Environmental Technology & Innovation 41 (2026) 104815

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Quantitative profiling of bacterial community restructuring and functional potential variation across upstream and downstream sediments of the Three Gorges Dam

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ARTICLE INFO

Keywords:
Three Gorges Dam
Bacterial communities
Quantitative microbiome profiling
Functional genes
Sediment

ABSTRACT

Although numerous studies have shown that dam construction alters microbial communities in aquatic ecosystems, most of these findings are based on relative microbiome profiling, which yields only compositional rather than absolute information. To address this limitation, we employed quantitative microbiome profiling on sediments collected from upstream (GDK, XXH, MH) and downstream (HLM, NJG) sites of the Three Gorges Dam to evaluate bacterial community and functional responses 21 years after dam operation commenced. Downstream sediments exhibited significantly lower levels of moisture, total nitrogen (TN), dissolved organic carbon (DOC), cation exchange capacity (CEC), and available potassium (AK) compared with upstream sediments ($p < 0.001$). These environmental changes were associated with a 56.49–64.45 % reduction in bacterial abundance ($p < 0.001$). α-diversity declined significantly, along with 88.16 % of ASVs, predominantly within Pseudomonadota (formerly Proteobacteria). Furthermore, overall bacterial community composition differed markedly between upstream and downstream sediments. Pseudomonadota dominated upstream communities (~2.32×10^{11} copies), whereas Desulfobacterota was most abundant downstream (~7.00×10^{10} copies). Our results indicated marked declines in the abundances of functional genes associated with inferred functional potential for carbon, nitrogen, sulfur, and phosphorus cycling in downstream sediments. Specifically, genes involved in carbon (e.g., carbohydrate degradation), nitrogen (e.g., nitrification, denitrification), sulfur (e.g., dissimilatory sulfate reduction), and phosphorus (e.g., purine metabolism, which involves organic phosphorus in nucleotides) cycling decreased significantly ($p < 0.001$). These findings suggest that dam construction may trigger cascading effects, in which reduced moisture and nutrient availability are associated with decreased

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https://doi.org/10.1016/j.eti.2026.104815
Received 8 December 2025; Received in revised form 28 January 2026; Accepted 10 February 2026
Available online 11 February 2026

microbial abundance, simplified community structure, and diminished biogeochemical functional potential.

1. Introduction

Reservoirs play multiple roles within watershed systems, including regulating water resources, controlling floods, enabling irrigation and water supply, generating power, and facilitating navigation (Null et al., 2024; Scola et al., 2014). As major infrastructures in river basins, they profoundly influence hydrological regimes and aquatic ecological processes. The Three Gorges Dam (TGD), located in the upper and middle reaches of the Yangtze River, is one of the world's largest hydroelectric projects (Guo et al., 2012). Since its construction, the TGD has substantially modified downstream flow regimes and sediment transport patterns, leading to cascading effects on nutrient dynamics and ecosystem functioning (Yang et al., 2007; Zhou et al., 2016). Sediments are essential to the ecological integrity of river systems, serving both as habitats for microbial life and as active zones for biogeochemical cycling. River sediments harbor highly diverse microbial communities and serve as critical sinks and processors for organic matter and nutrients (Chen et al., 2016; Wang et al., 2021, 2020a). Through processes such as adsorption-desorption, dissolution, resuspension, and microbial degradation, sediments regulate pollutant fate and drive essential nutrient transformations at the sediment-water interface (Gao et al., 2024; Pu et al., 2023; Yi et al., 2021). Consequently, understanding how dam-induced environmental changes impact sediment microbial communities is crucial for evaluating their implications for ecosystem functions.

Bacterial communities are a key component of sediment microbiota and are highly responsive to environmental changes, making them important indicators of ecosystem variation (Li et al., 2024; Tang et al., 2024; Wang et al., 2023). For example, in freshwater sediments of Taihu Lake, bacteria respond more strongly than fungi to variations in pH, organic carbon, and nitrogen (Wang et al., 2023). However, despite their recognized environmental sensitivity, the impacts of damming on sediment bacterial diversity remain unclear and actively debated. Some studies have observed increased bacterial α-diversity associated with reduced sediment grain size following dam construction (Wu et al., 2013), whereas others have reported pronounced downstream declines, possibly linked to nutrient depletion or habitat disruption (Liu et al., 2018). Such contradictory findings may stem from differences in sampling locations, hydrological regimes, and sediment physicochemical heterogeneity, underscoring the need to elucidate the mechanisms driving bacterial community responses to damming. Beyond altering community α-diversity, dam-induced reductions in nutrient inputs and hydrodynamic energy can also limit bacterial biomass. For instance, phospholipid fatty acid (PLFA) analyses have shown marked downstream declines in bacterial biomarker concentrations in sediments of the Three Gorges Dam reservoir (Sun et al., 2018). Concurrently, alterations in sediment physicochemical properties (e.g., sediment organic carbon, pH, grain size) have been associated with pronounced taxonomic turnover in bacterial communities. In large river systems such as the Yangtze and Lancang, upstream sediments are often dominated by Pseudomonadota, Bacteroidetes, and Firmicutes, whereas downstream communities exhibit higher relative abundances of Actinobacteria, Planctomycetes, and Cyanobacteria (Liu et al., 2018; Luo et al., 2020). However, these insights are largely derived from relative microbiome profiling (RMP) techniques, including amplicon and shotgun metagenomic sequencing, which may not capture true changes in bacterial abundance. Given that methodological constraints can obscure or distort ecological interpretations, it is critical to evaluate how data type and analytical approaches influence our understanding of sediment bacterial responses to damming.

RMP data are inherently compositional, meaning each taxon's abundance is constrained by the total read count within a sample, yielding only relative proportions (Gloor et al., 2017; Lin and Peddada, 2020). This compositional effect introduces spurious correlations: an apparent increase in one taxon may reflect decreases in others rather than a true ecological signal (Hardwick et al., 2018; Vandeputte et al., 2017). Thus, many reported associations between environmental parameters (e.g., organic carbon, TN, pH) and bacterial groups may be overestimated or misinterpreted, as they often rely on relative abundance data that can mask underlying dynamics. For instance, positive correlations between organic carbon and certain taxa, such as α-/γ-Proteobacteria or Bacteroidetes—which have been widely documented in aquatic microbial studies (Kunihiro et al., 2011; Sinkko et al., 2013; Yang et al., 2025; Zhang et al., 2015)—could instead be influenced by declines in other microbial groups. To address these limitations, quantitative microbiome profiling (QMP) has emerged as a powerful approach that incorporates absolute abundance data derived from microbial biomass estimations (e.g., internal standards) (Tkacz et al., 2018; Wang et al., 2024, 2020b). Unlike RMP, QMP enables accurate assessment of microbial abundance and reveals real compositional and functional shifts in microbial communities (Knight et al., 2018; Wang et al., 2024). Despite technical challenges, QMP has demonstrated greater sensitivity in detecting microbial responses to environmental perturbations, making it particularly relevant for sediment systems where nutrient availability and biomass are closely linked (Xiao et al., 2025).

