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Comparing Sediment and Water eDNA Metabarcoding for Monitoring Aquatic Biodiversity in a Plateau Lake

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ABSTRACT

Environmental DNA (eDNA) metabarcoding is a powerful tool for monitoring aquatic biodiversity in extreme environments like the Qinghai–Tibet Plateau (QTP), where traditional methods face significant challenges. However, key knowledge gaps remain, particularly in selecting optimal sampling substrates for target taxonomic groups and understanding substrate-specific detection differences. Here, using eDNA metabarcoding with five primer sets (targeting mitochondrial, nuclear, and nucleoid sequences), we systematically compared biodiversity detection between sediment and water samples collected from five sites in the Dongjicuona Lake across the tree of life (encompassing archaea, bacteria, algae, fungi, plants, protozoa, invertebrates, fishes, birds, and mammals). The results showed that sediment eDNA exhibited significantly higher amplicon sequence variant (ASV) richness compared to water eDNA for most taxonomic groups. In contrast, the opposite pattern was observed for fishes, while no significant differences were observed for fungi and birds. Community composition differs significantly between sediment and water samples for most taxonomic groups, except for mammals. Across all taxonomic groups, 18.0% of ASVs were shared between sediments and water, with fishes showing the highest overlap (42.6%) and archaea the lowest (4.5%). Additionally, the percentages of ASVs contributing to major community dissimilarity (cumulatively accounting for 80%) ranged from 6.4% (invertebrates) to 17.4% (protozoa), with most taxa exhibiting distinct habitat preferences. These findings support water sampling as an effective method for monitoring fish diversity, while emphasizing the need for a multi-substrate approach to comprehensively monitor other taxonomic groups in plateau lakes.

1 | Introduction

Biodiversity in freshwater ecosystems has been profoundly impacted by human activities and climate change, attracting widespread attention in recent decades (Butchart et al. 2010; Reid et al. 2019). Biodiversity conservation and management relies on comprehensive monitoring data of biological communities. Conventional methods for monitoring aquatic organisms (e.g., netting, trapping and electrofishing) are time-consuming, labor-intensive, environmentally disruptive, and require specialized taxonomic knowledge (Jia et al. 2024). In recent years, environmental DNA (eDNA) techniques have emerged as a promising alternative approach that has the potential to overcome these limitations of conventional methods (Rees et al. 2014; Thomsen and Willerslev 2015; Wang et al. 2021). Environmental DNA is a mixture of genomic DNA derived from organisms present in environmental samples, thus it can be applied for species-specific detection via polymerase chain reaction (PCR) methods (e.g., quantitative PCR and digital droplet PCR) and for community survey using metabarcoding (Taberlet et al. 2018). Though PCR amplification of DNA barcode regions for multiple taxa of interest, coupled with high-throughput sequencing and bioinformatic processing, eDNA metabarcoding can identify community

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composition in environmental samples (Thomsen et al. 2012). This approach has demonstrated high efficacy in biodiversity monitoring across prokaryotic, protistan, and metazoan communities in both freshwater and marine ecosystems (Feng, Li, et al. 2023; Jensen et al. 2021; Li et al. 2023; Stat et al. 2017).

Effective eDNA metabarcoding for monitoring aquatic biodiversity critically depends on selecting appropriate sampling substrates that must adequately represent the local taxonomic communities (Koziol et al. 2019). The primary sampling substrate used for aquatic eDNA metabarcoding is water, because it allows for a simple and noninvasive sampling process and easy adjustment of filtration volume (Tagliabue et al. 2023). However, sediment eDNA has an increasingly important role in aquatic eDNA metabarcoding, as it has a lower decay rate and higher DNA concentration of organisms (e.g., microbes and fishes) than water eDNA (Corinaldesi et al. 2011; Sakata et al. 2020; Turner et al. 2015). Some studies compared results of eDNA metabarcoding between water and sediment samples in marine ecosystems and found higher OTUs (operational taxonomic units) richness in sediments and significant differences in community composition (Antich et al. 2021; Brandt et al. 2021; Holman et al. 2019; Tagliabue et al. 2023; Zhu et al. 2023). However, knowledge on similar comparisons remains very limited in freshwater ecosystems, although a few studies collected samples of water and sediment simultaneously (Holman et al. 2019; Sakata et al. 2021). These studies primarily focused on single taxonomic groups (e.g., fishes) and were conducted in river environments. Significant differences in community composition were also observed in rivers, but no significant differences in alpha diversity were found, contrasting with results from marine environments. Although lakes are also freshwater ecosystems, their flow rates are markedly lower than those of rivers, resembling marine systems more closely. However, there remains a critical lack of systematic research directly comparing water and sediment eDNA metabarcoding across different taxonomic groups in lakes.

The Qinghai–Tibet Plateau (QTP), known as the "Asian water tower", has the world's largest and highest lake group and serves as the headwater region for multiple major Asian rivers (Feng et al. 2019). Aquatic biodiversity and ecosystems on the QTP are profoundly impacted by human activities (e.g., hydropower development and biological invasion) and climate change, and they exhibit particular vulnerability and sensitivity to such perturbations due to their unique evolutionary history and extreme environmental conditions (Feng, Liu, et al. 2023; Fu et al. 2021; Jia et al. 2025; Yao et al. 2022). However, the monitoring data of aquatic organisms on the QTP remain extremely limited, posing challenges to conservation efforts. Given the extreme conditions (e.g., cold climate, hypoxia and limited resources) on the QTP, eDNA metabarcoding, as an effective alternative to traditional survey methods, is crucial for aquatic biodiversity monitoring and conservation in this region. Although a few studies have investigated water eDNA metabarcoding in rivers in this region (Feng, Li, et al. 2023; Gao et al. 2023; Lu et al. 2023), research on lake water and sediment eDNA metabarcoding, as well as their differences, remains completely lacking.

In this study, using eDNA metabarcoding with five primer sets (targeting ribosomal DNA and mitochondrial sequences) for sediment and water samples collected from five sites in the Dongjicuona Lake, we investigated differences in biodiversity monitoring between the two sample types across the tree of life, encompassing archaea, bacteria, algae, fungi, plants, protozoa, invertebrates, fishes, birds, and mammals. Differences in taxonomic diversity and community composition for each taxonomic group were evaluated between the sediment and water samples. Furthermore, taxa primarily contributing to community dissimilarity between the two substrates were identified within each taxonomic group. Based on the characteristics of eDNA in sediments and water, along with the low mobility of lake water, we hypothesized that: (i) taxonomic diversity would be higher in sediments than in water; (ii) there would be significant differences in community composition between the two substrates; and (iii) for most taxonomic groups (except birds and mammals), these differences should be driven by taxa with distinct habitat preferences.

