zhang et al. 2019a). Moreover, microbial population dynamics serve as sensitive indicators of aquatic environmental changes. Given the extreme acidity and high heavy metal content of AMD, it creates a hostile environment for most microorganisms. As a result, only acidophilic and metal-tolerant microorganisms, including *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Nitrospirae*, and *Acidobacteria,* can survive (Chen et al. 2021). Similar patterns have been observed in the Aha River Basin in China and in the waters and sediments from the Lancaster Acid Mine Drainage Dam in South Africa (Sun et al. 2015; Lukhele et al. 2019). Microorganisms commonly found in AMD environments exhibit specialized ecological functions that enable them to thrive under such challenging conditions. For instance, Zhang et al. (2019b) observed that AMD influx rapidly selects for acid-tolerant, metal-reducing, and sulfate-cycling microbial assemblages, highlighting their bioremediation potential. Likewise, Wang et al. (2023) identified sulfur and Fe^{3+} concentrations as key abiotic drivers of microbial community dynamics, particularly for iron- and sulfur-cycling taxa.

Located in the karst region of southeastern Guizhou Province, the Yudong River Basin has been severely contaminated by acid mine drainage (AMD) generated from nearly a hundred abandoned coal mines. The Pinglu River section represents the most heavily polluted segment. However, understanding of how AMD influences microbial communities in both water and sediments under such specific karst hydrogeological conditions remains limited. This study aims to fill this knowledge gap by investigating microbial response mechanisms within this unique environment.

This study investigates the Pinglu reach of the Yudong River Basin to (1) assess changes in river water quality following the coal mine drainage discharge into the river (2) characterize planktonic and benthic microbial communities under AMD stress, and (3) elucidate the effects of water quality on microbial community composition. By integrating water and sediment analyses, we aim to advance understanding of AMD's ecological impacts and inform bioremediation strategies.

Materials and methods

Study area

Yudong Spring serves as a major pollution source in the Pinglu River of the Yudong River Basin. From Yudong Spring downstream to the Jiangkou River, the water exhibits a persistent brownish-yellow to reddish-brown discoloration, accompanied by elevated turbidity and suspended particulate matter with yellowish-red hues (Fig. 1b), indicative of severe AMD contamination.

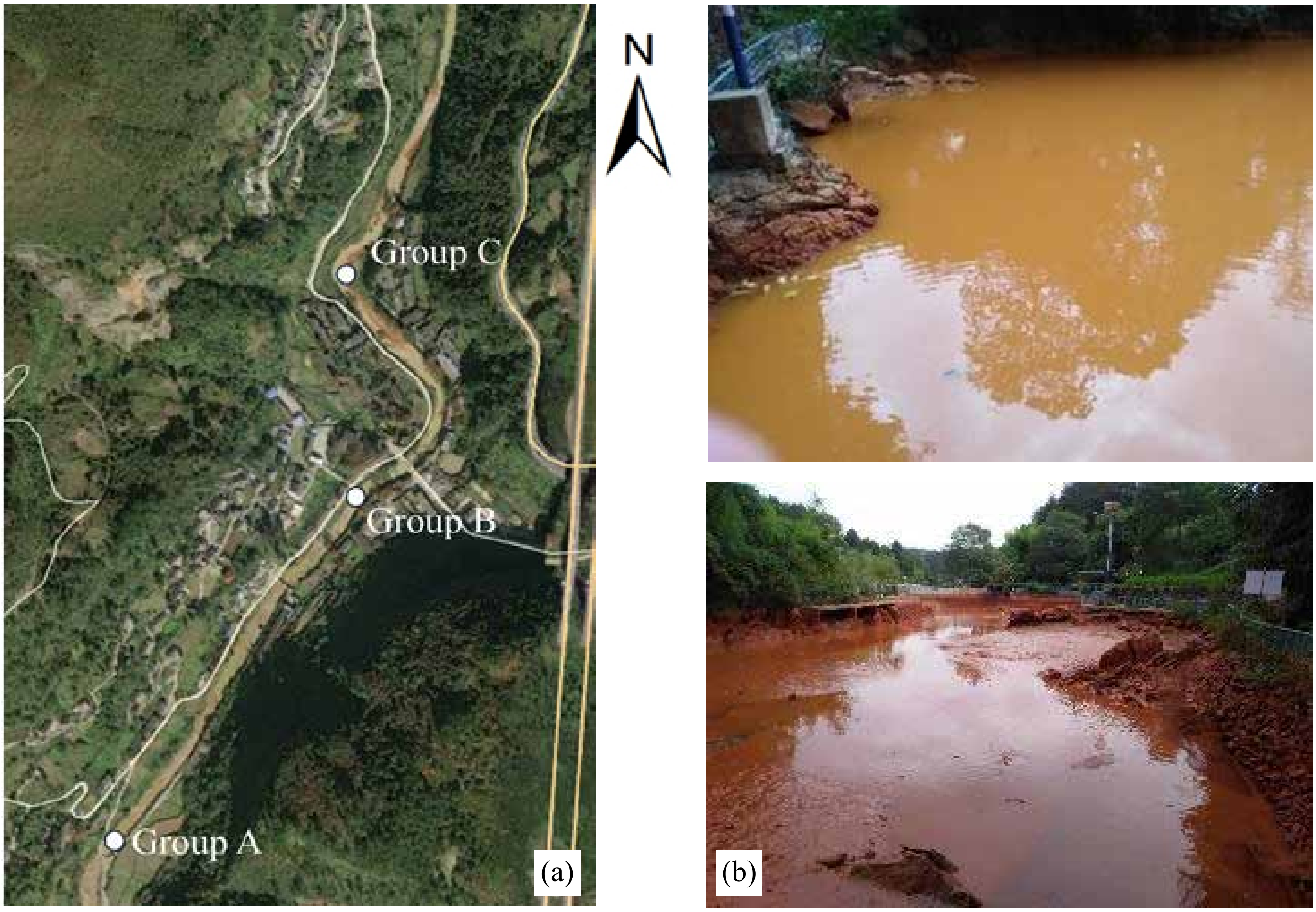

Fig. 1. Overview of study area. (a) Sampling point location; (b) River pollution caused by AMD

Sample collection and analysis

In July 2024, three sampling sites were established along the outflow of Yudong Spring, with three parallel samples collected at each station (Fig. 1a), representing upstream (Group A, sample 1-3), discharge point (Group B, sample 4-6), and downstream (Group C, sample 7-9) locations. Both water and sediment samples were collected at each site (18 samples in total). The upstream sites were located approximately 10 km from the spring outlet, and downstream sites about 8 km away. Water samples were labeled W1-W9, and sediment samples S1-S9. For archaeal community analysis, samples were further annotated as AW1-AW9 (water) and AS1-AS9 (sediment).

Water samples were collected in sterile 1-L polyethylene bottles at a depth of 20 cm below the water surface, following China's Technical Specifications for Surface Water Environmental Quality Monitoring. Sediment samples (top ~5 cm, approximately 500 g) were obtained using a grab sampler. Within a 1 m² area centered at the diagonal midpoint, five equidistant subsamples were collected following a quincunx sampling pattern, homogenized into a composite sample, and stored in sterile polyethylene zip-lock bags. All water samples were immediately stored at 4 °C and processed within 24 h. For molecular analysis, samples were preserved at 4 °C on-site, transported on dry ice, and stored at -80°C until analysis (Li et al. 2025).

Water pH was measured in situ using a portable pH meter. The collected water samples were transported to the laboratory as soon as possible and filtered through 0.45 µm polyester fiber filter membranes. Fe was determined using o-phenanthroline spectrophotometry; Mn by oxidative photometry with potassium periodate; COD by dichromate method; SO_4^{2-} by barium chromate spectrophotometry, and NH_3-N was determined by Nessler's reagent spectrophotometric method (Han et al. 2020). Blank and parallel samples were included in each batch and all analyses were conducted in triplicate. Final results represent the average of three replicate analyses, with an error range below 10%. All analyses followed the Chinese National Standard (HJ/T 91-2002).

