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Effects of non-random sources of alteration on biodiversity and ecosystem functioning

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Effects of non-random sources of alteration on biodiversity and ecosystem functioning

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Editorial: Effects of non-random sources of alteration on biodiversity and ecosystem functioning

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Editorial on the Research Topic
Effects of non-random sources of alteration on biodiversity and ecosystem functioning

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Exploring the relationship between biological diversity and ecosystem functioning has long been recognized as one of the main concerns in modern ecology (Loreau et al., 2001; Loreau, 2010). This is especially true in the recent context of global changes induced by human activities such as water abstraction, flow regulation, shifts in agricultural practices, contamination, climate change, and biological invasions (Gaston et al., 2010; Bellard et al., 2012). Although many previous studies have demonstrated that these changes can affect biodiversity, ecosystem functioning, and the biodiversity-ecosystem functioning (BEF) relationship (Strayer, 2012; Zhao et al., 2019; Stewart et al., 2022), our understanding of how genetics, individuals, populations, communities, and ecosystems respond to such changes is limited. Moreover, trophic structure is considered the link between biodiversity and ecosystem functioning (Cardinale et al., 2006), connecting species, populations, communities, and ecosystems. Therefore, understanding the response of food webs to perturbations can help ecologists better understand the relationship between biodiversity and ecosystem functioning. In the present Research Topic, we collected 16 research papers and two review papers related to this topic. These studies focused on a wide range of biological groups, such as microbes, algae, invertebrates, fish, and marine mammals, covering a variety of ecosystems, including soil, karst bedrock, freshwater, and marine waters. Several different data collection and analysis protocols were used in these studies, including stable isotopes, environmental DNA, genetic sources, and ecological models. Importantly, these studies involved in several non-natural sources of alteration induced by global changes such as contamination, habitat fragmentation and degradation, climate change, and biological invasions.

Contamination has received considerable attention because it affects not only biological diversity but also human health. Using six fish and two crustacean species as models, Ji et al. demonstrated the pollution characteristics of 16 polycyclic aromatic hydrocarbons (PAHs) in marine organisms in the East China Sea. They found that the main source of PAHs in the study animals was coal combustion and that gasoline combustion, oil combustion, crude oil spills, and vehicle emissions were important contributors. Therefore, the potential risk of lifetime cancer from the consumption of seafood should be considered and monitored over a long period. Eutrophication is another form of pollution that occurs in freshwater ecosystems. Zhu et al. revealed that the eutrophication level differs in Taihu Lake, China, with the level being higher in summer and in the southern region. Interestingly, microbial diversity was negatively correlated with the degree of water eutrophication, whereas its abundance was positively correlated with nutrient levels. Because the function of microorganisms indicates their participation in the migration and transformation of nutrients, more attention should be paid to these organisms when purifying lakes. Eutrophication may also be associated with cyanobacterial blooms. Wang et al. analyzed the effects of light on the diversity of cyanobacteria and coexisting microorganisms. Moreover, changes in related genes, functional structures, and internal metabolism involved in nitrogen cycling were revealed using 16S rRNA and non-targeted metabolomics sequencing technologies. Their results indicated that natural light conditions could regulate the diversity, abundance, and metabolites of cyanobacteria and coexisting microorganisms. Eutrophication is correlated with the composition of specific fish communities. Guo et al. found that fish communities differed significantly among zones with different nutrients in lakes. Accordingly, they suggested that reasonable stocking of piscivorous fish could be a potential approach for regulating lake ecosystem functioning.

Habitat change is another key factor that affects biological diversity. In lakes, this could be due to watershed land-use changes, which have been investigated by Du et al., affecting the traits and functional diversity of phytoplankton in lakes in Northeast China. Specifically, five of the 18 functional trait categories (i.e., flagella, filamentous, unicellular, mixotrophic, and chlorophyll *c*) can be considered potential indicators of the intensity of watershed land-use change. Functional richness decreases with intensive agricultural and urban land use, which is associated with an increase in functional homogenization. Moreover, soil fauna biodiversity can be regulated by urbanization. Yu et al. observed that soil physicochemical characteristics changed dramatically along an urbanization gradient. Subsequently, they detected variance using Shannon's diversity and Pielou's evenness indices. Although soil fauna abundance, taxon number, and community structure also exhibited different patterns along an urbanization gradient, their variance contributed slightly to the change in soil physicochemical characteristics. In terms of stream ecosystems, Li et al. found that native fish alpha diversity in the streams of four basins in the Wannan Mountains could decline because of the effects of low-head dams, which may in turn favor the colonization of native-invasive fish. Therefore, the fish community structure and beta diversity can also be modified. Similar results were reported by Gu et al., who found that small hydropower stations in the upper reaches of the Yangtze River have an impact on freshwater biodiversity such as fish, benthic invertebrates, plankton, and microorganisms. Liao et al. demonstrated that dams can also affect fish diversity in reservoir cascades connected to rivers. However, the response patterns of fish taxonomic, functional, and phylogenetic diversity were distinct in different reservoirs and were associated with their impounding age and longitudinal location. All the above studies provide essential references and implications for freshwater biodiversity conservation under habitat change conditions.

Climate change is a hot topic when discussing human-induced perturbations that cause environmental changes in ecosystems, such as lakes and streams. In our Research Topic, B-Béres et al. focused on flow intermittence in streams located in Hungary. They found that the drying of streams has a complex influence on benthic diatoms, with taxonomic diversity, functional diversity, and diatom-based quality indices exhibiting different responses. Taxonomical and functional redundancies can compensate for the negative effects of short-term flow intermittency on diatom assemblages. Lentic and lotic environments also induce a shift in the microeukaryotic communities of rivers. For instance, Wang et al. found that the network and stability of lotic areas are strongly determined by a microeukaryotic network. In contrast, the microeukaryotic network is highly fragmented in lentic areas, causing the loss of key functions in the microeukaryotic community, thus decreasing the stability and resilience of ecosystems. Organisms located at high-elevational sites are more easily affected by climate change. This was implied by the observations of Wang et al., who found that temperature and salinity were the key factors driving benthic macroinvertebrate elevational diversity patterns in lakes through three potential mechanisms: climate/productivity, environmental heterogeneity, and dispersal/history. Climate change may also affect vertebrates such as fish. Liu et al. indicated that the trade-off between maximum metabolic ability and energy efficiency is important before fish migration. Moreover, Wang et al. suggested that the trophic traits of fish, such as food and habitat, are correlated with resource utilization. As these performances can be affected by climate change, subsequent responses in the physiological and ecological traits of fish can be expected.

Biological invasion is a threat to global biodiversity, causing the decline and even local extinction of native populations. Based on genetic sources and diversity, Zhong et al. indicated that the genetic origins of some paddy field carp populations in South China are complex. More importantly, *Cyprinus carpio carpio* genetic resources have invaded paddy field carp populations in South China and should be given more attention in the future to preserve native germplasm resources. Biological invasions can even occur in the karst bedrock. In the review prepared by Li et al., they summarized the habitat characteristics and invasion status of karst bedrock to biological invasions. They found that the number of invasive species increased over time through competition, mutualism, allelopathy, and phenotypic plasticity. Interestingly, invasive species in karst bedrock usually have specific biological traits, such as strong fecundity, rapid growth rate, strong environmental adaptability, strong phenotypic plasticity, and high genetic diversity. These traits may help them to colonize the karst bedrock more easily. Finally, they indicated that some areas in China might face a higher risk of invasion, providing useful information for the management of invasive species. To survey invasive species, traditional approaches include field sampling using gillnets and traps. In Lin et al., environmental DNA (eDNA) metabarcoding was found to be a better approach than multi-mesh gillnets sampling approach in assessing fish diversity because it is sensitive, effective, and noninvasive. More importantly, eDNA technology can detect species that cannot be collected using traditional approaches; thus, it is particularly useful for rare, enigmatic, invasive, and endangered species.

In addition to focusing on the indirect effects of human activity, Li et al. reviewed the direct effects of human whale hunting on deep-sea biodiversity. Indeed, whale fall is beneficial for the diffusion and succession of deep-sea organisms, supporting the survival and evolution of specific fauna. However, the microbial processes, reproductive strategies, population genetics, and biogeography contributing to whale falls remain unclear. As the largest vertebrates in the deep sea, whales should be protected to preserve the deep-sea environment.

Overall, this Research Topic made a significant contribution to our understanding of how global changes in environmental characteristics can affect biodiversity, ecosystem functioning, and the BEF relationship. Studies on this topic have also provided suggestions to manage and compensate for these effects. However, further studies are required to better understand the mechanisms underlying these effects. Additionally, effective measures should be developed for policy managers.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Functional Responses of Phytoplankton Assemblages to Watershed Land Use and Environmental Gradients

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Watershed land-use changes have been identified as major threats to lake fauna, subsequently affecting ecosystem functioning. In this study, the functional-based approach was used to examine the effects of land use and environmental changes on phytoplankton communities in four selected lakes in Northeast China. We also identified the sensitive functional traits as indicators of environmental stressors. The integration of RLQ analysis with the fourth-corner approach significantly identified five of 18 functional trait categories, including flagella, filamentous, unicellular, mixotrophic, and chlorophyll *c*, as potential indicators to changes in watershed land-use intensity and environmental gradients. Significant relationships between traits and land use and water quality highlighted the consequential indirect impact of extensive agricultural and urban development on phytoplankton via allochthonous nutrient inputs and various contaminants. In addition, the functional richness of phytoplankton assemblages generally increased along with surface area and forests, but decreased along with intensive agricultural and urban land use, implying that functional homogenization may cause a reduction in ecosystem productivity and reliability to land-use intensity. Given the superior performance of the functional-based approach, our findings also highlighted the importance of the application of both the biological traits and functional diversity index in monitoring programs for lake ecosystems.

Keywords: algae, community structure, eutrophication, functional-based approach, human disturbance

INTRODUCTION

Agriculture and urbanization have drastically transformed complex natural ecosystems into simplified managed ecosystems in many locations (Flynn et al., 2009; Hooke and Martín-Duque, 2012). By changing contaminant, sediment, nutrient, and organic matter loading (Williamson et al., 2008), land-use changes have far-reaching consequences for aquatic ecosystems (Foster et al., 2003). Lakes can be sensitive to land-use changes because they often occupy low points in landscapes, effectively accumulating effects across watersheds (Arbuckle and Downing, 2001;

Stachelek et al., 2020). For example, intensive and extensive agricultural practices, and specifically the application of fertilizer or manure within watersheds, are strongly responsible for lake eutrophication (Bennett et al., 2001; Carpenter, 2005), in conjunction with the proliferation of harmful algal blooms, decrease in dissolved oxygen (DO) levels, and loss of aquatic biodiversity (Carpenter et al., 1998), while watershed urban development is associated with several environmental consequences, including nutrient pollution, and sewage, pathogen and other contaminants (Bai et al., 2018). The strong linkage between lake ecosystems and their watershed (Williamson et al., 2008) highlights the effects of watershed land use exerted on lake habitats and biota and their consequential indirect impact on ecosystem functioning.

Several studies described the decline in species diversity as a consequence of habitat degradation due to watershed development (Lenat and Crawford, 1994; Dodson et al., 2005; Xu et al., 2019). Changes in the structure and composition of regional habitats may lead to local extinction through changes in nutrient loading and runoff of pollutants from surrounding areas (Ricciardi and Rasmussen, 1999; Meador and Goldstein, 2003). Moreover, as watershed land-use types are likely to influence greenhouse gas emissions that can affect the regional climate (Smith et al., 2008), community structure is expected to respond significantly to differences in thermal conditions (Dell et al., 2011). Species should be shaped by environments based on their niches, which are better conceptualized by their morphological, behavioral, or life-history traits that impact their fitness via growth, reproduction, and survival, rather than their taxonomical identity (McGill et al., 2006). Thus, functional trait-based approaches can be informative for understanding the biotic communities in response to environmental gradients (Mouillot et al., 2013).

Phytoplankton, the foundation of aquatic food webs, are a highly diverse group of eukaryotes and cyanobacteria. As phytoplankton in aquatic systems have fast growth rates and short-generation times (Sommer, 1989), they are highly influenced by a multitude of factors, including nutrients, temperature, light, carbon dioxide (CO_2), and grazers (Sarnelle, 1992; Elliott et al., 2006; Burson et al., 2018), and they are known to be sensitive and predictive to environmental changes. Ecological studies investigating the effects of environmental gradients often assess phytoplankton community changes based on taxonomy-based approaches such as species composition, biomass, or diversity. Some evidence suggests that urban and agricultural land use within a watershed, indirectly representing increased nutrient inputs and various contaminants (Carpenter et al., 1998; Nielsen et al., 2012; Beaver et al., 2014), may influence the composition of phytoplankton assemblages by favoring the growth of cyanobacteria toward the agricultural and artificial land-use types (Sánchez et al., 2021). Phytoplankton biomass and production in the lakes surrounded by urban areas or agricultural land tend to be increased by anthropogenic nutrient enrichment (Peng et al., 2021). Despite substantial progress in the understanding of the changes in phytoplankton communities due to watershed land-use changes, studies using trait-based approaches and exploring the consequential functional outcomes following land-use change are still scarce.

In this study, we evaluate how environmental stress gradients, representing a range of land uses and trophic conditions, affect phytoplankton functional traits and the diversity of lakes in northeast China. We used RLQ and fourth-corner analyses to assess the bivariate relationships between environmental stress gradients and species traits. The aims of this study were to: (1) examine the patterns of species composition and trait distribution of phytoplankton in the selected lakes in relation to environmental changes and (2) identify the sensitive functional traits as indicators of environmental stressors. We hypothesized that the phytoplankton communities in lakes vary along a land use and environmental gradient. The assumed variation in communities could be promoted by environmental filtering for specific traits. In addition, we hypothesized with increasing agricultural and urban land-use pressure and nutrient enrichment, there would be a corresponding decrease in functional diversity of phytoplankton communities.

MATERIALS AND METHODS

Study Area

The study was conducted in four natural lakes in northeast China: the Chagan, Jingpo, Longhupao, and Wudalianchi (**Figure 1**), all belonging to the Songhua River Basin. The region is characterized by a temperate continental monsoon climate, with a mean annual temperature of 2.8°C and average annual precipitation of 531.1 mm. During the last several decades, the region has undergone massive changes in land cover and a large amount of land has formed sparse grassland and reed land which has become a place for local farmers to graze (Liu et al., 2005). Lake Chagan and Longhupao belong to the lowland part of the Songhua River Basin with loamy soils as the dominant geological features, while Lake Wudalianchi and Jingpo are lava-dammed lakes with sandy and loamy soils. The selected lakes varied in both development in their catchments and represented different morphometric and water quality conditions (**Table 1**).

Land Use and Environmental Gradients

The land-use variables comprised proportions of the forest, grassland, wetland, and agricultural and urban areas within lake watersheds. These were determined at the whole topographic catchment scale. The Geographic Information System (GIS) program ArcGIS (version 10.7) was used to analyze the percentage of different land cover types. Environmental gradients were described in terms of lake morphology and water quality. Lake morphological variables comprised surface area and maximum depth. The surface area was drawn with ArcGIS software based on physical barriers and topographical features. Maximum water depth was obtained from Jing et al. (2014). Water quality variables comprised DO, water temperature, and pH that were measured in site at the subsurface (∼0.5 m) using the YSI Professional Plus Multi-meter (YSI Incorporation, Yellow Springs, OH, United States) and variables related to trophic status including total nitrogen (TN), total phosphorus (TP), and

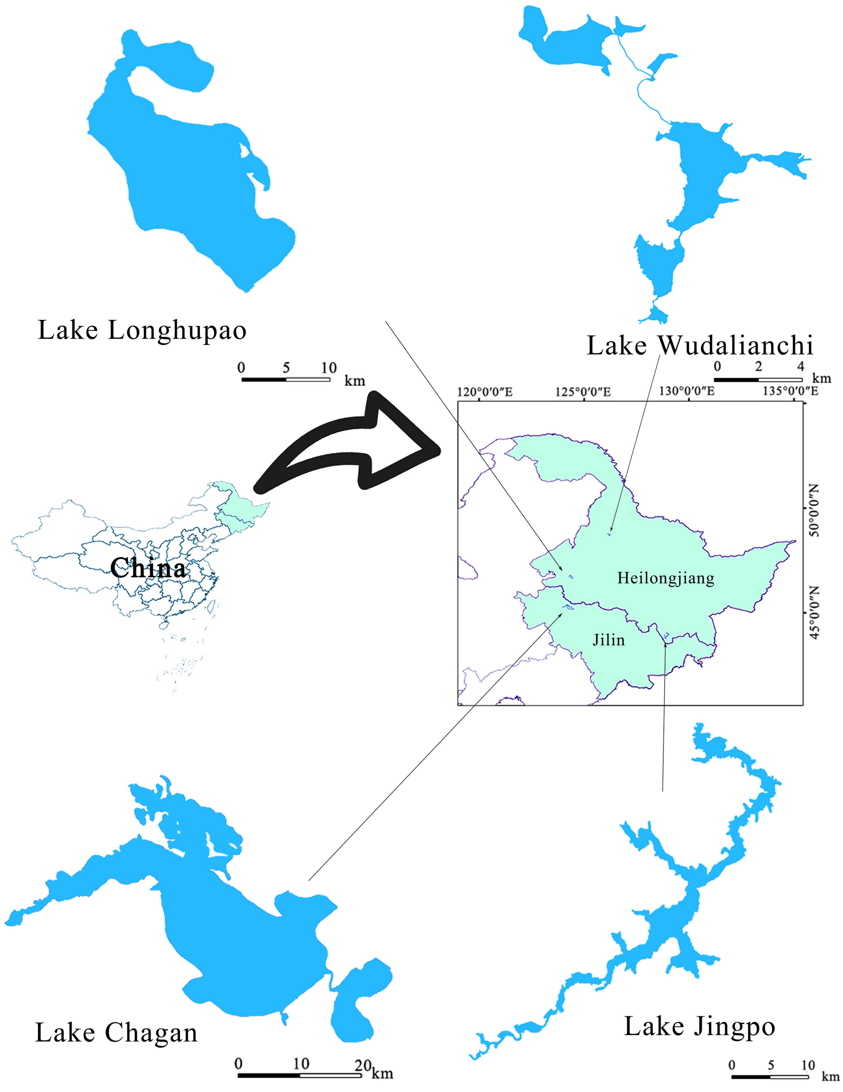


FIGURE 1 | Location of the four selected lakes in Northeast China.

chemical oxygen demand (COD). TN and TP were analyzed using the persulfate digestion method and COD was determined with open reflux method (American Public Health Association [APHA], 1992).

Functional Trait Selection

Phytoplankton were collected from the four lakes seasonally in May (spring), August (summer), November (autumn) 2019, and February (winter) 2020. Composite water samples combining euphotic depth-integrated subsamples were collected for phytoplankton at 12 locations in each lake. Phytoplankton counts and cell volume estimates were performed using the inverted microscope method (Utermöhl, 1958). Phytoplankton was identified at the species or genus level according to Hu and Wei (2006). A total of 18 functional traits (**Table 2**) that are considered crucial for reproduction, resource acquisition, and predator avoidance (Weithoff, 2003; Litchman and Klausmeier, 2008) were assigned for each of the 279 phytoplankton species. Cell size and greatest axial linear dimension (GALD) (μm) were obtained by averaging measurements from at least 10 individuals for each taxa. Other traits were compiled based on scientific literature, web search, and expert knowledge (Jones, 2000; Weithoff, 2003; Hu and Wei, 2006; Litchman and Klausmeier, 2008; Klais et al., 2017; Loewen et al., 2021).

Statistical Analysis

To demonstrate the location of different algal groups in relation to their functional traits, principal component analysis (PCA) was conducted on trait matrix of species. Species in the PCA plot were phylogenetically grouped in major taxonomic groups (phyla): chlorophytes, chrysophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, and euglenophytes.

Two complementary multivariate analyses, the RLQ analysis (Dolédec et al., 1996) and the fourth-corner approach

TABLE 1 | Information on watershed land use and environmental gradient parameters in four selected lakes.

Variables	Lake Chagan	Lake Longhupao	Lake Jingpo	Lake Wudalianchi
Surface area (km^2)	366.8	128.4	84.6	20.7
Maximum depth (m)	3.5	3.5	48	12
Agricultural area (%)	46.51	28.55	25.22	54.92
Urban area (%)	2.63	1.20	0.96	1.21
Forest (%)	0.04	0.13	67.43	20.41
Grassland (%)	28.80	49.25	4.07	14.33
Wetland (%)	1.54	1.36	0.90	0.00
Water temperature (°C)	14.9	14.7	14.1	11.8
Dissolved oxygen (mg/L)	8.96	10.38	9.43	10.17
pH	9.18	8.58	8.35	8.60
Total phosphorus (mg/L)	0.229	0.289	0.147	0.162
Total nitrogen (mg/L)	1.617	1.003	2.124	1.783
Chemical oxygen demand (mg/L)	8.189	5.889	7.420	7.650

TABLE 2 | Phytoplankton functional traits used in this study.

Traits	Abbreviation
Cell size $< 100\ \mu m^3$	Small
Cell size 100–1,000 μm^3	Medium
Cell size $> 100\ \mu m^3$	Large
Greatest axial linear dimension $> 35\ \mu m$	GALD $> 35\ \mu m$
Unicellular life form	Unicellular
Colonial life form	Colonial
Filamentous life form	Filamentous
Coenobium life form	Coenobium
Mucilaginous-producing	Mucilage
Nitrogen fixation	N-fixation
Silica requirement	Si-requirement
Chlorophyll *b*-containing	Chlorophyll *b*
Chlorophyll *c*-containing	Chlorophyll *c*
Phycobilins-producing	Phycobilins
Mixotrophy	Mixotrophy
Toxin-producing	Toxin
Flagellar motility	Flagella
Buoyancy-regulating	Buoyancy

(Dray and Legendre, 2008; Dray et al., 2014), were conducted to investigate the covariation between environmental characteristics and functional traits. RLQ (R, sites by environmental variables; L, sites by species abundance data; Q, species by traits) analysis performs a double inertia analysis between the Q and R through L ordination (Dolédec et al., 1996). Thus, it is possible to identify the effects of land use and environmental gradients on the traits based on the phytoplankton community structure. As suggested by Dray and Legendre (2008), we combined two permutation models for both the analyses tested the null hypotheses that "species assemblages are randomly attributed to sites, irrespective of the site characteristics" (model 2) and that "species are distributed according to their preferences for site conditions, but irrespective of their traits" (model 4). Finally, the fourth-corner approach was used to evaluate the specifically predictive role of functional traits in watershed land-use intensity and environmental gradients.

We calculated three multidimensional functional diversity indexes: functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv). Increases in these three index values indicate that more niche space is occupied by species, a more evenly abundance distribution in niche space, and a larger divergence in the abundance distribution in niche space, respectively (Mason et al., 2005). The relationships between functional diversity index and land use and environmental gradients were examined using Pearson correlation.

All the analyses and graphics were performed in R version 4.0.3 (R Core Team, 2020) with the *ade4* (Dray and Dufour, 2007), *vegan* (Oksanen et al., 2020), *FD* (Laliberté and Legendre, 2010), *corrplot* (Wei and Simko, 2021), and *ggfortify* (Tang et al., 2016) packages.

RESULTS

The first two axes of the PCA performed on phytoplankton functional traits explained 26.11 and 20.07% of the variation, respectively (**Figure 2**). Of the four general trait groups, the first was dominated by species that are small to medium sized and chlorophyll *b*-producing and form colonies or coenobiums (i.e., chlorophytes); the second comprised filamentous and toxin-producing species with the ability of buoyancy regulation and nitrogen fixation (i.e., cyanobacteria); the third consist of large-sized species that are unicellular, chlorophyll *c*-producing, and require silica (i.e., diatoms); the fourth contained species that are mixotrophic and flagellate (i.e., dinoflagellates, crypto-, and euglenophytes).

The RLQ analysis revealed that land use and environmental gradients influence the distribution of phytoplankton species with specific traits (model 2, $p < 0.001$) and that the composition of phytoplankton assemblages is dependent on the land use and environmental gradients of studied lakes and influenced by species traits (model 4, $p = 0.028$). The first two RLQ axes accounted for 97.44% of the variation in relationships between land use and environmental gradients and functional traits. The first RLQ axis was positively correlated with urban and agricultural land use, surface area, pH, and TN. Grassland, wetland, DO, and TP loaded positively onto the second RLQ axis, while forest cover, maximum depth, and water temperature loaded negatively onto the second RLQ axis (**Figure 3A**). The traits filamentous, phycobilins, toxin-producing, and nitrogen fixation occurred in cyanobacteria and the traits chlorophyll *b*-producing, medium sized, and coenobium generally occurred in chlorophytes positively correlated with the first RLQ axis, whereas the traits unicellular, mixotrophic, and chlorophyll *c*-producing negatively correlated with the first RLQ axis. The traits flagella, large sized, and the silica requirement were negatively related to the second RLQ axis (**Figure 3B**).

The fourth-corner analysis provided the significant interactions between individual traits and land use and environmental gradients. Of the 18 traits examined in this study, five were deemed indicators of morphological, land

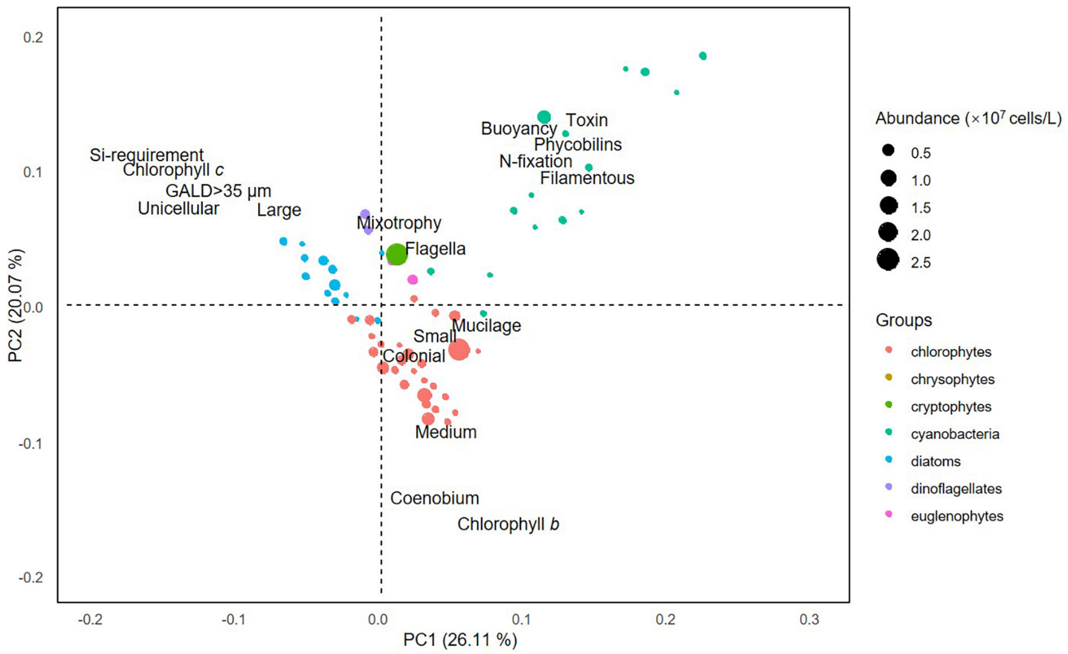


FIGURE 2 | Results of principal component analysis performed on the functional traits of phytoplankton species.

use, and environmental variables (**Figure 4**). Filamentous taxa possessed flagella were significantly positively associated with surface area, maximum depth, and urban areas, whereas unicellular, mixotrophic, chlorophyll *c*-containing taxa were significantly negatively associated with surface area, agricultural, and urban land use.

Functional richness of phytoplankton assemblages generally increased along with surface area and forests, but decreased along with intensive agricultural and urban land use (**Figure 5**). There were no relationships between FEve and morphological and environmental variables. FDiv was positively correlated with wetland and higher TP, but negatively with agricultural land use (**Figure 5**).

DISCUSSION

Lakes provide for a wide range of valuable ecosystem services, including provisioning, regulation, and maintenance, as well as cultural services for instance, by improving human well-being (Reynaud and Lanzanova, 2017; Custódio et al., 2020). To this end, conventional approaches to lake restoration and preservation have typically focused on the mechanisms and factors regulating species assemblages. The observable functional differentiation of the traits of phytoplankton community revealed that of the stress gradients studied, human land-use activities had a consistent and strong influence upon phytoplankton functional structure via their consequences for nutrient loading. In addition, land-use transformations due to extensive agricultural and urban development cause a reduction of the FRic in phytoplankton assemblages.

Our results indicate significant relationships between traits and environmental stress variables, which provide a framework to link phytoplankton communities of lakes to environmental filtering. More specifically, increased agricultural and urban areas around lakes associated with changes in physicochemical properties, such as the increase in nitrogen, pH, or decrease of DO generally favored filamentous and toxin-producing algae as opposed to mixotrophic algae. These results are in agreement with other studies, which found that agricultural or urban land use could increase nutrient inputs from the watershed and in turn influence phytoplankton functional traits (Phillips et al., 1978; Carpenter and Waite, 2000; Bosch et al., 2009; Loewen et al., 2021). However, different anthropogenic land-use types have changing effects on lake N and P stoichiometry, with agricultural runoffs generally having a higher nitrogen-to-phosphorus ratio than those from urban areas or grasslands (Carpenter et al., 1998). In this study, agricultural lands were positively associated with TN, but negatively associated with trait nitrogen fixation. These results suggest a reduction of N:P ratio under generally eutrophic conditions that may favor the dominance by nitrogen-fixing cyanobacteria due to their competitive advantage under nitrogen-limited conditions (Smith, 1983; Sharpley and Withers, 1994). We also found that increased phosphorus inputs from grassland and wetland were positively associated with toxic cyanobacterial species, providing further support for the role of phosphorus load in fueling toxic cyanobacterial blooms (Bormans et al., 2016). As the water sources of Lake Chagan and Longhupao pass through manured grassland and a large number of wetlands were transferred to irrigation ditches for rice fields (Liu et al., 2021), a higher level of phosphorus loading to lakes and low N:P ratios can be expected.

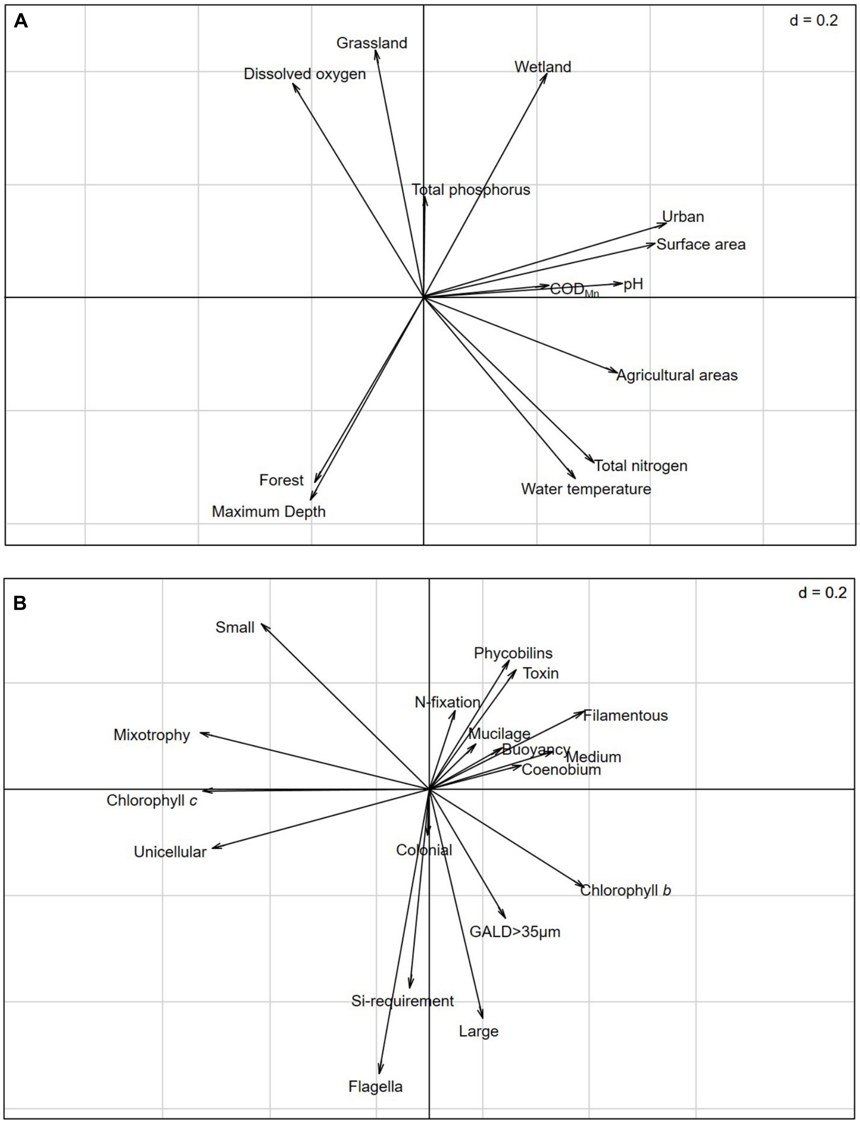
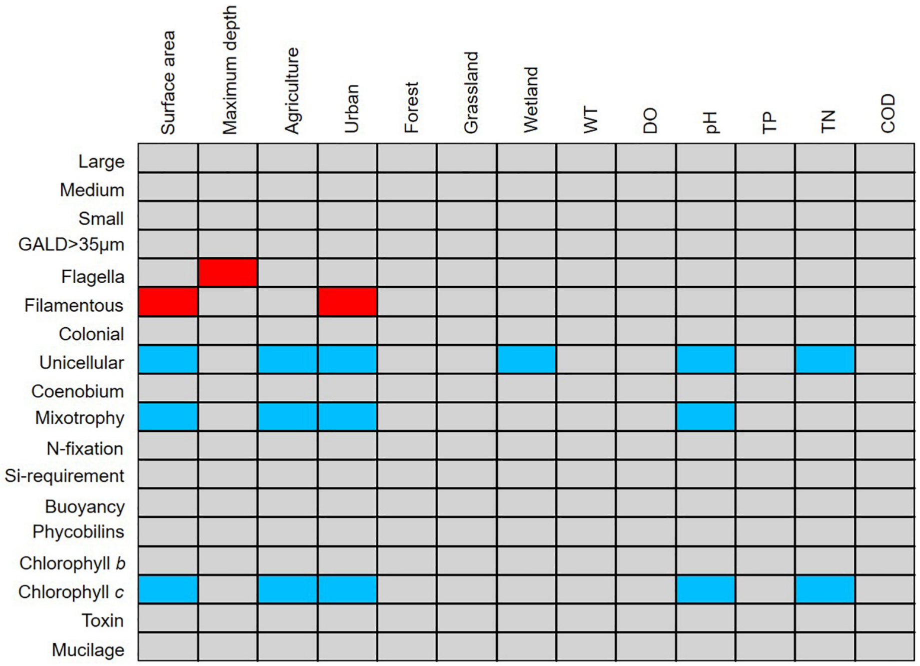


FIGURE 3 | RLQ analysis revealing the correlations between land use and environmental gradient variables (R table 101 × 13) and functional traits (Q table 279 × 18), constrained by species abundance (L table 101 × 279): **(A)** Coefficients for the land use and environmental gradient variables and **(B)** coefficients for the functional traits.

Our results also showed that land use and environmental gradients could potentially influence resource acquisition traits of phytoplankton. Mixotrophy, which is an important trait related to nutrient acquisition (Litchman and Klausmeier, 2008), is advantageous under low nutrient conditions (Troost et al., 2005). Mixotrophic taxa increased in lakes with relatively low land-use intensity and nutrient conditions, suggesting a competitive advantage of these taxa under low nutrient supply. In addition, differences in pigment composition allow differences in spectral utilization patterns that can provide opportunities for species coexistence under light limitation (Stomp et al., 2004). Experimental studies with chlorophyll *c*-containing species *Stephanodiscus hantzschii* have shown negative effects of light intensity on chlorophyll *c*, especially under high nutrient conditions (Marzetz et al., 2020). We observed the opposite pattern with high chlorophyll *c*-containing species under nitrogen-limitation conditions. This might be due to decreasing light availability in deeper lakes with more forested dominated watershed, which favors the growth of chlorophyll *c*-containing species, as has been found in other north-temperate lakes (Loewen et al., 2021).

We revealed significant morphological factors (maximum depth and surface area) constraining the composition of phytoplankton communities. Our results indicate deeper water favoring flagellated algae. These results further demonstrate that the physical factors related to lake morphology influence functional strategies to survive in terms of functional traits related to motility (Wentzky et al., 2020). For instance, lake



	Surface area	Maximum depth	Agriculture	Urban	Forest	Grassland	Wetland	WT	DO	pH	TP	TN	COD
Large													
Medium													
Small													
GALD>35μm													
Flagella		🟥											
Filamentous	🟥			🟥									
Colonial													
Unicellular	🟦		🟦	🟦			🟦			🟦		🟦	
Coenobium													
Mixotrophy	🟦		🟦	🟦						🟦			
N-fixation													
Si-requirement													
Buoyancy													
Phycobilins													
Chlorophyll *b*													
Chlorophyll *c*	🟦		🟦	🟦						🟦		🟦	
Toxin													
Mucilage													

FIGURE 4 | Results of the fourth-corner analysis revealing the correlation between phytoplankton traits and land use and environmental gradient variables in the selected lakes. Red cells indicate significant positive correlations ($p < 0.05$) and blue cells indicate significant negative correlations ($p < 0.05$).

depth exerts a strong influence on the stability of the water column (e.g., mixing and thermal stratification) and thus on the light and oxygen condition and the internal nutrient loading from sediment into the hypolimnion (O'Brien et al., 2003; Yang et al., 2016). The flagellates possess active motility that enable them to alter position within the water column to alleviate any spatial limitation or segregation of resources and select optimum conditions (Salmaso and Padisák, 2007; Litchman and Klausmeier, 2008; Wentzky et al., 2020). Deep lakes where turbulence and internal nutrient loading were relatively low that may give a competitive advantage of flagellated algae to overcome nutrient deficiency and sedimentation losses by adjusting the position in the water column. In contrast, the negative association between maximum depth and traits nitrogen fixation and toxin production is possible because the increasing internal phosphorus loading with decreasing N:P ratios in shallow lakes is the main driver of the nitrogen-fixing cyanobacterial blooms (Smith, 1983; Sharpley and Withers, 1994). We also observed links between water temperature and algal traits in deep lakes. In this study, the higher water temperature may increase the prevalence of flagellated algae. Similar to water depth, the water temperature could influence the vertical distribution of oxygen and upward mixing of nutrients (Straile et al., 2003) and thus mediate physiological processes and behavioral responses of phytoplankton. The other morphological factor that was positively associated with the filamentous nitrogen-fixing algae is lake surface area. As larger lakes in this study are common in urban areas and have relatively shallow sloped basins and high phosphorus loadings, their phosphorus-rich conditions and conducive environment for germination of akinetes may promote the proliferation of nitrogen-fixing cyanobacteria (Kovács et al., 2012).

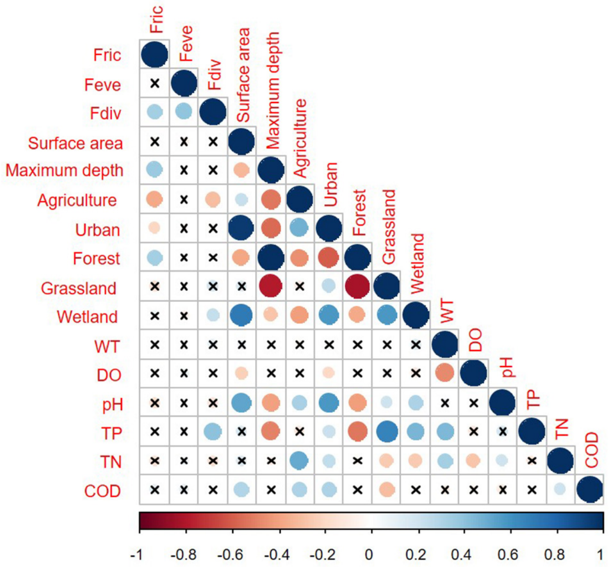


FIGURE 5 | Pearson correlation matrix for the functional diversity indices and morphometric and environmental descriptors. Non-significant correlations ($p > 0.05$) are represented by the crossed-out points.

In aquatic systems, land-use changes are major driving forces of biodiversity loss (Allan, 2004). Increasing environmental stress gradient in our study, representing intensive urbanization or agricultural practices generally reduce FRic. Several studies have previously identified the significant effect of environmental disturbance upon freshwater biodiversity, and land-use changes have been identified as further significant factors influencing lake ecosystems (Arbuckle and Downing, 2001; Light and Marchetti, 2007; Nielsen et al., 2012; McGoff et al., 2013). Watershed land-use development has often been cited as having a negative effect upon the biodiversity of lakes (Dudgeon et al., 2006; van Soesbergen et al., 2019). Functional diversity has been shown with potential applications for predicting environmental disturbance. In this study, FRic of phytoplankton communities decreased with increasing land-use intensity. Similar patterns of functional changes of community in response to environmental disturbance have been reported, for instance, a reduction in functional diversity of aquatic insects in southeastern Brazil (Castro et al., 2018), and of fishes in Malaysia (Wilkinson et al., 2018), and of benthic diatoms in Italy (Falasco et al., 2021). Potentially higher functional diversity represented much higher levels of productivity and reliability (Dìaz and Cabido, 2001; Vallina et al., 2017). Our result of a significant decline in FRic with increasing land-use intensity suggests that homogenization may cause a reduction in ecosystem productivity and reliability to human disturbance. Given the importance of functional diversity to productivity and reliability (Mason et al., 2013; Gherardi and Sala, 2015), we suggest that management at the watershed and local scales such as mitigating grazing and cultivation practices can help to ensure a stronger determinant of ecosystem processes in face of anthropogenic disturbances.

Functional richness represents the amount of functional space occupied by a species assemblage (Mouchet et al., 2010). We suggest that the significant decrease in FRic along the agricultural and urban land-use gradients may imply a low degree of niche differentiation among species, reflecting the occupation by species with similar traits inhabiting the area, in response to extensive habitat degradation due to increased intensity of land use. Although increasing nutrient loadings from watershed are likely to favor cyanobacteria bloom (Doubek et al., 2015; Richardson et al., 2019), the results suggest that increasing land-use intensity is to the detriment of FRic, indicating that the functional community structure of phytoplankton is susceptible to agriculture and urbanization-driven disturbances. In this study, a possible explanation for a reduced functional diversity along the environmental stress gradient is eutrophication and anthropogenic contamination by agriculture and urbanization. For example, eutrophication of lakes is characterized by a potential deficit of oxygen, blooms of cyanobacteria, decrease water transparency, and deterioration of water quality (Carpenter et al., 1998). Other detrimental consequences of converting natural land covers include pesticides and other toxic chemicals from agricultural and urban areas. For instance, urban runoffs are widely recognized as transport vectors of salts, pharmaceuticals, and trace metals such as cadmium, copper, and lead (Müller et al., 2020), while agricultural runoffs are widely recognized as transport vectors of pesticides and phosphorus and nitrogen in forms of fertilizers and manure (Carpenter et al., 1998; Munz et al., 2017). These contaminants will lead to the loss of biodiversity directly through simple toxicity effects on physiological, developmental, and reproductive processes (Burant et al., 2018) or indirectly by altering the environmental conditions (Yang et al., 2022).

CONCLUSION

In this study, we considered the effect of land-use changes and environmental gradients upon the functional response of phytoplankton assemblages. The integration of RLQ analysis with the fourth-corner approach significantly identified functional traits as potential indicators to aquatic environments. Furthermore, anthropogenic disturbance such as agricultural and urban land use can modify phytoplankton traits which consequentially decreased functional diversity of phytoplankton assemblages. Moreover, declines in FRic representing functional homogenization may cause a reduction in ecosystem productivity and reliability. Given the superior performance of functional-based approach, our findings also highlighted the importance of the application of both the biological traits and functional diversity index in monitoring programs for lake ecosystems.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XD and DS designed and orchestrated the research and wrote up the findings. KM, JY, and XJ providing help for field sampling. HW, HL, LW, and CZ provided review and comments and edited the manuscript. TH revised the content of the manuscript and responsible for the overall submission. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.819252/full#supplementary-material

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Inconsistent Patterns of Soil Fauna Biodiversity and Soil Physicochemical Characteristic Along an Urbanization Gradient

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Urbanization has induced substantial changes in soil physicochemical characteristic, which plays an important role in regulating soil fauna biodiversity in forests and grasslands. However, less is known about the urbanization effect on soil fauna biodiversity and how soil physicochemical changes mediate this effect. Along an urbanization gradient in the city of Guangzhou, we established four sites with different urbanization intensities, including an urban site, two suburban sites, and a rural site, and then studied their soil physicochemical characteristic and soil fauna biodiversity. The soil physicochemical characteristic dramatically changed along the urbanization gradient. In contrast, the soil fauna biodiversity exhibited a very different pattern. Soil fauna abundance was highest in the suburban sites. Moreover, there were significant changes of Pielou's evenness and community structure in the suburban sites. Soil fauna biodiversity property in the urban site was similar to that in the rural site, except that the rural site was characterized by Enchytraeidae while the urban site was not characterized by any taxa. Our linear and canonical correspondence analysis models suggested that soil physicochemical characteristic only contributed a little to the variance of soil fauna abundance (19%), taxa number (27%), and community structure (12%). In contrast, soil physicochemical characteristic explained about half of the variance in Shannon's diversity and Pielou's evenness. However, with urbanization intensity increasing, soil physicochemical changes could both increase and decrease the diversity and evenness. Thus, our results revealed an inconsistent pattern between soil fauna biodiversity and soil physicochemical characteristic along an urbanization gradient. This study suggested that soil physicochemical change was less important as expected in regulating soil fauna biodiversity pattern under an urbanization context. To elucidate the effect of urbanization on soil fauna biodiversity, further studies should take other urbanization agents into account.

Keywords: soil biodiversity, urban biodiversity, soil invertebrate, global change, urbanization

INTRODUCTION

Urbanization is among the most dramatic changes in terrestrial ecosystem surface, resulting in a series of substantial abiotic and biotic changes (McIntyre et al., 2001; Veresoglou et al., 2015; Eisenhauer et al., 2019). Soil fauna is a megadiverse kingdom providing an array of ecosystem services (Wardle et al., 2004) and has been harnessed as a tool in monitoring ecosystem health (Fountain and Hopkin, 2005; Bispo et al., 2009; Gerlach et al., 2013). Soil fauna biodiversity is generally regulated by population dispersal limitation, environmental influence, and biotic interaction (Vellend, 2010; Gao M. et al., 2020). Urbanization has induced substantial changes in soil physicochemical characteristic, but less is known about its effects on soil fauna biodiversity. In natural and semi-natural ecosystems, soil physicochemical characteristic plays a vital role in regulating soil fauna biodiversity (Coleman et al., 2004). However, it is unclear that in human-dominant urban ecosystems, how soil physicochemical changes will mediate the effects of urbanization on soil fauna biodiversity.

There are many studies on urban soil physicochemical characteristic changes, drawing a conclusion that urbanization substantially altered soil physicochemical characteristic. In urban areas, soils are usually served as a supporting for green space and embedded in the context of city constructions such as buildings, roads, and public squares. Such landscape background would increase soil temperature (Shi et al., 2012) and exacerbate soil moisture fluctuation (Butler and Davies, 2011). Urban soils may be trampled, covered, dug, or removed and thus losing its natural structure and texture, showing higher bulk density, incomplete vertical structure, and broken texture (Gilbert, 1989; Banat et al., 2005; Wei and Yang, 2010). Artificial product wastes, such as broken bricks, glass and china, and kitchen refuse, are buried into soils during city constructions and residential daily life in urban areas. In addition, artificial wastes could also enter into soils in the form of dust, through rainfall water flow and dry deposition. As a result, increased soil pH (Jim, 1998; Pouyat et al., 2015; Asabere et al., 2018) and changed element contents (e.g., C, N, P, and Ca) (Pouyat et al., 2002; Trammell et al., 2020) in urban areas are widely reported. Notably, traffic, industry, and garden management may produce wastes rich in metal elements, such as Cd, Cr, Cu, Pb, and Zn, which are harmful to most lives and hard to be removed from soils once being polluted (Wei and Yang, 2010).

In contrast, the effects of urbanization on soil fauna biodiversity are far from a conclusion. There are some studies suggesting that soil fauna biodiversity is higher in less disturbed ecosystems under an urbanization context (Fiera, 2009; Szlavecz et al., 2018; Liu et al., 2019), while some studies find contradictory results (Sterzynska et al., 2018; Joimel et al., 2019). For example, by summarizing 758 studies, Joimel et al. (2017) concluded that there are higher soil fauna biodiversity in downtown and industrial areas. We still knew little why results of urbanization effects on soil fauna contradict.

At present, there are more studies suggesting that urban soil fauna biodiversity is influenced by factors other than soil physicochemical characteristic under an urbanization context. Habitat landscape characteristic, such as habitat area, connectivity, and diversity, are important in determining soil fauna biodiversity (Bolger et al., 2000; Milano et al., 2018; Xie et al., 2018; Gao M. et al., 2020), mostly because they could affect extinction rate, individual and gene exchanges, and taxa co-occurrence, thus shaping community assembly processes (LaPoint et al., 2015; Lepczyk et al., 2017). Transport and cross-region tourism would help soil fauna overcoming geographic isolation and entering into potential urban habitats, resulting in direct changes in soil fauna taxa composition (Gray, 1989; Tothmeresz et al., 2011; Horvath et al., 2012; Chatzinikolaou et al., 2018). In urban green space, garden management and visits of residents will expose soil fauna under continuous anthropogenic disturbances (Norton, 2011; Tresch et al., 2018, 2019). These facts raised a question that how important the soil physicochemical property change is in mediating urbanization effect on soil fauna biodiversity.

In this article, by studying the soil fauna communities and soil physicochemical characteristics in four urbanization gradient sites, we aimed at exploring the following: (1) whether soil fauna biodiversity changes consistently with soil physicochemical characteristic as urbanization intensity increases and (2) how soil physicochemical characteristic regulates soil fauna biodiversity across the urbanization gradient.

MATERIALS AND METHODS

Sites Along an Urbanization Gradient

Four urbanization gradient sites were established in Guangzhou City (22°26′ to 23°56′N and 112°57′ to 114°03′E), which is the capital of Guangdong Province in South China. The climate is a subtropical, marine monsoon, with an annual average temperature of 21.5–22.2°C and an annual average precipitation of 1,623.6–1,899.8 mm. Natural vegetation covering the city is tropical monsoon forest. The typical soil in natural ecosystems is latosolic red soil.

With urbanization intensity decreasing, the four sites are urban site (URB), suburban site 1 (SUB1), suburban site 2 (SUB2), and rural site (RUR), located at Yuexiu Park, Tianlu Lake Forest Park, Maofeng Mountain Forest Park, and Shimen Forest Reserve, respectively (**Figure 1A**). The Yuexiu Park (where URB is located) developed from the Sun Yat-sen Memorial Park established in 1920s. At present, Yuexiu Park is embedded in Guangzhou downtown and surrounded by residential buildings and business districts with a dense human population. The vegetations in the park are either native woodland or managed garden. Soil ecosystems in Yuexiu Park were highly fragmented by impervious roads and highly disturbed by tourists. The Tianlu Lake Forest Park (where SUB1 is located) is about 19 km away from the Yuexiu Park. The Tianlu Lake Forest Park is at the edge of Guangzhou downtown, laying between natural hilly forests in the north and residential areas in the south. The vegetations are integrated secondary broadleaf forests and plantations. The Maofeng Mountain Forest Park (where SUB2 is located) is about 27 km away to the Yuexiu Park and 9.4 km to the Tianlu Lake Forest Park. The park is surrounded by hilly forests and

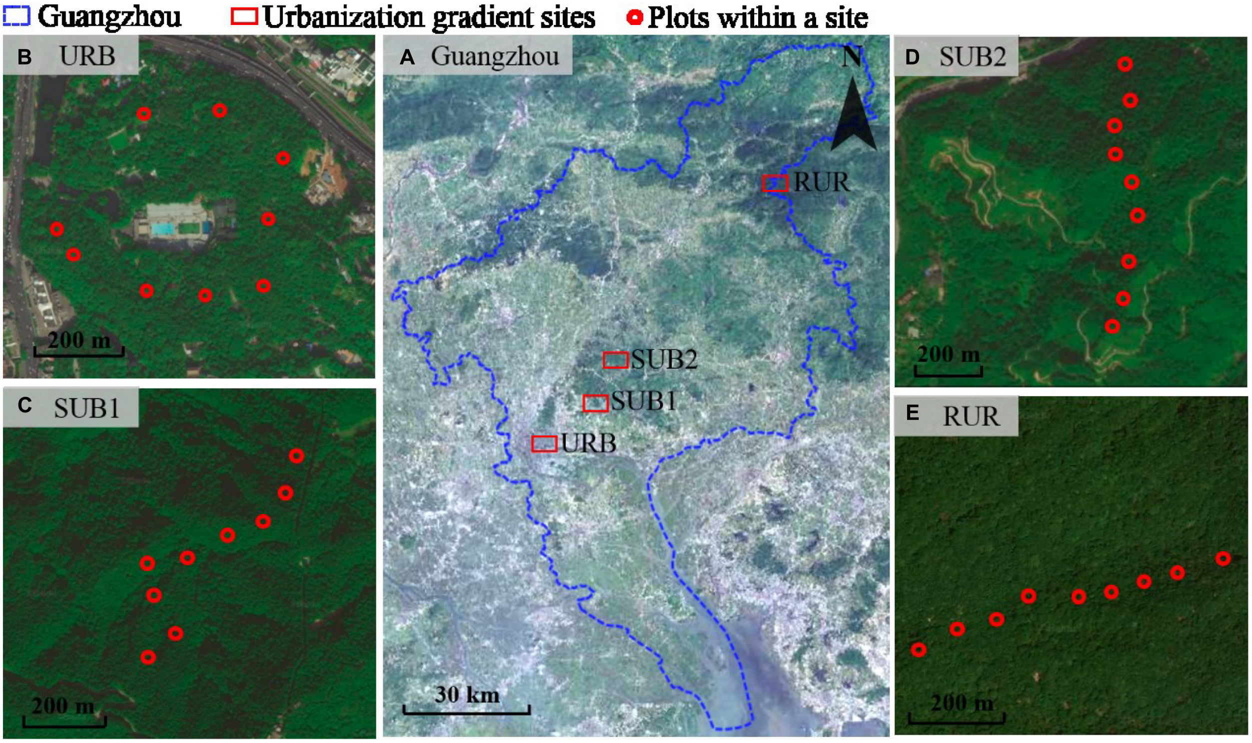

FIGURE 1 | (A) The site location in Guangzhou City along an urbanization gradient. **(B–E)** The locations of nine plots within each site. The urbanization gradient sites are urban (URB), suburban 1 (SUB1), suburban 2 (SUB2), and rural (RUR) sites.

only has a roadway to connect with Guangzhou downtown. The vegetations are also secondary broadleaf forests and plantations. The Shimen Forest Reserve (where RUR is located) is at the Northeastern Guangzhou, about 75 km away from the Yuexiu Park. The Reserve is mainly covered by subtropical evergreen broadleaf forests and, in the mountain feet, by plantations.

Soil Fauna Samplings and Identifications

To obtain represented soil fauna samples, we made samples in a 1,000-m-long transect in each urbanization gradient site to cover as much environmental habits as possible in plant-covered areas (**Figures 1B–E**). We planned to take samples in 9 plots lying at a straight line. However, it was hard to make it in these subtropical hilly forests, where there were so many ridges and valleys, while the Yuexiu Park was not large enough. Soil fauna sampling was performed only once in each plot of the four sites during November 2020 to January 2021. We established three subplots in each plot, and the subplots were 5 m away from each other. To acquire a sample, plant litter was first removed from the surface. Then a soil core was taken in a subplot with a 5 cm diameter cylinder, to 10 cm depth. Soils collected in a plot were pooled and mixed to generate a soil sample. Immediately after collections, the samples were transported to our laboratory, and soil fauna were extracted using Tullgren dry funnels for 48 h. All specimens were sorted mainly according to Yin (1998) and counted using a dissecting microscope (Leica, German) and a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan).

In each plot, soil fauna abundance (individual number from a sample) and taxa number (taxa number from a sample, S) were recorded and used to calculate Shannon's diversity index (H) as follows (Shannon, 1948):

$$H = -\Sigma P_i log_2 P_i$$

where P_i is the ratio between the abundance of group i and the total number of soil fauna. Evenness was evaluated according to the Pielou's evenness index (J) as follows (Pielou, 1969):

$$J = H/log_2 S$$

Soil Samplings and Physicochemical Analyses

Similar to the soil fauna sampling, another three soil corers were taken using a 3 cm diameter steel corer in each plot to generate a soil sample for soil physicochemical characteristic analysis. Soil bulk density (BD) was measured following the methodology described in Maynard and Curran (2007). In brief, the soil samples were dried at 105°C for 48 h and were weighed. BD (in g/cm^3) corresponds to the dry weight divided by the volume of samples. Soil pH was measured using a 1:2.5 soil-water suspension with the potentiometric method. Gravimetric soil water content (SWC) was measured on 20 g soil dried at 105°C for 48 h. Soil organic matter (SOM) content was determined using H_2SO_4-$K_2Cr_2O_7$ oxidation method. Soil total nitrogen content (TN) was quantified by the Kjeldahl acid digestion method. Soil total phosphorus content (TP) was quantified using the molybdate blue method after acid digestion (Hou et al., 2014). Soil heavy metal concentration (i.e., Cd, Cr, Cu, Pb, and Zn) was analyzed with graphite furnace atomic absorption spectrophotometry method after digestion in a mixture of nitric acid, perchloric acid, and hydrogen peroxide.

Statistical Analysis

Since species richness tends to vary with sampling intensity and fauna individual number, we used rarefaction to compare species richness among the urbanization gradient sites. We randomly sampled 41 individuals (the lowest value in the soil fauna sampling) from each plot to estimate taxa richness. One-way ANOVA was used to assess the differences in soil fauna abundance, taxa richness, *H*, and *J* among the urbanization gradient sites. Where the overall analysis was significant ($p < 0.050$), we used Tukey's HSD *post hoc* tests for pairwise comparisons. The same procedures were also used to test the differences in BD, SWC, pH, SOC, TN, TP, Cd, Cr, Cu, Pb, and Zn among the sites.

We assessed the changes in soil fauna community structure using multivariate analysis. Using taxa and abundance data, principal coordinate analysis (PCoA) with Jaccard's dissimilarity was employed to visualize the separation of communities among the four urbanization gradient sites. Furthermore, we tested the differences in soil fauna communities among the sites using permutational multivariate analysis of variance (PERMANOVA). The *p*-value was obtained based on 999 permutations. The same procedure was used to assess the overall differences in soil physicochemical characteristic among the sites using PCoA and PERMANOVA. To examine the heterogeneity of soil fauna biodiversity and soil physicochemical characteristic within each site, the homogeneity of multivariate dispersions was calculated. Then the homogeneities were compared using one-way ANOVA and Tukey's HSD *post hoc* tests (Anderson, 2006). Indicator species of each gradient site were determined using Dufrene-Legendre indicator (Dufrêne and Legendre, 1997).

To determine the relationships between soil physicochemical variables and soil fauna biodiversity (i.e., taxa number, abundance, *H*, and *J*), we used linear models with all-subset selection of *a priori* explanatory variables (i.e., BD, SWC, pH, SOC, TN, TP, Cd, Cr, Cu, Pb, and Zn). We then explored the influence of soil physicochemical characteristic on soil fauna community structure using canonical correspondence analysis (CCA) with backward stepwise selection of *a priori* explanatory variables (i.e., BD, SWC, pH, SOC, TN, TP, Cd, Cr, Cu, Pb, and Zn).

All analyses were performed using R version 4.02 (R Core Team, 2020). One-way ANOVA, Tukey's HSD, and linear regression model were performed using the stats package (R Core Team, 2020). All-subset selection for the best linear model was performed using the leap package (Lumley and Miller, 2020). Rarefaction, PCoA, PERMANOVA, CCA, backward stepwise selection for the best CCA model, and homogeneity of multivariate dispersions were conducted using the vegan package (Oksanen et al., 2017). Indicator species analysis was done using the labdsv package (Roberts, 2013).