2 | Materials and Methods

2.1 | eDNA Sampling and Extraction

Sediment and water samples were manually collected from five sites of Donggicona Lake in July 2023 (35°10′–35°30′ N, 98°20′–98°50′ E; Figure 1), with three replicates at each site. Among these sites, two (G1 and G3) were densely vegetated with aquatic plants and had muddy substrates, while the other three (G2, G4, and G5) lacked aquatic vegetation and featured sandy/rocky substrates. Additionally, site G4 was positioned at the outflow channel with a lotic condition, while other sites (lakeshore zone) were typical lentic systems. Water samples were collected using an organic glass hydrophore and stored in sterile 1.0 L plastic bottles, combining equal volumes from surface (10 cm depth), mid-depth, and near-bottom (10 cm above sediment) layers. Within 6 h of collection, water samples were filtered through 0.45 μm mixed cellulose ester (MCE) membranes, which were then immediately transferred to sterile 2 mL microcentrifuge tubes and stored in liquid nitrogen until transferred to an ultracold freezer in the laboratory. Two blanks were collected by filtering 500 mL of bottled mineral water. Surface sediment samples (0–2 cm depth, about 10 g aliquots) were collected using sterile disposable spatulas, placed in sterile 15 mL centrifuge tubes and stored in liquid nitrogen. All reused equipment was sterilized by soaking in commercial 10% bleach solution and rinsed with sterile water and the water from the sampling site.

DNA extraction and library preparation were conducted in dedicated laboratories, with all workspaces decontaminated via UV irradiation and DNA-off reagents before use. Sediment DNA was extracted from 5 g of each sediment sample using the DNeasy PowerMax Soil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Water DNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) applying four times more ATL buffer and proteinase K compared with the manufacturer's protocol and two rounds of elution with 100 μL sterilized ddH_2O (Jensen et al. 2023; Sigsgaard et al. 2017). An extraction blank was included for both water and sediment DNA extractions by adding no sample.

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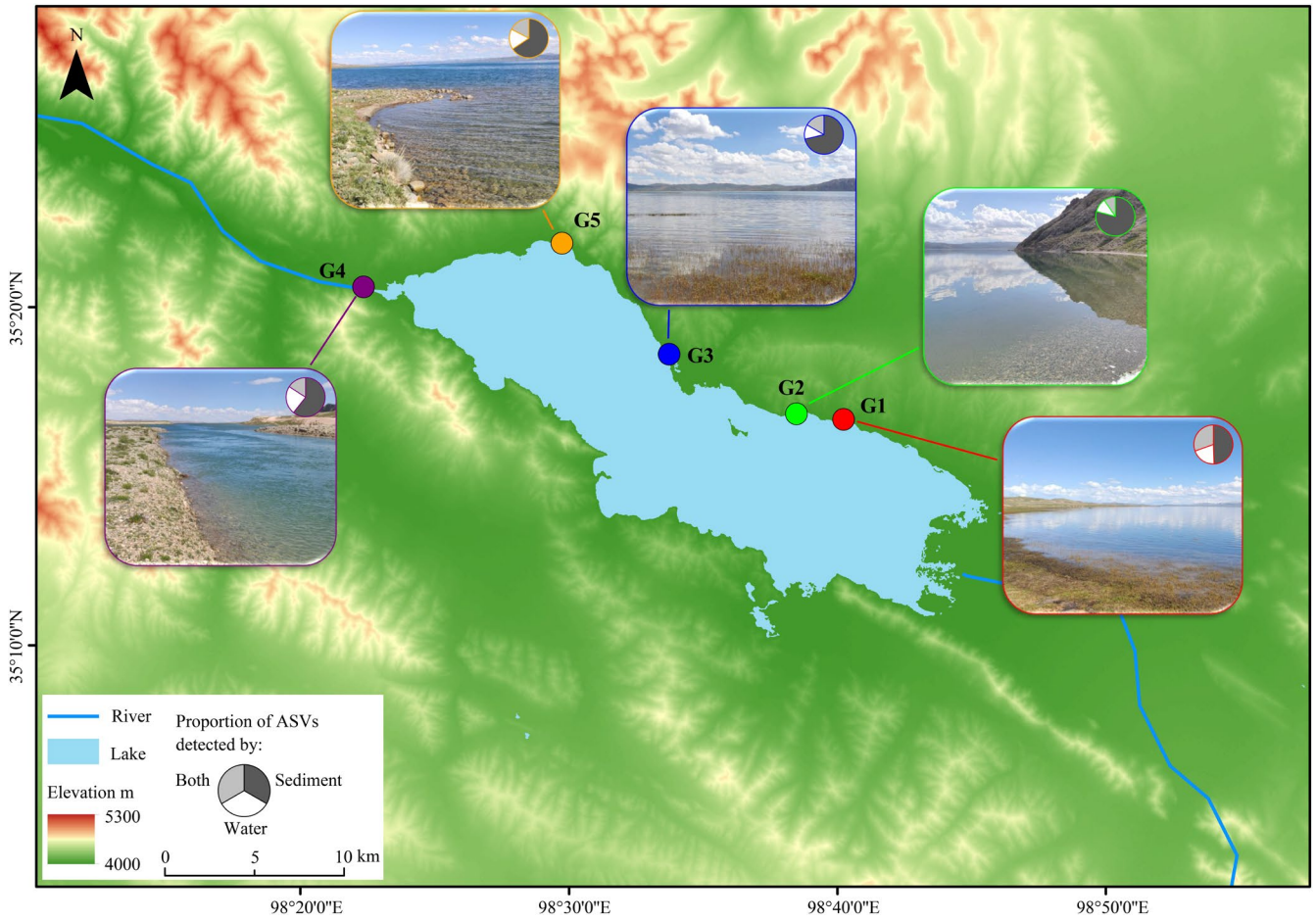

FIGURE 1 | Map of sampling sites in the Dongjicuona Lake. Each sampling site was represented by a distinct color, with accompanying photographs depicting typical environments. A pie chart for each site illustrated the proportion of ASVs detected exclusively in sediments, water, or both sample types.

