

Assessing riverine fish community diversity and stability by eDNA metabarcoding

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ABSTRACT

Monitoring and evaluating river ecosystems are of paramount importance in addressing ecological and environmental challenges. While eDNA (environmental deoxyribonucleic acid)-based method has emerged as a valuable tool for understanding these issues, its application in assessing phylogenetic diversity, community stability, and the identification of key species within river systems has been limited. In this study, we conducted eDNA metabarcoding to assess fish community phylogenetic diversity, species composition, assembly variations, and their relationship with hydrologic characteristics. We also explored ecological stability and the roles of key species in a river system comprising a mainstem (MS) and a tributary (TB) with distinct hydrologic features. Our findings uncovered previously unknown endemic fish species diversity, exemplified by the discovery of *Triplophysa*. We identified disparities in diversity, community composition, and assembly between the MS and TB. Furthermore, we established significant associations between water-level variations, water flow, and changes in community composition. *Triplophysa*, with its high diversity, played a pivotal role in the ecological network, contributing significantly to community stability. The loss of *Triplophysa* increased fragility in co-occurrence patterns. Comparing the ecological network of fish communities between the MS and TB, we found that the MS displayed greater robustness and lower vulnerability, with increased species competition likely promoting network stability. Overall, this study highlights the potential of integrating eDNA metabarcoding data for monitoring phylogenetic diversity, community structure, and variations in stability within fish communities.

1. Introduction

Rivers, despite covering less than 1 % of earth's landmasses, pose a formidable challenge for biodiversity conservation and the provision of essential ecosystem services (Deiner et al., 2016). One of the major challenges is the effective monitoring and preservation of these vital but limited ecosystems. Fish species, serving as key indicators in assessing the health of river ecosystems (Li et al., 2023), provide valuable insights into the intricate web of life within these dynamic environments. Understanding fish populations is essential not only for comprehending complex nutrient cycling but also for enhancing the overall functioning and resilience of aquatic ecosystems (Darling & Côté, 2018; Holmlund & Hammer, 1999). Fish, occupying roles as prey, predators, and competitors, create an intricate balance within these ecosystems. However, the persistent decline of native fish species presents a significant challenge,

as it can lead to cascading impacts on the entire ecosystem (Berger et al., 2020; Cook, 2008). Therefore, the effective monitoring and conservation of fish communities within river ecosystems are of paramount importance, given the challenges posed by declining species and the need to maintain the integrity and sustainability of these critical environments.

In recent years, the use of eDNA has emerged as a promising tool for studying ecological communities and addressing complex environmental questions (Collins et al., 2022). In particular, eDNA metabarcoding of fish communities has become widely adopted for describing, modeling, and predicting fish populations in dynamic freshwater habitats (Hallam et al., 2021; Lecaudey et al., 2019; Sales et al., 2021; Wang et al., 2021). Among various eDNA metabarcoding primers, Mifish primers have demonstrated exceptional species resolution and the ability to detect a greater number of fish species (Li et al.,

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2023; Miya et al., 2015; Wang et al., 2021; Zhang et al., 2020). Studies have shown that eDNA metabarcoding, using Mifish primers, surpasses traditional sampling methods in discriminating assemblage compositions and providing extensive spatial data on freshwater fish biodiversity (Cilleros et al., 2019). Additionally, eDNA analysis has proven effective in accurately reconstructing phylogenetic and phylogeographic patterns of fishes (Tsuji et al., 2023). Compositional data derived from eDNA metabarcoding can be leveraged to assess community structure, identify network associations and trophic interactions, and understand how communities respond to water types, water quality, and landscape variables (Blabolol et al., 2020; Valentini et al., 2016; West et al., 2020; Zhang et al., 2022).

Rivers typically exhibit a dendritic network structure, wherein hydrologic connectivity plays a crucial role in governing the dispersal pathway from upstream to downstream (Vannote et al., 1980). Therefore, it is crucial to investigate the influence of various hydrologic characteristics on fish communities within river networks. The concept of ecological stability encompasses multiple dimensions, and ecological networks have emerged as a valuable tool for predicting interrelationships and community organization (Deng et al., 2012; Zhang et al., 2022). Understanding how complex ecological networks affect community stability is a fundamental issue in community ecology. The topological properties and complexity of networks can serve as indicators of ecological stability (de Vries et al., 2018), and various indexes such as robustness, vulnerability, and cohesion provide valuable insights in this regard (Liu et al., 2022). In network theory, a higher level of robustness and lower vulnerability suggest a more stable network (Deng et al., 2012; Wu et al., 2021). Moreover, the identification of key species and their roles within the community is an effective approach for studying community stability (Liu et al., 2022). However, the understanding of ecological stability in fish communities and the identification of key species in river systems remain limited.

The Jing River is one of the ten major tributaries of the Yellow River, and the specific branch of the Jing River represents the largest watershed area, longest river length, and highest total water resources within the region (Zhao et al., 2023). Notably, the hydrological characteristics of the Jing River and its tributaries, particularly the Rui River, exhibit significant variations, providing an exceptional opportunity to explore the impact of these hydrological differences on riverine fish communities. Moreover, the Jing River system is renowned for its remarkable fish biodiversity. Both the mainstem of the Jing River and the Rui River, one of its major tributaries, serve as primary habitats for a diverse range of fish species (Xu et al., 2022). This high diversity offers a unique platform for investigating the intricate interactions among fish species and their responses to environmental changes. In addition, the Jing River system has been a subject of historical hydrological and ecological research (Xu et al., 2015; Zhao et al., 2022), which facilitates our ability to conduct long-term analyses and assess changes in fish communities over time.

The primary objective of this study was to assess the phylogenetic diversity, community structure, and ecological stability of fishes within a river system. Additionally, we aimed to investigate the species that contribute to the stability of the fish community. While previous studies might have explored aspects of eDNA metabarcoding and fish community structures, the present manuscript delves deeper into the relationship between the hydrologic characteristics of river systems and the stability of fish communities. To accomplish these objectives, we conducted a comprehensive analysis of the fish community in the study area. We collected eDNA samples and reconstructed the phylogenetic relationships among the fish species present. Furthermore, we investigated the community structure by analyzing the composition, abundance, and distribution patterns of fish species in both the MS and TB. To evaluate ecological stability, we examined various aspects of the ecological networks, including their topological properties and complexity, as well as indexes such as robustness, vulnerability, and cohesion. We had two main hypotheses: (1) Differences in diversity,

assembly, and stability of fish communities would exist between the MS and TB due to their distinct hydrologic conditions; and (2) Specific key species would be essential for maintaining community stability within river systems.

2. Material and methods

2.1. Site description and sample collection

We conducted eDNA metabarcoding sequencing to characterize fish communities along an approximately 110 km stretch of a river. The sampling was carried out in July 2022 on the Jing River mainstem and its tributary, the Rui River (Fig. 1), both of which belong to the Yellow River basin in China. The mainstem and tributary presented distinct hydrologic conditions. For the sampling process, we collected a total of 41 water samples from 15 different locations. This included 8 sites (with 21 samples) in the mainstem and 7 sites (with 20 samples) in the tributary.