DNA extraction and PCR amplification were performed using conventional methods (Shang et al. 2020). Briefly, genomic DNA was extracted from the samples, and barcode-labeled primers targeting the V3 to V4 regions of the 16S rRNA gene were used for amplification. Universal primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) were employed for bacterial amplification, while archaeal sequences were amplified with primer pairs 524F10extF (TGYCAGCCGCCGCGGTAA) and Arch958RmodR (YCCGGCGTTGAVTCCAATT). Amplification libraries were generated, and the amplification protocol consisted of an initial denaturation at 95 °C for 3 minutes, 27 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds; followed by a final extension at 72 °C for 10 minutes. The libraries were stored at 4 °C using an ABI GeneAmp® 9700 PCR machine. PCR products are purified using the AxyPrep DNA Gel Extraction Kit (AXYGEN, USA) and quantified using the QuantiFluor™ -ST Blue Fluorescence Quantification System (Promega, USA). Equimolar amounts of purified products were mixed and subjected to PCR-mediated ligation of Illumina adapter sequences to the target regions, thereby constructing Illumina sequencing libraries. Sequencing was conducted on the Illumina MiSeq platform (Illumina, San Diego, USA). Raw sequencing data were quality-filtered and optimized before operational taxonomic unit (OTU) clustering. Subsequent analyses included OTU diversity, microbial community composition (at the phylum and genus levels), and correlations between microbial communities and environmental factors.

Data treatment and analysis

Fast software was used to conduct quality control of the original double-ended sequencing reads, and FLASH software was employed for splicing the sequences. Bases with quality scores below 20 in the read tail were filtered. A 50 bp sliding window was applied, and the average mass value in the window was lower than 20. The backend bases were cut off from the window, and the reads shorter than 50 bp after trimming or containing N bases were removed. Operational taxonomic units (OTUs) were clustered using the UPARSE v7.1 software at a 97% similarity threshold, and chimeric sequences were removed. The RDP Classifier was used to compare the Silva 16S rRNA gene database (v138) for OTU species taxonomic annotation. The confidence threshold was 70% (Wang et al. 2007), and the community composition of each sample was calculated at different species classification levels. All statistical analyses were performed using R packages for statistical analysis and data visualization. Mantel tests were conducted to evaluate correlations between microbial communities and environmental factors.

Results and Discussion

Change in water quality index

The water quality indices of the Pinglu River are shown in Fig. 2. Acid mine drainage (AMD) from the abandoned coal mines significantly increased river acidity, reducing the pH from 7.63±0.06 (upstream) to 3.22±0.04. Downstream, pH gradually increased due to dilution, consistent with the pattern reported by Zou et al. (2025) in AMD-affected rivers. AMD generation in abandoned coal mines is primarily driven by pyrite oxidation in mine strata, releasing large quantities of Fe, SO_4^{2-}, and H^+ (Acharya and Kharel, 2020). Pollutant concentrations peaked at the Yudong Spring outfall and declined downstream, highlighting the pronounced impact of AMD on river water quality. It should be noted, however, that this study reflects conditions during the rainy season only. The extent of dilution and downstream recovery is likely strongly influenced by seasonal hydrological variations, warranting future investigation across multiple temporal scales.

Microbial community composition

Alpha diversity analysis

Alpha diversity analysis was used to assess the abundance and diversity of microbial communities in river water and sediments affected by AMD. As shown in Table 1, sequencing coverage was sufficient to detect most microbial taxa present. The Shannon index indicated higher bacterial diversity downstream (1.695±0.031) than upstream (1.379±0.685) or at the Yudong Springs outfall (1.346±0.071), whereas archaeal diversity was highest upstream (3.656±0.117) and lowest at the outfall (3.101±0.152). The Ace index suggested greater bacterial abundance at the outfall but lower species richness upstream.

Sediment analyses followed a similar trend, showing the lowest bacterial diversity at the outfall (1.692±0.089) and the highest archaeal diversity downstream (3.437±0.065). AMD pollution altered microbial populations, favoring pollution-tolerant species while reducing pollution-intolerant ones, leading to the lowest diversity near the outfall. However, bacterial abundance remained relatively high, suggesting adaptation to polluted conditions and the formation of stable ecological niches **(Jia et al. 2023)**. These results demonstrate AMD's significant impact on microbial communities and its implications for ecosystem restoration and bioremediation strategies.

PCoA Analysis

Principal co-ordinates analysis (PCoA) was employed to assess the divergence of bacterial and archaeal communities between water and sediment samples. For bacteria (Fig. 3a), the first two PCoA axes explained 57.17% of the total variance (PCoA1: 30.74%; PCoA2: 26.43%), indicating significant

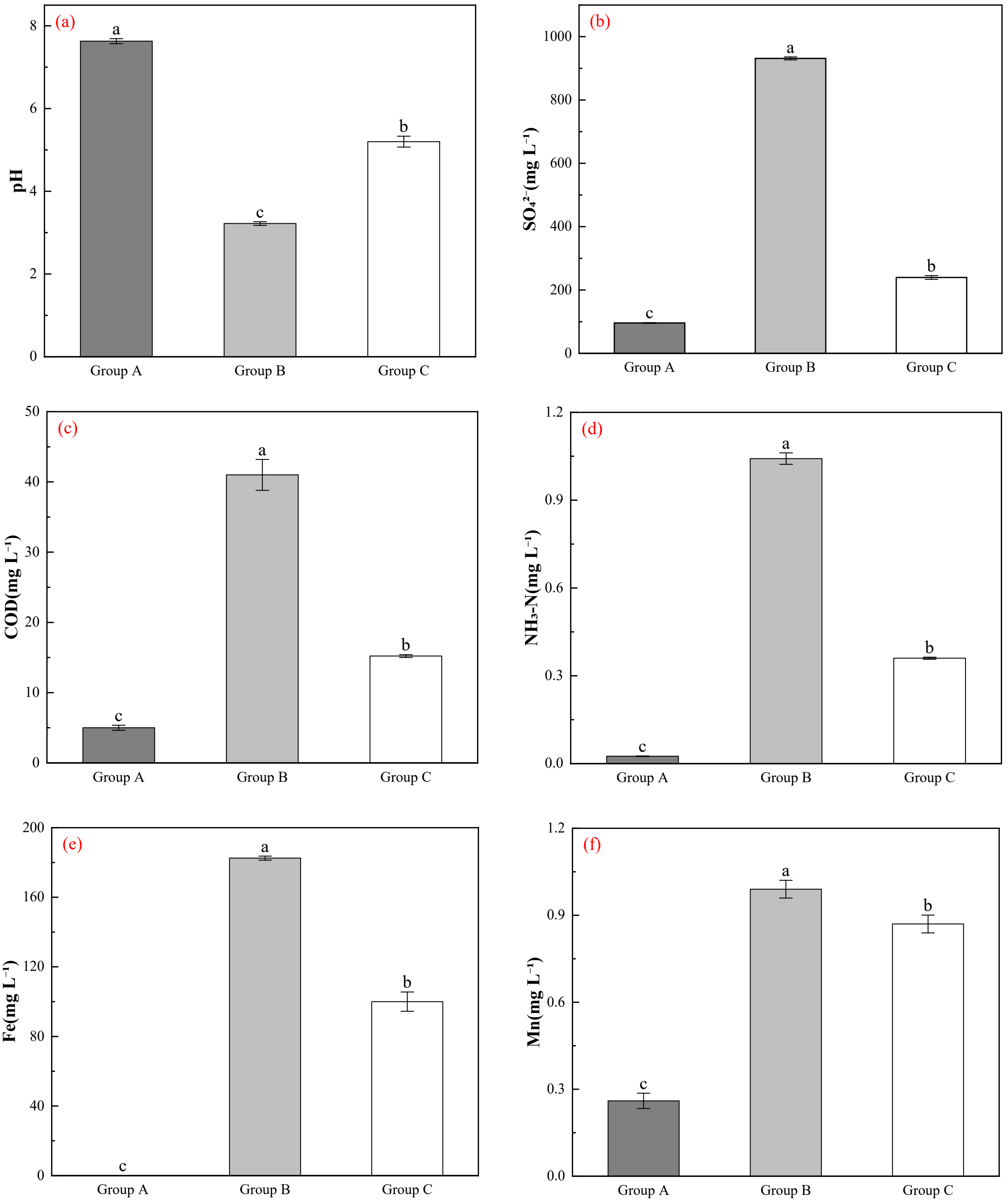

Fig. 2. River water quality change

compositional differences. In water samples, the upstream (W1) and downstream (W3) sites clustered closely together, whereas the AMD-impacted Yudong Spring outfall sample (W2) was distinctly separated. Sediment bacterial communities exhibited even greater spatial heterogeneity, with all samples (S1, S2, S3) widely dispersed. The most contaminated outfall samples (W2, S2) displayed the most distinct bacterial compositions, underscoring the primary role of AMD-induced environmental gradients in shaping community structure.