2.2 | Library Preparation and Sequencing

Five primer pairs were used to amplify mitochondrial, nuclear, and nucleoid gene sequences for all eDNA samples and blanks. Among them, Bact02 was used to target the 16S ribosomal RNA (rRNA) gene region of prokaryotes (Taberlet et al. 2018). Euka02, targeting the eukaryotic nuclear 18S rRNA gene region, was used to amplify algae and fungi (Guardiola et al. 2015). For plants, protozoa, and invertebrates, the modified mlCOIintF/jgHCO2198 (Feng et al. 2025; Leray et al. 2013) was used to amplify the mitochondrial cytochrome c oxidase I (COI) gene region. For fishes and mammals, Tele02 was used to amplify the mitochondrial 12S rRNA gene region (Taberlet et al. 2018). The primers 12Sa/12Sh were used to amplify birds' mitochondrial 12S rRNA gene region (Saenz-Agudelo et al. 2022). Unique tags (i.e., 2–3 N bases and a unique eight-nucleotide sequence) were added to the 5′ ends of both forward and reverse primers for each sample, enabling the identification of individual PCRs and avoiding tag jumps (Coissac 2012; Jensen et al. 2021).

PCR amplifications for each primer set were carried out in quadruplicate, with each replicate containing PCR reactions for 30 samples and four filter and extraction blanks, and two blank PCR reactions (36 uniquely tagged reactions total). Each PCR reaction was conducted in 25 μL volumes containing 12.5 μL AceTaq Master Mix (Vazyme, Nanjing, China), 0.5 μL of each forward and reverse primer (10 μM), 2.0 μL DNA template, 1 μL bovine serum albumin (BSA) (20 mg/mL) and 8.5 μL ddH_2O. Thermocycling parameters were: 95°C for 3 min, 35 or 40 cycles (35 cycles for Bact02 and 40 cycles for others) of 95°C for 30 s, 54 or 52°C (54°C for Tele02 and 12Sa/12Sh and 52°C for others) for 30 s, 72°C for 1 min, and a final extension of 72°C for 8 min. Fragment sizes were verified on 1.0% agarose gel stained with ethidium bromide. The 36 PCR products from each replicate were pooled in equal volumes, generating a total of 20 pools (four PCR replicates per primer set), which were then individually purified using the TaKaRa MiniBEST DNA Fragment Purification Kit Ver.4.0. Twenty libraries were constructed using the Hieff NGS MaxUp II DNA Library Prep Kit for Illumina (Yeasen, Shanghai, China) and sequenced on an Illumina NovaSeq 6000 platform with paired-end reads (2×250 bp for Bact02 and mlCOIintF/jgHCO2198, and 2×150 bp for Euka02, Tele02 and 12Sa/12Sh).

2.3 | Bioinformatic Processing and Taxonomic Assignment

The raw paired-end sequencing data were filtered using fastp v0.20.0 to remove low-quality reads and correct mismatched base pairs in overlapping regions (Chen et al. 2018). High-quality reads were demultiplexed and primer-trimmed using cutadapt

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v4.6 (Martin 2011), yielding sequencing data for each individual PCR product. The DADA2 pipeline v1.28.0 was used to generate amplicon sequence variant (ASV) tables for each primer set, recording the abundance of each ASV in individual PCR products (Callahan et al. 2016).

The SILVA (Quast et al. 2013) and MIDORI2 (Leray et al. 2022) reference databases were widely used for taxonomic classification of ASVs, since they provide comprehensive and regularly updated datasets of aligned rRNA (16S/18S/23S/28S) sequences and mitochondrial sequences, respectively. The IDTAXA algorithm in the R package *DECIPHER* (Murali et al. 2018), a novel approach for accurate taxonomic classification of microbiome sequences (e.g., 16S/18S rRNA genes), employs a machine learning model trained on curated databases (e.g., SILVA) to reduce overclassification errors. Given that mitochondrial genes have a higher evolutionary rate than nuclear or nucleoid rRNA genes, BLAST is often the practical choice for their taxonomic classification. Therefore, this study employed these two reference databases and their respective tools for the taxonomic classification of ASVs. Specificly, ASVs derived from the 16S rRNA (Bact02) and 18S rRNA (Euka02) amplicon datasets were taxonomically assigned using the IDTAXA algorithm (default confidence threshold of 60), against the SILVA (release 138) reference database. Such analysis provides a statistical confidence score at each taxonomic level, allows it to stop classifying at the level where certainty drops, preventing overclassification of novel organisms. For ASVs derived from the COI (mlCOIintF/jgHCO2198) and 12S rRNA (Tele02 and 12Sa/12Sh) amplicon datasets, taxonomic assignment was performed using BLASTn against the MIDORI2 (GenBank265 version) unique nucleic acid sequence of eukaryotic mitochondrial COI and srRNA databases, and the best hits were extracted from the outfile. Only alignment results with query coverage more than 90% and sequence similarity more than 80% remained. Different taxonomic ranks were assigned based on the following similarity thresholds: ≥97% for species, ≥94% for genus, ≥85% for family, and ≥80% for order (Yarza et al. 2014). Any taxonomic rank that could not be assigned was labeled as "NA." After taxonomic assignment, ASVs were firstly filtered to remove those with fewer than 10 total reads or no phylum-level assignment.

All ASVs were classified into ten taxonomic groups following the seven-kingdom system (Ruggiero et al. 2015): (1) archaea and bacteria were assigned according to their kingdom-level classifications, (2) algae, fungi, protozoa and plants were classified according to their phylum-level classifications under Chromista, Fungi, Protozoa and Plantae, respectively, (3) invertebrates were identified based on the phylum-level classifications (excluding Chordata) under Animalia, (4) fishes, birds and mammals were assigned according to their class-level classifications of Actinopteri, Aves and Mammalia, respectively, (5) taxonomic assignments were also verified using the web tools AlgaeBase (https://www.algaebase.org/) and World Register of Marine Species (https://www.marinespecies.org/). Thus, ten independent ASV tables were generated and further filtered to (1) exclude ASVs with total relative abundances below 0.001% in each table, and (2) remove ASVs showing higher read counts in blank controls than in any samples. To ensure detection reliability, an ASV was considered valid only when it occurred in at least two out of four PCR replicates per sample; otherwise, it was regarded as absent in that sample. To evaluate representativeness of the sequencing per PCR of each sample, rarefaction curves were generated using the *rarecurve* function in the R package *vegan* (Oksanen et al. 2020), and showed high consistency and sufficient sequencing depth for the PCR replicates (Figure S1). For each sample, the four PCR replicates were individually rarefied to the median read depth of all PCR replicates (de Cárcer et al. 2011), and then aggregated by summing the read counts.