RESULTS

Soil Physicochemical Characteristics Along the Urbanization Gradient

There were substantial differences among the urbanization gradient sites in soil BD, soil pH, SOC, TN, TP, gravimetric SWC, and total soil Cd, Cu, Cr, Pb, and Zn content (ANOVA: all $p < 0.050$) (**Figure 2**). With urbanization intensity increasing, gradual changes were observed in BD (**Figure 2A**), SWC (**Figure 2F**), Cu (**Figure 2H**), and Pb (**Figure 2J**). Soil physicochemical characteristic in the site URB was very different from other sites and had the highest BD (1.33 ± 0.04 g/cm^3), soil pH (5.04 ± 0.29), TP (0.584 ± 0.060 g/kg), Cu (18.2 ± 1.3 mg/kg), Cr (49.1 ± 7.7 mg/kg), and Pb (162.8 ± 46.3 mg/kg), but lowest SWC (13.1 ± 1.6%). In contrast, the site RUR had the lowest BD (0.78 ± 0.04 g/cm^3), TP (0.299 ± 0.055 g/kg), Cu (4.15 ± 0.17 mg/kg), and Pb (32.8 ± 1.14 mg/kg), but the highest SOC (36.8 ± 2.2 g/kg), TN (2.72 ± 0.20 g/kg), and SWC (25.6 ± 1.2%). Apart from total soil Zn, differences between RUR and URB in all measured soil physicochemical characteristics were significantly different (Tukey's HSD: $p < 0.050$). Notably, total soil Pb, Cu, and Cr in the site URB were as high as 5.0 (Tukey's HSD: $p = 0.002$), 4.4 (Tukey's HSD: $p < 0.001$), and 3.2 (Tukey's HSD: $p = 0.037$) times higher than those in the site RUR. Values of soil physicochemical characteristic in the two suburban sites, namely, SUB1 and SUB2, were usually between the sites RUR and SUB. The SUB1 and SUB2 had similar soil physicochemical characteristic values and only significantly differed in BD (Tukey's HSD: $p = 0.017$).

The results of PCoA on soil physicochemical characteristic based on Jaccard's dissimilarity were consistent with the variance analysis. All plots could be grouped into three categories with a clear separation (**Figure 3A**). Plots within URB were quite dissimilar with each other, and betadisper analysis showed that heterogeneity within the URB was the highest (**Figure 4A**). However, the ordination ellipses of URB did not overlap on any other sites. Pairwise PERMANOVA showed that soil physicochemical characteristic of URB significantly differed with SUB1, SUB2, and RUR (all adjusted $p = 0.006$) (**Supplementary Table 1**). Plots within the site RUR were highly clustered and did not overlap with any other sites either. Pairwise PERMANOVA showed that RUR was significantly different from other sites (all adjusted $p = 0.006$). In contrast, SUB1 and SUB2 had similar soil physicochemical characteristic, and the plots of these two sites were highly blended (pairwise PERMANOVA: $p = 0.996$).

Soil Fauna Biodiversity Along the Urbanization Gradient

There were not significant differences in taxa number, *H*, and *J* (**Figure 5**). Interestingly, the SUB1 with intermediate

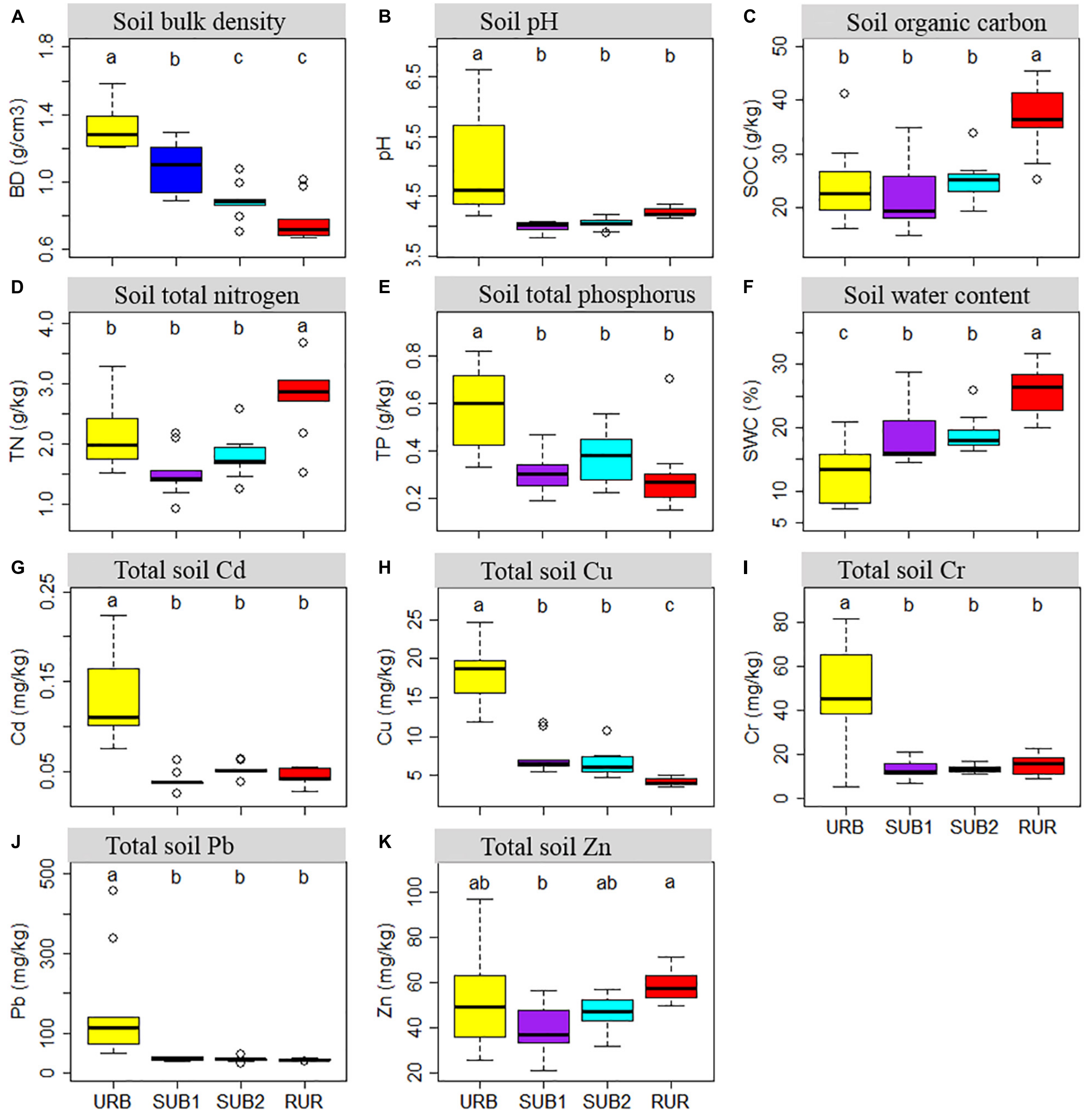

FIGURE 2 | Boxplots showing soil physicochemical characteristic in urbanization gradient sites: **(A)** Soil bulk density; **(B)** Soil pH; **(C)** Soil organic carbon content; **(D)** Soil total nitrogen content; **(E)** Soil total phosphorus content; **(F)** Gravimetric soil water content; **(G)** Total soil Cd content; **(H)** Soil total Cu content; **(I)** Soil total Cr content; **(J)** Soil total Pb content; **(K)** Total soil Zn content. The urbanization gradient sites are urban (URB), suburban 1 (SUB1), suburban 2 (SUB2), and rural (RUR) sites. Each boxplot is shown for nine samples. Different letters represent statistically significant differences at $p = 0.050$.

urbanization intensity had the highest soil fauna abundance (272.8 ± 47.0 ind.), which was significantly higher than that of the URB (121.1 ± 24.1 ind.) (Tukey's HSD: $p = 0.042$) and the RUR (122.6 ± 13.4 ind.) (Tukey's HSD: $p = 0.039$), but similar to the SUB2 (250.9 ± 53.5 ind.) (Tukey's HSD: $p = 0.977$) (**Figure 5C**). The SUB1 also had the highest taxa number (10.8 ± 1.2) (**Figure 5A**) and lowest J (0.51 ± 0.02) (**Figure 5E**). However, the differences in taxa number (ANOVA: $p = 0.156$) or Pielou's evenness index (ANOVA: $p = 0.131$) among the four sites were not significant. There was only minor difference in Shannon's diversity index among the four sites (3.51–3.67) (ANOVA: $p = 0.963$).

Principal coordinate analysis based on Jaccard's dissimilarity showed that soil fauna community structure did not vary substantially among the urbanization gradient sites. Generally, soil fauna community structure of the four urbanization gradient sites was blended, and the ordination ellipses for each site were largely overlapped (**Figure 3B**). Soil fauna community

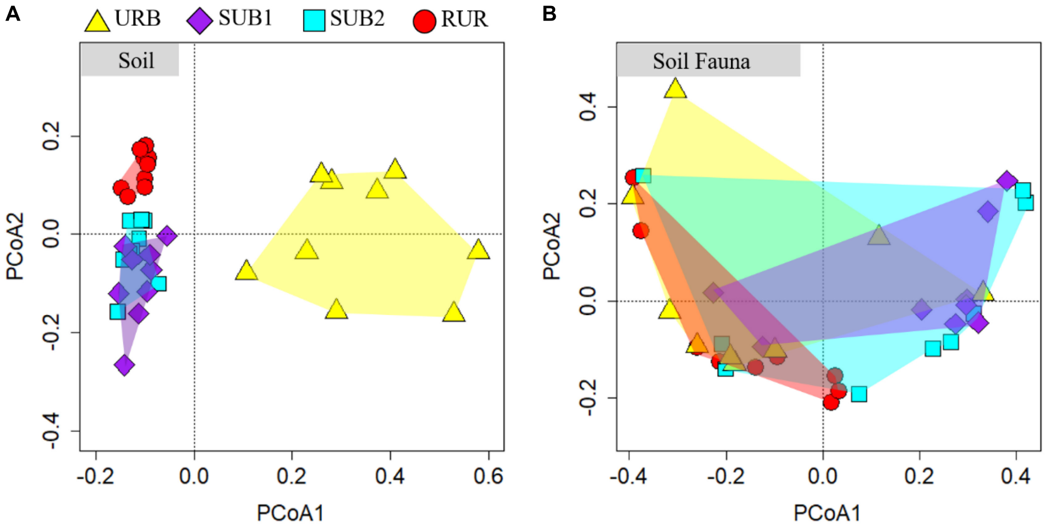

FIGURE 3 | Principal coordinate analysis plots of **(A)** soil physicochemical characteristic and **(B)** soil fauna community structure in urbanization gradient sites. The urbanization gradient sites are urban (URB), suburban 1 (SUB1), suburban 2 (SUB2), and rural (RUR) sites.

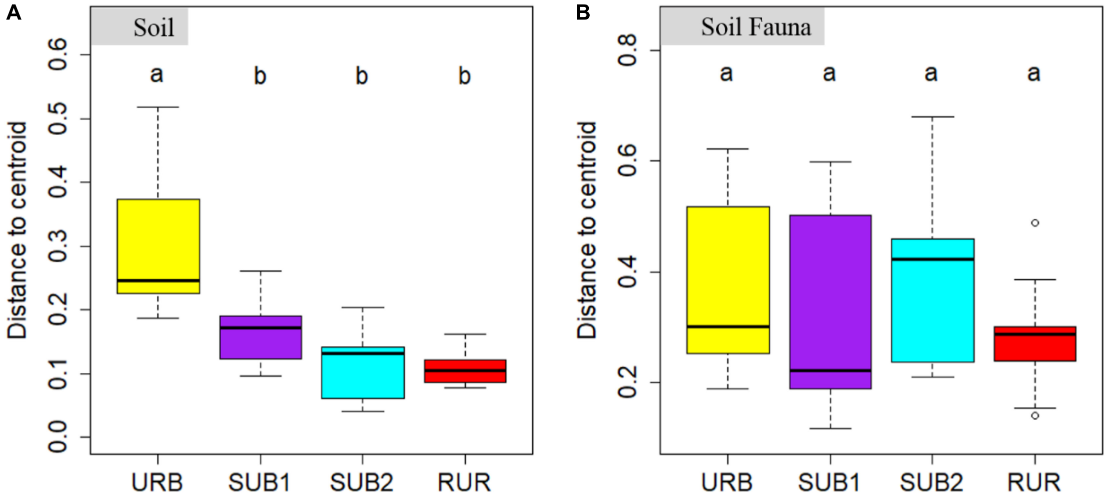

FIGURE 4 | Boxplots showing the Jaccard's dissimilarity of **(A)** soil physicochemical characteristic and **(B)** soil fauna community in urbanization gradient sites. The urbanization gradient sites are urban (URB), suburban 1 (SUB1), suburban 2 (SUB2), and rural (RUR) sites. Each boxplot is shown for nine samples. Different letters represent statistically significant differences at $p = 0.050$.

structure was highly dissimilar within the URB and SUB2. The heterogeneity within each urbanization gradient site could also be seen in betadisper analysis, showing that average distance to centroids was 0.370, 0.331, 0.387, and 0.283 for URB, SUB1, SUB2, and RUR, respectively (ANOVA: $p = 0.503$) (**Figure 4B**). The plots within the SUB1 were slightly clustered, while two plots were very similar to the RUR plots. Pairwise PERMANOVA showed that soil fauna community structure in the site SUB1 significantly differed from RUR (adjusted $p = 0.018$) and tended to be differed from URB (adjusted $p = 0.072$) (**Supplementary Table 1**).

Indicator species analysis revealed that SUB1 was characterized by Pauropoda, SUB2 was characterized by Diplura, and RUR was characterized by Enchytraeidae (**Figure 6**). The analysis did not identify any taxa with high indicator value for the URB.

Relationships Between Soil Physicochemical Characteristics and Soil Fauna Biodiversity Across the Urbanization Gradient Site

Linear model analysis suggested that across the 36 plots, soil physicochemical characteristic contributed little to the variation in soil fauna abundance and taxa number, but played an important role in regulating both *H* and *J*. The best linear model

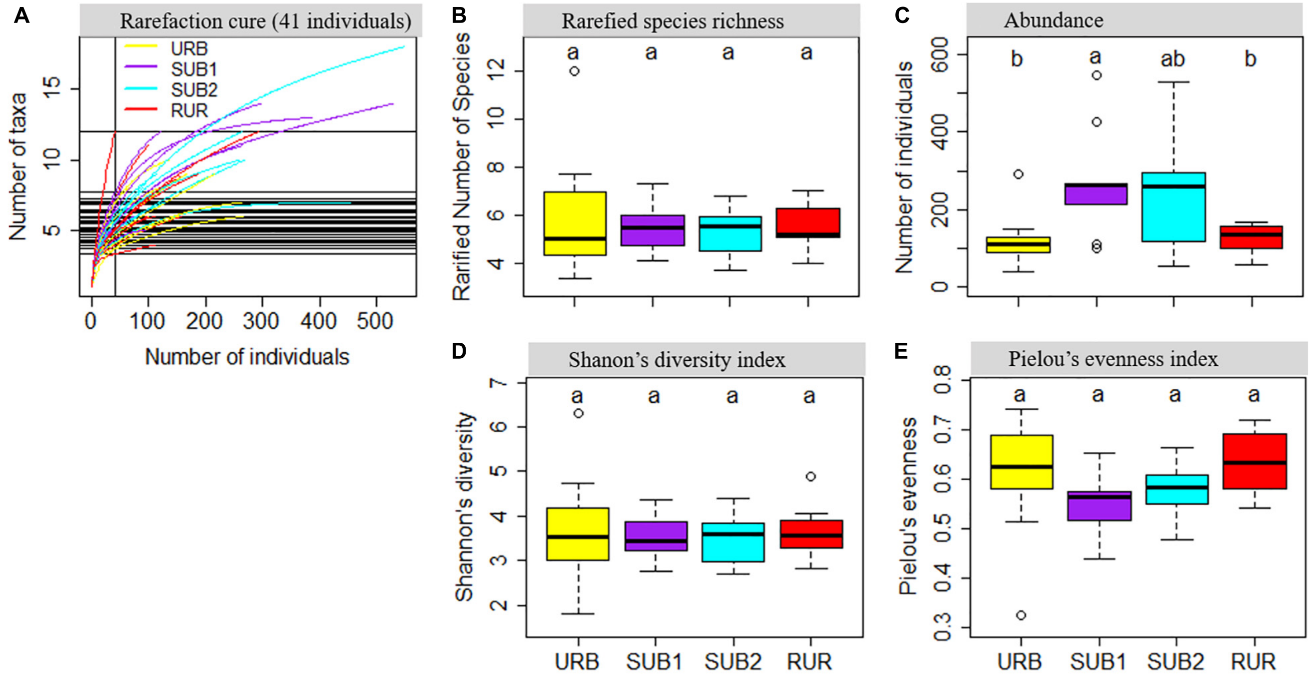

FIGURE 5 | Soil fauna biodiversity properties in urbanization gradient sites. **(A)** Species rarefaction curve based on 41 individuals (the lowest value in the soil fauna sampling). Boxplots showing **(B)** the rarefied number of species, **(C)** the number of collected individuals, **(D)** Shannon's diversity index, and **(E)** Pielou's evenness index. The urbanization gradient sites are urban (URB), suburban 1 (SUB1), suburban 2 (SUB2), and rural (RUR) sites. Each boxplot is shown for nine samples. Different letters represent statistically significant differences at $p = 0.050$.

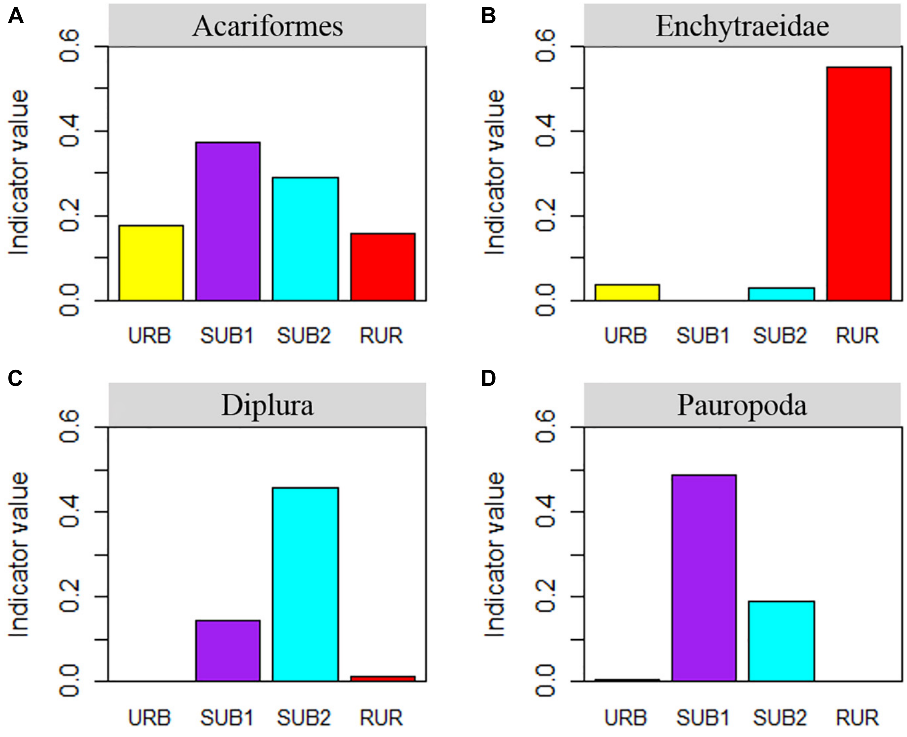

FIGURE 6 | Significant indicator taxa for each urbanization gradient site as determined by the Dufrene-Legendre indicator species analysis. The significant indicator taxa are **(A)** Acariformes, **(B)** Enchytaeidae, **(C)** Diplura, **(D)** Pauropoda. The urbanization gradient sites are urban (URB), suburban 1 (SUB1), suburban 2 (SUB2), and rural (RUR) sites.

for soil fauna abundance explained only 18.5% of the variance across all plots ($p = 0.046$) (**Table 1**). The independent variables included in the model were Cr ($r = 0.62$), SOC ($r = –0.41$), TN ($r = –0.29$), BD ($r = –0.47$), and Cd ($r = –0.58$) (all $p > 0.050$). A set of variables including Cu, SOC, and Cr explained 26.8% of the variance in soil fauna taxa number ($p = 0.004$). Total soil Cr content had an extremely negative effect on taxa number ($r = –0.92$, $p = 0.002$), while Cu had a significant positive effect ($r = 0.63$, $p = 0.032$). Linear model analysis also suggested that soil physicochemical characteristic played an important role in regulating H ($R^2 = 0.535$, $p < 0.001$) and J ($R^2 = 0.456$, $p < 0.001$). Contents of heavy metal, i.e., Cr ($r = –0.86$, $p = 0.005$), Pb ($r = –0.28$, $p = 0.097$), and Zn ($r = –0.48$, $p = 0.026$), had negative effects on H, while soil pH ($r = 0.69$, $p = 0.014$) and TN ($r = 0.56$, $p < 0.001$) had positive effects on H. The soil Pb content ($r = –0.64$, $p = 0.009$) was negatively correlated with J, while Cd ($r = 0.76$, $p < 0.001$) and TN ($r = 0.63$, $p < 0.001$) were positively correlated with J.

The best CCA model indicated that soil physicochemical characteristic included SWC ($p = 0.001$), Cu ($p = 0.013$), and Cr ($p = 0.018$), explaining only 12.1% variance of the soil fauna community structure ($p = 0.021$) (**Figure 7**).

DISCUSSION

Effects of Urbanization on Soil Physicochemical Characteristic

In accordance with previous studies, our findings suggested that soil physicochemical characteristic substantially changed along the urbanization gradient. With urbanization proceeding, soil habitats in urban areas may be patched, disturbed, and polluted (Forman, 2009). Changes in urban soil physicochemical characteristic are reported worldwide. The higher soil pH in our urban site could be attributed to crowded buildings (Jim, 1998; Pouyat et al., 2015; Asabere et al., 2018). Busy traffic in urban areas may account for the heavy metal accumulation in urban soils (Wei and Yang, 2010). Soil structure and texture destroy induced by topsoil cover and removal, heavy equipment compaction, or walking could result in the higher soil BD (Kissling et al., 2009). Kitchen refuse and other organic waste being buried into soil, as well as fertilizer application in garden areas, will induce an increase in SOM content and thus the increases of soil organic carbon, nitrogen, and phosphorus (Pouyat et al., 2002; Trammell et al., 2020). The soil moisture decline in the urban soils maybe due to aboveground litter removal and soil texture destruction (Butler and Davies, 2011). This study clearly showed the changes of soil physicochemical characteristic in urban soils, which is happening in cities around the world.

TABLE 1 | Results from all-subset linear regression analysis relating soil fauna biodiversity to soil physicochemical characteristic.

Dependent variables	Independent variables	R^2	P
Number of individuals	Cr·, TN, *SOC·*, *BD·*, *Cd·*	0.185	0.046
Number of taxa	Cu*, *SOC*, *Cr***	0.268	0.004
Shannon's diversity	pH*,TN***, Cu, *BD*, *Pb·*, *SWC*, *Zn**, *Cr***	0.535	<0.001
Pielou's evenness	Cd**, TN***, *Zn*, *Cr*, *Pb****	0.456	<0.001

*Initial models included all soil physicochemical variables measured in this study (refer to **Figure 6** for details). The independent variables included in the best models were standardized and exhibited in a descending order, where variables with negative effect is in italic font. · indicates p < 0.100; *p < 0.050; **p < 0.010; ***p < 0.001. Refer to **Figure 6** for the abbreviations of the independent variables.*

Effects of Urbanization on Soil Fauna Biodiversity

Our results did not support the intermediate disturbance hypothesis, in which the suburban sites failed to indicate a significant increase in taxa richness and diversity. Indeed, it is not clear whether the hypothesis is valid in urban ecosystems, as studies reported contradictory results. While some studies find intermediate urbanization levels favor soil fauna biodiversity (Bogyo et al., 2015; Yu et al., 2021), some others suggest soil fauna biodiversity decreases with increasing urbanization intensity (Gray, 1989; Nagy et al., 2018; Lovei et al., 2019). Interestingly, soil fauna abundance in this study peaked under a medium urbanization intensity, as showed in both sites SUB1 and SUB2, which was in accordance with our previous study in the downtown and surrounding region of Guangzhou (Yu et al., 2021) and some other studies (Papastefanou et al., 2015).

We still did not know much why these results contradicted. However, studies on aboveground organisms suggest that urbanization may either facilitate or inhibit biodiversity. Urbanization could be harmful to organism as it induced direct habit loss, soil pollution, and strong disturbance (McKinney, 2006; Knop, 2016; Sanchez-Bayo and Wyckhuys, 2019). In contrast, urban habitats provide many resources for soil fauna, such as water from irrigation, food from garbage, and refuge sites in gardens and under paving stones (Jones and Leather, 2012; Hornung et al., 2018; Szlavecz et al., 2018). Soil fauna biodiversity response to urbanization maybe largely a tradeoff between the negative and positive effects of urbanization. Therefore, there should be efforts to investigate the soil fauna biodiversity response to each specific urban environmental change on soil fauna in order to improve the understanding on the urbanization effects on soil fauna biodiversity.

The response to urbanization is usually taxonomic-dependent, which may contribute to decreasing Pielou's evenness index in the suburban sites, and is also a potential mechanism to maintain taxa number and diversity. Urbanization could be destructive to organisms specialized to inner and continuous habitats, but favorable to opportunists and cosmopolitan species (Knop, 2016; Diego Ibanez-Alamo et al., 2017). Soil biodiversity is especially diverse, and even functional and taxonomic redundant (Wolters, 2001; Allison and Martiny, 2008). What is more, global human footprint has dramatically accelerated species dispersal and thus alien species invasion, favoring opportunists and cosmopolitan species, which could flourish in human-dominant urban areas (Gray, 1989; Tothmeresz et al., 2011; Horvath et al., 2012; Chatzinikolaou et al., 2018). These would easily cause changes in soil fauna community structure and taxa evenness (Ge et al., 2019;

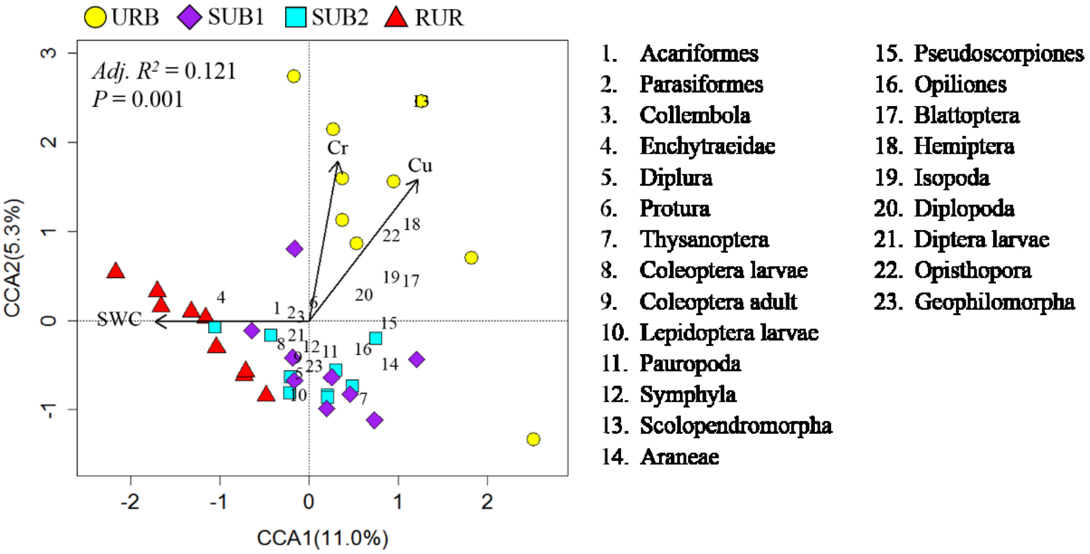

FIGURE 7 | Canonical correspondence analysis (CCA) ordination plot using soil physicochemical characteristic as the environmental data matrix and the abundance of soil fauna taxa as response variable. The CCA model is the best model selected by a backward stepwise procedure. Explained variance (R^2) is the sum of all canonical eigen values and adjusted by Bonferroni method (Zuur et al., 2007). The *P*-values are based on a permutation test (999 permutations). The urbanization gradient sites are urban (URB), suburban 1 (SUB1), suburban 2 (SUB2), and rural (RUR) sites.

Tresch et al., 2019; Abrego et al., 2020). However, under such a situation, taxa number and diversity could remain unchanged as some species may fill blank niches or occupy new niches created by urbanization (Morelli et al., 2016; Nielsen et al., 2019).

Indicator taxa for the four urbanization sites were different (the urban site did not have significant indicator species). Diplurans are small, elongate, delicate, primitive invertebrates, predating other small arthropods or ingest fungal mycelia and detritus (Ferguson, 1990). Most diplurans are euedaphic, but some are nocturnal cryptozoans, hiding under stones or bark during the day. Pauropods are tiny, true euedaphic terrestrial myriapods (Scheller, 2002). They inhabit in the lower litter layers, F-layers, and mineral soil in forests. It is generally assumed that pauropods are fungus feeders, but they may also be predaceous. In contrast, Enchytraeidae are typically 10–20 mm in length, bigger than both diplurans and pauropods. Enchytraeidae are commonly found in moist soils rich in organic matter (van Vliet, 2000), which probably made them to be the indicator species for the rural forest soil fauna community in this study. More efforts should be made to understand why they could be indicator species for the soil fauna communities in these ecosystems. Nevertheless, these taxa may have a potential to indicate urbanization effects on soil ecosystem.

Roles Soil Physicochemical Characteristics Play in Mediating Urbanization Effects on Soil Fauna Biodiversity

Across the 36 plots in this study, soil physicochemical characteristic only contributed little to the variances in fauna abundance, taxa number, and community structure, but is quite important in regulating Shannon's diversity and Pielou's evenness.

We found soil heavy metal contents, including Cd, Cr, Cu, Pb, and Zn, were very important factors in influencing soil fauna biodiversity. Negative relationship between soil fauna biodiversity and heavy metal content may be attributed to the universal heavy metal toxicity to organism growth and reproduction (Crommentuijn et al., 1993; Didden and Rombke, 2001; Herbert et al., 2004). In urban field studies whether or not heavy metals could inhibit soil fauna biodiversity (Fiera, 2009; Santorufo et al., 2012; Sterzynska et al., 2018), the results may depend on the resistance of study organism and the range of heavy metal content. The metals Cr and Cu were the key factors contributing to the divergence of urban soil fauna community structure from other sites, indicating that the accumulated heavy metals in urban soils played an important role in mediating urbanization effects on soil fauna community structure. In contrast, soil water availability was important in maintaining the rural soil fauna community structure and was strongly correlated with Enchytraeidae, the indicator species for the soil fauna community in the rural site. Soil in rural area was less disturbed, and with a higher SOM and lower BD, thus having a better water holding capacity, which may be important to resist to the negative effect of urbanization on soil fauna biodiversity.

Soil fauna biodiversity is not only affected by soil physicochemical characteristic but also regulated by habitat landscape, aboveground vegetation, direct disturbance, and so on. Soil physicochemical characteristic is very important in regulating soil fauna biodiversity in natural forests, grasslands, and farmlands (van Straalen, 1998; Bispo et al., 2009; Gerlach et al., 2013). In urban ecosystems, habitat landscape properties, including area, connectivity, and landscape heterogeneity, can significantly affect soil fauna biodiversity (Braaker et al., 2014; Milano et al., 2018; Xie et al., 2018). In addition, garden management (Byrne and Bruns, 2004) and land use history (Smetak et al., 2007; Francini et al., 2018) can also influence soil fauna biodiversity. These environmental factors that influence soil fauna biodiversity may not vary consistently, thus decoupling the apparent correlation between soil fauna biodiversity and physicochemical characteristic, which should be taken into account in further studies.

This study suggested that soil physicochemical changes due to urbanization could either increase or decrease soil fauna biodiversity, which was similar to studies on aboveground organisms suggesting urbanization could either facilitate or inhibit biodiversity through different urbanization agents (McKinney, 2006; Jones and Leather, 2012; Hornung et al., 2018). Consequently, though soil physicochemical characteristic explained about half of the variances in Shannon's diversity and Pielou's evenness across the plots, neither of them exhibited a pattern similar to the soil physicochemical characteristic along the gradient. These results suggested that under an urbanization context, the effects of soil physicochemical changes on soil fauna biodiversity may be indirect and complicated.

LIMITATIONS OF THIS STUDY

It should be noted that sampling was performed only once in this study. Traditionally, in subtropical ecosystems, soil fauna biodiversity is different between wet and dry seasons, which is found in Guangzhou City (Qin et al., 2009; Wang and Tong, 2012) and other subtropical ecosystems (Zhao et al., 2011). Soil fauna biodiversity responses to environmental changes could also differ between wet and dry seasons. For example, Gao D. et al. (2020) found the response pattern of soil nematodes to land use changes in wet season is different from that in dry season in a subtropical forest. Therefore, there is a possibility that soil fauna biodiversity pattern along the urbanization gradient in wet season will be different from the one shown in this study. Moreover, soil fauna could be substantially influenced by low soil water availability (Liu et al., 2015) and low air temperature (Meyer-Wolfarth et al., 2021), thus less sensitive to other environmental changes in the mid-dry season. Nevertheless, during the sampling period, the air temperature ranged from 10 to 27°C with a mean of 18°C, which may not depress soil fauna growth and reproduction. The SWC ranged from 7 to 31% during the experiment. However, SWCs lower than 10% were only observed in the urban site, which could be attributed to the urbanization effect. Therefore, this study should produce reliable results on the relationships between soil fauna biodiversity and soil physicochemical characteristics.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

GX and ZFW designed the experiment. CL, ZJW, ZL, ML, XF, and YL contributed to the field samples and analyzed the soil physicochemical properties. XC identified the soil fauna. SY and ZJW conducted the statistical analysis and wrote the manuscript. GX, ZFW, and CL revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.824004/full#supplementary-material

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Distribution and Assemblage Variation of Benthic Macroinvertebrates: A Uniform Elevational Biodiversity Pattern Among Different Groups?

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Biodiversity patterns along the elevational gradient of vertebrates have been widely focused on in previous studies, but they are still insufficient on invertebrates in lakes to a wide elevational extent. Based on field samplings and literature, we compared biodiversity patterns among different taxonomic groups of benthic macroinvertebrates in 104 lakes of China and India along an elevational gradient of 2–5,010 m a.s.l. and revealed the key driving factors, and then, we discussed the key mechanisms underlying elevational biodiversity patterns. We found that elevational biodiversity patterns of different taxonomic groups were not uniform, e.g., an exponentially decreasing pattern of Bivalvia, a first horizontal and then decreasing pattern of Gastropoda, and a linear decreasing pattern of Oligochaeta and Insecta. Elevation and elevation-controlled variables (temperature and salinity) were the key driving factors to biodiversity patterns. Their effects were strongest on Bivalvia and less on Gastropoda, whereas they were relatively weak on Oligochaeta and Insecta. Finally, we discussed three important mechanisms that shaped elevational biodiversity patterns and assemblage variations of benthic macroinvertebrates by linking our results with the classic hypotheses about biodiversity patterns, including climate/productivity, environmental heterogeneity, and dispersal/history. These results could improve our understanding of biodiversity patterns and biodiversity conservation.

Keywords: elevational gradient, distribution variation, elevational mechanism, macroinvertebrates, Chinese and Indian lakes

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INTRODUCTION

Understanding the distribution patterns and their underlying mechanisms of biodiversity is one of the central objectives in biogeography and ecology (Gaston, 2000; Lomolino, 2001; Mittelbach et al., 2007; Laiolo et al., 2018). Large-scale spatial biodiversity patterns have recently received extensive attention, especially in the context of global climate change (Collen et al., 2014; Birrell et al., 2020). Along elevational gradients, organisms generally show a decreasing trend in species richness, and this pattern has been verified in several taxonomic groups (Bryant et al., 2008; Teittinen et al., 2017). Learning this elevational biodiversity pattern is helpful not only for determining the underlying drivers of biodiversity but also for predicting the biological response to global climate change, which is very important for biodiversity maintenance and protection (Lomolino, 2001; Rahbek, 2005; Liu et al., 2021).

In the previous studies, it was widely thought that biodiversity decreased monotonically with increasing elevation. However, recent studies showed that elevational biodiversity may show either a monotonically decreasing or a hump-shaped pattern (McCain, 2005; Rahbek, 2005; Fu et al., 2006; Beck et al., 2017; Teittinen et al., 2017). Whatever the distribution pattern has shown, climatic, area, and local environmental variables are the most cited driving factors (Lomolino, 2001; Hawkins et al., 2003; McCain, 2004, 2007, 2009; Birrell et al., 2020). Besides those explanatory factors above, biodiversity distribution along elevational gradient also varies due to specific taxonomic groups (Bryant et al., 2008; Kessler et al., 2011; Nunes et al., 2020; Pandey et al., 2021). Therefore, it is necessary to study elevational biodiversity patterns on different taxonomic groups.

Biodiversity patterns along elevational gradient have been well-examined for vertebrates, such as mammals (Brown, 2001; McCain, 2005, 2007; Gebert et al., 2019), birds (McCain, 2009; Neate-Clegg et al., 2021), reptiles (McCain, 2010; Jins et al., 2021), amphibians (Fu et al., 2006; Wang et al., 2019), and fishes (Bhatt et al., 2012; Qian et al., 2021). However, we have insufficient data about invertebrates (Sanders et al., 2003; Beck et al., 2017; Albrecht et al., 2018; Nunes et al., 2020; He et al., 2021), especially benthic macroinvertebrates in lakes (de Mendoza and Catalan, 2010; Wang et al., 2011). This limits our general understanding of biodiversity distribution. Benthic macroinvertebrates are diverse and abundant and play an important role in ecosystem functions (Lin et al., 2020). Meanwhile, benthic macroinvertebrates are sensitive to environmental changes, which makes them useful as good indicators for disturbances and pollution (Edegbene et al., 2021).

In this article, we studied benthic macroinvertebrates (i.e., Bivalvia, Gastropoda, Oligochaeta, and Insecta) in 104 lakes along an elevational gradient of 2∼5,010 m a.s.l. in China and India. Our objectives were to compare elevational biodiversity patterns among different benthic macroinvertebrate groups and to unravel whether the effects of elevation on the biodiversity patterns were uniform. Through explaining the relationship between elevation and elevation-related variables, we tried to reveal the mechanisms underlying these patterns. Our study could enrich the cognition about elevational biodiversity patterns of different benthic macroinvertebrate groups to a complete and wide extent and verify that those patterns were primarily determined by climate/productivity factors.

MATERIALS AND METHODS

Data Source and Distribution of Studied Lakes

Study areas are located in China and India. A total of 104 lakes were studied with an elevational gradient of 2–5,010 m a.s.l. (**Figure 1**). In our study, benthic macroinvertebrates from 41 lakes in the Tibetan Plateau were collected from our field investigations in 2015 and 2016. To ensure the comparability of data, referring to our field investigations, we filtered the studies that used similar collecting methods and summarized the distribution data of benthic macroinvertebrates from 63 lakes, including 25 lakes from the Eastern Plain of China, 10 lakes from the Inner Mongolia-Xinjiang Plateau of China, 11 lakes from the Yunnan-Guizhou Plateau of China, three lakes from the Tibetan Plateau of China, and 14 lakes from the mainland of India (PRISMA flow diagram for literature searching and filtering is presented in **Appendix 1**). Locations, elevation and collecting methods, and data sources of studied lakes are presented in **Appendix 2**. To avoid the incomparability of taxonomic information from different sampling efforts among studies, we classified benthic macroinvertebrates groups into the uniform taxonomic level. Oligochaetes, gastropods, and bivalves were unified into genus. Insects were unified into family or genus.

Field Investigations and Source of Collected Environmental Variables

The macroinvertebrates sampling habitats included pelagic zones and littoral zones in each lake. In pelagic zones, six samples in two different stations were collected with a weighted Petersen grab ($0.0625\ m^2$). In littoral zones, different habitats in two stations were randomly selected along the shoreline between depths of 15–30 cm, and two samples were collected using a D net (0.25 × 2 m, with 420 μm mesh). All the samples were sieved with a 420 μm sieve in the field. Specimens were manually picked from sediment with a white porcelain plate in the field and preserved in a 10% formalin solution. All specimens were brought back to the laboratory for microscopic examination, counting, and weighing. Benthic macroinvertebrates were identified to the uniform taxonomic level according to professional references (Liu et al., 1979; Morse et al., 1994; Epler, 2001; Wang, 2002).

In the field investigations, elevation was measured with a handheld GPS produced by GARMIN, USA. Water dissolved oxygen (DO), pH, and salinity were measured with a YSI ProPlus (Yellow Spring Inc., USA). Water transparency (transparency) was measured with a Secchi disk. Total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_4^+), nitrate nitrogen (NO_3^-), and chemical oxygen demand (COD) were measured according to the environmental quality standards for surface water of China (GB 3838-2002; Wei et al., 1989). The DO, pH, salinity, transparency, TN, TP, NH_4^+, NO_3^-, and COD of collected lakes were extracted from the literature.

Drainage area, lake area, lake length, lake width, recharge coefficient, average water depth, and precipitation were collected from in the book of *Lakes of China* (Wang and Dou, 1998). Annual mean temperature (Temp_mean), maximum temperature of the warmest month (Temp_max), minimum temperature of the coldest month (Temp_min), temperature annual range (Temp_range), and evaporation were downloaded from WorldClim website (http://www.worldclim.org/; Fick and Hijmans, 2017), and values were extracted at each lake scale in ArcGIS 10.1.

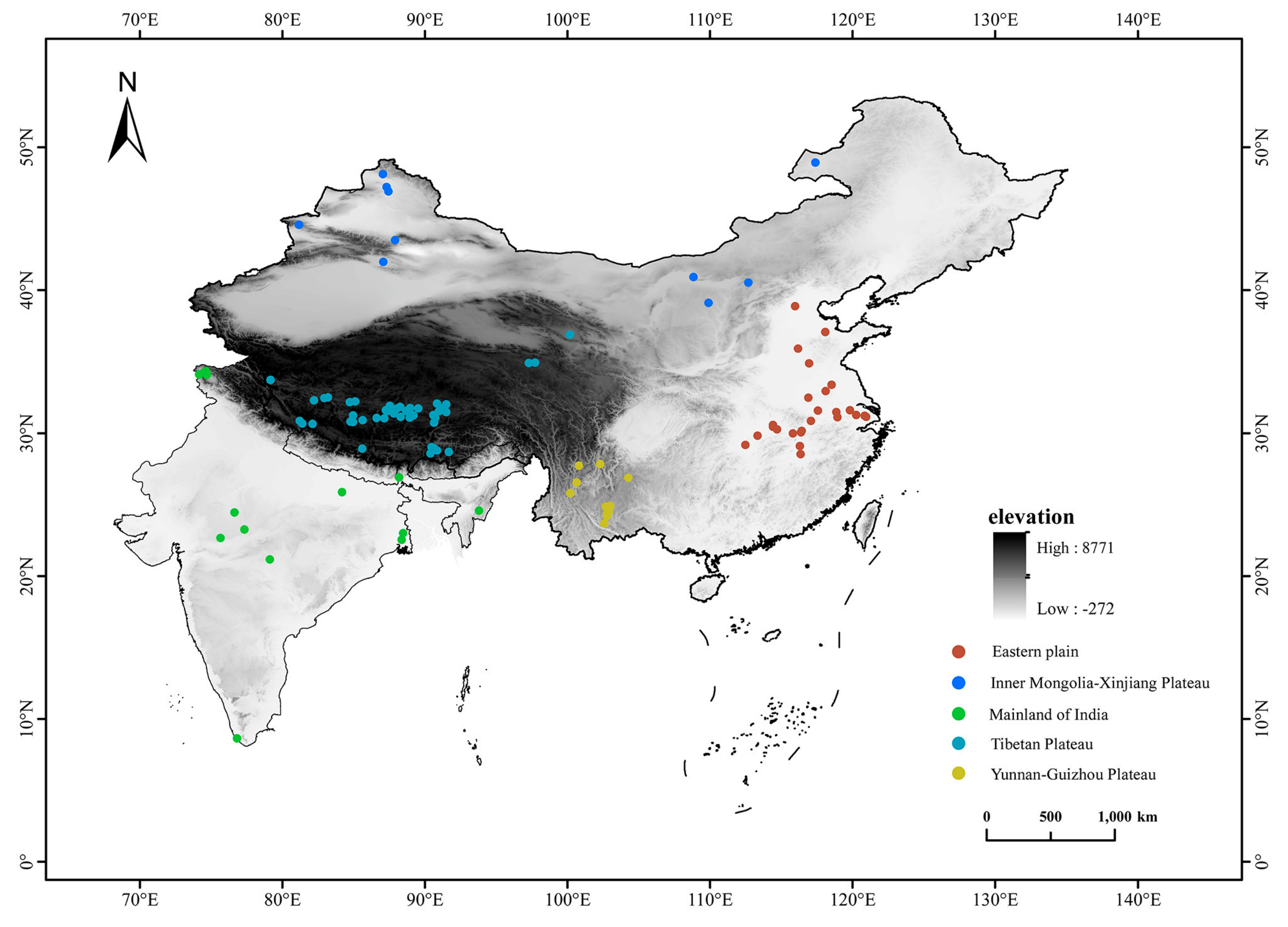

FIGURE 1 | Geographical position of 104 studied lakes distributed in China and India along an elevational gradient of 2–5,010 m a.s.l.

Statistical Analyses

First, we examined the relationship between elevation and other environmental variables with Spearman's rank correlation analysis to explore whether other variables were significantly correlated with elevation. Principal component analysis (PCA) was used to analyze the relationship among 22 environmental variables. According to the relationships above, we divided environmental variables into two groups, namely, elevation-related variables (significant correlation with elevation), and elevation-unrelated variables (no significant correlation with elevation). Then, by exploring the relationships between species richness (uniform genus or family level) of different groups (Bivalvia, Gastropoda, Oligochaeta, and Insecta) and elevation by regression analysis and quantile regression with the packages of quantreg (Koenker, 2021) in R 3.4.3 (R Core Team, 2017), we aimed to understand the distribution variations of different groups along the elevational gradient. Canonical correspondence analysis (CCA) was used to examine the relationship between macroinvertebrates composition and the explanatory variables. Forward selection with Monte Carlo permutation tests (999 permutations) was used to select a parsimonious set of explanatory variables under the cutoff point of $p < 0.05$. Partial canonical correlation analysis (pCCA) was used to analyze the relative importance of elevation-related variables and elevation-unrelated variables. Then, the total variation in macroinvertebrate composition was partitioned into four independent fractions: elevation-unrelated variance, elevation-related variance, shared variance, and unexplained variance. Some environmental variables were log-transformed before analyzing. PCA, CCA, and pCCA analyses were performed using the software CANOCO for Windows 5.0 version (Ter Braak and Šmilauer, 2012).

RESULTS

Environmental Variables Along the Elevational Gradient

A PCA of 22 environmental variables showed that the first ordination axis was driven by elevation and elevation-related variables (mainly climatic variables) and explained 28.6% of the variance. The second ordination axis was mainly related to the area and other environmental variables and explained 16.0% of the variance (**Figure 2**). Temp_max, Temp_mean, precipitation, NH_4^+, Temp_min, longitude, DO, TN, COD, and

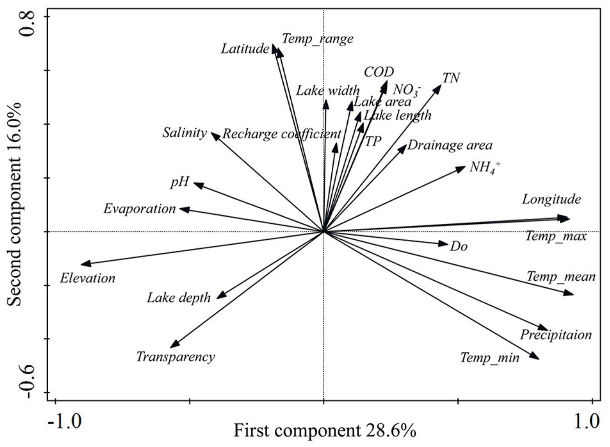

FIGURE 2 | Plot of the principal component analysis for the 104 lakes with 24 environmental variables in China and India. COD, chemical oxygen demand; DO, water dissolved oxygen; NH_4^+, ammonium nitrogen; NO_3^-, nitrate nitrogen; Temp_mean, annual mean temperature; Temp_max, maximum temperature of the warmest month; Temp_min, minimum temperature of the coldest month; Temp_range, temperature annual range; TN, total nitrogen; TP, total phosphorus.

TP showed negative correlations with elevation ($p < 0.05$). Conversely, transparency, evaporation, average water depth, pH, and salinity showed positive correlations with elevation ($p < 0.05$). Other environmental variables including lake length, lake width, latitude, recharge coefficient, lake area, drainage area, Temp_range, and NO_3^- showed no significant correlation with elevation ($p > 0.05$). Minimum, maximum, and mean value of environmental variables and their relationships with elevation are given in **Appendix 3**.

Taxa Composition and Distribution Patterns Along Elevational Gradient

A total of 638 taxa belonging to four classes and 106 families were recorded in our studied lakes. Insecta was the most diverse group with 353 taxa belonging to 76 families. Gastropoda was also a diverse group with 137 taxa belonging to 17 families. There were fewer taxa of Oligochaeta and Bivalvia, which were recorded as 78 taxa belonging to five families and 70 taxa belonging to eight families, respectively.

Taxa distributions along the elevational gradient were not uniform among different taxonomic groups of benthic macroinvertebrates (**Figure 3**). Insecta and Oligochaeta were the widely distributed groups, and some taxa only occurred in high-elevational lakes. The distributions of Bivalvia and Gastropoda were relatively narrow.

The responses of benthic macroinvertebrate incidence to elevation were mostly negative, and only a few taxa showed a positive response (see **Appendix 4** for details). Species richness of benthic macroinvertebrates showed decreasing tendency along elevation. However, these decreasing patterns were different among taxonomic groups: Bivalvia showed an exponentially decreasing pattern that showed a rapid decline in biodiversity at low elevation; Gastropoda richness showed a horizontal and then decreasing pattern and presented a significant decline over 2,000 m a.s.l.; and Oligochaeta and Insecta showed a linear decreasing pattern (**Figure 4**).

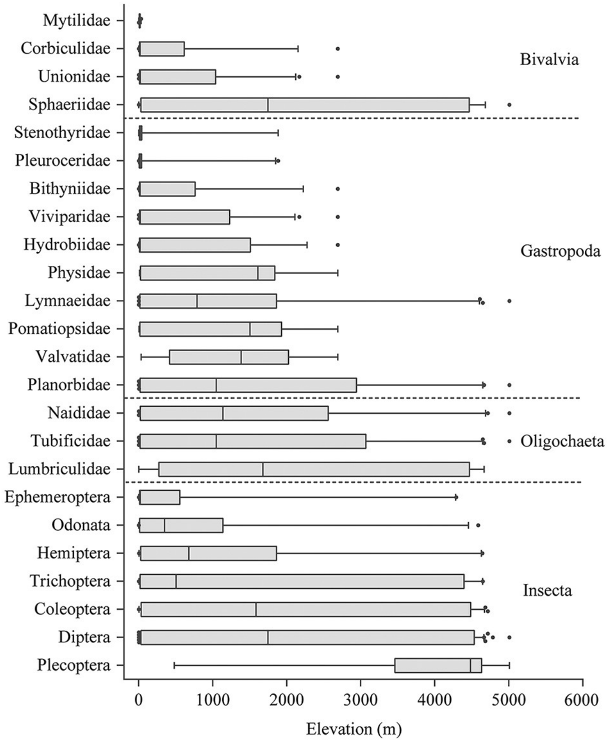

FIGURE 3 | Distributions of different groups of benthic macroinvertebrates along elevational gradient in lakes of China and India.

Explanatory Variables of Assemblage Variation

Climatic variables were the most important explanatory factors for assemblage variation of benthic macroinvertebrates. Variables controlled by climate such as temperature, precipitation, salinity, and pH have significant effects on taxa distribution (**Table 1**). Area variables were also important factors in explaining assemblage variations, such as watershed area and water depth, which showed a significant impact on the distributions of Bivalvia and Gastropoda. Moreover, the watershed area showed a significant impact on the distribution of Insecta (**Table 1**). Compared with climatic variables and area variables, the impacts of other environmental variables on assemblage variation were relatively weak (**Table 1**). In general, elevation and elevation-controlled variables were the key driving factors for assemblage variation of benthic macroinvertebrates (**Table 1**).

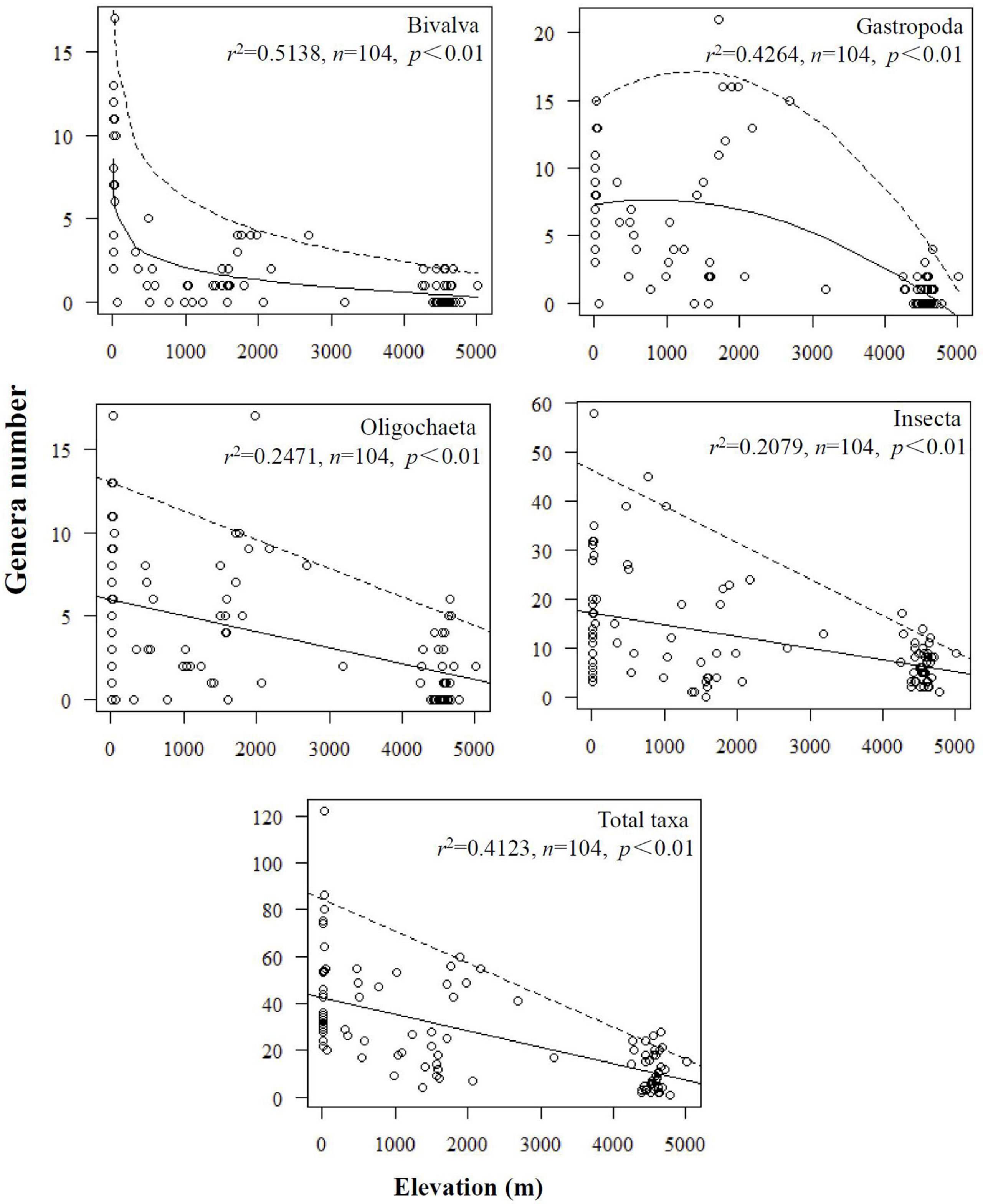

FIGURE 4 | Biodiversity patterns along elevational gradient in different groups of benthic macroinvertebrates in lakes of China and India. Oligochaetes, gastropods, and bivalves were identified to genus or species. Insects were identified to family or genus. The solid lines represent the best regression curve using the least-squares method (statistical significance of r^2 values is shown). The dashed lines represent the quantile regression curve of 95th percentiles.

TABLE 1 | Explanations (%) and order of entry of significant explanatory variables in the forward selection with Monte Carlo permutation tests to the assemblage variation of benthic macroinvertebrates ($\alpha = 0.05$).

Variables	All species	Oligochaeta	Bivalvia	Gastropoda	Insecta
Elevation	3.2%$^{(2)}$	4.5%$^{(2)}$	25.5%$^{(1)}$		4.0%$^{(1)}$
Temp_mean*	4.9%$^{(1)}$	7.3%$^{(1)}$	3.9%$^{(5)}$	7.0%$^{(2)}$	2.9%$^{(3)}$
Temp_max*	2.6%$^{(5)}$	3.3%$^{(3)}$		8.9%$^{(1)}$	
Temp_min*	2.7%$^{(6)}$			4.1%$^{(4)}$	2.4%$^{(5)}$
Temp_range					2.8%$^{(6)}$
Precipitation*	1.6%$^{(14)}$			2.2%$^{(7)}$	
Evaporation*	1.6%$^{(16)}$				1.8%$^{(16)}$
Salinity*	3.0%$^{(3)}$		4.4%$^{(4)}$		2.2%$^{(7)}$
pH*	1.7%$^{(13)}$		2.7%$^{(6)}$		1.7%$^{(14)}$
DO*	1.8%$^{(11)}$	2.8%$^{(4)}$			1.8%$^{(13)}$
TN*	2.0%$^{(7)}$				2.1%$^{(8)}$
TP*	1.7%$^{(15)}$			3.9%$^{(5)}$	1.8%$^{(12)}$
NH_4^{+*}	1.7%$^{(12)}$				2.1%$^{(15)}$
NO_3^-	1.9%$^{(8)}$				2.1%$^{(9)}$
COD*	1.8%$^{(10)}$				2.5%$^{(4)}$
Drainage area	3.0%$^{(4)}$		5.4%$^{(3)}$	3.4%$^{(6)}$	3.4%$^{(2)}$
Lake area	1.8%$^{(9)}$				1.8%$^{(11)}$
Lake width					1.9%$^{(10)}$
Water depth*			6.3%$^{(2)}$	4.4%$^{(3)}$	
Lake length					

"" means the environmental variable correlates significantly with elevation. The numbers in the brackets mean orders of entry of significant explanatory variables.*

COD, chemical oxygen demand; DO, water dissolved oxygen; NH_4^+, ammonium nitrogen; NO_3^-, nitrate nitrogen; Temp_mean, annual mean temperature; Temp_max, maximum temperature of the warmest month; Temp_min, minimum temperature of the coldest month; Temp_range, temperature annual range; TN, total nitrogen; TP, total phosphorus.

Relative Contributions of Elevation-Related and Elevation-Unrelated Variables on Assemblage Variance

Elevation-related variables explained much more variation than elevation-unrelated variables. However, the proportions of assemblage variation explained by elevation-related variables and elevation-unrelated variables were different among taxonomic groups (**Figure 5**). Bivalvia showed the largest proportion of variation explained by the pure effect of elevation-related variables, followed by Gastropoda, while Oligochaeta and Insecta showed a relatively small proportion of variation explained by the pure effect of elevation-related variables (**Figure 5**). In total, the effect of elevation on taxa distribution was strongest in Bivalvia and less strong in Gastropoda, whereas it was relatively weak in Oligochaeta and Insecta.

DISCUSSION

Elevational Biodiversity Patterns of Different Biological Groups

It is a comprehensive study that explored the biodiversity patterns of multiple taxonomic groups of benthic macroinvertebrates (including Bivalvia, Gastropoda, Oligochaeta, and Insecta) along such an extensive elevational gradient at a large geographical scale. Our results elucidated that species richness of benthic macroinvertebrates declined with increasing elevation, but the patterns varied among taxonomic groups, which showed an exponentially decreasing pattern of Bivalvia, a horizontal and then decreasing pattern of Gastropoda, and a linear decreasing pattern of Oligochaeta and Insecta.