To prevent cross-contamination, we ensured the sterility of the sampling equipment by washing it with a 30 % commercial bleach solution containing less than 3 % sodium hypochlorite. Subsequently, the equipment was thoroughly rinsed with distilled water after each sampling site. The water flow measurements at each location were obtained using a Global FP111 flow probe from Geo scientific Ltd., based in Vancouver, British Columbia, Canada. We collected 1 L of water for each sample, which was immediately placed in a cool box filled with ice packs to maintain a temperature of approximately 4 °C during transportation and storage. In the laboratory, within 24 h of sample collection, each water sample was filtered using a 142 mm diameter polycarbonate membrane with a pore size of 0.22 µm. Subsequently, the filters were securely stored in a sterile freezer at −80 °C until further processing. These sampling and filtration procedures were implemented to ensure the preservation and integrity of the eDNA samples, allowing us to accurately analyze the fish communities present in the river system.

2.2. eDNA extraction and high-throughput sequencing

For total eDNA extraction, we followed the protocol provided by the Qiagen DNeasy Blood & Tissue Kit (Collin et al., 2019). The concentration and quality of the extracted DNA were assessed using a Qubit 3.0 Fluorometer (ThermoFisher, USA). To construct the sequencing library, PCR (Polymerase Chain Reaction) amplicons with unique indexes were generated. The MiFish-F (5'-GTCGGTAAACTCGTGCCAGC-3') and MiFish-R (5'-CATAGTGGGGTATCTAATCCCAGTTTG-3') primer pair was utilized to amplify a ~170 bp fragment of the mitochondrial 12S rRNA (ribosomal Ribonucleic acid) gene from fish mitochondrial genomes (Miya et al., 2015). Custom 8 bp barcodes were incorporated into the primers to differentiate between different samples. In the first PCR amplification, each reaction was carried out in a 30 µL volume, comprising of 15 µL of 2 × Hieff® Robust PCR Master Mix (Yeasen Biotechnology, Shanghai, China), 1.5 µL of each primer (10 µM), 1 µL of DNA template (10 ~ 20 ng), and 11 µL of ddH₂O. The PCR conditions consisted of an initial denaturation step at 94 °C for 3 min, followed by 5 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 20 s, extension at 65 °C for 30 s, and then 28 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. Each sample was subjected to three technical replicates, and the replicates were combined to minimize PCR stochasticity. The pooled PCR products were visualized on a 2 % agarose gel stained with GelRed (Cambridge Bioscience, UK). The purified PCR products served as the template DNA for a second round of PCR, which involved attaching Illumina indexes. The second PCR was performed in a 30 µL reaction volume, containing 15 µL of 2 × Hieff® Robust PCR Master Mix, 1.5 µL of each primer (10 µM), 1 µL of the purified product, and 11 µL of ddH₂O (double-distilled water). The PCR conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 8 cycles

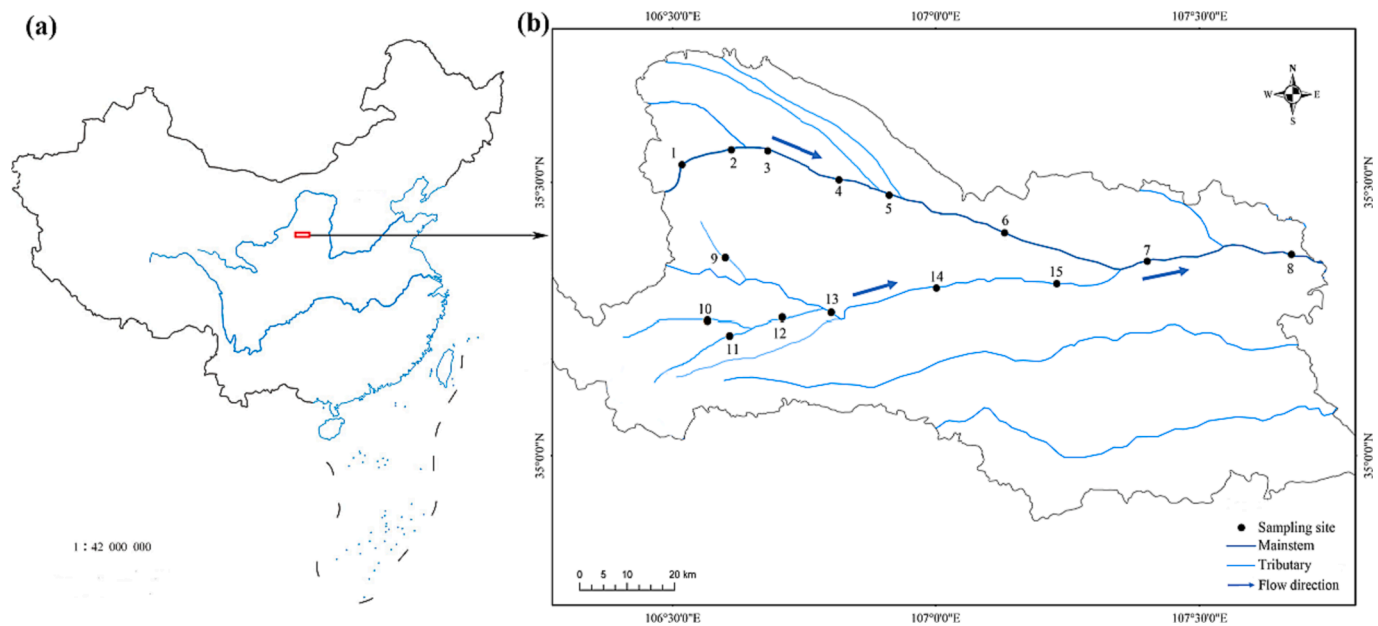


Fig. 1. (a) Locations of rivers sampled within China and (b) sampling sites of the eDNA in MS and TB.

of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR duplicates were performed, and the replicates were pooled prior to visualization on a 2 % agarose gel stained with GelRed. High-throughput sequencing was conducted on the Illumina NovaSeq 6000 PE250 platform (Illumina, San Diego, USA) at Novogene Co., Ltd. Following these procedures, we successfully generated sequencing data that accurately represented the fish communities present in the river system.

2.3. Sequence preprocessing and bioinformatics approaches

The raw sequencing data underwent initial processing steps within the QIIME (Quantitative Insights Into Microbial Ecology) pipeline (Caporaso et al., 2010). This included demultiplexing, trimming of barcode and primer sequences from paired-end reads, and subsequent merging using FLASH. To ensure the retention of high-quality data, reads shorter than 200 bp were excluded. Subsequently, operational taxonomic units (OTUs) were delineated at a 97 % similarity threshold using the UPARSE (Ultra-fast Pipeline for Amplicon Sequence Analysis) algorithm (Edgar, 2013). OTUs were subsequently annotated using a local blast search against the Nucleotide database GenBank in NCBI and MitoFish (Mitochondrial Genome Database of Fish). The blast search returned 10 optimal hits from each of the aforementioned databases, and taxonomic assignments were confirmed after comparative screening. To determine the presence of fish species, we gathered information on historical fish species in the river basin. This information was used as a reference to validate the identified fish species. Negative controls were included in the analysis to control for potential contamination. Following thorough bioinformatics analysis, no valid sequences were identified in the negative controls, and they were subsequently excluded from further analysis. By implementing these steps, we ensured the reliability and accuracy of our data for subsequent analyses and interpretation.