Archaeal communities (Fig. 3b) showed a similar pattern, with PCoA1 and PCoA2 explaining 73.65% of the variance (47.28% and 26.37%, respectively). In both water and sediment samples, the outfall (AW2/AS2) and downstream (AW3/AS3) sites formed distinct clusters, clearly separated from the upstream samples (AW1/AS1). Notably, the upstream water (AW1) and sediment (AS1) samples from the pristine zone plotted in close proximity, suggesting a convergent archaeal community in the absence of AMD contamination.

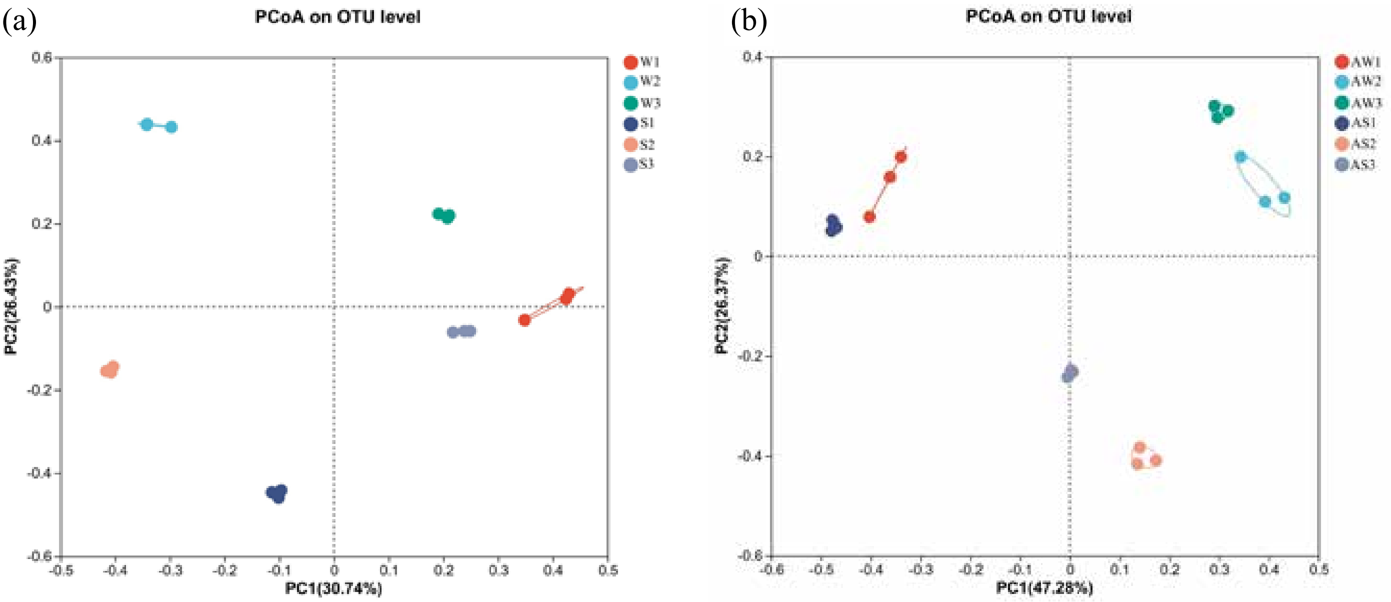

Fig. 3. PCoA analyses of water and sediment (a) Bacterial (b) Archaea

Table 1. Alpha diversity indices of microbial communities

Microbial community	Group	OTUs	Shannon	Ace	Coverage
Bacterioplankton	A	269±9	1.379±0.685	21.034±1.960	0.998±0.0006
	B	228±16	1.346±0.071	26.287±6.020	0.998±0.0012
	C	390±9	1.695±0.031	25.142±2.063	0.999±0.0006
Benthic bacteria	A	738±37	2.094±0.124	42.284±9.150	0.996±0.0006
	B	351±28	1.692±0.089	25.045±1.034	0.998±0
	C	496±43	2.073±0.095	26.805±5.504	0.998±0.0010
Planktonic archaea	A	106±7	3.656±0.117	191.894±12.187	0.999±0.0001
	B	57±22	3.101±0.152	99.242±44.625	0.999±0
	C	205±21	3.112±0.115	339.647±41.929	0.999±0.0002
Benthic archaea	A	227±26	3.336±0.118	373.908±37.328	0.999±0.0001
	B	102±19	2.579±0.536	190.387±34.807	0.999±0.0001
	C	322±11	3.437±0.065	526.162±24.960	0.999±0

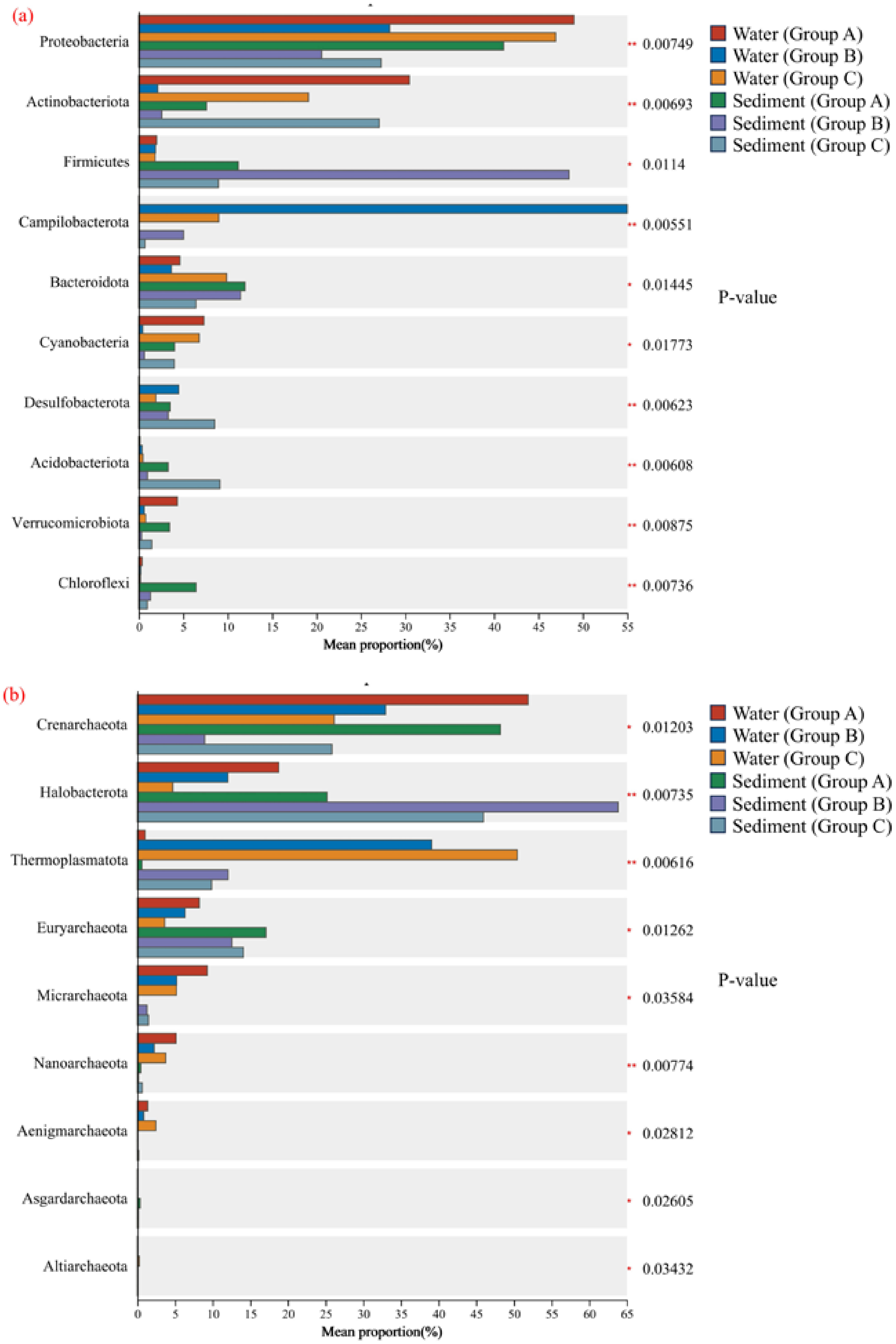

Fig. 4. Microbial intergroup difference significance tests (a) Bacteria (b) Archaea

Significance testing of intergroup microbial differences

Fig.4 demonstrates the disparities in the mean relative abundance of shared species across various groups, clearly depicting the pronounced variation in species abundance among them. The findings indicate that both bacterial and archaeal species across all groups exhibited statistically significant differences in abundance ($P<0.05$), revealing environmentally specific adaptations of microorganisms in response to AMD contamination. Notably, even low-abundance taxa, including *Chloroflexi*, *Altiarchaeota*, and *Asgardarchaeota*, exhibited statistically significant differences among groups ($P<0.05$), suggesting that environmental pollution may also affect less abundant taxonomic groups.