Rahbek (2005) summarized 204 data sets from 140 studies on elevational biodiversity patterns, including plants, vertebrates, and invertebrates, and assigned them to one of five patterns, namely, monotonic decreasing; horizontal, then decreasing; hump-shaped; increasing, and other. Among the patterns above, a hump-shaped elevational biodiversity pattern was more typical (c. 50%) than a monotonic decreasing pattern (c. 25%). However, the elevational range of previous studies was not wide enough (e.g., <500 m, or more than 2,000 m) to reflect the continuous biodiversity pattern. For example, a hump-shaped pattern would be concealed if elevations below 500 m were excluded (Neate-Clegg et al., 2021). And if the elevation was over 4,000 m, the monotonic increasing pattern would often hardly appear.

Our results showed a decreasing elevational biodiversity pattern in species richness, rather than the more typical hump-shaped elevational biodiversity pattern in the literature, which may be caused by the studied groups. For example, nonvolant small mammals showed a clear hump-shaped elevational biodiversity pattern in species richness (McCain, 2005; Gebert et al., 2019). The hump-shaped pattern was also typical in vertebrates such as birds (McCain, 2009; Neate-Clegg et al., 2021), reptiles (McCain, 2010), and amphibians (Fu et al., 2006; Wang et al., 2019), and terrestrial invertebrate species, such as

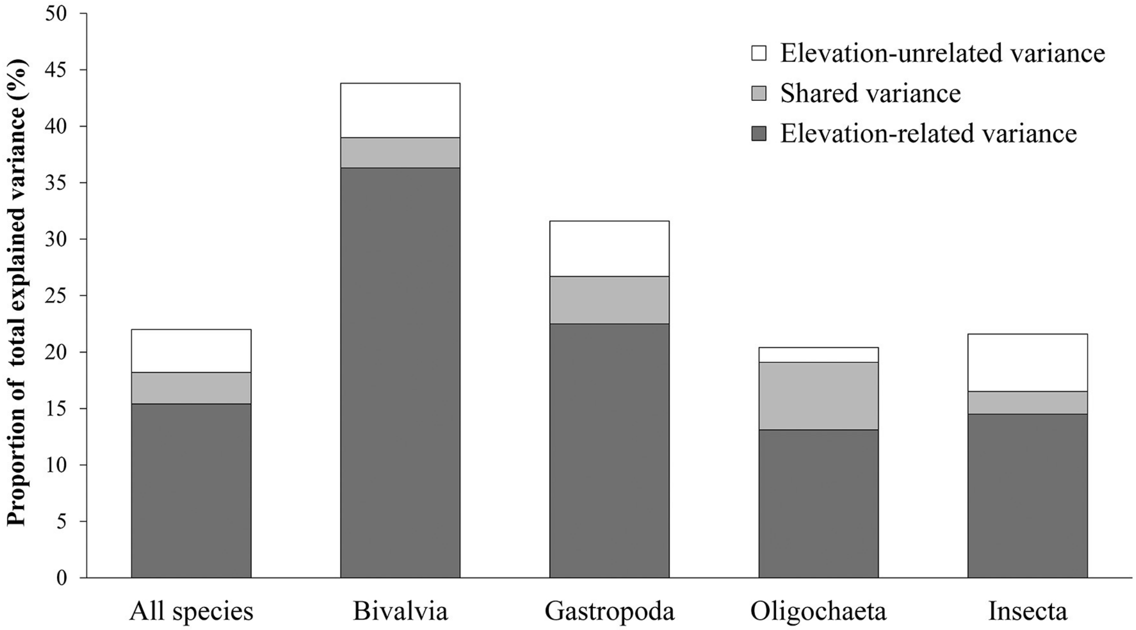

FIGURE 5 | Proportion of total explained variance related to elevation-related variance, shared variance, and elevation-unrelated variance in different groups of benthic macroinvertebrates. Oligochaetes, gastropods, and bivalves were identified to genus or species. Insects were identified to family or genus.

beetles (da Silva et al., 2018; McCabe and Cobb, 2020), moths (Beck et al., 2017), and ants (Szewczyk and McCain, 2016; Nunes et al., 2020). However, aquatic biological groups often showed a decreasing elevational biodiversity pattern in species richness, such as fish (Fu et al., 2004; Bhatt et al., 2012; Qian et al., 2021), crustacean (Hessen et al., 2007; Ramos et al., 2021), rotifer (Obertegger et al., 2010), aquatic plants (Jones et al., 2003; Stefanidis et al., 2021), phytoplankton and cyanobacteria (Jankowski and Weyhenmeyer, 2006; Teittinen et al., 2017).

Key Mechanisms of Elevational Biodiversity Patterns

Three important hypotheses, namely, climate/productivity, environmental heterogeneity, and dispersal/history, have been most mentioned to explain the biodiversity patterns (Field et al., 2009; He et al., 2020). In our study, elevation-related variables (i.e., temperature and salinity) and elevation play a dominant role in biodiversity patterns, which could be corresponded with the three hypotheses above. Among them, temperature, which represents the climate/productivity hypothesis, is the most important explanatory variable in explaining assemblage variation for macroinvertebrates and different groups (Bivalvia, Gastropoda, Oligochaeta, and Insecta; **Table 1**). Temperature has been mostly recognized as a primary factor to shape biodiversity patterns for the groups to adjust quickly to changing climate (He et al., 2020). Temperature monotonically declines with elevation, so some tropical species find it hard to survive at high elevational areas, which leads to the decrease of species richness (Fu et al., 2004; Wang et al., 2019). In addition, low temperature could limit the species richness in two ways. One way is to reduce metabolic rates, thereby increasing generation times, and then hinder evolutionary processes (Rozen-Rechels et al., 2019). The other way is to lead to foraging restrictions in ectotherms and reduce their ability to utilize food resources (Sanders et al., 2003; Classen et al., 2015). All in all, the temperature has important impacts on almost all biological processes, such as interspecific relationships, gene mutation, speciation, extinction, and evolution. Through those ways, it affects the biodiversity patterns (Rozen-Rechels et al., 2019).

Salinity, which represents the environmental heterogeneity hypothesis, is also an important explanatory variable to explain assemblage variation in this study. Elevation generally influences the precipitation and evaporation and ultimately determines the salinity of the lakes and finally affects the biodiversity patterns (Williams et al., 1990). Several studies have shown that salinity is an ecological barrier to determining geographical distribution. With salinity increased, biodiversity declined exponentially. Lin et al. (2017) invested zooplankton communities in 45 lakes of the Tibetan Plateau and found that species richness declined significantly with salinity. In addition, our unpublished data of benthic macroinvertebrates in 40 lakes of the Tibetan Plateau showed that the taxa composition in saline lakes was significantly different from that in freshwater lakes. Oligochaeta, Gastropoda, and Bivalvia were generally distributed in freshwater lakes with salinity below 3 g/L. Chironomidae were distributed in saline lakes with salinity up to 35 g/L. Only a few taxa of Insecta (i.e., Ephydridae and Hydrophilidae) can survive in lakes with salinity over 35 g/L.

Besides the influences of temperature and salinity, elevation, which represents the dispersal/history hypothesis, also plays an important role in determining biodiversity patterns (Currie et al., 2004; Hagen et al., 2021). Elevational patterns in China were

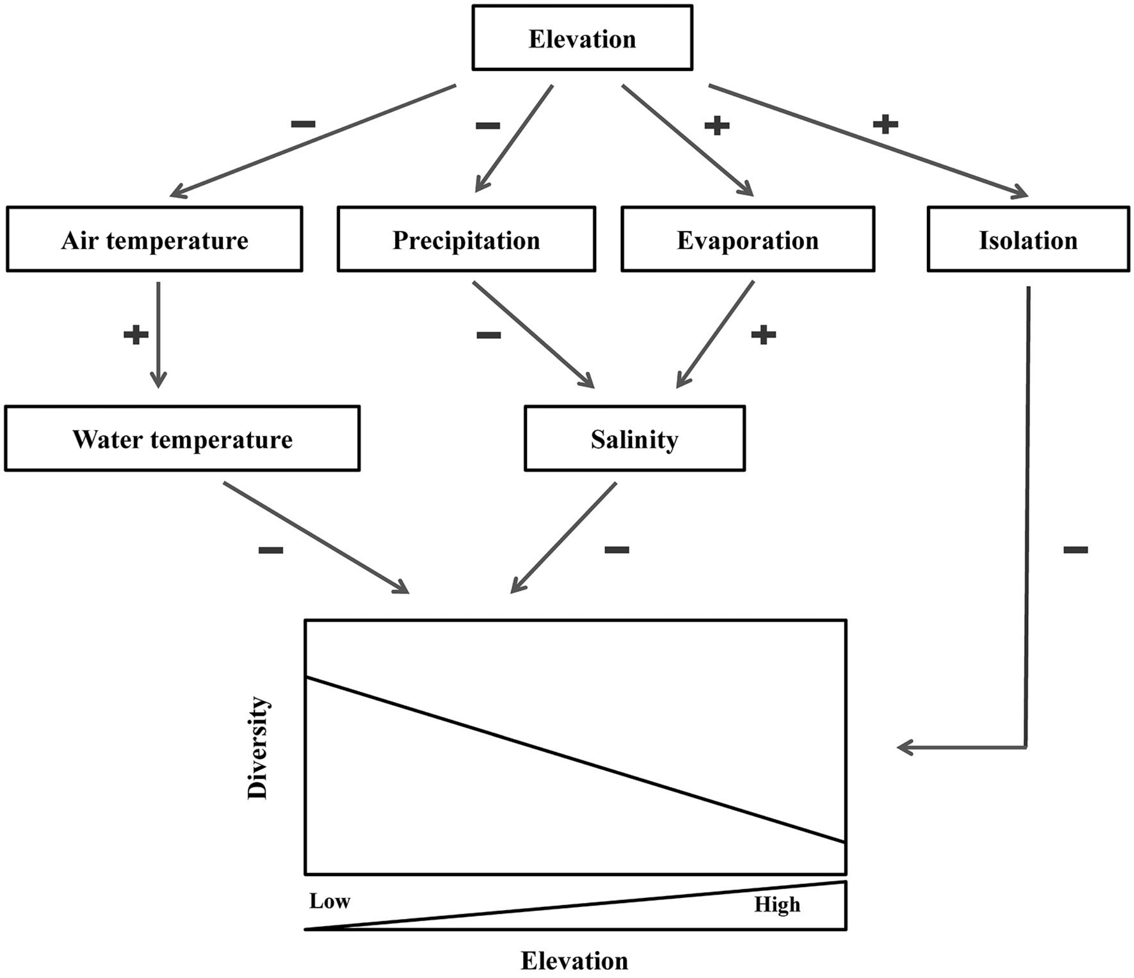

FIGURE 6 | Mechanisms of elevational effects on benthic macroinvertebrates biodiversity in lakes of China and India. "±" indicate whether there is a positive or a negative tendency between two variables.

characterized by complex geological tectonic histories since the Cenozoic (Favre et al., 2015). Dispersal ability is a key condition for species distributions (e.g., Heino, 2013; Siefert et al., 2015). Island biogeography studies have shown that weak dispersers are often absent from distant islands or habitat fragments, leading to a decrease in species richness with isolation (Prugh et al., 2008; Sandel et al., 2020). Our results confirmed that this phenomenon also occurs in lake ecosystems. Most bivalves with passive movement ability are absent from high altitude areas, leading to the poor biodiversity of bivalves in Tibetan Plateau lakes. Furthermore, we have also investigated two genera of small bivalves (*Pisidium* and *Sphaerium*) in Tibetan Plateau lakes, as small bivalves may be easier to be transferred from the lowland to high plateau areas by waterbirds (Cai et al., 2018). Compared with strong aerial dispersers with flying adults (i.e., Insecta), weak and passive dispersers with aquatic adults (i.e., Gastropoda and Bivalvia) are more sensitive to elevation restrictions (Heino, 2013). This is consistent with our study. In addition, McCulloch et al. (2017) studied New Zealand's entire plecopteran fauna (100 species), observed a positive correlation between geographical range and relative wing length, and found that even small reductions in wing size could significantly influence geographical range.

In summary, the impacts of elevation on biodiversity patterns of benthic macroinvertebrates may be controlled through three important mechanisms (**Figure 6**): (i) with the elevation increasing, the air temperature drops sharply, leading to decreasing of the water temperature and ultimately reducing the biodiversity of benthic macroinvertebrates; (ii) precipitation and evaporation are significantly affected by the increasing elevation, which eventually increases the salinity of lakes and thereby reduces the biodiversity of benthic macroinvertebrates; (iii) due to isolation, elevation hinders the exchange and diffusion of species and ultimately affects biodiversity.

Our study concluded different biodiversity patterns for different groups of benthic macroinvertebrates along the elevational gradient. Climatic factors played a key role in the elevational biodiversity patterns in all the groups. Insecta distribute along a wider elevational gradient than Gastropoda and Bivalvia. Finally, we revealed three mechanisms that shaped the biodiversity patterns of benthic macroinvertebrates.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

BW and YC put forward the scientific hypothesis and participated in the investigations in the fields. BW, YZ, and YH identified the macrozoobenthos. BW and YH collected the literature data and analyzed the data. BW wrote the manuscript. YC, YH, and YZ edited the manuscript. All authors read and approved the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.817708/full#supplementary-material

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Flow Intermittence Drives the Benthic Algal Composition, Biodiversity and Diatom-Based Quality of Small Hilly Streams in the Pannonian Ecoregion, Hungary

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Climate change is putting increasing pressure on flowing waters. Drastic water level fluctuations in rivers or drying up of small and medium-sized streams all contribute to the biodiversity crisis threatening freshwater ecosystems. Benthic diatoms are important elements of biofilm in small streams. However, knowledge on the relationship between benthic diatoms and flow intermittence is incomplete, especially in regions recently impacted by recurrent drying. Thus, we investigated benthic diatom flora of small intermittent, hilly streams in the warm temperate region of Europe (the Pannonian Ecoregion). Our hypotheses were addressed to compositional changes, biodiversity loss and diatom-based ecological assessment. The results revealed clear flow intermittence-induced differences in taxa and trait composition of diatoms. Altogether six species for the dry phase and three species in the aquatic phase were identified as indicative ones by using indicator value analyses. In contrast to water regime induced changes in assemblages, there was a seasonal overlap in taxa and trait composition. During the study period, the drying up of streams did not result in significant biodiversity loss either at taxa or trait levels. Functional dispersion, however, reduced significantly by summer. Overall, neither the hydrological regime nor seasonal changes had a significant effect on diatom-based quality indices, except for the Rott trophic index (TID index). The TID index values were significantly lower in dry phases than in aquatic ones. These results suggested that the drying up of streams has a very complex influence on benthic diatoms. It seems that taxonomical and functional redundancy can reduce the negative impact of short-time flow intermittence on assemblages. As a practical benefit, the results are the first to support the use of diatom-based quality indices in the assessment of flow intermittence in the temperate region.

Keywords: flow intermittence, small hilly streams, temperate region, water quality indices, aquatic ecosystem, compositional changes

INTRODUCTION

Over the past decades, biodiversity loss has become a major problem facing humanity and as it is constantly worsening it threatens ecosystems functioning and human well-being (Díaz et al., 2006; He et al., 2019; Crabot et al., 2021; Thompson et al., 2021). Although freshwaters are one of the most diverse ecosystems, integrity loss of communities here is an increasing trend that poses a challenge to biodiversity conservation (Dudgeon et al., 2006; Reid et al., 2019). Climate change and its impact on freshwater biodiversity is one of the 12 threats named by Reid et al. (2019) endangering the entire ecosystem from specimen to community level resulting in, inter alia, reduction in cell/body size, changes in phenology, changes in species distribution, spread of invasive or non-native species or even disease outbreaks (see more in Reid et al., 2019). Rapid and extreme changes in water regime, increasing water temperature, drying of small water bodies and nutrient concentration during drying are just some of the influences that can result in irreversible transformations in freshwater ecosystems (see more in Magand et al., 2020).

As climate change, water abstraction or even land use resulted in a shift of perennial watercourses becoming intermittent ones in the last 5 decades, small streams all around the world are increasingly threatened by flow cessation (Messager et al., 2021). According to the latest IPCC report (IPCC, 2021), there is high probability of increase in extreme climatic events, including severe droughts will occur in the near future. These events will result in an increase in the number of intermittent streams and the duration of non-flow periods. In the Mediterranean regions characterized by an intra-annual moisture deficit, a number of intermittent streams can be considered as natural elements of the landscape (Sabater et al., 2016; Acuña et al., 2017). In contrast, the intermittent shift of formerly perennial streams to an intermittent state is a relatively new phenomenon in the warm temperate region, where the summers are hot and there are no significant seasonal differences in precipitation[1] (B-Béres et al., 2019; Várbíró et al., 2020). Independently of the historical background of intermittent streams, i.e., drying is a new phenomenon or it dates back to the ancient times, these watercourses constitute a very large fraction of river networks and their number is probably underestimated (Sabater et al., 2016).

Intermittency is a strong pressure on organisms. Populations living in intermittent streams are either able to adapt to dry conditions or they disperse to a new area or become extinct from the given ecosystem (Falasco et al., 2016a; Sabater et al., 2016; Stubbington et al., 2017). Thus, these watercourses can be usually considered as lower diversity areas during the aquatic phase compared to permanent waters. However, appearance of lentic and terrestrial species in assemblages during intermittence can result in higher taxonomical and functional variability here (Stubbington et al., 2017). But intermittence is a strong environmental pressure that induces diversity changes during drying of streams. The replacement of taxa is quite intense then, the appearance of lentic and terrestrial taxa in assemblages first results in an increase of diversity in pools (Stubbington et al., 2017; Novais et al., 2020). But finally the increase in biotic interactions within these assemblages and the ongoing drying of pools lead to the decrease in biodiversity (Stubbington et al., 2017).

Freshwater benthic diatoms are one of the most abundant algal groups in the biofilm of streams (Pan et al., 1999), thus they play a key role in these ecosystems as primary producers and also as a food source for herbivores (Kireta et al., 2012). Since they are sensitive indicators of environmental stressors (McCormick and Cairns, 1994), they provide valuable information on the ecological quality of waters (Water Framework Directive – EC, 2000). In addition, in the last few years, an increasing number of studies have shown that benthic diatoms respond sensitively to drying of waters (Novais et al., 2014, 2020; Falasco et al., 2016a; Piano et al., 2017; B-Béres et al., 2019; Stubbington et al., 2019; Várbíró et al., 2020; Tornés et al., 2021). Previous studies pointed out that diatom indices can be useful metrics comparing the ecological quality of intermittent streams in different phases (Barthès et al., 2015; Novais et al., 2020). In a comprehensive study, Novais et al. (2020) validated that values of diatom-based metrics from dry and from previous aquatic phases are comparable and showed no significant differences. There is no doubt, however, that drying of streams results in compositional modifications within assemblages (Novais et al., 2014, 2020; Falasco et al., 2016a; Piano et al., 2017; B-Béres et al., 2019; Várbíró et al., 2020; Tornés et al., 2021). Flow cessation and drying of streams create very harsh conditions for benthic algae and finally result in a decrease in biodiversity (Sabater et al., 2017; B-Béres et al., 2019; Novais et al., 2020; Várbíró et al., 2020; Tornés et al., 2021). Although taxonomical or functional redundancy seems to be able to balance this negative effect (Novais et al., 2020; Lukács et al., 2021), latest studies suggest that this phenomenon is highly dependent on the duration of the dry periods not only in the case of diatoms (Tornés et al., 2021), but also in macroinvertebrate assemblages (Crabot et al., 2021). Others pointed out that the time scale of dry periods is crucial in determining the extent of compositional changes. Drying of streams has a stronger negative influence on benthic diatom assemblages in regions where the flow intermittence is a new phenomenon than in regions with historical occurrence of intermittent streams (Várbíró et al., 2020). Our knowledge of flow intermittence – benthic diatom relationship, however, is incomplete in regions recently impacted by recurrent drying. It is especially true in the Pannonian Ecoregion, the largest part of which belongs to Hungary. Approximately 10,000 watercourses are registered here, but less than 10% of them are monitored regularly by the Water Framework Directive-related (WFD) National Biomonitoring System[2] (Stubbington et al., 2018). Very small streams with a catchment area less than 10 km^2 are not included in this biomonitoring system. Thus, we have no information on how many of these small streams are affected by drying in the region. Our knowledge of how these streams contribute to the maintenance of biodiversity of benthic flora is also incomplete. Therefore, we started to study benthic diatom

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[1] http://koeppen-geiger.vu-wien.ac.at

[2] www.vizeink.hu

assemblages of these very small intermittent streams in the last few years focusing first on the southern hilly part of the region. Our main aim was to investigate the impact of drying up on stream diatom assemblages. We hypothesized the following:

> (H1) Assemblages composition – Dry and aquatic phases of streams can be characterized by compositional differences at both taxonomical and trait levels. It is also assumed that indicator taxa could be identified in both phases.
>
> (H2) Biodiversity – Drying of streams coincides with the loss of diversity.
>
> (H3) Diatom-based assessment of water quality – Only slight differences in diatom-based quality are assumed between the different water regimes.

MATERIALS AND METHODS

Sampling Area and Environmental Parameters

A total of 30 diatom samples were collected from 13 sampling sites on 8 very small intermittent streams in spring and summer in 2019 (**Figure 1** and **Supplementary Table 1**). The streams are located in the southern part of Hungary within the Mecsek Mountains. The local climate is quite special here since Mediterranean impacts can also prevail beside continental ones resulting in a moderately cool and moderately dry climate in the region (Ács et al., 2015). Thus, this area can be characterized by 650–750 mm of mean annual precipitation, 7–10°C of mean annual temperature and more than 2000 h of total annual sunshine duration (Ács et al., 2015; Mezősi, 2017).

The catchment areas of each studied stream are less smaller than 10 km^2. They are hilly streams, their bedrock is calcareous or red sandstone (Petőczi stream) and the sediment size is coarse to medium. In all but one sampling site (Szentkatalin), the cover of aquatic macrovegetation is also negligible. Most of the streams run through forests; where characteristic tree taxa are mostly beech, oaks, poplars, birch and robinia.

In this study, altogether 11 samples were collected from dry streambed. Hereinafter they will be referred to as the dry phase. There was flowing or standing water in the streambed in 19 sampling events. After we proved that there were no differences in composition and in diversity and also in ecological status indices between these two conditions (**Supplementary Table 2**; see details in Statistical analyses subchapter) we handled these 19 sampling events together as an aquatic phase.

At sampling sites where surface water could be found in the streambed (aquatic phase), a total of 10 physical and chemical parameters were measured. Water temperature (T - °C), pH and conductivity (COND - μS cm^{-1}) were measured in the field with a portable-multi-parameter digital meter (HQ30d, Germany). The water samples were kept at 4°C in a cooler bag during transportation to the laboratory for further analyses. Nutrient and chloride contents were determined according to international and national standards (NO_3^--N in mg L^{-1} – MSZ 1484-13:2009, 2009; NO_2^--N in μg L^{-1} – MSZ 1484-13:2009, 2009; NH_4^+-N in μg L^{-1} – MSZ ISO 7150-1:1992, 1992; PO_4^{3-}-P in mg L^{-1} – MSZ EN ISO 6878:2004, 2004 and Cl$^-$ in mg L^{-1} – MSZ 1484-15:2009, 2009). Total suspended solids (TSS in mg L^{-1} – MSZ 12750-6:1971, 1971) and total dissolved solids (TDS in mg L^{-1} – Németh, 1998) were measured according to national standard and proposal. Based on the nutrient concentrations and other physical and chemical parameters of the streams, the anthropogenic impact on them was negligible (**Supplementary Table 3**).

Sample Collection, Preparation and Identification

In flowing conditions, sampling was done according to the European standard (EN 13946, 2014). In pools, when more than one pool was detected, composite samples were collected with the proviso that at least 5 cobbles should be used as substrate per pool. In the dry phase, 5–10 cobbles were sampled from the middle of the streambed. Independently of the aquatic states, when cobbles were not found in the streambed, we used the most characteristic substrates for collecting diatom samples (pebbles, emergent or submerged macrophytes, branches). Substrates were cleaned by toothbrushes and diatom samples were preserved by Lugol's solution according to the recommendation of European standard (EN 13946, 2014). Diatom valves were prepared by hot hydrogen-peroxide method (EN 13946, 2014) and valves were embedded into Naphrax® (EN 14407, 2014). At least 400 valves were counted and identified using a Leica DMRB microscope (1000–1600-fold magnification). The identification of diatoms was carried out using up-to-date and relevant literatures: Krammer and Lange-Bertalot (1997a,b, 2004a,b), Potapova and Hamilton (2007); Bey and Ector (2013), Stenger-Kovács and Lengyel (2015).

Data Processing and Statistical Analyses

Diatom taxa were classified into 27 trait categories including cell size according to Berthon et al. (2011), guild, life-form and attachment type according to Rimet and Bouchez (2012) and length and width (L/W) ratio according Stenger-Kovács et al. (2018); **Supplementary Table 4**). We applied moisture preference of taxa proposed by van Dam et al. (1994) updated in 2011 (**Supplementary Table 4**). Additionally, taxa were referred to as pioneer according to Rimet and Bouchez (2012; **Supplementary Table 4**). The number of categories within traits were 5, 4, 3, 3, 6, and 5, respectively; **Table 1**.

The number of taxa observed in the samples was considered as taxa richness (Taxa_S). Effective Shannon's H values (Jost, 2006) was calculated to compare the "true diversities" of assemblages (Beck and Schwanghart, 2010; Stuart-Smith et al., 2013; Morris et al., 2014). Effective Shannon's H is the exponential of Shannon's H. Taxa richness and Shannon's H were calculated by using the Past software package (version 4.08; Hammer et al., 2001).

The main components of diversity metrics as functional richness (FRich), functional evenness (FEve), functional divergence (FDiv) and functional dispersion (FDis) were calculated by using "FD" R software package in R

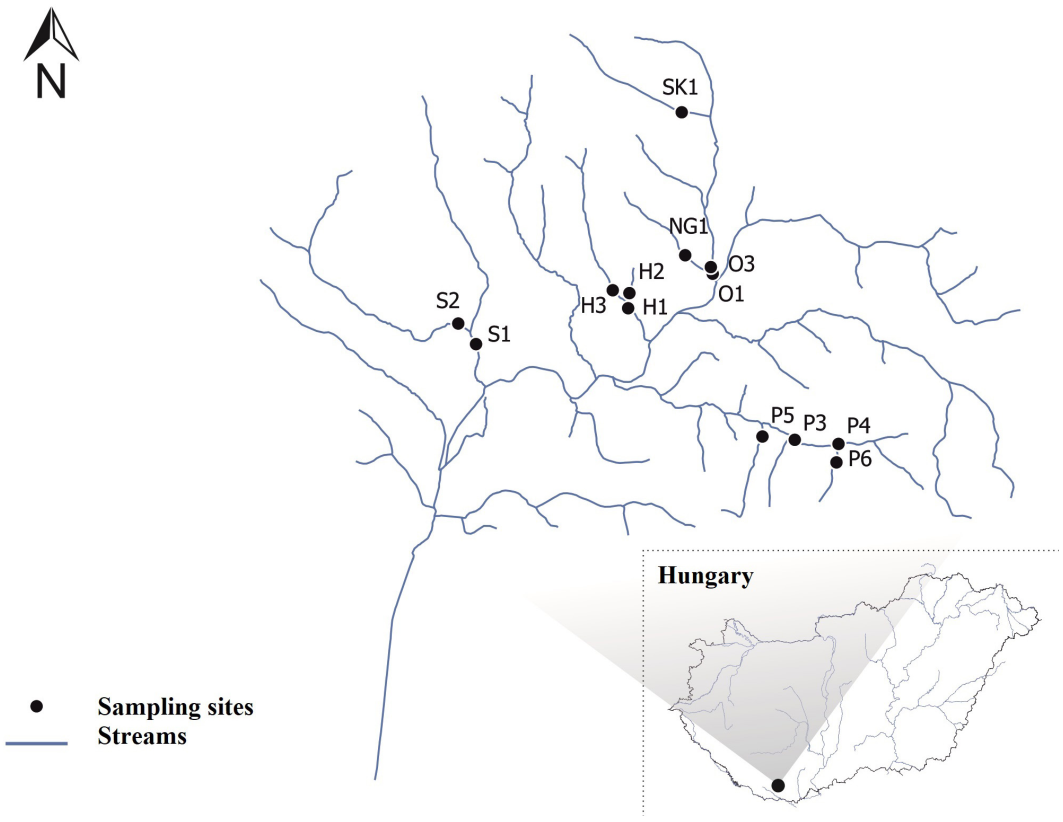

FIGURE 1 | Map of the study area in the Pannonian Ecoregion (Hungary), the watercourses are marked with blue lines. The sampling sites were marked with black circles. Abbreviations of sampling sites are found in **Supplementary Table 1**.

(Laliberté and Legendre, 2010; Laliberté et al., 2014; version 3.5.2; R Core Team, 2019).

To identify the indicative species of sites' groups with similar drying characteristics, we performed indicator value analysis based on relative abundances of taxa (IndVal; Dufrêne and Legendre, 1997). For the calculation of these values, R package "indicspecies" was used (v1.7.9; De Cáceres and Legendre, 2009).

Altogether four indices were calculated to assess the diatom-based ecological quality of streams: Specific pollution sensitivity index (IPS; Coste, 1982), Rott's trophic index (TID; Rott et al., 1999), Rott's saprobic index (SID; Rott et al., 1997) and the Hungarian phytobenthos metric (IPSITI; Várbíró et al., 2012). While SID can assess organic load, TID is sensitive for trophic level. The IPS and IPSITI aim to assess both trophic and saprobic states. All of them are based on the weighted average equation of Zelinka and Marvan (1961) modified by Coste (1982).

$$index = \frac{\sum_{j=1}^{n} p_j \times s_j \times v_j}{\sum_{j=1}^{n} p_j \times v_j}$$

p_j: relative abundance of counting units of "j" taxon in sample.

s_j: sensitivity of "j" taxon (optimum).

v_j: tolerance/indicator value of "j" taxon.

Diatom indices IPS, SID, and TID were calculated by using OMNIDIA software package (version 5.2; Lecointe et al., 2003), while ISPITI is the arithmetic mean of these three indices. The final values of indices vary between 1 (worse quality) to 20 (best quality).

In the first step, we applied Mann-Whitney test for testing significant differences in diversity and diatom metrics between flowing and standing conditions. We also performed Non-metric Multi-dimensional Scaling (NMDS) with Bray-Curtis distance by applying CANOCO 5.0 software package to compare the taxa and trait composition of streams in flowing and standing aquatic conditions (ter Braak and Šmilauer, 2002). These tests proved that there were no differences in either the studied metrics or the composition of these streams. Thus, as we mentioned above, these two conditions were merged and sampling events were handled as aquatic phase in further analyses.

Comparing the taxa and trait composition in dry and aquatic phases, and also in spring and summer, four NMDSs with Bray-Curtis distance were performed. Here, we used the relative abundances of taxa and the community weighted mean (CWM) matrix, in which the main values of traits in the community were weighted by the relative abundances of the taxa matrix.

One-way ANOVA tests were used to compare the diversity characteristics and also diatom-based water qualities between dry and aquatic phases and also between the two seasons. The fixed factors were the phase-type or the season, while the dependent

TABLE 1 | Applying functional traits and their categories and key references.

Traits	Trait categories		References
Cell size (μm^3)	S1	5–99	Berthon et al., 2011
	S2	100–299	
	S3	300–599	
	S4	600–1499	
	S5	≥1500	
Guilds	Low profile		Rimet and Bouchez, 2012
	High profile		
	Motile		
	Planktic		
Life-forms	Unicellular		
	Filamentous		
	Colonial		
Attachment	Weakly		
	Moderately		
	Strongly		
Length/width ratio	LW1	<2	Stenger-Kovács et al., 2018
	LW2	2–3	
	LW3	4–5	
	LW4	6–11	
	LW5	12–19	
	LW6	≥20	
Moisture preference	Strictly aquatic	AQU	van Dam et al. (1994) updated in 2011
	Occasional aerophile	AOC	
	Aquatic-subaerial	WSA	
	Aerophile	AER	
	Terrestrial	TER	
Spreading	Pioneer		Rimet and Bouchez, 2012

variables were taxa_S, effective Shannon's H, FRich, FEve, FDiv, FDis and the diatom indices as IPS, TID, SID, and IPSITI.

RESULTS

Taxa Composition

A total of 208 diatom taxa were identified in the samples: 206 to species and 2 to genus level (**Supplementary Table 5**).

According to the taxonomy-based NMDS analysis (stress value: 0.089), there was only partial overlap in diatom taxa pools between dry phase and aquatic phase (**Figure 2A**). While altogether 106 taxa were recorded in both water types, 24 taxa were found exclusively during dry phase and 78 taxa were identified exclusively during aquatic phase (**Supplementary Table 5**). The different hydrological regime of small streams explained 82.17% of the variance in the taxa composition of benthic diatom assemblages for the first two canonical axes (**Figure 2A**). The eigenvalues of the first and second axes were 0.577 and 0.245. The diatom taxa *Diadesmis contenta*, *Meridion circulare*, *Planothidium lanceolatum*, *Reimeria sinuata* and *R. uniseriata* characterized the assemblages of streams with dry streambed, while *Cocconeis* species, *Navicula* and *Nitzschia* sensu lato species, *Gomphonema pumilum* and *Halamphora* species as *H. montana* and *H. normanii* characterized the assemblages of streams where water was present (**Figure 2A**). *Diadesmis contenta*, *Meridion circulare*, *Navicula veneta*, *Frustulia vulgaris*, *Luticola ventricosa* and *Nitzschia liebetruthii* were significant indicators of dry-phase assemblages ($p < 0.05$), while *Gomphonema pumilum*, *Gomphonema angustatum* and *Navicula lanceolata* were indicative of aquatic phase ($p < 0.05$, in case of *G. pumilum*: $p < 0.01$).

According to the NMDS analysis, a considerable seasonal overlap in taxa composition was found (**Figure 2B**). From those taxa, which were recorded in spring, 39 taxa were not found in summer. In contrast, altogether 74 taxa were recorded exclusively in summer. Some of them were dominant in summer such as *Cocconeis placentula*, *Halamphora normanni*, *Placoneis paraelginensis* and *Sellaphora pseudopupula*.

Trait Composition

Based on the trait composition of diatom assemblages the NMDS (stress value: 0.088) analysis revealed clear differences between dry phase and aquatic phase (**Figure 3A**). The eigenvalues of NMDS were 0.559 and 0.238, while the hydrological regime and trait composition correlation was 79.74 with the first two canonical axes. The dry phase was characterized by trait categories as high profile guild, S2 size, colonial, moderate or weak attachments and aerophilic, but surprisingly, non-aerophilic category was also characteristic here. In contrast, low profile guild, small (S1) or medium size (S3), unicellular, pioneer and more or less rounded forms (LW1 and LW2) were dominant in aquatic phase. In addition, here, WSA (aquatic-subaerial) and AOC (occasional aerophile) spreading trait groups characterized this phase (**Figure 3A**).

Similarly to the taxonomy-based analysis, a less pronounced difference in trait composition was found between spring and summer (**Figure 3B**). All trait categories were found in both seasons, only their proportion was different. In summer, the following trait categories characterized the assemblages: high profile guild and/or S2 size and/or colonial category and/or low profile guild and/or LW1 category.

Biodiversity and Diatom Indices

Although both taxa and trait composition of benthic assemblages of the two phases clearly separated, hydrological regimes studied here did not result in significant differences either in the taxonomy-, or in the trait-based diversity metrics ($p > 0.05$; **Figure 4**). Despite the seasonal overlap in taxa and trait composition, the FDis was reduced significantly by summer ($p = 0.012$). The other diversity metrics, however, did not show seasonal differences ($p > 0.05$; **Supplementary Figure 1**).

In the case of TID index, we found strong positive correlation between diatom-based ecological quality and hydrological regime: it was significantly lower in streams with dry streambed than in aquatic phase ($p = 0.0122$; **Figure 5B**). Overall, neither hydrological regime nor seasonal changes resulted in significant

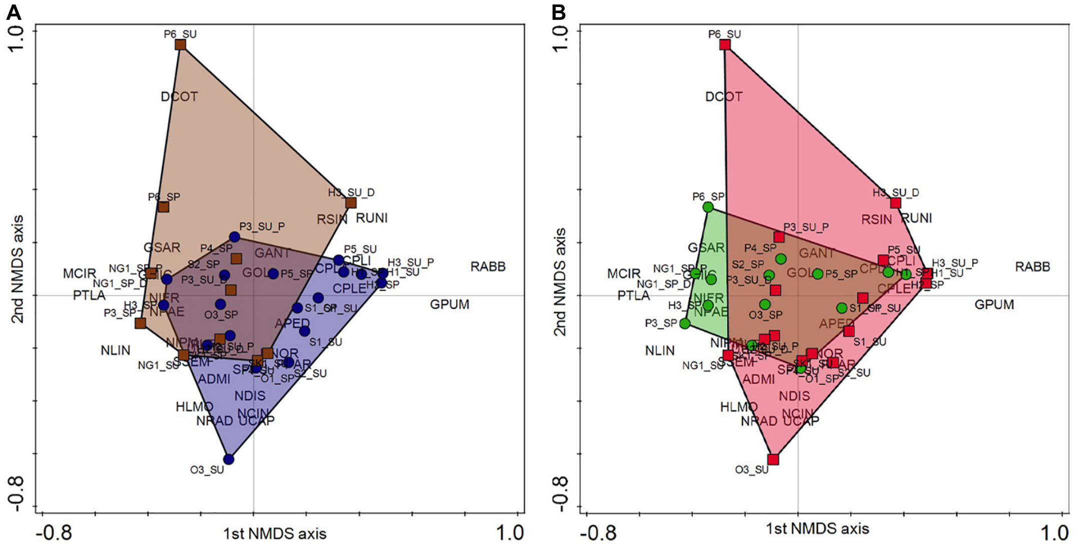

FIGURE 2 | Results of the species-based Non-metric Multi-dimensional Scaling (NMDS) analysis of benthic diatom assemblages. Four letter codes indicate the 208 taxa identified in the samples (see list and codes in **Supplementary Table 5**). **(A)** Dry phase is marked with brown squares (their covered area is brown). Aquatic phase is marked with blue circles (their covered area is blue). **(B)** Spring period is marked with green circles (their covered area is green). Summer period is marked with red squares (their covered area is red).

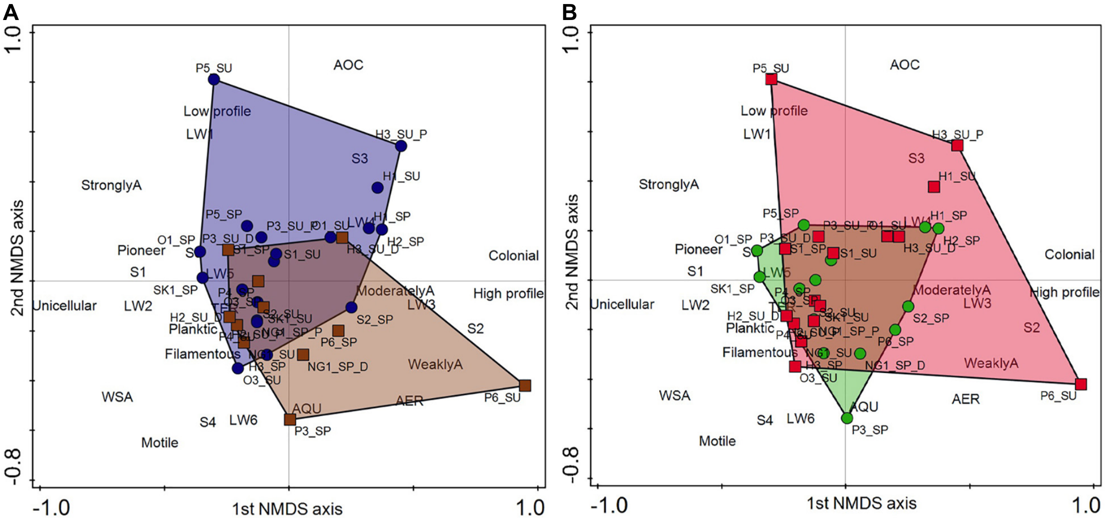

FIGURE 3 | Results of the trait-based Non-metric Multi-dimensional Scaling (NMDS) analysis of the mean trait profiles of diatom assemblages. Trait categories and their abbreviations are found in **Table 1**. **(A)** Dry phase is marked with brown squares (their covered area is brown). Aquatic phase is marked with blue circles (their covered area is blue). **(B)** Spring period is marked with green circles (their covered area is green). Summer period is marked with red squares (their covered area is red).

differences in the other indices ($p > 0.05$; **Figure 5** and **Supplementary Figure 2**).

DISCUSSION

Compositional Changes

Intermittent small streams provide a variety of services to human communities and they contribute to the integrity of river networks (see more in Magand et al., 2020). The assemblages living here are special compared to permanent flowing waters, since lentic and terrestrial species appear regularly beside lotic taxa during intermittence (Stubbington et al., 2017). Additionally, intermittence requires special adaptation strategies, thus, functional composition of diatom assemblages continuously changes during drying up of streams (Sabater et al., 2017).

Here, we hypothesized that there would be clear compositional differences between streams with dry streambed (dry phase)

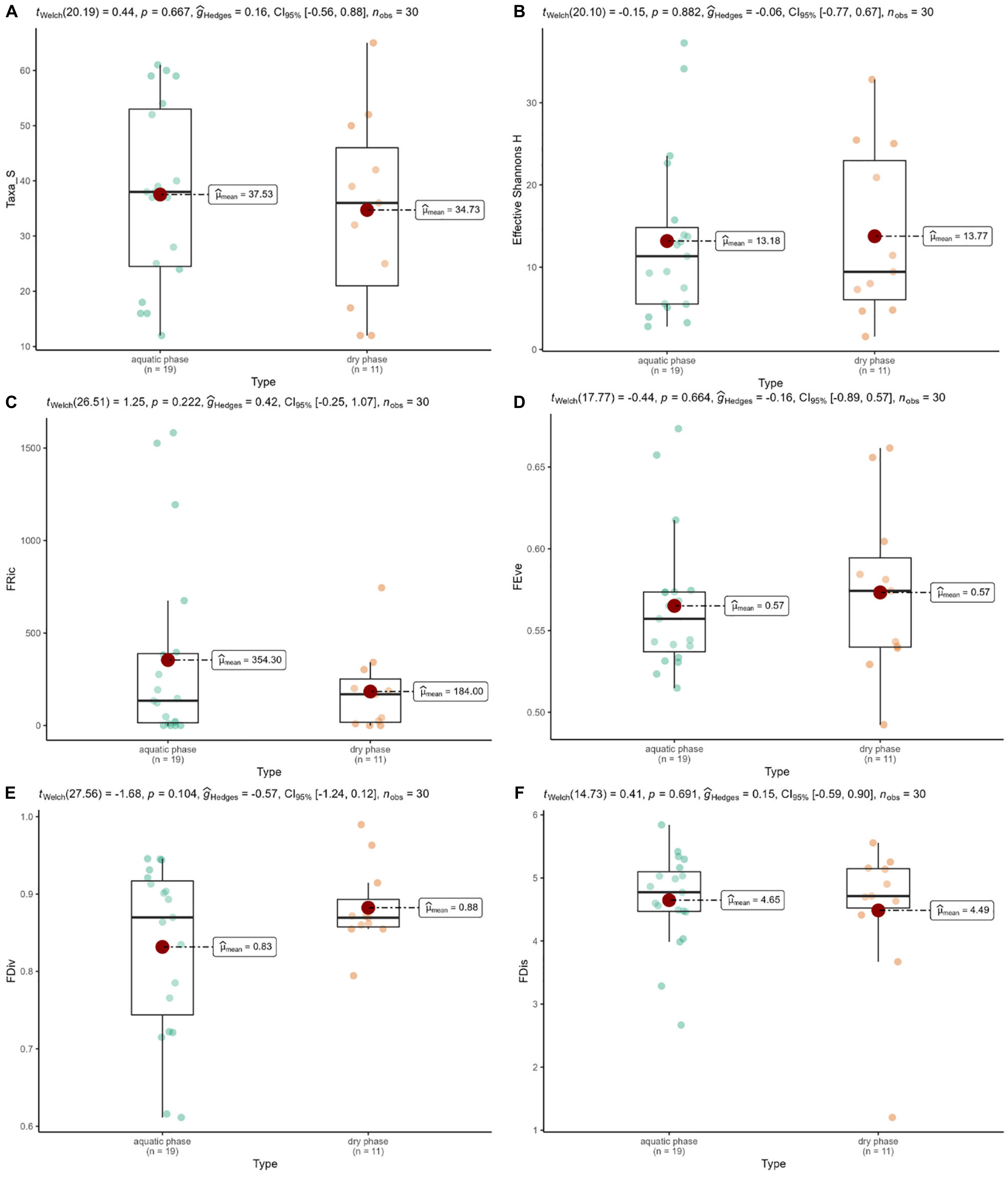

FIGURE 4 | Taxa_S **(A)**, effective Shannon's H **(B)**, functional richness **(C)**, functional evenness **(D)**, functional divergence **(E)**, and functional dispersion **(F)** in the dry and aquatic phases. Results of ANOVA tests: $p > 0.05$ in all cases.

and streams in aquatic phase both in terms of taxonomical and trait levels. We also assumed that indicator taxa could be identified in both phases. The results supported our hypotheses. We found clear, hydrological regime induced compositional differences both at taxonomy and trait levels and indicative species could be recorded too. Periphytic algae, including diatoms

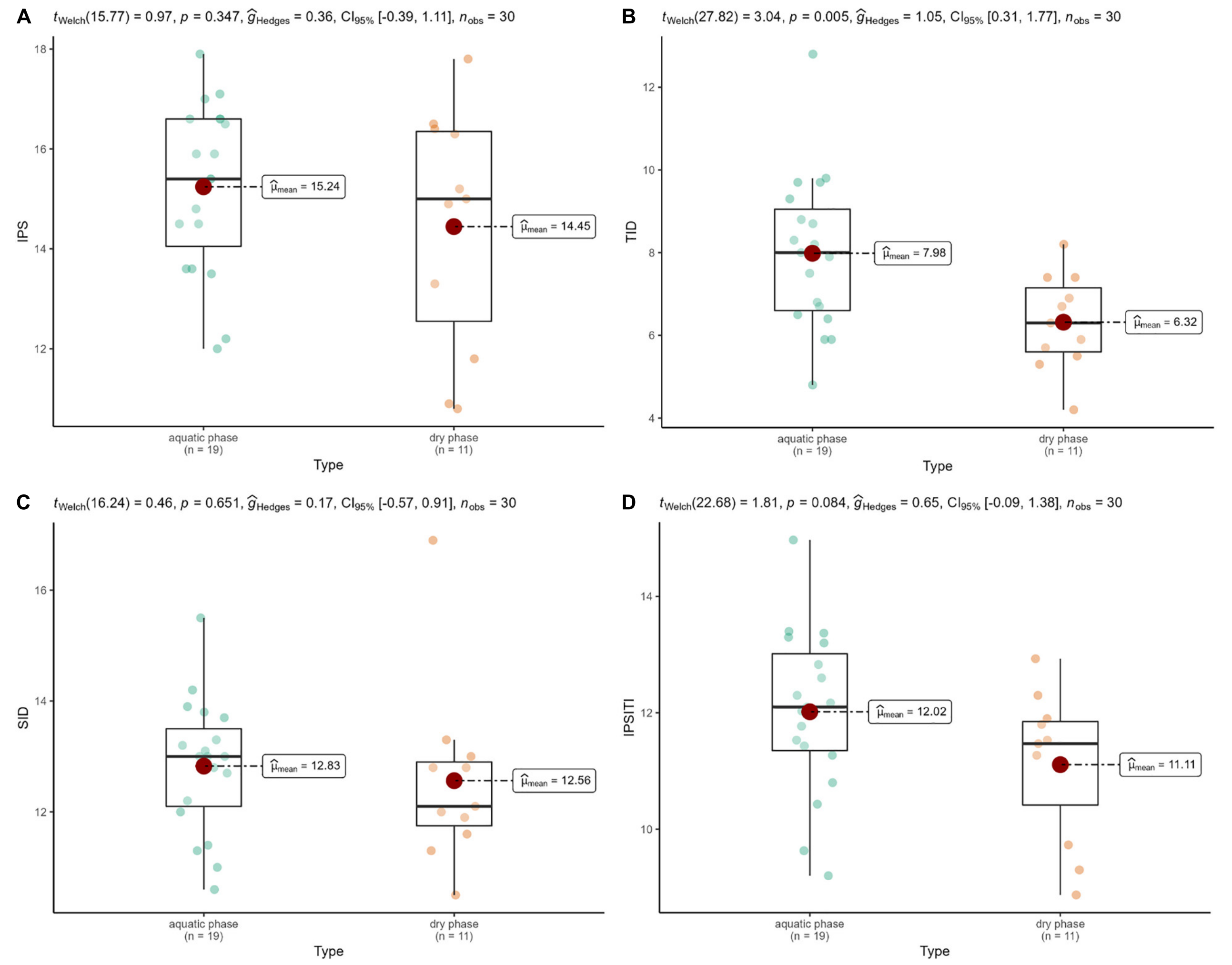

FIGURE 5 | IPS **(A)**, TID **(B)**, SID **(C)**, and IPSITI **(D)** diatom indices in the dry and aquatic phases. Results of ANOVA tests: $p = 0.005$ in the case of TID, $p > 0.05$ in the other cases.

sensitively indicated the intermittence independently of the ecoregion (Novais et al., 2014, 2020; B-Béres et al., 2016, 2019; Falasco et al., 2016a; Várbíró et al., 2020; Lukács et al., 2021).

In this study, mainly colonial taxa belonging to high profile guild dominated the dry phase (but see *Meridion circulare* – low profile guild taxa). Current evidences about the dominance of high profile guild and colonial forms in intermittent streams are inconsistent among studies (Falasco et al., 2016a; Novais et al., 2020; Várbíró et al., 2020). These traits can support survival in permanent waters, where obviously the environmental conditions, especially the water regime, are more stable than in intermittent streams (Várbíró et al., 2020). Novais et al. (2020) also found, that the relative abundances of the high profile guild are higher during aquatic (flow) phase than in dry phase.

In contrast to high profile guild, colonial lifestyle, especially in case of those colonial taxa which form mucous tube can be advantageous in intermittent streams (Falasco et al., 2016a) and during dry phase (Sabater et al., 2017). In our study, mucilage-forming taxa were not dominant during the dry phase, only non-tube forming colonial taxa such as *Diadesmis contenta*. Although *Frustulia vulgaris*, which forms a mucous tube, was one of the indicator species of dry phase, this species was never dominant in samples.

Beside the above mentioned taxa, aerophilous and/or motile species were revealed as indicative species in dry phase. Appearance of aerophilous taxa in intermittent streams is a well-known phenomenon (Novais et al., 2014; Falasco et al., 2016a; Sabater et al., 2016, 2017; B-Béres et al., 2019). Similar to high profile guild and colonial taxa, it is not easy to ascribe the members of motile guild into a water regime phase or type, i.e., they are diverse in their habitat preferences with respect to water phase. Motile taxa can be considered as physical disturbance indicators, since their dominance within assemblages usually increases with siltation and in depositional habitats (Falasco et al., 2016b; Novais et al., 2020), which are common during intermittence. Additionally, motile taxa are able to migrate from the sediment surface to the deeper, wet layers during drying. After rewetting, they return to the surface and colonize it relatively fast (McKew et al., 2011). Our previous studies, however, pointed out that intermittence is a strong selective pressure for motile taxa independently from the ecoregions (Várbíró et al., 2020) and this guild is characteristic for permanent streams in the Pannonian Ecoregion (B-Béres et al., 2019). Here, indicator species of dry phase belonging to motile guild were small sized and aerophilous or subaerial taxa. These characteristics together and their additive ecological benefits made survival possible for diatoms bearing these traits.

A further indicator species was the low profile and colonial *Meridion circulare* during dry phase, which was often dominant in streams with dry streambed (max. 36%). This species is usually found in small streams in winter and spring (Stenger-Kovács et al., 2013). In this study, *M. circulare* was more abundant in spring than in summer. But this species, as a member of the low profile guild, tolerates the physical disturbances which can be characteristic during intermittence. We believe these two characters (favor low temperature and tolerate disturbances) lead to the high number of *M. circulare* in streams with dry streambed in spring. In summer, its relative abundance decreased in the dry phase too, pointing out a more significant temperature-dependent than intermittence-dependent appearance of this taxon.

In this study, streams in aquatic phase were dominated mostly by pioneer category (e.g., *Achnanthidium minutissimum* and *Amphora pediculus*) and/or low profile guild, small and medium sized, unicellular taxa (e.g., *Planothidium lanceolatum*, *Reimeria sinuata*, and *R. uniseriata*) belonging usually to WSA or AOC spreading trait groups. It is a well-known phenomenon that physical disturbance is indicated by small sized, unicellular, low profile taxa (Berthon et al., 2011; B-Béres et al., 2019; Novais et al., 2020). In intermittent streams right after the rewetting of the streambed, these traits, i.e., small size, low profile guild, unicellular and/or pioneer, support the fast spreading of species and colonization of habitats. In our study, the relative lack of aquatic macrovegetation can enhance the chance for spreading of species bearing these traits.

Moisture preference should be an important trait in intermittence studies, since it clearly refers to the tolerance of water shortage. However, Novais et al. (2020) pointed out that most taxa they found in Portuguese streams do not have clear moisture preference. Here, more than 71% of the taxa related to WSA or AOC moisture categories meaning that these taxa are able to tolerate aerophilic conditions for a while. These taxa were characteristic in aquatic phase, while true aerophilic taxa preferred dry phase, as we mentioned above.

Mostly unicellular, stalk-forming *Gomphonema* species were indicative of aquatic phase. Long peduncle i.e., stalk provides an opportunity for taxa to live in the upper layers of biofilm. This life-form subserves an efficient access to light and nutrients but it exposes taxa to high flow disturbances (Passy, 2007). Here, most of the studied streams run through forests. Thus, there is no significant anthropogenic impact on them and their nutrient content is relatively low. In addition, flow conditions in the aquatic phase are comparatively stable. These circumstances, as we mentioned above, favor stalk-forming taxa.

Biodiversity Changes

We hypothesized that biodiversity would be reduced in the dry phase. The results, however, did not confirm our assumption. Neither taxonomical nor functional diversity metrics changed significantly due to drying. These results suggest that taxonomical and functional redundancies were able to compensate for biodiversity loss. Although these results are in accordance with observations of Novais et al. (2020), they contradict the very recent results of studies by Crabot et al. (2021) and Tornés et al. (2021). Crabot et al. (2021) and Tornés et al. (2021) highlighted that functional redundancy decreases with flow intermittence. But this decrease is time-dependent, the longer the dry period the smaller the protective effect of the functional redundancy on assemblages. In our study, the duration of the dry period of streams was very variable (from 7 to 266 days), however, in most cases, it was lower than 50 days, which is lower than the threshold of duration resulting in diversity reduction in the paper of Tornés et al. (2021). They found a significant decrease in biodiversity only after 50 days. Crabot et al. (2021) also noted that any 10% increase in flow intermittence results in a 5% decrease in functional redundancy. In our case, the annual flow intermittence does not reach the 10% in most cases.

Independently of the water regime, a slight decrease in FDis was revealed in summer. The FDis is strongly connected to the dominance of the trait values (Laliberté and Legendre, 2010). Because other metrics like FDiv and FRich did not change significantly, decrease in FDis did not reveal a shift of assemblages' composition to extreme traits, and traits did not disappear, only their proportions changed. This was probably due to the biotic interactions that might be stronger during summer. Since proportion of other algal groups, e.g., green algae, cyanobacteria, usually increases in summer (Biggs and Stokseth, 1996; Suren et al., 2003) resulting in an enhanced resource competition and thus, a biotic pressure on diatoms.

Changes in Diatom Indices

Almost 10,000 watercourses are registered in Hungary (Stubbington et al., 2018), but less than 10% of them are monitored regularly[2]. This means that there is no information about the risk of vulnerability or the risk of intermittence as well as the ecological quality for more than 9000 small Hungarian watercourses. These ecosystems, however, are strongly endangered both by anthropogenic and natural impacts including climatic extremes. To the best of our knowledge, this paper is the first where diatom-based ecological quality of very small intermittent hilly streams were studied in the Pannonian Ecoregion. Since there is no diatom index for evaluating the status of these streams, altogether four diatom indices regularly used in WFD-based biomonitoring in Hungary (Várbíró et al., 2012) were analyzed here. Based on former studies by Falasco et al. (2016b) and by Novais et al. (2020), only slight differences in diatom indices were hypothesized between aquatic and dry phases. The results supported our hypothesis, since drying up of streams had significant negative influence only on TID, while water regime had no impact on the other three diatom indices. The stability of the studied indices points out their applicability in ecological status assessment of intermittent streams. However, it has to be highlighted that much more sampling sites including permanent ones as well as differently loaded sites should be involved in the analyses. Our study is only the first step in this way, but the results are encouraging. These diatom indices were developed mostly for assessing anthropogenic impact on permanent waters with larger catchment areas than the waterbodies studied here. Nevertheless, our results suggest that these indices are stable enough to assess the status of very small permanent streams even in dry phase. We think that the higher values of TID in the aquatic phase were attributable to the higher proportion of small, pioneer taxa considered as indicators of good or excellent waters. These taxa, as we mentioned above, are good colonizers and tolerate physical disturbances caused by rewetting. Analyzing this diatom index together with the others can be a good way to indicate both nutrient supply and climate-induced influences on benthic diatom assemblages in small intermittent streams in the Pannonian Ecoregion.

CONCLUSION

Here, we examined the influence of flow intermittence on benthic diatom communities in very small hilly streams (catchment area <10 km^2) in the Pannonian Ecoregion. While the results revealed strong water regime dependent differences in taxa and trait composition of assemblages, no seasonal separation was found. However, our findings have highlighted that short-term drying up of streams might not necessarily cause biodiversity loss. It seems that taxonomical and functional redundancy could prevail. In contrast, decrease in functional dispersion in summer suggests proportional changes in trait values. But it has to be stressed that traits did not disappear from the system, and assemblages were not pushed to dominance of extreme traits. In this study, we first analyzed the usability of diatom-based quality indices in the assessment of drying up of small streams in this region. We showed that these metrics are useful tools for ecological evaluation of streams even in the dry phase. As we noted, these streams are strongly endangered to become episodic. However, it is not a simple shift from permanence to intermittency, the lengthening of the dry-phase in the future might result in irreversible compositional changes and significant diversity loss, threating the entire aquatic ecosystem not just benthic algae. Thus, any knowledge, which supports their ecological role in this region, can help to save them and their valuable microflora.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

VB-B: conceptualization, data curation, project administration, supervision, and roles/writing – original draft. ZK, GV, GM, and VB-B: formal analysis and visualization. VB-B, PB, and ZC: funding acquisition. ZK, VB-B, IB, PB, and BP: investigation. VB-B, IB, and GV: methodology. PB, GB, ZC, and VB-B: resources. VB-B, PB, ZC, BP, GB, GV, and IB: writing – review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Occurrences, Sources, and Human Health Risk Assessments of Polycyclic Aromatic Hydrocarbons in Marine Organisms From Temperate Coastal Area

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The pollution characteristics of 16 polycyclic aromatic hydrocarbons (PAHs) in marine organism species (6 species of fishes and 2 species of crustaceans) from the coastal area of the East China Sea were determined. The concentrations of 16 PAHs in the studied organisms ranged from 29.73 to 87.02 ng/g dw and 2- and 3-ring PAHs were the most abundant compounds in the aquatic organisms. The habitat, diet and predator-prey relationship have posed potential effects on the PAH accumulation in marine organisms. The source identification of PAHs was performed by using the molecular diagnostic ratios and principal component analysis (PCA). The results showed that the main sources of PAHs in the marine organisms were coal combustion, followed by mixture of gasoline combustion, oil combustion, crude oil spill and vehicle emissions. The incremental lifetime cancer risk of human *via* ingestion process of marine organisms in this sea area was also estimated and the assessment showed that it posed an acceptable but non-negligible risk to human health.