2.4. Ecological, phylogenetic and statistical analysis

Statistical analyses were conducted using SPSS software. Group differences were assessed using ANOVA (Analysis of Variance), a parametric statistical method chosen for its suitability in comparing means across multiple river sections, each representing distinct groups characterized by their unique hydrological features. To further investigate

specific group differences, post-hoc tests were employed, including the LSD (Least Significant Difference) test and Tukey's test. The significance level was set at $P < 0.05$, indicating statistical significance. Phylogenetic tree construction was carried out using MEGA software (v11.0.10) (Tamura et al., 2021). We employed the Maximum Likelihood (ML) method with the Nearest-Neighbor Interchange (NNI) algorithm and bootstrapping ($n = 1000$) for tree inference. This approach best suits our goal of accurately depicting fish species relationships. ML balances computational efficiency and tree optimization, while NNI efficiently explores tree space. Bootstrapping ($n = 1000$) ensures robust branch support.

NMDS (Non-metric multidimensional scaling) analysis was used to visualize and compare the community compositions. Dissimilarity tests, including Jaccard dissimilarity distance matrix, were employed to examine the dissimilarity between groups. PERMANOVA (Permutational multivariate analysis of variance) was employed to rigorously assess dissimilarity significance. These widely accepted ecological methods were chosen for their ability to handle complex multivariate data, compare similarity matrices, and analyze various experimental designs. We employed one-sample Student's t-tests to assess the characteristics of each treatment pair, including the disparities between observed and random networks. The relationship between fish communities and river course characteristics was evaluated using the mantel test, which calculated the correlation between the community data and the environmental variables. To identify specialist taxa associated with different communities, LefSe (Linear discriminant analysis Effect Size) analysis based on LDA (Linear Discriminant Analysis) score was performed at the OTU level. This method is widely acknowledged for its effectiveness in identifying OTUs that contribute significantly to differences between predefined groups. Specifically, we applied a criterion of an LDA score of 3.0, coupled with a significance level of 0.05, to pinpoint OTUs that were significantly enriched or depleted in either the MS or TB communities. The LDA score serves as a quantitative measure of the effect size, enabling us to prioritize the most influential OTUs in shaping community distinctions. To further elucidate the most distinguishing taxa between the MS and TB communities, we harnessed the power of the Random Forests algorithm. This machine learning ensemble method, implemented using the RF package v.4.6–14 with default parameters (Liaw and Wiener, 2002; Statnikov et al., 2008), excels in feature selection and classification tasks. In our study, it played a pivotal role in identifying key taxa that contributed to the

differentiation between these two ecological niches.

To understand the relative importance of different community assembly processes, the iCAMP (infer community assembly mechanism by phylogenetic-bin-based null model analysis) approach was utilized (Ning et al., 2020). By employing these analytical methods, we aimed to comprehensively investigate and interpret the patterns and dynamics of fish communities in relation to hydrological conditions and community assembly processes.

2.5. Ecological network stability analysis

We employed MENA (Molecular Ecological Network Analysis) pipelines to construct MENs (Molecular Ecological Networks). MENs provide a valuable framework for exploring ecological interactions within complex microbial communities. In our analysis, we utilized a RMT (Random Matrix Theory)-based approach, as detailed by Deng et al. (2012). Specifically, MENs were independently constructed for both the mainstem (MS) and tributary (TB) communities. Gephi 0.9.2 software was employed for network visualization.

To assess network robustness, the abundance-weighted mean inter-

action strength of each node ($wMIS_i$) was calculated using the equation:

$$wMIS_i = \frac{\sum_{j \neq i} b_j s_{ij}}{\sum_{j \neq i} b_j} \quad (1)$$

Here, b_j represents the relative abundance of species j , and s_{ij} denotes the association strength between species i and j , measured by the Pearson correlation coefficient. Nodes with $wMIS_i$ values ≤ 0 were removed from the network, and the proportion of remaining nodes was reported as the network robustness.

Vulnerability was evaluated using the equation:

$$\max\left(\frac{E-E_i}{E}\right) \text{ and } E = \frac{1}{n(n-1)} \sum_{i \neq j} \frac{1}{d_{ij}} \quad (2),$$

where E represents the global efficiency of the network and E_i denotes the global efficiency after removing node i and its associated links. Vulnerability measures the impact of removing node i and its connections on the overall network performance.

Two cohesion values (positive and negative) to measure the degree of cooperative behaviors or competitive interactions correlation (Herren and McMahon 2017), which could indirectly represent the complexity, were calculated as the following equations:

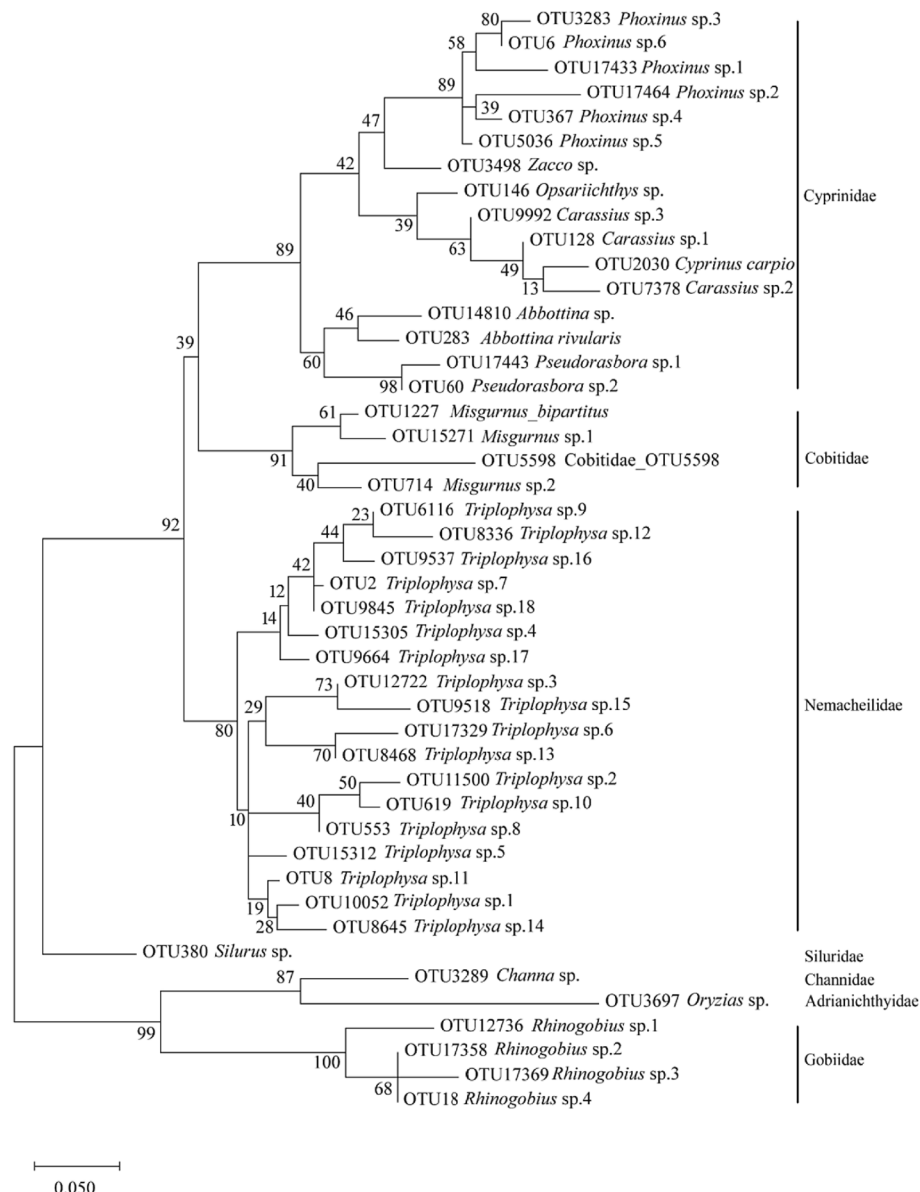


Fig. 2. Maximum likelihood phylogenetic tree of 12 S rRNA (MiFish) gene sequences. The numbers at the nodes indicate bootstrap probabilities.

$$c_j^{pos} = \sum_{i=1}^n a_i \bullet \bar{r}_{i,j} > 0 (\text{Positive Cohesion}) \quad (3)$$

And

$$c_j^{neg} = \sum_{i=1}^n a_i \bullet \bar{r}_{i,j} < 0 (\text{Negative Cohesion}) \quad (4)$$

Where a_i is the abundance of OTU i in the sample j and $\bar{r}_{i,j}$ is the connectedness. By employing these calculations and equations, we were able to assess the MENs' robustness, vulnerability, and cohesion, providing insights into the interactions within the MS and TB communities.