2.4 | Statistical Analyses

All statistical analyses were performed using R based on ASV data. Alpha and beta diversity analysis was conducted with the R package *vegan* (Oksanen et al. 2020). The relative abundances were obtained by total sum scaling normalization with the function *decostand*. Alpha diversity (ASV richness, Shannon, Simpson and Pielou's indexes) was calculated with the function *diversity*. The Wilcoxon signed-rank test (for paired samples) was used to assess differences in alpha diversity between sediment and water samples. Community compositional differences were visualized by principal coordinate analysis (PCoA) with the function *pcoa*, based on the Bray–Curtis dissimilarities calculated with the function *vegdist*. To assess the significance of community compositional differences between sediment and water samples, a permutational multivariate analysis of variance (PERMANOVA) was performed using the function *adonis2* with 999 permutations. Similarity percentages (SIMPER) analyses were conducted to identify taxa mainly contributing to dissimilarity between sediment and water samples, using the *simper* function in the R package *vegan* (Clarke 1993). Such analysis was not applied to birds and mammals because they do not primarily live in water and were absent at some sites.

3 | Results

3.1 | Sequencing Results and Taxonomic Annotation

A total of 147.85 million 16S rRNA (Bact02) raw reads, 236.62 million 18S rRNA (Euka02) raw reads, 164.46 million COI (mlCOIintF/jgHCO2198) raw reads, 63.27 million 12S rRNA (Tele02) raw reads, and 241.12 million 12S rRNA (12Sa/12Sh) raw reads were obtained across 30 samples and six blank controls. After quality filtering, demultiplexing, trimming, and read merging, the average number of sequences per PCR was: 315,007 for samples and 46,565 for blank controls for Bact02, 360,598 and 4739 for Euka02, 268,354 and 473 for mlCOIintF/jgHCO2198, 43,779 and 2038 for Tele02, and 201,420 and 16 for 12Sa/12Sh.

A total of 75,152, 10,926, 26,049, 984, 1696 ASVs with taxonomic assignments were primitively obtained from the libraries for Bact02, Euka02, mlCOIintF/jgHCO2198, Tele02 and 12Sa/12Sh, respectively. Among them, 25, 20, 3, 6, and 0 ASVs were detected in blank controls, representing 4.73%, 0.10%, 0.02%, 1.48%, and 0% of total read counts, respectively. These ASVs were dominated by Sphingomonas, Bacillariophyceae, Onychiuridae and human sequences, and were removed because they were either exclusive

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to blank controls or more abundant in controls than in samples. After taxonomic assignment and removal of extremely low abundance ASVs, a total of 298 archaeal, 7174 bacterial, 1228 algal, 160 fungal, 273 plant, 447 protozoan, 828 invertebrate, 31 fish, 435 avian, and 21 mammalian ASVs were retained for downstream analysis. The dominated taxa (phylum, class or family level) were: Crenarchaeota in archaea, Proteobacteria in bacteria, Ochrophyta in algae, Chytridiomycota in fungi, Florideophyceae in plants, Discosea in protozoa, Eurotatoria in invertebrates, Nematheilidae in fishes, Anatidae in birds, and Bovidae in mammals (Figure 2).

3.2 | Alpha Diversity and Community Structure

The ASV richness significantly differed by the sample type for most taxonomic groups: with sediment samples exhibiting higher

FIGURE 2 | ASV relative abundances collapsed at the main taxon levels (phylum, class, or family) for ten taxonomic groups (A: archaea, B: bacteria, C: algae, D: fungi, E: plants, F: protozoa, G: invertebrates, H: fishes, I: birds, J: mammals) in each sample.

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diversity than water samples for archaea, bacteria, algae, plants, protozoa, invertebrates, and mammals (Wilcoxon test, V = 113–120, Benjamini–Hochberg (BH) adjusted $p < 0.01$), while water samples showed higher diversity than sediment samples for fishes (Wilcoxon test, V = 1.5, BH adjusted $p < 0.01$; Figure 3). For fungi and birds, no significant difference in richness was detected between sediment and water samples (Wilcoxon test, V = 95.5 and 64, BH adjusted $p > 0.05$). Similar patterns were observed for other alpha diversity indices (e.g., Shannon; Figure S2; Table S1), further supporting the broader diversity captured in sediment samples for most taxonomic groups.

The PCoA plots separated the communities between sediment and water samples for most taxonomic groups, except for mammals (Figure 4). The PERMANOVA indicated significant effects of the sample type on community composition for most taxonomic groups, including archaea ($F_{1,23} = 6.45$, $R^2 = 0.219$), bacteria ($F_{1,28} = 18.74$, $R^2 = 0.401$), algae ($F_{1,28} = 14.79$, $R^2 = 0.346$), fungi ($F_{1,28} = 12.31$, $R^2 = 0.305$), plants ($F_{1,28} = 12.52$, $R^2 = 0.309$), protozoa ($F_{1,28} = 6.65$, $R^2 = 0.192$), invertebrates ($F_{1,28} = 6.36$, $R^2 = 0.185$), fishes ($F_{1,28} = 3.46$, $R^2 = 0.110$), and birds ($F_{1,20} = 3.84$, $R^2 = 0.161$), with all BH-adjusted $p < 0.01$ except fishes (< 0.05). However, the mammalian community composition showed no significant differences between sediment and water samples, with the sample type explaining only 9.4% of the variance (PERMANOVA $F_{1,18} = 1.86$, BH adjusted $p > 0.05$).