Keywords: polycyclic aromatic hydrocarbons, marine organisms, source identification, health risk assessment, temperate coastal area

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) have been widely concerned because of their teratogenicity, carcinogenicity and mutagenicity. With the development of social economy, PAHs from industrial production, agricultural production, transportation and domestic pollution sources are increasing gradually (Hu et al., 2008; Jiao et al., 2012; Yoo et al., 2014; Tong et al., 2019). They can enter marine environment through various ways, such as wastewater discharge, atmospheric deposition, surface runoff and crude oil leakage (Heemken et al., 2000). It is worth noting that PAHs tend to accumulate in aquatic organisms through the biological lipid membrane (Billiard et al., 2002) since their lipophilic and hydrophobic properties, and their toxicity can be amplified through the food chain/web, finally causing toxic effects on human health *via* ingestion (Charles et al., 1992; Chen et al., 2002).

Located in a temperate zone with humid climate and abundant precipitation, the coastal area of the East China Sea owns massive biological resources, especially Zhejiang coastal waters. Zhejiang Province covers a sea area of ∼260,000 square kilometers and its continental coastline and island shoreline extend for ∼6,500 km, accounting for 20.3% of the total length of China's coastline. The convergence zone of coastal current and Taiwan warm current makes the coastal waters of Zhejiang rich in fishery resources because of its low salinity, great seasonal changes and abundant nutrients. From 2017 to 2019, the average annual marine fishing yield of Zhejiang Province accounted for more than 27% of the that in China (CSF, 2019, 2020). However, the increasing human maritime activities have caused more concerns about the quality of marine environment. As reported in a previous study (Wang X. Y. et al., 2015), the sum of 16 PAH concentrations in the surface sediments samples collected from Zhoushan Archipelago and Xiangshan Harbor ranged from 3.67 to 31.30 ng/g and 11.58 to 481.44 ng/g, respectively. To date, there are still few studies and information which address the current situation of PAH pollution in the marine organisms of this sea area and its potential risk to human health.

The main tasks of this study can be described as follows: (1) Marine organisms will be collected from the temperate coastal area of the East China Sea and used to determine the residual level of 16 priority PAHs; (2) Diagnostic ratios and Principal Component Analysis (PCA) will be applied for identification of the possible sources of PAHs; (3) The safety risk to human health *via* ingestion of these aquatic products will be clearly evaluated. The purpose of this study is to further understand the level of pollutants in coastal aquatic products in the East China Sea, to provide useful information for the prevention and control of PAH pollution and an assessment for the potential risk of consuming PAH-contaminated seafood in this area.

MATERIALS AND METHODS

Sample Collection

The study area was set in Zhejiang coastal waters in the East China Sea (**Figure 1**) and the marine organisms were sampled by bottom trawl at nine sites in April 2019. Eight kinds of high-economic-value marine organisms were selected, including six fishes and two crustaceans. The basic biological information is shown in **Supplementary Table 1**. After collection, the samples were washed with ultrapure water, stored in PE sealed bags and then transported to the laboratory at –20°C until analysis.

Experimental Section

Materials

Silica gel (60∼100 mesh for chromatography column), anhydrous sodium sulfate, 98% concentrated sulfuric acid, acetone, dichloromethane, n-hexane and other chromatographic pure solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The silica gel needs to be activated for use according to previous protocol (Wang et al., 1994). The 16 USEPA priority PAH standard mixture including Naphthalene (Nap), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Fl), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flu), Pyrene (Pyr), Benzo[*a*]Anthracene (BaA), Chrysene (Chr), Benzo[*b*]fluoranthene (BbF), Benzo[*k*]fluoranthene (BkF), Benzo[*a*]pyrene (BaP), Indeno[1,2,3-*cd*]pyrene (InP), Dibenz[*a*,*h*]anthracene (DahA), Benzo[*g*,*h*,*i*]perylene (BghiP) and internal standard (*p*-Terphenyl-d_{14}) were purchased from Supelco, Inc. (Bellefonte, PA, United States).

Sample Pretreatment

The sample pretreatment method was based on the determination of specified sixteen polycyclic aromatic hydrocarbons in aquatic products by gas chromatography-mass spectrum in Chinese aquatic industry standards (SC/T 3042–2008) and previously established protocols (Yu et al., 2016, 2019). In brief, muscle tissue (0.2–5 g, depending on amount available) was freeze-dried for 24 h, grinded, mixed with 18 mL n-hexane/dichloromethane (2:1, v/v), transferred into 50 mL centrifuge tubes, and vortex mixed for 1 min. Samples were then extracted in an ultrasonic extractor for 30 min, and centrifuged at a rate of 4,800 rad/min for 3 min. The extraction solution was concentrated to 5 mL, followed by washing with sulfuric acid solution (60%, v/v) for derosination and centrifuged at a 4,800 rad/min rate for 3 min, then transferred into a chromatographic column filled with 3 g silica gel and 1 g anhydrous sodium sulfate. The columns were eluted with 12 mL n-hexane/dichloromethane (1:1, v/v). The PAH-containing eluents were collected and placed in a nitrogen stream; the final solution was adjusted to 1 mL by hexane and spiked with *p*-terphenyl-d_{14} as an internal standard before instrument analysis.

Gas Chromatography-Mass Spectrometry Conditions

Gas chromatography–mass spectrometry (GC-MS) (Agilent 7890A-5975C) was used for the determination of 16 PAHs. A DB-5 capillary column (15 m × 0.25 mm × 0.25 μm) was used for PAH mixture separation. The inlet temperature was set at 250°C. The initial temperature of GC oven was set at 50°C, held for 3 min, increased to 180°C at a rate of 15°C/min, heated to 300°C at a 6°C/min rate, and held for 2 min. The electron impact (EI) ion source and quadrupole temperatures were set at 230 and 150°C, respectively. The 16 PAHs were detected by selected ion monitoring (SIM) mode and the target ions of PAH compounds were selected based on our previous study (Yin et al., 2015, 2021). Helium (purity > 99.9%) was used as a carrier gas with a flow rate of 1 mL/min. Pulsed splitless injection of 1 μL of the sample solutions was performed.

Quality Assurance/Quality Control

The linear regression coefficients for calibration curves based on five standard concentrations (10, 50, 100, 200, and 500 ng/mL) were greater than 0.99. The limit of detection (LOD) was defined as the concentrations of target compounds that gave rise to a peak with a signal-to-noise ratio (S/N) of 3 (Chen et al., 2007), which varied from 0.1 and 1.9 ng/g. The results showed that no target compounds were detected (below LOD) in the procedure blanks. The measured recoveries of matrix-spiked samples were 61.4 ± 8.8%, 70.9 ± 3.4%,

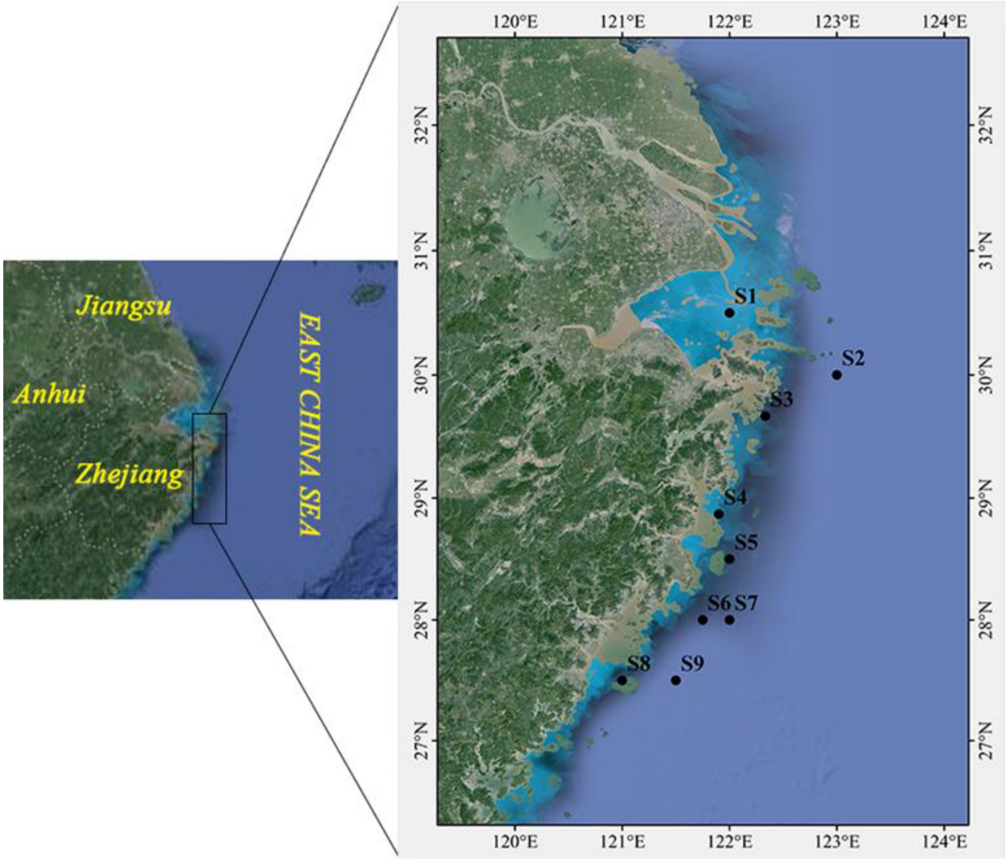

FIGURE 1 | Descriptive map of the sampling sites.

74.8 ± 6.9%, 122.7 ± 8.2% and 118.6 ± 6.5% for Naphthalene-d_8, Acenaphthene-d_{10}, Phenanthrene-d_{10}, Chrysene-d_{12} and Pyrene-d_{12}, respectively.

RESULTS AND DISCUSSION

Concentrations and Composition Patterns of Polycyclic Aromatic Hydrocarbons

The PAH concentrations in marine organisms from the coastal area of Zhejiang in the East China Sea ranged from 29.73 ng/g dw in *Konosirus punctatus* to 87.02 ng/g dw in *Cynoglossus robutus*, with an average concentration of 53.35 ng/g dw. Detailed data were summarized in **Supplementary Table 2**. And the ranges of the concentration levels of Σ16PAHs were illustrated in **Figure 2**. Varied concentrations of PAHs in the studied marine organisms could be observed among different species and even among the individuals of the same species. According to previous studies, the species-specific differences and individual differences may be attributed to the different feeding behavior, metabolic capability, trophic level and biomagnification ability of each species and individuals (Rahmanpour et al., 2014; de Albergaria-Barbosa et al., 2018; Santana et al., 2018; Yu et al., 2019). As shown in **Table 1**, the average concentration of Σ16PAHs (53.35 ng/g) in the marine organisms of this study was close to those from Nansha and Xisha Islands (67.79 and 71.69 ng/g, Li et al., 2019), lower than those from South China Sea (289.86 ng/g, Ke et al., 2017), Ghana coast of West Africa (192 ng/g, Bandowe et al., 2014c), Tanmen of China (161.46 ng/g, Li et al., 2021) and Dongfang and Yachen gas fields and the Pearl River Delta of China (386 ng/g, Yu et al., 2019), but higher than those from Zhuhai of China (31.21 ng/g, Li et al., 2021) and the northern Bering Sea Shelf and Chukchi Sea Shelf (34.97 ng/g, Ma et al., 2020). The comparison of PAH concentrations with this respect indicated that the marine organisms from the coastal area of Zhejiang in the East China Sea were moderately contaminated by PAHs.

The composition pattern of PAHs in the marine species was presented in **Figure 3**. The relative compositions of PAHs were similar to each other, 2- and 3-ring PAHs were predominant in the marine organism tissues, accounting for 42.69 and 49.46%, respectively; followed by 4- and 5-ring PAHs, accounting for 5.90 and 1.93%, respectively. It is reported that low molecular weight polycyclic aromatic hydrocarbons (LMW PAHs) have higher gill-water transfer efficiency due to higher water solubility, while high molecular weight polycyclic aromatic hydrocarbons (HMW PAHs) tend to combine with particles (Baumard et al., 1999;

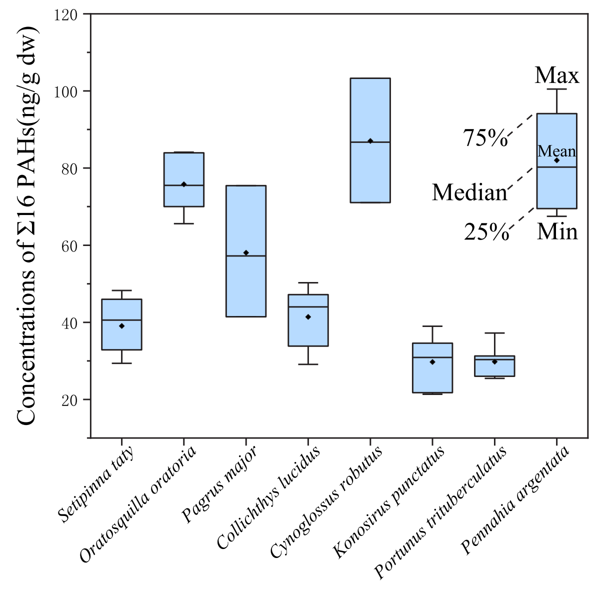

FIGURE 2 | Concentrations of Σ16PAHs in the marine organisms.

Sun et al., 2016). Also, previous studies have shown that the removal or biotransformation rate of HMW PAHs is higher than that of LMW PAHs (Baumard et al., 1998). This may explain the reason why marine organisms are prone to the occurrence of LMW PAHs. Furthermore, it is stated that the habitats can be an important factor to affect the distribution characteristics of PAHs in marine organisms (Snyder et al., 2015). The coastal areas of Zhejiang Province including Zhoushan Archipelago and Xiangshan Harbor, and some nearby cities with high industrialization, such as Shanghai and Nantong, have built many oil refineries, coal power plants and petrochemical plants (Wang X. Y. et al., 2015). And the pollution emissions from these plants will affect the composition of PAHs in the water bodies and surface sediments of the surrounding sea. Jiang et al. (2014) sampled seawater and surface sediments from Zhoushan coastal area, and their results showed that 2- and 3-ring PAHs in seawater and sediment samples were predominant. This probably leads to higher accumulation of LMW PAHs in the studied aquatic products in this region (Badreddine et al., 2016; Margherit et al., 2018; Webster et al., 2018). Our results showed the composition of PAHs in the marine organism samples posed a potential correlation between the compositions of PAHs in the organisms and their habitat. Differences in physiology and diet also likely account for the differences in PAH exposure for aquatic organisms (Snyder et al., 2015). As shown in **Supplementary Table 1**, *C. robutus*, which has the highest PAH concentrations, is a typical benthic fish that is in contact with bottom sediments for long periods of time. Some studies have shown that soil and sediment are the greatest sinks for PAHs (Huang et al., 2018), hence *C. robutus* may accumulate more PAHs from sediments. This is consistent with some existing findings that benthic organisms in contact with the sediments tend to have a stronger accumulation capacity for PAHs than aquatic organisms in contact with the water (Nakata et al., 2003). Furthermore, the least abundant PAHs was detected in *K. punctatus*, which mainly feed on phytoplankton, and higher PAH contents were detected in carnivorous fishes that feed on other small fishes. It may suggest that the carnivorous fish species exhibited higher PAH concentrations than the herbivorous ones. This result is consistent with a previous study on the accumulation potential of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) in freshwater fish species (Eqani et al., 2013). The food web also affects the concentration levels of PAH compounds. For example, Nap accounted for the highest proportion in the two crustaceans, which may cause a relatively high proportion of Nap in *Pennahia argentata* that feed on crustaceans.

Source Appointment

Molecular Diagnostic Ratios

The molecular diagnostic ratio method has been commonly used to discriminate the source of PAH pollution. Since PAH

TABLE 1 | Comparison of PAH concentrations (ng/g dw) in the marine organisms from sea areas.

Sea area	Sampling year	Organism tissue	Concentration range (Avg.)	References
Coastal area of Zhejiang Province	2019	Muscle tissues	29.73∼87.02 (53.35)	This study
South China Sea	2015	Muscle tissues	94.88∼557.87 (289.86)	Ke et al., 2017
Ghana Coast, West Africa	2010	Muscle and gut + gill tissues	71∼481 (192)	Bandowe et al., 2014c
Zhuhai, China	2018	Muscle tissues	13.74–42.59 (31.21)	Li et al., 2021
Tanmen, China	2018	Muscle tissues	24.39–684.83 (161.46)	Li et al., 2021
Nansha Islands, China	2016	Muscle tissues	32.71–139.09 (67.79)	Li et al., 2019
Xisha Islands, China	2016	Muscle tissues	12.79–409.28 (71.69)	Li et al., 2019
The northern Bering Sea Shelf and Chukchi Sea Shelf	2014	Muscle tissues	32.2–128.1 (34.97)	Ma et al., 2020
Dongfang and Yachen gas fields and the Pearl River Delta, China	2013	Muscle tissues	190–606 (386)	Yu et al., 2019

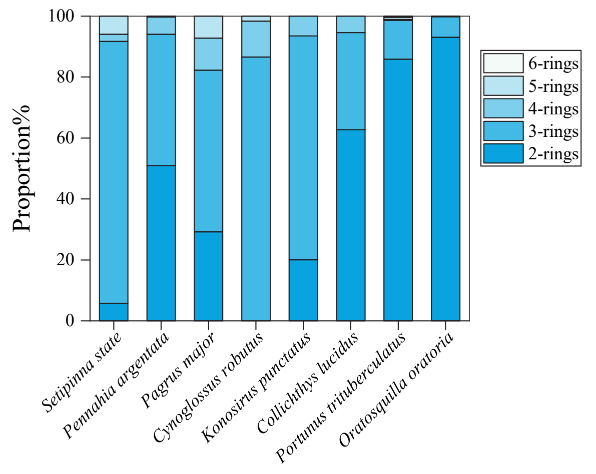

FIGURE 3 | Composition patterns of PAHs in the marine organisms.

compounds that are isomers often have similar thermodynamic partition coefficients and kinetic mass transfer coefficients, the content ratio of single-component compounds of PAHs has been widely used to identify the sources of PAH contamination in environmental samples (Hellou et al., 2005; Nasr et al., 2010; Tobiszewski and Namiesnik, 2012; Zhang et al., 2021), even these ratios may not be stable in organisms due to the complexity of metabolic processes and the particularity of different hydrocarbons.

The commonly used molecular diagnostic ratios include Ant/(Phe + Ant) and Flu/(Flu + Pyr). When Ant/(Phe + Ant) < 0.1 or Flu/(Flu + Pyr) < 0.4, the main source of PAHs is petroleum source; when Ant/(Phe + Ant) > 0.1, the main source of PAHs is combustion source; when $0.4 <$ Flu/(Flu + Pyr) < 0.5, the main source of PAHs is natural gas, diesel, oil and other combustion; when Flu/(Flu + Pyr) > 0.5, the main sources of PAHs are biomass and coal combustion (Yunker et al., 2002). The cross plot of Ant/(Phe + Ant) and Flu/(Flu + Pyr) was selected to draw the PAH source of the studied samples. As indicated in **Figure 4**, all of the collected fish samples had PAHs mainly from combustion. Since the samples were collected in the nearshore of Zhejiang coastal area of the East China Sea, they are susceptible to land-based sources of production and domestic pollutant emissions.

Principal Component Analysis

PCA is a multivariate analytical tool for receptor modeling in environmental source identification studies, which can represent the total variability of the original PAH data with a minimum number of factors (Liu et al., 2009). It provides a dimensionality reduction method to reduce the number of variables, reflecting most of the information of the original variables (Javed et al., 2008). The main advantage of this method is that it requires little information of the pollutant source or predetermined characteristics of the source (Sofowote et al., 2008). The concentrations of PAH variables can be considered as a linear combination of many potential factors, and the samples are identified by classifying them through several comprehensive characteristic indicators and comparing the loadings to infer the source of contamination (Liu et al., 2009). In this study, source analysis of the 11 detected PAHs was performed by using IBM SPSS Statistics 25.0 to identify three principal components (PC1, PC2, and PC3), calculate the cumulative variance contribution of the principal components, and obtain the correlated factor loading matrix (see **Supplementary Table 3**).

The results of PCA in **Figure 5** and **Supplementary Table 3** showed that the cumulative contribution of the three factors reached 81.487%. The contribution of PC1 was 54.698%, where Ace, Phe, Ant, Flu, Pyr, and BbF had large loading values, and Phe, Ant, Flu and Pyr are mainly emitted by coal combustion (Harrison et al., 1996; Simoneit, 2002; Yunker et al., 2002; Li et al., 2006; Tian et al., 2009), indicating that PC1 was coal combustion. The contribution of PC2 was mainly composed of Fl and BaP, explaining 16.064% of the total variance. Fl is an important tracer of coking (Sofowote et al., 2008; Wang C. H. et al., 2015), and BaP is mainly from gasoline combustion (Simoneit, 2002; Yunker et al., 2002; Sofowote et al., 2008) and vehicles (Randolph and Joel, 2003; Li et al., 2006), hence PC2 is a mixed source of coking, gasoline combustion, and vehicle emissions. PC3 only has a high percentage of Nap, and studies show that Nap is a characteristic indicator of crude oil spill (Patrolecco et al., 2010; Deng et al., 2013) and oil combustion (Dong and Lee, 2009; Khairy and Lohmann, 2013). Therefore, PC3 was identified as a mixed source of oil combustion and crude oil spill.

Combining the analysis results of two PAH source identification methods above, it is found that the results of these two methods are basically consistent. The results determined that the most important source of PAHs in the near-shore marine organisms in the study area was coal combustion, followed by a mixture of gasoline combustion, oil combustion, crude oil spill, and vehicle emissions. A possible explanation for this result was that marine organisms in the coastal waters of Zhejiang province were affected by the pollution from coal-powered plants, petroleum refineries and petrochemical plants in surrounding cities. This finding was consistent with previous field investigations in the highly industrialized harbor and sea areas (Bandowe et al., 2014a; Benali et al., 2017). It should be noted that most of the marine organisms captured by trawling was not migratory but stationary. The observed results of PAHs in the marine organisms may reflect the pollution situation around the study site.

Health Risk Assessment

Diet is one of the main ways that people are exposed to PAHs in their daily life. Numerous international studies have evaluated the combined carcinogenic effect of PAHs to human health, and the Incremental Lifetime Cancer Risk (ILCR) has been widely used to evaluate the risk of PAHs. To gain the carcinogenic risk level, the toxic equivalent factor (TEF) of 16 PAHs to BaP have been commonly used to assess the risk of PAHs in organisms by calculating their toxic equivalent concentration (TEQ) of BaP. The commonly applied TEFs have been proposed by Nisbet and

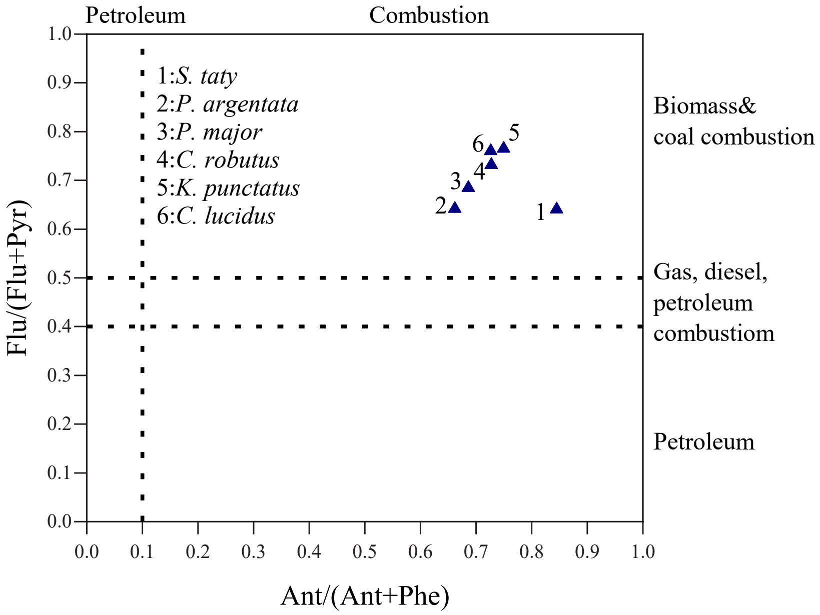

FIGURE 4 | Cross plot of the molecular diagnostic ratios of Flu/(Flu + Pyr) and Ant/(Phe + Ant) in the marine organisms.

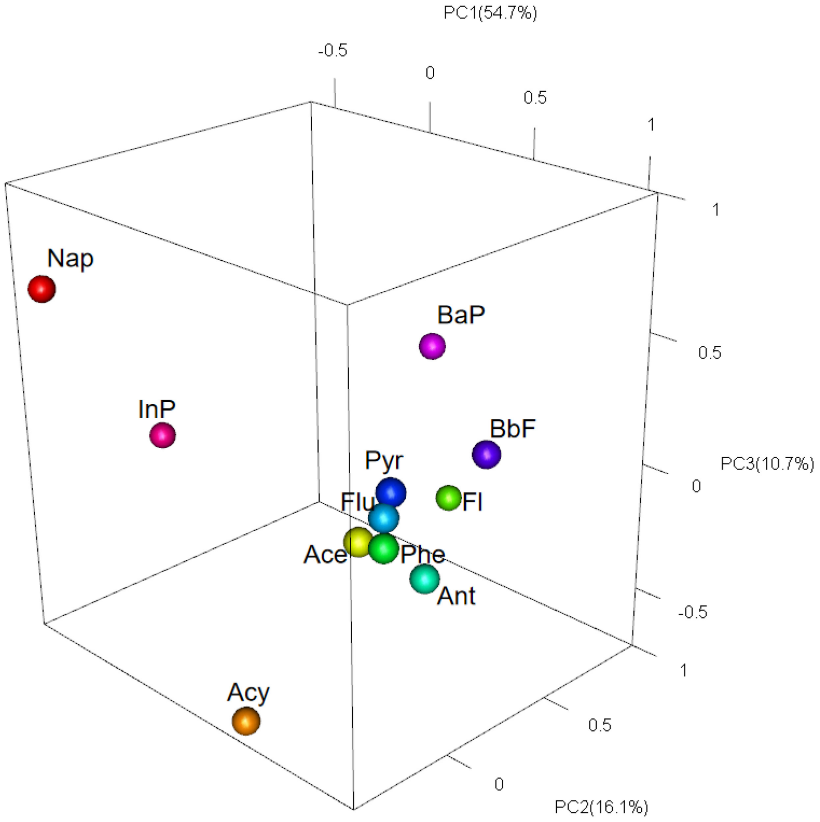

FIGURE 5 | The PCA results of PAHs in the marine organisms.

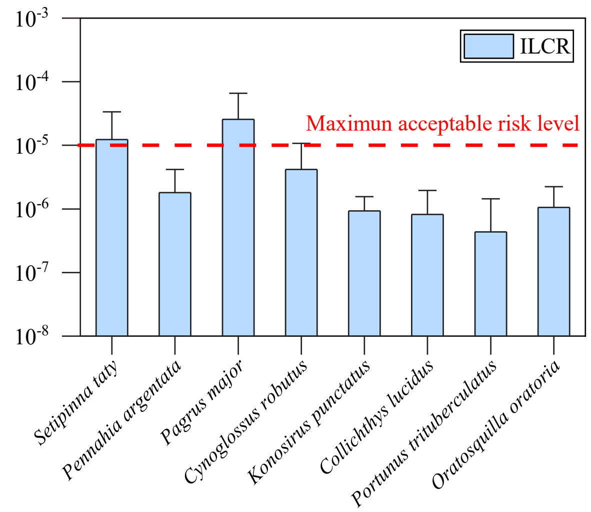

FIGURE 6 | Ranges of the estimated ILCRs by consuming the marine organisms collected from this study area.

LaGoy (Nisbet, 1992; see **Supplementary Table 4**). The equations for *TEQ* and *ILCR* are shown as follows:

$$TEQ = \sum_{i=1}^{n} TEF_i \times C_i \tag{1}$$

where TEF_i is the toxicity equivalence factor of this compound *i* relative to that of BaP; C_i is the concentration of an individual PAH compound *i* in marine organisms tissue.

$$ILCR = \frac{TEQ \times CSF_{ingestion} \times IR \times EF \times ED}{BW \times AT} \tag{2}$$

where *CSF* is the oral cancer slope factor of BaP of 7.3 mg/kg/day; *IR* is daily intake rate of different organisms. The average daily consumption is assumed to be 59, 35, and 49 g/day for fish, mollusk, and crustacean species, respectively (Zhang et al., 2013). *EF* is exposure frequency (365 days/year); *ED* is exposure duration, taken as the average human life span of 70 years (Bandowe et al., 2014b). *BW* is average bodyweight, assumed to be 60 kg (Adomako et al., 2011). *AT* is average exposure time, assumed as 25,550 days in this study.

After calculation, the *ILCR* values for marine organisms from the coastal area of the East China Sea are shown in **Figure 6**, ranging from 4.32×10^{-7} (*P. trituberculatus*) to 2.54×10^{-5} (*P. major*). According to the fish advisory committees and safe eating guidelines from the USEPA, an ILCR of 1×10^{-5} is regarded as the maximum acceptable risk level (ARL), while the priority risk level of 1×10^{-4} is considered as serious (USEPA, 2000). Except *S. taty* and *P. major*, the values of other samples in the present study were well below the ARL, indicating low carcinogenic risk to local residents near the East China Sea.

CONCLUSION

This study investigated the concentrations and compositional patterns of 16 PAHs in the marine organisms from the coastal area of the East China Sea. The PAHs with 2- and 3-ring were the dominance, then followed by 4-ring. The marine organisms in the study area were moderately contaminated by PAHs when compared with the marine organisms from different sea areas. The PAH accumulation in marine organisms has shown discernable connections with their habitats, diets and predator-prey relationships. The results of the source identification showed that PAH pollution in marine organisms from the Zhejiang coastal area of the East China Sea mainly originated from coal combustion. The incremental lifetime cancer risk from PAH-contaminated seafood consumption was significantly lower than the priority risk level (10^{-4}), but potential risks still should be carefully considered in the study sea area. The results are expected to provide useful baseline information of the PAH pollution in the coastal aquatic products from the temperate coastal area of China, and to further support the control of PAH pollution and environmental management in this area.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SJ: conceptualization, methodology, data curation, writing—original draft, and writing—review and editing. FY: resources, writing—review and editing, supervision, and funding acquisition. WZ: writing—review and editing. ZS: data curation. BQ: investigation. PS: resources. JZ: writing—review and editing, resources, and supervision. DK: writing—review and editing and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.850247/full#supplementary-material

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A Comparison of Fish Diversity in Rocky Reef Habitats by Multi-Mesh Gillnets and Environmental DNA Metabarcoding

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This study was undertaken in order to explore the practical effectiveness of the environmental DNA (eDNA) metabarcoding approach in evaluating fish composition and diversity in a high heterogeneous rocky reef habitat. We assessed the fish composition and diversity characteristics of the rocky reef habitat at Dachen Islands, Taizhou and the Zhejiang Province in China in November 2020 by comparing two methods: multi-mesh gillnets and eDNA. A comparative analysis was carried out on the fish composition and diversity characteristics gained under the two methods by using taxonomy, ecotypes and diversity indices. The results showed that there were 28 species of fish collected through gillnets, distributed under 24 genera, 19 families, 6 orders and one class. Among them, 4, 18, and 6 species of near-surface, near groundfish and groundfish were found, respectively, with *Thryssa mystax, Johnius belangerii,* and *Sebastiscus marmoratus* being the dominant species in each water layer. A total of 81 species of fish detected by eDNA metabarcoding belonging to 67 genera, 46 families, 15 orders and 2 classes. The near-surface, near groundfish and groundfish species were 17, 42, and 22, with *Thryssa vitrirostris, Benthosema pterotum, Harpadon nehereus,* and *Dasyatis akajei* being the dominant species in each water layer. Twenty species (71.4%) and 41 species (50.6%) of reef fish were counted by gillnets and eDNA, respectively. The results showed that multi-mesh gillnets can accurately obtain information on fish composition in rocky reef habitats, but with some selectivity. The eDNA technology can detect species not collected by gillnets, but the number of species detected in areas with fast water velocity is significantly less than other eDNA stations where the water velocity is slow. In summary, the combination of traditional nets and eDNA will provide more information on taxonomic diversity and population biomass, transforming natural resource management and ecological studies of fish communities on a larger spatial and temporal scale.

Keywords: eDNA, multi-mesh gillnets, rocky reef habitat, fish diversity, evaluation

INTRODUCTION

Rocky reef habitats host numerous fisheries with their limited space and are one of the natural habitats that uphold high fish diversity (Darling et al., 2017). High heterogeneity within rocky reefs and the complex hydrodynamic effects around them create habitat for coastal fishes, especially reef fishes, while the combined hydrodynamic forces such as tides and upwellings in shallow seas provide abundant bait organisms in rocky reef waters. As a result, rocky reef habitats naturally become binding sites for fish and other nekton to breed, develop, spawn, feed and avoid predators. Typically, a dynamic pattern of fish assemblage in these areas become essential parts of coastal communities, which are dominated by ground reef-dwelling fishes. Other migratory fish select this habitat periodically to complete their specific life histories. Due to the high spatial heterogeneity and temporal variability of some reef habitats, it is often difficult to conduct a comprehensive and systematic survey of fish communities. Hence, a better understanding of fish assemblage patterns and their diversity in rocky reef habitats is essential to reveal anthropogenic disturbance in a large spatiotemporal scale, which may play key roles in regional fisheries management. Undoubtedly, it is needed to build a more rational and efficient assessment type to fulfill this scientific aim.

The diversity of fish species in rocky reef habitats has been investigated in a very long history and in various ways, especially since the 1990s in coastal countries such as Italy, Spain, the United Kingdom and Australia (Underwood and Chapman, 1996; Azzurro et al., 2010). For the past decade, several reports on fish diversity in rocky reef waters in China called upon scholars' attention to this topic (Wang et al., 2012, 2013; Liang et al., 2014; Li et al., 2020). In general, these studies on fish diversity assessment were mainly based on destructive sampling tools such as bottom trawling, gillnetting, longlining and crab traps (Juza et al., 2018). Among these conventional assessment methods, gillnets are widely used for fish monitoring in complex habitats such as rocky reefs, but the probability of fish contacting and being trapped in the net increases with swimming distance, speed and discontinuity in body contour, and is therefore passive and selective (Silvano et al., 2017; Grimaldo et al., 2019). The size distribution of the fish is also asymmetrical and, when nets are encountered, catching is less efficient due to the slower speed of the smaller nets and their less flexible nature. However, its disadvantage of high selectivity can be significantly reduced by a changed triple gillnet with multiple mesh combinations, which is more representative for evaluating fish diversity in rocky reef habitats (Olsen et al., 2019). When tidal currents and estuary water meet and mix in coastal area of the East China Sea, the rocky reef habitat shows rather poor visibility, low transparency and strong currents, making underwater observation, such as scuba diving, ineffective and impossible. The sampling methods such as multi-mesh trammel nets can take full advantage of their high flexibility and low selectivity. However, fishes belonging to order Anguilliformes, family Sparidae and Serranidae, can hardly be trapped by sampling gillnets, due to their slender and smooth body, strong swimming power and strong sight, such as *whitespotted conger*, snappers and groupers. Given this, a relatively new effective method, eDNA metabarcoding, can be introduced besides multi-mesh trammel nets, in order to do a comprehensive survey on fish diversity in rocky reef habitats.

Environmental DNA is the small amount of DNA that an organism releases into its surroundings, leaving a genetic footprint of skin, scales, waste and other tissues (Pawlowski et al., 2020). eDNA metabarcoding is a sensitive, effective, non-invasive biodiversity monitoring method for detecting rare, enigmatic, invasive and endangered species with high certainty, low cost and minimal stress on the aquatic organisms. It also estimates biodiversity in terrestrial, aquatic and marine environments and acts as a proxy signal for the biomass of fish species and populations (Takahara et al., 2012). It has been successfully used in amphibians (Tews et al., 2010; Malekian et al., 2018; Bailey et al., 2019; Ficetola et al., 2019; Brys et al., 2020; Fediajevaite et al., 2021), fish (Pont et al., 2018; Jo et al., 2019; Stoeckle et al., 2020), insect larvae, crustaceans and mammals (Pont et al., 2018) and has proven to be an effective genetic monitoring tool for the presence of species in ecosystems. At present, the eDNA metabarcoding approach is gradually becoming popular in the study of fish in China. Fish eDNA has been detected in various aquatic environments, including ponds (Fujiwara et al., 2016; Harper et al., 2019a,b), streams and rivers (Doi et al., 2017; Rice et al., 2018; Pont et al., 2019; Shogren et al., 2019) and marine waters (Thomsen et al., 2012; Port et al., 2016), very rare studies have reported combining traditional netting and eDNA techniques for fish diversity investigations in rather complex habitats such as rocky reefs. Few researchers have focused on rocky reef habitats, and, to our knowledge, no one has compared the efficiency of eDNA vs. multi-mesh gillnets to measure fish diversity.

During the investigation of fish diversity in the reef habitat of Dachen Islands in the East China Sea in October 2020, we made a serendipitous observation that the DNA of *Dasyatis akajei* and *Harpadon nehereus* were detected in the water sample but were not captured by gillnets. Given this observation, we wondered if eDNA metabarcoding detection could be more abundant than the results collected by gillnets in the reef habitat. If so, diversity analysis of reef habitat fish using eDNA metabarcoding could provide more accurate information on important reef-dwelling fish with strong swimming abilities and superior underwater perceptions. To test the concept and compare the effects of eDNA metabarcoding and multi-mesh gillnets on fish diversity assessment, we aimed to firstly compare the differences and similarities of the composition of fish species and their ecological groups, and the influencing factors of diversity between the two methods, and, secondly, analyze the advantages and disadvantages of the two methods to provide optimization strategies for fish biodiversity investigation in complex habitats.

MATERIALS AND METHODS

Study Area and Sites Layout

Data for this study was obtained from a background survey of fisheries in rocky reef habitats in November 2020. The study area is located in the sea area of Dachen Islands, near the upper and lower islands, in the eastern part of the Jiaojiang District, Taizhou City and the Zhejiang Province in China. The sea area is located between 27 and 29° degrees off the East China Sea near the coast, located in the mid-latitude zone. It belongs to the subtropical monsoon climate. Affected by the intersection of three major water systems, such as Taiwan warm current, Zhejiang coastal current and mainland runoff, the annual average temperature is 16.7℃, the perennial water temperature is between 10.5 and 27.9℃, the water depth is 18–20 meters, the water quality is fertile, the temperature and salt are suitable, and the bait is rich. Its location advantages and geographical conditions provide an ideal habitat and breeding place for many fish.

Due to the large amount of sediment in the north side of Shang Dachen Island and the need to ensure that the extraction of water DNA can be completed within the effective time, the stations were located in the south of Shang Dachen Island and around Xia Dachen Island. Eleven stations (S1–S11) were set up, of which stations S7 and S8 were near the cage aquaculture area and stations S9 and S11 were near the mussel culture area. The specific survey sites were set up as shown in **Figure 1**. Fish and water samples were collected in the following order: S11, S6, S7, S10, S4, S9, S2, S5, S3, S8. The details of the latitude and longitude coordinates of each sampling site and the time of the water sample collection is shown in **Supplementary Table 1**.

Sampling Methods and Sample Handling

Fish Sample Collection and Biological Identification

Gillnets were placed at 11 sites between 8:00 a.m. and 12:00 p.m. on the 24th of November 2020. The height of the gillnets is 1.5 m, the outer mesh coat is 26 cm, and the inner mesh coat consists of four spliced sections with the mesh of 25, 35, 50, 60, and 70 mm, ensuring a sample strip length of approximately 120 m per site and a release time of about 18 ± 6 h per release. All stations were then closed before low tide on the 25th—the low tide was at 13:12. The fish were returned to the laboratory within 2 h of collection and species identification was carried out within 6 h. Identification was based on the Nelson system (Nelson, 1976). At the same time, biological measurements were made, including length, body weight, feeding intensity and gonadal maturity. All standards were based on the Marine Survey Code (GB12763-2007) (China State Bureau of Quality and Technical Supervision, 2007a) and the Marine Monitoring Code (GB17378-2007) (China State Bureau of Quality and Technical Supervision, 2007b) and the species of the fish were identified according to the Zhejiang Marine Fish Journal.

Water Sample Collection and Environmental DNA Enrichment

Twenty-two samples were collected from the surface and bottom layers of the seawater at each of the 11 stations before each net release. All samples were collected before low tide and then placed in sterile sealable wide-mouth bottles. In particular, surface water samples were taken approximately 3–5 m from the surface and 3–5 m from the bottom, and the sampling locations were synchronized with the gillnet fish sampling stations to collect water samples before each retrieval. The water samples were filtered immediately after collected. A mixed fiber membrane of 47 mm in diameter and 0.45 μm pore size was used for filtration and negative control was set up for each sample per filtration. All filtered material was rinsed once with a 10% bleach solution and washed three times with distilled water (Shu et al., 2020). After filtration, the membranes were sealed in sterilized 10 ml lyophilisation tubes and stored at –20°C until DNA extraction. The extraction of the eDNA was achieved using the E.Z.N.A^{TM} Mag-Bind Soil DNA Kit (Omega Bio-Tek). The extraction procedure was based on the kit's instructions and was repeated three times for each sample. All steps were taken to avoid contamination at each stage of the experimental process. CTD thermohalimeter was used to measure water depth, flow velocity, temperature, pH levels, turbidity, salinity, dissolved and oxygen at each survey station. At the same time, two 500 ml parallel water samples were collected at each sampling point to determine the following nutrient indexes: nitrate, nitrite, ammonia nitrogen, silicate and phosphate.

DNA Amplification

The MiFish-U-F:(5′-GTCGGTAAAACTCGTGCCAGC-3′) and MiFish-U-R:(5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′), which target the 12S rDNA region of the mitochondria gene, were used to amplify 170 bp fragments of the extracted eDNA samples (Miya et al., 2015). The 30 μL reaction system includes 15 μL of 2 × PCR Buffer, 1 μL of dNTP (10 mmol/L), 1 μL of forwarding primer (10 pmol/μL), 1 μL of reverse primer (10 pmol/μL), 5 μL of DNA template and 36.5 μL of sterilized double-distilled water. Reaction conditions were: 95°C pre-denaturation 3 min; 94°C denaturation 20 s, 55°C annealing 20 s, 72°C extension 30 s (5 cycles), and 72°C extension 75 min, 10°C.

Library Preparation and Sequencing

Illumina Miseq^{TM} was used for DNA sequencing library construction. Library quality was evaluated using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, United States) and Agilent Bioanalyzer 2100 (Agilent Technologies Inc., California, United States). The library was then sequenced on the Illumina Miseq platform (Shanghai Bioengineering Co. Ltd.).

Data Processing and Analysis Methods

Bioinformatics Data Processing

The data obtained by off-line sequencing is double-ended sequence data, and the sequencing sequence contains barcode sequence, primer and linker sequence added during sequencing. Firstly, the primer splice sequence needs to be removed, and then the paired reads are spliced into a sequence according to the overlapping relationship between PE reads, and then the samples are identified and distinguished according to the barcode tag sequence to obtain each sample data. Finally, the quality of each sample data is filtered by quality control to obtain the effective data of each sample.

OTU clustering and annotation are carried out according to sequence similarity ≥ 97%. After that, the OTU representative sequence is compared with Mito fish[1] and NCBI.[2] The database

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[1] http://mitofish.aori.u-tokyo.ac.jp/

[2] https://www.ncbi.nlm.nih.gov/

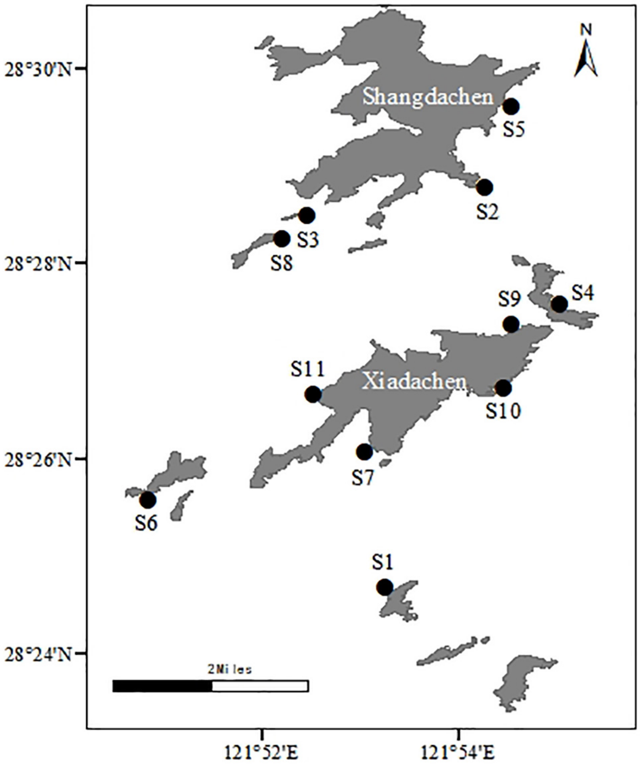

FIGURE 1 | Distribution of rocky reef habitat survey sites on Dachen Islands.

is compared, classified and annotated, and the corresponding OTU abundance table is obtained. In the parallel samples of each sampling point, the unmatched species sequences are eliminated, and the number of matched species sequences is processed according to the mean value. The raw data were deposited in the NCBI BioProject database[3] with the accession number of PRJNA808626.

Species Composition Analysis

After removing the data information compared to non-fish (such as bacteria, birds, amphibians and mammals), screen out OTUs compared to fish with identity value $\geq$ 97% and E-value $\leq$ 10–5, and then merge OTUs compared to the same species. If there are OTUs that cannot be compared to the species level, statistics shall be made up to the next higher level, such as genus, family, etc. Count the proportion of effective sequence number of each kind of fish in each sample in Excel, and refer to FishBase database, notes of inland fish species and distribution in China and marine fish records of Zhejiang to improve fish taxonomy information.

Fish Reefing and Habitat Layer

According to their dependence on the reef, fish are classified as reef-touching, reef-trending, reef-attracting and pelagic fish species. Reef-touching fish are sedentary fish that depend on rocky reefs for their entire life history and can only be collected in reef habitats such as *Sebastiscus marmoratus.* Reef-touching fish such as *Hapaloyenys mucronatus* are stage-dependent on reefs for breeding, feeding or larval shelter. Reef-attracting fish prefer to stay around the reef but are not directly dependent on it, such as Engraulidae and Clupeidae. Pelagic fish are those that do not depend on the reef at all for their survival. All fish are classified into three ecological types based on their water layers: near-surface fish, near groundfish and groundfish (Wang et al., 2012).

Calculation of Fish Biodiversity Indices

The Shannon-Wiener diversity index (Tikadar et al., 2021), the Simpson diversity index (Duijndam et al., 2020), the Margalef richness index (Van Loon et al., 2018) and the Pielou evenness index (Tikadar et al., 2021) were used to analyze the diversity of gillnet fish species based on the ecological characteristics of the coastal waters of Dachen and the fishery resources data obtained. Based on the eDNA high-throughput sequencing results, the Shannon and Simpson indices were used to assess the diversity of fish community distribution. The Chao1 and ACE indices were used to calculate the abundance of fish community distribution. Shannon and Simpson diversity indices are commonly used to reflect alpha diversity indices. The greater the Shannon value, the higher the community diversity. Simpson is used to estimate one of the microbial diversity indexes in samples, which is often used to quantitatively describe the biodiversity of a region in ecology. The greater the Simpson index value, the lower the community diversity. The Pielou index reflects the evenness of the distribution of different species

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[3] https://www.ncbi.nlm.nih.gov/bioproject/

in the community. The Margalef index reflects the richness of species in the community. A species diversity index is a comprehensive index of richness and uniformity. The specific equations are set out below.

(a) $H = \sum(Pi{*}Log_2 Pi)$Pi is the probability of drawing the ith individual from among the N individuals.
(b) $D = 1 - \sum P^2 i$,
(c) $D = \frac{(S-1)}{LnN}$ is the number of species in the ecosystem, and N is the total number of individuals.
(d) $J = \frac{H}{H_{max}}$ H is the measured diversity, and Hmax is the maximum diversity.
(e) $H' = -\sum_{i=1}^{S_{obs}} \frac{ni}{N} ln \frac{ni}{N}$ Sobs denotes the number of OTUs observed. ni represents the number of sequences contained in the ith OTU and N denotes the total number of sequences
(f) $D' = \frac{\sum_{i=1}^{S_{obs}} ni(ni-1)}{N(N-1)}$.
(g) $S_{chao1} = S_{obs} + \frac{n1(n1-1)}{2(n2+1)}$ hao1 indicates the estimated number of OTUs.

$$(h) \quad S_{ACE} = \begin{cases} S_{abund} + \frac{S_{rare}}{C_{ACE}} + \frac{n1}{C_{ACE}} \hat{\gamma}^2_{ACE}, & for \hat{\gamma}^2_{ACE} < 0.80 \\ S_{abund} + \frac{S_{rare}}{C_{ACE}} + \frac{n1}{C_{ACE}} \hat{\gamma}^2_{ACE}, & for \hat{\gamma}^2_{ACE} \geq 0.80 \end{cases}$$

$$N_{rarre} = \sum_{i=1}^{abund} in_i$$

$$C_{ACE} = 1 - \frac{n1}{N_{rare}}$$

$$\hat{\gamma}^2_{ACE} = max\left[\frac{S_{rare}}{C_{ACE}} \frac{\sum_{i=1}^{abund} i(i-1)ni}{N_{rare}(N_{rare}-1)} - 1, 0\right]$$

denotes the number of OTUs containing i sequences. Srare indicates the number of OTUs containing "abund" entries or less than "abund." Sabund = number of OTUs with more than "abund" entries. abund indicates the threshold value of the "dominant" OTU, the default is 10.

SPSS 22.0 data analysis software was used for analysis, and Prism was used to make box charts.

RESULTS

Fish Species Composition

A total of 186 fish were collected through gillnets, with a total biomass of 10,809.83 g, belonging to 6 orders, 19 families and 24 genera, with 28 species (**Table 1**), all of which are in the order of the Clupeiformes, which were the most numerically dominant at the order level, with 43.5% of the total catch in 2 families, 3 genera and 4 species (indicating percentages of numbers, the same as that below). The next largest group was the Perciformes, with 11 families, 15 genera and 18 species, accounting for 36% of the caught species. Once again, the Scorpaeniformes were divided into 1 family, 1 genus and 1 species, accounting for 15.1%. At the bottom was the Anguilliformes at 2.6%, the Tetraodontiformes at 1.6% and the Siluriformes at 1.0%, respectively. The Engraulidae accounted for 39.2% of the total catch at the family level, followed by the Sciaenidae (20.4%) and Sebastidae (15.0%), with the rest of the families accounting for less than 5%.

A total of 81 species of fish were detected by eDNA, of which 76 species of the Spoked Finiformes belonged to 12 orders, 43 families and 64 genera, accounting for 93.8% of the total number of species, which is overwhelmingly dominant in the taxonomy (**Table 1**). At the order level, similar to the gillnet statistics, the Perciformes accounted for 56.7% of the total number of species, with 45 species in 40 genera and 23 families, the Clupeiformes accounted for 11.1%, with 9 species in 7 genera and 3 families, and the Tetraodontiformes accounted for 7.4%, with 6 species in 3 families and 3 genera. The Pleuronectiforme, Scorpaeniformes and Anguilliformes all accounted for 3.7%, with the former two belonging to 3 families, 3 genera and 3 species, and the latter to 2 families, 2 genera and 3 species. At the family level, the Sciaenidae, as the dominant family, accounted for 18.5% of all species, followed by the Engraulidae at 7.4%, the Gobiidae at 6.1%, the Tetraodontidae at 4.9% and the Dasyatidae at 3.7%. The Trichiuridae, Sparidae, Kyphosidae, Clupeidae, Muraenesocidae, Polynemidae, and Blenniidae were tied in sixth place, accounting for 2.4% of all species. There are 5 cartilaginous fishes in 3 orders, 3 families and 3 genera, accounting for 6.1%. The Myliobatiformes accounted for 40% of the total number of chondrichthyans and the Myliobatiformes and Carcharhiniformes accounted for 20%.

A total of 96 species of fish were counted by both gillnets and eDNA, with the number of points by station shown in **Figure 2**. The minimum number of species collected in the gillnet was 2 for sites S1 and S9, and the maximum number of species was 10 for site S4. The highest number of fish species detected by eDNA was 55 species each at sites S10 and S11, and the lowest was 11 species at site S3, with more than half of the total number of species at sites S6–S11. The statistics showed that 12 of the same species were counted both ways, with *Johnius belangerii* and *Muraenesox cinereus* being detected at 6 locations. In addition to the species commonly found in local waters, five species of fish that have not been recorded in China were also detected (**Table 2**).

Fish Reefing Statistics

Of the 96 fish species, 12 were reef-touching, 6 were collected by gillnetting, 9 were detected by eDNA and 3 were counted by both methods, with *Sebastiscus marmoratus* being the most frequently occurring species (**Table 1**). There were 9 reef-trending species, 2 species collected in gillnets and 7 species detected by eDNA, with *Lateolabrax maculatus* being the most frequently detected species by eDNA. There were 27 reef-attracting species, with 11 collected by gillnet and 23 detected by eDNA, the *Johnius belangerii*, *Larimichthys crocea*, and *Muraenesox cinereus* being the most frequently occurring species counted in both ways (**Table 1**). There were 49 distant reef species, 10 species collected by gillnets and 43 species detected by eDNA. The *Benthosema pterotum*, *Dasyatis akajei* and *Harpadon nehereus* were the most frequently occurring species

TABLE 1 | Fish species statistics of the study sites.

Fish types	Latin name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Reef-touching	*Chaetodon modestus*						▲					
	Cheilodactylus quadricornis				▲		■	■	■		■	■
	Entomacrodus stellifer										■	
	Epinephelus akaara				■	■	■		■			■
	Girella punctata									■		
	Goniistius zonatus				▲						▲	
	Oplegnathus fasciatus						■		■			
	Parablennius yatabei						■	■	■	■	■	■
	Sebastiscus marmoratus		▲	■	▲	▲	■	▲■	▲■	■	▲■	▲■
	Siganus canaliculatus									■		■
	Siganus fuscessens	▲			▲	▲		▲			▲	▲
	Strigatus							■		▲		▲
Reef-trending	*Acanthopagrus schlegelii*						■			■		
	Choerodon zosterophorus										■	
	Coradion chrysozonus		■			■	■		■	■	■	■
	Hapalogenys analis						■				■	■
	Hapaloyenys mucronatus				▲							
	Kareius bicoloratus											■
	Lateolabrax maculatus	■			■	■	■	■	■	■	■	
	Pagrus major					▲				■		
	Parapristipoma trilineatum											▲
	Plotosus lineatus		■								■	■
	Stephanolepis cirrhifer								▲			■
Reef-attracting	*Argyrosomus japonicus*	▲		▲								
	Cheilodactylus quadricornis				▲		■	■	■		■	■
	Conger myriaster		■			■	■	■	■	■	■	■
	Decapterus maruadsi								■			
	Johnius belangerii	■	▲	▲	■	▲■	▲	▲■	■	▲■	▲■	▲■
	Johnius grypotus				■					■	■	
	Lagocephalus inermis									■		■
	Lagocephalus lunaris						■			■		
	Lagocephalus spadiceus							■	■			■
	Larimichthys crocea	■	■	▲■	▲■	■	▲■	▲■	▲■	■	▲■	■
	Miichthys miiuy		■				■	■	■	■	■	■
	Mugil cephalus					■	■	■	■	■	■	
	Muraenesox bagio	■										
	Muraenesox cinereus	■	▲■	▲■	▲■	■	▲■	▲■	▲■	■	■	■
	Nibea albiflora					▲	■	▲■	■		▲■	■
	Nibea miichthioides						■	■	■		■	■
	Paralichthys olivaceus						■	■	■	■	■	■
	Platycephalus indicus								▲			
	Plotosus anguillaris					▲						
	Polydactylus sextarius			▲			▲	■	■	■	■	
	Rhinoptera bonasus	■					■	■			■	
	Sardinella zunasi											▲
	Sillago sinica				■				■	■	■	■
	Takifugu fasciatus				■	■	■	■	■	■	■	■
	Takifugu flavidus				■	■	■	■	■	■	■	■
	Thryssa kammalensis	■	■		▲		■	■		■	■	▲
	Thryssa_vitrirostris		■		■	■	■	■	■	■	■	■
Pelagic fish	*Amblychaeturichthys hexanema*	■	■		■	■	■	■	■	■	■	■
	Amblychaeturichthys sciistius				■	■		■	■	■		■
	Amoya chusanensis							■		■		

(Continued)

TABLE 1 | (Continued)

Fish types	Latin name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
	Atrobucca nibe							■				
	Benthosema pterotum	■	■	■	■	■	■	■	■	■	■	■
	Bregmaceros atlanticus	■					■					
	Chelidonichthys kumu								■			
	Chrysochir aureus						■	■	■	■	■	■
	Coilia brachygnathus							■			■	■
	Coilia mystus						■	■				
	Collichthys lucidus	■	■		■	■	■	■	■	■	■	■
	Collichthys niveatus	■	■		■	■	■	■	■	■	■	■
	Cynoglossus lineolatus							■				
	Dasyatis akajei	■	■	■	■	■	■	■	■	■	■	■
	Dasyatis centroura										■	
	Eleutheronema tetradactylum				■	■	■		■		■	■
	Harpadon nehereus	■	■	■	■	■	■	■	■	■	■	■
	Himantura microphthalma						■				■	■
	Hoplosebastes armatus						▲					
	Ilisha elongata	■			■		■	■	■			
	Jaydia_lineata							■				
	Johnius borneensis	■				■	■	■	■	■		■
	Konosirus punctatus						■			■	▲	■
	Konosirus punctatus						■			■	▲	■
	Larimichthys polyactis										▲	
	Lepturacanthus savala										■	
	Malakichthys wakiyae			▲								
	Minous monodactylus								■			
	Odontamblyopus lacepedii		▲			▲						
	Pampus argenteus	■	■		■	■	■	■	■	■	■	■
	Paratrypauchen microcephalus						■				■	■
	Pennahia anea								■			■
	Pennahia argentata				▲■			■	■	■	▲■	■
	Pennahia argentata				▲■			■	■	■	▲■	■
	Pennahia macrocephalus							■	■	■	■	■
	Platycephalus cultellatus	■			■	■	■	■			■	
	Psenopsis anomala									■	■	
	Sarda orientalis											■
	Sardinella lemuru						■		■	■	■	
	Sciaenops ocellatus						■		■		■	■
	Scoliodon macrorhynchos									■		
	Scomber japonicus				■		■			■	■	■
	Setipinna melanochir				■	■	■	■	■	■	■	■
	Stolephorus teguhi		■	■			■	■	■	■	■	■
	Takifugu alboplumbeus							▲				
	Thryssa mystax				▲						▲	▲
	Trichiurus japonicus				■	■		■	■	■	■	■
	Tridentiger barbatus						■	■				
	Trypauchen vagina	■			■	■	■	■	■	■		■

The ▲ indicates fish collected by gillnets, the ■ indicates fish detected by eDNA metabarcoding approach.

detected. The results from each site showed that 8 sites had more reef-touching species than reef-trending species, but all had fewer reef-attracting and pelagic fish species (**Table 1**). Overall, reef-attracting species were the most species collected through gillnets, followed by pelagic fish species, then reef-touching species, and finally reef-loving species. The pelagic fish species were the most species collected by eDNA, followed by the reef-attracting species, then the equally diverse reef-attracting and reef-loving species. In terms of the substrate characteristics of their habitats, the number of fish species preferring sandy and muddy habitats reached half, both by gillnetting and eDNA testing.

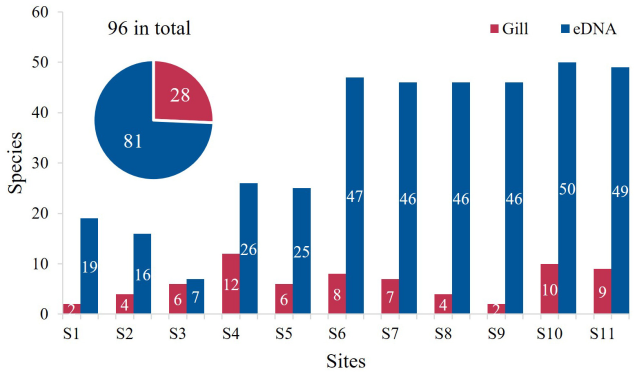

FIGURE 2 | The number of fish species at each site. The pie chart shows the number of fish species counted in two ways.