3. Results

3.1. Fish diversity, composition and community assembly

A total of 2,786,920 filtered reads were obtained from the 41 samples, with an average of 67,974 reads per sample (range: 32,545 to 117,962). We observed 3 to 27 OTUs in each sample. Among these reads, 45 OTUs were identified as fish species, representing a total of 149,302 reads. These OTUs belonged to 7 families and 11 genera, including 16 OTUs in *Cyprinidae*, 4 OTUs in *Cobitidae*, 18 OTUs in *Nemacheilidae*, 1 OTU in *Siluridae*, 1 OTU in *Channidae*, 1 OTU in *Adrianichthyidae*, and 4 OTUs in *Gobiidae* (Fig. 2).

At the genus level, differences in fish community compositions were observed between the MS and TB (Fig. 3a). In the MS, *Triplophysa* accounted for 85 % of the fish eDNA sequences, while *Phoxinus* accounted for 12 % (Fig. 3a). In the TB, *Triplophysa* represented 34 % of the fish eDNA sequences, *Phoxinus* accounted for 26 %, and *Rhinogobius* accounted for 13 % (Fig. 3a). NMDS analysis based on Jaccard distance and dissimilarity analysis using Bray-Curtis distance confirmed significant differences in community compositions between the MS and TB (Fig. 3b, Table S1). To understand the assembly processes of fish communities, we employed the iCAMP method, which indicated that stochasticity played a crucial role in driving fish community assembly in both the MS and TB (Fig. 3c). However, the percentage of stochasticity was higher in the MS (84.1 %) compared to the TB (71.1 %) (Fig. 3c).

These findings demonstrate notable differences in fish community compositions between the MS and TB, as well as the dominant role of stochastic processes in shaping fish community assembly in the studied river system.

3.2. Relationship between hydrological characteristics and fish community

Three hydrologic characteristics were considered to assess their influence on the fish community in the MS and TB of the river. The water flow was found to be 0.55 ± 0.11 m/s in the MS and 0.37 ± 0.14 m/s in the TB. Regarding water level, it was measured as 43.38 ± 23.13 m in the MS and 18.88 ± 9.29 m in the TB. Additionally, the river width was determined to be 22.5 ± 14.47 m in the MS and 15.80 ± 12.86 m in the TB. Pairwise comparisons were conducted to examine the differentiation of water flow, water level, and river width between the MS and TB (Figure S1). The results indicated significant differences ($P < 0.05$) in water flow and water level between the two locations. To explore the relationship between hydrologic characteristics and fish community composition, correlations were analyzed. The mantel test was performed to examine the associations between the number of reads per OTU and the hydrologic variables. The results revealed significant correlations between water level (Bray-Curtis $r = 0.1440$, $P = 0.011$; Jaccard $r = 0.2591$, $P = 0.001$) and water flow (Bray-Curtis $r = 0.1296$, $P = 0.011$; Jaccard $r = 0.2114$, $P = 0.003$) with the distribution of fish communities (Table S2). These findings highlight the significant influence of water flow and water level on the composition and distribution of fish communities in the studied river system.

3.3. Key species required for maintaining stable communities

To explore the inter-relationships within fish communities, the process of constructing molecular ecological networks was implemented. Notably, *Triplophysa* species occupied a core position in these interactive networks (Fig. 4). In the MS, the nodes with the highest degree were *Triplophysa* sp.1, *Triplophysa* sp.9, and *Triplophysa* sp.16, while in the TB, it was *Triplophysa* sp.8 (Table S3). Furthermore, *Triplophysa* sp.1 and *Misgurnus* sp.2 had the highest betweenness and stress centrality, respectively, in both MS and TB (Table S3). The nodes with the highest eigenvector centrality were *Triplophysa* sp.16 in MS and *Triplophysa* sp.8 in TB (Table S3). Regarding closeness centrality, *Triplophysa* sp.1, *Triplophysa* sp.9, and *Triplophysa* sp.16 had the highest values in MS, while *Triplophysa* sp.8 had the highest value in TB (Table S3).

At the OTU level, the LEfSe analysis identified 13 specialist OTUs characterizing the MS communities and 7 specialist OTUs characterizing the TB communities (Fig. 5a). Among the specialists in MS, there were *Triplophysa* species and *Phoxinus* species, while specialists in TB included *Abbottina rivularis*, *Carassius* species, *Opsariichthys* species, *Pseudorasbora* species, *Rhinogobius* species, and *Silurus* species (Fig. 5a). Using the random forests machine learning algorithm, 21 OTUs were identified as the most important biomarkers in both MS and TB (Fig. 5b). *Triplophysa*

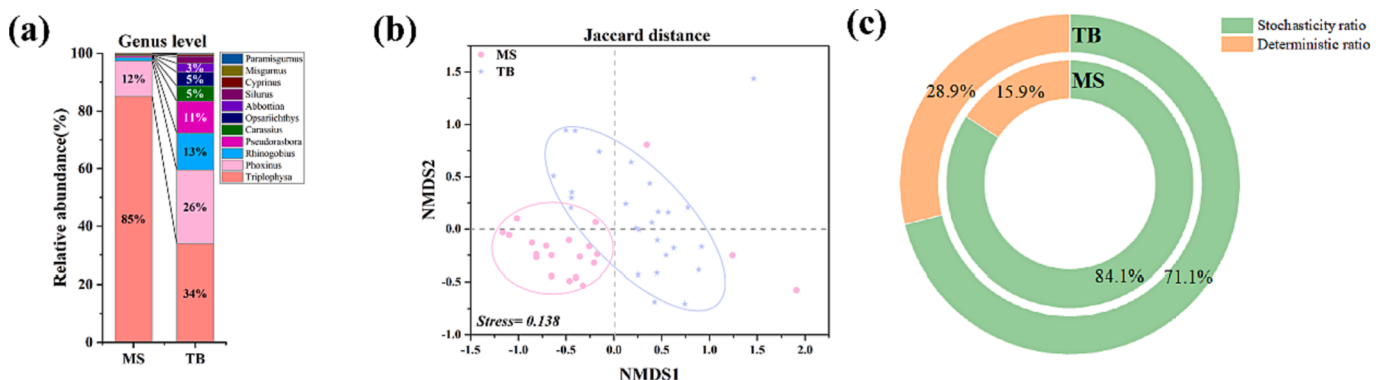


Fig. 3. Differences in community composition and assembly in MS and TB. (a) Relative sequence abundances of identified genera in MS and TB. (b) NMDS plot of fish species community compositions at different eDNA sampling sites. The community was significantly differentiated into two groups. (c) Ecological processes of fish community assembly based on infer Community Assembly Mechanisms by Phylogenetic-bin (iCAMP) in MS and TB. Different colors represent the different assembly processes.