In this study, we applied QMP to investigate the responses of sediment bacterial communities to dam-induced environmental changes in the Three Gorges Reservoir region. We collected sediment samples from five upstream and downstream sites at two depth intervals (0–10 cm and 10–20 cm) to address three fundamental questions: 1) How does bacterial abundance and diversity respond to TGD-induced environmental changes? Given nutrient depletion in downstream sediments (Yang et al., 2007; Zhou et al., 2016), we hypothesized a significant decline in bacterial abundance and α-diversity. 2) Which environmental factors (e.g., TN, pH, DOC) govern the absolute abundance and assembly of dominant bacterial taxa? We expected sediment organic carbon and DOC to be major drivers, alongside pH. 3) How are the abundances of genes associated with major bacterial functions, particularly those involved in carbon, nitrogen, and phosphorus cycling, affected by shifts in bacterial abundance and composition? We predicted that the abundances of genes predominantly associated with heterotrophic microorganisms (e.g., those involved in carbohydrate degradation, nitrification,

denitrification, and purine metabolism) would show the most pronounced decline under nutrient-limited conditions. By integrating QMP with functional metagenomic analysis, our study aims to elucidate the true ecological responses of sediment microbiomes to large-scale hydrological engineering.

2. Materials and methods

2.1. Site characteristics and sampling

Sediment samples were collected in 2024 from five sites along the Yangtze River, upstream and downstream of the Three Gorges Dam (110°18'–111°16' E, 30°45'–31°02' N; Fig. S1). This sampling was conducted 21 years after the dam began full operation in 2003. As the world's largest hydroelectric dam, it impounds a reservoir extending approximately 700 km along the river's mainstem and covers about 632 km^2 of flooded area. The study sites experience a mean annual temperature of 16.6–17.9 °C and precipitation of 1200–1535 mm (Table S1). Sampling was conducted during May–June 2024 at three upstream locations—Guandukou (GDK), Xiangxi River (XXH), Miao River (MH)—and two downstream locations—Huangling Temple (HLM), Nanjinguan (NJG). The sampling water depths varied between sites, with upstream reservoir sites (GDK, XXH, and MH) ranging from 80 to 100 m and downstream sites (HLM and NJG) ranging from 25 to 30 m (Table S1). The different water depths may result in varying hydrostatic pressures between sites, which could potentially influence sediment properties. At each site, five sediment cores (approximately 20 cm deep and 5 cm in diameter, yielding ~100 g per section) were collected along a 50-m transect with cores spaced ~10 m apart to ensure spatial representation. The cores were immediately sectioned on-site into 0–10 cm (surface) and 10–20 cm (subsurface) layers, resulting in five replicates per layer and a total of 10 samples per site (50 samples overall). Each section was then split into two subsamples: one was transported to the laboratory for air-drying and stored at 4 °C for subsequent physicochemical analyses; the other was placed in sterile tubes, flash-frozen on-site using liquid nitrogen, and transferred to −80 °C storage upon return to the laboratory for microbial DNA extraction.

2.2. Physicochemical analysis

Sediment pH was measured at a 1:2.5 sediment-to-water ratio using a ST3100/F pH meter (USA) (Sun et al., 2018). Moisture content was determined gravimetrically (wet basis). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) were extracted with 2 M KCl and quantified using a Skalar SAN Plus segmented flow analyzer (Skalar, the Netherlands). Total nitrogen (TN) was measured by the Kjeldahl method. Sediment organic carbon (SOC) was determined via potassium dichromate oxidation, and dissolved organic carbon (DOC) using a Shimadzu TOC analyzer (Japan) (Nelson and Sommers, 1996). Total phosphorus (TP) was quantified using sodium carbonate fusion followed by molybdenum–antimony colorimetric analysis, while available phosphorus (AP) was determined by the Olsen method. Total potassium (TK) and available potassium (AK) were assessed via flame photometry after sodium hydroxide and ammonium acetate extraction, respectively. Cation exchange capacity (CEC) was measured using 1 M ammonium acetate at pH 7.0.

2.3. DNA extraction and quantitative microbiome profiling (QMP)

DNA was extracted from all 50 sediment samples. Briefly, well-mixed sediment samples (0.6 g) were analyzed using the Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quality of DNA extracts was determined by spectrophotometry (OD-1000 +, OneDrop Technologies, China). DNA extracts were considered of sufficient quality if the ratio of OD260 to OD280 (optical density, OD) and the ratio of OD260 to OD230 were approximately 1.8. All eligible DNA samples were stored at −80°C.

Nine synthetic DNA sequences, distinct from natural microbial gene sequences, were designed and synthesized by GeneCowin Biotechnology Inc. (Shanghai, China). These sequences were grouped into four concentration gradients (differing by 10-fold each) based on their copy number content. DNA extracts were spiked with internal standard spike-in DNA (GeneCowin, China) at 0.1 % total DNA by mass. DNA mixed with internal standard spike-in DNA was sheared using the Covaris® system (Covaris, USA). DNA libraries were prepared using a ligation-dependent method with a DNA library preparation kit (GeneCowin, China). The 200–600 bp range of sequencing libraries was size-selected and recovered using MagicPure® Size Selection DNA Beads (Transgen, Beijing). DNA libraries were qualified on the Agilent 2100 Bioanalyzer (Agilent, USA) and quantified with the Kapa Library Quantification kit (Kapa Biosystems, Wilmington, MA) using the ABI 9700HT Fast Real-Time PCR system (Thermo Fisher Scientific). Libraries were sequenced on the NovaSeq 6000 platform (Illumina USA) and generated 10 G bps of 2 × 150 bp pair-end reads.

Raw reads were filtered using fastp (version 0.23.2) (Chen et al., 2018b) software to remove (1) sequences containing > 1 N bases, (2) sequences with a Phred score of ≥ 20<60 %, (3) sequences containing residual sequencing adapters/barcodes, and (4) sequences with length < 100 bp. Clean reads were subsequently filtered to remove sequences of human origin using Bowtie 2 (with the "–end-to-end –very-fast" command) against the human genome reference sequence (GRCh38) from the University of California, Santa Cruz. Read quality was checked using FastQC (version 0.11.9) (Cock et al., 2010).

In the sequencing data, internal standard spike-in DNA sequences were first identified and removed to create standard curves. Standard curves for each sample were made with the logarithmic value of read-count per unit length as the horizontal coordinate and the copy number logarithmic value of each gradient sequence in the internal standard spike-in DNA sequence as the vertical coordinate. Based on these standard curves, the absolute copy number of each gene fragment per sample was calculated.