Fish Habitat Layer Statistics

According to the fish inhabiting water layer, we made statistics based on the fish collected by gillnets and eDNA technology (**Figure 3**). The results showed that there was more groundfish than near groundfish and near-surface fish at S2 and S8 stations among fish collected by gillnets. No groundfish was collected at sites S1 and S9. The number of groundfish and near groundfish is equal at S3, S5, S6 and S7 stations. Near groundfish fish dominated at S4 and S10 stations, while near-surface fish dominated at S11 stations.

A total of 28 species were collected through the gillnets, with 4 near-surface species, 18 near groundfish and 6 groundfish. Eighty-one fish species were detected by eDNA, with 17 near-surface species, 42 near groundfish and 22 groundfish. In the process of ranking the frequency of the occurrence of the dominant fish species in each stratum counted, the *Thryssa mystax*, *Johnius belangerii* and *Sebastiscus marmoratus* were found to be the near-surface fish, near-groundfish and groundfish in the gillnet collection, occurring at stations 3, 8, and 7, respectively. The *Thryssa vitrirostris*, *Benthosema pterotum*, *Harpadon nehereus* and *Dasyatis akajei* were the near-surface fish, near groundfish and groundfish detected by eDNA at 9 sites, 11 sites and 11 sites, respectively. In addition to this, the gillnets statistics showed that *Siganus fuscessens* and *Larimichthys crocea* tended to be more abundant near the ground and the *Muraenesox cinereus* was more abundant at the bottom. The results of the eDNA metabarcoding showed that the *Stolephorus teguhi*, *Coradion chrysozonus* and *Lateolabrax maculatus* were more abundant in the near-surface layer, with the *Collichthys lucidus*, *Collichthys niveatus*, *Larimichthys crocea* and *Pampus argenteus* being more abundant near the ground and the *Amblychaeturichthys hexanema* and *Muraenesox cinereus* more abundant at the ground.

TABLE 2 | Common fishes counted by gillnets and eDNA, and species that have not appeared in the East China Sea detected by eDNA.

Classification	Species
Fish counted jointly by both methods (according to frequency of occurrence of sites)	*Johnius belangerii*(11)
	Larimichthys crocea(11)
	Muraenesox cinereus(11)
	Sebastiscus marmoratus(10)
	Thryssa kammalensis(8)
	Cheilodactylus quadricornis(6)
	Nibea albiflora(6)
	Polydactylus sextarius(6)
	Pennahia argentata(6)
	Konosirus punctatus(4)
	Strigatus(3)
	Pagrus major(2)
	Stephanolepis cirrhifer(2)
Species not recorded in the East China Sea	*Choerodon zosterophorus*
	Coradion chrysozonus
	Johnius borneensis
	Scoliodon macrorhynchos
	Stolephorus teguhi

Brackets indicate that several sites have appeared in total.

Species Diversity Index

Diversity indices for gillnet statistics are expressed as Shannon's diversity index, Simpson's diversity index, Margalef's richness index and Pielou's evenness index. The eDNA statistics are expressed as Shannon's diversity index, Simpson's diversity index, Chao1's, and Ace's. The Shannon diversity index combines the evenness and richness of the population with higher values corresponding to higher diversity in the community, and the Simpson diversity index is the opposite. The higher the Chao1 index the more species are present in the community (**Figure 4**).

The distribution trends of the Shannon diversity index and the Simpson diversity index obtained in both ways were generally consistent. The spiny web statistics showed that the Shannon diversity index ranged from 0.50 to 2.18. The highest index site was S10, the lowest was S1 and S9, and 6 sites had above-average diversity indices. The Simpson's diversity index ranged from 0.05 to 0.60, with sites S1 and S9 having the highest indices, site S5 having the lowest and 5 sites were above the mean. The Margalef richness index ranged from 0.62 to 3.25, with site S10 having the highest index, sites S1 and S9 having

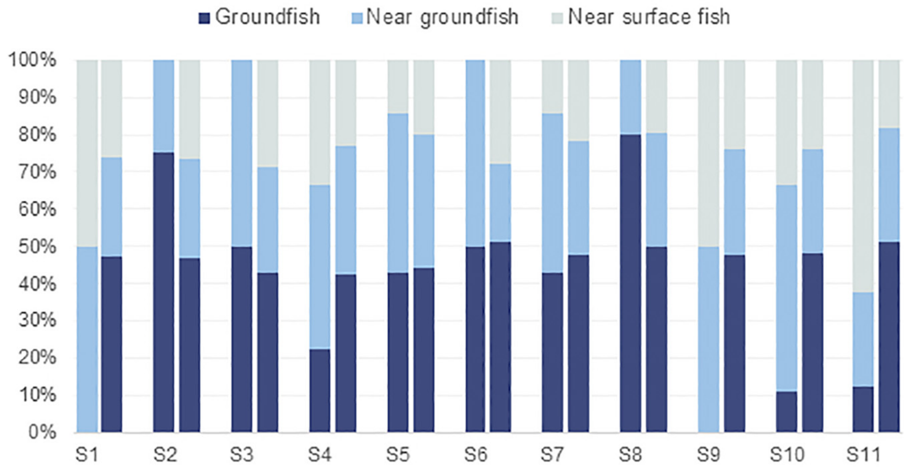

FIGURE 3 | Percentage of fish populations in different water layers at each site. Among the two columns at each site, the left represents fish counted by gillnets, and the right represents fish detected by eDNA.

the lowest index and 6 sites were above the mean. The Pielou evenness index ranged from 0.63 to 0.98, with site S5 having the highest index, site S6 having the lowest index and 4 sites were above the mean.

In contrast, the alpha diversity index shown by eDNA high-throughput sequencing results showed a range of 1.12–3.00 for the overall Shannon diversity index at each site. The S7 site had the highest index, S3 had the lowest index and 6 sites were above the mean. The surface range is 1.26–2.39, with the highest index at site S10 and the lowest index at site S8. The bottom range is 0.64–3.00, with site S7 having the highest index and site S3 having the lowest index. The overall Simpson diversity index for each site ranged from 0.07 to 0.38, with site S3 having the highest index, site S5 having the lowest index and 4 sites having indices above the mean. The surface range is 0.02–0.42, with site S8 the highest and site S7 the lowest. The bottom layer ranged from 0.08 to 0.54, with site S3 the highest and sites S5 and S7 the lowest. High throughput sequencing of all samples showed that Chao1 indices ranged from 2 to 52, with the highest S9 site index of 30 and the lowest S1 site index of 6 in the surface samples, and the highest S10 site index of 52 and the lowest S3 site index of 2 in the bottom samples. Ace indices ranged from 2 to 55, with the highest S7 site index of 55 and the lowest S1 site index of 8.75 in the surface samples, and the highest S10 site index of 52 and the lowest S3 site index of 2 in the bottom pieces, with the two indices following the same trend.

Environmental Factors

The analysis of environmental factors showed that factors other than the water flow had little effect on the method (**Figure 5**). Therefore, we deeply analyzed the relationship between the flow rate of water bodies and the monitoring results (**Table 3**). The data showed that the bottom values of the Shannon diversity index were higher than the surface values at all sites except sites S2 and S3. The number of fish species detected in the bottom samples at several sites S5–S10 exceeded 40 species and sites S1 and S2 were below 10. Of the surface samples, site S9 yielded the highest number of species detected at 18, with S1, S2, S8, S10, and S11 all under 10 species.

DISCUSSION

Analysis of Differences in Fish Composition Between Sampling Methods

The two methods, gillnetting and eDNA high-throughput sequencing, yielded significantly different results in fish species composition. According to the survey data of Liang et al. (2019) and Han et al. (2020), the gillnets collected in this study were indeed species found in the local waters in that season. In addition, dominant species not collected such as *Chrysochir aureus*, *Harpodon nehereus,* and *Collichthys lucidus* were also recorded to be active in the area, which was confirmed by the results of our survey of open waters away from the islands and reefs using crab cages and trawls in the same period—see **Supplementary Table 2** for the results. The results of the eDNA test showed that 68 species were not collected by gillnets, including the above species that were active in the sea but not collected by gillnets. We also conducted fish collection experiments in November 2019 by gillnetting, ground cages, and longline fishing, where 16, 2, and 8 species of fish were collected by the 3 gears, for a total of 20 species. Thirteen of these species were consistent with the 2020 gillnet collection and 11 were consistent with the eDNA test results. This experiment collected the leader fish by gillnets, which verified the accuracy of the eDNA. Meanwhile, 5 fish species were detected that have not been recorded in the East China Sea: *Choerodon zosterophorus* (Wang et al., 2015), *Coradion chrysozonus* (Zuo, 2011), *Johnius borneensis* (Kar et al., 2017),

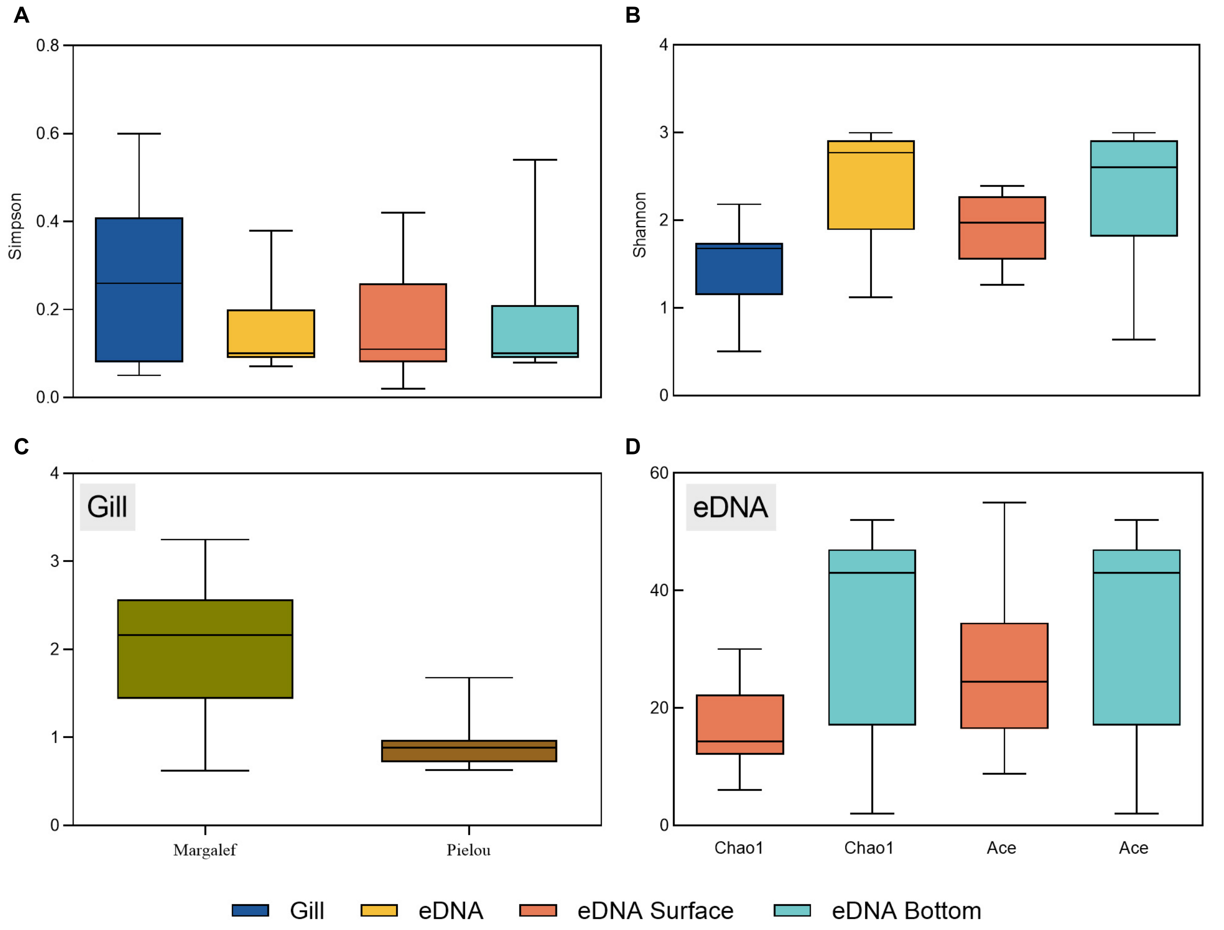

FIGURE 4 | Box plots of diversity indices for each site using gillnets and eDNA metabarcoding—**(A,B)** shows the Shannon diversity index and Simpson diversity index which includes surface and bottom water layers detected by eDNA, **(C)** shows the Margalef diversity index and Pielou diversity index calculated by gillnets and **(D)** shows the Chao1 diversity index and the Ace diversity index calculated by eDNA metabarcoding.

Scoliodon macrorhynchos (Liu et al., 2020), and *Stolephorus teguhi* (Kimura et al., 2009). The above results may be due to three reasons: firstly, the essential differences between the two sampling methods lead to different results. The actual operation of multi-mesh gillnets can obtain visual information on fish species but, due to the selectivity of the nets themselves, the spatial constraints of sampling, environmental factors (e.g., water depth, transparency and season) and the size, behavior and physiological characteristics of the fish, means that other species inhabiting the local area are inevitably missed. In contrast, eDNA is detected only through the collection of seawater. The seawater's horizontal and upward flow characteristics lead to eDNA migration, which may bring fish DNA information from distant open waters (Takahara et al., 2012; Mauvisseau et al., 2017). After upwelling, mixing surface and bottom layers are also more likely to enrich fish DNA from different water layers. It has been shown that flowing water may lead to concentrations of eDNA within hundreds of meters independent of the presence of local species, but further identification by additional means is also needed in practical studies (Tillotson et al., 2018). Secondly is the influence of the fish organism's factors and environmental factors. As fish are swimmers, they can swim freely in the water overcoming the resistance of the current and there are also the seasonal and reproductive migrations; individuals vary in size so the probability of being trapped by the mesh of a gillnet will vary. In contrast, DNA information from certain migratory fish shed in the water may happen to be collected during the collection of water samples but the fish may not actually be in the vicinity for long periods and therefore not collected by the gillnets. Thirdly, the influence of primers used during eDNA detection causes differences in comparison databases. For example, species from the South China Sea and the Indian and Pacific Oceans were detected in this study. The first reason for this is presumed to be inaccurate identification results due to insufficient primer precision or PCR amplification preferences (Liu et al., 2016). The second reason may be due to the existence of closely related homologues or species taxonomic redundancy in the region. The third reason may be related to warming currents in Taiwan or global warming. Previous surveys of fish diversity in rocky reef habitats on the

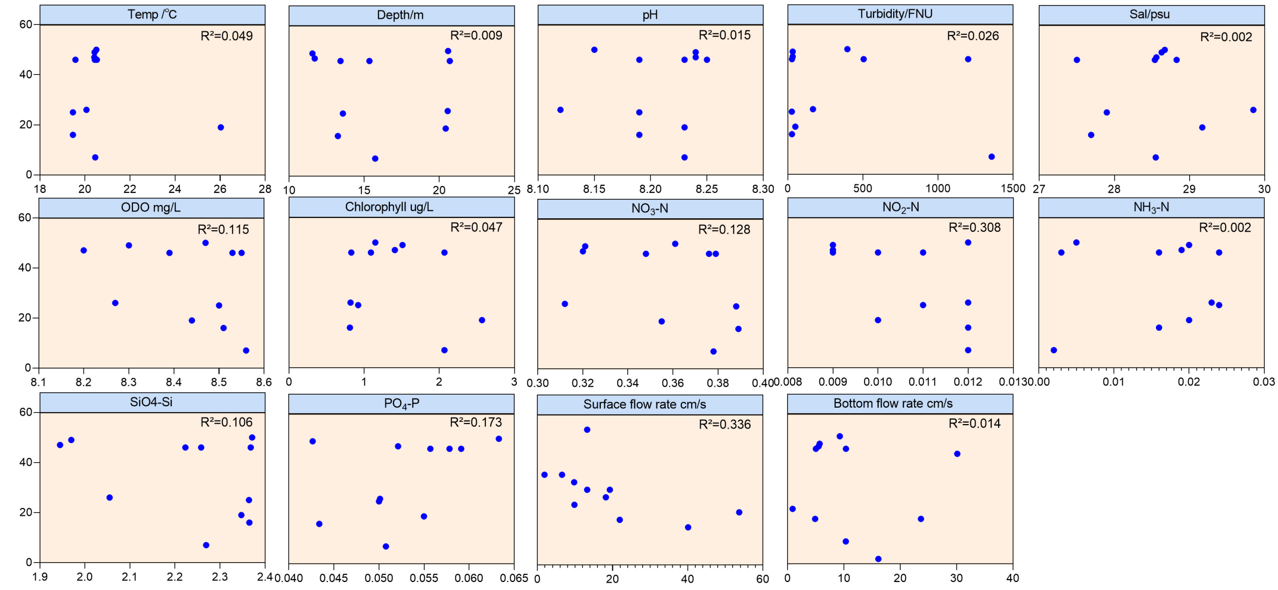

FIGURE 5 | Correlation graph between each environmental factor and the number of fish species detected by eDNA.

TABLE 3 | Surface and bottom flow rates and fish species detected by eDNA at the time of water harvesting at each site.

Sites	Water sample collection time	Surface flow rate cm/s	Bottom flow rate cm/s	Number of species detected on the surface	Bottom detection number of species	Total number of detections
S1	7:31	40.122	23.679	5	18	19
S6	8:11	9.795	5.533	11	47	47
S11	8:47	9.857	5.705	8	48	49
S7	9:19	10.884	5.023	10	46	46
S10	9:52	13.239	9.288	18	51	50
S4	10:13	1.873	0.897	12	22	26
S9	10:27	18.201	10.369	9	46	46
S2	11:15	19.277	10.351	10	9	16
S5	11:26	6.540	4.900	12	18	25
S3	12:17	21.912	16.131	6	2	7
S8	12:24	53.703	30.119	7	44	46

Saddleback Islands also identified several unlisted fish species (Wang et al., 2013).

In terms of numbers and ecotypes of fish, 186 fish were collected by gillnets, mostly reef-attracting species inhabiting sandy mud substrate habitats. The number of fish OTUs detected by eDNA high-throughput sequencing was generally below 50, with low overall abundance and mostly pelagic fish species preferring sandy mud substrates. Both methods yielded more than half of the total number of fish species counted near the ground, followed by groundfish and the fewest near-surface fish. Analytically, as gillnetting is an actual physical operation and eDNA testing is a biological tool, the two are bound to result in differences in the exact number of fish visualized, but the generally low number of fish OTUs obtained from eDNA testing may be due to several reasons. Firstly, the biomass of the fish population and the amount of DNA it releases through excretion processes can significantly affect detection abundance. The eDNA concentration has been shown to be positively correlated with actual biomass by Takahara et al. (2012) and Mauvisseau et al. (2017). Secondly, environmental factors such as currents, tides, wind and salinity can affect the degradation rate and persistence of eDNA in seawater (Thomsen et al., 2012; Barnes et al., 2014), which can lead to variations in DNA concentrations in the collected water samples and thus affect quantitative analysis results. Thirdly, the actual location of sampling and different sampling times may also cause fluctuations in eDNA. Due to the random nature of fish movement and human sampling, areas rich in fish DNA may be missed. Sampling at different times of the year can also affect eDNA concentrations due to fish swimming times and factors such as waves and currents. The overall trend in the number of fish counted in different habitat layers was consistent between the two approaches. This is probably because the multi-mesh gillnets are used to capture fish by fits, stinging and entanglement (Wang et al., 2013) combined with the location of the drop underwater, and therefore are more likely to collect near-bottom fish. In contrast, the water samples tested by eDNA may be mixed up and down by wave impact, so species from other water layers can also be detected at different collection locations. In the present study, *Thrissa kammlensis*, which prefer to inhabit the pelagic zone, were detected in bottom water samples from sites S1, S2, S6, S7, S9, and S10. The groundfish *Dasyatis akajei* were also detected in surface water samples from sites other than S6 and S8. Therefore, further refinement and full consideration of environmental influences are needed to detect species in different water layers using eDNA.

Analysis of the Factors Influencing Fish Diversity

In addition to the apparent differences between the results of the two approaches in terms of fish species composition, the box plot shows that the diversity of fish species obtained by eDNA detection is generally higher than that collected by gillnets, as can be seen by comparing the diversity index values for all of the sites. The Shannon diversity index, for example, was 2.48 for the whole study area obtained by gillnet sampling, with the highest index of 2.18 for site S10 and the lowest index of 0.5 for sites S1 and S9. Several sites were near reefs, but the opening of an ecotourism area near site S10 and many of the reefs has led to a gradual increase in fish abundance. Six reef-touching species, 5 reef-trending species and 18 reef-attracting species were counted using two sampling methods. Among the 11 stations, the diversity indices of S3, S4, S5, S7, S10 and S11 were above the overall mean value, and several stations were mainly distributed around the lower Dachen Islands, probably due to the more transparent water quality in this area compared to the northern Jiaojiang estuary area. The overall diversity of the 8 sites was consistent with the bottom value. Three sites were not significantly different, so it can be assumed that the variety of fish in the bottom waters of the area largely determined the diversity of fish at the same vertical spatial scale. The Shannon diversity index value for the whole study area obtained by the eDNA technique was 3.03, with the highest overall diversity of 3.00 for site S7 and the lowest overall diversity of 1.12 for site S3, which was higher than the lowest index obtained with the gillnet statistics, but the overall diversity of sites S5, S6, S7, S9, S10 and S11 were all higher than that of the whole study area by the gillnet statistics. The majority of these sites were also centered on the Xia Dachen Islands, with nearly 67% of the sites agreeing with the gillnet statistics. The amount of OTU abundance of fish species can be represented by the Chao1 and Ace indices. The two characteristic index trends of 2–52 and 2–55 in the results are generally consistent, but there are considerable differences between individual samples. This may be due to the effect of individual samples (S3 surface layer) on the whole, presumably related to the quality of eDNA and sampling site differences in the marine samples.

As can be seen, the overall effect of the eDNA technique in detecting species diversity in rocky reef habitats was significantly higher than that of the multi-mesh gillnets, but there were also instances where the indices from the same site varied considerably. For example, the number of fish species collected by gillnetting at site S3 was 6, which is in the middle range of fish counts for all sites but was the lowest diversity index detected by the eDNA technique, most likely due to the effect of water velocity and is discussed below. Although there were site-specific differences, the overall range of diversity indices obtained by the two methods converged in terms of overall extent, suggesting the applicability of both sampling methods to the assessment of fish diversity in rocky reef habitats.

In addition to the differences in the number and composition of fish caused by the choice of survey methods, the physical characteristics of the reef habitat itself and the external environmental factors are important reasons for the richness of fish species diversity. Firstly, in terms of the physical parts of the reef itself, island rocks can provide suitable substrates for fish to attach to and serve as important habitats for them to roost, grow, breed and avoid enemies. This has attracted some reef-tending fishes such as *Sebastiscus marmoratus* to become sedentary species in rocky reef habitats and migratory fish like *Siganus fuscessens* and *Muraenesox cinereus* have made seasonal use of the rocky reef waters (Wang and Wu, 2015; Zeng et al., 2016). Secondly, in terms of external environmental factors, the sea where the study area was located is subject to the actions of the coastal waters of Jiangsu and Zhejiang, the surface water and deep water of the warm Taiwan current, the mixed water of the Yellow Sea (Zhang and Wang, 2004) and the spatial and temporal variations of the hydrological elements are dramatic. The deepwater of the warm Taiwan current with its low temperatures and high salinity characteristics, when superimposed on the surface water of the current, will allow the mixing of sediment deposits and the transport of nutrient salts. It will promote the growth of phytoplankton or intertidal macroalgae, leading to an increase in phytoplankton or macroalgae feeding consumers, thus attracting more secondary consumers of fish (Guidetti, 2000; Zhang et al., 2019).

In general, the water samples collected from the bottom had higher community richness. This may be due to three factors; the first being that environmental factors such as substrate type, nutrition and the fish's physiological habits have led to a greater preference for rocky and muddy habitats. Secondly, DNA sedimentation due to factors such as water exchange or DNA migration cannot be collected because the water flows too fast. Thirdly, the upward pulling process of the bottom water samples after collection may have mixed in the upper water column and may have collected DNA information from fish active in the upper water column. To address this phenomenon, this study used FVCOM to simulate the flow velocity of the water column at the time of sampling at each site and compared it with the high-throughput sequencing results (**Supplementary Table 3**), and found that the surface flow velocity reached 0.4 m/s at S1 and 0.5 m/s at S8 at the time of sampling, while the actual abundance of fish detected in the surface layer at these two sites was low, with 5 and 7 species, respectively.

Similarly, bottom water flow velocities in S1 reached 0.23 m/s and only 18 fish species were detected. Therefore, this study speculates that the number and abundance of fish species detected by eDNA may be related to the flow rate of the water column. Of course, as fish are migratory species, their DNA imprinting in the water column changes position as the water moves. When the water is flowing too fast, it may accelerate the rate of dilution of the DNA in the water, thus preventing the collection of water samples containing high levels of fish DNA.

This study also collected water samples at a randomly selected station in the open sea, far from the reef with a high-water flow rate. The eDNA high-throughput sequencing results showed no fish species. Of course, it cannot be ruled out whether this was due to the fish's swimming range or other factors. We also conducted a simultaneous trawl and eDNA survey in this area and will further investigate the relationship between eDNA detection results and water flow rates.

Strengths and Limitations of This Study

The eDNA technology allows exploration of fish diversity in complex habitats, such as areas where trawling is inconvenient, which complements methods that have previously been used with traditional nets. However, some limitations should be borne in mind when interpreting these findings. Firstly, when conducting research, it is more robust to conduct repeat experiments at different dates and seasons. This study verified the usability of the method and continued to combine traditional means with eDNA technology in the later trawl fishery resource surveys. The influence of the complexity of the environment on the detection results of eDNA technology is also fully considered, to provide information to support the conservation of fish diversity. Secondly, eDNA technology may not be able to monitor the species living in the study area in sea areas with fast currents. However, at the same time, the transmission of currents also brings information on the DNA of species living in the oceans, which needs to be discussed in relation to the local historical accumulated fish data in a targeted manner. However, this also emphasizes the validation of the effect of water flow on eDNA in this paper. We will use this study as a basis to consider more biotic and abiotic effects in future experiments to better improve them.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in the NCBI BioProject database with the accession number of PRJNA808626.

AUTHOR CONTRIBUTIONS

YL: conceptualization, formal analysis, writing—original draft, and writing—review and editing. JL: funding acquisition. ZW: conceptualization, investigation, and writing—review and editing. SZ: conceptualization, writing—review and editing, and funding acquisition. KW: investigation. XL: software. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Insights Into Energy Accumulation and Allocation Strategy of Reproductive Migration of Black Amur Bream (*Megalobrama terminalis*) in the Pearl River Basin, China

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Energy allocation is an important component of the reproductive cycle, and as such, it will affect survival, migration, and reproductive success. To reduce the risk of reproductive migration failure and to optimally allocate a limited amount of energy, it is vital to first understand the trade-off between reproduction and somatic growth in migrating fish. In this study, we chose *Megalobrama terminalis*, an endemic species residing in the Pearl River basin with relatively high migratory potential, as a candidate to investigate energy accumulation and allocation strategy during reproductive migration. The analysis used a quantitative assessment of biochemical composition and energy density in somatic and visceral tissues of *M. terminalis* females during the reproductive cycle. The results indicated that stage III to stage IV of *M. terminalis* was the vital migration-launching period. The asynchrony of development was confirmed in energetic relationships in muscle and ovary. Specifically, there was a regulatory mechanism for allocating lipids to each tissue reasonably during the breeding migratory preparation period (stage III). The significant change in lipid content of the ovary is considered as a crucial physiological index, which reflects the readiness for breeding migration of *M. terminalis.* In addition, the negative energy density relationship between somatic and reproductive tissues indicated a trade-off between maximum metabolic ability and energy efficiency before migration launching in *M. terminalis*. The present findings provide effective information for initiating further research on the ecological adaptation of migrating fish species.

Keywords: reproduction, energy allocation, migration, *Megalobrama terminalis*, proximate composition

HIGHLIGHTS

- Current study is the first demonstration of energy accumulation and allocation strategy in *Megalobrama terminalis* during the breeding migration.
- The remarkable change of lipid content in ovary is considered as a crucial physiological index reflecting the readiness for breeding migration of *M. terminalis*.
- A regulatory mechanism to allocate the lipid of each tissue reasonably in the breeding migratory preparation period of *M. terminalis* has been found in our research.

INTRODUCTION

Almost half of all vertebrate species are fish, and they encompass a wide spectrum of physiological and ecological adaptations. Generally speaking, fish life-history theory predicts that organisms will balance their energy allocation among maintenance, storage, growth, migration, and reproduction to maximize their fitness (Roff, 1983). As a vital aspect of the life history, reproductive behavior is highly relevant to optimal energy accumulation and allocation (Alonso-Fernández and Saborido-Rey, 2012; Villegas-Ríos et al., 2014). Specifically, females are recognized as allocating more energy to gonad development than males (Saborido–Rey et al., 2004). In addition, fish reproductive migration is a highly energy-consuming activity. In the course of reproductive migration, fish are likely to face various risks, for example, less effective avoidance of predation and energy shortages. Therefore, fish regulate the types and levels of energy stored in various organs to meet the energy requirements of reproductive migration (Caudill et al., 2007). The previous research has indicated that body size, osmotic pressure regulation, swimming speed, and sexual maturity contribute to fish migratory preparation (McCormick et al., 1998; Saborido–Rey et al., 2004). Usually, the migrating fish with large body size and high fertility rates have a clear “profit” as concerning the consumption of energy during reproduction migration (Wysujack et al., 2010; Barneche et al., 2018). Moreover, it has been hypothesized that storing sufficient energy may be necessary for migratory fish reproduction migratory (Jonsson and Jonsson, 2003; Bureau et al., 2007).

Barbour (1985) proposed the hypothesis that fish need to reserve enough energy for migration during the migratory preparation period. Certain relevant research points supported this hypothesis (Thorpe et al., 1998). Nevertheless, the preparation period that differs among individuals within a population is considered discrepantly (Jonsson and Jonsson, 2003). Relevant research has indicated that individuals with higher energy initiated the migration earlier (Colombier et al., 2007). The contribution of energy molecules stored in different fish tissues is unevenly distributed during reproductive migration (Kiessling et al., 2004), although information related to the fish migratory preparation process is lacking. Energy density is identified as one of the most important indexes used to assess the stored energy capacity of fish, because it depends on the contents carrying molecules such as proteins, fat, and carbohydrates (Mourente et al., 2002; Penney and Moffitt, 2014). In the recent years, energetics related to fish migration has become a focus in fish ecology (Penney and Moffitt, 2014; Thomas and Johan, 2018; Lennox et al., 2019; Tamario et al., 2019). However, to the best of our knowledge, a very few studies have been conducted on the mechanism of energy reallocation in fish tissues in relation to digestion, growth, reproduction, and other major functions during the maturing process. Thus, the study of the biochemical composition of wild fish populations is an important approach for understanding the variation in energy allocation among life-history processes (Connell, 1975).

The black Amur bream (*Megalobrama terminalis*) is a migratory species inhabiting the lower reaches of the Pearl River. The species is regarded as a good candidate for studying the energy allocation of migratory fish (Chen et al., 2020). During spawning seasons, *M. terminalis* migrate nearly 250 km upstream from a drainage network to the spawning grounds (Luopangjiang and Qingpeitang). Owing to the continuous enhancement of human activities (e.g., water conservancy projects, waterway dredging, water pollution, and overfishing), serious decreases in wild populations of *M. terminalis* have been reported in the Pearl River basin during this decade (Li et al., 2014, 2018). In our previous research, it was demonstrated that the *M. terminalis* spawning migration occurs from late June to mid-July, a pattern that is different from historical records (Liu et al., 2021b). In the recent studies, *M. terminalis* was deemed to be an omnivorous fish with strong ecological adaptability (Liu et al., 2020, Liu et al., 2021b). The amount of energy allocated between growth and reproduction is deemed to be the critical factors for the survival and reproduction of *M. terminalis* in natural ecosystems. Related research suggests that there is a shift in the diet of black Amur bream during the gonad development period, with different food preferences of both immature and mature individuals (Xia et al., 2017, 2020). Further research has provided evidence that *M. terminalis* regulates activities of the gut microbiome and degradation enzymes to digest foods with higher nutrition to supply energy for the spawning migration (Liu et al., 2021a). However, the overall effect on variation in energy allocating during *M. terminalis* reproduction migratory has been difficult to evaluate.

To date, most previous research on *M. terminalis* has been related to larval resources, feeding habits, ecological investigation of spawning grounds, and digestive function (Tan et al., 2009; Wang et al., 2010; Xia et al., 2017; Liu et al., 2020), whereas a very few studies have reported the pattern of energy accumulation and reproductive investment for *M. terminalis* in the course of the reproduction cycle. To fill this knowledge gap, we attempted to investigate the utilization efficiency of energy storage, energetic trade-offs between somatic and reproductive growth and differences in energy accumulation, and allocation for gonadal maturation and spawning migration of *M. terminalis* females. A quantitative assessment method that involves biochemical composition and energy density was utilized to analyze somatic and visceral tissues of *M. terminalis* females in the spawning ground, the fattening ground, and migration routes during the reproductive cycle. The aim was to characterize the process of energy accumulation and allocation during reproductive cycle of *M. terminalis*. In parallel, through analysis of fine-scale variation in proximate composition and energy content in the course of gonad development, the study aimed to provide data supporting further exploration of fitness in wild population of *M. terminalis*. Additionally, this study also collects data relevant to energetic ecology of fish migration.

MATERIALS AND METHODS

Ethics Approval

The methods that involve animals in this study were conducted in accordance with the Laboratory Animal Management Principles of China. All experimental protocols were approved by the Ethics Committee of the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

Fish Sampling

A total of 350 female specimens of black Amur bream from five localities were collected using circular cast nets (16 m diameter, mesh size 3 cm) in the Pearl River during 2019. The sampling variables that include date of collection, location, and environment information are provided in **Table 1**. According to the corresponding sampling sites and sampling times, sampling in sites S1–S2 was conducted from July 9 to 21. Sampling in sites S3–S5 was conducted from June 18 to July 8 to ensure the distinction between migratory and non-migratory *M. terminalis* (**Table 1**). The sampling sites are shown in **Figure 1**. For each sample, total body weight in g (W_t, measured to the nearest 1 g) and standard length (SL, to the nearest 1 mm), eviscerated weight (EW, to the nearest 1 g), and gonad and liver weights (GW and LW, respectively, to the nearest 0.01 g) were measured. The sex and stage of sexual maturity were identified for all individuals based on the morphological characteristics, as described by Nikolsky (1963): I = immature, II = developing, III = maturing, IV = mature, V = spawning and post-spawning. The gonadosomatic index (GSI = 100 × GW/EW) was estimated as an indicator of the fish reproductive period. The hepatosomatic index (HSI = 100 × LW/EW) and fatness ($K = 100 \times W_{t/}SL^3$) were measured as bioenergetic indices to evaluate fish conditions.

Histological Observations

Fish selected in different reproductive periods were anesthetized using MS-222 ($0.2\ g\ L^{-1}$ MS-222 + $0.2\ g\ L^{-1}$ $NaHCO_3$) and then stunned and quickly decapitated. For histological analysis, the left ovaries of fish were dissected out and fixed with Bouin's fixative for 24 h at°C and embedded in paraffin wax, and then, the tissues were cut to 5-mm thicknesses sections and stained with hematoxylin–eosin (H&E).

Biochemical Assays

To investigate the fish biochemical composition and energy density, 120 fish tissue samples were randomly selected for analysis. Each specimen was dissected, and organs and muscles (without skin) were removed from a location posterior to the insertion of the dorsal fin. The liver and ovaries were taken from each fish stored in plastic bags on ice and frozen (−80°C) until processing. For biochemical analysis, each tissue of each sampled fish was homogenized and freeze-dried for 24 h at −80°C to a constant weight. The resulting dry tissue was cooled and weighed, and the moisture percentage was calculated as (100–%dry tissue). Determination of crude protein content was measured by the Kjeldahl method (Hach et al., 1985); determination of crude lipid content was measured by the chloroform–methanol extraction method (Folch et al., 1957); ash content was determined by the method of Penney and Moffitt (2014). The energy value was measured by a Phillipson Microbomb Calorimeter (Gentry Instruments Inc., Aiken, SC, United States). All biochemical analyses were performed in triplicate.

Statistical Analysis

The data were analyzed using STATISTICA 6.0 (StatSoft, Inc., Tulsa, OK, United States). The normality of the data and homogeneity of variance were assessed with the Kolmogorov–Smirnov test and Levene's test, respectively. The GSI, HSI, fatness, composition, and energy density were analyzed by one-way ANOVA to evaluate the differences among groups. All data were expressed as means ± SD, and Tukey's *post-hoc* test was used as necessary. A p-value below 0.05 was used to determine statistical significance. Principal component analysis (PCA) was used to obtain principal coordinates and their visualization from the complex biochemical composition of the ovary. To better understand the relationship between biochemical content of the fish ovary and the habitat environment and biological indices associated with different groups, a redundancy analysis (RDA) was conducted. Here, we used the R implementation of the procedure (version 1.1.3).

RESULTS

Changes During Reproductive Migration

The biochemical composition changes in ovary of *M. terminalis* during the reproductive migration period are shown in **Figure 1**. The proportion of sexually mature (stages IV and V) individuals increased significantly in the main stem (S3, S4, and S5), whereas immature ovaries were dominant in the estuary (S1 and S2). The ovary of *M. terminalis* gradually matures during reproductive migration. Lipid constituents of the ovary of the black Amur bream rose from the ovary from S1 to S5, whereas water content showed an opposite pattern of variability (**Supplementary Figure 1**). To compare the similarity of proximate composition among different sample sites, PCA ordination revealed a clear separation of the proximate composition of the ovary between estuary and main stem populations. The proximate composition of ovary between the S1 and S2 groups on PCA scores showed greater similarity, separated from the proximate composition of the ovary in the S4 and S5 groups that formed a cluster (**Figure 2A**). Samples in the S3 group were located between non-migrating (S1, S2) and migrating populations (S4, S5)

TABLE 1 | Basic environmental information and biological information of gut microbial community pertaining for the different groups studied of *Megalobrama terminalis*.

		Estuary		**Main stem**		
		S1	**S2**	**S3**	**S4**	**S5**
Environmental information	Sample period	July 15 to 21	July 9 to 15	July 2 to 8	June 25 to July 1	June 18 to 24
	Temperature (°C)	28.7 ± 0.2	28.4 ± 0.4	28.8 ± 0.4	28.4 ± 0.3	28.7 ± 0.3
	Salinity (‰)	0.09 ± 0.03^{b}	0.05 ± 0.02^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
	pH	8.0 ± 0.1	7.9 ± 0.2	8.1 ± 0.2	8.2 ± 0.2	7.9 ± 0.2
	DO (mg/L)	6.7 ± 0.1^{a}	6.8 ± 0.2^{ab}	7.2 ± 0.1^{b}	7.7 ± 0.2^{b}	7.5 ± 0.2^{ab}
Biological information	n	50	50	50	50	50
	SL ± SD	182 ± 15.7^{a}	235 ± 21.2^{ab}	247 ± 16.4^{b}	271 ± 20.4^{b}	262 ± 16.1^{b}
	W_t ± SD	101.8 ± 19.1^{a}	259 ± 25.1^{b}	363 ± 22.5^{c}	403 ± 32.3^{c}	372 ± 23.1^{c}
	GSI (%)	0.7 ± 0.1^{a}	4.1 ± 1.3^{b}	6.9 ± 1.4^{b}	9.2 ± 2.3^{b}	6.6 ± 0.9^{b}
	HSI (%)	1.5 ± 0.12^{a}	2.5 ± 0.13^{c}	2.2 ± 0.12^{bc}	1.2 ± 0.10^{a}	1.0 ± 0.10^{a}
	K	1.8 ± 0.13^{a}	2.1 ± 0.20^{ab}	2.3 ± 0.22^{b}	2.4 ± 0.21^{b}	2.2 ± 0.14^{b}

Different superscript letters indicate significant differences in different groups, $p < 0.05$.
DO, dissolved oxygen; GSI, gonadosomatic index; HSI, hepatosomatic index; K, fatness; SL, standard length; W_t, body weight.

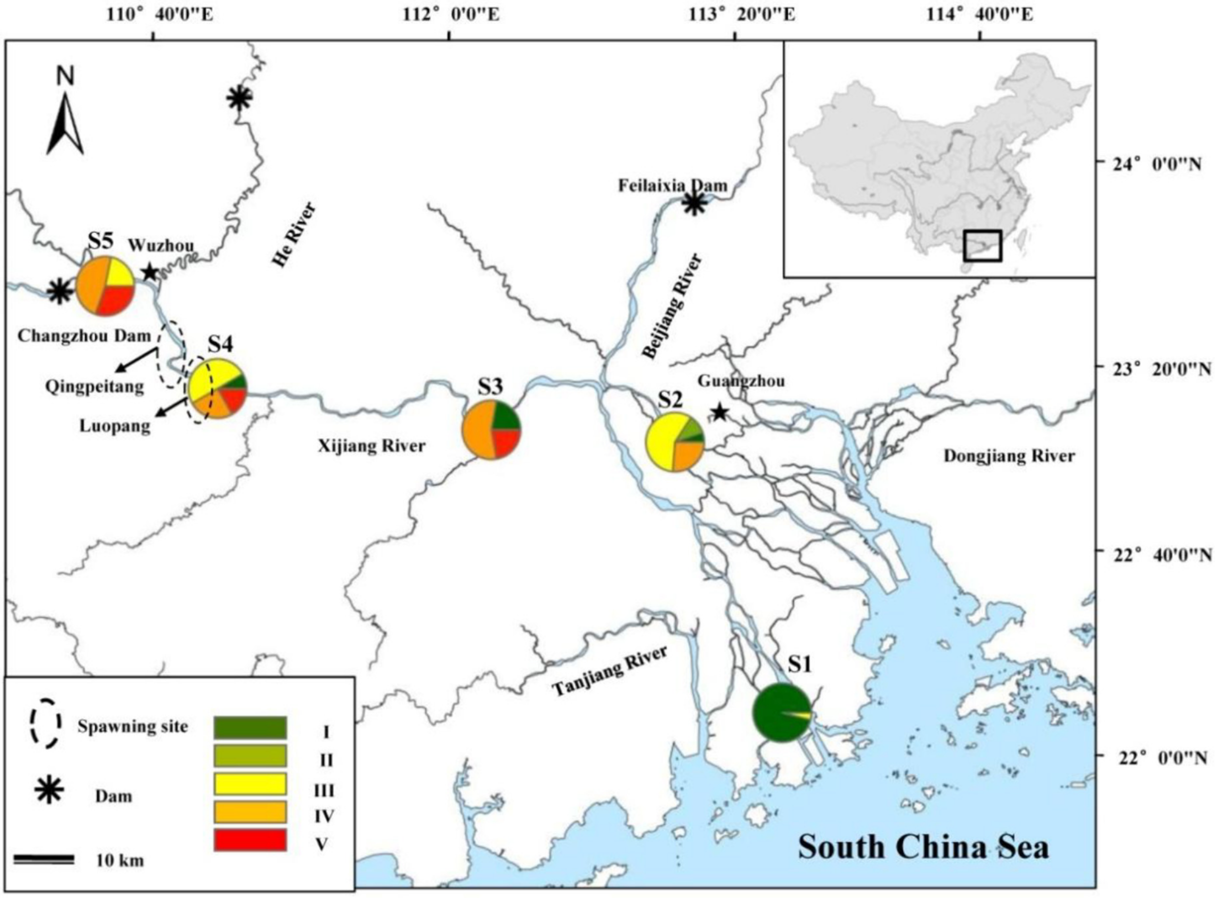

FIGURE 1 | Sampling map showing the sample locations of *Megalobrama terminalis*. Rate of each ovary development stage of *M. terminalis* in sample sites was shown during spawning migration period. Black circles were shown as spawning ground of *M. terminalis* in Pearl River.

(**Figure 2A**). The proximate composition of ovary in each group was closely related to their habitat and biological index (**Figure 2B**). The proximate composition of S4 and S5 groups was more closely related to dissolved oxygen, fatness, and GSI. In contrast, the proximate composition of S1 and S2 was correlated with salinity and HSI.

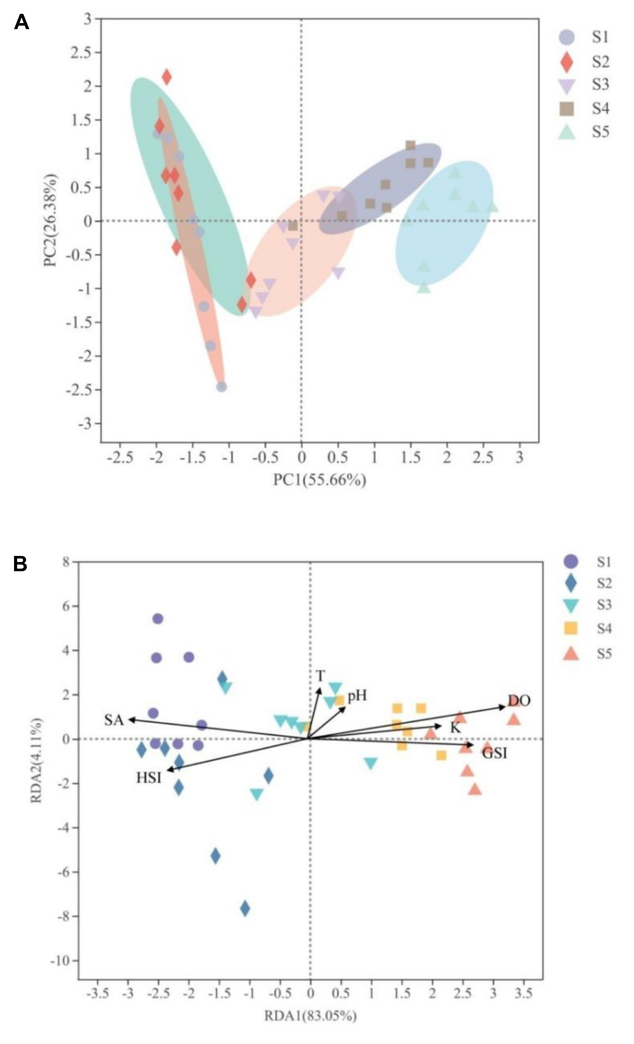

FIGURE 2 | Comparison of the main biochemical compositions in the different groups studied of *Megalobrama terminalis*. **(A)** Principal coordinate analysis (PCA) of main biochemical compositions in *M. terminalis* ovary from different groups. The individual samples are color-coordinated according to the different locations. **(B)** Redundancy analysis (RDA) showed the correlation between the biochemical compositions of the different groups studied of *M. terminalis* and their biological indices and habitat environment factors.

Morphometry and Histology

The distribution of the morphometrical parameters across maturity stages is shown in **Figure 3**. It is clear that there were increases in length and weight from the immature stage to the mature stage (**Figures 3A,B**). Although this increase was gradual throughout the maturation for the somatic measurements, the GSI showed a sudden peak at stage IV (**Figure 3C**). However, HSI showed a peak at stage II and then gradually decreased (**Figure 3D**). Histological observations of the *M. terminalis* ovary during different developmental stages are shown in **Figure 4**. Oocytes varied markedly with ovary development, and the description of histological changes in *M. terminalis* is given shown in **Table 2**.

Proximate Composition: Variability in Different Tissues

Biochemical changes across different maturity stages in each tissue of female *M. terminalis* are presented in **Figure 5**. During sexual maturation, water contents in the ovary and liver decreased from stages I to III and then increased from stages III to V (**Figures 5A,C**). In the contrast, water content in muscle had no obvious changes from stages I to V (**Figure 5B**). For total ash, a slight downward trend was observed in ovary tissues from stages I and II (**Figure 5D**), whereas muscle and liver tissues remained practically unaffected (**Figures 5E,F**). Concerning protein content, this constituent increased in the ovary from stages I to III and dropped significantly in stage IV, which shows an opposite pattern compared to water content in ovary tissue (**Figure 5G**). In the muscle tissue, total protein content declined from stages III to V, whereas less variation was observed in the liver tissue during maturation (**Figures 5H,I**). Lipid content in the ovary and liver gradually went up from stages I to IV and then dropped off significantly in spawning and post-spawning (stage V) (**Figures 5J,L**). Similarly, lipid content in the muscle tissues increased and subsequently decreased during maturation with a maximum value in stage III (**Figure 5K**).

Energy Accumulation and Allocation

The weights of trunk, ovary, and liver were positively correlated with body mass from stages III to IV (**Figures 6A–C** and **Supplementary Table 1**). The rate of increase of ovary mass rose sharply from stages III to IV, whereas the rate of increase of trunk mass declined. The proportions of protein and lipid in muscle were positively correlated ($R^2 = 0.152$; $p < 0.01$) (**Figure 6D**), and the proportions of proteins and lipids were negatively related in the ovary and liver tissues from stages III to IV ($R_{ovary}{}^2 = 0.312$; $P_{ovary} < 0.01$; $R^2{}_{liver} = 0.284$; $p_{liver} < 0.01$) (**Figures 6E,F**). The variation in energy density across maturity stages for each tissue is shown in **Figures 7A–C**. In the muscle, no significant change in energy density was perceived from stages I to V (**Figure 7A**). In contrast, there was a clear pattern related to reproduction, since maxima were observed in ovary and liver at female stage IV (**Figures 7B,C**), and there was a sharp drop reaching a minimum of energy density at stage V. Some statistically significant negative relationships were observed between ovary and muscle energy density from stages III to IV ($R^2 = 0.215$; $p < 0.01$) (**Figure 7D**). The muscle energy density decreased rapidly, whereas the ovary increased slowly in stage III. In contrast, energy density of muscle dropped slightly and ovarian energy density rose dramatically in stage IV. Ovary and liver energy density showed a positive relationship ($R^2 = 0.246$; $p < 0.01$) (**Figure 7E**). By contrast, muscle and liver energy density had a negative relationship from stages III to IV ($R^2 = 0.193$; $p < 0.05$) (**Figure 7F**).

DISCUSSION

In this study, we found that the ovary of migrating black Amur bream gradually matured during movement from the estuary (fattening ground) to the main stem (spawning ground) during the breeding season (**Figure 1**). Maturity stages III to IV of the

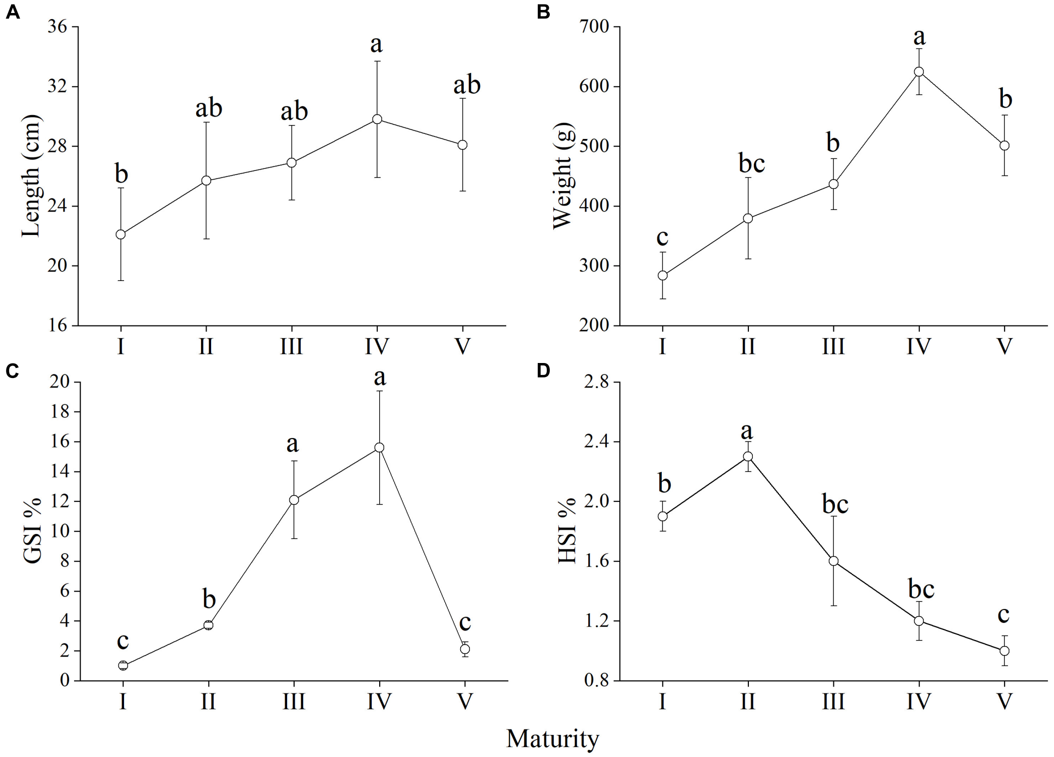

FIGURE 3 | Line chart showing the distribution of **(A)** body length, **(B)** body length data across *Megalobrama terminalis* maturity stages. Shown are also the patterns in **(C)** gonadosomatic index (GSI), and **(D)** hepatosomatic index (HSI) of *M. terminalis*. Different superscript letters indicate significant differences of component in different maturity stage, $p < 0.05$.

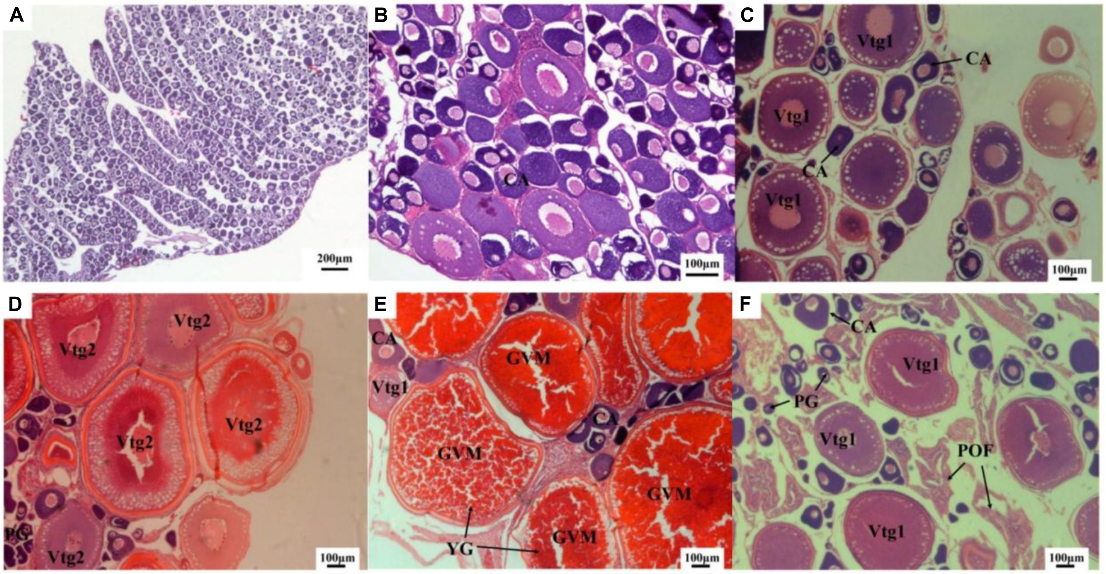

FIGURE 4 | Transverse sections of ovary of the black Amur bream **(A,B)** immature female ovary; **(C)** developing female ovary; **(D)** maturing female ovary; **(E)** mature female ovary; **(F)** spawning and post-spawning female ovary; CA, cortical alveolar oocyte; GVM, germinal vesicle migration; PG, primary growth oocyte; POF, post-ovulatory follicle; Vtg1, primary vitellogenic oocyte; Vtg2, secondary vitellogenic oocyte.

TABLE 2 | Histological descriptions of the stages in the reproductive cycle of female *Megalobrama terminalis*.

Stage	Description
I (Immature)	Small ovaries, often clear, blood vessels indistinct. Only oogonia and PG oocytes present.
II (Developing)	Enlarging ovaries, blood vessels becoming more distinct. PG, CA, and Vtg1 oocytes present.
III (Maturing)	The number of nucleolus increased and irregular deformation occurred close to the inner edge of the nuclear membrane. PG, CA, Vtg1, and Vtg2 oocytes present.
IV (Mature)	Large ovaries, blood vessels prominent. Individual oocytes visible macroscopically. Oocytes undergoing late GVM.
V (Spawning/post-spawning)	Flaccid ovaries, blood vessels prominent. Atresia (any stage) and POFs present. Some CA and/or vitellogenic (Vtg1, Vtg2) oocytes present.

black Amur bream were observed as a vital migration-launching period. Meanwhile, significant differences in environmental factors and biological indices were observed between estuary and main stem populations, especially in salinity, dissolved oxygen, GSI, HSI, and fatness (**Table 1**). A clear separation of the proximate composition of the ovary was detected between estuary and main stem populations (**Figure 2A**). Secor (1999) indicated that not all individuals of migratory populations participated in the breeding migration. Non-migrating and migrating individuals demonstrated significant differences in maturity and energy reserves of fish reproductive organs. It is widely recognized that lipids and proteins are high energy substances for fish storage (Zaboukas et al., 2006; Sieiro et al., 2020). The recent study has illustrated that migratory individuals are evolving toward larger body size and higher fertility than non-migratory individuals (Burns and Bloom, 2020). In this study, we found that migrating population in the main stem had larger body size and higher GSI than that of non-migrating population (**Table 1**). The proximate composition of ovary in the groups S4 and S5 had a close relationship with biological indices, especially in fatness and GSI (**Figure 2B**). Lipid content in the ovary of the black Amur bream gradually increased from the estuary to the main stem, which indicates that migrating

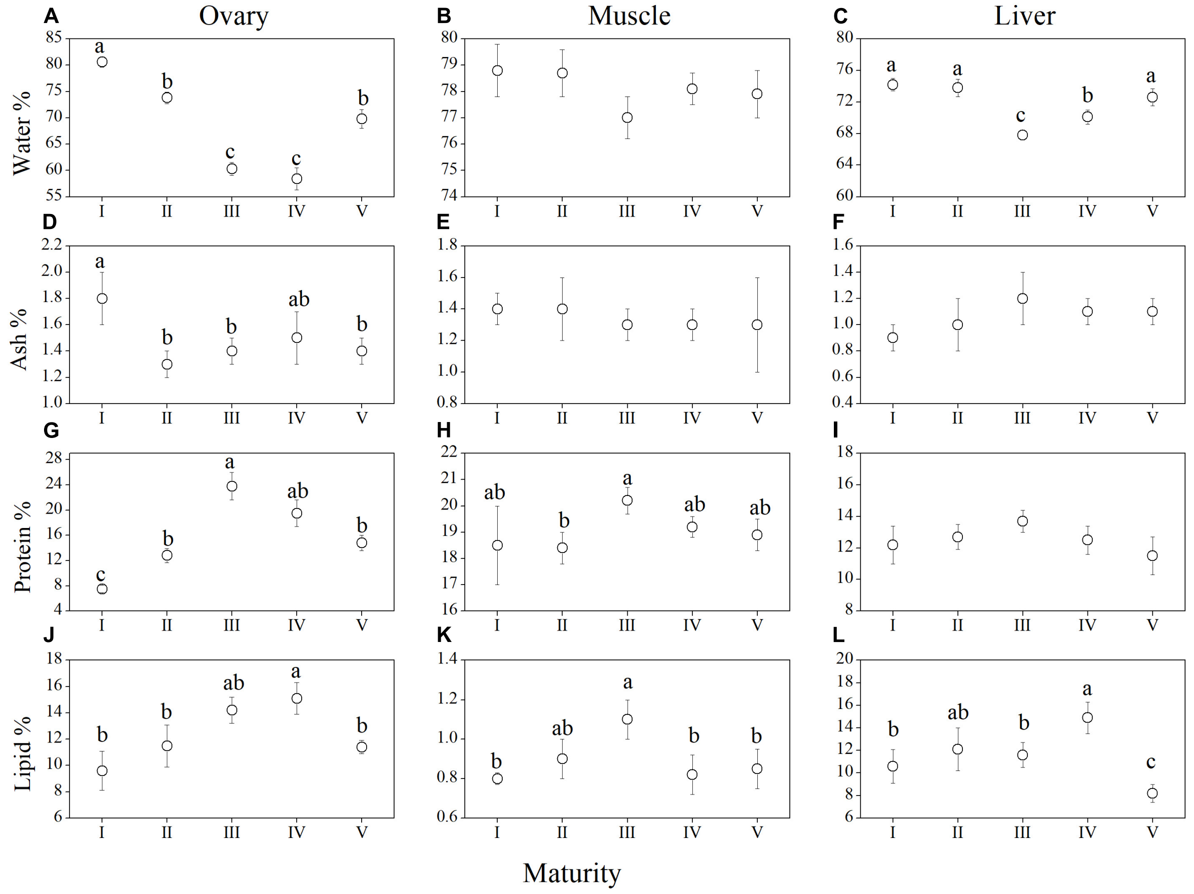

FIGURE 5 | Four main biochemical constituent proportion of different tissues in female *Megalobrama terminalis* from maturity stages I to V. Water content in the ovary **(A)**, in the muscle **(B)**, and in the liver **(C)**; ash content in the ovary **(D)**, in the muscle **(E)**, and in the liver **(F)**; protein content in the ovary **(G)**, in the muscle **(H)**, and in the liver **(I)**; lipid content in the ovary **(J)**, in the muscle **(K)**, and in the liver **(L)**. Different superscript letters indicate significant differences of component in different maturity stage, $p < 0.05$.