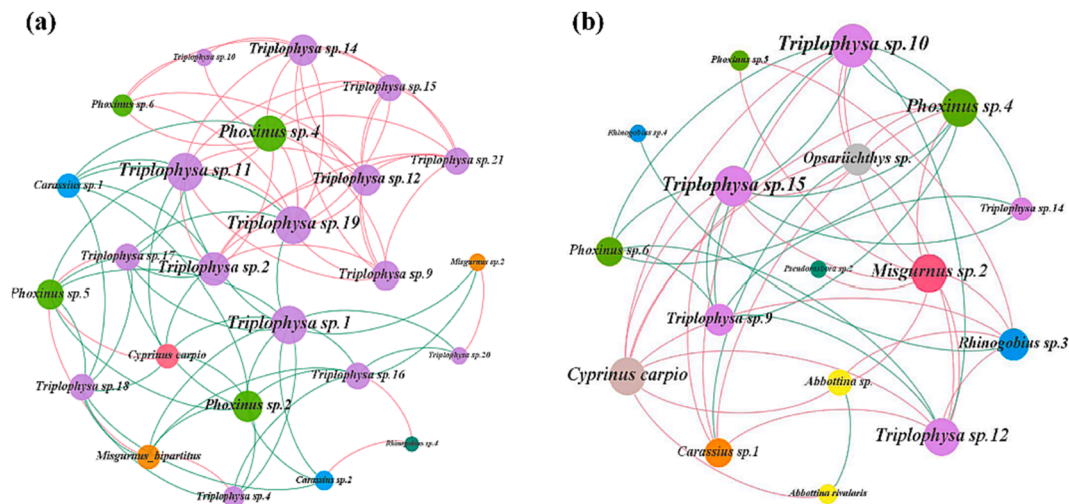


Fig. 4. Molecular ecological networks of fish communities in (a) MS and (b) TB. The size of the node represents the node degree of the OTUs (The number of connections among OTUs). Node color represents taxonomic groups within each network. Lines connecting nodes (edges) represented positive (red) and negative (green) interactions. The networks were visualized using Gephi 0.9.2 software. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

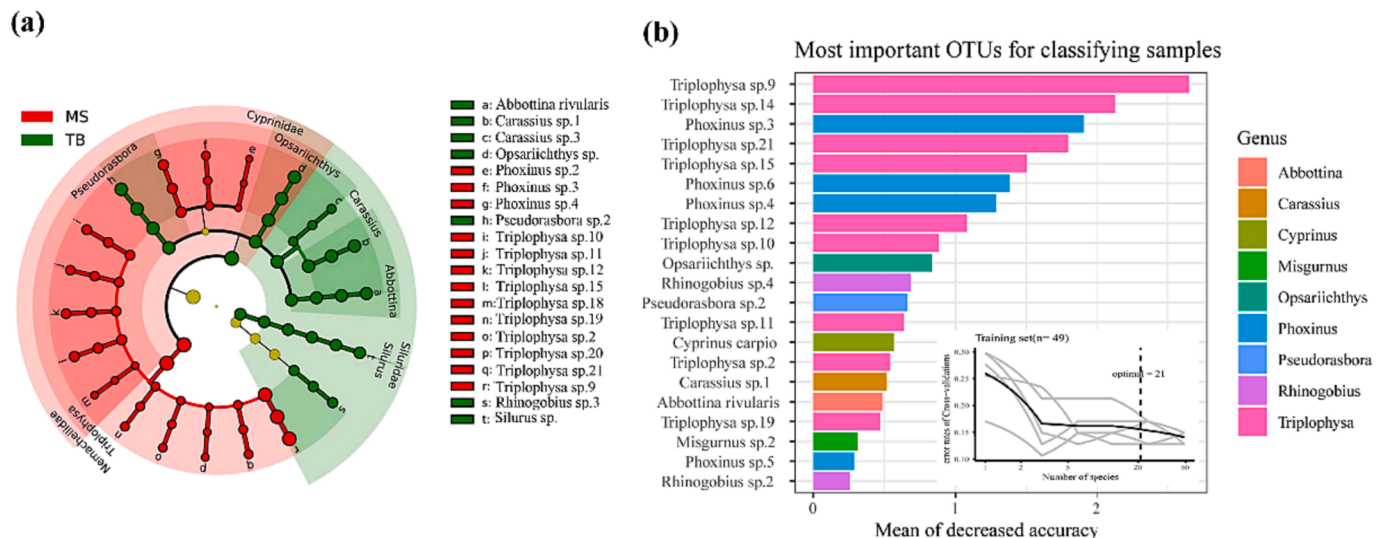


Fig. 5. Key species screening for maintaining communities in MS and TB. (a) LefSe analysis of fish abundance between MS and TB. (b) The top 21 biomarker bacterial OTUs are accurately identified by Random-forest model. The biomarkers are ranked in descending order of importance to the accuracy of the model. The inset represents 10-fold cross-validation error as a function of the number of input OTUs used to differentiate MS and TB community ranked by variable importance.

sp.9 was particularly important in differentiating between MS and TB communities (Fig. 5b).

Assessing the vulnerability of co-occurrence relationships within fish communities after species loss, it was found that the network became more vulnerable with the decrease of *Triplophysa* species (Table S4). Specifically, the loss of *Triplophysa* sp.1 and *Misgurnus* sp.2 would have the greatest negative impact on the coexistence of MS and TB, respectively (Table S4). These findings highlight the significance of *Triplophysa* species in the molecular ecological networks and their potential impact on the overall stability and dynamics of fish communities in the studied system.

3.4. Networks stability, vulnerability and complexity

The stability of the fish community's topology was evaluated by calculating the robustness and vulnerability of the MENs using empirical data. Robustness refers to the proportion of species that remain in the

network after random or targeted removal of nodes (Deng et al., 2012). In our study, random species removal simulations involved the random removal of 50 % of the nodes. The results demonstrated that the MS exhibited significantly higher robustness compared to the TB ($P < 0.05$) (Fig. 6a). Moreover, the vulnerability of the MS was lower than that of the TB (Fig. 6b), indicating a higher level of topological stability in the MS.

To further investigate the extent of cooperation and competition within the fish communities, a novel index called cohesion was employed (Fig. 7). This index provides insights into the collective interactions among species. The analysis showed that the MS had a lower negative cohesion value (indicating higher competition) compared to the TB (Fig. 7a), while the positive cohesion value (indicating cooperation) in the MS was lower than that in the TB (Fig. 7b). These findings suggest that the fish community in the MS exhibited higher resistance and lower cooperation compared to the TB. In summary, these results indicate that the fish community in the MS demonstrated higher

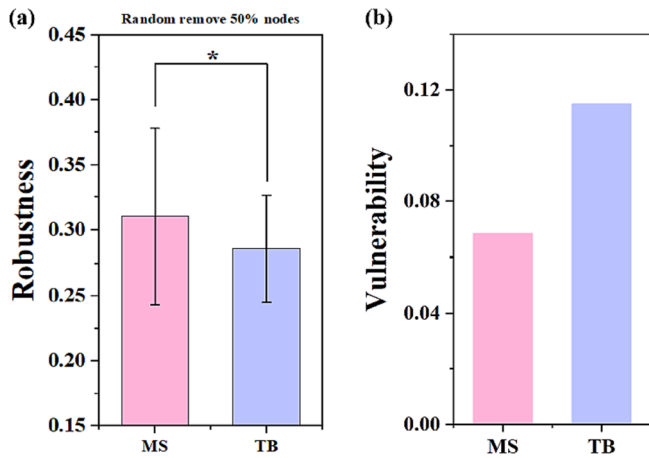


Fig. 6. Stability including (a) Robustness and (b) vulnerability analyses for fish communities in MS and TB. Robustness measured as the proportion of taxa remaining after 50 % of the taxa are randomly removed from each of the empirical MENs. “*” mean the significantly differed at the level of $P < 0.05$.