Microbial community composition at phylum level

High-throughput sequencing revealed the distribution of dominant bacterial taxa in the water and sediments of the Pinglu River. The top 10 phyla, ranked by relative abundance, are depicted in Fig. 5a. Upstream water was dominated by *Proteobacteria* (49.0 ± 1.3%) and *Actinobacteriota* (30.5 ± 2.0%), while *Actinobacteriota* declined to 19.1 ± 2.7% downstream. AMD influx introduced *Campylobacterota*, which became dominant at the Yudong Spring outfall (55.0 ± 1.5%). In sediments, *Firmicutes* surged to 48.4 ± 5.6% at the outfall, contrasting with the decline of *Proteobacteria* (20.6 ± 1.8%), highlighting AMD-driven microbial restructuring.

Bacterial community composition and abundance differed significantly between water and sediments at the phylum level, demonstrating the substantial impact of abandoned coal mine drainage on microbial communities. A notable finding was the dominant presence of *Campylobacterota* at the Yudong Spring outfall, contrasting with the prevalence of *Acidobacteria* documented in other studies (Feng et al. 2023). Although investigations of this phylum in AMD environments are scarce, its potential role in the nitrogen cycle of karst systems has been suggested (Huang et al. 2021). We therefore propose that the extensive underground river networks in this typical karst terrain may harbor and enrich *Campylobacterota*, which are then discharged at the spring, leading to their observed dominance. The high relative abundance of *Firmicutes* in outfall sediments - a phylum known for pollution resilience - further indicates elevated contamination levels. Members of *Firmicutes* possess numerous metal resistance and reduction genes (Hesse et al. 2018), making them key bioindicators of metal pollution.

Both water and sediment samples exhibited similar bacterial community structures. *Proteobacteria* and *Actinobacteriota* dominated river water, whereas *Proteobacteria* and *Bacteroidota* were more abundant in sediments, consistent with previous findings (Feng et al. 2023). Members of *Proteobacteria*, primarily facultative or obligate aerobes (Rosselló-Mora et al. 1999), play crucial roles in carbon, nitrogen, and phosphorus cycling, contributing significantly to the self-purification of water and sediments (Basińska et al. 2020). *Actinobacteriota*, often associated with carbon and nitrogen cycling, thrive under neutral to alkaline conditions (Jia et al. 2020), which may explain their decline following AMD contamination. *Bacteroidota*, important in phosphorus cycling and organic matter degradation (Kozich et al. 2013; Rosselló-Mora et al. 1999), are also correlated with metal content and possess metal resistance genes (Hesse et al. 2018). The dominance of *Bacteroidota* and *Firmicutes* at the most polluted outfall supports the hypothesis proposed by Aoyagi et al. (2017) which suggests that their coexistence stimulates sulfate-reducing bacterial (SRB) metabolism and is vital in AMD remediation. This further underscores their importance in maintaining the stability of the microbial ecosystem under extreme environmental stress.

Archaeal community analysis revealed distinct phylum-level variations (Fig. 5b). Upstream water was dominated by *Crenarchaeota* (51.9 ± 3.2%) and *Halobacterota* (18.8 ± 8.3%), whereas *Thermoplasmatota* became prevalent at the AMD-impacted outfall (39.1 ± 6.3%) and increased downstream (50.5 ± 4.0%). Sediments exhibited similar trends, with *Halobacterota* peaking at the outfall (63.8 ± 3.3%) and remaining dominant downstream (46.0 ± 1.7%) .

At the phylum level, archaeal communities showed consistency across water and sediment samples. *Crenarchaeota*, a foundational lineage in archaeal phylogeny (Woese et al. 1990), maintained high relative abundance in both environments. These archaea likely play key roles in geochemical cycling (Spear et al. 2005; Stahl et al. 2012). Their prevalence in the Yudong River Basin, a karst-dominated region with complex hydrology, abundant groundwater, and mineral-rich subsurface connections, may reflect adaptations to inorganic nutrient fluxes.

Archaeal communities differed markedly between water and sediment samples. In water, *Thermoplasmatota* dominated at the outfall and downstream sites, consistent with its known acidophilic adaptations (Bargiela et al. 2023). This dominance highlights its tolerance to AMD-induced acidity. In contrast, *Halobacterota* were the predominant sediment archaea across all sampling sites, which is a key finding of this study. We propose that this dominance results from hypersaline microenvironments generated by the unique karst geology, which selectively favor halophilic archaea. Collectively, these results indicate that the distinctive geochemical background of karst systems drives unique ecological adaptations in microbial communities.

Microbial community composition at genus level

Genus-level analysis provides deeper insights into potential ecosystem functions(Fig. 6a). Both water and sediment samples exhibited high bacterial diversity but low relative abundances for most genera, with no dominant taxa observed except at the Yudong Spring outflow. The outflow water was characterized by a high abundance of *Sulfuricurvum* (54.6 ± 1.5%), a sulfur-oxidizing bacterium within the *Campylobacterota*, which plays a pivotal role in sulfate redox transformations, primary productivity, and the emission of volatile sulfur compounds in AMD environments (Whaley-Martin et al., 2023). In contrast, the sediment community was dominated by *Clostridium_sensu_stricto_1* (17.8 ± 2.1%) and *Terrisporobacter* (17.5 ± 2.8%), both belonging to the *Firmicutes*. *Terrisporobacter* is an acetogen capable of degrading diverse carbon sources (Yin and Wang, 2019), whereas *Clostridium_sensu_stricto_1* is associated with nitrogen compounds and linked to the nitrogen cycle (Yan et al., 2023; Zheng et al., 2022). The co-dominance of these taxa indicates that sulfur and nitrogen cycling are key microbial processes at this AMD discharge site.

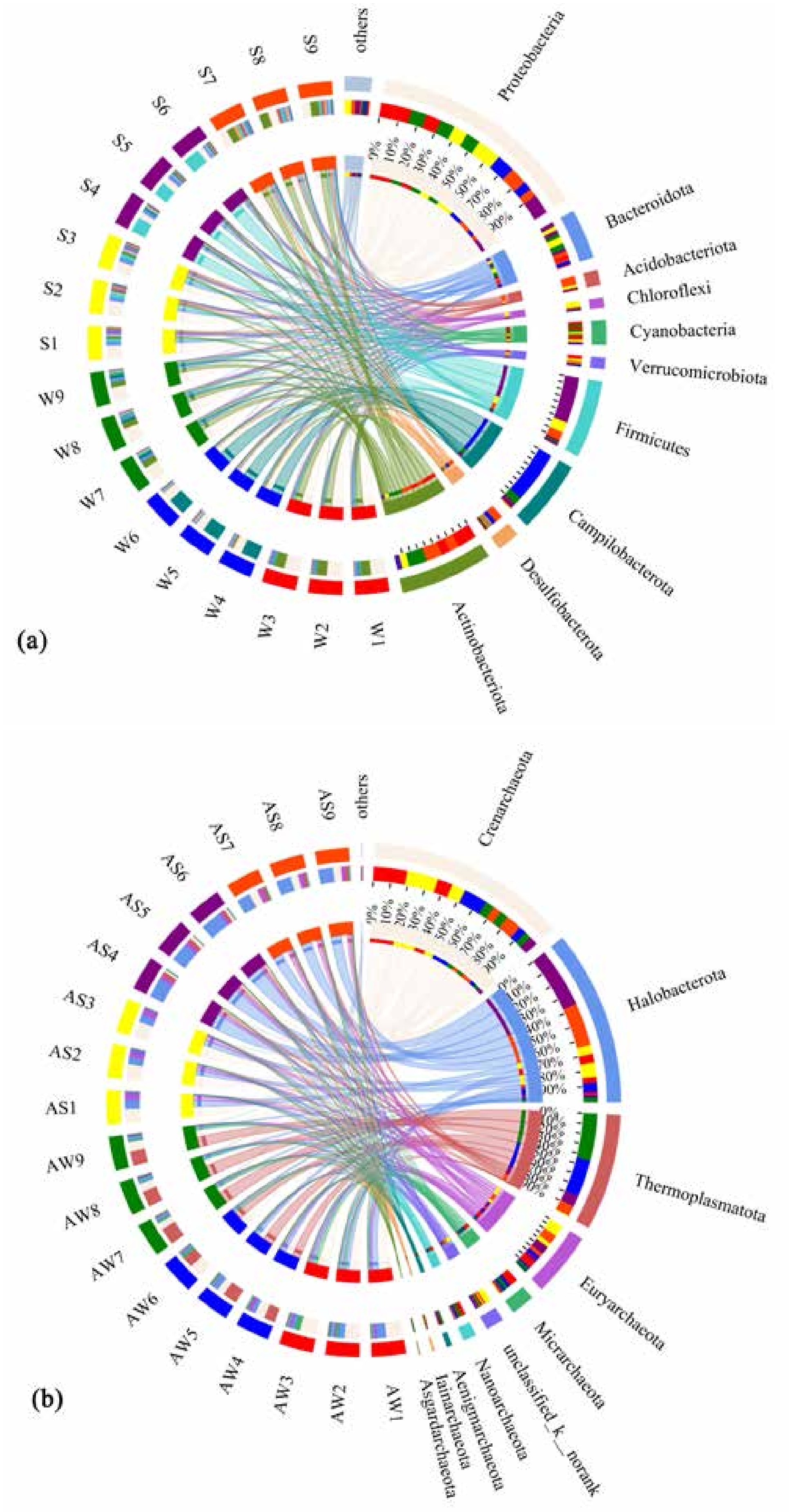

Fig. 5. Microbial community structure at the phylum level. (a) Bacterial phylum composition in water and sediments. (b) Archaeal phylum composition in water and sediments