Clean reads from each sample were assembled using MEGAHIT (version 1.2.9) (Li et al., 2015) using the –presets meta-large

command, and contigs with length ≥ 500 bp were selected as assembly results. All redundant contigs were removed using MMseqs2 (version 15.6f452) (Steinegger and Söding, 2017) with parameters "–min-seq-id 0.95 –cov-mode 1 -c 0.9". Prodigal (version 2.6.3) was used to annotate assembled contigs (Hyatt et al., 2010). High-quality target genes with length ≥ 99 bp were clustered using MMseqs2 with parameters "–min-seq-id 0.95 –cov-mode 1 -c 0.9" to obtain a non-redundant set. Genome binning was performed using MetaWRAP (version 1.2.3) with default parameters (Uritskiy et al., 2018). Bacterial gene sets were functionally annotated against the National Center for Biotechnology Information (NCBI) NR and GenBank databases using DIAMOND (version 2.0.14) (Buchfink et al., 2015).

2.4. Data analysis

All statistical analyses and plotting in this study were completed in R (version 4.5.1) (https://www.r-project.org/). Prior to parametric analyses, data normality was assessed using the Shapiro-Wilk test and homogeneity of variance was tested using Levene's test. When assumptions were met ($p > 0.05$), one-way ANOVA followed by Fisher's LSD post hoc test was performed. For data not meeting these assumptions, non-parametric Kruskal-Wallis tests followed by Dunn's post hoc tests were used. Differential analysis used the LSD.test function in the "agricolae" package (version 1.3–7) for post hoc testing (Mendiburu, 2023). Data processing (such as calculating means, variances, and sums) and plotting used the "tidyverse" package (Wickham et al., 2019). To explore the effects of all detected sediment physicochemical properties (such as moisture, DOC, CEC, and TN) on bacterial α-diversity (characterized using the Shannon-Wiener index) and dominant bacterial abundance (such as Pseudomonadota, Desulfobacterota), this study constructed random forest models using the "randomForest" package (version 4.7–1.2) (Liaw and Wiener, 2002). Subsequently, this study used the rf.significance function (999 permutation tests) in the "rfUtilities" (version 2.1.5) (Altmann et al., 2010) and "rfPermute" packages (version 2.5.5) (Archer, 2021) and the rfPermute function to evaluate the explanatory power of random forest models for target variables (i.e., bacterial α-diversity and dominant bacterial abundance), model significance, and the importance of different physicochemical factors. We used the cca function in the "vegan" package (version 2.7–1) (Oksanen et al., 2022) for canonical correspondence analysis (CCA) to determine the effects of sediment physicochemical factors on bacterial community compositional changes and their explanatory power. Meanwhile, to assess whether individual factors significantly affect bacterial community compositional changes, Monte Carlo permutation tests (999 permutations) were used. To explore correlations between different physicochemical factors and their relationships with significantly changed ASVs, this study used the mantel_test function in the "linkET" package (version 0.0.7.4) (Huang, 2021) for Pearson correlation analysis and Mantel tests, respectively. To compare methodological differences between QMP and RMP, co-occurrence network analysis was performed using both quantitative and relative abundance data. To ensure comparable statistical power while accounting for differences in data distribution, ASVs ranking in the top 1 % by absolute abundance (~312 ASVs) were selected for QMP-based networks, while ASVs ranking in the top 3 % by relative abundance (~559 ASVs) were included for RMP-based networks. Only ASVs present in at least 50 % of samples (≥25 of 50 samples) were retained for network construction. Pairwise associations between ASVs were calculated using Spearman correlation, and only strong correlations ($|r| > 0.95$, $p < 0.001$) were retained as network edges. Network topological properties, including node number, edge number, positive/negative edge ratio, average density, transitivity, diameter, and average path length, were calculated using the "igraph" package (version 2.0.3) in R.

2.5. Data and code availability

All metagenomic sequencing data in this study are deposited in the National Microbiology Data Center (NMDC) with accession number NMDC20311573 (https://nmdc.cn/resource/genomics/sample/detail/NMDC20311573). The R scripts and analysis workflow used in this study, including code for statistical analysis and figure generation, are publicly available on Zenodo at https://doi.org/10.5281/zenodo.18408285 to ensure the reproducibility and transparency of our results.

3. Results

3.1. Changes in sediment physicochemical properties among different sampling sites

Analysis of sediment physicochemical properties from upstream (GDK, XXH, MH) to downstream (HLM, NJG) of the Three Gorges Dam revealed two distinct distribution patterns. First, for specific indicators (moisture, CEC, DOC, TN, AK), differences among the three upstream sites within the same sediment layer (0–10 cm or 10–20 cm) were relatively minor. Similarly, differences between the two downstream sites were minor and generally not statistically significant ($p > 0.05$ for most comparisons; Fig. S2). However, these indicators were significantly lower at downstream sites compared to upstream sites ($p < 0.001$; Fig. S3). For example, in the 0–10 cm surface layer, sediment moisture at upstream sites ranged from 48.62 % to 56.97 %, while at downstream sites it ranged from 28.51 % to 31.85 %. In the 10–20 cm subsurface layer, the moisture content decreased from 38.73 % to 46.35 % upstream to 29.34 %-30.63 % downstream, corresponding to reductions of approximately 41.44 % and 27.75 %, respectively. These results indicate that the dam has a substantial impact on these parameters, particularly in surface sediments. Second, other indicators, including pH, SOC, NH_4^+-N, NO_3^--N, TP, and TK, also differed significantly among sites but did not follow a consistent upstream-downstream pattern. These differences may be influenced by site-specific hydrological or ecological factors, not solely the dam.