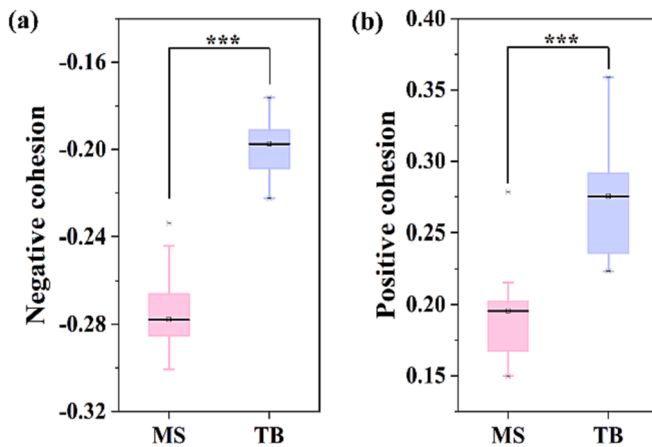


Fig. 7. Difference in cohesion value of fish communities in MS and TB. The difference was calculated by ANOVA analysis, and the same letter indicates no difference ($P < 0.05$). (a) The community negative cohesion, and (b) the community positive cohesion. “***” mean the significantly differed at the level of $P < 0.001$.

topological stability and resistance compared to the TB, as evidenced by their higher robustness, lower vulnerability, and higher competition.

4. Discussion

To effectively manage and conserve fish biodiversity in river systems, it is crucial to understand species composition, assembly mechanisms, and stability variations (Sales et al., 2021). Traditional biological monitoring methods are often biased, while the use of eDNA has emerged as a promising approach for accurate species identification (Wang et al., 2021). However, to date, the eDNA method's application in studying the stability of riverine fish communities has been limited. In our study, we employed eDNA metabarcoding to comprehensively assess various aspects of fish communities in the MS and TB, including phylogenetic taxonomy, community structure, assembly processes, stability, and key species. By utilizing eDNA, we overcame the limitations associated with traditional monitoring methods, obtaining a more accurate and unbiased understanding of fish biodiversity. While we observed differences in stability between the mainstem (MS) and tributary (TB), with the MS demonstrating higher topological stability and resistance, it's important to note that these findings provide valuable

insights into the ecological dynamics of riverine fish communities. Understanding such variations is essential for effective resource management and conservation efforts.

4.1. Enhanced understanding of biodiversity through fish eDNA metabarcoding

In our study, we harnessed the power of eDNA metabarcoding, a cutting-edge technique that offers several advantages for assessing biodiversity at the ecosystem level. This method allows us to identify a wide range of taxa from easily collectible environmental samples (He et al., 2022). Recent studies have also highlighted the remarkable accuracy of eDNA analysis in reconstructing phylogenetic relationships among fish species (Tsuiji et al., 2023). One of the standout features of eDNA metabarcoding is its ability to uncover hidden diversity and resolve taxonomic uncertainties (McClenaghan et al., 2020). This becomes especially valuable when dealing with taxa that pose challenges in species-level identification based solely on morphological characteristics. In our case, eDNA proved to be a game-changer, significantly improving taxonomic resolution compared to traditional morphological identification methods. A particular focus of our study was the genus *Triplophysa*, a group of small to medium-sized fish inhabiting river bottoms within the family Nemacheilinae, order Cypriniformes (Zhang et al. 2009). The river system we investigated serves as a biodiversity hotspot for *Triplophysa* species. This genus, however, has long concealed a substantial amount of unknown biodiversity, with many species yet to be formally recognized or described (Wang et al., 2020). Previous research endeavors had employed DNA barcoding to shed light on the cryptic diversity within *Triplophysa* (Wang et al., 2020). In this context, our study stands as a pioneering effort, harnessing the capabilities of eDNA metabarcoding to unveil the previously undocumented diversity of endemic *Triplophysa* species. The high taxonomic resolution achieved through eDNA metabarcoding was instrumental in revealing this hidden species richness within the *Triplophysa* genus. Not only does this advance our understanding of biodiversity patterns, but it also sheds light on the pressing conservation needs of riverine ecosystems. In essence, our findings underscore the potency of eDNA metabarcoding as an invaluable tool for assessing biodiversity. This approach excels, especially in the detection and characterization of cryptic and endemic taxa. By transcending the limitations of traditional morphological identification methods, eDNA metabarcoding provides a comprehensive and accurate view of biodiversity dynamics within riverine ecosystems. As we continue to refine and apply eDNA approaches, the potential for advancing our knowledge of poorly described taxa, like the diverse *Triplophysa* genus, is considerable. This breakthrough methodology promises to play a pivotal role in our ongoing efforts to conserve and manage the rich biodiversity of river ecosystems.

4.2. Fish communities' structure and assembly were different between MS and TB

Spatial and temporal dynamics in hydrology have been widely acknowledged as crucial factors influencing the dynamics of fish assemblages (Magoulick et al., 2021). Previous research has indicated that alterations in water levels can have a substantial impact on fish abundance and contribute to spatial and temporal variations in fish communities (Thomaz et al., 2007; Mims and Olden, 2013; Couto et al., 2018). Additionally, river discharge has been identified as a potential factor contributing to differences in fish community structure across river systems (Li et al., 2023). Consistent with these findings, our study revealed distinct species and community compositions between the MS and TB, which can be attributed to the pronounced differences in hydrologic features. While our study primarily focused on hydrological conditions, it's acknowledged that environmental factors, especially water quality, land use, and the impacts of climate change, can exert significant influence on community assembly. These factors, in

conjunction with hydrological conditions, shape the dynamics of fish communities. Further research is warranted to investigate these factors and their interplay with hydrological conditions to provide a more comprehensive understanding of riverine fish community dynamics in the Yellow River Basin.

Previous research has demonstrated that the relative contributions of deterministic and stochastic assembly processes can vary across different systems (Giam and Olden, 2016; Jia et al., 2021). Our findings demonstrated that fish community assembly in both the mainstem (MS) and tributary (TB) was influenced by a combination of deterministic and stochastic processes, with stochastic processes exerting a predominant influence. These findings align with similar community assembly patterns observed in various organisms across different ecosystems. For instance, wetland ecosystems have shown similar patterns in the assembly of vegetation, macroinvertebrates, and birds (Daniel et al., 2019), while lakes have exhibited comparable assembly patterns in aquatic plants, macroinvertebrates (Heino and Tolonen, 2017), and fishes (Liu and Wang, 2018; Jia et al., 2021). The co-occurrence of both deterministic and stochastic assembly processes highlights the complexity of fish community dynamics and underscores the importance of considering multiple ecological mechanisms in understanding community assembly. While deterministic processes, such as environmental filtering and niche differentiation, may shape the presence of certain fish species in response to specific habitat conditions, stochastic processes, including dispersal and chance colonization events, also play a significant role in shaping community composition (Zhou and Ning, 2017). By incorporating both deterministic and stochastic processes into our understanding of fish community assembly, we can develop more accurate models and management strategies for preserving and conserving fish biodiversity in river systems. Future research in the Yellow River Basin should prioritize the integration of hydrological data with environmental factors, such as water quality, land use, and climate change. Such an integrated approach will offer a holistic view of the factors influencing fish communities in the region. By combining traditional hydrological assessments with these environmental parameters, we can achieve a more nuanced and accurate understanding of biodiversity dynamics, community assembly, and stability in riverine ecosystems.