At the genus level (Fig. 6b), archaeal relative abundances varied significantly across sites. The upstream river water harbored a diverse archaeal community dominated by *norank_f__Nitrososphaeraceae* (15.9 ± 2.2%), *Candidatus_ Nitrocosmicus* (11.6 ± 0.5%), and *Methanosarcina* (10.6 ± 4.1%). Similar trends were observed in upstream sediments, with relative abundances of 16.9 ± 1.6%, 22.1 ± 0.9%, and 16.0 ± 3.5%, respectively. Following AMD inflow, *norank__f__ norank__o___BSLdp215* became the dominant taxon in the Yudong Spring outfall (24.5 ± 6.5%) and downstream river water (43.2 ± 4.3%). The "norank" designation indicates sequences lacking taxonomic classification and warrants further functional studies. In contrast, *Methanocorpusculum* dominated outfall (46.2 ± 2.6%) and downstream sediments (28.1 ± 0.6%). The high abundance of this strictly anaerobic archaeon underscores the prevalence of anoxic conditions within the sediments and its potential role in carbon cycling under heavy metal stress.

Contrasting microbial communities in karst and non-karst AMD systems

Microbial communities in karst-associated AMD systems demonstrate distinctive ecological adaptations that contrast sharply with those in non-karst environments. While classical AMD systems, such as the Richmond Mine, are typically dominated by iron- and sulfur-oxidizing taxa like *Acidithiobacillus* and *Leptospirillum* under extreme acidic conditions (Teng et al. 2017), the Yudong karst system exhibits a unique microbial assemblage. In this system, *Campylobacterota*, particularly *Sulfuricurvum*, dominate waters at the discharge point, reaching relative abundances of 54.6 ± 1.5%. Sediments show a marked proliferation of *Firmicutes*, including *Clostridium* and *Terrisporobacter*, suggesting enhanced heterotrophic processes under carbonate-buffered conditions. The presence of carbonate rocks in karst rivers significantly alters heavy metal migration and transformation in AMD aquatic environments **(Qin et al. 2024)**, resulting in notable niche differentiation among archaeal communities. Within sediments, *Halobacterota* dominate (63.8 ± 3.3%), whereas *Thermoplasmatota* prevail in the aqueous phase. Despite these taxonomic distinctions, the core functional groups responsible for sulfur and iron transformation are conserved, which underscores that karst hydrogeological conditions, while selectively shaping microbial community structures, also sustain the fundamental biochemical functions of AMD systems.

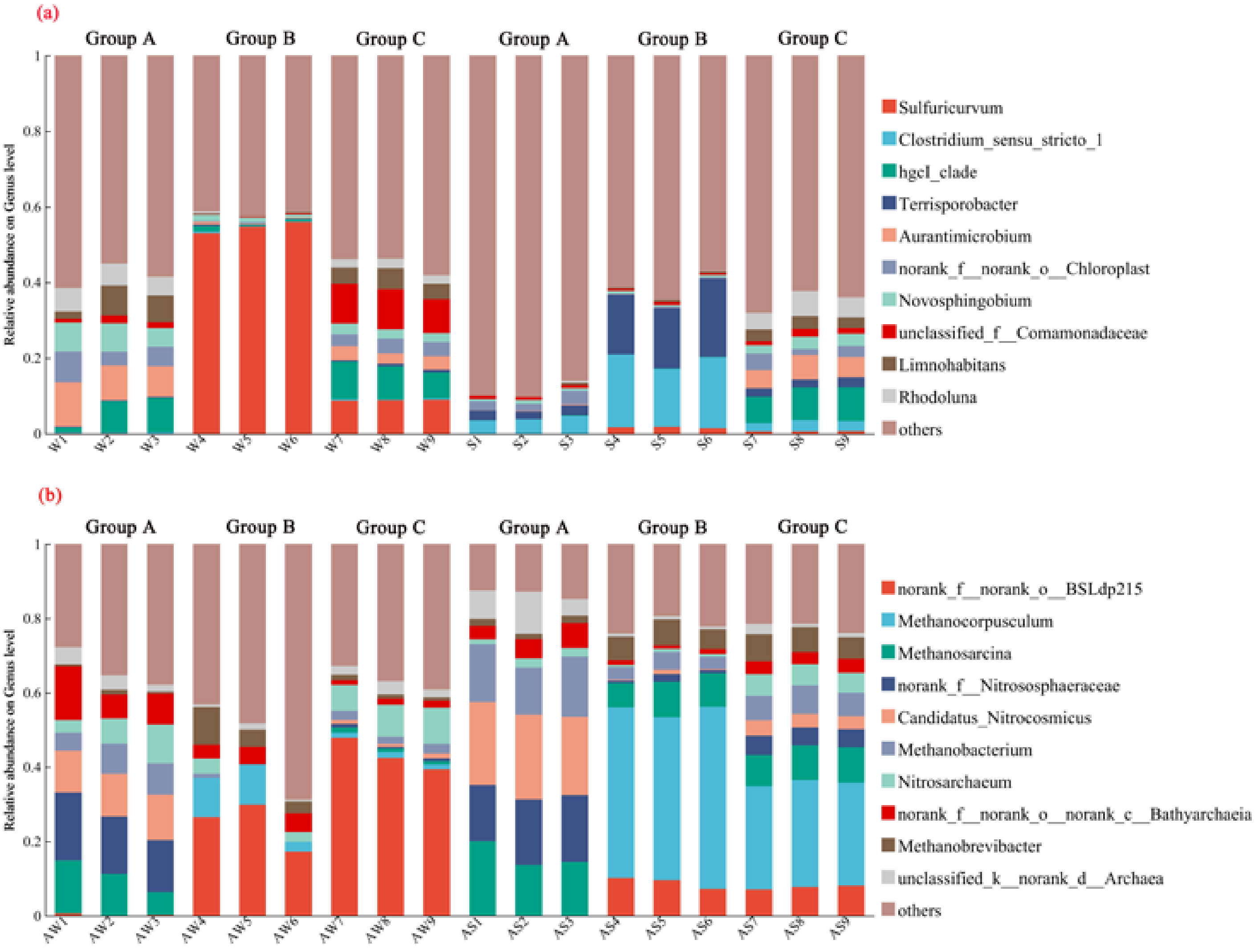

Fig. 6. Microbial community composition at the genus level. (a) Dominant bacterial genera in water and sediments. (b) Dominant archaeal genera in water and sediments

Influence of water quality index on microbial community composition

The relationship between water quality and microbial community

Microbial diversity is closely associated with environmental factor fluctuations. Mantel tests revealed significant distance-corrected relationships between environmental factors and microbial communities (Fig. 7). While the overall influence of environmental factors on riverine microbial communities remained consistent under varying conditions, distinct patterns emerged. Both planktonic (water) and benthic (sediment) bacterial communities showed strong positive correlations with all measured water parameters ($r > 0.59$, $p < 0.01$), particularly with sulfate and pH ($r > 0.84$, $p < 0.01$). Archaeal communities in water and sediments similarly showed strong correlations with all environmental factors ($r > 0.7$, $p \leq 0.01$), with exceptionally high correlations observed for NH_3-N, Fe, Mn, and COD ($r > 0.96$, $p < 0.01$).