3.3 | Taxa Mainly Contributing to Dissimilarity

Overall, 18.0% of ASVs were shared between sediment and water samples. These shared ASVs accounted for 21.9% of detected ASVs in sediment and 51.1% in water samples, while representing 42.4% and 70.0% of read counts in sediment and water samples, respectively. The proportion of shared ASVs varied among taxonomic groups (Figure 5), with the highest overlap observed in fishes (42.6%), followed by fungi (32.5%), algae (22.8%), birds (22.8%), protozoa (22.4%), plants (19.9%), bacteria (16.6%), invertebrates (15.8%), mammals (6.4%), and archaea (4.5%). Shared ASVs made high contributions to the relative abundance in fishes (sediment: 95.8%; water: 77.7%) and fungi (87.9% and 78.1%), moderate contributions in algae (51.5% and 63.5%), birds (37.7% and 55.7%), protozoa (41.2% and 39.0%), plants (47.4% and 17.7%), and bacteria (39.8% and 67.2%), while low contributions in invertebrates (13.0% and 43.5%), mammals (18.0% and 31.3%), and archaea (6.7% and 35.1%).

The SIMPER analysis was used to explore taxa that contributed most to dissimilarity between sediment and water samples. Ranked by contribution from high to low, in archaea, bacteria, fungi, algae, plants, protozoa, invertebrates, and fishes, there were 50 (16.8%), 1110 (15.5%), 86 (7.0%), 25 (15.6%), 22 (8.1%), 78 (17.4%), 53 (6.4%), and 5 (16.1%) ASVs, respectively, cumulatively contributing to 80% of the total (Figure S3; Table S2).

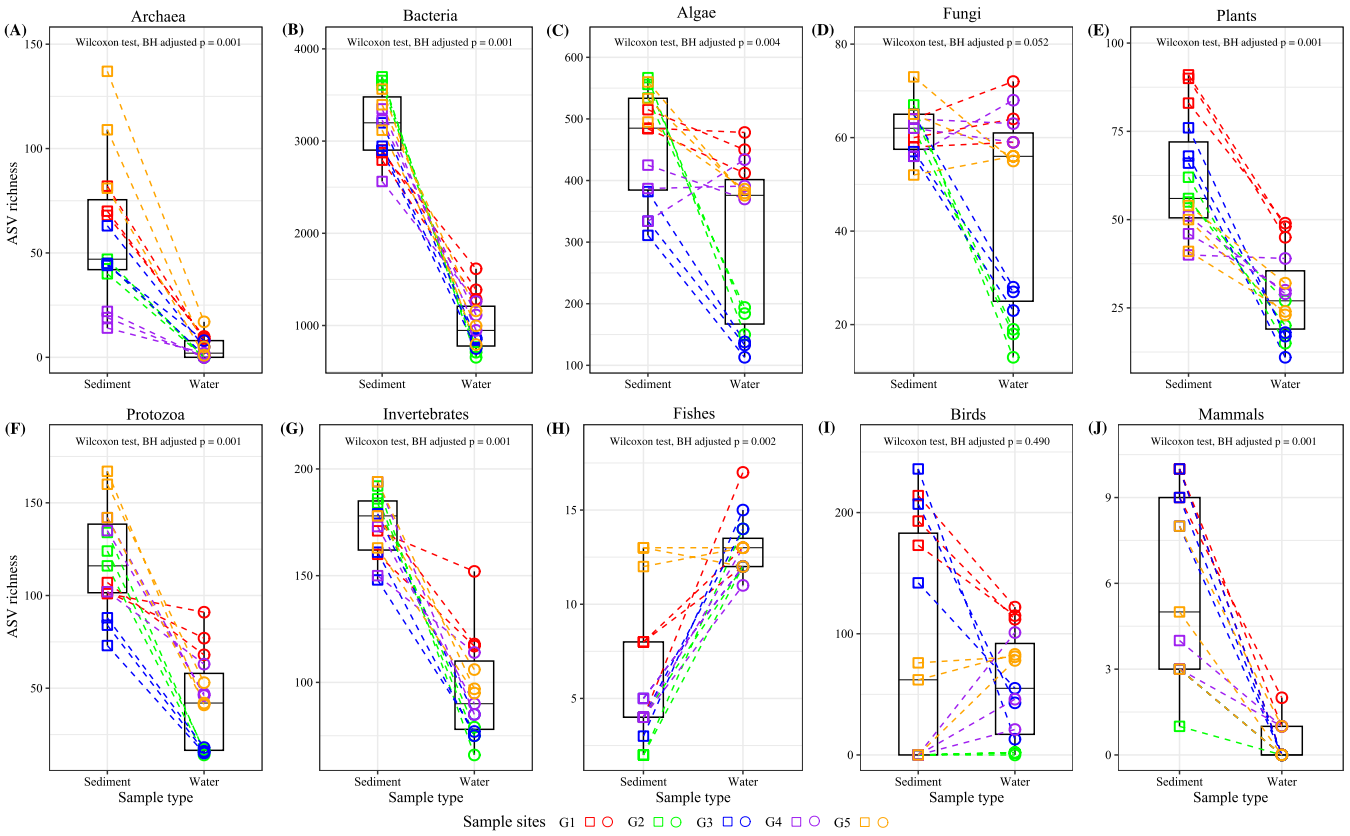

FIGURE 3 | Box plots of ASV richness of different taxonomic groups (A: archaea, B: bacteria, C: algae, D: fungi, E: plants, F: protozoa, G: invertebrates, H: fishes, I: birds, J: mammals). Each sampling site was represented by a distinct color. Square and circular symbols represent sediment and water samples, respectively. Wilcoxon test Benjamini–Hochberg (BH) adjusted *p*-values indicating significance of ASV richness differences between sediment and water samples were shown in each plot.