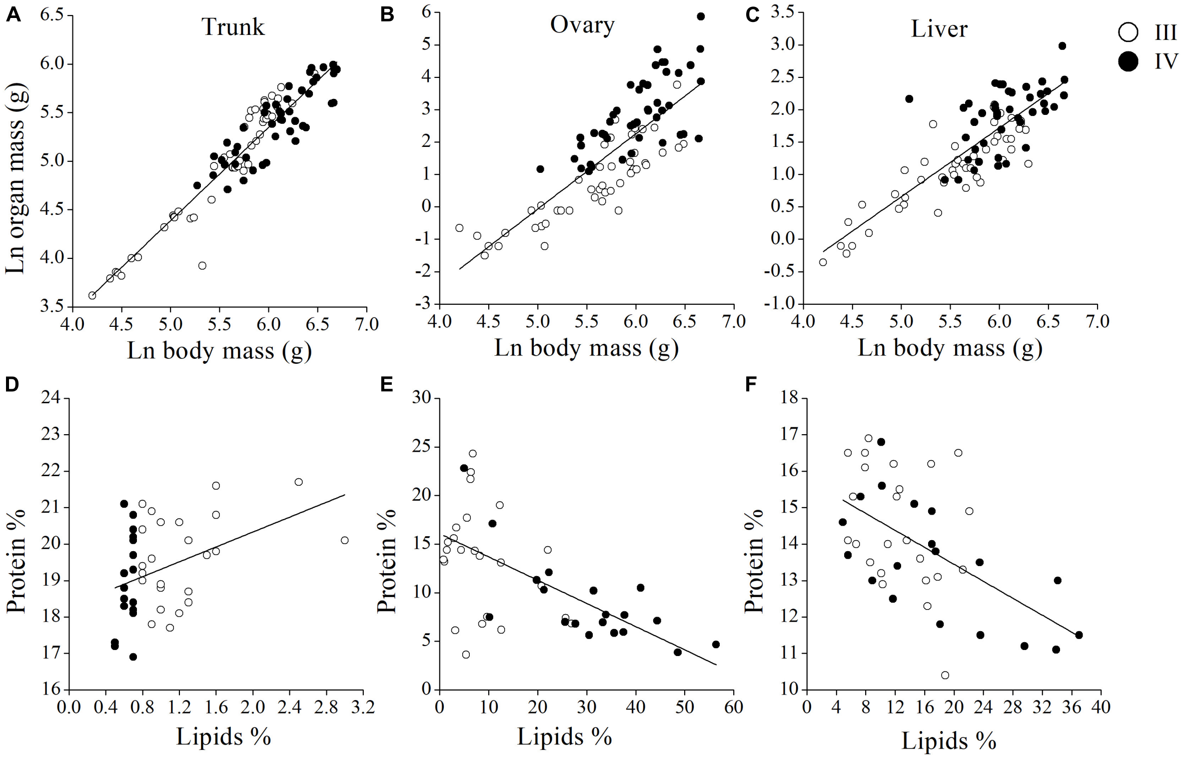

FIGURE 6 | Scatter plots indicating the relationships between the organ and body mass for maturity stage III and IV of *Megalobrama terminalis* female in the trunk **(A)**, in the ovary **(B)**, and in the liver **(C)**. Scatter plots showing the relationships between the proportion of total lipids and proteins for *M. terminalis* female in maturity stage III and IV in the muscle **(D)**, in the ovary **(E)**, and in the liver **(F)**. Lines show regression fits with the exceptions.

population stored more energy for ovary development. The high lipid content of tissues also signified the need for high energy biomolecules to perform the energy-consuming locomotion (Zaboukas et al., 2006). Relevant studies have pointed out that fish utilize lipids to regulate their energy expenditure for gonad development (Takama et al., 1985). Thus, the remarkable change in lipid content of the ovary might be considered as a crucial physiological index, which reflects the readiness for breeding migration of *M. terminalis*.

In order to make an effective assessment of *M. terminalis* ovary development, it is imperative to describe the process of energy accumulation and oocyte recruitment during ovary development. According to our study, it appears that *M. terminalis* follows a similar pattern of oocyte developmental stages as in most one-time spawning type fish species (Murua and Saborido–Rey, 2003; Costa et al., 2015). The variation tendon of the proximate composition in muscle and ovary tissues was due to increases in the protein content from stages I to III (**Figures 5G,H**). The protein content is essential to maintaining major body organic structure and function of migrating fish during reproductive migration (Lin et al., 2017; Serrat et al., 2019). In addition, somatic tissue is regarded as primarily location of fish energy reserves, and this is commonly determined by the protein content throughout reproduction (Lin et al., 2019). Lipid content in the ovary gradually increased during the migration-launching period, whereas that in the muscle slightly declined. This indicated that energetic investment of *M. terminalis* concentrated on reproduction, whereas there was a more intense reduction in the lipid contents of the somatic tissues (Zaboukas et al., 2006). Furthermore, decreases in the lipid contents of fish somatic tissues are mainly devoted to supplying energy for reproductive migration (Doucett et al., 1999; Kiessling et al., 2004). During the migration-launching period, the accumulation of lipids in the ovary proceeds until the onset of vitellogenesis; this is interpreted as initiation of the synthesis of yolk-forming molecules. The production of abundant eggs during the relatively short period of reproduction is very energy intensive (Tocher, 2003). In the course of this period, the proportions of protein and lipid in the ovary showed a negative correlation, which suggests that lipids were more important than proteins in the *M. terminalis* ovary during advanced maturity stages. Studies present have shown that lipids are important in the ovaries of marine fish during maturity stages (Rosa et al., 2002; Serrat et al., 2019). Related research reveals that lipids predominate in the liver and ovary in the common octopus (Sieiro et al., 2006). Despite lipid content of somatic and reproductive tissue being of vital importance, the lipid content in tissues of *M. terminalis* rose to a certain level and remained stable instead of increasing blindly during the migratory reproductive preparation period (stage III). Relevant research has indicated that excessive lipids limited fish swimming speed during migration (Slotte et al., 2000). Consequently, lipids may have diverse effects in different tissues of the black Amur bream, and there may be a regulatory mechanism for allocating the lipids to each tissue to

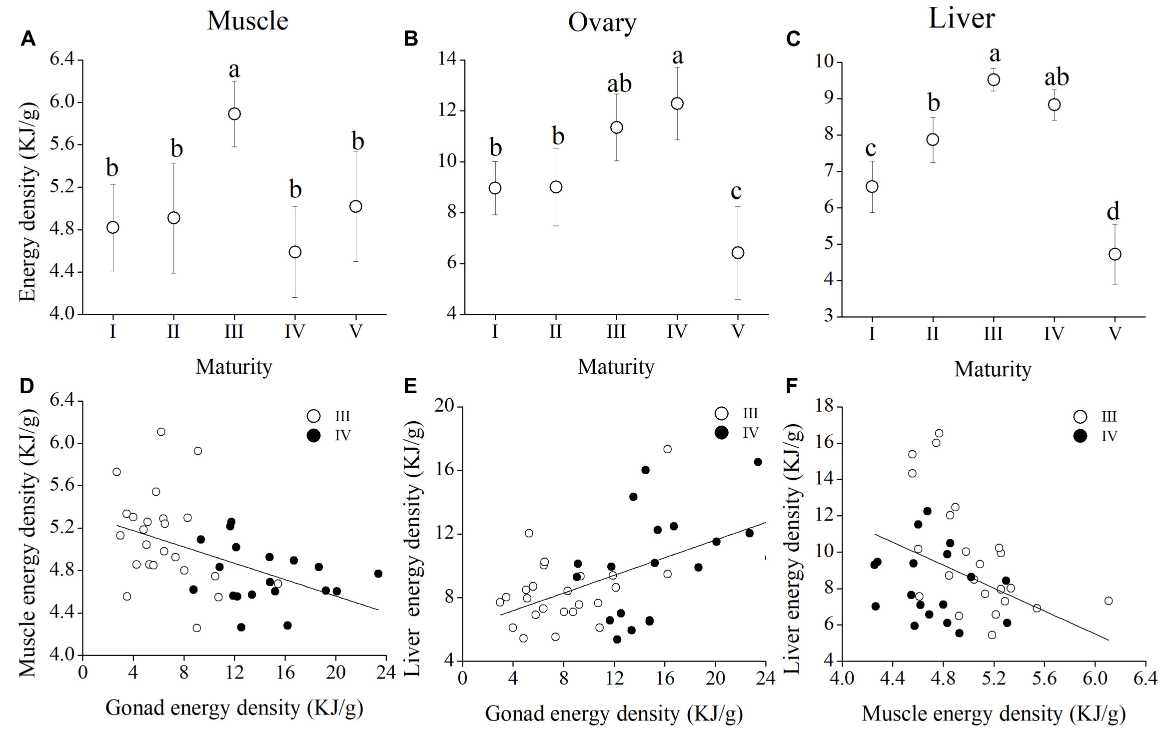

FIGURE 7 | Changes in energy density across *Megalobrama terminalis* female maturity stages in the muscle **(A)**, ovary **(B)**, and liver **(C)**. Scatter plots indicating the relationships of energy density in specific tissues [muscle **(D)**, ovary **(E)**, and liver **(F)** of *M. terminalis* female in maturity stages III and IV. Different superscript letters indicate significant differences of energy density in different maturity stages, $p < 0.05$.

reduce the risk of migration failure in the breeding migratory preparation period.

This study demonstrated that ovary mass gain is accelerated, while somatic mass grows more slowly during the vital migration-launching period. Relevant studies have indicated that the relationship between organ and body size can be applied to explain the allometric variation at the metabolic level (Oikawa and Itazawa, 1992). Some studies have manifested that the somatic tissues accumulated energy continuously until the physiologically maturing stage, whereas the reproductive organs had a drastic energy accumulation throughout the sexual maturation period (Lahti et al., 2001, 2002). The energy density in *M. terminalis* somatic tissues increased initially after declining owing to fluctuating contents of total protein and lipid during the maturation process. More specifically, the rise in the energy density of somatic tissues was due to the body growth of *M. terminalis* from stages I to III. Rapid body growth in fish results from intensive protein synthesis and low protein degradation (Houlihan et al., 1990; Sieiro et al., 2020). Owing to the high level of protein in the somatic tissues, the energy metabolism is mainly protein-based (Navarro et al., 2014). However, a negative energy relationship between somatic and reproductive tissues was observed in *M. terminalis* during the vital migration-launching period. The downtrend of energy density in somatic tissues after stage III was related to the decline of lipid content. The previous research has suggested that somatic tissues of migrating fish are applied to provide energy for the migration process (Kiessling et al., 2004). Moreover, lipids are crucial energy substances with high energy efficiency in fish somatic tissues (Hinch et al., 2002). Higher variability of energy density in the ovary and liver was observed during a vital migration-launching phase due to enhanced lipid contents. This ascent of energy density in ovary associated with yolk accumulation in stage IV has been found in some fish species (Fernández et al., 2009; Wu et al., 2017). Specifically, rapid synthesis of yolk materials in the developing oocytes during the migration-launching phase is conducive to the energy substances being rapidly synthesized in the later physiological maturity stages (Alonso-Fernández and Saborido-Rey, 2012; Lin et al., 2017). Not all individuals in migratory fish populations participate in the migration (Secor, 1999). Non-migrating and migrating individuals presented obvious discrepancies in body size and lipid storage status. Our previous results suggested that lipid metabolism in migrating population was higher than that in non-migrating population, which provides a line of evidence for that *M. terminalis* consuming lipids to supply energy for spawning migration (Liu et al., 2021a). Similarly, it has been proposed that Atlantic salmon maturation was linked with growth rate and lipid metabolism (Herbinger and Friars, 1991). Barneche et al. (2018) revealed that larger females disproportionately reproduce more than smaller females whether measured by fecundity or by total reproductive energy. Larger females had a higher metabolic ability factor that is significant for fish survival, energy consumption, and migration (Priede, 1985; Hinch and Rand, 2000). The higher metabolic ability requires a more complex organizational structure, which inevitably leads to higher energy consumption and lower energy efficiency (Weiner, 1993; Chappell et al., 1999; Hinch and Rand, 2000). In fact, energy accumulation is closely linked to the co-evolved life-history traits favored for optimal energetic allocation (Lin et al., 2017). Therefore, there may be a trade-off between maximum metabolic ability and energy efficiency before migration launching in the black Amur bream.

CONCLUSION

Above all, stages III to IV of the black Amur bream were observed as a crucial migration-launching period. The asynchrony of development was confirmed in energetic relationships in somatic and ovary tissues. In addition, the negative energy relationship between somatic and reproductive tissues clarified a trade-off between maximum metabolic ability and energy efficiency before the migration launching of *M. terminalis*. The lipid content displayed diverse effects in different tissues of *M. terminalis*, and there was a regulatory mechanism for allocating the lipid content of each tissue reasonably to reduce the risk of migration failure during the breeding migratory preparation period. The results demonstrated that there is a dependency between somatic and reproductive tissue growth in *M. terminalis* females during migratory preparation that serves to improve fitness. Finally, these findings illustrate that the high energy metabolism demands of reproduction of *M. terminalis* are regarded as a key factor driving the variation in energy accumulation and allocation. The findings facilitate further research on ecological adaptations of migrating fish during the reproductive cycle.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The methods involving animals in this study were conducted in accordance with the Laboratory Animal Management Principles of China. All experimental protocols were approved by the Ethics Committee of the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

YaL: conceptualization, data curation, and writing–original draft. XL: funding acquisition. JL: funding acquisition and supervision. YuL: formal analysis, writing, reviewing, and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Microeukaryotic Community Shifting Along a Lentic-Lotic Continuum

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As an important regulator of ecosystem functions in river systems, microeukaryotes play an important role in energy and material conversion, yet little is known about the shift along a lentic-lotic continuum. In this study, the 18S rRNA genes sequencing was used to identify the microeukaryotic communities at 82 sites along a lentic-lotic continuum with the aim of understanding the impact of upstream inlet river on microeukaryotic communities in Baiyang Lake (BYD) and its downstream. Our results showed that the upstream inlet river affected the diversity and community composition of microeukaryotes in BYD and downstream rivers, and environmental variables greatly affected the composition of microeukaryotic community. The community composition in BYD had lower variabilities. Co-occurrence network analysis revealed that the network was non-random and clearly parsed into three modules, and different modules were relatively more abundant to a particular area. As keystone taxa, some nodes of the upstream microeukaryotic network played an important role in structuring network and maintaining the stability of the ecosystem. In BYD and downstream, the microeukaryotic network was highly fragmented, and the loss of keystone taxa would have an adverse impact on the integrity and function of the microeukaryotic community. Microeukaryotes had strong tendencies to co-occur, which may contribute to the stability and resilience of microeukaryotic communities. Overall, these findings extend the current understanding of the diversity and community composition of microeukaryotic along a lentic-lotic continuum.

Keywords: high-throughput sequencing, community composition, microeukaryotes, co-occurrence network, phytoplankton

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INTRODUCTION

As an important regulator of ecosystem functions in river systems, microeukaryotes play an important role in energy and material conversion, and are used as biological indicators for water quality assessment (Zancarini et al., 2017; Cai et al., 2020). Eukaryotic plankton can quickly respond to the changes of water environment, because of its short life cycle and sensitive to the physicochemical changes of water body. The abundance, biomass, species diversity, and community composition of phytoplankton are severely affected by changes in water quality (Jeppesen et al., 2005; Xu et al., 2010; Wu et al., 2021). Several studies have shown that the structure and function of phytoplankton communities are directly affected by changes in water quality (Buchanan, 2020; Amorim and Moura, 2021). A recent study showed that when there is a significant environmental gradient in a polluted flowing water ecosystem, the structure of the microenvironmental phytoplankton community is largely determined by the species classification caused by local pollution, and the difference in community composition increases with the increase of the Euclidean distance of environmental variables (Xu et al., 2020). Eukaryotic plankton are key components in the trophic web structure, and play a vital role in the energy flow and nutrient cycle of the aquatic ecosystems (Boenigk and Arndt, 2002; Zubkov and Tarran, 2008). The impact of external disturbances (such as environmental factors, spatial processes and grazing pressure) on the phytoplankton community has always been of interest in ecological research (Guo et al., 2019, 2020; Wijewardene et al., 2021; Wu et al., 2022). However, there are few studies on how environmental conditions shape eukaryotic plankton communities (Caron and Hutchins, 2013).

Previous studies on phytoplankton communities were mostly based on morphological recognition, which were highly dependent on the identification experience of researchers. This method limits the research on phytoplankton diversity to a certain extent. The emergence of high-throughput sequencing makes it possible to quickly and accurately obtain the composition of the microbial community in samples (Amao et al., 2019). The high-throughput sequencing of environmental samples has the potential to detect rare, small and fragile species, and can be used to monitor and quantify biodiversity (Gao et al., 2020). Recently, the use of diatom to detect water quality and environmental changes in the freshwater system of the Han River in South Korea has been reported. Researchers have used 18S rRNA to analyze the molecular profiling of seasonal diatoms (Muhammad et al., 2021). Through high-throughput sequencing to explore the effects of nutrient and stoichiometry gradients on the microbial communities of Erhai Lake and its input rivers, the results showed that nutrients (phosphorus) were key drivers for the community structure dominated by Cyanobacteria (Liu et al., 2019). Overall, these studies provide a better understanding of the diversity and composition of microeukaryotic communities.

A lentic-lotic continuum links hydrology and ecology, and understanding the community shifts along a lentic-lotic continuum is conducive to our further understanding of hydro-ecological links to freshwater systems (Jones et al., 2017; Qu et al., 2018). However, there are still few studies on the shift of phytoplankton communities in the lentic-lotic continuum system. Along the lentic-lotic continuum, the composition and dissimilarities of phytoplankton are shaped more by local hydrological and physicochemical variables than species dispersal factors (Qu et al., 2018). But the study of shifts in the microeukaryotic community along a lentic-lotic continuum is still missing. The Baiyang Lake (BYD) is a flood slowing and detention area in the middle reaches of the Daqing River system in the Haihe River Basin, which is responsible for the flood regulation and storage of nine rivers. BYD and its upstream tributaries together constitute the southern branch of the Daqing River system in the Haihe River Basin. BYD has vast waters and rich resources of aquatic animals and plants. It is an important freshwater breeding base in northern China. Understanding how the upstream inlet river affects the microeukaryotic communities in BYD and downstream of the water quality gradient is essential for predicting the response of river ecosystems to global changes and human activities.

In this study, we examined how do microeukaryotic communities shift along the water quality gradient in a lentic-lotic continuum catchment (i.e., the BYD Basin) (**Figure 1**). We had three main research questions: (i) Does upstream inlet river affect the diversity of microeukaryotes in BYD and downstream rivers? (ii) Does upstream inlet river affect the community composition of microeukaryotes in BYD and downstream rivers? (iii) How does the upstream inlet river change the microeukaryotic communities of BYD and the downstream river? The change of environmental variables will directly affect the community structure of microeukaryotes. Thus, we hypothesized that: (H1) The alpha diversity in the upstream should be higher than that in BYD and downstream rivers along the water quality gradient; (H2) The upstream microeukaryotic community network should be the most complex; and (H3) Upstream inlet river could change the microeukaryotic communities of BYD and downstream rivers by changing environmental variables. In order to answer these questions and test these hypotheses, we analyzed the microeukaryotic communities in BYD and its upstream and downstream areas using high-throughput gene sequencing. By understanding the composition and changes of microeukaryotic communities in BYD and its upstream and downstream, this study attempts to fill the gaps in microeukaryotes changes along the water quality gradient in a lentic-lotic continuum catchment, and provides insights for the BYD Basin's water environment management.

MATERIALS AND METHODS

Study Area

This research was carried out in the BYD Basin (**Figure 1**). BYD (38°44′–38°59′ N, 115°45′–116°07′ E) is the largest lake in Hebei Province. It belongs to the Haihe River Basin and is the general term for the 143 interconnected large and small lakes at the junction of Baoding and Cangzhou. BYD is located in a warm temperate semi-humid continental monsoon climate zone. The four seasons are distinct and the precipitation is mostly concentrated in summer. The annual average temperature is 12.2°C, and the annual average precipitation is 529.7 mm (Tong et al., 2021). BYD has vast waters and rich resources of aquatic animals and plants. It is an important freshwater aquaculture base in northern China.

The main river entering BYD on the west side is the Fu River, and its water quality is poor (Brauns et al., 2016), leading to poor water quality on the west side and good water quality on the east side of BYD. The Juma River, another upstream river, is mainly distributed in mountainous areas, where the pollution mainly comes from tourism, and the water quality is relatively good. The Juma River with better water environment was selected for the upstream sampling. The samples in BYD

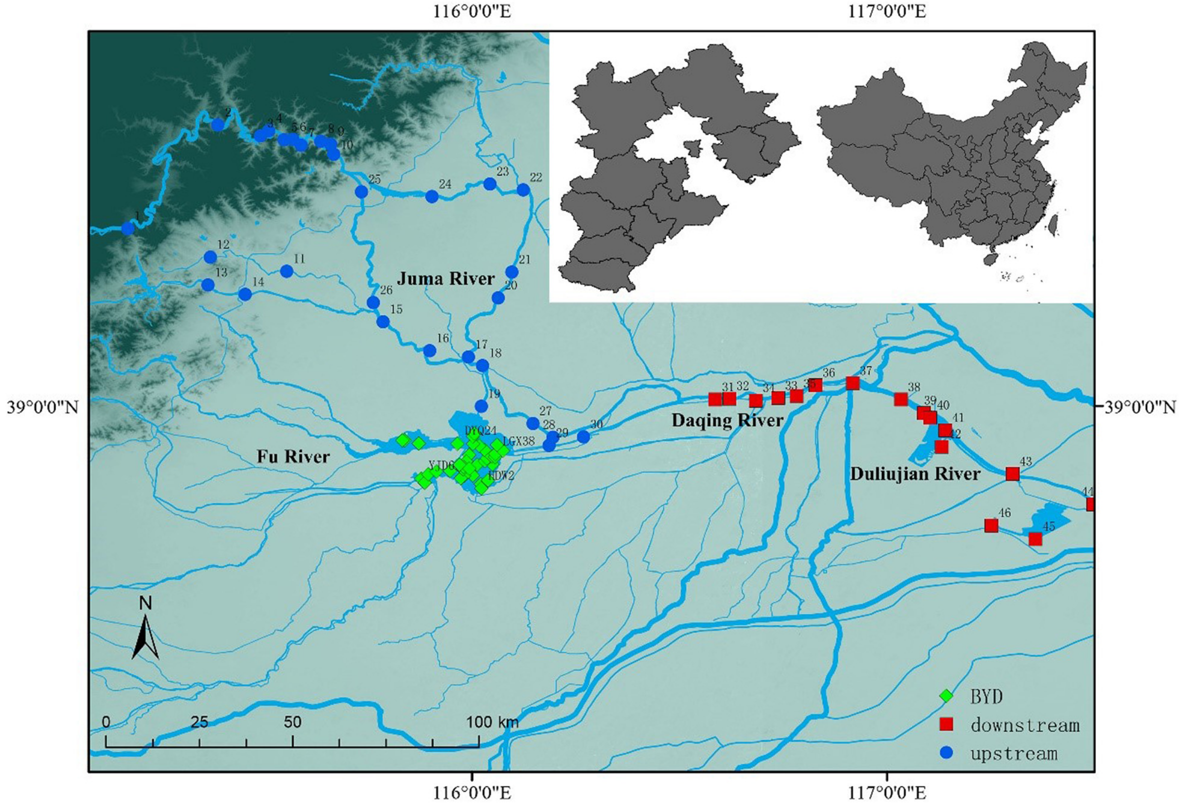

FIGURE 1 | The distributions of 82 sampling sites located in the BYD Basin, northern China. The different shapes and colors in the figure represent different freshwater areas. Blue: upstream; green: BYD; red: downstream.

were set up according to the gradient of water quality from west to east, covering the main lake of BYD. The Daqing River and Duliujian River are located in the downstream, both of which pass through urban areas. The physicochemical conditions of the river ecosystems have undergone great changes, and the water bodies have shown a certain degree of eutrophication and pollution (Chen et al., 2021).

Field Sampling and Chemical Analyses

The sample points covered the upstream, BYD, and downstream to reflect the differences in the characteristics of the overall microeukaryotes in the basin. A total of 82 sampling points were set up in the BYD Basin, including 30 samples in the upstream Juma River, 36 samples in BYD, and 16 samples in the downstream Daqing River and Duliujian River (**Figure 1**). At each sampling point, a YSI handheld meter (model 80; YSI, Yellow Springs, OH, United States) was used to measure water temperature (Temp), pH and conductivity (Cond). Latitude and longitude were measured using a Global Positioning System (GPS) unit (Triton 500, Magellan, Santa Clara, CA, United States). A total of 500 ml water, which was pre-filtered through a plankton net to remove larger multicellular organisms, was filtered with Whatman GFF filters using a vacuum pump. These filters were then wrapped in tin foil and stored at –80°C until DNA extraction and subsequent analyses. Water samples were acid-fixed and transported to the laboratory at 4°C for chemical analyses. Total nitrogen (TN) was analyzed using ion chromatography after persulfate oxidation. Total phosphorus (TP) was quantified using the ammonium molybdate method. Turbidity was measured using a portable turbidity meter HACH2100Q (HACH, Loveland, CO, United States). Chemical Oxygen Demand (COD) was determined using the rapid digestion spectrophotometry (HJ/T 399-2007).

DNA Extraction, PCR, Sequencing, and Sequence Processing

DNA was extracted using the E.Z.N.A.® Soil DNA Kit according to the manufacturer's instructions, and was quantified using QuantiFluor^{TM}-ST (Promega, Madison, WI, United States). The highly variable V4 region of the 18S rRNA were amplified using the forward primer containing sequence "GGCAAGTCTGGTGCCAG" and the reverse primer containing sequence "ACGGTATCTRATCRTCTTCG." PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. DNA libraries were validated by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States), and quantified by using QuantiFluor^{TM}-ST (Promega, Madison, WI, United States). DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument according to manufacturer's instructions (Illumina, San Diego, CA, United States). Sequencing was performed using a 2 × 300 paired-end configuration; image analysis and base calling were conducted by the MiSeq Control Software embedded in the MiSeq instrument.

Raw sequences were demultiplexed and quality-filtered using QIIME (version 1.17). The operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 18S rRNA gene sequence was analyzed by RDP Classifier against the silva database 138.

Data Analyses

Alpha diversity indexes, including Chao1, Observed OTUs, Shannon, and Phylogenetic Diversity, were calculated using QIIME (Caporaso et al., 2010). Beta diversity was calculated using Bray–Curtis distance and visualized by principal coordinate analysis (PCoA). The chord diagram was used to show the sum of relative abundances of dominant lineages (the lineages with a relative abundance >1%) of three freshwater areas. Three non-parametric statistical methods based on Adonis (Hartman et al., 2018), ANOSIM (Gao et al., 2019), and MRPP (Gao et al., 2019) were used to analyze the differences in the composition of microeukaryotic communities in the upstream, BYD and downstream using the *vegan* package (Oksanen et al., 2007). Distance-based redundancy analysis (db-RDA) was conducted using R package *vegan* 2.5-5 to assess the relationships between environmental variables and microeukaryotic community structure. The R package *igraph* was used to evaluate and visualize the co-occurrence interactions between OTUs (OTUs with an average relative abundance higher than 0.01% and exist in more than half of the samples) to reveal the complexity of microeukaryotic communities and the potential interrelationships between members of microeukaryotic communities. For the construction of co-occurrence networks, the Spearman correlations between all OTU pairs was calculated, and the P-values were adjusted for false discovery using FDR adjustment (Benjamini and Hochberg, 1995). We only considered strong (Spearman's correlation coefficient $r > 0.80$ or $r < -0.80$) and significant ($P < 0.05$) correlations. Topological properties of the networks were calculated. The module structure of the networks was analyzed and the topological roles of the OTUs were assessed by calculating the connectivity of each OTU in the network, including within-module connectivity (Zi: within-module degree Z-score of node i) and among-module connectivity (Pi: participation coefficient of node i). The nodes with Zi $\geq$ 2.5 and/or Pi $\geq$ 0.62 were identified as keystone taxa (Deng et al., 2012).

RESULTS

Physicochemical variables varied widely across the three areas (upstream, BYD and downstream) (**Supplementary Table 1**). For example, the turbidity ranged from 0.72 to 63 NTU at the time of sampling, the conductivity ranged from 264 to 10710 μs/cm, TN ranged from 0.63 to 5 mg/L, TP ranged from 0.01 to 0.75 mg/L, and COD ranged from 0.32 to 71.10 mg/L. The upstream had the lowest average turbidity (4.74 NTU), conductivity (517.32 μs/cm), and COD (14.63 mg/L) than BYD and downstream areas. The average pH (6.48), TN (0.37 mg/L), and TP (0.03 mg/L) of BYD were the lowest among the 3 areas. The average turbidity (19.68 NTU), conductivity (4014.69 μs/cm), TP (0.12 mg/L), COD (43.20 mg/L) were the highest in downstream than in other areas.

Alpha Diversity

Overall, 1,140,450 high-quality sequences were yielded through the sequencing of 18S rRNA genes and 1904 OTUs were detected at the 97% similarity level. Venn diagram showed that 451 OTUs were shared among the upstream, BYD, and downstream (**Figure 2A**). Downstream had the lowest alpha diversity indexes while the upstream had the highest value of Chao1 and Observed OTUs (**Figure 2B**). The results answered our first question (*question i*). Correlation analysis showed that alpha diversity indexes were negatively correlated with turbidity, temperature, conductivity, pH, COD, TP, TN, and positively correlated with the TN:TP ratio (**Supplementary Figure 1**).

Community Composition and Its Association to Environmental Factors

The results showed that the community composition of microeukaryotes in upstream, BYD, and downstream were differed significantly with each other (**Figures 2C,D**), which was further supported by the results of three non-parametric statistical approaches of Adonis, ANOSIM, and MRPP (**Table 1**). There were 14 lineages which had an average relative abundance above 1% in at least one area (**Figure 2D**). Ten of the lineages were significantly different among the three areas. Chlorophyta, Cryptomonas, and Metazoa were dominant (with an average relative richness > 5%) in all three areas. As shown by the mean Bray–Curtis distance between all pairs of samples, microeukaryotic communities in BYD had a significant lower mean Bray-Curtis distance value than that in upstream and downstream (**Supplementary Figure 2**), suggesting lower variabilities of the community composition (**Figure 2C**).

Results of db-RDA showed that the shift of microeukaryotic communities in this Basin was significantly ($P < 0.05$) associated with all of the tested environmental factors (**Figure 3A**). Specifically, community shift in upstream was significantly ($P < 0.05$) associated to turbidity, temperature, conductivity, pH, COD, and the TN:TP ratio (**Figure 3B**). Community shift in BYD was significantly ($P < 0.05$) associated to conductivity, COD, TN, and TP (**Figure 3C**). Community shift in downstream area was significantly ($P < 0.05$) associated to conductivity and the TN:TP ratio (**Figure 3D**).

Co-occurrence Networks

For the microeukaryotic communities in the whole study area, the resulting network consisted of 442 nodes (OTUs) linked by 3591 edges (significant correlation relationships), with more

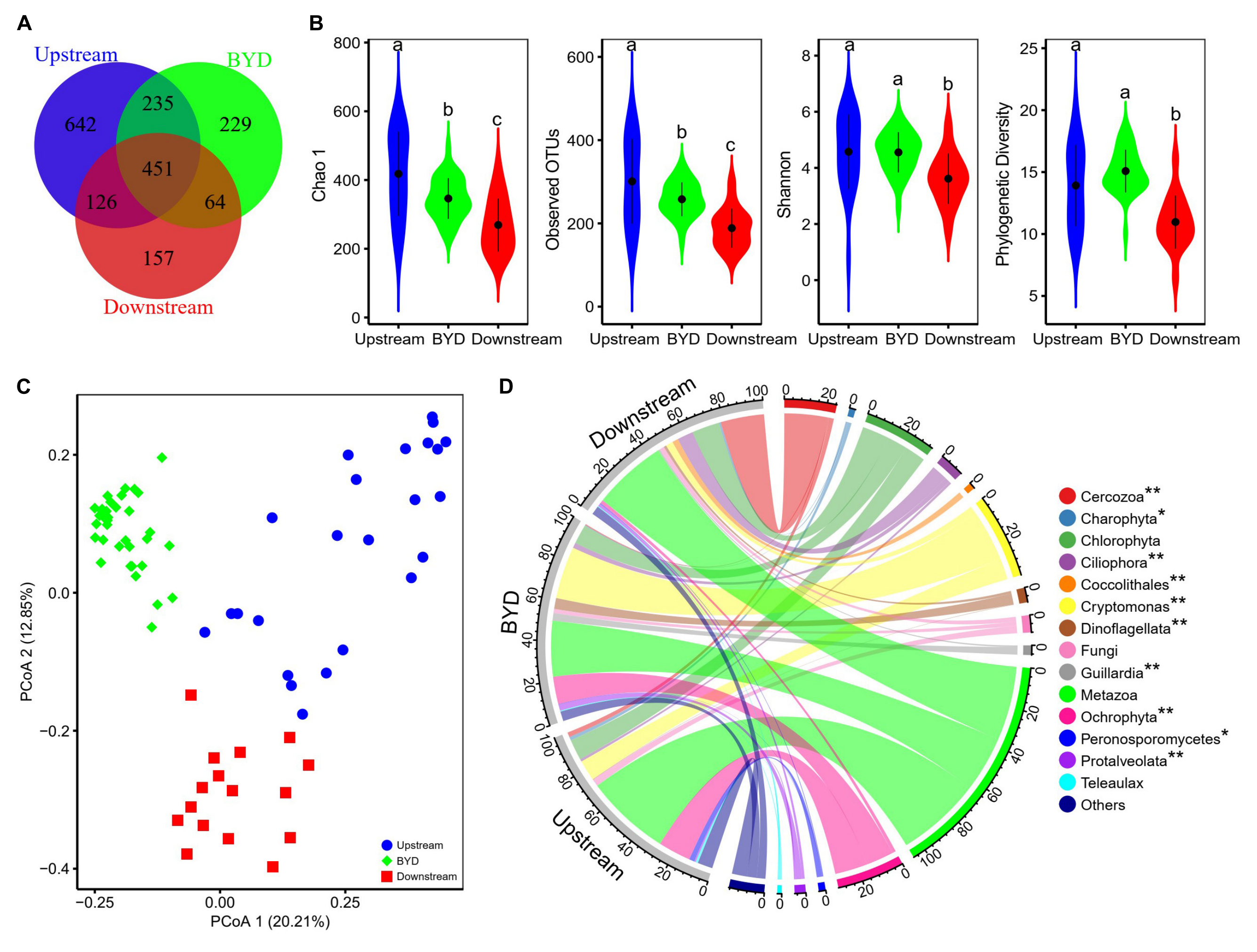

FIGURE 2 | Basic differences of microeukaryotic communities along the lentic-lotic continuum in all three areas, including upstream, BYD, and downstream. **(A)** Venn diagram showing the unique and shared OTUs within and between three study areas. **(B)** Alpha diversity differences among three study areas. Different letters indicate the significant level at $P < 0.05$ determined by ANOVA. **(C)** Principal coordinates analysis (PCoA) based on Bray–Curtis distance in terms of the relative abundance of OTUs. **(D)** Chord diagram showing taxonomic composition and its differences of the microeukaryotic communities in three study areas. Only the lineages with an average relative abundance > 1% are shown. The significant differences were shown in "*" ($p < 0.05$) and "**" ($p < 0.01$).

than 98% of the edges were positive (**Figure 4A**). The average path length, clustering coefficient, centralization betweenness, and modularity were all greater than those of the respective random network (**Supplementary Table 2**), suggesting that the network was non-random and had "small-world" properties and a significant modular structure. The entire network was clearly parsed into three modules (**Figure 4B**). Module-A, -B, and -C accounted for the majority in upstream, BYD, downstream sites, respectively (**Figure 5A**). Ternary plot showed that most of the OTUs from the module-A, -B, and -C had higher relative abundances in upstream, BYD, and downstream sites, respectively, indicating that different modules were specific (relatively more abundant) to a particular area. Moreover, these three modules composed of OTUs which were primarily occupied by Metazoa in module-A, by Cryptomonas and Chlorophyta in module-B, and by Cercozoa and Ochrophyta in module-C (**Figures 5B–D**), suggesting that taxonomic relatedness played a key role in determining the modular structure. Based on the connectivity (within-module connectivity and between module connectivity), the co-occurrence network of microeukaryotic communities in the whole study area had three connectors and nine module hubs (**Supplementary Figure 3**). The connectors belonged to other modules, and the modules hubs came from module-A, -B, and -C.

For each area specifically, the co-occurrence network of microeukaryotic communities consisted of 374 nodes and 4837 edges in upstream, 215 nodes and 558 edges in BYD, and 158 nodes and 290 edges in downstream, respectively (**Figure 6**). The microeukaryotic network in upstream had the highest average degree and clustering coefficient, and the lowest average pathlength and centralization betweenness, suggesting that microeukaryotic communities in upstream had the most complex network (**Supplementary Table 2**). This answered our second question (*question ii*). Moreover, according to the modularity,

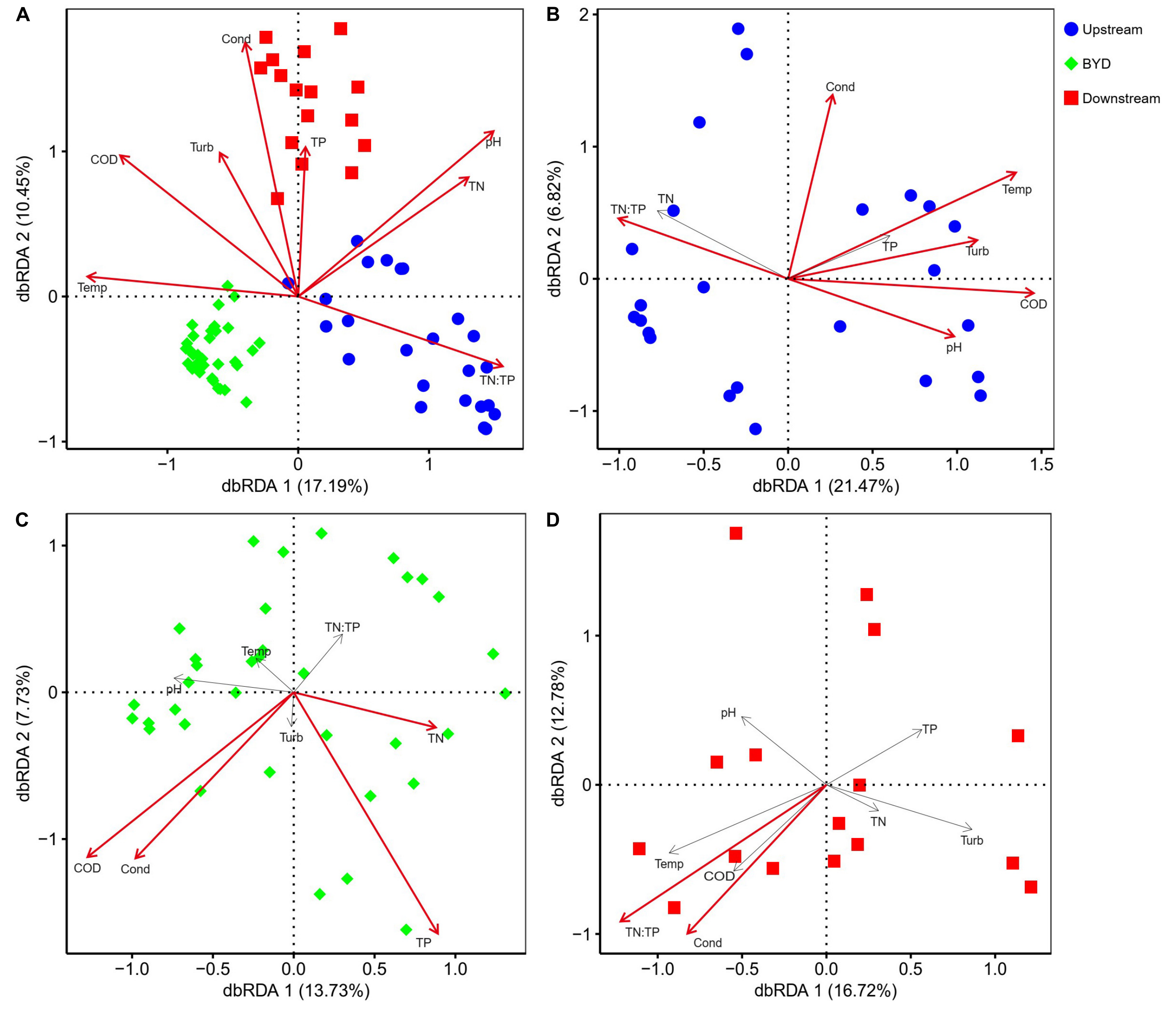

FIGURE 3 | Distance-based redundancy analyses (db-RDA) reveal the relationships between environmental variables (arrows) and microeukaryotic communities (points) in **(A)** the all three areas, **(B)** upstream, **(C)** BYD, and **(D)** downstream. Significant variables ($P < 0.05$ by envfit function) are shown in red.

the network of upstream microeukaryotic communities had many keystone taxa playing as network hubs, module hubs, and connectors but do not have a well modular structure (**Supplementary Table 2** and **Figure 6A**). On the contrary, the networks of BYD and downstream microeukaryotic communities had a highly modular structure with a few modular hubs and connectors (**Supplementary Table 2** and **Figures 6B,C**).

TABLE 1 | Results of significant differences of microeukaryotic communities between the sample areas with three non-parametric statistical approaches.

	Adonis		ANOSIM		MRPP	
	R^2	*P*	*R*	*P*	Delta	*P*
Upstream vs. BYD	0.177	0.001	0.604	0.001	0.741	0.001
BYD vs. Downstream	0.204	0.001	0.785	0.001	0.682	0.001
Upstream vs. Downstream	0.131	0.001	0.414	0.001	0.853	0.001

DISCUSSION

Changes in Microeukaryotic Biodiversity

As hypothesized (H1), the downstream alpha diversity was significantly lower than that of the upstream, indicating that the diversity of microeukaryotes decreased significantly with the severity of water quality damage in the lentic-lotic continuum catchment. The decrease of downstream diversity may be due to the excessive interference of urban and agricultural activities on the water environment (higher TN and TP)

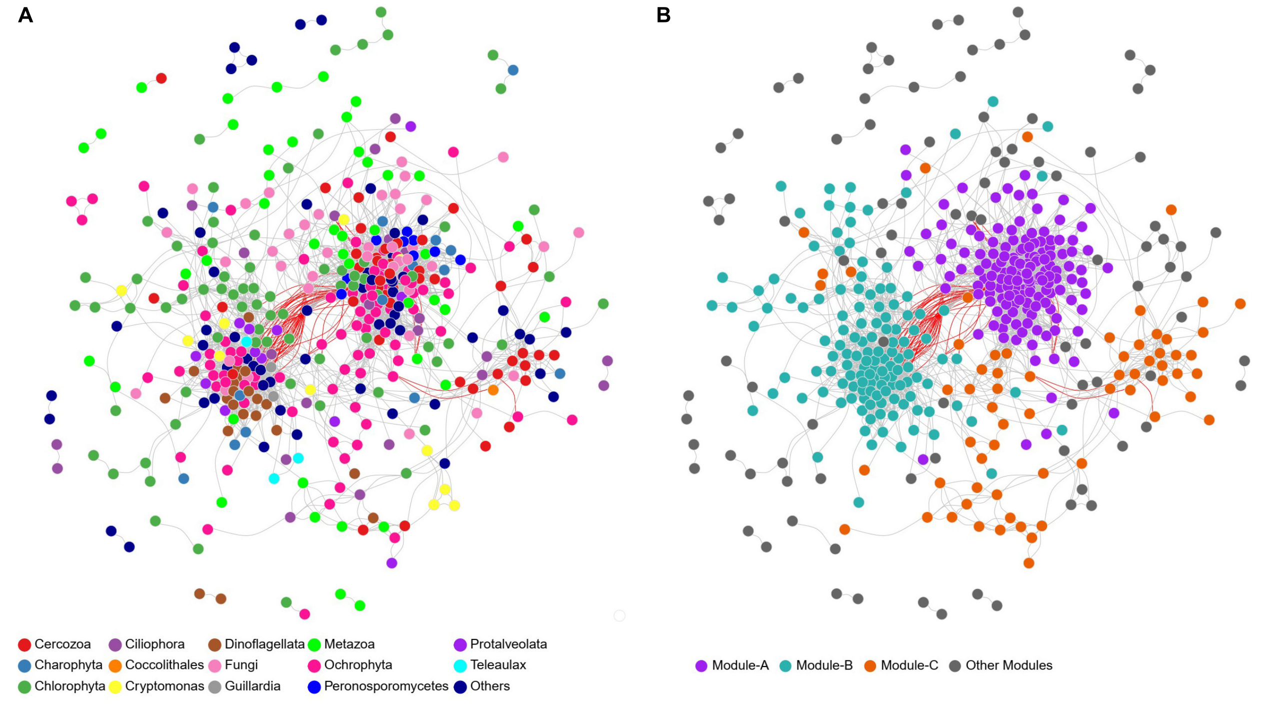

FIGURE 4 | Co-occurrence network of microeukaryotic communities in the whole area colored by **(A)** major lineages and **(B)** major modules (modules have more than 50 nodes). Circle nodes represent OTUs. Edges represent Spearman correlations (only strong and significant relationships with Spearman's $r > 0.8$ or $r < -0.8$ and $P < 0.05$ are shown). The gray and red lines indicate positive and negative correlations, respectively.

(Teng et al., 2011; Hao et al., 2012; Hosen et al., 2017; Santos et al., 2017; Lopes et al., 2021). The variabilities of the community composition in BYD were significantly lower than that of upstream and downstream communities, reflecting significant changes in relative abundances of various taxa in upstream and downstream. Community composition is determined by environmental factors and spatial (distance) factors (Moran-Ordonez et al., 2015; Ptatscheck et al., 2020; Uritskiy et al., 2020). The upstream Juma River has pollution from tourism, while the downstream Daqing River has pollution influx along the way causing water quality degradation, which may affect the diversity of microeukaryotic communities in the lentic-lotic continuum catchment, resulting in high variations of alpha diversity in upstream and downstream samples. Because BYD is rich in fishery resources and human-made disturbances are serious, it may cause homogeneity between habitats, resulting in high similarity of microeukaryotic communities among samples in similar environments (Vilar et al., 2014; Parreira de Castro et al., 2018). In addition, the relative abundance of microeukaryotic communities in each sample site was not the same, which in turn reflected the high heterogeneity between different points in the lentic-lotic continuum catchment, indicating that the community composition of each sample site is unique to the ecosystem (Burgos-Caraballo et al., 2014). Environmental factors have a significant impact on the microeukaryotic community (Yuksek et al., 2006; Dunstan et al., 2012; Logares et al., 2020). Local environmental constraints such as environmental pollution caused by human activities can shape the local community structure of eukaryotes, which supports the species ordering hypothesis in river ecosystems (Lake, 2000; Huber et al., 2002). Since phytoplankton respond quickly and strongly to environmental disturbances, they have been used as an indicator of environmental changes and ecosystem status in freshwater ecosystems (Bianchi et al., 2003; Lepisto et al., 2004; Moustaka-Gouni et al., 2006; Li et al., 2015; Liu et al., 2015). Our results consistent with this view.

Changes in the Network Structure of Microeukaryotes

The co-occurrence network showed that the microeukaryotic community in the entire study area was non-random, with "small world" characteristics and a significant modular structure. The entire network was clearly parsed into three modules, which had different taxonomic composition. Several parameters representing the network topology were calculated. Compared with BYD and downstream, the upstream network had higher average degree and clustering coefficient in the lentic-lotic continuum catchment, while modularity and average path length showed the opposite trend (**Supplementary Table 2**). As hypothesized (H2), the upstream network showed higher transitivity than BYD and downstream networks (a measure of connectivity within a cluster). When the environment is disturbed by the outside world, it will quickly affect the entire network, causing the upstream network structure to be unstable, indicating that the network has a low ability to

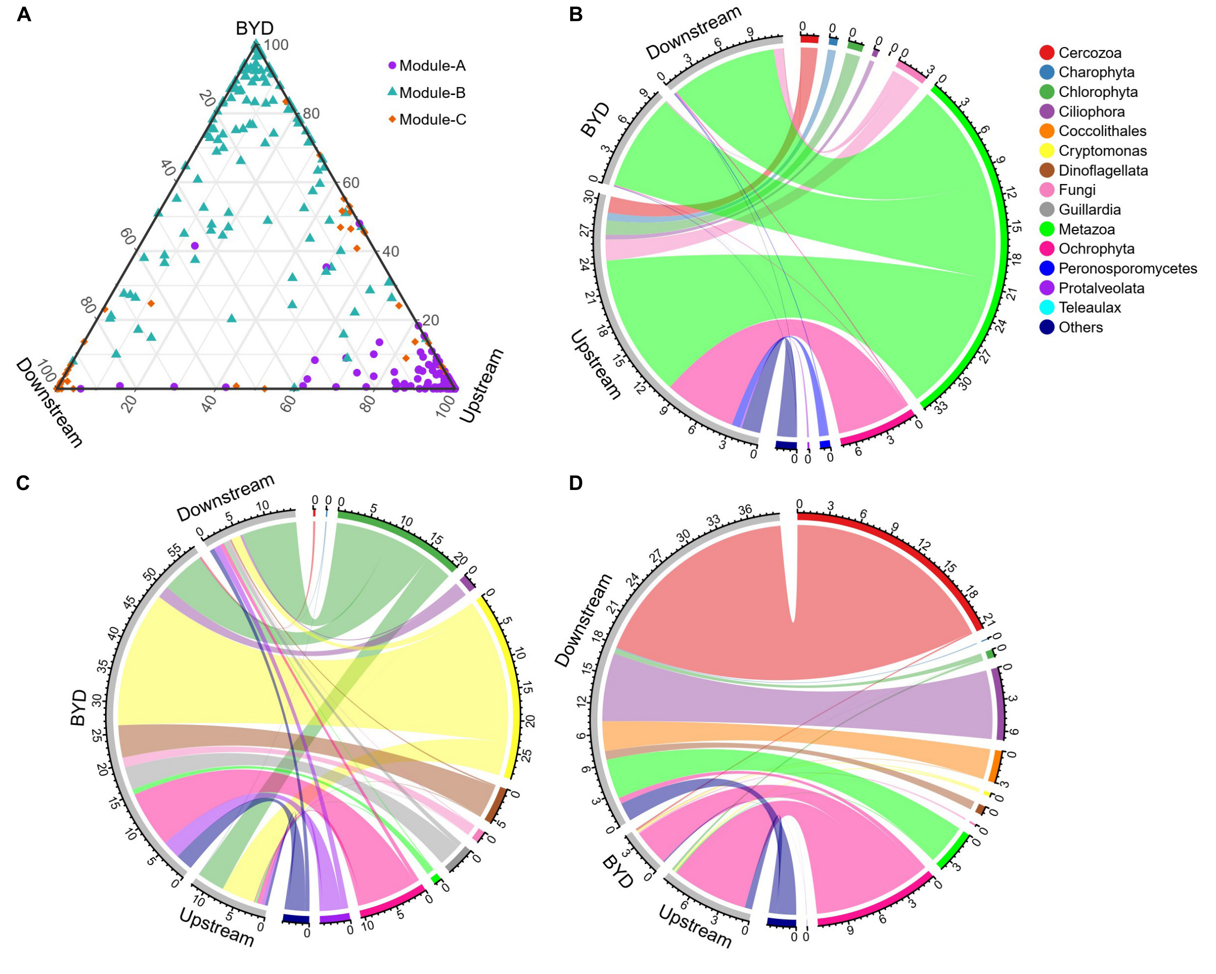

FIGURE 5 | (A) Ternary plot of the OTUs in major modules of the co-occurrence network for the whole microeukaryotic communities of three area. Chord diagrams showing the taxonomic composition of **(B)** module-A, **(C)** module-B, and **(D)** module-C.

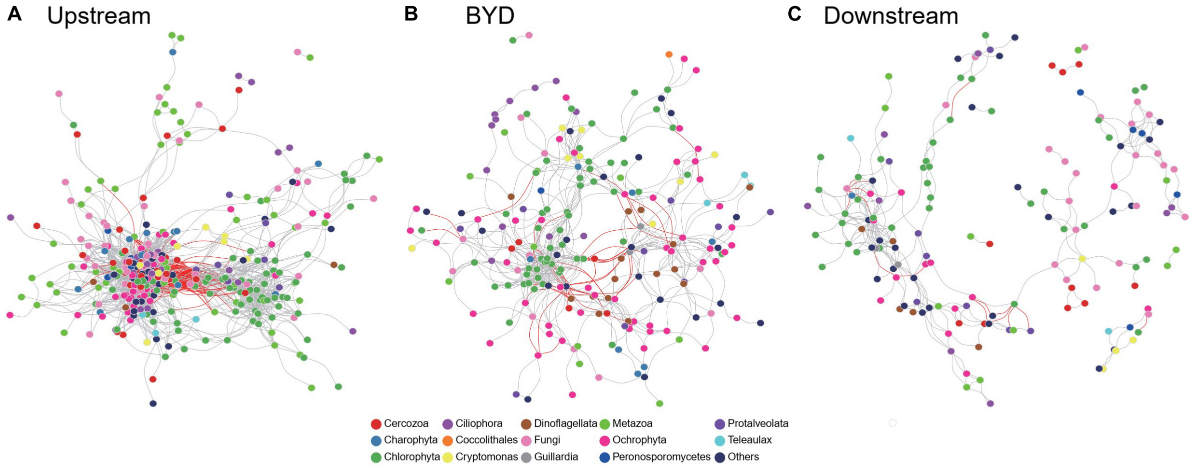

FIGURE 6 | Co-occurrence network of microeukaryotic communities at **(A)** upstream, **(B)** BYD, and **(C)** downstream. Circle nodes represent OTUs. Edges represent Spearman correlations (only strong and significant relationships with Spearman's $r > 0.8$ or $r < -0.8$ and $P < 0.05$ are shown). The gray and red lines indicate positive and negative correlations, respectively.

buffer environmental changes (Chen et al., 2021). These results indicated that the microeukaryotic OTUs were more connected upstream than BYD and downstream.

Compared with upstream networks, BYD and downstream networks had fewer OTUs. The inflow of microeukaryotes from the upstream can affect the structure of the microeukaryotic communities in BYD and downstream (Zhulidov et al., 2011). These inputs, to some extent, explained the overlap of the identified OTUs between the upstream, BYD and downstream (**Figure 2A**). Some of the microeukaryotes in BYD and the lower reaches may come from the upper reaches. However, the taxonomic composition of these communities was subsequently modified by local environments. In the lentic-lotic continuum catchment, microeukaryotes had strong tendencies to co-occur (93% positive edges in upstream, 92% positive edges in BYD, and 94% positive edges in downstream, **Supplementary Table 2**). The relatively high proportion of positive correlations in microeukaryotic networks indicated that in a given environment, these taxa were interdependent or complementary, and cooperative relationships are strong (Fernandez et al., 2015). Under the gradient of water quality changes along the lentic-lotic continuum, from upstream to BYD and downstream, the network gradually became smaller and there were fewer connections. This may be due to the greater amplitude or frequency of disturbance events in the middle and lower reaches. In BYD and downstream, environmental variables (such as temperature, hydrology) may be more variable and extreme than upstream. The microeukaryotic co-occurrence network structure responded to environmental disturbances, which may explain the difference in scale and structure of upstream, BYD and downstream networks along the lentic-lotic continuum (Yang et al., 2022). The composition and dissimilarities of phytoplankton were more affected by local hydrological and physicochemical variables along the lentic-lotic continuum (Qu et al., 2018).

Module structures can reveal more ecological properties of complex communities and shed light on the different groups of nodes performing different functions (Xiong et al., 2018). The way in which water quality affected the river's eukaryote community not only represents the local environmental impact, but also represents the regional process. For instance, module-A was dominated by Metazona, which has a certain role in in promoting water purification. Module-B was dominated by Cryptomonas and Chlorophyta. Studies have shown that Chlorophyta can absorb nitrogen and phosphorus sources in the water body (Carrick and Lowe, 1989). Cryptomonas were also found to be favored by reductions of nutrient loading in shallow lakes (Jeppesen et al., 2007). This was consistent with the nutritional status of BYD. According to module structure, some nodes of the upstream microeukaryotic network were identified as keystone taxa indicating their important roles in structuring network and maintaining the stability of the ecosystem (Banerjee et al., 2018). There were only a few modular hubs and connectors in BYD and downstream (**Supplementary Figure 3**), indicating the high fragmentation of the network and the strong independence of modules. The loss of these keystone taxa will increase the fragmentation of the network, and adversely affect the integrity and function of the microeukaryotic community (Widder et al., 2014; Banerjee et al., 2018).

Environmental Factors Driving the Variation of Microeukaryotic Communities

Revealing the spatial distribution characteristics of microeukaryotic communities and their environmental driving factors is crucial to clarify the stability and function of the ecosystem (Ren et al., 2020). In the upstream, water from the Juma River, the Fu River and the South-to-North Water Diversion Project are collected. In addition to the Duliujian River, there are other rivers flowing downstream. The overlap ratio of the three freshwater areas is not high. This may explain the low similarity of microeukaryotic communities in the three freshwater areas. Our results also showed the main changes of microeukaryotic community composition along the lentic-lotic continuum. The upstream water body is flowing, BYD is a shallow lake, and the downstream is intercepted by dams. In the lentic-lotic continuum catchment, the fluidity of water body is not strong. Moreover, the downstream area has a high salinity because it is close to the estuary to the sea. There were many species found only downstream. In the lentic-lotic continuum, different water body states may have an impact on the microeukaryotic community (Fraisse et al., 2013; Dzhayani, 2020). As expected in H3, these changes in community composition can be attributed to changes in local environments (Hooper et al., 2005; Yuksek et al., 2006; Dunstan et al., 2012; Logares et al., 2020). The diversity of phytoplankton is mainly directly affected by river physicochemical and hydrodynamic variables (Padisak et al., 2006; Isabwe et al., 2018).