3.2. Shifts in sediment bacterial community composition

To assess how the dam affects bacterial communities, we analyzed differences in bacterial copy numbers, α-diversity, and β-diversity across depths and locations. In the surface layer, bacterial copy numbers were statistically similar among upstream sites (GDK, XXH, MH; $p > 0.05$) and among downstream sites (HLM, NJG; $p > 0.05$) (Fig. 1a). This pattern was consistent in the subsurface layer. However, bacterial copy numbers at downstream sites were markedly lower than at upstream sites, decreasing by 64.45 % in the surface layer and 56.49 % in the subsurface layer ($p < 0.001$). Similar trends were observed in bacterial α-diversity metrics, including Shannon-Wiener index, richness, and Pielou evenness (Fig. 1b-d). For instance, in the surface layer, the average species count was 31,305 upstream versus 30,836 downstream, representing a loss of 469 species ($p < 0.001$; Fig. 1c). In the subsurface layer, richness declined by approximately 403 species. These findings indicate that dam construction significantly reduced both bacterial abundance

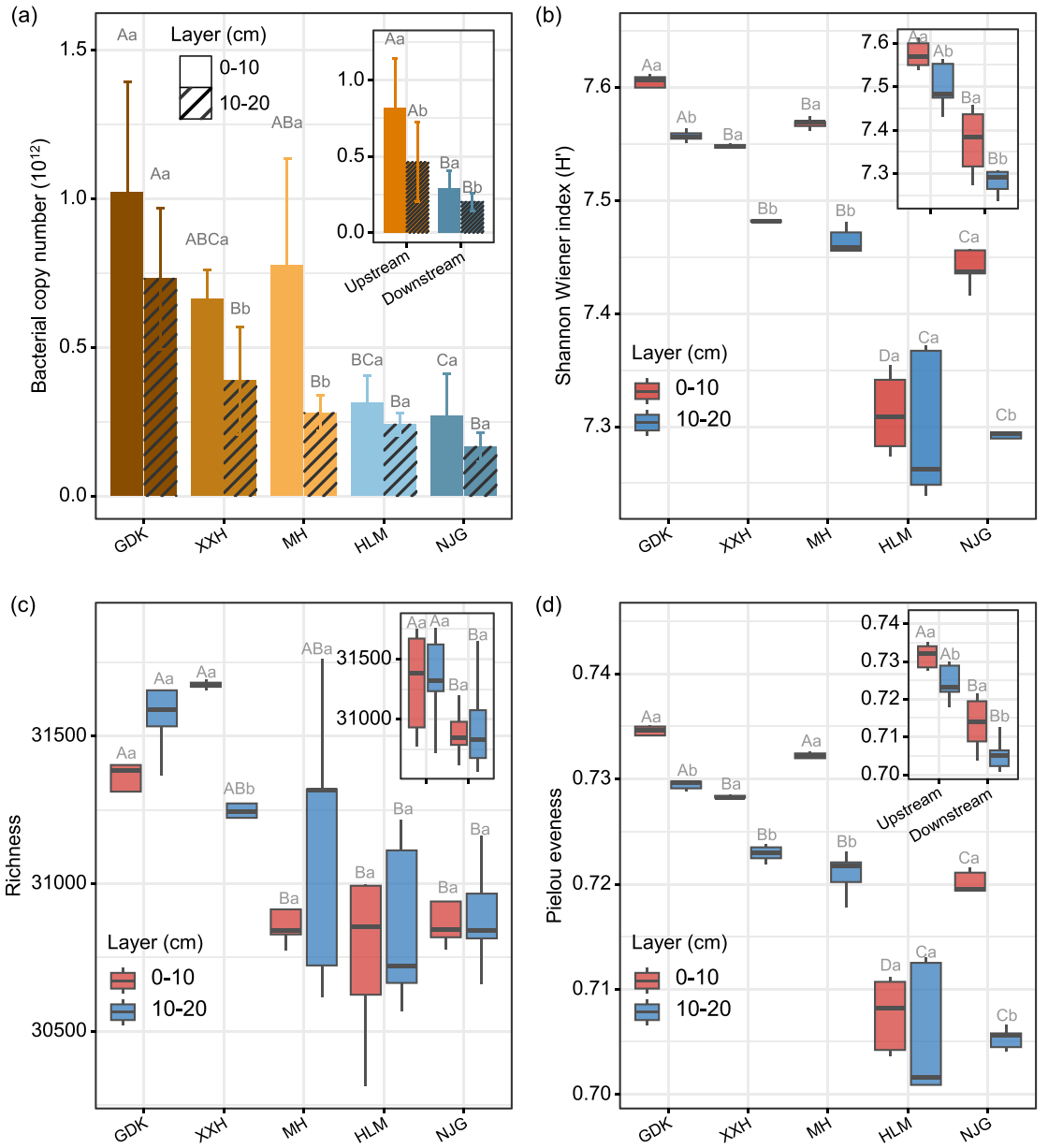

Fig. 1. Comparisons of bacterial abundance and α-diversity across upstream (GDK, XXH, MH) and downstream (HLM, NJG) sites of the Three Gorges Dam. Bacterial copy number (a), Shannon-Wiener index (b), Richness (c) and Pielou evenness index (d). Different lowercase letters indicate significant differences among sediment layers (0–10 vs. 10–20 cm) at the same sampling site, while different uppercase letters indicate significant differences among sampling sites within the same sediment layer. The same letters indicate no significant differences between groups. Significance was determined at $p < 0.05$.

and diversity in downstream sediments.

We further analyzed bacterial community composition. Twelve dominant bacterial phyla accounted for 96.08 % of the total abundance. In upstream samples (0–10 cm), the most abundant group was Pseudomonadota (~2.32 $\times 10^{11}$ copies), followed by Desulfobacterota (~1.23 $\times 10^{11}$) and Chloroflexota (~1.20 $\times 10^{11}$) (Fig. 2a). In contrast, downstream samples were dominated by Desulfobacterota (~7.00 $\times 10^{10}$), followed by Pseudomonadota (~5.30 $\times 10^{10}$) and Chloroflexota (~4.69 $\times 10^{10}$). Similar dominance patterns were observed in the 10–20 cm layer. Among the 31,870 ASVs detected, 88.16 % exhibited a significant decrease downstream ($p < 0.05$), 0.48 % exhibited a significant increase, and 11.35 % showed no significant change (Fig. 2b). The 154 ASVs that increased were mainly affiliated with Chloroflexota, while the 28097 ASVs that decreased belonged predominantly to Pseudomonadota (Fig. 2c). Notably, parallel analysis using RMP yielded contrasting patterns: several taxa including Bacteroidota, Spirochaetota, Desulfobacterota, Planctomycetota, and Chloroflexota appeared to increase in relative abundance downstream (Table S2), whereas QMP revealed that all these taxa actually declined in absolute abundance (Fig. S12). Furthermore, RMP-based richness estimates were approximately 46 % lower than QMP values (16,696 vs. 31,305 ASVs in surface sediments) due to rarefaction constraints (Fig. 1 and Table S3).