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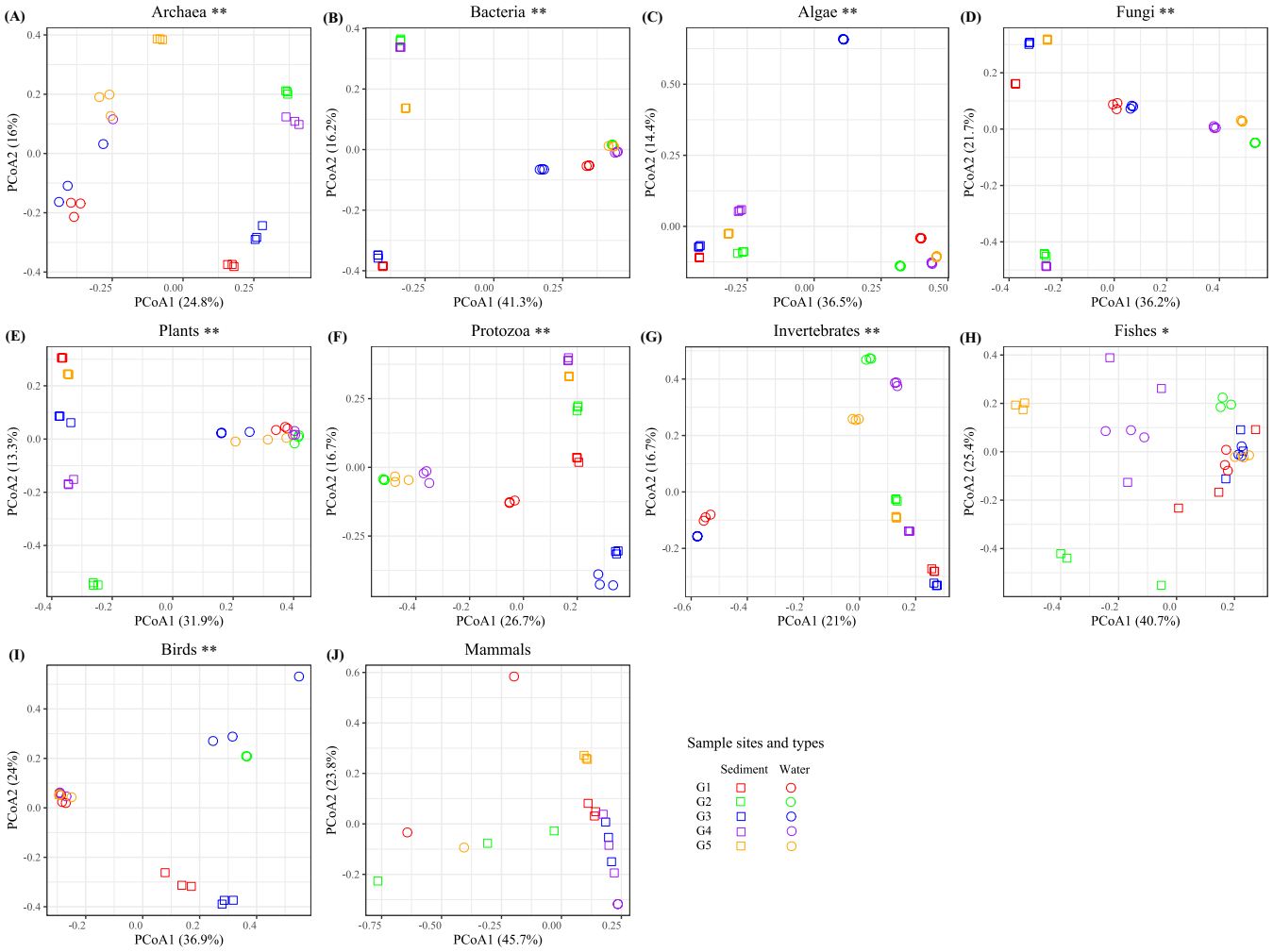

FIGURE 4 | Principal coordinate analysis (PCoA) plots based on Bray–Curtis dissimilarities for different taxonomic groups (A: archaea, B: bacteria, C: algae, D: fungi, E: plants, F: protozoa, G: invertebrates, H: fishes, I: birds, J: mammals). The PERMANOVA significance (sediment vs. water) was shown as follows: *, Benjamini–Hochberg (BH) adjusted $p < 0.05$; **, BH adjusted $p < 0.01$.

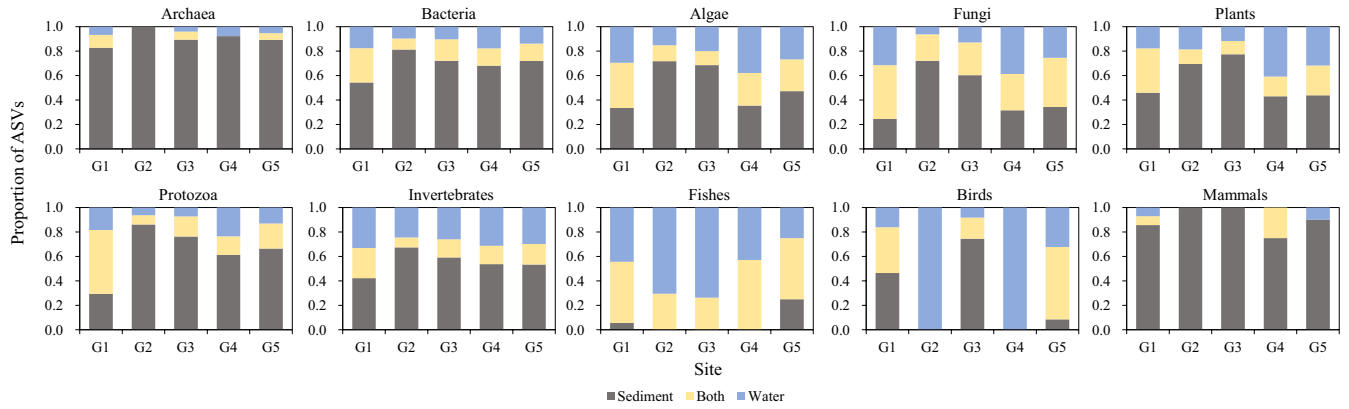

FIGURE 5 | Stacked bar chart representing the proportion of ASVs detected exclusively in eDNA from sediment, water, or both sample types.