4.3. Key endemic species played an active role in regulating fish community stability

White et al. (2020) demonstrated that individual species can have both positive and negative contributions to ecosystem stability. Consistent with this, our findings from interactive networks, LefSe, and RF analyses also indicate that *Triplophysa* plays a crucial role as a key species in maintaining the stability of fish communities. LefSe analysis revealed that *Triplophysa* exhibited habitat specialization in the MS, which is known to contribute to the stability of ecological communities (Gad et al., 2020; Kolasa and Li, 2003; Liao et al., 2016). The analysis of interactive networks and vulnerability further supported the importance of *Triplophysa* species for the overall stability of fish communities, particularly in the MS. The exclusion of these key taxa would lead to unstable networks, highlighting their crucial role in preserving the overall stability of the community. Therefore, the management and conservation efforts should prioritize the protection of *Triplophysa* species to ensure the stability and resilience of the fish community. These findings highlight the importance of considering individual species and their ecological roles in promoting ecosystem stability. By focusing on key species like *Triplophysa*, managers and conservationists can implement targeted measures to preserve their habitats and protect the stability of fish communities. While dominance can be indicative of a species' influence, our approach goes beyond dominance to identify key species based on their ecological roles, ensuring a more comprehensive understanding of their significance in ecosystem stability. Future research should delve deeper into understanding the specific mechanisms through which *Triplophysa* contributes to stability, enabling more

effective conservation strategies and sustainable management of riverine ecosystems.

4.4. Fish community stability varied significantly between MS and TB

To the best of our knowledge, no previous studies have investigated fish community stability using eDNA monitoring data. Given the distinct hydrologic conditions in the MS and TB, we hypothesized that the stability of fish communities might differ between the two areas. Ecological networks offer a valuable approach for characterizing species interactions and assessing ecological stability (Yuan et al., 2021). Our results revealed that the network robustness was higher in the MS compared to the TB, while the network vulnerability was lower in the MS. Previous research has indicated that a lower ratio of negative-to-positive cohesion indicates unstable properties under stress (Hernandez et al., 2021). In ecological systems, a substantial proportion of negative correlations within communities is essential for upholding stability (Liu et al., 2020). In our study, we used the ratio of negative to positive cohesions as an indicator of community stability (Fig. 7). We found that the fish communities in the MS, characterized by higher water levels and faster water flow (Figure S1), potentially exhibited overlapping niches, leading to increased competition (Pastore et al., 2021). Consequently, the resistance of fish communities in the MS and TB to external stressors showed significant differences. Preserving fish community interactions, particularly the key role of *Triplophysa* within the community, may be crucial for enhancing the resistance of fish communities in the MS. These findings highlight the importance of considering fish community stability in the context of different hydrologic conditions and ecological interactions. By understanding the mechanisms underlying stability and the role of key species like *Triplophysa*, conservation efforts can be targeted to protect and sustain fish communities in riverine ecosystems. Future studies should further investigate the specific mechanisms driving community stability and explore management strategies that promote the resilience of fish communities under changing environmental conditions.

5. Conclusion

In conclusion, our study demonstrates the potential of eDNA metabarcoding analysis for assessing the phylogenetic diversity, community structure, and ecological stability of riverine fish communities. The use of eDNA metabarcoding allowed us to accurately reconstruct the phylogenetic relationships of fishes and uncover hidden endemic species. We observed distinct differences in community compositions, community assembly processes, and ecological stability between the MS and TB, which can be attributed to the unique hydrologic features of each system. Importantly, our findings highlight the key role of *Triplophysa* as an indicator species for community stability. These endemic fish species play a significant role in maintaining the stability of fish communities in regional rivers. Therefore, conserving and managing the populations of *Triplophysa* species is essential for promoting regional biodiversity conservation efforts. However, it's important to acknowledge our study's limitations include its focus on the specific Jing River system, caution is needed when applying our findings to other river ecosystems. Future research should expand sampling, use complementary methods, and study diverse riverine environments for a broader understanding and more robust conservation strategies. Overall, our work underscores the utility of eDNA metabarcoding data in evaluating the phylogenetic diversity, community structure, and stability variations of fish assemblages in river systems. This approach provides valuable insights for sustainable management and conservation strategies aimed at preserving the integrity and stability of riverine ecosystems. Further research should continue to explore the potential applications of eDNA metabarcoding in assessing the dynamics of fish communities and informing conservation practices in riverine environments.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2023.111222>.