The changes of nutrient concentrations such as TN and TP are the main reasons driving the spatial changes of phytoplankton community structure (Parkes and Duggan, 2012; Wang et al., 2015; Xiong et al., 2016). The microtrophic community is mainly determined by the level of nutrients, and the increase of nutrients will change the community composition of phytoplankton, macroinvertebrates and fish in the water body (Chessman et al., 1999; Weijters et al., 2009; Zhang et al., 2021). The microeukaryotic community was mainly composed of Chlorophyta, Cryptomonas and Metazoa (with a relative richness $>5\%$) in our study areas. The higher relative abundance of Chlorophyta may be partly due to the higher nitrogen and phosphorus values downstream. Studies have shown that within a certain range of nutrient salt concentration, filamentous Chlorophyta can absorb 90% of the available nitrogen sources and more than 80% of the phosphorus sources in the water body (Carrick and Lowe, 1989). This has a certain contribution to reduce the level of lake eutrophication, improving the self-purification capacity and transparency and restoring high aquatic vegetation communities. The water body at the downstream sampling point showed a certain degree of eutrophication, and the high abundance of Chlorophyta might have a beneficial effect on mitigating eutrophication in this area. Some studies have found that the existence of Cryptomonas are beneficial to the reduction of nutrient loading in shallow lakes (Jeppesen et al., 2007). Therefore, the presence of Chlorophyta and Cryptomonas suggested that the composition of microeukaryotic communities might be a response to the increased nutrient content in these rivers. Such species have ecological significance in the environment of eukaryotes. The change of temperature will indirectly affect the community composition of eukaryotes by affecting the physicochemical properties of the water body. The zooplankton microfauna are all chemoheterotrophic microorganisms, so the decrease in DO caused by warming can inhibit their diversity (Fortney et al., 2018). In BYD and downstream, the higher temperature may lead to the lower relative abundance of the Metazoa. At the same time, higher temperature will promote the increase of nitrate nitrogen, provide nitrogen source for phytoplankton, and may promote the increase of the abundance of Chlorophyta and Cryptomonas, thereby changing the community composition of eukaryotes. The increase in turbidity will affect the light penetration, resulting in the light tilt of phytoplankton growth (Shi et al., 2017). The lower abundance of Cryptomonas in the downstream may be due to the higher turbidity, TN and TP because, low light and high salinity conditions are harmful to the growth of these pigmented phyla in freshwater (Domingues et al., 2011). DO is the main driving force of nutrient and energy flow patterns in aquatic ecosystems (Diaz and Rosenberg, 2008). The DO of upstream and BYD was significantly higher than that of downstream, indicating that the water self-purification ability of upstream and BYD is better. There are many functional protozoa and micrometazoa in BYD and downstream, indicating that the water body has a high degree of self-purification (Li et al., 2019). The changes in the abundance of Metazoa may be a response to the degree of water quality degradation, which is consistent with the water quality in all three areas. COD marks the content of organic pollutants in the water (Margot et al., 2013). In short, the downstream water quality was worse, the water body eutrophication and organic pollution were more serious, which may have an impact on the microeukaryotic community composition of the downstream samples (Duan et al., 2009; Wang et al., 2011; Yang et al., 2012; Buchanan, 2020; Amorim and Moura, 2021).

Water diversion projects are playing an increasingly important role in improving the water quality of rivers. However, water transfers will also affect the concentration of nutrients and other physicochemical variables, which will have a certain impact on the water environment, thereby affecting the diversity and community composition of phytoplankton (Yin et al., 2011; Dai et al., 2020; Xu et al., 2021; Yang et al., 2021). At the same time, the short-period hydrological conditions brought by the water diversion project have also shaped the community composition of biofilms to a certain extent (Guo et al., 2020). In summary, the relative abundance of certain phyla, Chlorophyta and Cryptomonas were significantly different between upstream, BYD and downstream sample points, indicating that the variabilities of microeukaryotic communities are related to environmental factors. At present, there are many large-scale water diversion projects, especially in China, such as the South-to-North Water Diversion Project, BYD is also its water receiving lake. Large-scale water diversion projects are needed not only for meeting human demand for water, but also for maintaining the healthy development of the ecosystem. However, the construction of large-scale water diversion projects will undermine the concept of natural cascading reservoir continuum and increase the degree of homogeneity among water bodies (Barbosa et al., 1999; Nekola and White, 1999). From our results, the water quality and phytoplankton community of BYD in the lentic-lotic continuum system were all affected by the incoming water, so we suggest that river managers take some measures to reduce the impact of water diversion projects on water quality, such as dredging sediment, increasing water flow, and controlling sources of pollution.

CONCLUSION

Our results clearly demonstrated that the upstream incoming water had a certain impact on the diversity and community composition of microeukaryotes in BYD and downstream rivers by influencing the water environment. The lower variabilities of BYD's community composition might be due to the homogeneity among habitats. Co-occurrence network analysis further revealed that microeukaryotes had a strong tendency to co-occur, which may help maintain community stability and ecological function. In addition, microeukaryotic networks was non-random and had "small-world" properties and a significant modular structure. Some nodes of the upstream microeukaryotic network, as keystone taxa, played an important role in structuring network and maintaining the stability of the ecosystem. In BYD and downstream, the microeukaryotic network was highly fragmented, and the loss of keystone taxa would be detrimental to the integrity and function of the microeukaryotic community. In summary, our results can provide a better understanding of the composition and diversity of microeukaryotes communities in the lentic-lotic continuum system, and provides insights for BYD's water environment management. In addition, the study has a potential guiding significance for large-scale water conservancy diversion.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, SAMN26210067.

AUTHOR CONTRIBUTIONS

YW: methodology, data curation, formal analysis, writing – original draft, writing – review and editing. ZR: methodology, data curation, and formal analysis. PH: conceptualization, methodology, writing – review and editing. JX: conceptualization and methodology. DL: methodology. CL: conceptualization and methodology. BL: writing – review and editing.

NW: conceptualization, methodology, supervision, writing – review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.887787/full#supplementary-material

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Effects of Nutrient Levels on Microbial Diversity in Sediments of a Eutrophic Shallow Lake

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Microorganisms can both indicate the water quality characteristics and the health of the aquatic environment, which have an important influence on the cycling of organic carbon, nitrogen (N), and phosphorus (P) in nature. In this study, we took Taihu Lake, a typical eutrophic lake in China, as the research object, and monitored the northern (Changzhou City) and southern (Changxing County) regions of Taihu Lake for three consecutive years (2019–2021), respectively. We also analyzed the microbial diversity in sediments, and then summarized the effects of different nutrient environments on microorganisms in the aquatic environment. The results showed that the pollution level in the northern part of Taihu Lake was higher than that in the southern region of Taihu Lake, where the pollution was mainly in summer (June–July). The pollution in the southern region of Taihu Lake is relatively stable between 2019 and 2021; the changes in the northern part of Taihu Lake are relatively significant. Microbial diversity in the study area was negatively correlated with the degree of eutrophication of water bodies; microbial abundance was positively correlated with nutrient levels. The functional difference analysis indicated that the microorganisms in the sediments of Taihu Lake in the study area were involved in the nutrient transport and transformation, and played an important role in the purification of the lake water body. This study reveals the relationship between water eutrophication and microbial diversity, and then provides a theoretical basis for the management of eutrophic lakes.

Keywords: nutrient levels, microbial diversity, sediments, eutrophic shallow, lake

INTRODUCTION

The concept of eutrophication was first introduced by Lindeman in 1942, and the study concluded that the eutrophic stage of a water body is the last stage of the natural evolution of a normal lake (Lindeman, 1942). The water body reaches the eutrophication level when the nutrient total phosphorus (TP) $>$ 0.02 mg/L and nitrate nitrogen (NO_3^--N) $>$ 0.3 mg/L (Namsaraev et al., 2018). Eutrophication of water bodies was once compared to "ecological cancer," which destroys the ecosystem of water bodies and causes the ecological imbalance of water bodies by making the community structure homogeneous (Ramachandra and Solanki, 2007). Freshwater eutrophication is a widespread global environmental problem and a consequence of intense human activities (Wang et al., 2019). Currently, 54% of Asian lakes, 53% of European lakes, 48% of North American lakes, 41% of South American lakes and 28% of African lakes are eutrophic (Zhang et al., 2020).

According to the process of eutrophication formation, there are two types of eutrophication, natural and man-made (Conty and Bécares, 2013). Natural eutrophication mainly refers to the continuous deposition of soil erosion, evaporation, precipitation, river entrained alluvium, and aquatic debris at the bottom of water caused by natural environment without human interference, and the enrichment of nutrients in water bodies, which in turn leads to the development of water bodies from poor nutrients to eutrophication, gradually evolving into swamps and eventually into land. The process of eutrophication under natural conditions is very slow and takes hundreds or even tens of thousands of years to complete (Cui et al., 2021; Padedda et al., 2021). Nutrients contained in industrial, agricultural, and domestic wastewater have caused the eutrophication of lake water, degrading the quality of the water and the aquatic ecosystem (Liu and Wang, 2016). From the perspective of primary production of water bodies, eutrophication can be further classified into the following four categories: hypertrophic, eutrophic, mesotrophic, and oligotrophic (Kim et al., 2021; Wang et al., 2021). N and P were regarded as the limiting factors to the density of aquatic organisms and the main reduction objects especially for the cultural eutrophic lakes (Yuan et al., 2011). The sources of nutrients include two categories: external sources include the discharge of urban living and production wastewater (point source pollution) and lake runoff, farmland surface runoff, lake precipitation, dustfall, farming bait, etc. (non-point source pollution); internal sources are mainly the dead decay of aquatic plants and animals, and nutrients released from sediments. In addition, the destruction of the water body ecosystem is also one of the reasons for the deterioration of the water body. A good biological community structure of the aquatic environment is not only a good indicator of the health of the water, but also a guarantee of the self-purification of the water (Schindler et al., 1987; Madsen and Cedergreen, 2002; Rahaman and Sinha, 2013; Coluccio et al., 2021; Younes et al., 2021).

As an important part of the water ecosystem, microorganisms play a very important role in the migration and transformation of nutrients, organic matter and other materials at the water-sediment interface. Microorganisms can influence the nutrient status in the water environment, and when the external environment in the aquatic environment changes, the species and abundance of the microbial community will also change (Moshiri and Crumpton, 1977; Phelps et al., 1994; Jonah et al., 2015; Lakshmipathi et al., 2019; Vincent et al., 2021). At present, studies on microbial communities have mainly focused on the vertical distribution characteristics of the microorganisms in sediments within a specific region, and relatively few studies have been conducted on the dominant populations and microbial community structure under different trophic states of the environment (Sangakkara, 1997; Powlson et al., 2001; Haller et al., 2011; Liu et al., 2015).

In this study, the water quality and nutrient content in sediments of Taihu Lake, a typical eutrophic lake in China, were monitored for three consecutive years in the northern (Changzhou City) and southern (Changxing County) regions of Taihu Lake, respectively, and the microbial diversity in sediments was analyzed. And then the effects of different nutrient environments on microorganisms in the aquatic environment were summarized. This study can provide valuable experience for the study of biodiversity in eutrophic lakes.

MATERIALS AND METHODS

Sample Collection

Taihu Lake is the third largest freshwater lake in China, which is located in the core area of Yangtze River Delta. In recent years, with the rapid economic development and population growth as well as frequent human activities, industrial wastewater, and domestic sewage continuously flow into the lake, making the eutrophication problem of Taihu Lake more and more serious. In this study, lake water and surface sediment samples were collected from six points (N1–N6; S1–S6) each in North and South Taihu Lake for subsequent water quality analysis and microbiological analysis from 2019 to 2021, respectively, and the sample points were collected as shown in **Figure 1**.

Analysis of Indicators

At the sampling site, a portable dissolved oxygen meter was used to determine the dissolved oxygen (DO) and water temperature (WT). And then a pH meter was used to determine the pH of the water body. The rest of the physical and chemical properties were determined in accordance with the national standard method within 3 days after sampling, mainly including: permanganate index (COD_{Mn}), total nitrogen (TN),

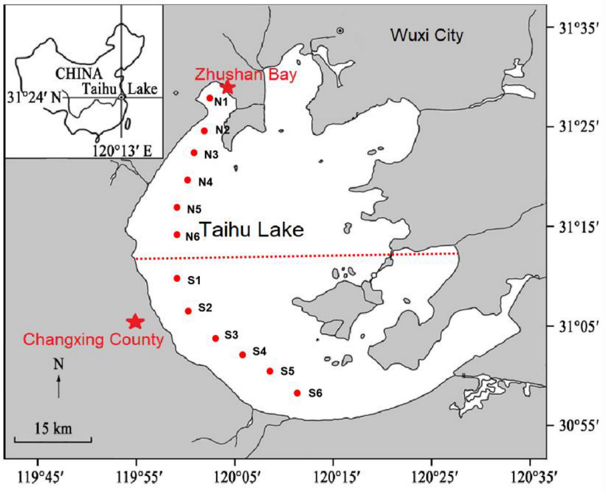

FIGURE 1 | Schematic diagram of sampling locations.

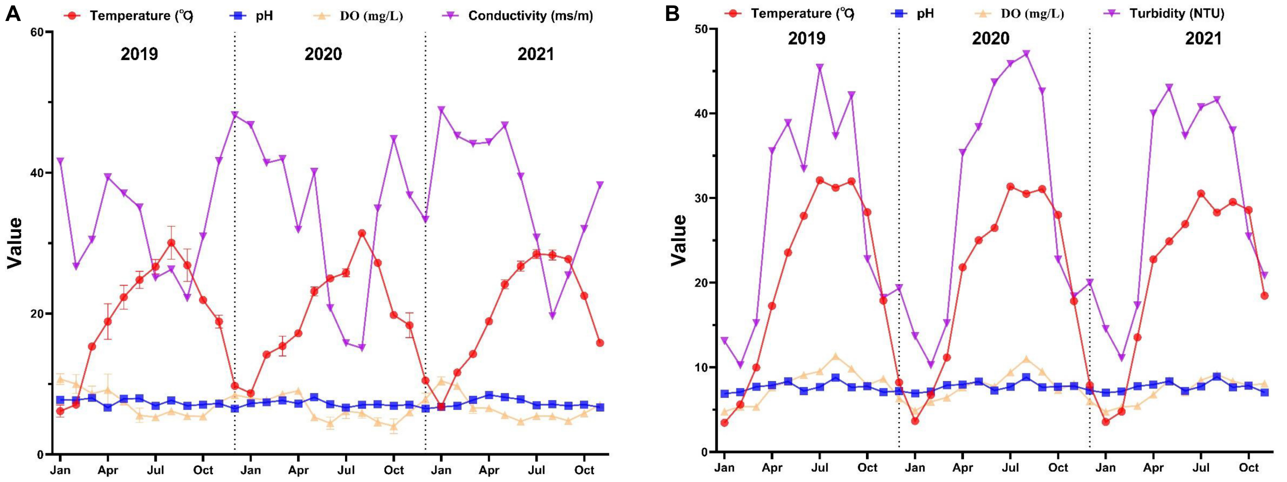

FIGURE 2 | Water quality of South **(A)** and North **(B)** Taihu Lake.

total phosphorus (TP), nitrate nitrogen (NO_3^--N), ammonia nitrogen (NH_3-N). According to the national standard, the determination of COD_{Mn} was done by the acidic method (GB/T11892-1989), the determination of TN was done by the alkaline potassium persulfate extinction UV spectrophotometric method (GB/T11894-1989), the determination of TP was done by the ammonium molybdate spectrophotometric method (GB/T11893-1989), the determination of NO_3^--N was done by the phenol disulfate method (GB/T7480-1987), and the determination of NH_3-N was done by the nano reagent colorimetric method (GB/T7479-1987).

Analysis of Microbial Community

DNA Extraction and PCR Amplification

Total DNA extraction was performed according to the EZNA soil kit (Omega Bio-tek, Norcross, GA, United States) instructions. DNA concentration and purity were determined using NanoDrop2000, and DNA extraction quality was measured by 1% agarose gel electrophoresis; 5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers for polymerase chain reaction (PCR) amplification of the V3–V4 variable region. The amplification procedure was: pre-denaturation at 95°C for 3 min, 27 cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s), and extension at 72°C for 10 min (PCR: ABI GeneAmp 9700). The amplification system was 20 µl, 4 µl 5*FastPfu buffer, 2 µl 2.5 mM dNTPs, 0.8 µl primer (5 µM), 0.4 µl FastPfu polymerase; 10 ng DNA template (Zhang et al., 2020).

Illumina MiSeq Sequencing

The PCR product was recovered using a 2% agarose gel, purified using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States), eluted with Tris–HCl, and detected by 2% agarose electrophoresis. Detection quantification was performed using QuantiFluorTM-ST (Promega, Madison City, Wisconsin, United States). The purified amplified fragment was constructed into a library of PE 2*300 according to the standard operating protocol of the Illumina MiSeq platform (Illumina, San Diego, CA, United States). The steps of constructing the library: (a) connecting the "Y" shaped liknker; (b) using magnetic beads to remove the self-ligating fragment of the linker; (c) enriching the library template by PCR amplification; (d) denaturing the sodium hydroxide, producing single-stranded DNA fragments. Sequencing using Illumina's MiSeq PE300 platform (Zhang et al., 2020).

Statistical Treatment

Statistical analysis was performed using Origin Pro 9.0 software for Windows (OriginLab, Northampton, MA, United States). An analysis of the structure and abundance of the bacterial community distribution in surface sediments was performed using the free online platform Majorbio I-Sanger Cloud Platform (Zhang et al., 2020).

RESULTS AND DISCUSSION

Water Quality Physical and Chemical Indicators Monitoring

The changes in water quality monitored from January 2019 to November 2021 are shown in **Figure 2**. The values of WT in South and North Taihu Lake are 6.2℃–31.4℃ (mean value 19.7℃) and 3.5–32.1℃ (mean value 20.1℃), respectively. There is no significant difference between the WT in South and North Taihu Lake, but the temperature difference is large in all quarters, with certain characteristics of time and space distribution. The highest temperature of each year is concentrated in July–September, and the lowest temperature in January. The values of pH are 6.49–8.45 (mean value 7.29) and 6.89–8.88 (mean value 7.66), with a small trend of pH variation, and no obvious pH exceedance was found. The DO levels ranged from 4.00 to

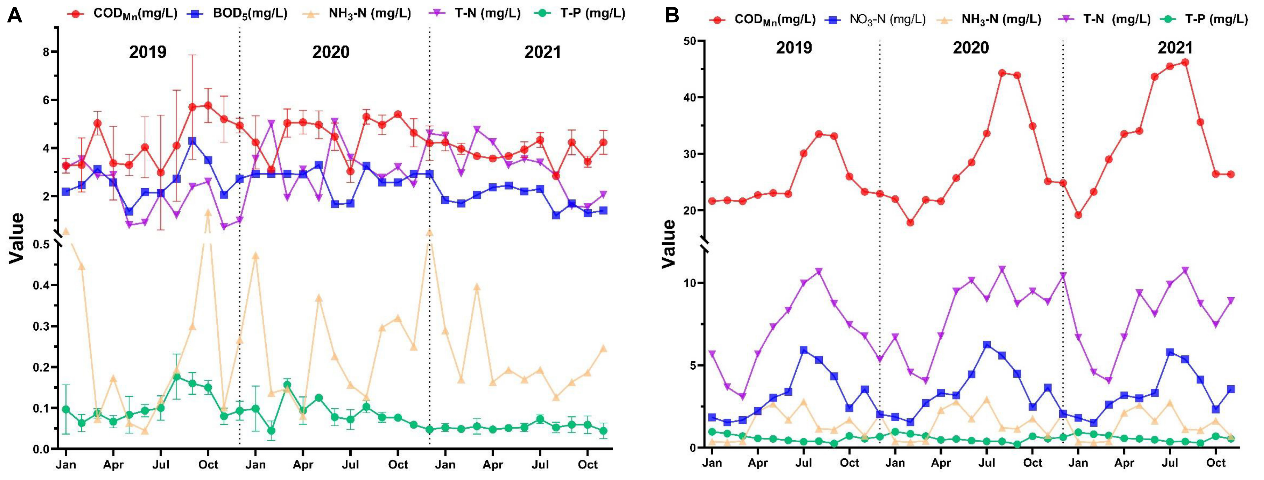

FIGURE 3 | Nutrient concentrations in water of South **(A)** and North **(B)** Taihu Lake.

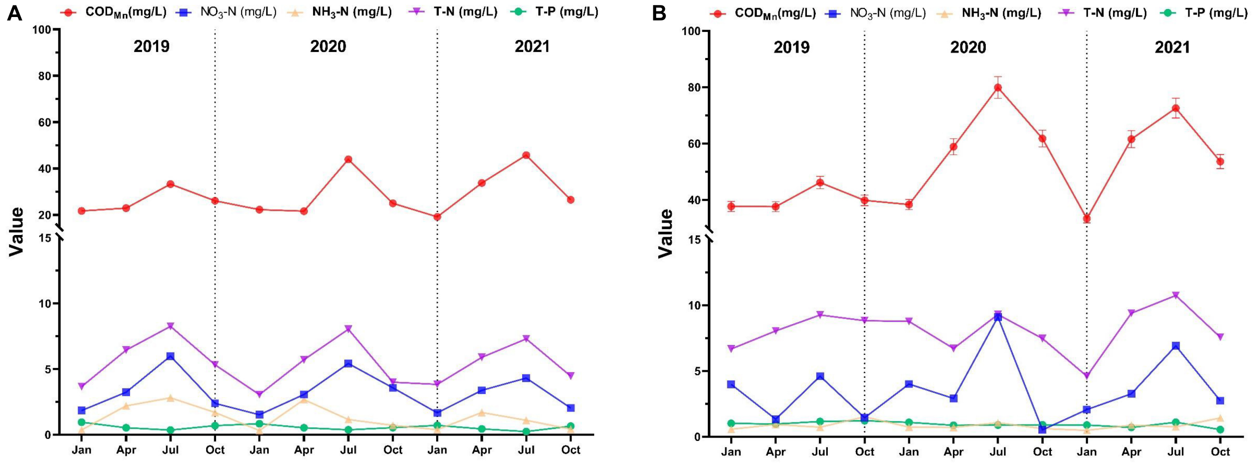

FIGURE 4 | Nutrient concentrations in sediment of South **(A)** and North **(B)** Taihu Lake.

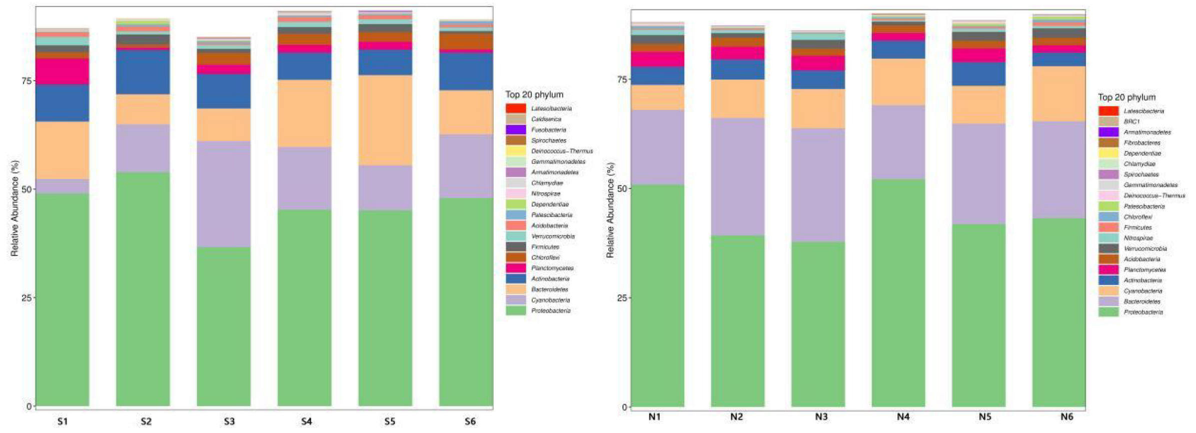

FIGURE 5 | Changes in microbial communities at the phylum level in the sediments of South (S) and North Taihu Lake (N).

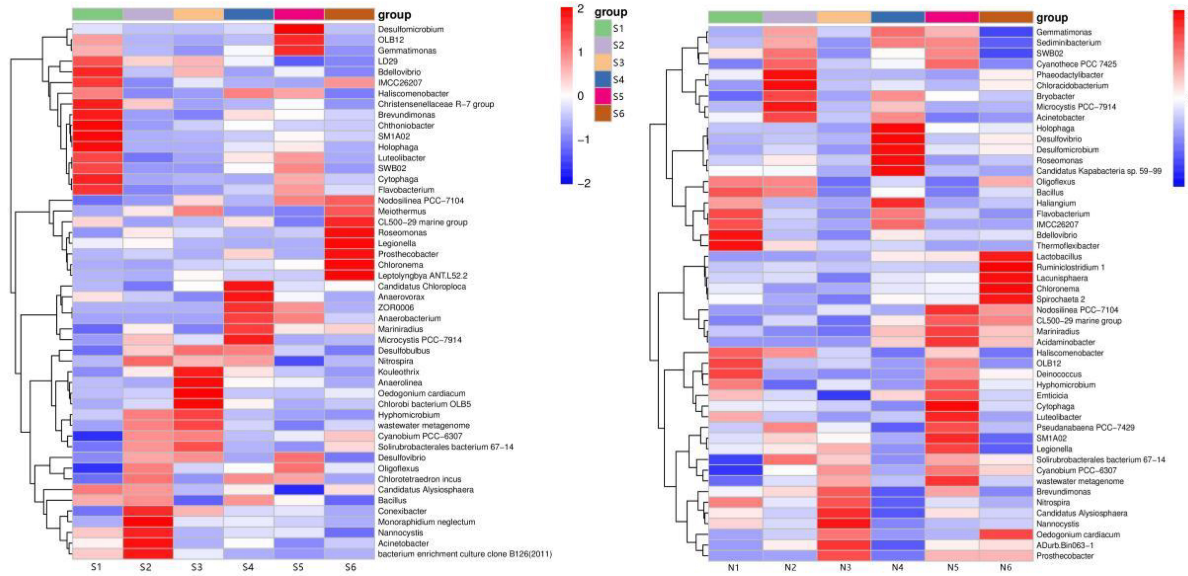

FIGURE 6 | Genus-level heat map-cluster analysis of microbial communities in sediments from South (S) and North Taihu Lake (N).

10.71 mg/L (mean value 6.85 mg/L) and 4.55 to 11.33 mg/L (mean value 7.48 mg/L), respectively. The maximum concentration of DO in South Taihu Lake in winter and the lowest in summer and autumn, and the minimum concentration of DO in North Taihu Lake in winter and the maximum in summer. The conductivity of South Taihu Lake ranged from 15.1 to 48.9 ms/m (average value 34.9 ms/m), and the conductivity fluctuated irregularly from year to year, with the lowest conductivity in autumn. The turbidity of North Taihu Lake ranged from 10.22 to 47.01 NTU (mean value 28.98 NTU), and the overall turbidity was high, especially in summer and autumn, indicating that the water body was turbid.

Water Quality Nutrient Level and Change

The changes of water quality nutrient salts in the southern and northern regions of Taihu Lake, observed with the data monitored from January 2019 to November 2021, are shown in **Figure 3**. The annual COD_{Mn} in South and North Taihu Lake ranged from 2.833 to 5.767 mg/L (mean value 4.214 mg/L) and 17.817 mg/L to 46.208 mg/L (mean value 23.838 mg/L), respectively; the amount of NH_3-N ranged from 0.045 to 1.337 mg/L (mean value 0.259 mg/L), 0.315 to 2.937 mg/L (average value 1.417 mg/L); the amounts of TN were 0.720–5.083 mg/L (average value 2.830 mg/L), 3.075–10.815 mg/L (average value 7.625 mg/L); the amounts of TP were 0.044–0.177 mg/L (average value 0.082 mg/L), 0.204–0.964 mg/L (average value 0.574 mg/L); NO_3-N in North Taihu Lake is 1.514–6.255 mg/L (average value 3.298 mg/L).

According to the Environmental Quality Standard for Surface Water (GB3818-2002), the amounts of COD_{Mn} and TN in North Taihu Lake exceeded the limits of Class V water standard. The amount of NH_3-N in spring and summer exceeds the standard limit for Class V water, and the amount of TP also exceeds the standard limit for Class V water most of the time. The amount of TP in South Taihu Lake exceeds the standard limit of Class V water, and the amount of TN also exceeds the standard limit of Class V water most of the time. It can be seen that both South and North Taihu Lake are polluted to different degrees. While comparing the nutrient levels of water in North and South Taihu Lake, it can be found that North Taihu Lake is more polluted and has higher N and P content. This is consistent with the conclusion of higher pollution in the northern part of Taihu Lake mentioned in previous studies (Cheng et al., 2005; Chen et al., 2018; Luo et al., 2020).

Sediment Nutrient Levels and Trends

The data monitored from January 2019 to November 2021 were used to observe the changes of sediment nutrient salts in the southern and northern regions of Taihu Lake. In **Figure 4**, it can be obtained that the annual COD_{Mn} in South and North Taihu Lake ranged from 19.143 to 45.764 mg/L (mean value 28.472 mg/L) and 33.370 to 79.924 mg/L (mean value 51.796 mg/L), respectively; the amount of NH_3-N ranged from 0.324 to 2.819 mg/L (mean value 1.299 mg/L) and 0.483–1.515 mg/L (mean value 0.869 mg/L); the amounts of NO_3-N were 1.529–5.994 mg/L (mean value 3.207 mg/L), 0.524–9.297 mg/L (mean value 3.597 mg/L); the amounts of TN were 3.058–8.259 mg/L (mean value 5.500 mg/L), 4.608–10.744 mg/L (mean value 8.114 mg/L); the amounts of TP were 0.242–0.953 mg/L (mean value 0.577 mg/L), 0.548–1.212 mg/L (mean value 0.947 mg/L). The TP in South Taihu Lake exceeded the standard limit of Class V water except for July every year, and the TP in North Taihu Lake exceeded the standard limit of Class V water. It can be

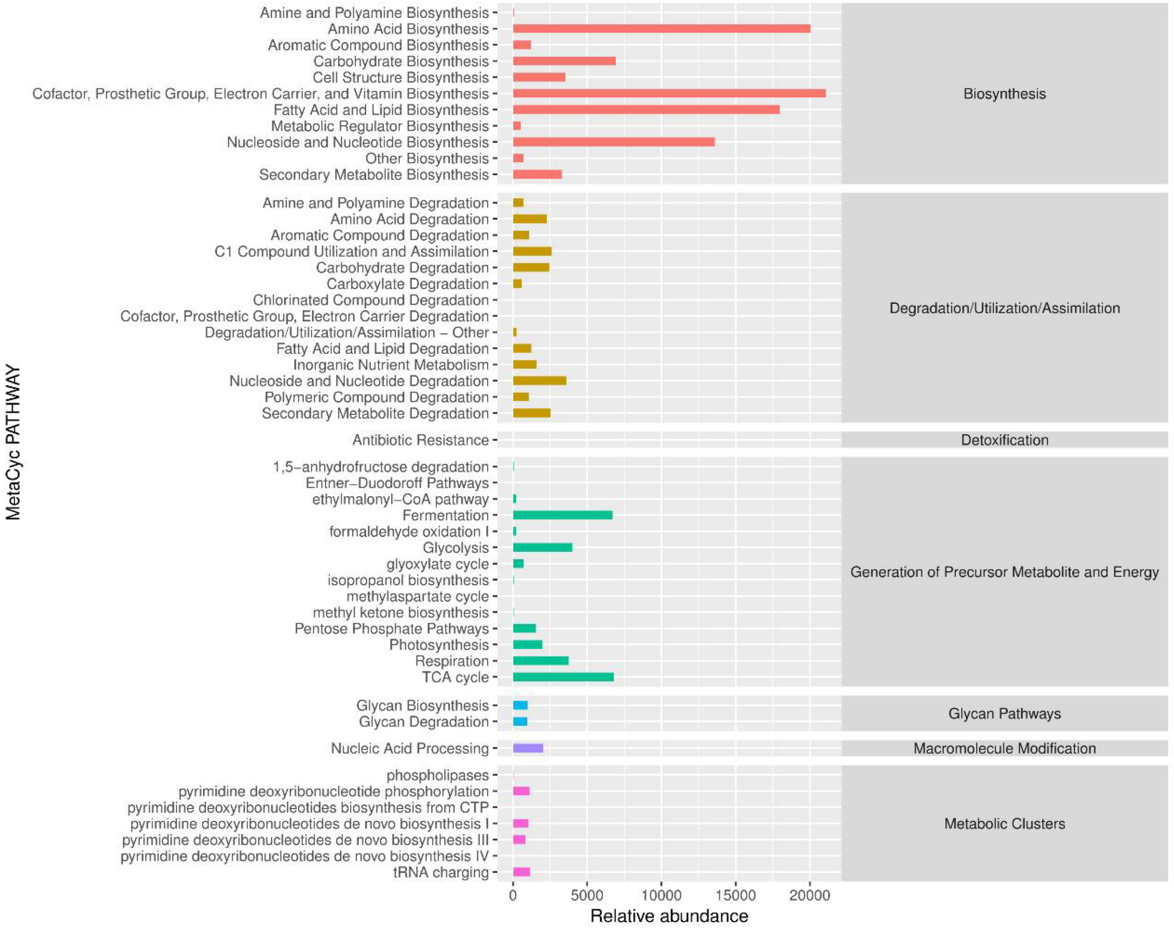

FIGURE 7 | KEGG metabolic pathway abundance statistics.

seen that South and North Taihu Lake have been polluted to some extent, and the contents of COD_{Mn}, TN and TP in North Taihu Lake are basically higher than those in South Taihu Lake, indicating that the pollution in North Taihu Lake is more serious.

Evaluation of Microbial Diversity in Sediments

Types and Roles of Microorganisms

The community structure of various samples at different taxonomic levels in the sampling sites of North and South Taihu Lake was analyzed statistically. The top 20 phyla of relative abundance of bacteria in the samples at the phylum level are shown individually, and the relative abundance distribution of the dominant bacterial groups in the sediments of South (S) and North (N) Taihu Lake at the phylum level is shown in **Figure 5**. Among them, the phyla with greater abundance are *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Actinobacteria,* etc. The sum of the relative abundance of these phyla accounted for more than 80% in each sampling site, and the first phylum in relative abundance in each sampling site was Proteobacteria, indicating that the species composition of microorganisms in the sediment samples of South and North Taihu Lake at the phylum level had some similarity.

The main flora of S1 in the southern part of Taihu Lake were *Proteobacteria* (49.03%), *Bacteroidetes*(13.22%), *Actinobacteria*(8.52%); S2 were *Proteobacteria* (53.93%), *Cyanobacteria*(11.01%), *Actinobacteria*(10.21%); S3 were *Proteobacteria* (36.66%), *Cyanobacteria*(24.44%), *Actinobacteria* (7.96%); S4 were *Proteobacteria* (45.26%), *Bacteroidetes*(15.49%), *Cyanobacteria*(14.47%); S5 were *Proteobacteria* (45.15%), *Bacteroidetes*(20.78%), *Cyanobacteria*(10.36%); S6 were *Proteobacteria* (47.96%), *Cyanobacteria*(14.70%), *Bacteroidetes* (10.14%).

The sampling sites N1–N6 located in the northern part of Taihu Lake were all in the phylum *Proteobacteria* > *Bacteroidetes* > *Cyanobacteria*, with relatively low diversity, and the proportions of the three main phyla were N1 (50.90, 17.13, 5.70%); N2 (39.29, 26.87, 8.79%); N3 (37.80, 25.99, 8.99%); N4 (52.13, 16.97, 10.65%); N5 (41.86, 22.98, 8.67%); N6 (43.26, 22.13, 12.61%). Although the distribution of dominant phyla was the same at each sampling site, the relative abundance content of each phylum varied significantly. And the species composition of microorganisms in sediment samples from North and South Taihu Lake at the phylum level was somewhat different; the microbial diversity in South Taihu Lake was higher than that in North Taihu Lake, indicating that microbial diversity was negatively correlated with the concentration of nutrients. However, there are evidence that the abundance and biomass of various microbial components increases with the eutrophication of aquatic ecosystems (Conty and Bécares, 2013).

Microbial Community Diversity

Microbial diversity is a key element to respond to the state of the ecosystem, and there are significant differences in microbial community diversity in different ecological environments (Wu et al., 2015; Pastorelli et al., 2021). In order to obtain more information about the bacterial community, the standardized processing method of row processing was used based on the Euclidean distance between the relative abundance of dominant species at each sample genus level, and the species clustering algorithm was average, based on the pheatmap package to draw the dominant. The clustering heat map of the dominant species based on the pheatmap package is shown in **Figure 6**.

At the genus level in South Taihu Lake, *SM1A02*, *Holophaga* and *Acinetobacter*, Bacterium enrichment culture clone *B126* were preferentially clustered, and the abundance composition of the six samples between these two groups was similar, with *SM1A02* and *Holophaga* having the highest abundance at S1. And the highest abundance was found at S2 for *Bacteroides* and Bacterium enrichment culture clone *B126*. At the genus level in North Taihu Lake, *Candidatus Alyslosphaera* and *Nannocystis* clustered preferentially before clustering with *Nitrospira spp*. The content of these three genera was mainly concentrated in N3, reflecting a high similarity.

Prediction of Functional Metabolism of Microbial Communities

Combined with the results of PICRUST2 analysis, the metabolic pathway abundance statistics were plotted using the normalized pathway abundance table, as shown in **Figure 7**. The right side of the graph shows the first level pathway to which the pathway belongs, the left side shows the second level pathway, and the bar graphs in the figure express the abundance of each pathway. The first level pathways include Biosynthesis, Degradation/Utilization/Assimilation, Detoxification, Generation of Precursor Metabolite and Energy, Glycan Pathways, Maclromolecule Modification and Metabolic Clusters. The metabolic pathways involved in microorganisms in the sediments of the Taihu Lake study area were mainly biosynthetic, including Amino Acid Biosynthesis, Carbohydrate Biosynthesis, Cofactor/Prosthetic Group/Electron Carrier/Vitamin Biosynthesis, Fatty Acid and Lipid Biosynthesis, Nucleoside and Nucleotide Biosynthesis are the main metabolic pathways of biosynthesis.

Among the Degradation/Utilization/Assimilation pathways, most of the secondary pathways were in balanced abundance, with the highest abundance of Nucleoside and Nucleotide Degradation. In the pathway of precursor metabolite and energy production, fermentation and TCA cycle (tricarboxylic acid cycle) were in higher abundance. The above functional differences suggest that sediment microorganisms in Taihu Lake in the study area are involved in nutrient transport and transformation, and play an important role in lake water purification.

CONCLUSION

The physical and chemical indexes such as pH, TN, TP, and COD_{Mn} of the Taihu Lake show that the pollution degree in the north of Taihu Lake is higher than that in the south. And the pollution mainly occurred in summer. The pollution in southern Taihu Lake was relatively stable from 2019 to 2021. The microbial communities in sediments of the northern and southern Taihu Lake were studied. The results showed that the microbial community structure in sediment samples showed obvious spatial diversity and similarity. The difference is that the relative abundance content of each phylum is different at each sampling point. The diversity of microorganisms was inversely proportional to the degree of eutrophication, while the abundance of microorganisms was positively correlated with the level of nutrients in the study area. The functional differences indicate that the microorganisms in the sediments of Taihu Lake in the study area participate in the migration and transformation of nutrients, which plays an important role in the purification of lake.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

WZu: conceptualization, data curation, and writing – original draft. JL: methodology and software. QL: conceptualization, methodology, and resources. XG: investigation and data curation. LW: investigation and supervision. YG: conceptualization and resources. JS: methodology and data curation. ZZ: conceptualization and supervision. PG: conceptualization, methodology and supervision. WZa: conceptualization, methodology and supervision. All authors contributed to the article and approved the submitted version.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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Review of the Impact of Whale Fall on Biodiversity in Deep-Sea Ecosystems

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"Whale Fall" is a collective term for the whale carcass, the process of dead whale fall, and the formed deep-sea ecosystem. The whale fall process produces a lot of unstable organic matter that has a significant impact on deep-sea ecosystems. Scientists speculate that organic matter input is the source of energy and material for organisms in deep-sea ecosystems. In the seafloor of the North Pacific, whale fall supports the survival of at least 12,490 organisms of 43 species, contributing to the prosperity of deep-sea life. Due to the specificity of the time and space of the formation of whale fall, there are few studies on whale fall and its impact on the deep-sea ecosystem. This article summarizes and analyses the current research status on the distribution of whale fall and its impact on the deep-sea ecosystem at home and abroad. The results show that the current distribution of whale fall is mainly concentrated in the Pacific and Atlantic regions, and the research on the impact of whale fall on deep-sea ecosystems focuses on the formation process, degradation rate and impact on deep-sea biological systems. This article has some significance to the understanding of biodiversity and ecosystem succession in the deep-sea "desert area."

Keywords: whale fall, deep-sea, impact, biodiversity, ecosystems

INTRODUCTION

Whale fall refers to the phenomenon in which whales die and sink to the seafloor of the marine to form an ecosystem. When a whale dies in the ocean, its carcass sinks to the bottom of the ocean. And biologists named this process after the whale fall. Whale fall, together with cold seeps and hydrothermal vents, is called an "oasis" for deep-sea life (Wang, 2020). Normally, the carcass of a whale can support a decomposing-dominated circulatory ecosystem for up to 100 years.

The unique geological features of the deep sea create a variety of habitats that support chemoautotroph communities on the seafloor (Schuller et al., 2004). The deep-sea environment has long been considered to be low-energy, nutrient-poor because the organic inputs are achieved by only a small amount of carbon produced by photosynthesis. However, there are some local areas with high microbial biomass and activity in the deep-sea, in which whale fall is like an "oasis" in the "ocean desert" and has become an important geographical station for the evolution and development of life in extreme deep-sea environments (Jorgensen and Boetius, 2007; Wang, 2021).

Most deep-sea sediments trap about 2–10 g of particulate organic carbon flux per year (Lutz et al., 2007). However, the soft tissue of a 30 t whale carcass contains about 1.2×10^3 kg of active organic carbon that is equivalent to the background organic carbon flux of 100 m^2 of the deep seafloor in 1,000 years (Smith C. R. et al., 2014). It is also estimated that whale carcasses transport organic matters to the deep sea 2,000 times faster than the supply of marine snow. Marine snow is a kind of organic matter production activity mainly composed of organic matter debris, originating from the euphotic layer of the upper ocean. In the deep sea, detritus made up of organic matter is falling like snowflakes, so the process is called "marine snow." Because sunlight cannot reach the deep sea, its creatures rely heavily on marine snow as a source of energy, so marine snow is considered as the foundation of deep-sea ecosystems (Van Dover, 2000; Smith and Baco, 2003).

Based on the studies, the sulfate reduction and sulfide levels in sediments around 0.5 m from 30 t whale carcasses were significantly increased over a period of at least 7 years (Treude et al., 2009a). The large organic inputs produced by whale fall not only show up in lipid-rich skeletons, but also create sulfur-rich habitats in the sediments surrounding the carcasses. The species richness of the biomes formed by whale fall is as high as 407 species, which is slightly lower than that of hydrothermal vents (469 species), but far greater than that of cold seeps (230 species) (Smith and Baco, 2003). Thus, whale fall may fundamentally contribute to deep-sea biodiversity and the dispersal of thiophilic species between sulfur-rich habitats (e.g., hydrothermal vents, cold seeps) and anoxic basins. Whale fall also promotes the transportation of organic matters from the upper ocean to the middle and lower layers of the ocean, playing an important role in the ocean carbon cycle (Smith et al., 1989; Bennett et al., 1994; Butman et al., 1995; Scheltema, 1996).

What is the major distribution of whale falls that have been found in the extreme environment of the deep sea? How do whale falls participate in the formation of the deep-sea ecosystem? How do the organisms interact and how much do they contribute? These are common concerns among whale fall researchers. This article systematically sorts out the relevant research results, summarizes the research related to the distribution of whale fall, analyses the research progress of the impact of whale fall on the distribution of marine organisms, deepens people's understanding of whale fall, and provides certain basis and direction for further scientific research.

DEFINITION OF WHALE FALL

"Where there is a whale falling, there is a birth." Regarding the "whale fall," it itself contains a literary flavor. However, there has never been a clear definition of it in the Chinese scientific community. In 2020, the Institute of Deep-Sea Science and Engineering, Chinese Academy of Sciences discovered a whale carcass about 3 m long in the South China Sea. This is the first time that China has discovered such an ecosystem as whale fall. In other related popular science articles, whale fall is defined as the carcass or remains of a whale; it is also described as a phenomenon or process that when a whale dies, the carcass will eventually sink to the seafloor. Therefore, the definition of whale fall can be expressed as follows: "Whale fall" is a general term for the whale carcass, the process of falling, and the formed deep-sea ecosystem.

Studies have shown that each whale that reaches the seafloor represents the input of energy (Lundsten et al., 2010b). In 1987, the manned submersible "Alvin" discovered a 21-m-long blue whale skeleton during a routine dive. Scientific researchers found a "biological carpet" on its skeleton, including bacteria and worms. The ecosystem that came to be known as "whale fall" was first discovered. The study found that biomes grew and thrived in sulfur-rich sediment around rotting whale carcass or remain (Smith et al., 1989). And whale falls have been found in the deep sea of the Pacific and Atlantic oceans and in fossils from the northeast Pacific Ocean 30 million years ago (Smith and Baco, 2003). Whale fall is considered a stepping stone to the growth of deep-sea creatures (Distel et al., 2000). Studies since the 1850s have shown that whale fall can support a large population of organisms. In the seafloor of the North Pacific, whale fall supports the survival of at least 12,490 organisms of 43 species, contributing to the prosperity of deep-sea life (Liu, 2015). With further research on whale fall, researchers have also carried out a series of exploration experiments. For example, try to artificially implant a whale carcass to further study the effects of whale fall on the deep-sea ecosystem.

THE CHARACTERISTICS AND DISTRIBUTION OF WHALE FALL

Whale's soft tissues and bones hold huge reserves of oil. From prehistoric times to the industrial age, humans have been hunting whales for biofuels. Studying data collected by scientists during the heyday of industrial whaling, Higgs et al. (2011) found differences in the lipid content of bones in different parts of whales. Analysis of the skeletal composition of several large whales found that most of the lipids are concentrated in the skull and caudal vertebrae, while the thoracic vertebrae have relatively little lipid content. In addition, it also was found that the lipid content of whale skeleton varies greatly with the maturity and skeleton of the whale. The skeletal lipid content of young whales is much lower than that of adults. The jaw, skull, and caudal vertebrae are high in lipid (approx. 20–84%); the ribs, scapula, sternum, and lumbar vertebrae are middle (approx. 15–30%); and the thoracic and cervical vertebrae are relatively low (approx. 5–20%). In addition, seasonal changes in whales' physiology and feeding can affect their fat storage. This is evident in the records of whales caught in different seasons. And high-lipid skeleton attracts a large number of thiophilic micro-organisms. This skeletal lipid gradient also corresponds to sites of microbial bioerosion and thus plays an important role in studying whale fall-associated microbial communities (Higgs et al., 2011).

As an energy-rich ecosystem, whale fall has several distinctive characteristics. (a) Whales usually enter the marine food web as relatively intact carcasses after death. (b) Lipid- and protein-rich whale fall contributes a large amount of energy for deep-sea organisms. (c) Whale fall is unevenly distributed in space and time.

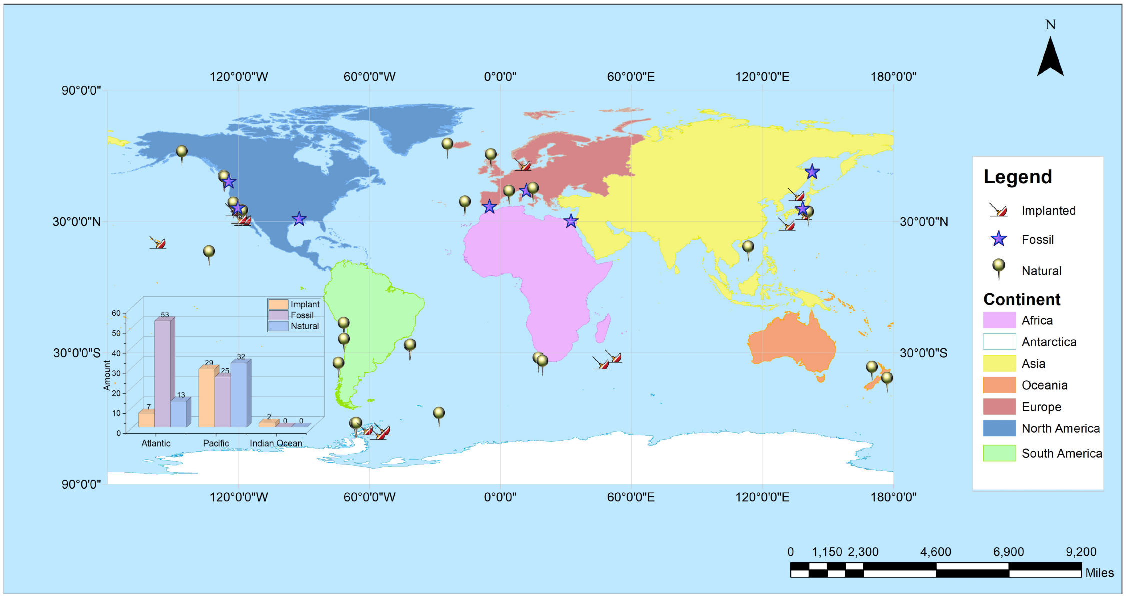

FIGURE 1 | The distribution of currently known whale falls in the world.

Over the past 200 years, whaling has severely reduced the population of large cetaceans, especially between 1860 and 1986. The number of all large whales has decreased sharply, and some species have vanished or are facing extinction (e.g., the North Atlantic gray whale). Whaling has dramatically changed the probability and geographic distribution of whales sinking into the deep sea (Butman et al., 1995, 1996). The decline in whale falls has reduced species diversity and may have contributed to the extinction of species in deep sea ecosystems ranging from whale falls to hydrothermal vents (Hecker, 1985; Mclean, 1985; Craddock et al., 1995). It is estimated that whaling in the 19th and 20th centuries has reduced whale fall habitats by as much as 95%, potentially exterminating up to half of marine basin species that feed on whale carcasses. Some insight into the impact of fluctuations in whale carcass numbers can be gained by studying whale fall ecology and biogeography. However, even such studies fail to shed light on the characteristics of species endangered by whaling. If we hope to explore deep sea wonders that understand ecology and evolution, it is essential to explore distant and unknown deep sea ecosystems to further reduce the impact of anthropogenic factors on marine ecosystems, such as pollution and overfishing. The distribution of currently known whale falls is shown in **Figure 1**. As seen in the Figure, whale falls are mainly distributed in the Atlantic Ocean and the Pacific Ocean. Among the oceans, the Atlantic Ocean has the largest number of whale falls including natural whale falls, implanted whale falls, and fossil whale falls. And the inset shows that the distribution of whale falls in the Atlantic Ocean has a significantly higher number of fossil whale falls than the others; in the Pacific Ocean, the number of whale falls of all three properties is approximately the same, with a higher number of natural whale falls; while the number of whale falls in the Indian Ocean is low, and so far there is no report of whale fall in the Arctic Ocean. With the progress of science and technology, whale falls have been discovered continuously (Smith et al., 1989, 2015; Mclean, 1992; Baco and Smith, 2003; Smith and Baco, 2003; Goffredi et al., 2004; Braby et al., 2007; Lundsten et al., 2010a,b; Smith C. R. et al., 2014; Smith K. E. et al., 2014; Alfaro-Lucas et al., 2017). At the same time, scientists have also implanted whale carcasses to study the community ecology and phylogenetics of the whale fall to further investigate the species supported by whale falls and their impact on deep-sea ecosystems.

EFFECTS OF WHALE FALL ON THE DEEP-SEA BIOMES

It takes approximately 2 years for the soft tissues of whale carcasses to decompose completely. However, exposed skeletons can support specific populations for decades due to their high lipid content (Baco and Smith, 2003; Schuller et al., 2004). The duration for which whale falls maintain their chemosynthetic fauna depends on their lipid content, supporting specific organisms for about 10 years in an oxygen-rich environment and up to 50 years or more in an oxygen-poor environment (Smith and Baco, 2003; Fujiwara et al., 2007; Glover et al., 2010; Lundsten et al., 2010b). Whale skeletons are decomposed by bacteria, producing sulfide that continues to flow into the surrounding seawater and sediments (Treude et al., 2009b). Sulfides provide energy for the chemoautotrophic fauna living on or around whale remains. Whale falls act as carriers for the spread and succession of these organisms.

Smith and Baco (2003), pointed out that there were differences in whale falls formed by different growth stages. The fauna found in young whale skeletons does not appear to be as dependent on chemical autotrophy as larger skeletons (Smith and Baco, 2003). Related studies have shown that the distribution of red bone marrow is inversely proportional to the lipid content of the skeleton. There are more red bone marrow in young whales, and the decrease of lipid content in it will shorten the survival time of whale (Ohe, 1950). Feltmann et al. (1948) pointed out that fragmented whale vertebrae showed "blood spots," which are red bone marrow, the site of mammalian blood cell production, and that bones filled with yellow bone marrow had high lipid content. Tont et al. (1977) also showed that vertebrae filled with red bone marrow had a low lipid content. Honda et al. (1984a,b) found that in young whales, even the caudal vertebrae contained red bone marrow, but as the whales aged, the red bone marrow was gradually replaced by yellow bone marrow and the process started in the caudal vertebrae.

Relevant studies have shown that the enrichment capacity of whale skeleton to nearby sediments is lower than that of other tissues, and that skeletons inhibit microbial lipase activity and reduce bioturbation rates in their surroundings, but increase species diversity of larger animals (Smith et al., 1998). It is worth noting that some deep-sea animals can change their own metabolism, growth rate, feeding behavior, and reproduction to better utilize whale carcasses (Gage and Tyler, 1991; Levin, 2000). The decomposition of whale carcasses delivers large amounts of organic matters to the seafloor and serves as a unique habitat for deep-sea life, contributing to the complexity and biodiversity of the deep-sea environment (Levin, 2000).

Smith et al., collected a total of 2,649 macrofauna individuals from 17 whale fall samples discovered in 1988, of which 143 species were identified, consisting of 1% of macrofauna gathered around the skeleton. Seven of these species were not collected in previous intensive sampling of sediment macrofauna in the Santa Catalina Basin (SCB) and are presumed to be possible alien species, only *Mitrella permodesta* was collected on the whale bones, and the other six species were found within 1 m of the whale skeleton, suggesting the response to the habitat conditions created by the whale fall (Bennett et al., 1994). The average abundance of the macrofaunal community was found to be the highest among the whale fall samples; species diversity showed a clear pattern around the whale skeleton: species richness increased with decreasing distance from the skeleton (Pettibone, 1993).

The local diversity of background sediments in the SCB is low, while the biodiversity near the whale skeleton is significantly increased, with a significant increase in rare species diversity (Connell, 1978; Petraitis et al., 1989). Stockton and DeLaca (1982) hypothesized that concentrated organic inputs from whale falls would lead to the local development of dense communities on the deep seafloor, producing characteristic species structures. Such change in the local benthic population takes many years (Stockton and DeLaca, 1982). So far, only 21 species larger deep-sea animals are found around the whale fall, including 11 species identical to hydrothermal vents and 20 species identical to cold seeps. In addition to the typical molluscan and serpulid communities, whale fall has recorded many new species and evolutionary peculiarities, including *Osedax*, gastropods, and a variety of animals living on sulfur bacteria. The ecosystem formed by whale fall facilitated the development of a deep-sea characteristic fauna, and high species abundance could be reached and maintained for decades.

Biologists have found that scavengers on the seafloor also use lipid-rich bones from whale carcasses or remains for organic synthesis, including many organisms that have been discovered for the first time in science, some of which can directly utilize whale bones as food. Sipuncula worm Phascolosoma saprophagicum feed on lipids in the skeleton (Gibbs, 1987), limpet of *Osteopeltidae* feeds on bacteria that grow on bone tissue (Marshall, 1987), *Osedax* degrades bones for nutrients with the help of endosymbiotic heterotrophic bacteria (Rouse et al., 2004; Goffredi et al., 2010).

EFFECTS OF WHALE FALL ON THE DEEP-SEA MICROBES

Microorganisms account for a significant portion of the Earth's biodiversity (Van Der Heijden et al., 2008). Due to evolutionary pressures, they exist in almost all known environments, some of which are harsh, forcing them to adapt to specific ecological niches (Coughlan et al., 2015). The deep-sea ecosystem is unusual in that it is a high-pressure, low-temperature, low-oxygen environment, where organic matter is mainly low concentrations of stable carbon. Despite the physical and biochemical limitations of the deep-sea, microbial communities still exist in the deep-sea sediments (Li et al., 1999; Vetriani et al., 1999; Dhillon et al., 2005; Knittel et al., 2005; Inagaki et al., 2006). Whale falls deliver large amounts of organic material to the seafloor, and the microbes of the deep-sea habitat into which this organic material enters have unique ecological potential, particularly in terms of interactions between microbial populations and specific pathways that facilitate nutrient cycling. Whale falls provide a source of material for the deep sea. At the same time, the organic enrichment produced by the fall of oil-rich whales stimulates the degradation of oil by bacteria in the sediment (Schuller et al., 2004). To assess the effects of organic enrichment on deep-sea microbial communities, (Goffredi et al., 2010), investigated bacterial diversity in sediments around two whale colonies located in Monterey Canyon at depths of 1,820 m and 2,893 m. Bacteroidetes, *Epsilonproteobacteria* and *Firmicutes* were found mainly in the sediments where the whales landed, compared with the surrounding control sediments 20 m away. In comparison with control sediments 20 m away, *Bacteroidetes*, *Epsilonproteobacteria,* and *Firmicutes* were found mainly in the sediments below the whale fall, while *Gammaproteobacteria* and *Planctomycetes* were found mainly in the control sediments. A large number of *Deltaproteobacteria* were found in both sediments, with *Desulfobacteraceae* and *Desulfobulbaceae* mainly distributed under the whale fall. The bacterial community at 1,820 m depth 7 months after whale fall deposition was less diverse than the reference sediment, with *Deltaproteobacteria*, *Epsilonproteobacteria,* and *Bacteroidetes* accounting for 89% of the community biodiversity (Goffredi and Orphan, 2010). At 70 months, bacterial diversity in the reference sediment near the whale fall at a depth of 2,893 m decreased. In the long term, the impact of the whale fall was also manifested by an increase in total organic carbon and enhanced protein hydrolysis activity, which lasts for at least 17–70 months. The analysis found no significant differences between the bacterial communities gathered around the two whale falls, but differed from the control group, suggesting that the deposition of whale fall biomass has a greater impact on the deep-sea microbial community than a specific benthic location (Smith et al., 1998). Whale falls, as discrete resource blocks, are thought to contribute significantly to deep-sea habitat heterogeneity and may contribute to the proliferation of unique microbial assemblages (Grassle and Morse-Porteous, 1987; Baco and Smith, 2003).

The amount of organic carbon available in most deep-sea microbial communities is very low. However, the level of organic carbon obtained by microorganisms was significantly higher than the amount of primary organic matter produced in surface waters in the local habitats of whale fall. Whale falls thus provide a unique ecological niche that dynamically affects microbial diversity and activity in the associated sediments over time. Whale falls and surrounding macrofaunal communities reinforce the bioturbation of their surroundings. Bacteria are commonly enriched around whale fall habitats, including *Bacteroidetes*, *Firmicutes*, *Epsilonproteobacteria*, *Desulfobacteraceae,* and *Desulfobulbaceae* of the *Deltaproteobacteria*, suggesting that eutrophic deep-sea bacterial communities may lead to biotic community changes. Due to the high oxygen consumption of microorganisms, which is conducive to the formation of anoxic conditions for anaerobic processes such as sulfate reduction and methanogenesis. Whale fall and its surrounding sediments are suitable habitats for sulfide chemical synthesis communities and sulfate reduction and methanogenesis bacteria (Allison et al., 1991). Microorganisms found in deep-sea whale falls are mainly heterotrophic, such as *Vesicomyid clams*, *Bathymodioline mussels,* and *Vestimentiferan tubeworms*, which cover the skeletal surface of whales and form "oases of life" similar to deep-sea hydrothermal vents (Bennett et al., 1994; Deming et al., 1997; Goffredi et al., 2004). The initial formation of whale falls can cause great perturbations in natural environmental conditions, even resulting in a short-term reduction in surrounding diversity (Danise et al., 2012). Over time, the impact of nutrients released from whale carcasses or remains expanded laterally, with some researchers observing an increase in bacterial diversity and total organic carbon (TOC) content at distances of up to 20 m from the whale bones (Goffredi et al., 2010). Related studies have shown that, methanogenic bacteria are predominantly archaea (98%) in whale fall sediments, including *Methanomicrobiales* and *Methanosarcinales* (Onishi et al., 2018). Temporal changes in this archaeal community included the early establishment of methylotrophic methanogens followed by development of methanogens thought to be hydrogenotrophic, as well as members related to the newly described methanotrophic lineage, ANME-3. Chemical analysis revealed elevated methane and depleted sulfate concentrations in the sediments under the whale-fall, as compared to surrounding sediments. Carbon was enriched (up to 3.5%) in whale-fall sediments, as well as the surrounding sea floor to at least 10 m, forming a "bulls eye" of elevated carbon (Goffredi et al., 2008). Studying the diversity and distribution of natural microbial communities in sediments of permanently low-temperature deep-sea environments is crucial to our understanding of global biogeochemical cycles, especially the decomposition and cycling of organic carbon.