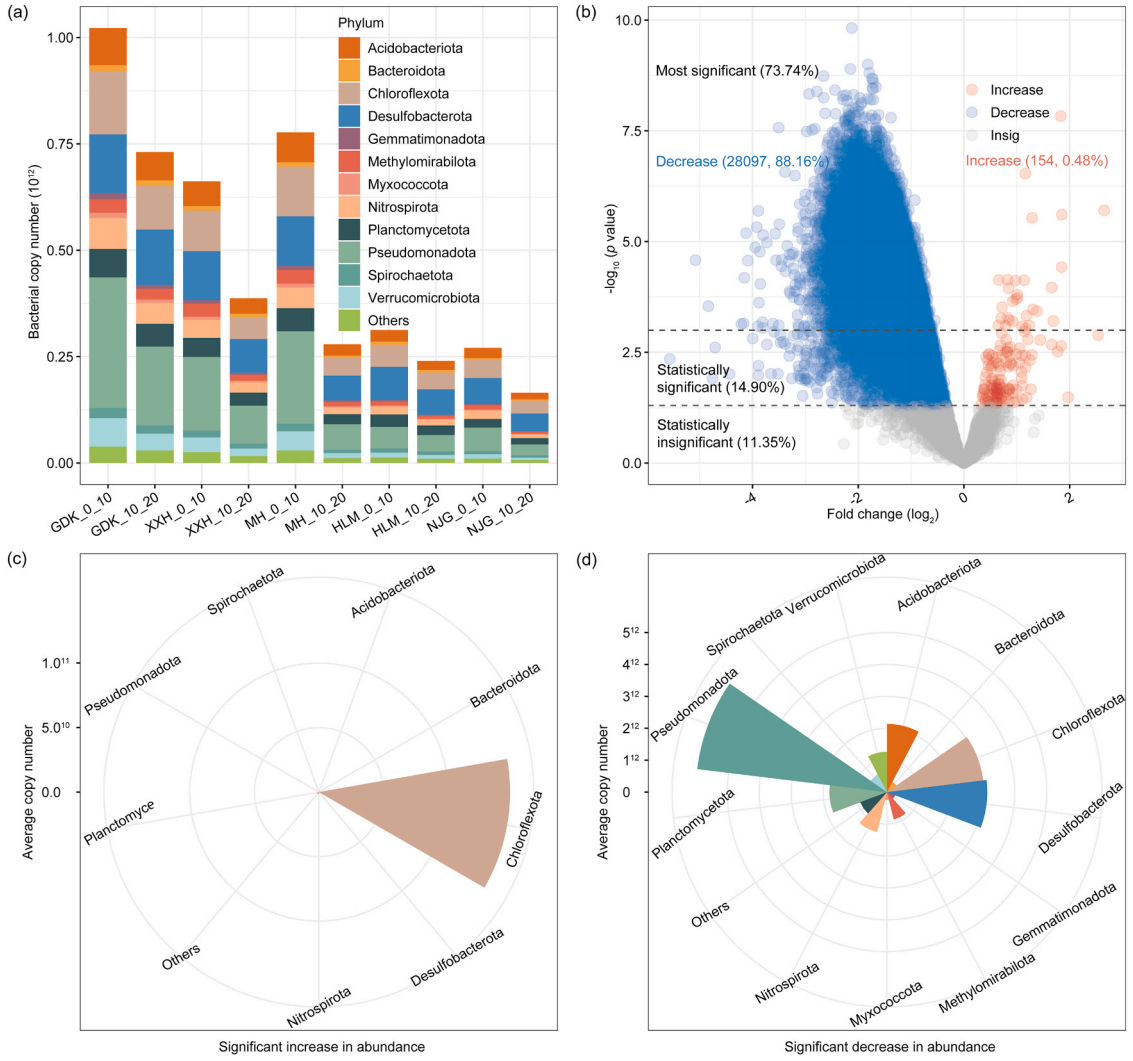

Fig. 2. Changes in bacterial community composition upstream and downstream of the dam. Absolute abundance composition of bacteria in different sampling sites (a). Differential changes in bacterial taxa abundance in sediments upstream and downstream of the dam (b). Significance is presented as $p < 0.05$ (statistically significant), $p < 0.001$ (most significant). and non-significant $p > 0.05$ (statistically insignificant) results. Significantly increased bacterial taxa in downstream sediments compared to upstream sediments (c); Significantly decreased bacterial taxa in downstream sediments compared to upstream sediments (d).

3.3. Relationships between physicochemical factors and microbial community composition

To identify potential drivers of bacterial diversity loss, we employed random forest modeling. The model, incorporating moisture, TN, NH_4^+-N, AK, CEC, and DOC as predictors, explained 94.06 % of the variation in the Shannon-Wiener index ($p < 0.001$; Fig. 3a). Among these, moisture had the highest importance (18.19 %), followed by TN, NH_4^+-N, AK, CEC, and DOC. Linear regression analysis showed that these factors were significantly positively correlated with the Shannon-Wiener diversity index ($p < 0.001$, Fig. S4). Canonical correspondence analysis (CCA) showed that the first two axes explained 89.04 % of the variation in community composition. Key environmental drivers included DOC, CEC, AP, AK, TP, and moisture (Fig. 3b). Samples clustered clearly into upstream and downstream groups, suggesting distinct bacterial assemblages shaped by dam construction. These results also indicate that the primary drivers for α-diversity and β-diversity differ. For dominant bacterial phyla, Spearman correlation analysis showed that their abundance was significantly positively correlated with TN, AK, DOC, moisture, and CEC ($p < 0.01$; Fig. 3c). Random forest models explained over 60 % of the variation in abundance for Pseudomonadota, Verrucomicrobiota, Myxococcota, and Gemmatimonadota, but only 30.03 % for Desulfobacterota. Mantel analysis revealed that all measured physicochemical variables were strongly associated with the compositional changes observed in ASVs that significantly decreased downstream. In contrast, these variables showed limited associations with unchanged ASVs and no association with ASVs that significantly increased (Fig. 3d).