Mantel tests confirmed a strong correlation between overall microbial community structure and environmental variables. This statistically robust relationship indicates that AMD-induced changes in water quality are the primary drivers of microbial shifts. Specifically, following AMD discharge into the river, the abundance of microorganisms

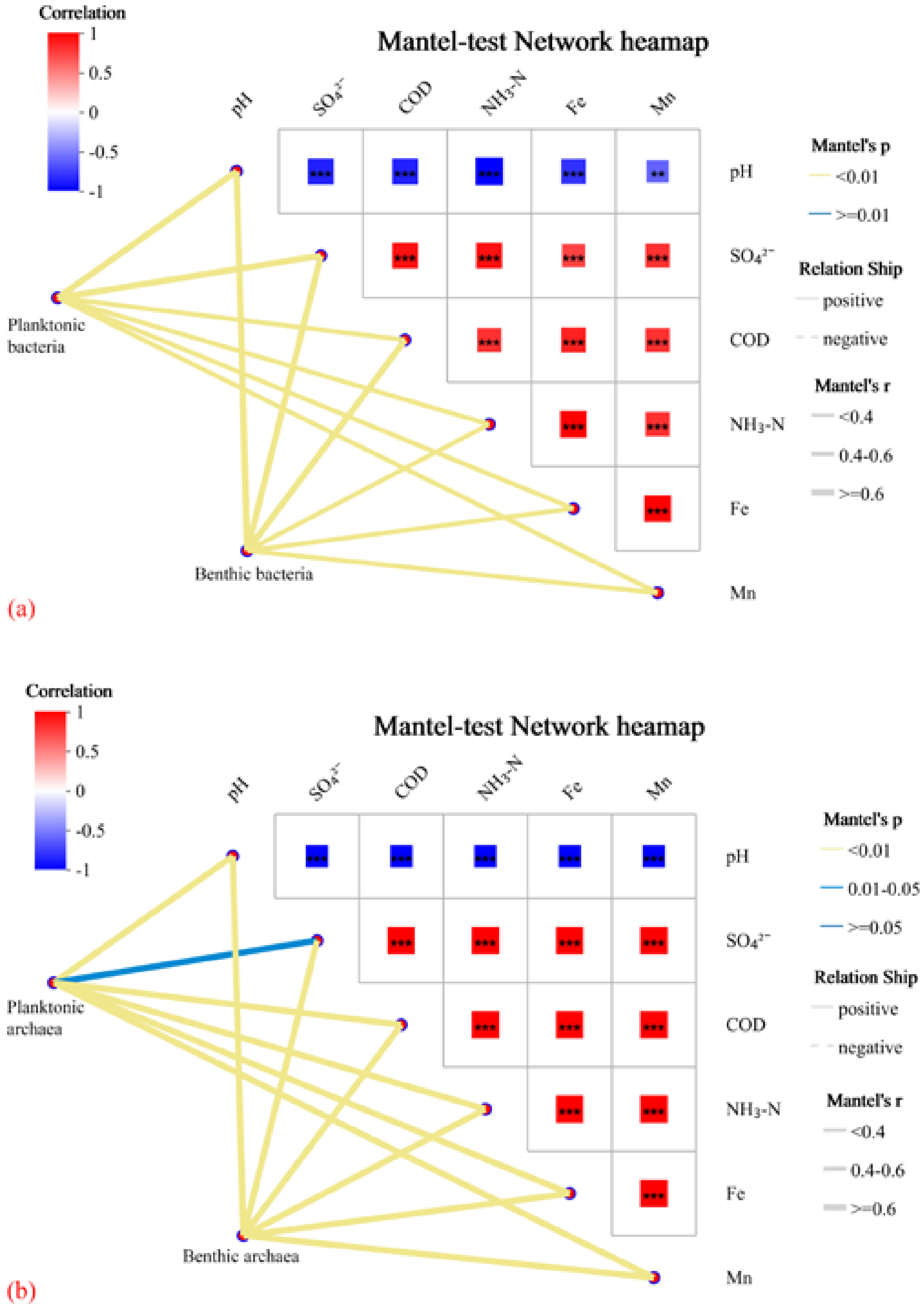

Fig. 7. Mantel test analysis of microbial community-environment interactions. (a) Bacterial communities; (b) Archaeal communities. (Lines indicate community-environment factor correlations; heatmaps show inter-factor correlations. Significance levels: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ (Mantel's $|r|$))

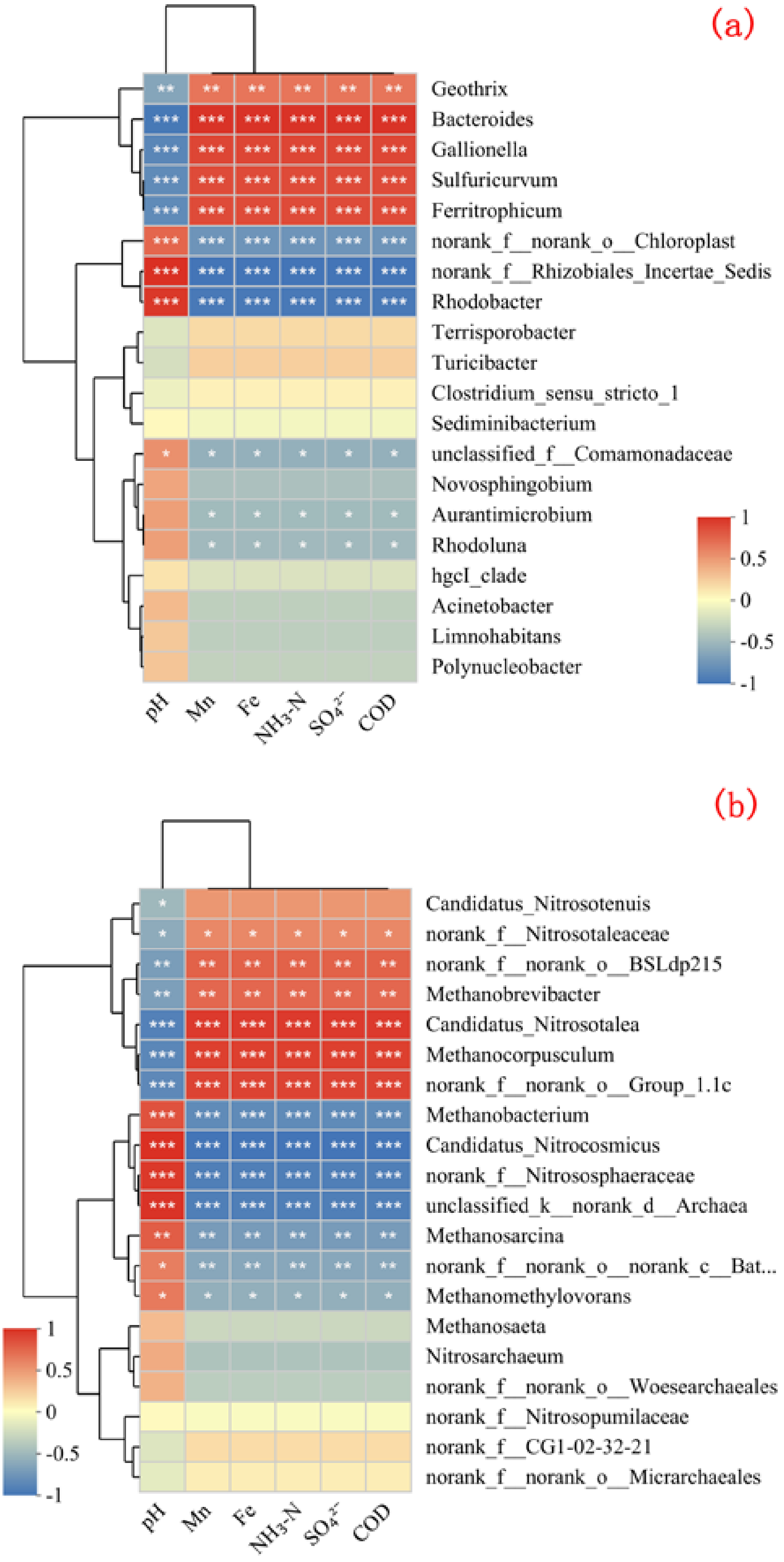

Fig. 8. Spearman's correlation heatmap of environmental factors with genus level (a) bacteria (b) archaea (different colours denote R-values, * denotes P ≤ 0.05, ** denotes P ≤ 0.01, *** denotes P ≤ 0.001)

capable of processing the introduced contaminants increases, thereby helping maintain ecosystem stability under elevated pollution loads.