Among them, in archaea, Nitrososphaerales (14 ASVs) and Woesearchaeales (29 ASVs) were the main contributing taxa, with contributions of 0.348 and 0.309, respectively, and their read counts in sediment were significantly higher than those in water samples (Figure 6). In bacteria, eight orders each contributed over 0.02, among which five orders showed significantly higher read counts in water than sediment samples. In algae, the six classes that collectively contributed over 0.72 exhibited significant enrichment in divergent sample types, with Bacillariophyceae and Glissomonadida in sediments, and Dinophyceae, Intramacronucleata, and Cryptophyceae in water samples. In fungi, Cryptomycota and Chytridiomycota were the main contributing taxa, with the former showing no significant difference in read counts, and the latter showing significantly

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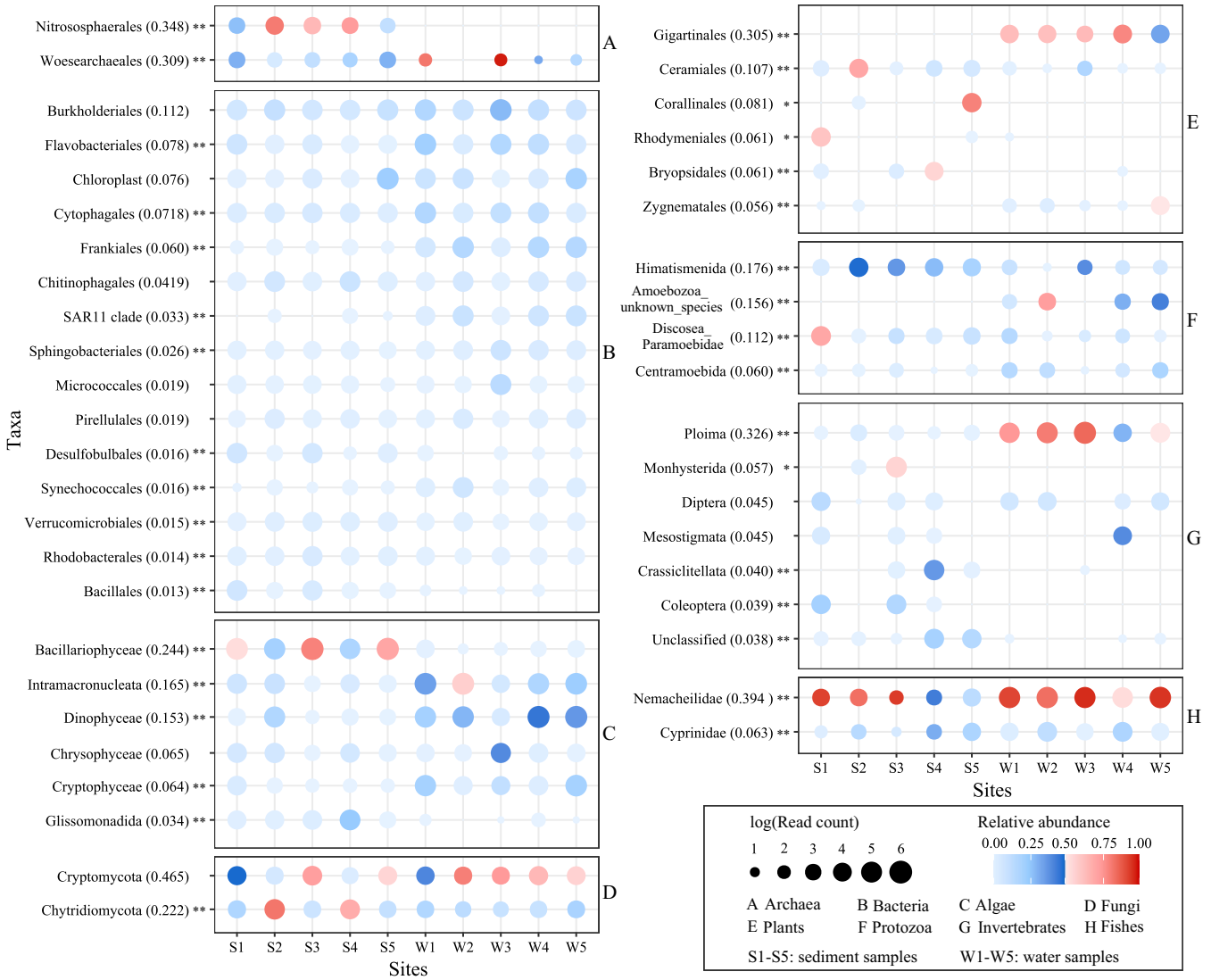

FIGURE 6 | Taxa contributing most to community dissimilarity between sediment and water samples based on SIMPER analyses. The taxonomic levels used for exhibition varied among different taxonomic groups, including phylum (fungi), class (algae), order (archaea, bacteria, plants, protozoa, and invertebrates), and family (fishes). * and ** indicated significant differences in read counts between sediment and water samples (Wilcoxon test, Benjamini–Hochberg adjusted $p < 0.05$ and $p < 0.01$, respectively).

higher read counts in sediments. In plants, six orders collectively contributed over 0.67 and exhibited significant enrichment in different sample types, with Ceramiales, Corallinales, Rhodymeniales, and Bryopsidales in sediment samples, and Gigartinales and Zygnematales in water samples. In protozoa, the two ASVs with the greatest contribution, differing by only two base pairs and likely belonging to the same species, collectively contributed 0.156. They were annotated only to the Amoebozoa without further taxonomic details, and were exclusively detected in water and absent from sediment samples. The taxa contributing most to invertebrate dissimilarity was the rotifer order Ploima (0.326), showing significantly higher read counts in water samples, while other major contributing taxa, including Monhysterida (0.057), Crassiclitellata (0.040), and Coleoptera (0.039), all exhibited inverse distribution patterns with sediment preference. All ASVs in fishes were classified into the families Nemacheilidae and Cyprinidae, and exhibited significantly higher read counts in water than in sediment samples.

4 | Discussion

The present study investigated the differences in biodiversity monitoring using sediment and water eDNA metabarcoding across the tree of life from prokaryotes to vertebrates in a plateau lake. Sediment eDNA demonstrated significantly higher diversity compared to water eDNA for most taxonomic groups, including archaea, bacteria, algae, plants, protozoa, invertebrates, and mammals, while the opposite pattern was observed for fishes. In addition, we found significant differences in community composition for most taxonomic groups, except for mammals. Finally, this study identified taxa that contributed most to dissimilarity between sediment and water samples for each taxonomic group.