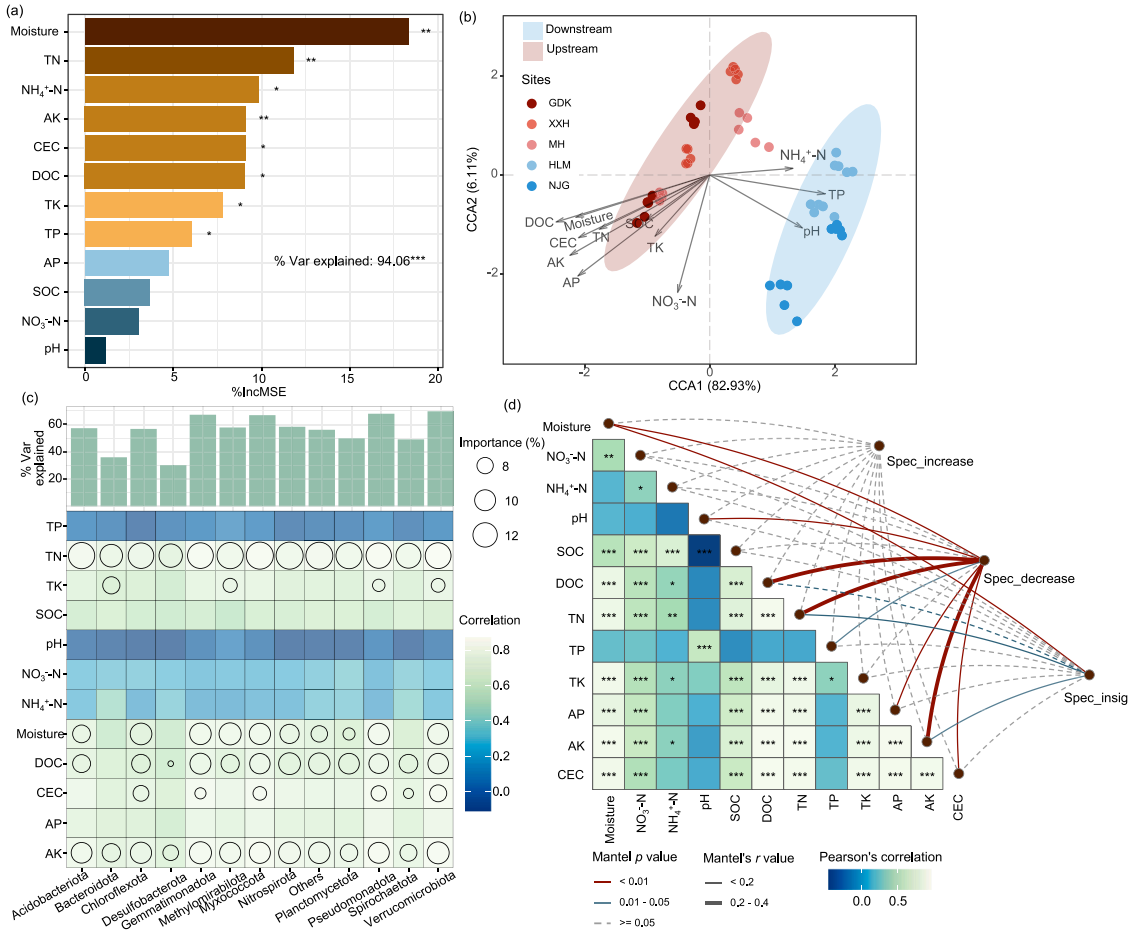

Fig. 3. Relationships between sediment physicochemical properties and bacterial communities. Random Forest analysis of physicochemical drivers of the Shannon-Wiener index (a). Canonical Correspondence Analysis (CCA) of physicochemical effects on community composition (first two axes shown) (b). Random Forest analysis of physicochemical drivers for major bacterial taxa abundance; heatmap shows Spearman correlations between properties and bacterial abundance (c). Circle size represents the degree of importance, and only variables with significant importance are shown. The heatmap indicates Spearman correlations between physicochemical properties and bacterial abundance, revealing that most factors were positively correlated with bacterial abundance. Pearson correlations among physicochemical properties and Mantel tests between these properties and bacterial communities categorized as (d): Spec_increase (higher abundance downstream), Spec_decrease (lower abundance downstream), and Spec_insig (no significant difference). Statistical significance is indicated as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

3.4. Impacts of bacterial community changes on sediment multifunctionality

To evaluate the impact of altered bacterial communities on sediment multifunctionality, we assessed the association of significantly altered taxa with functional gene profiles involved in carbon (C), nitrogen (N), sulfur (S), and phosphorus (P) cycling. Downstream-enriched microbes were associated with ~143 C cycle genes, and their Shannon-Wiener index significantly increased compared to upstream ($p < 0.05$; Fig. S5a). These genes were primarily involved in nucleotide and amino acid biosynthesis (organic biosynthesis), carbohydrate degradation (organic degradation), and CO_2 fixation or release (carbon fixation/release pathways) (Fig. S6a). In contrast, ~1229 C cycle genes were associated with downstream-depleted microbes. In the 0–10 cm layer, both the Shannon-Wiener and richness indices for these genes significantly declined downstream ($p < 0.05$; Fig. S5b). These genes included pathways for carbohydrate degradation, CO_2 flux, and biosynthesis of amino acids, cofactors, and nucleotides (Fig. 4a).

For N, S, and P cycles, significantly increased downstream microbes had no effect on N or S gene diversity but significantly increased the Shannon-Wiener index of P cycling genes (Fig. S7a-9a). These microbes enhanced pathways such as nitrogenous organic degradation and synthesis, and S reduction within S cycling (Fig. S6b and c). Notably, they significantly enriched purine and pyrimidine metabolism pathways, including synthesis, transformation, and degradation (Fig. S6d). Conversely, bacteria that significantly declined downstream reduced the Shannon-Wiener index of N, S, and P cycle genes (but not richness; Fig. S7b-9b). These declines were associated with reduced capacity for nitrogenous organic degradation, nitrification, dissimilatory nitrate reduction, denitrification (Fig. 4b), dissimilatory sulfur redox processes (Fig. 4c), and purine/pyrimidine metabolism as well as transporter functions (Fig. 4d). Overall, dam-induced bacterial compositional shifts appear to impair sedimentary C, N, S, and P cycling potential, particularly those functions driven by heterotrophic microbes.