The effects of water quality parameters on bacterial and archaeal communities

Spearman's correlation heatmap (Fig. 8) illustrates the relationships between the top 20 planktonic and benthic microbial genera (by abundance) and river physicochemical parameters. Environmental factors were identified as pivotal drivers of microbial composition ($p < 0.05$). Among bacteria, *Sulfuricurvum*, *Ferritrophicum*, *Gallionella*, *Bacteroides*, and among archaea, *Candidatus_ Nitrosotalea*, *Methanocorpusculum*, *norank_f__norank_o__ Group_1.1c*, *norank_f__norank_o__BSLdp215* showed strong positive correlations with Fe, Mn, SO_4^{2-}, NH_3-N, and COD ($r > 0.65$), but negative correlations with pH ($r < -0.6$). In contrast, *norank_f__Rhizobiales_Incertae_ Sedis*, *Rhodobacter*, chloroplast-related bacterial groups, *Methanobacterium*, *Candidatus_Nitrocosmicus*, *norank__f__ Nitrososphaeraceae*, and unclassified*__d__Archaea* exhibited opposite trends, correlating negatively with pollutants but positively with pH.

This study highlights the high sensitivity of aquatic microbial communities to environmental changes, identifying AMD as the primary driver of microbial restructuring. Significant alterations in microbial diversity induced by AMD underscore its potential as a robust bioindicator for assessing environmental quality in the Yudong River Basin. AMD influx drastically lowers pH and elevates concentrations of heavy metals and sulfate. In response, *Sulfuricurvum*, a sulfur-oxidizing chemoautotroph, exhibited the most pronounced response, dominating the AMD-impacted Yudong Spring outflow, consistent with elevated SO_4^{2-} concentrations (Giordani et al. 2019). Furthermore, the oxidation of Fe^{2+} and subsequent formation of $Fe(OH)_3$ precipitates coincided with significant enrichment of *Clostridium_sensu_stricto_1* in sediments, a genus positively correlated with Fe. This organism can directly reduce Fe^{3+} or indirectly support other Fe^{3+} reducers by supplying substrates such as readily degradable organic acids (Fan et al. 2018). Consequently, the abundance of these key taxa can serve as proxies for predicting environmental contamination levels. These microbial shifts also have profound implications for ecosystem stability: acid- and metal-tolerant taxa dominated at the discharge point, while microorganisms negatively correlated with water quality indicators were more prevalent upstream and downstream. Therefore, system stability is maintained not by a static community composition but through the dynamic reorganization of dominant species along pollution gradients.

Limitations and future prospects

This study has several limitations, reflecting challenges common to similar research. First, the karst geology of the study area, marked by fissures, caves, and subterranean rivers, complicates the tracing AMD sources. Although Yudong Spring was identified as the primary drainage outlet for nearby closed coal mines, the exact origin of AMD remains unresolved due to inaccessible subsurface pathways. Second, the influence of dissolved organic matter (DOM) on microbial communities was not assessed using advanced analytical tools, leaving DOM-microbe interactions unexplored. Third, the analysis focused solely on microbial taxonomic structure, without considering the distribution of functional genes.

Future studies should employ multi-spatiotemporal sampling and high-throughput sequencing to capture microbial community dynamics across seasonal and spatial gradients. Integrating geochemical modeling with multi-omics approaches could further clarify the linkages between microbial functions and AMD-driven environmental stressors.

Conclusion

This study demonstrates that AMD from abandoned coal mines significantly degrades water quality and reshapes microbial communities in the karst Yudong River Basin. Extreme acidification (pH 3.22±0.04) and elevated pollutant concentrations (Fe, Mn, SO_4^{2-}) were observed at discharge points, with partial recovery downstream. Microbial diversity sharply declined near AMD inputs, with *Campylobacterota* dominating acidic water (55.0 ± 1.5%) and *Firmicutes* prevailing in sediments (48.4 ± 5.6%), reflecting habitat-specific adaptations to metal stress. Strong genus-level correlations ($r > 0.65$) with pollutants were observed, particularly for *Sulfuricurvum* (sulfur cycling) and *Clostridium_sensu_ stricto_1* (iron/nitrogen cycling), underscoring their roles in elemental transformations. Archaeal communities exhibited niche partitioning, with *Thermoplasmatota* dominating acidic waters and *Halobacterota* thriving in saline sediments, highlighting microenvironmental specialization. While these findings reveal the ecological resilience of specialized taxa, their bioremediation potential requires validation through functional assays. This study enhances our understanding of microbial responses to AMD in karst systems but identifies critical knowledge gaps, particularly regarding seasonal dynamics and metabolic pathways. Future research should integrate metagenomics and geochemical modeling to elucidate microbial metabolic networks and optimize remediation strategies for mining-impacted karst waterways.

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References

Acharya, B.S. & Kharel, G. (2020). Acid mine drainage from coal mining in the United States–An overview. *Journal of Hydrology*, 588, 125061. DOI:10.1016/j.jhydrol.2020.125061

Aoyagi, T., Hamai, T., Hori, T., Sato, Y., Kobayashi, M., Sato, Y., Inaba, T., Ogata, A., Habe, H. & Sakata, T. (2017). Hydraulic retention time and pH affect the performance and microbial communities of passive bioreactors for treatment of acid mine drainage. *Amb Express*, 7(1), 142. DOI:10.1186/s13568-017-0440-z

Bargiela, R., Korzhenkov, A.A., McIntosh, O.A., Toshchakov, S.V., Yakimov, M.M., Golyshin, P.N. & Golyshina, O.V. (2023). Evolutionary patterns of archaea predominant in acidic environment. *Environmental Microbiome*, 18(1), 61. DOI:10.1186/s40793-023-00518-5

Basińska, A.M., Reczuga, M.K., Gąbka, M., Strózecki, M., Luców, D., Samson, M., Urbaniak, M., Lesny, J., Chojnicki, B.H., Gilbert, D., Sobczynski, T., Olejnik, J., Silvennoinen, H., Juszczak, R. & Lamentowicz, M. (2020). Experimental warming and precipitation reduction affect the biomass of microbial communities in a Sphagnum peatland. *Ecological Indicators*, 112, 106059. DOI:10.1016/j.ecolind.2019.106059

Bu, C., Li, X., Li, Q., Li, L. & Wu, P. (2024). Spatiotemporal distributions, sources, and health risks of heavy metals in an acid mine drainage (AMD)-contaminated karst river in southwest China. *Applied Water Science*, 14(12), 251. DOI:10.1007/s13201-024-02317-w

Chen, D., Feng, Q. & Liang, H. (2021). Effects of long-term discharge of acid mine drainage from abandoned coal mines on soil microorganisms: Microbial community structure, interaction patterns, and metabolic functions. *Environmental Science and Pollution Research*, 28(38), pp. 53936-53952. DOI:10.1007/s11356-021-14566-2

Chen, D., Zhang, Y., & Feng, Q. (2023). Hydrochemical characteristics and microbial community evolution of Pinglu River affected by regional abandoned coal mine drainage, Guizhou Province, China. *Environmental Science and Pollution Research*, 30(27), pp. 70671-70687. DOI: 10.1007/s11356-023-27403-5

Fan, X., Ding, S., Gong, M., Chen, M., Gao, S., Jin, Z. & Tsang, D.C. (2018). Different influences of bacterial communities on Fe (III) reduction and phosphorus availability in sediments of the *Cyanobacteria*-and macrophyte-dominated zones. *Frontiers in microbiology*, 9, 2636. DOI:10.3389/fmicb.2018.02636

Feng, L., Zhang, Z., Yang, G., Wu, G., Yang, Q. & Chen, Q. (2023). Microbial communities and sediment nitrogen cycle in a coastal eutrophic lake with salinity and nutrients shifted by seawater intrusion. *Environmental Research*, 225, 115590. DOI:10.1016/j.envres.2023.115590

Giordani, A., Rodriguez, R.P., Sancinetti, G.P., Hayashi, E.A., Beli, E. & Brucha, G. (2019). Effect of low pH and metal content on microbial community structure in an anaerobic sequencing batch reactor treating acid mine drainage. *Minerals Engineering*, 141, 105860. DOI:10.1016/j.mineng.2019.105860

Han, X., Schubert, C.J., Fiskal, A., Dubois, N. & Lever, M.A. (2020). Eutrophication as a driver of microbial community structure in lake sediments. *Environmental microbiology*, 22(8), pp. 3446-3462. DOI:10.1111/1462-2920.15115

Hesse, E., O’Brien, S., Tromas, N., Bayer, F., Luján, A.M., van Veen, E.M., Hodgson, D. & Buckling, A. (2018). Ecological selection of siderophore-producing microbial taxa in response to heavy metal contamination. *Ecology letters,* 21(1), pp. 117-127. DOI:10.1111/ele.12878