References

- Cilleros, K., Valentini, A., Allard, L., Dejean, T., Etienne, R., Grenouillet, G., Brosse, S., 2019. Unlocking biodiversity and conservation studies in high diversity environments using environmental DNA (eDNA): a test with Guianese freshwater fishes. *Mol. Ecol. Resour.* 7, 1–20.
- Collins, R.A., Baillie, C., Halliday, N.C., Rainbird, S., Sims, D.W., Mariani, S., Genner, M. J., 2022. Reproduction influences seasonal eDNA variation in a temperate marine fish community. *Limnol. Oceanogr. Lett.* 7, 443–449.
- Couto, T.B.D.A., Zuanon, J., Olden, J.D., Ferraz, G., 2018. Longitudinal variability in lateral hydrologic connectivity shapes fish occurrence in temporary floodplain ponds. *Can. J. Fish. Aquat. Sci.* 75 (2), 319–328.
- Darling, E.S., Côté, I.M., 2018. Seeking resilience in marine ecosystems. *Science* 359, 986–987.
- de Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., Girlanda, M., Gweon, H.S., Hallin, S., Kaisermann, A., Keith, A.M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K. E., Oliver, A., Ostle, N., Prosser, J.I., Thion, C., Thomson, B., Bardgett, R.D., 2018. Soil bacterial networks are less stable under drought than fungal networks. *Nat. Commun.* 9, 3033.
- Deiner, K., Fronhofer, E.A., Mächler, E., Walser, J.C., Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyor belts of biodiversity information. *Nat. Commun.* 7:12544.
- Deng, Y., Jiang, Y.H., Yang, Y.F., He, Z.L., Luo, F., Zhou, J.Z., 2012. Molecular ecological network analyses. *BMC Bioinf.* 13, 113.
- Gad, M., Hou, L., Li, J., Wu, Y., Rashid, A., Chen, N., Hu, A., 2020. Distinct mechanisms underlying the assembly of microeukaryotic generalists and specialists in an anthropogenically impacted river. *Sci. Total Environ.* 15 (748), 141434.
- Giam, X., Olden, J.D., 2016. Environment and predation govern fish community assembly in temperate streams. *Glob. Ecol. Biogeogr.* 25 (10), 1194–1205.
- Hallam, J., Clare, E.L., Jones, J.I., Day, J.J., 2021. Biodiversity assessment across a dynamic riverine system: A comparison of eDNA metabarcoding versus traditional fish surveying methods. *Environmental DNA* 3, 1247–1266.
- He, W.L., Xu, D.P., Liang, Y.D., Ren, L., Fang, D.A., 2022. Using eDNA to assess the fish diversity and spatial characteristics in the Changjiang River-Shijiu Lake connected system. *Ecol. Ind.* 139, 108968.
- Heino, J., Tolonen, K.T., 2017. Untangling the assembly of littoral macroinvertebrate communities through measures of functional and phylogenetic alpha diversity. *Freshwater Biol.* 62 (7), 1168–1179.
- Hernandez, D.J., David, A.S., Menges, E.S., Searcy, C.A., Afkhami, M.E., 2021. Environmental stress destabilizes microbial networks. *ISME J.* 15, 1722–1734.
- Holmlund, C.M., Hammer, M., 1999. Ecosystem services generated by fish populations. *Ecol. Econ.* 29 (2), 253–268.
- Jia, Y.T., Jiang, Y.H., Liu, Y.H., Sui, X.Y., Feng, X., Zhu, R., Li, K.M., Chen, Y.F., 2021. Unravelling fish community assembly in shallow lakes: Insights from functional and phylogenetic diversity. *Rev. Fish Biol. Fish.* 32, 623–644.
- Lecaudey, L.A., Schletterer, M., Kuzovlev, V.V., Hahn, C., Weiss, S.J., 2019. Fish diversity assessment in the headwaters of the Volga River using environmental DNA metabarcoding. *Aquat. Conserv. Mar. Freshwat. Ecosyst.* 29 (10), 1785–1800.
- Li, Z.Y., Jiang, P.W., Wang, L.X., Liu, L., Li, M., Zou, K.S., 2023. A comparison of seasonal composition and structure of fish community between environmental DNA technology and gillnetting in the Pearl River Estuary. *China. Ecological Indicators* 147, 109915.
- Liu, X., Wang, H., 2018. Contrasting patterns and drivers in taxonomic versus functional diversity, and community assembly of aquatic plants in subtropical lakes. *Biodivers. Conserv.* 27 (12), 3103–3118.
- Liu, S.W., Yu, H., Yu, Y.H., Huang, J., Zhou, Z.Y., Zeng, J.X., Chen, P.B., Xiao, F.S., He, Z. L., Yan, Q.Y., 2022. Ecological stability of microbial communities in Lake Donghu regulated by keystone taxa. *Ecol. Ind.* 136, 108695.
- Magoulick, D.D., Dekar, M.P., Hodges, S.W., Scott, M.K., Rabalais, M.R., Bare, C.M., 2021. Hydrologic variation influences stream fish assemblage dynamics through flow regime and drought. *Scientific Reports* 11, 10704.
- McClenaghan, B., Fahner, N., Cote, D., Chawarski, J., McCarthy, A., Rajabi, H., Singer, G., Hajibabaei, M., 2020. Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. *PLoS One* 15, e0236540.
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., Iwasaki, W., 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species Subject Category: Subject Areas: R. Soc. Open Sci. 2, 150088.
- Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., et al., 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nat. Commun.* 11 (1), 4717.
- Pastore, A.L., Barabás, G., Bimler, M.D., et al., 2021. The evolution of niche overlap and competitive differences. *Nat. Ecol. Evol.* 5, 330–337.
- Sales, N.G., Wangenstein, O.S., Carvalho, D.C., Deiner, K., Præbel, K., Coscia, I., McDevitt, A.D., Mariani, S., 2021. Space-time dynamics in monitoring neotropical fish communities using eDNA metabarcoding. *Sci. Total Environ.* 754, 142096.
- Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis Version 11. *Mol. Biol. Evol.* 38, 3022–3027.
- Thomaz, S.M., Bini, L.M., Bozelli, R.L., 2007. Floods increase similarity among aquatic habitats in river floodplain systems. *Hydrobiologia* 579, 1–13.
- Tsuji, S., Shibata, N., Inui, R., Nakao, R., Akamatsu, Y., Watanabe, K., 2023. Environmental DNA phylogeography: Successful reconstruction of phylogeographic patterns of multiple fish species from cups of water. *Mol. Ecol. Resour.* 00, 1–16.
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.* 25, 929–942.
- Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. *Can. J. Fish. Aquat. Sci.* 37 (1), 130–137.
- Wang, S.P., Yan, Z.G., Hänfling, B., Zheng, X., Wang, P.Y., Fan, J.T., Li, J.L., 2021. Methodology of fish eDNA and its applications in ecology and environment. *Sci. Total Environ.* 755, 142622.
- Wang, T., Zhang, Y.p., Yang, Z.y., et al., 2020. DNA barcoding reveals cryptic diversity in the underestimated genus *Triplophysa* (Cypriniformes: Cobitidae, Nemacheilinae) from the northeastern Qinghai-Tibet Plateau. *BMC Evol. Biol.* 20, 151.
- White, L., O'Connor, N.E., Yang, Q., Emmerson, M.C., Donohue, I., 2020. Individual species provide multifaceted contributions to the stability of ecosystems. *Nat. Ecol. Evol.* 4, 1594–1601.
- Wu, M., Chen, S., Chen, J., Xue, K., Chen, S., Wang, X., Chen, T., Kang, S., Rui, J., Thies, J.E., Bardgett, R.D., Wang, Y., 2021. Reduced microbial stability in the active layer is associated with carbon loss under alpine permafrost degradation. *PNAS* 118.
- Xu, B., Qi, B., Ji, K., Liu, Z., Deng, L., Jiang, L., 2022. Emerging hot spot analysis and the spatial-temporal trends of NDVI in the Jing River Basin of China. *Environ. Earth Sci.* 81, 55.
- Xu, L., Shi, Z., Wang, Y., Zhang, S., Chu, X., Yu, P., Xiong, W., Zuo, H., Wang, Y., 2015. Spatiotemporal variation and driving forces of reference evapotranspiration in Jing River Basin, northwest China. *Hydrol. Process.* 29, 4846–4862.
- Yuan, M.M., Guo, X., Wu, L.W., Zhang, Y., Xiao, N., Ning, D., Shi, Z., Zhou, X., Wu, L., Yang, Y.F., Tiedje, J.M., Zhou, J.Z., 2021. Climate warming enhances microbial network complexity and stability. *Nat. Clim. Chang.* 11, 343–348.
- Zhang, Z., Li, H., Shen, W.L., Feng, K., Li, S.Z., Gu, S.S., Zhou, Y.Q., Peng, X., Du, X.F., He, Q., Wang, L.L., Zhang, Z.Z., Wang, D.R., Wang, Z.J., Deng, Y.e., 2022. The stability of phyto-zooplanktonic networks varied with zooplanktonic sizes in Chinese coastal ecosystem. *mSystems* 7, 1–15.
- Zhang, S., Zhao, J., Yao, M., 2020. A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. *Methods Ecol. Evol.* 11, 1609–1625.
- Zhao, X., Fang, K., Chen, F., Martin, H., Roig, F.A., 2023. Reconstructed Jing River streamflow from western China: A 399-year perspective for hydrological changes in the Loess Plateau. *J. Hydrol.* 621, 129573.
- Zhou, J., Ning, D., 2017. Stochastic Community Assembly: Does It Matter in Microbial Ecology? *Microbiol. Mol. Biol. Rev.* 81.