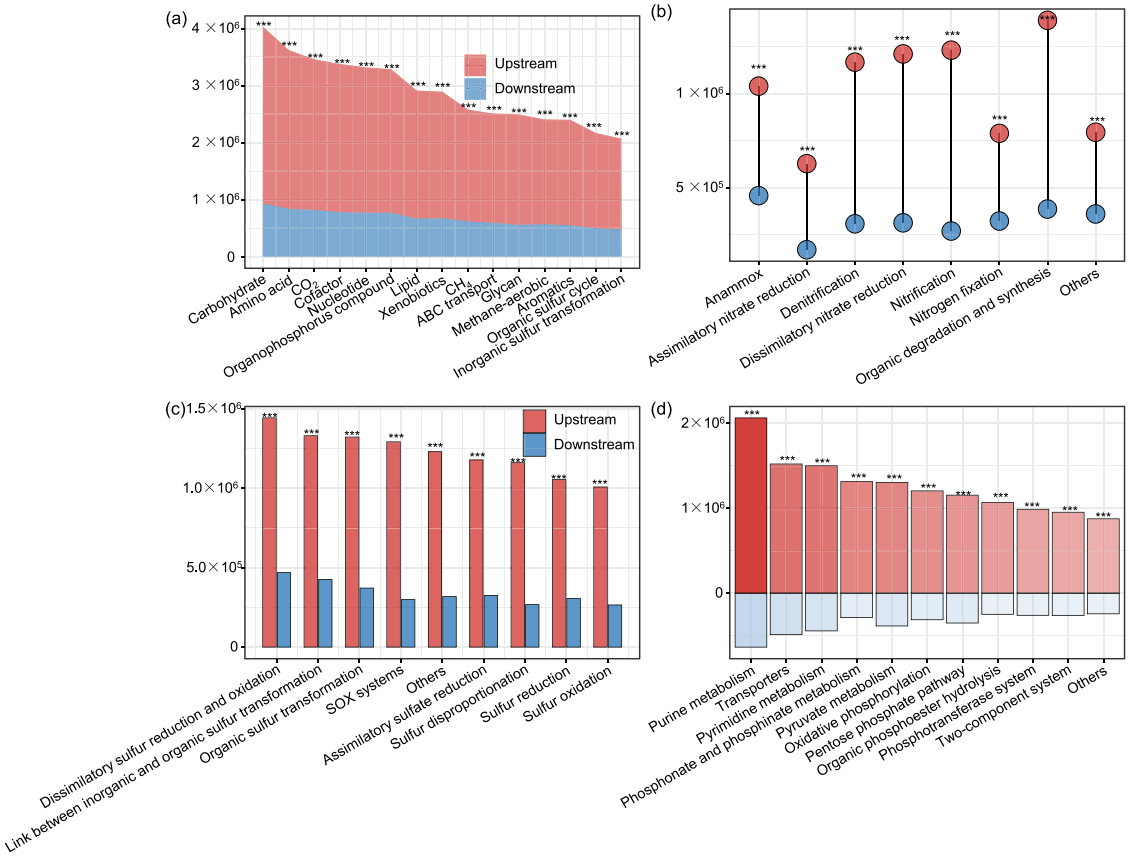

Fig. 4. Differences in major functional gene categories inferred from significantly decreased bacterial taxa (as shown in Fig. 2d) between upstream and downstream regions. Panel (a) shows carbon cycling genes including carbohydrate degradation pathways such as glycolysis, TCA cycle, and various polysaccharide degradation enzymes. Panel (b) displays nitrogen cycling genes including nitrification (amoA, hao) and denitrification (narG, nirK, norB, nosZ) pathways. Panel (c) presents sulfur cycling genes including dissimilatory sulfate reduction (dsrAB) and sulfur oxidation pathways. Panel (d) shows phosphorus cycling genes including nucleotide metabolism pathways (primarily purine metabolism) rather than direct inorganic phosphorus transformation. Statistical significance is indicated as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

4. Discussion

Since the Three Gorges Dam (TGD)—one of the world's largest hydroelectric projects—began operation in 2003 (Zhou et al., 2016), extensive research has investigated its ecological impacts on the Yangtze River basin (Guo et al., 2012; Wang et al., 2012; Yu et al., 2023). However, most studies relied on relative microbiome profiling, which obscures absolute abundance dynamics. Here, we applied quantitative microbiome profiling (QMP) to examine how dam-associated shifts in sediment physicochemical properties may be linked to bacterial community restructuring and functional potential variation across upstream and downstream sites.

Twenty-one years after dam operation commenced, substantial differences in sediment properties were observed between upstream and downstream sites (Fig. S2-S3). Downstream sediments exhibited markedly lower moisture, CEC, DOC, TN, and AK ($p < 0.001$), reflecting the interception of suspended particles and reduced fluxes of particulate matter and organic inputs by the TGD (Chen et al., 2018a; Zhou et al., 2016). For instance, within the influence range of the TGD, the average sediment load at Zhicheng decreased by ~90 % after impoundment (2003–2014) compared with the pre-dam period (1951–2002) (Zhou et al., 2016). The markedly lower moisture content in downstream sediments is primarily attributed to dam-induced sediment coarsening. The TGD traps fine particles ($d \leq 0.125$ mm) in the reservoir while releasing sediment-depleted water that erodes fine materials from the downstream riverbed (Dai and Liu, 2013; Zhang et al., 2025). Since fine-grained sediments possess higher water-holding capacity due to their greater surface area, the progressive coarsening of downstream sediments directly reduces their moisture retention (Kondolf, 1997). These reductions indicate that downstream sediments have become drier and less capable of retaining nutrients (Baldwin and Mitchell, 2000; House, 2003), which may partly explain the substantial microbial changes observed.

Consistent with this nutrient depletion and reduced sediment quality, bacterial abundance and diversity declined significantly downstream. Copy numbers decreased by ~65 % in the 0–10 cm layer and ~57 % in the 10–20 cm layer, accompanied by sharp reductions in richness and Shannon diversity ($p < 0.001$, Fig. 1). Similar declines have been reported in other dam-regulated rivers (Li et al., 2024; Tang et al., 2024; Chen et al., 2018a; Liu et al., 2018; Luo et al., 2020; Sun et al., 2018), but our QMP results suggest that dam construction is likely associated with microbial loss, particularly in surface sediments. Both CCA (Fig. 3b) and ASV distribution patterns (Fig. S10-S11) further suggest that these changes may be primarily dam-associated rather than solely the result of local variability.

Bacterial community composition also shifted markedly. Upstream sediments were dominated by Pseudomonadota, Desulfobacterota, and Chloroflexota, whereas Desulfobacterota emerged as the most abundant phylum downstream, followed by Pseudomonadota and Chloroflexota (Fig. 2a). Previous studies based on relative abundance consistently identified Pseudomonadota as dominant (Liu et al., 2018; Luo et al., 2020; Yu et al., 2023), often highlighting Pseudomonadota, Bacteroidota, Actinobacteriota, and Bacillota as major groups. By contrast, our QMP results revealed Bacteroidota at < 1 % and Bacillota outside the top 95 % of taxa. This discrepancy underscores the limitations of relative quantification, which can distort microbial dominance patterns, whereas QMP provides a more accurate depiction of ecological restructuring (Knight et al., 2018; Vandeputte et al., 2017; Wang et al., 2020b).

To further validate the distinct analytical capacities of QMP versus RMP, we conducted a parallel analysis of our dataset using both approaches (Table S2–S4). The RMP-based analysis substantially underestimated species richness due to rarefaction, yielding values approximately 46 % lower than QMP estimates (16,696 vs. 31,305 ASVs in surface sediments; $p < 0.001$). Notably, compositional analyses based on RMP suggested that several taxa, including Bacteroidota, Spirochaetota, Desulfobacterota, Planctomycetota, and Chloroflexota, appeared to increase in relative abundance downstream (Table S2). However, QMP revealed that the absolute abundances of all these taxa actually declined, demonstrating how compositional constraints inherent in relative data can mask true ecological patterns. Co-occurrence network analysis further highlighted these methodological differences (Fig. S13 and Table S4): the QMP-derived network (312 nodes, 23,847 edges) exhibited exclusively positive correlations with high density (0.49) and transitivity (0.91), whereas the RMP-derived network (559 nodes, 19,869 edges) contained 32.7 % spurious negative correlations (6501 edges) with substantially lower density (0.13) and transitivity (0.73). These findings underscore that compositional artifact inherent in RMP can generate false negative associations and obscure genuine microbial co-occurrence patterns, supporting recent studies advocating for absolute quantification in microbial ecology (Hardwick et al., 2018; Vandeputte et al., 2017; Wang et al., 2024; Xiao et al., 2025).