The present study revealed significantly higher ASV richness in sediment eDNA compared to water eDNA across most taxonomic groups (e.g., archaea, bacteria, algae, plants, protozoa,

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and invertebrates), consistent with findings from two other studies in marine environments (Brandt et al. 2021; Holman et al. 2019). However, these findings differed from those in riverine studies, where no significant difference in species or ASV richness was found between sediment and water samples (Naro-Maciel et al. 2022; Sakata et al. 2021). This discrepancy may be attributed to the different hydrological regimes of rivers and lakes. Previous studies have shown that eDNA concentrations are higher and degradation rates are slower in sediments compared to water (Corinaldesi et al. 2011; Sakata et al. 2020; Turner et al. 2015). The accumulation of sedimentary eDNA may be driven by the precipitation of eDNA-bearing particles from the water column, including the fecal matter containing viable epithelial cells and abundant mitochondrial DNA (Wotton and Malmqvist 2001). The slow DNA decay in sediments may result from the adsorption of both DNases and DNA molecules, combined with inhibitory effects of low-oxygen environments (Corinaldesi et al. 2011; Pietramellara et al. 2009). However, in rivers, the accumulation, spatial distribution, and preservation time of sedimentary eDNA are significantly altered by hydrodynamic scouring, differing markedly from conditions in lakes or deep-sea settings. Moreover, the distinct hydrological regimes of rivers and lakes may lead to fundamental differences in water eDNA dynamics: eDNA signals of lake samples tend to be highly localized, while eDNA samples in rivers may represent a spatially integrated signal from the upstream (Littlefair et al. 2023).

Our results showed significantly higher fish diversity detected in water samples compared to sediment samples, which aligned with previous studies (Sakata et al. 2020; Zhu et al. 2023). This finding appears contradictory to the observation that fish eDNA is more concentrated in sediments than surface water (Turner et al. 2015). One possible explanation is that, for fishes, the detection via eDNA metabarcoding is fundamentally governed by the organisms' activity ranges (e.g., mobility) and physiological processes (e.g., excretion). Fishes are mobile and primarily release eDNA into the water column through mucus, feces, or gametes. Water is a dynamic medium where eDNA from fishes is continuously released, transported, and mixed by currents, tides, and other hydrological processes. This allows eDNA from a broader spatial and temporal range to be captured in water samples, reflecting a higher diversity of fishes. In contrast, sediments act as a sink, accumulating eDNA over time but primarily reflecting local or historical inputs, which may limit the detection of transient (e.g., fishes) or less abundant species (Turner et al. 2015). Such an explanation can also account for the detection patterns observed in birds. In vegetated muddy habitats (G1 and G3), prolonged avian resting and foraging activities resulted in continuous fecal deposition and eDNA accumulation in sediments, leading to higher detected taxonomic diversity than water samples. In contrast, bare sandy/rocky areas without vegetation (G2, G4, and G5) support only transient bird visits, explaining the absence of avian eDNA in sediments and minimal detection in water samples.

The proportion of ASVs shared between different sample types was relatively low (overall 18.0%), but their contribution to read counts was relatively high (42.4% in sediment samples and 70.0% in water samples). This revealed a fundamental pattern: although a small subset of core taxa was widely distributed and abundant in both substrates, the major biodiversity was unique to each substrate. This also explained why the community composition of all aquatic taxonomic groups showed significant differences between the two substrates. The SIMPER analysis provided critical insights into the specific taxa responsible for the major community differences. The strong preference of archaeal orders Nitrososphaerales and Woesearchaeales for sediments aligned with their known roles in benthic nitrogen and carbon cycling (Luo et al. 2024). In algae, the enrichment of Bacillariophyceae (diatoms) in sediments was expected due to their siliceous frustules preserving in benthic environments (Westacott et al. 2021). In contrast, the predominant detection of Dinophyceae and Cryptophyceae in water could be attributed to their planktonic lifestyle (Sas et al. 2022). The contrasting patterns observed in key invertebrate taxa were particularly illustrative. The order Ploima (rotifers) was a major contributor associated with the water, consistent with their planktonic nature (Williamson 1983). In comparison, the orders such as Monhysterida, Crassiclitellata, and Coleoptera are typically benthic or substrate-associated (Snelgrove 1999), explaining their preference for sediments.

Overall, this study provided a comprehensive comparative assessment of sediment and water eDNA metabarcoding for monitoring biodiversity across the tree of life in a plateau lake. First, alpha diversity was significantly higher in sediments than in water for most taxonomic groups, whereas fish exhibited the opposite pattern. Second, all aquatic taxonomic groups exhibited significant differences in community composition between substrates. Finally, the ASVs primarily responsible for community dissimilarity largely exhibited distinct substrate preferences. These findings justified water sampling as an adequate method for fish monitoring, while underscoring the necessity of a multi-substrate approach for a comprehensive evaluation of other taxonomic groups. A critical consideration when using sediments for eDNA metabarcoding is that sediment eDNA profiles may not reflect real-time biodiversity, due to prolonged persistence of eDNA in sediments (Turner et al. 2015), creating a component of historical biological communities.

Author Contributions

X.F. and Y.C. conceptualized and designed the study. X.F., X.W., R.Z., Y.J., and X.S. conducted fieldwork and contributed to the acquisition of environmental samples. X.F., X.W., and Z.Q. conducted the laboratory work, analyzed and interpreted the data. X.F., X.W., R.Z., Y.J., X.S., Z.Q., and Y.C. wrote and revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequencing data have been deposited in the Genome Sequence Archive (GSA: http://gsa.big.ac.cn/) under accession number CRA028973. The ASV tables representing taxonomic information and the relative read abundance of ASVs for various taxonomic groups have been deposited at Figshare (https://doi.org/10.6084/m9.figshare.29908553).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** ASV rarefaction curves on individual PCR reactions per sample. Each sampling site was represented by a different color. Sediment samples are represented by solid lines, while water samples are shown with dashed lines. **Figure S2:** Box plots of Shannon index of different taxonomic groups (A: archaea, B: bacteria, C: algae, D: fungi, E: plants, F: protozoa, G: invertebrates, H: fishes, I: birds, J: mammals). Wilcoxon test Benjamini–Hochberg (BH) adjusted *p*-values indicating significance of ASV richness differences between sediment and water samples are shown in each plot. **Figure S3:** Cumulative contribution curve of ASVs to community dissimilarity

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between sediment and water samples. **Table S1:** Alpha diversity (ASV richness, Shannon, Simpson and Pielou's indexes) of ten taxonomic groups in each sample. **Table S2:** Results of simper analysis on ASV contribution to dissimilarity between sediment and water samples. ASVs were included in a surrogate set in order of decreasing percentage contribution to the average similarity until the cumulative contribution was 80%. ava, average abundances in the sediment group; avb, average abundances in the water group; average, ASV contribution to average between group dissimilarity; cusum, ordered cumulative contribution; ratio, average to sd ratio; sd, standard deviation of contribution.

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