Huang, L., Bae, H., Young, C., Pain, A.J., Martin, J.B. & Ogram, A. (2021). *Campylobacterota* dominate the microbial communities in a tropical karst subterranean estuary, with implications for cycling and export of nitrogen to coastal waters. *Environmental Microbiology*, 23(11), pp. 6749-6763. DOI:10.1111/1462-2920.15746

Jia, B., Li, Y., Zi, X., Gu, X., Yuan, H., Jeppesen, E. & Zeng, Q. (2023). Nutrient enrichment drives the sediment microbial communities in Chinese mitten crab Eriocheir sinensis culture. *Environmental Research*, 223, 115281. DOI:10.1016/j.envres.2023.115281

Jia, L., Liu, H., Kong, Q., Li, M., Wu, S. & Wu, H. (2020). Interactions of high-rate nitrate reduction and heavy metal mitigation in iron-carbon-based constructed wetlands for purifying contaminated groundwater. *Water research*, 169, 115285. DOI:10.1016/j.watres.2019.115285

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. & Schloss, P.D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and environmental microbiology*, 79(17), pp. 5112-5120. DOI:10.1128/AEM.01043-13

Li, J., Wang, L., Wu, B., Wang, J., Yu, Y., Kuzyakov, Y., Ding, S. & Xu, X. (2025). Convergence and divergence of microbial communities in river-Qinghai lake sediment continuum on Tibetan Plateau. *Water Research*, 282, 123757. DOI:10.1016/j.watres.2025.123757

Li, X., Ren, H., Xu, Z., Chen, G., Zhang, S., Zhang, L. & Sun, Y. (2023). Practical application for legacy acid mine drainage (AMD) prevention and treatment technologies in karst-dominated regions: A case study. *Journal of Contaminant Hydrology,* 258, 104238. DOI:10.1016/j.jconhyd.2023.104238

Lukhele, T., Selvarajan, R., Nyoni, H., Mamba, B.B. & Msagati, T.A.M. (2019). Diversity and functional profile of bacterial communities at Lancaster acid mine drainage dam, South Africa as revealed by 16S rRNA gene high-throughput sequencing analysis. *Extremophiles*, 23, pp. 719-734. DOI:10.1007/s00792-019-01130-7

Ma, L., Banda, J.F., Wang, Y., Yang, Q., Zhao, L., Hao, C. & Dong, H. (2024). Metagenomic insight into the acidophilic functional communities driving elemental geochemical cycles in an acid mine drainage lake. *Journal of Hazardous Materials*, 466, 133070. DOI:10.1016/j.jhazmat.2023.133070

Malá, J., Hübelová, D., Schrimpelová, K., Kozumplíková, A. & Lejska, S. (2022). Surface watercourses as sources of karst water pollution. *International Journal of Environmental Science and Technology*, 19(5), 3503-3512. DOI: 10.1007/s13762-021-03440-w

Naghoum, I., Edahbi, M., Melián, J.A.H., Doña Rodriguez, J.M., Durães, N., Pascual, B.A. & Salmoun, F. (2025). Passive Treatment of Acid Mine Drainage Effluents Using Constructed Wetlands: Case of an Abandoned Iron Mine, Morocco. *Water,* 17(5)*,* 687. DOI:10.3390/w17050687

Qin, S., Li, X., Huang, J., Li, W., Wu, P., Li, Q. & Li, L. (2024). Inputs and transport of acid mine drainage-derived heavy metals in karst areas of Southwestern China. *Environmental Pollution*, 343*,* 123243. DOI:10.1016/j.envpol.2023.123243

Rosselló-Mora, R., Thamdrup, B., Schäfer, H., Weller, R. & Amann, R. (1999). The response of the microbial community of marine sediments to organic carbon input under anaerobic conditions. *Systematic and Applied Microbiology*, 22(2), pp. 237-248. DOI:10.1016/S0723-2020(99)80071-X

Shang, Y., Wu, X., Wei, Q., Dou, H., Wang, X., Chen, J., Zhang, H., Ma, S. & Zhang, H. (2020). Total arsenic, pH, and sulfate are the main environmental factors affecting the microbial ecology of the water and sediments in Hulun Lake, China. *Frontiers in Microbiology*, 11, 548607. DOI:10.3389/fmicb.2020.548607

Spear, J.R., Walker, J.J., McCollom, T.M. & Pace, N.R. (2005). Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. *Proceedings of the National Academy of Sciences of the United States of America*, 102(7), pp. 2555-2560. DOI:10.1073/pnas.0409574102

Stahl, D.A. & De La Torre, J.R. (2012). Physiology and diversity of ammonia-oxidizing archaea. *Annual review of microbiology*, 66(1), pp. 83-101. DOI:10.1146/annurev-micro-092611-150128

Sun, W., Xiao, T., Sun, M., Dong, Y., Ning, Z., Xiao, E., Tang, S. & Li, J. (2015). Diversity of the sediment microbial community in the Aha watershed (Southwest China) in response to acid mine drainage pollution gradients. *Applied and Environmental Microbiology*, 81(15), 4874-4884. DOI:10.1128/AEM.00935-15

Teng, W., Kuang, J., Luo, Z. & Shu, W. (2017). Microbial diversity and community assembly across environmental gradients in acid mine drainage. *Minerals*, 7(6), 106. DOI:10.3390/min7060106

Wang, M., Wang, X., Zhou, S., Chen, Z., Chen, M., Feng, S., Li, J., Shu, W. & Cao, B. (2023). Strong succession in prokaryotic association networks and community assembly mechanisms in an acid mine drainage-impacted riverine ecosystem. *Water Research,* 243, 120343. DOI:10.1016/j.watres.2023.120343

Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. A*pplied and environmental microbiology,* 73(16), pp. 5261-5267. DOI:10.1128/AEM.00062-07

Whaley-Martin, K.J., Chen, L.X., Nelson, T.C., Gordon, J., Kantor, R., Twible, L.E., Marshall, S., McGarry, S., Rossi, L., Bessette, B., Baron, C., Apte, S., Banfield, J.F. & Warren, L.A. (2023). O_2 partitioning of sulfur oxidizing bacteria drives acidity and thiosulfate distributions in mining waters. *Nature Communications*, 14(1), 2006. DOI:10.1038/s41467-023-37426-8

Woese, C.R., Kandler, O. & Wheelis, M.L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America*, 87(12), pp. 4576-4579. DOI:10.1073/pnas.87.12.4576

Yan, P., Gu, X., Peng, Y., Fan, Y., Zhang, M., Sun, S. & He, S. (2023). Insight into the nutrient change in freshwater ecosystem under ferrous addition: Revealed by phosphorus, nitrogen, and microbial community. *Journal of Cleaner Production*, 433, 139874. DOI:10.1016/j.jclepro.2023.139874

Yin, Y. & Wang, J. (2019). Enhanced biohydrogen production from macroalgae by zero-valent iron nanoparticles: Insights into microbial and metabolites distribution. *Bioresource technology*, 282, pp. 110-117. DOI:10.1016/j.biortech.2019.02.128

Zhang, W., Gu, J., Li, Y., Lin, L., Wang, P., Wang, C., Qian, B., Wang, H., Niu, L., Wang, L., Zhang, H., Gao, Y., Zhu, M. & Fang, S. (2019a). New insights into sediment transport in interconnected river–lake systems through tracing microorganisms. *Environmental science & technology,* 53(8), pp. 4099-4108. DOI:10.1021/acs.est.8b07334

Zhang, X., Tang, S., Wang, M., Sun, W., Xie, Y., Peng, H., Zhong, A., Liu, H., Zhang, X., Yu, H., Giesy, J.P. & Hecker, M. (2019b). Acid mine drainage affects the diversity and metal resistance gene profile of sediment bacterial community along a river. *Chemosphere*, 217, pp. 790-799. DOI:10.1016/j.chemosphere.2018.10.210

Zheng, Y., Sun, Z., Liu, Y., Cao, T., Zhang, H., Hao, M., Chen, R., Dzakpasu, M. & Wang, X. (2022). Phytoremediation mechanisms and plant eco-physiological response to microorganic contaminants in integrated vertical-flow constructed wetlands. *Journal of Hazardous Materials*, 424, 127611. DOI:10.1016/j.jhazmat.2021.127611

Zou, S., Liu, P., Wang, J., Lu, H., Zhang, Y., Wang, Y. & Li, B. (2025). Characterizing and tracing the heavy metals' spatial distribution in karst surface river affected by acid mine drainage. *Environmental Engineering Research,* 30(2), pp. 144-159. DOI:10.4491/eer.2024.180