Notably, ~88.2 % of ASVs significantly declined downstream, predominantly within Pseudomonadota, while only ~0.5 % increased, mostly belonging to Chloroflexota (Fig. 2c). The absolute abundance of all major phyla decreased, especially in the 0–10 cm layer: Pseudomonadota (~74 %), Verrucomicrobiota (~73 %), Myxococcota (~72 %), and Gemmatimonadota (~72 %). These groups are typically copiotrophic, thriving in nutrient-rich environments (Chen et al., 2018a), and random forest analysis showed that > 60 % of their variation was explained by measured physicochemical parameters (Fig. 3). Moreover, many α-, β-, and γ-Proteobacteria are known to be copiotrophic, thriving in organic-rich conditions (Górska et al., 2024; Huang et al., 2021; Leff et al., 2015). This may explain why Pseudomonadota were dominant in nutrient-rich upstream sediments but lost their absolute dominance downstream, where nutrient depletion and sediment desiccation may have constrained their survival. In contrast, Desulfobacterota exhibited a smaller decline (~43 %), consistent with their adaptation to oligotrophic and anaerobic environments (Kadnikov et al., 2023; Rincón-Tomás et al., 2024). Likewise, the enrichment of Chloroflexota (e.g., Anaerolineaceae) reflects their capacity to degrade recalcitrant organic matter under anoxic, carbon-limited conditions (Sinkko et al., 2013). Together, these results suggest a restructuring of downstream bacterial communities toward taxa better adapted to nutrient-poor, anaerobic habitats.

Functional potential analyses revealed significant reductions in microbial capacity for biogeochemical cycling in downstream sediments compared with upstream sites, consistent with previous studies documenting the biogeochemical consequences of dam construction (Friedl and Wüest, 2002; Maavara et al., 2017). Our results showed that downstream-enriched taxa encoded only a restricted repertoire of carbon metabolism genes (~143), largely associated with biosynthesis and CO_2 fixation, and exhibited only marginal increases in gene diversity. In contrast, taxa that declined carried a much broader functional portfolio (~1229 genes),

including key pathways for organic matter degradation and CO_2 turnover. Comparable reductions were also observed for nitrogen cycling (e.g., nitrification, denitrification), sulfur cycling (e.g., sulfide oxidation/reduction), and phosphorus cycling (e.g., purine and pyrimidine metabolism), resulting in substantial losses of functional gene diversity with only limited compensatory gains. Collectively, this contraction of the downstream microbial gene pool suggests a potentially diminished capacity for sedimentary biogeochemical cycling, consistent with ecological theory that microbial biomass fundamentally constrains ecosystem-scale material turnover rates (Battin et al., 2008; Crowther et al., 2019; Han et al., 2025; Wang et al., 2025). Although our study employed a spatial comparison design rather than a temporal before-after approach, and the inferred functional potential reflects genetic capacity rather than measured metabolic activity, several lines of evidence support the robustness of our conclusions: (1) the consistent upstream-downstream patterns observed across multiple independent sites and two sediment depths, (2) the strong explanatory power of our random forest models (>60 % for major taxa, 94 % for Shannon diversity), and (3) the ecological coherence between observed physicochemical gradients, bacterial community shifts, and functional gene changes.

Nonetheless, several caveats warrant consideration. First, gene abundance reflects metabolic potential rather than directly measured process rates; actual biogeochemical cycling rates are further influenced by enzyme activity, substrate availability, and environmental conditions. Second, our sampling design, while providing adequate statistical power with three upstream and two downstream sites (each with five replicate cores per depth layer), represents a subset of the TGD's influence zone (~700 km of river length). Site-specific factors such as tributary inputs, local land use, and sediment heterogeneity may influence bacterial communities at scales not captured here. Future studies incorporating temporal monitoring, direct measurements of biogeochemical process rates, and broader spatial coverage would help validate both the causal relationships and functional inferences proposed in this study.

5. Conclusion

The reassembly of downstream bacterial communities—marked by the sharp decline of Pseudomonadota and the selective enrichment of Chloroflexota—may have reduced the genetic potential for carbohydrate degradation. This shift may suppress organic matter decomposition and CO_2 release, potentially enhancing sedimentary carbon sequestration. Although certain Chloroflexota taxa may partially compensate through specialized functions (e.g., degradation of recalcitrant carbon, sulfur reduction, purine metabolism), the overall reduction highlights profound ecological consequences of dam regulation. From a climate perspective, this restructuring could potentially reduce downstream greenhouse gas emissions, as the sharp decline of heterotrophic, copiotrophic Pseudomonadota may diminish microbial respiration and carbon mineralization. Importantly, these insights were only detectable through QMP, underscoring its unique power to reveal absolute shifts in microbial abundance and function that would otherwise be overlooked by relative approaches. However, we acknowledge that these interpretations are based on correlative spatial comparisons, and that gene abundance reflects metabolic potential rather than actual process rates. Future longitudinal studies incorporating direct measurements of biogeochemical process rates (e.g., respiration rates, nutrient fluxes) and experimental validation are needed to confirm the causal relationships between dam regulation and microbial community responses and to validate these functional inferences.

CRediT authorship contribution statement

Kaikai Zheng: Formal analysis, Writing. **Tong Liu:** Data curation. **Bao Qian:** Funding acquisition. **Xian Xiao:** Investigation. **Baocheng Wang:** Formal analysis. **Bingyi Zhou:** Data curation. **Ruilin Huang:** Investigation, Funding acquisition, Formal analysis, Writing, Conceptualization.

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.

Acknowledgements

The authors are grateful to the Editor and anonymous referees whose constructive comments substantially improved the manuscript. We would like to thank Dr. Haowei Ni for his help with the Concept diagram. We would like to thank Huangling Temple Hydrological Branch for his help with the sampling. This study was supported by the CRSRI Open Research Program (CKWV2025954/KY), the China Postdoctoral Science Foundation (2024M751482), the China Scholarship Council (202303340012), the Hydrology Bureau of Changjiang Water Resources Commission Innovation and Technology Fund (SWJ-24CJX05), and the Talent Introduction Project ZHYJ202302 of Anhui Science and Technology University.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2026.104815.

Data availability

To enhance the reproducibility and transparency of our study, we have made the R scripts and analysis workflow publicly available on Zenodo (https://doi.org/10.5281/zenodo.18408285).

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