CONCLUSION

Whale fall has a significant effect on the deep-sea biological community, which is beneficial to the diffusion and succession of deep-sea organisms. Whale fall also supports special fauna and provides the basis for the reproduction, survival and evolution of some deep-sea sulfur-loving microorganisms. And whale skeletons have played an important role in speciation and greatly enhance biodiversity. Despite great advances in whale fall research, there are still significant gaps in our understanding of the microbial processes, reproductive strategies, population genetics, and biogeography that contribute to whale fall. Therefore, it is important for us to explore a series of mysterious and unknown deep-sea ecosystems such as whale falls and understand the complex dynamics of community change in the deep-sea environment.

AUTHOR CONTRIBUTIONS

QhL: conceptualization, data curation, and writing – original draft. YL: methodology and software. GL: conceptualization, methodology, and resources. ZW: investigation and data curation. ZZ: investigation and supervision. YS: conceptualization and resources. NL: methodology and data curation. QiL: conceptualization and supervision. WZ: conceptualization, methodology, and supervision. All authors contributed to the article and approved the submitted version.

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Effects of Solar Radiation on the Cyanobacteria: Diversity, Molecular Phylogeny, and Metabolic Activity

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Cyanobacteria bloom is a global aquatic ecological problem that seriously threatens human health and social development. The outbreak of cyanobacteria bloom is affected by various environmental factors, among which light dose is an essential factor. In this study, the growth changes of cyanobacteria under different amounts of natural light were studied by simulating different depths of Taihu Lake, and we used 16S rRNA and non-targeted metabolomics for sequencing to reveal the effects of light on the diversity of cyanobacteria and coexisting microorganisms, and to analyze the changes of related genes, functional structures and internal metabolism involved in nitrogen cycling. The result shows that excessive and insufficient light could limit the growth, photosynthesis, and EPS secretion of cyanobacteria, resulting in an antioxidant stress response. At the same time, the amount of natural light affects the vertical distribution of cyanobacteria, and under the condition of 1/3 natural light, cyanobacteria first appeared to float. In addition, the amount of natural light affects the diversity, abundance, and metabolites of cyanobacteria and coexisting microorganisms, and the expression of *nifH*, *nirK*, and *nirS*, three nitrogen-fixing genes, is significantly different in different genera. This study provides valuable information on the molecular mechanism of the effects of the amount of natural light on cyanobacteria bloom.

Keywords: visible light, cyanobacteria bloom, metabolic activity, nitrogen-fixing gene, microbial diversity

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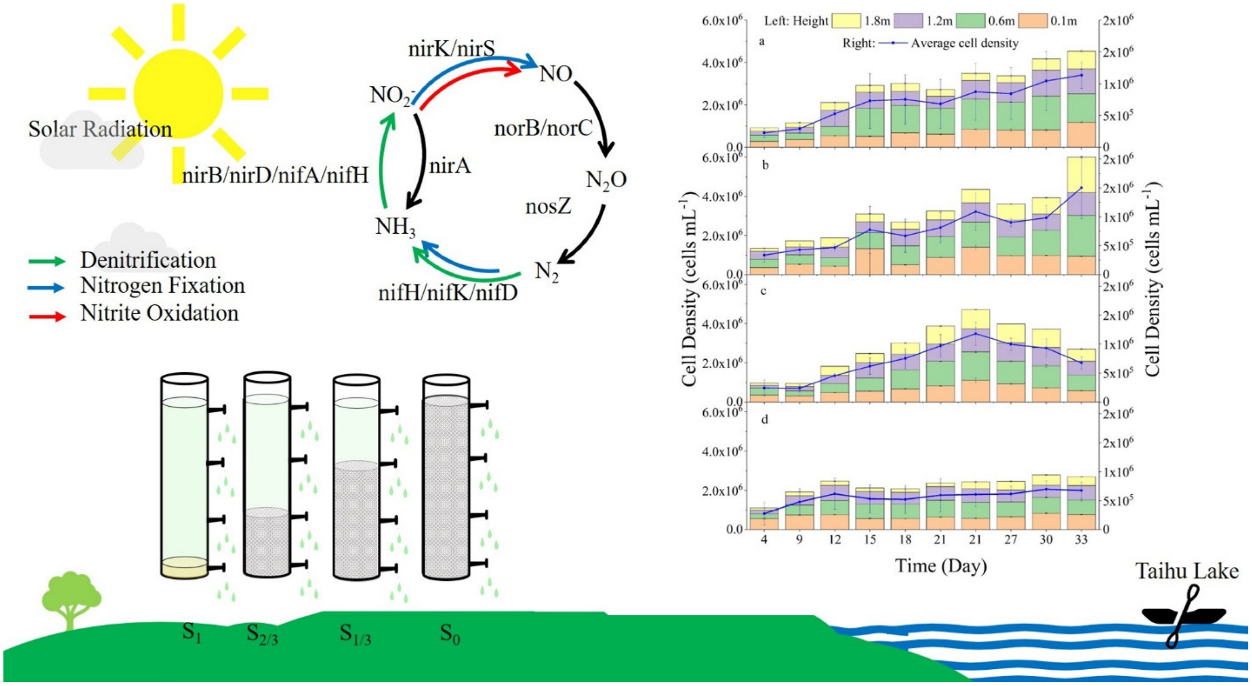
HIGHLIGHTS

- Insufficient and excessive light affects the growth of algae cells.
- Light dose affects the microbial community structure of symbiosis with algae.
- The amount of light affects the expression of metabolites and related nitrogen-fixing genes of microorganisms.

INTRODUCTION

Cyanobacteria are one of the earliest organisms on earth and are considered significant contributors to the formation and nitrogen fixation of the earth's atmosphere (Sylvain et al., 2013). Cyanobacteria have a series of unique physiological characteristics, such as nitrogen fixation mechanism, temperature and light adaptation mechanism, and suspension mechanism,



GRAPHICAL ABSTRACT |

enabling them to occupy a competitive advantage in a relatively harsh environment. Furthermore, cyanobacteria blooms are easy to form in freshwater lakes and reservoirs in summer (Hou et al., 2021). In recent years, the frequent occurrence of cyanobacteria blooms in Taihu Lake in China has seriously threatened ecosystem security and human health, making more research scholars want to uncover the underlying mechanism and explore more effective prevention measures.

The environmental factors affecting cyanobacteria bloom are complex, and light dose is considered to be one of the most critical environmental factors (Figueredo and Giani, 2009; Desortovaa and Pun Ocha, 2011). Early studies consider cyanobacteria to be the dominant species under high turbidity and insufficient light conditions (Havens et al., 2003) and confirm that some cyanobacteria could lie dormant for several months in the absence of light and grow rapidly immediately after light restoration (Montechiaro and Giordano, 2006). At the same time, the light will affect the vertical distribution of cyanobacteria in the water. Cyanobacteria through the continuous assembly and rupture of intracellular air sacs suspend in appropriate water depth to obtain the optical light conditions so that they can reproduce in large numbers (Wallace et al., 2000; Pinilla, 2006). At present, most studies are based on laboratory control of light dose to explore its influence on the growth of cyanobacteria (Kruger et al., 1981; Wiedner et al., 2003; El Semary, 2010; Salvador et al., 2016). However, the difference between the growth of cyanobacteria under natural light and indoor culture is obvious, so it is more practical to discuss the physiological and biochemical characteristics of algae under natural light.

Cyanobacteria is one of the most widespread nitrogen-fixing microorganisms, playing a pivotal role in nitrogen fixation in the water environment, and the energy needed for nitrogen fixation is mainly supplied by photosynthesis (Kuypers et al., 2018). Plants, eukaryotes, prokaryotes, and other organisms coexisting in the water environment where cyanobacteria live are also involved in the nitrogen fixation process. Plants can form chloroplasts for photosynthesis by forming endosymbiosis with cyanobacteria. Eukaryotes have also been reported to achieve nitrogen-fixing symbiosis with cyanobacteria. Cyanobacteria can sit on the cell wall of a kind of single-celled algae, donating fixed nitrogen and receiving fixed carbon in return (Thompson et al., 2012; Martínez-Pérez et al., 2016). It has also been widely reported that prokaryotic microorganisms participate in nitrogen fixation, nitrification, and denitrification in the process of nitrogen cycling, and the related links are mainly driven by microorganisms through regulating the corresponding enzymes encoded by their functional genes (Garnier et al., 2006; Zhong et al., 2017). For example, most ammonia-oxidizing bacteria belonging to *Betaproteobacteria* and *Gammaproteobacteria* can encode ammonia monooxygenase (AMO) to oxidize ammonia to hydroxyl amine (Hooper et al., 1997; Arp and Stein, 2003). *Proteobacteria* and *Verrucomicrobia* have a variety of methano-oxidizing bacteria that can encode hydroxylamine oxidoreductase (HAO) to convert hydroxyl amine into NO, which can be further oxidized to nitrous acid or reduced to N_2O. The diversity of microorganisms involved in the N cycle can be discovered in these relevant reports (Nyerges and Stein, 2009; Maalcke et al., 2014). Meanwhile, the responses of microbial N function genes to environmental changes in different ecosystems are different (Tu et al., 2016; Kou et al., 2019; Yu et al., 2019), which contains oxygen produced by photosynthesis may affect the activity of enzymes involved in nitrogen fixation (Berman-Frank et al., 2003). However, there is a lack of research on the effects of natural light on the expression of nitrogen-fixing genes and metabolites of cyanobacteria and coexisting microorganisms.

The research on cyanobacteria mainly focuses on the effects of nutrient salts and other factors on algal growth and bloom formation, while studies on the relationship between algae and light have mainly focused on experimental simulations, lacking the effects of natural outdoor light on algal growth. In particular, there is a lack of studies on the effects of light on the algae and algae symbiosis microbial diversity distribution, metabolites, and nitrogen fixation gene expression. Therefore, in this paper, we investigated under different amounts of natural light the physiological and biochemical responses of *Microcystis aeruginosa* at low concentrations as well as the expression changes of the diversity of coexisting microorganisms, metabolic process, and nitrogen-fixing process-related genes. The main research contents are as follows: (1) growth and photosynthesis of algae cells; (2) EPS secretion and protein synthesis of algal cells; (3) Changes in antioxidant enzyme system in algae cells; (4) Diversity analysis of coexisting microorganisms in the growth environment of algae cells; (5) Correlation analysis between metabolites and microorganisms; (6) Expression of genes related to microbial involvement in nitrogen fixation. This study reveals the influence mechanism of natural light on the growth of cyanobacteria and the formation of algal blooms.

MATERIALS AND METHODS

Experimental Materials

Microcystis aeruginosa is a microcystin-producing typical alga. The *Microcystis aeruginosa* (FACHB-905) used in this study was purchased from Wuhan Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Plexiglas columns were cleaned and filtered (filter membrane 0.45 μm) Taihu Lake water was added, followed by indoor cultures of *Microcystis aeruginosa.* The culture conditions were direct cultivation under natural field conditions.

Experimental Design

The experimental device is shown in **Figure 1**. The experiment was carried out in four plexiglass cylinders (height: 200cm, diameter: 20cm), with an injection port set at the bottom of cylinder, and four sampling outlets set at heights of 10, 60, 120, and 180 cm, respectively (the average water depth of Taihu lake is 1.8 m). The culture (100mL) is added to the experimental device in batches and inoculated *Microcystis aeruginosa* into a sterilized plexiglass water column (60 L) at an initial cell density of 1.0×10^4 cells/mL. Four experiments adopted four different natural light conditions (full light S_1, 2/3 amount of light $S_{2/3}$, 1/3 amount of light $S_{1/3}$, and no light S_0, respectively).

The experiment was repeated three times for each group, and the test cycle was 33 days. During the experiment, the average daytime light intensity at the location of the experimental device was 1.01 × 105 lx measured by an illuminometer (9:00–10:00 am, 0.38×10^5 lx; 11:00–12:00 am, 1.01×10^5 lx; 2:00–3:00 pm, 1.04×10^5 lx). To ensure the light dose and experimental accuracy, the experimental device was placed on the open field on the shore of Zhushan Bay in the northwest of Taihu Lake (120.087969°E, 31.469109°N). We took 30 mL water samples from each of the four sampling outlets every three days for analysis and evaluated the effects of light on diversity, molecular phylogeny, and metabolic activity of *Microcystis aeruginosa*.

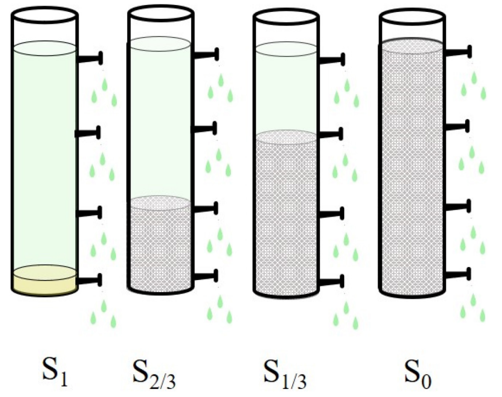


FIGURE 1 | Schematic diagram of the experimental device.

Measurements of Total Chlorophyll, Extracellular Polymeric Substances

The total chlorophyll content (chlorophyll-a) was measured by ethanol extraction according to the method of Chao (Chao et al., 2008). Extracellular polymeric substances include the soluble extracellular polysaccharides (sEPS) and the bound extracellular polysaccharides (bEPS). EPS extraction for protein quantification and analysis was performed as follows: 10 mL of sample solution were centrifuged at 2500 g for 15 min (VELOCITY 14R, Dynamica, Australia) and the supernatant was collected to measure sEPS content. Then, the precipitate was suspended in a 0.05% NaCl solution, Ultrasonicated (SB-3200D, SCIENTZ, China) at 150 W for 60 s, and centrifuged at 12000 g for 15min. The resulting supernatant was carefully collected for the measurement of bEPS. The concentrations of the two forms of EPS were analyzed using the phenol-sulfuric acid method.

Enzyme Activity and Total Protein Content Analysis

To establish the concentration and activity of plant enzymes, 20 mL algal cells were collected from each group and centrifuged at 2500 × g for 15 min. Then transferred into PBS (0.01m) and homogenized at 12000 r·min-1 for 2 min (FSH-2a, XINRUI INSTRUMENT, 3). Disrupted cells and centrifuged at 12000 *g* at 4°C for 15 min to obtain supernatants. The activity of the SOD, CAT, and POD was assessed in supernatants using a commercialized chemical assay kit: TP assay kit, SOD assay kit, CAT assay kit, POD assay kit (Nanjing Jiancheng Bioengineering Institute, China).

Sequencing and PCR Amplification

A sample of 200 mL was collected and filtered through a 0.45 μm fiber membrane, which was collected and used for testing. Total genomic DNA samples were extracted using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, United States), following the manufacturer's instructions, and stored at −20°C prior to further analysis. Then, the extracted genomic DNA was detected by 1% agarose gel electrophoresis. PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, United States). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2250 bp sequencing was performed using the Illumina NovaSeq platform with NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China). Referring to previous studies, the selection of amplification primer sequences was shown in **Table 1** (Cantera and Stein, 2007; Zhang et al., 2017).

Untargeted Metabolomics

A sample of 500 mL was collected and filtered through a 0.45 μm fiber membrane, which was collected and used for testing. Take a sample of the filter membrane and put it into a 2 mL EP tube, accurately add 3 mL of acetonitrile: isopropanol: Water (3:3:2, V/V/V) Mixed solution (−20°C), and add 3–4 2 mm zirconium beads; Put it into a high flux tissue grinder, shocked at 30 Hz for 20 s, allowed to stand for 10 s, cycled eight times, and sonicated in an ice water bath for 5 min; After centrifugation for 2 min, 500 μL of the supernatant solution is taken and added into a new 2 mL EP tube. The vacuum concentrator was concentrated to dryness (8-10 h), And the remaining supernatant was placed on a −80°C refrigerator for backup; Add 80 μL of 20 mg/mL MEOX solution for redissolution, vortex vibration for 30 s, and incubate at 60°C for 60 min; Finally, add 100 μL BSTFA-TMCS (99:1) reagent, react at 70°C for 90 min, emulsion at 14 000 RPM for 3 min, And add 90–100 μL of supernatant into the detection bottle; Samples were placed in sealed cuvettes to be tested and processed for GC-TOF upper detection within 24 h. Gas Chromatography was performed on a DB-5ms Capillary column (30 m × 250 μm I.D., 0.25 μm film thickness, Agilent J & W Scientific, Folsom, CA, United States) to separate the effects at a constant flow of 1 mL/min helium. 1 μL of the sample was injected in a split mode in A 1:10 Split ratio by the auto-sampler. The injection temperature was 280°C. The temperature of the transfer line ion source was 320°C and 230°C, respectively. The programs of temperature-rise were followed by an initial temperature of 50°C for 0.5 min, 15°C/min rate up to 320°C, and Staying at 320°C for 9 min. Mass spectrometry was performed using a full scan method with a scan rate of 10 spec/s, electron energy of −70 V, and a solvent delay of 3 min.

TABLE 1 | Primer information.

Gene	Forward primer	Reverse primer	Product length
nifH	AAAGGYGGWATCGGYAARTCCACCAC	TTGTTSGCSGCRTACATSGCCATCAT	458
nirK	GGMATGGTKCCSTGGCA	GCCTCGATCAGRTTRTGG	515
nirS	GTSAACGTSAAGGARACSGG	GASTTCGGRTGSGTCTTGA	407

Statistical Analysis

GraphPad Prism Software was used to process data, while data was analyzed using SPSS software (Version 22.0, SPSS Inc., Chicago, IL, United States). Comparisons between different treatments (flow and static) were performed by two-way analysis of variance (ANOVA) and subsequent Fisher LSD comparison tests. Both bacterial community structure and abundance in V. natans leaf biofilms were assessed using the online platform Majorbio I-Sanger Cloud Platform.[1] Each experiment was performed in triplicate and repeated at least 3 times.

RESULTS AND DISCUSSION

Changes of Cell Density and Chlorophyll A Content

Algal cell density is often used to measure the algae growth. To study the effect of the amount of natural light on the growth of *Microcystis aeruginosa*, we compared the cell density of *Microcystis aeruginosa* at different water depths (1.8, 1.2, 0.6, 0.1m) under four natural light conditions (**Figure 2**): full light (**Figure 2A**), 2/3 amount of light (**Figure 2B**), 1/3 amount of light (**Figure 2C**), and no light (**Figure 2D**). The results showed that the growth of *Microcystis aeruginosa* varied significantly with different amounts of light and different water depths. By comparing the average density of algae in the four groups, we found that the algae growth was the best under 2/3 amount of light in each water depth. On the 33rd day, the algae density from top to bottom reached 1.81×10^{6} cells/mL, 1.16×10^{6} cells/mL, 2.09×10^{6} cells/mL, and 0.94×10^{6} cells/mL, and the average density of algae was the highest among the four groups (1.50×10^{6} cells/mL). This indicated that the growth of algal cells is best under 2/3 amount of light, and excessive or insufficient natural light affects the growth of algae cells. Furthermore, we believe that the growth rate of algae cells in the lower layer is lower than that in the upper layer (0.1 m < 0.6 m < 1.2 m < 1.8 m deep water), which may be due to the blocking of natural light by the algae cells in the upper layer, affecting the photosynthesis of the algae cells in the lower layer (Havens et al., 2003). Under the condition of no light, the algae density in each water depth was lower than that in other groups and different from the other three groups, the algae density showed a trend of increasing first and then decreasing, and reached the maximum on the 21st day. This indicates that insufficient light would limit the growth of algae cells and accelerate the decay of algae cells.

Through culturing *Microcystis aeruginosa* under different light intensities, found that when the incident light dose was less

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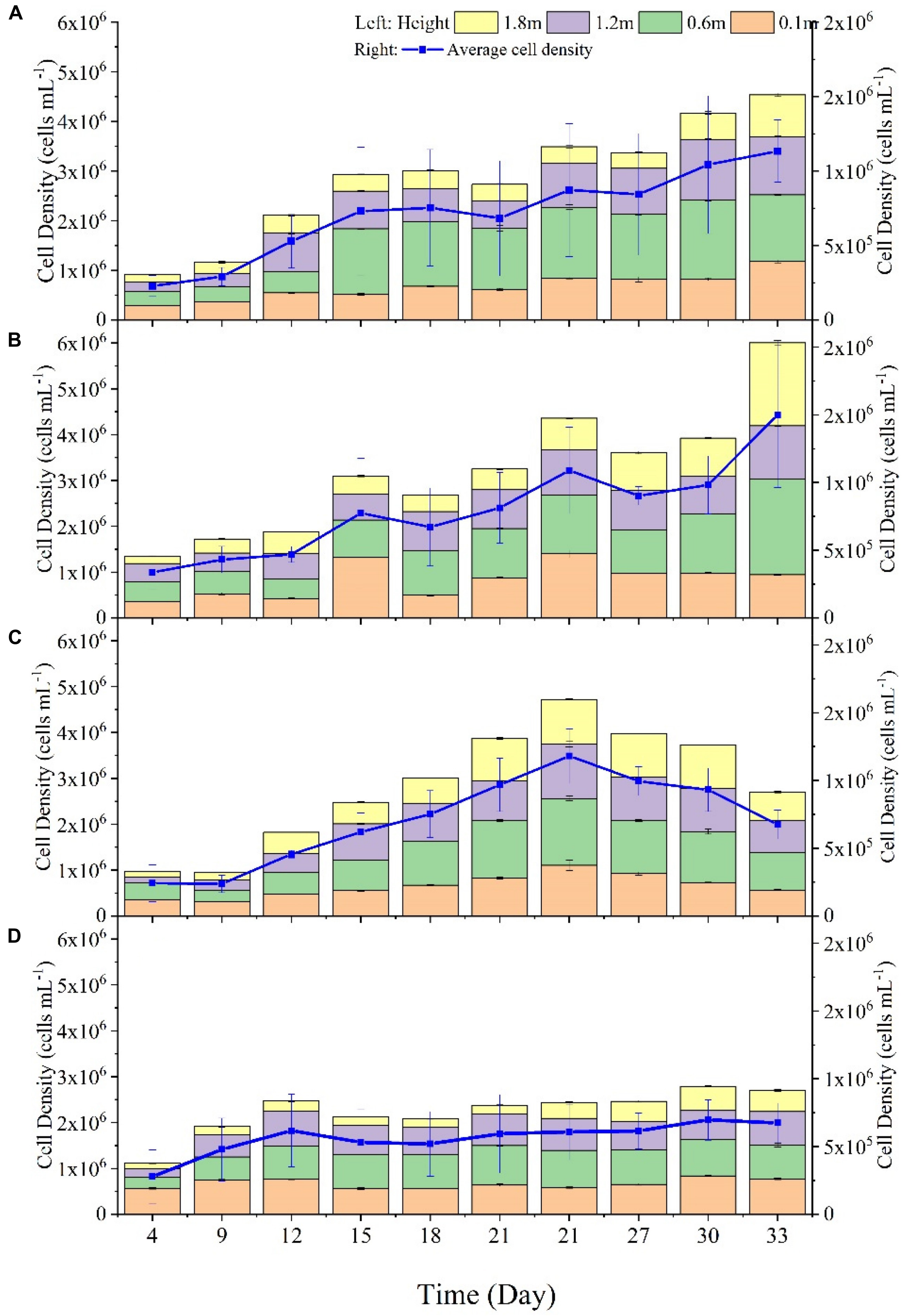

FIGURE 2 | The cell density of *Microcystis aeruginosa* at different water depths (1.8, 1.2, 0.6, 0.1m) under four natural light conditions: full light **(A)**, 2/3 amount of light **(B)**, 1/3 amount of light **(C)**, and no light **(D)**.

than 120 lx, the specific growth rate of *Microcystis aeruginosa* was negative, which means the extinction trend appeared (Chen et al., 2016). In the enclosure of Microcystis blooms in summer, Sun Yang-cai et al. (2007) shaded the water surface to reduce the light dose at 0.1 m depth to 800 lx, which makes the concentration of chlorophyll a in the enclosure decrease from 176.9 μg/L before

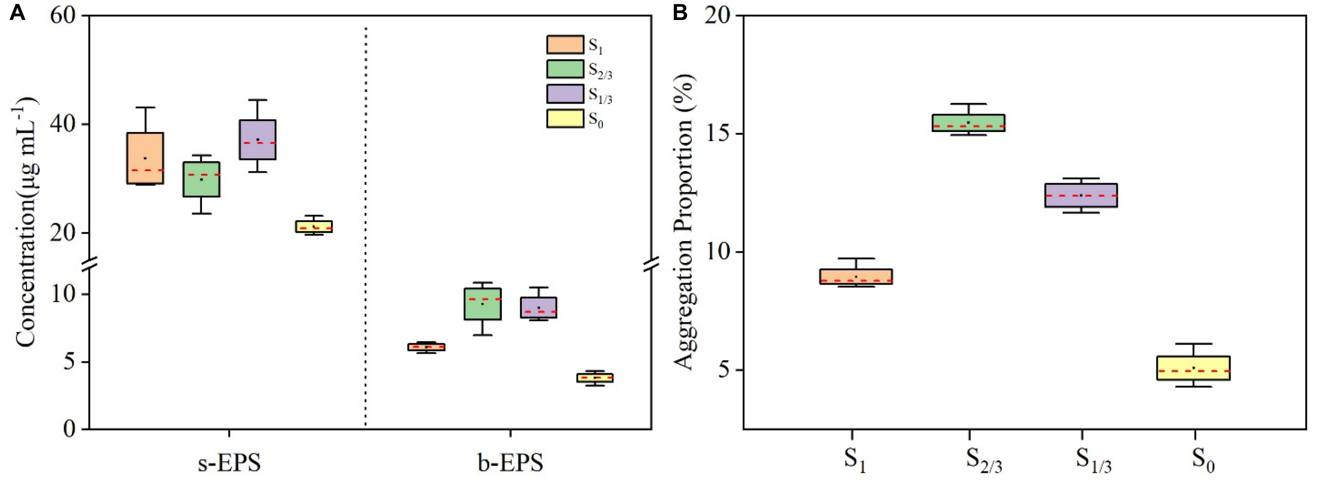

FIGURE 3 | Changes of soluble EPS (S-EPS) in the growth environment of algae and binding EPS (B-EPS) on the surface of algae cells contents **(A)** and aggregation proportion **(B)** in each group. The upper and lower boundaries represent the smallest observed value (lower edge) and the largest observed value (upper edge), the black dot in the middle is the mean, and the red dotted line is the median line.

shading to 21.4 μg/L after 7 days. In conclusion, the growth of algal cells is closely related to the amount of natural light, and both excessive and insufficient light can limit the growth and photosynthesis of algal cells (Havens et al., 2003).

Secretion Variation Characteristics of Extracellular Polysaccharides

Microcystis aeruginosa can secrete EPS to form a protective film on the cell surface to cope with external stimuli. EPS has strong viscosity and is closely related to the formation of the *Microcystis aeruginosa* community (Xiao et al., 2018). The main components of EPS in algae cells are polysaccharides and proteins, as well as a small amount of lipids and DNA (Phélippé et al., 2019). To further study the influence mechanism of the amount of natural light on the growth of *Microcystis aeruginosa*, on the last day, we measured the contents of soluble EPS (s-EPS) in the growth environment and binding EPS (b-EPS) on the surface of algae cells (**Figure 3A**) and the aggregation proportion of algae cells (**Figure 3B**). The results showed that the concentration of s-EPS was higher than that of b-EPS under different amounts of natural light, and the concentration of s-EPS was between 19.68 and 41.10 μg/mL, and that of b-EPS was between 3.25 and 10.86 μg/mL. The concentrations of the two EPS were significantly lower than those in other groups under the condition of no light (S_0 group). The amount of EPS secreted by algal cells will affect the size of cells aggregation proportion, thus changing the morphology of the cell community. We compared the agglomeration rate of algae cells in each group (**Figure 3B**) and found that under the condition of 2/3 amount of light ($S_{2/3}$ Group), the aggregation proportion of algae cells reached the highest, which was positively correlated with EPS concentration. Studies have shown that the acetyl group in polysaccharide components of EPS is an important factor affecting the hydrophobicity and adhesion of algae cells, which can promote the aggregation of algae cells (Chen et al., 2016). The aggregation proportion in the S_0 group was significantly lower than that of other groups, indicating that insufficient light could reduce the secretion of EPS, and the reduction of EPS could reduce the aggregation proportion of algae cells.

Changes of Antioxidant System and Malondialdehyde Concentration

In order to further explore the effects of different amounts of natural light on the growth of cyanobacteria cells, this study compared the levels of four common antioxidant stress indicators, including total reactive oxygen species (ROS), superoxide dismutase (SOD), peroxidase (POD) and malondialdehyde (MDA), in each group on the 30th day (**Figure 4**). The results showed that the concentrations of ROS and MDA were lowest under the condition of two-thirds amount of light, and all indexes reached the maximum values under no light condition, indicating that 2/3 amount of light was the most suitable for the growth of algae cells. The stress degree of algae cells is the most severe under no light condition, which can stimulate the activities of SOD and POD in the antioxidant enzyme system to remove ROS in cells and alleviate the oxidative damage of algae cells (Romeo et al., 2000). Other sources of ROS include the oxidation of glycolic acid and the degradation of fatty acids. Alpha Hydroxyl Acid, the final product of chloroplast photorespiration, enters the peroxisome and is oxidized to glycolic acid with the help of O_2 to produce H_2O_2. H_2O_2 is also produced when fatty acids are degraded by acyl-coA oxidase. ROS produced by peroxisomes are used for seed and pollen germination, fruit ripening, senescence, and stomatal movement (Anuj et al., 2020).

In addition, when the external stress exceeds the balance capacity of the cellular antioxidant system, the oxygen free

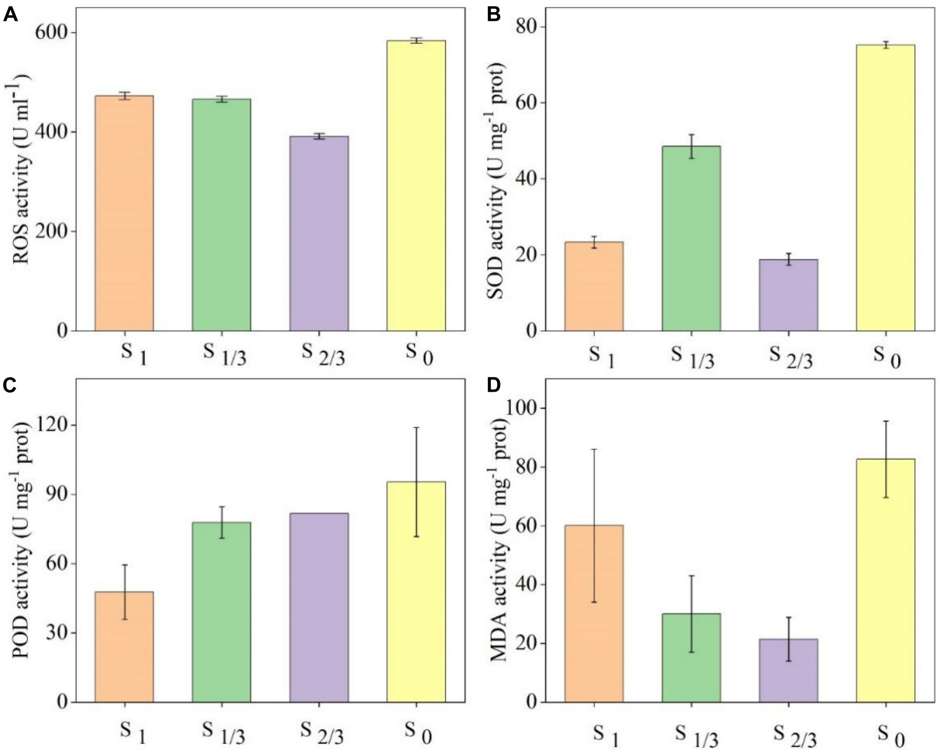

FIGURE 4 | Oxidative stress indexes of algae cells grown under different amounts of light: **(A)** ROS content, **(B)** and the concentration changes of superoxide dismutase (SOD), **(C)** peroxidase (POD), **(D)** malondialdehyde (MDA).

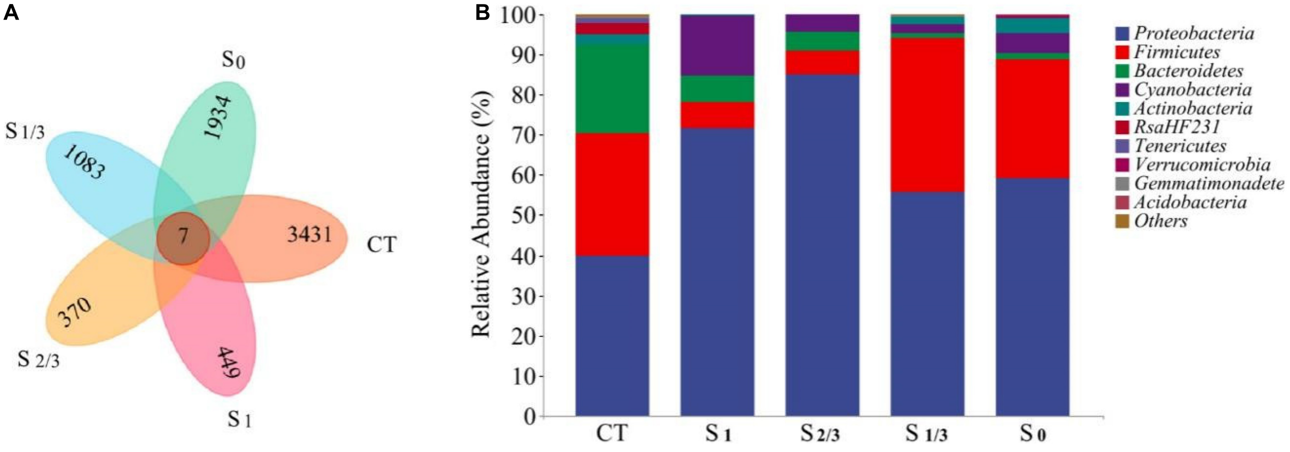

FIGURE 5 | Microbial community analysis of different samples: **(A)** Venn diagram of the distribution of OTUs. **(B)** The relative abundance of microbial communities at the phylum level.

radicals generated by the non-enzymatic system in algae cells will trigger lipid peroxidation to form lipid peroxides (Kaur et al., 2006), which can explain why the concentration of MDA in S_0 group is the highest.

Microbial Community and Function

In this paper, we used 16S rRNA sequencing to investigate the influence of different light levels on microbial community diversity in the growth environment of *Microcystis aeruginosa*, and the results are shown in **Figure 5**. The Venn diagram (**Figure 5A**) shows the distribution of the number of operational taxonomic units (OTUs) for each group of samples, and common and unique OTUs can be used to show composition similarity and overlap of different samples. The number of OTUs for each group was 449 (S_1), 370 ($S_{2/3}$), 1083 ($S_{1/3}$), and 1934 (S_0), indicating that different amounts of natural light had

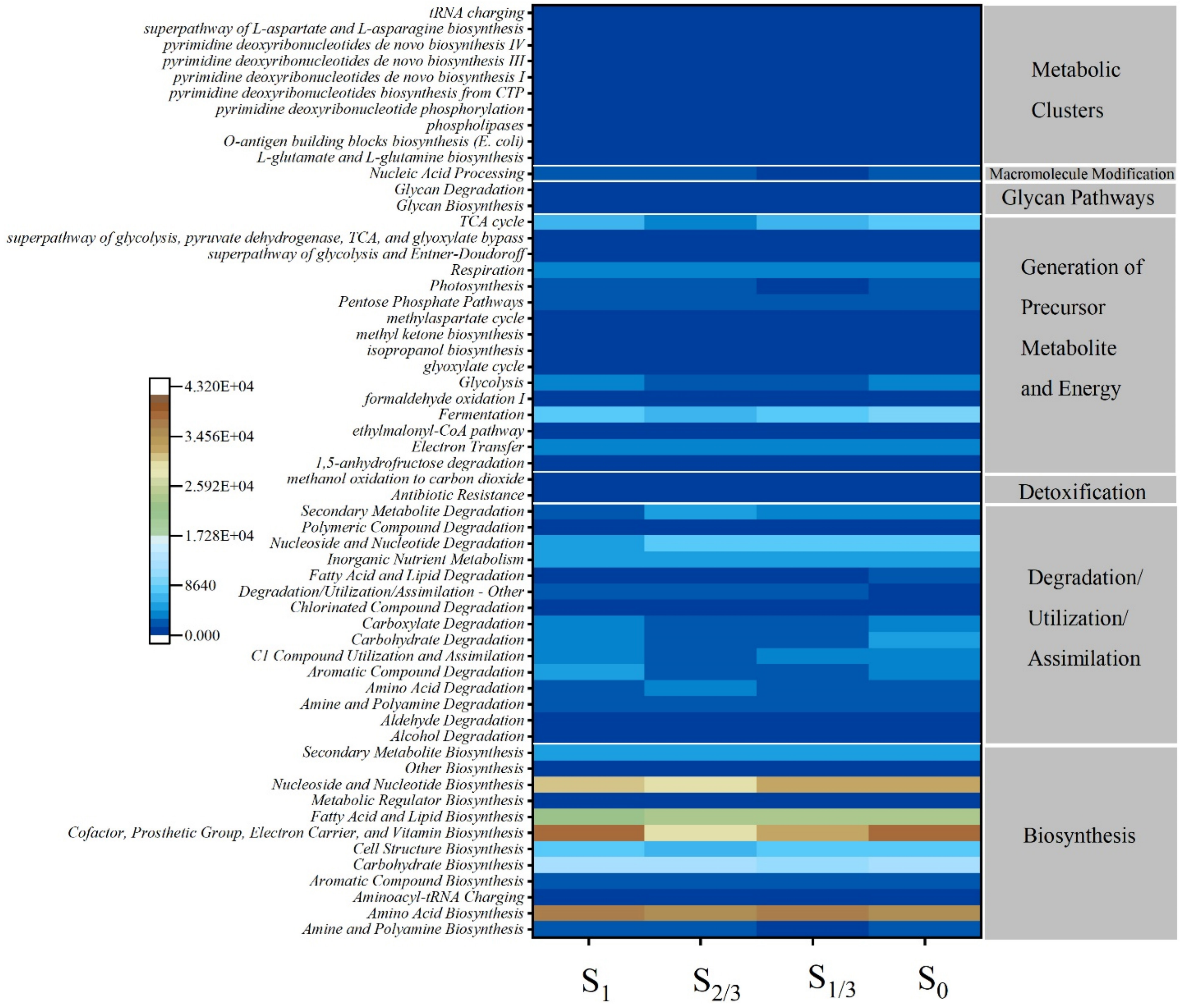

FIGURE 6 | Metabolic pathways of different functional categories predicted by PICRUSt algorithm based on the microbial composition of 16S rRNA. Legends represent the richness of the predicted readings, red for metabolic function with high richness and blue for metabolic function with low richness.

significant effects on microbial diversity in cyanobacteria growing environment. To further explore the richness and species variation of each microflora, we compared the relative abundance of microbial communities in each group at the level of phylum classification (**Figure 5B**). It turns out that at the phylum level, *Proteobacteria, Firmicutes, Bacteroidetes, Cyanobacteria,* and *Actinobacteria* were the dominant bacteria in the growth environment of cyanobacteria under different light conditions. These dominant bacteria have been confirmed to be ubiquitous in polluted water and can promote the stability of EPSs secretion (Lv et al., 2014; Wang et al., 2016; Liu et al., 2017). The relative abundances of these phyla changed significantly under different light conditions. *Proteobacteria and Firmicutes* reached the maximum relative abundance under 2/3 and 1/3 natural light, respectively. The relative abundances of *Bacteroidetes* and *Cyanobacteria* reached the maximum under full light conditions, while that of *Actinobacteria* reached the maximum under no light conditions. Cyanobacteria and bacteria usually interact in this microenvironment, including mutualism and mutual inhibition (Chen et al., 2007; Sun Yang-cai et al., 2007; Bai et al., 2016; Mehmeti et al., 2016). Among these bacteria interacting with cyanobacteria, many studies have shown that *Proteobacteria* are the most abundant bacterial group in the water environment of Taihu Lake (Ma et al., 2016; Zhu et al., 2019).

At the same time, *Bacteroidetes* is also a common bacterial species in the algal sphere, and a major component of hydrolytic acidification reactions (Ma et al., 2016). The microbial community composition in the study system also differed under different light conditions. In addition, they found that light dose had a significant impact on the microbial community through the study on lake surface water, which is consistent with the conclusions of our study (Paver and Kent, 2017).

The results of PICRUSt (**Figure 6**) showed that the main microbial functions in the growth environment

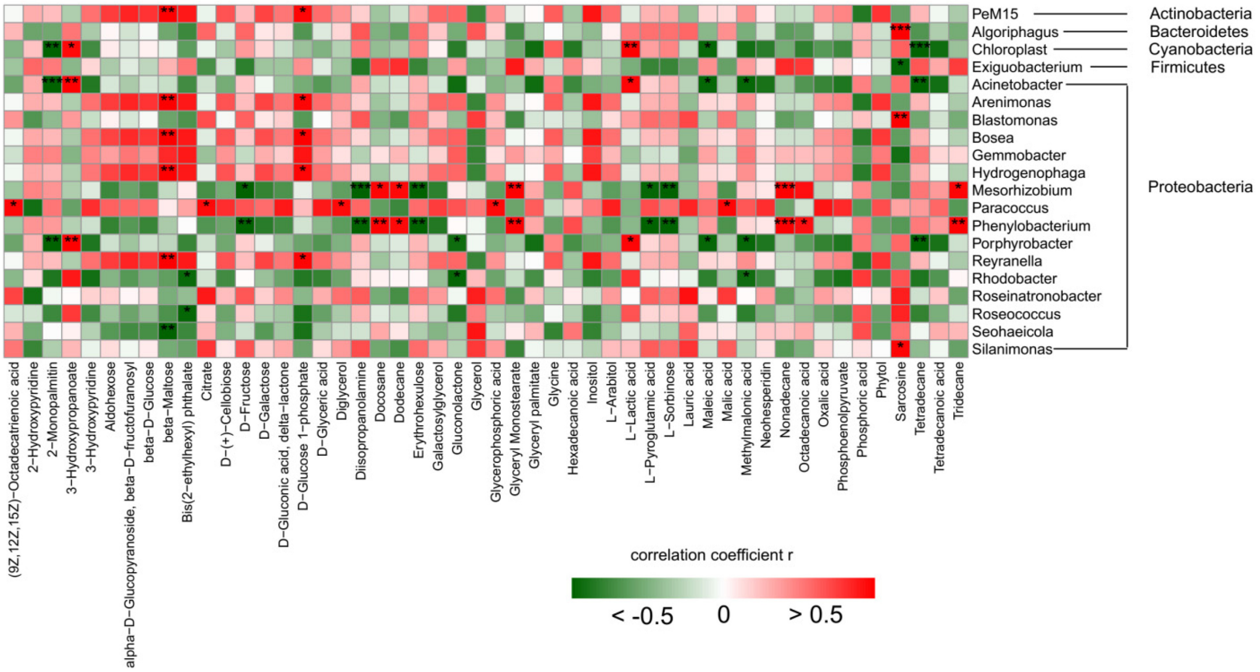

FIGURE 7 | Thermograms of correlation between bacteria and metabolites. The notes on the right are the top 20 bacteria genus; Below are the names of the top 50 metabolites in abundance. Correlation analysis: Pearson correlation coefficient and P value were calculated, and the number of * was marked according to the P value ($P < 0.001$, *** means extremely significant correlation; $P < 0.01$, ** means very significant correlation; $P < 0.05$, * means significant correlation; $P > 0.05$, no * means insignificant correlation; Red represents positive correlation, green represents negative correlation).

of *Microcystis aeruginosa* include biosynthesis, degradation/utilization/assimilation, detoxification, generation of precursor, metabolite, and energy, glycan pathways, macromolecule modification and metabolic Clusters (**Figure 6A**). Among them, amino acid biosynthesis occupies the most abundant biosynthesis function, which provides essential energy and carbon source for bacteria (Lopez-Gonzalez et al., 2015). Other studies have shown that during amino acid metabolism, bacteria can use ammonia to synthesize amino acids and humus, which is conducive to denitrification (Wu et al., 2017). In addition, the light dose can significantly affect other biosynthesis functions such as cofactor, prosthetic group, electron carrier, and vitamin biosynthesis. nucleoside and nucleotide biosynthesis, fatty acid and lipid biosynthesis, and carbohydrate biosynthesis. Bacteria can synthesize fatty acids using acetyl coenzyme a (Co A) as a precursor, and fatty acids are the main components of the cell membrane. By regulating the type and composition of fatty acids, bacteria can regulate cell membrane fluidity, and maintain membrane stability and normal physiological functions to adapt to adversity stress (Zhang and Rock, 2008).

To explore the effect of light on the relationship between cyanobacterial growth and microorganisms, we determined the metabolome of microorganisms in the environment using GC-MS. The thermography about the correlation between microflora and metabolites (**Figure 7**) showed that 26 of the 50 metabolites have at least one significant correlation with microorganisms. Most of the microorganisms in *Bacteroidetes* and *Cyanobacteria* were negatively correlated with the change of metabolites, while most of the microorganisms in *Actinomycetes* were positively correlated with the change of metabolites. Among *Proteobacteria*, *Acinetobacter, Blastomonas, Gemmobacter, Mesorhizobium,* and *Porphyrobacter* were all negatively correlated with metabolites, while *Arenimonas* and *Hydrogenophaga* were positively correlated with metabolites.

The genus of *Cyanobacteria* showed a significant negative correlation with 2-Monopalmitin, Maleic acid, and Tetradecane, while a significant positive correlation with L-Lactic acid and 3-Hydroxypropanoate. L-lactic acid is generated from the catalytic reduction of pyruvate by NAD-dependent lactate dehydrogenase (nLDH) (Goffin et al., 2004). This process involves glycolysis, gluconeogenesis, fructose and mannose metabolism, pyruvate metabolism, and other metabolic pathways. *Actinomycetes* showed a significant positive correlation with beta-Maltose, D-Gluconic acid, and delta-lactone. *Bacteroidetes* showed a significant positive correlation with Sarcosine, and *Firmicutes* showed a significant negative correlation with Phytol. In summary, metabolomics analysis showed that cyanobacteria and microorganisms in the environment produced a large number of metabolites under different light conditions, and the relative contents of metabolites in various bacteria were significantly affected by different amounts of light.

Microbial N Functional Genes

Microorganisms participate in nitrogen fixation, nitrification, denitrification, and other processes in the nitrogen cycle

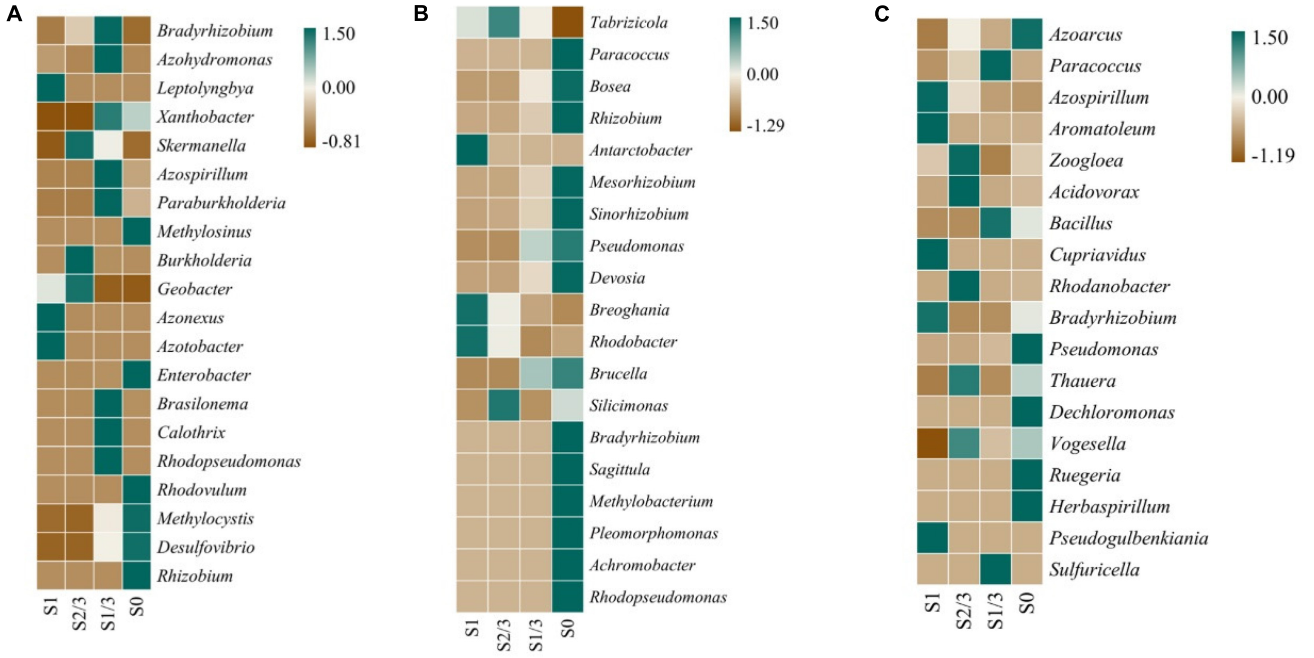

FIGURE 8 | Expression of nitrogen-fixing genes *nifH* **(A)**, *nirK* **(B)** and *nirS* **(C)** at bacteria genus.

by releasing enzymes encoded by their functional genes (Zhong et al., 2017). *NifH* is a marker gene commonly used to determine nitrogen-fixing microorganisms in the environment, which is involved in the process of transforming N_2 into NH_3 in the nitrogen-fixing cycle (Zehr et al., 2003). During the nitrogen cycle, nitrite reductase (Nir) encoded by *nirK* or *nirS* genes can catalyze NO_2^- to NO reduction, which is often used as a marker gene to characterize the diversity and abundance of denitrifying bacteria in the environment (Regan et al., 2017; Ma et al., 2019). To further explore the effect of light conditions, we analyzed the expression of genes related to nitrogen-fixing microorganisms in the growth environment of *Microcystis aeruginosa* at the end of the experiment (**Figure 8**).

The results showed that there were significant differences in the expressions of *nifH*(a), *nirK*(b), and *nirS*(c) genes in different strains under different light levels.

Among them, the *nifH* gene is mainly expressed in *Flavobacterium*, *Rhodopseudomonas*, *Desulfovibrio,* and *Rhizobium*. Several kinds of microbes, including *Desulfovibrio*, are characterized by an increase in *nifH* expression with the decrease of light dose (**Figure 8A**), while the expression of nitrogen-fixing gene *nirK* in *Pseudomonas*, *Alcaligenes*, *Rhizobium*, and other microorganisms gradually increased with the decrease of light exposure (**Figure 8B**). The *nirS* gene was expressed in various microorganisms including *Azotobacter*, *Azospirillum*, *Bacillus*, *Rhodobacteria*, *Bradyrhizobium*, *Pseudomonas*, *Dechlorococcus*, and *Thioalgae* (**Figure 8C**).

For example, *Proteobacteria*, *Anaerobic ammonia-oxidizing Bacteria*, and *Bacteroidetes* can reduce nitrite to nitric oxide (Maia and Moura, 2014). These microorganisms have been found in many hypoxic environments, and *nirK* and *nirS* are involved in the expression of nitrite reductase (Bartossek et al., 2010). However, these nitrogen-fixing genes have also been found in other bacteria, such as anaerobic ammonium-oxidizing bacteria, nitrite and methane-oxidizing bacteria, and ammonia-oxidizing bacteria and archaea. These bacteria do not contain *nirK* and *nirS* genes but may use other nitrite reductases to reduce nitrite to nitric oxide (Kartal and Keltjens, 2016).

In addition, some researchers have found that the expression of microbial N functional genes is affected by climatic factors (e.g., precipitation or temperature), and most of the environmental variables significantly related to N functional genes (e.g., precipitation, soil organic matter, etc.) are also closely related to basic metabolism of microorganisms (De Vries et al., 2012; Zhou et al., 2021). Consequently, the amount of natural light can be an important factor affecting the expressions of related genes of nitrogen-fixing microorganisms in the nitrogen cycle, which provides a more in-depth discussion and theoretical basis for the important role of coexisting microorganisms in the nitrogen-fixing process in the growth environment of cyanobacteria.

CONCLUSION

In conclusion, this paper deeply discusses the effects of natural light on the growth state of cyanobacteria individuals and communities, as well as the response of cyanobacteria molecular systems and cyanobacterial coexistence community structure, biosynthetic functions, metabolism, and nitrogen fixation processes. We conclude that both excessive and insufficient light can limit cyanobacterial growth, affect photosynthesis and EPS production of algae, and influence the vertical distribution of cyanobacteria in the water body. At the same time, the amount of light can alter the structure of the microbial communities and their metabolites in the cyanobacterial growth environment, and may also affect the expression of nitrogen-fixing genes associated with microorganisms that coexist with cyanobacteria. This study is of great significance to further study the potential mechanism of cyanobacterial blooms.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

ZW: conducting experiments, data analysis, and writing – original draft preparation. GL: data analysis and writing – review and editing. HH: curation and writing – review and editing. WZ: conceptualization and methodology. JW: investigation and resources. SH and ZZ: supervision, writing – review and editing, and funding acquisition. All authors contributed to the article and approved the submitted version.

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Effects of Small Hydropower Stations Along Rivers on the Distribution of Aquatic Biodiversity

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At present, there is little research on the impact of small hydropower stations on aquatic biodiversity. In order to investigate whether the existence of small hydropower stations has a significant impact on the aquatic biodiversity of their watersheds, we conducted a systematic study on the abundance of plankton, benthic animal, fish and microorganism in the watersheds of 15 small hydropower stations in Qionglai City. The results showed that 59 species of phytoplankton from 3 divisions, 16 species of zooplankton from 4 categories, 25 species of benthic animal from 3 phyla and 30 species of fish were found in the study basin. The analysis of the physical and chemical indicators of water bodies and the distribution characteristics of aquatic organisms found that the operation of small diversion-type power stations in Qionglai City changed part of the aquatic habitat in the basin, with a greater impact on the activities of large aquatic animals (fish) and a smaller impact on plankton and microorganism, and the intensity of the impact was shown as fish > benthic animal > plankton > microorganism. The small hydropower stations in this study have an impact but not significant on the aquatic biodiversity in the Baimo and Wenjing River in the Qionglai City, and this study provides a data reference for the comprehensive assessment of the environmental impact of small hydropower stations.

Keywords: small hydropower station, fish, plankton, benthic animal, microorganism

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INTRODUCTION

Since 1950s, as an important clean and renewable energy, hydropower has played an important role in electricity supply, poverty reduction, rural economy and social development (Li et al., 2022), which is an indispensable guarantee for achieving the goals of "carbon peaking" and "carbon neutrality". At the same time, small hydropower station projects are widely used in rural areas and remote mountainous areas because of their small investment, low risk, stable benefits and low operating costs (Fu et al., 2008). By 2021, more than 47,000 small hydropower stations have been built in China, with an annual power generation of 250 billion kilowatt-hours, equivalent to saving more than 70 million tons of standard coal and reducing carbon dioxide emissions by 180 million tons per year (Peng et al., 2022). It has also been pointed out that the construction of small hydropower stations has negative ecological impacts, such as affecting fish migration and reproduction (Cao, 2019); limiting the spread of animals and plants (Rivinoja et al., 2010); altering the hydraulic flow characteristics of the river (Guo et al., 2015); and changing the structure and genetic diversity of biological communities (Sundermann et al., 2011; Liu, 2020; Costea et al., 2021).

The impact of small hydropower stations on the aquatic environment can be monitored by aquatic biodiversity. As one of the main indicators of water environment assessment, aquatic biodiversity plays an important role in ecological restoration and health assessment of river basins (Sun et al., 2013; Gao et al., 2015), while the health of aquatic biota can directly or indirectly reflect the health of the water environment. Phytoplankton, zooplankton and large aquatic organisms are widely used in water environment monitoring and assessment. For example, Wei et al. (2012) analyzed the water quality of the Erhai Sea by investigating the species, number and distribution of phytoplankton; Zhao et al. (2008) evaluated and analyzed the status of the water ecological environment in the Huaihe River basin by investigating the composition and abundance of phytoplankton, zooplankton and benthic animal. Fish cover all trophic levels of consumer ecology and provide a powerful tool for assessing aquatic environments (Lin et al., 2021); plankton are an important link in the material and energy transfer of aquatic ecosystems, with phytoplankton playing a vital role in the food chain as the first trophic level (Zhang et al., 2010); zooplankton participate in the material cycle of aquatic ecosystems by feeding on phytoplankton, bacteria and other organisms (Bi et al., 2001; Li et al., 2004); benthic organisms are an essential part of the aquatic food web and provide the bait base for most fish in rivers, and are closely related to the ecological taxa and zonal composition of riverine fish (Šarauskienė et al., 2021); in addition, the species and abundance of microbial communities can reflect changes in aquatic ecosystems. Overall, the aquatic organisms mentioned above can indicate the health of the aquatic environment at a certain level (Xu, 1996; Araújo et al., 2000; Jakovčev-Todorović et al., 2005; Salmaso et al., 2006; Li, 2022).

At present, the assessment of aquatic ecological environment based on aquatic biodiversity is mainly focused on large and medium-sized reservoirs and hydropower stations (Otahelová and Valachovič, 2003; Bredenhand and Samways, 2009; Andrianova, 2020; Chen et al., 2020), and fewer studies have been reported on the disturbance of aquatic biodiversity by small hydropower stations. Therefore, it is meaningful to explore the impact of small hydropower stations on the characteristics, diversity and functional groups of aquatic communities to improve the ecological impact assessment index system of small hydropower stations. This study investigated the impacts of the construction of 15 small hydropower stations in Qionglai on the diversity of aquatic organisms (fish, plankton, benthic animal and microoganism). With a view to provide basic data and theoretical basis for the development of small hydropower stations and ecological environmental protection of river basins.

MATERIALS AND METHODS

Sample Collection

Qionglai City is located in Sichuan Province of western China, at the upper reaches of the Yangtze River, and has been "the first state in southern Sichuan" since ancient times. There are numerous small hydropower stations in the Baimo and Wenjing River basins in Qionglai.

Since the sampling conditions were available during the low-water seasons, in December 2021, we collected samples from a total of 16 points at 15 small hydropower stations and the confluence of watersheds in the Baimo and Wenjing River basins in Qionglai City. The river is divided into four watersheds (Q1-Q4) according to its connectivity and location (**Table 1**). Among them, S0 is the confluence of rivers, and the remaining 15 sampling points are all diversion-type power stations. The geographical distribution of sampling points is shown in **Figure 1**, S1-S4 belong to basin Q1, S5 belongs to basin Q2, S6-S9 belong to basin Q3 and S10-S15 belong to basin Q4.

Sampling Methods

Fish Collection and Identification

The collection of fish resources included field sampling and visiting surveys, on-site identification of fish specimens as far as possible, determination of biological basic data, looking through common fish atlases to determine fish species, and taking scales and other materials for age identification. For the identification of fish, reference the *Chinese Fish Atlas* (Li, 2015) to determine the species of fish.

Phytoplankton Collection and Identification

1 liter of water sample was collected with glass water sampler into polyethylene bottles, stored in 1.5% Lugol's solution and brought back to the experiment for analysis. Firstly, it needed to settle for 48 h, and then using a siphon device, the water sample was concentrated to 30 mL, 0.1 mL of the shaken concentrated water sample was taken on a plankton counting plate, and the morphological analysis of the algal species was observed by microscopic counting method under a light microscope. For the identification of algae, reference the *The system, taxonomy and ecology of Chinese freshwater algae* (Hu and Wei, 2006).

TABLE 1 | Sampling sites.

Watershed	Sample	Name
Q1	**S1**	Tang Shuimo Hydropower Station
	S2	Niu Xin Hydropower Station
	S3	Long Menkou Hydropower Station
	S4	Fu Xiang Hydropower Station
Q2	**S5**	Yan Zi Hydropower Station
Q3	**S6**	Zhen Xishan Hydropower Station
	S7	Ma Ping Hydropower Station
	S8	He Ping Hydropower Station
	S9	Xiang Shuitan Hydropower Station
Q4	**S10**	Xiao Jiawan Hydropower Station
	S11	Zheng Fa Hydropower Station
	S12	Tian Tai Hydropower Station
	S13	Tian Chepo five-stage Hydropower Station
	S14	Tian Chepo four-stage Hydropower Station
	S15	Hong Yan Hydropower Station
Watershed confluence	**S0**	Ma Qihukou

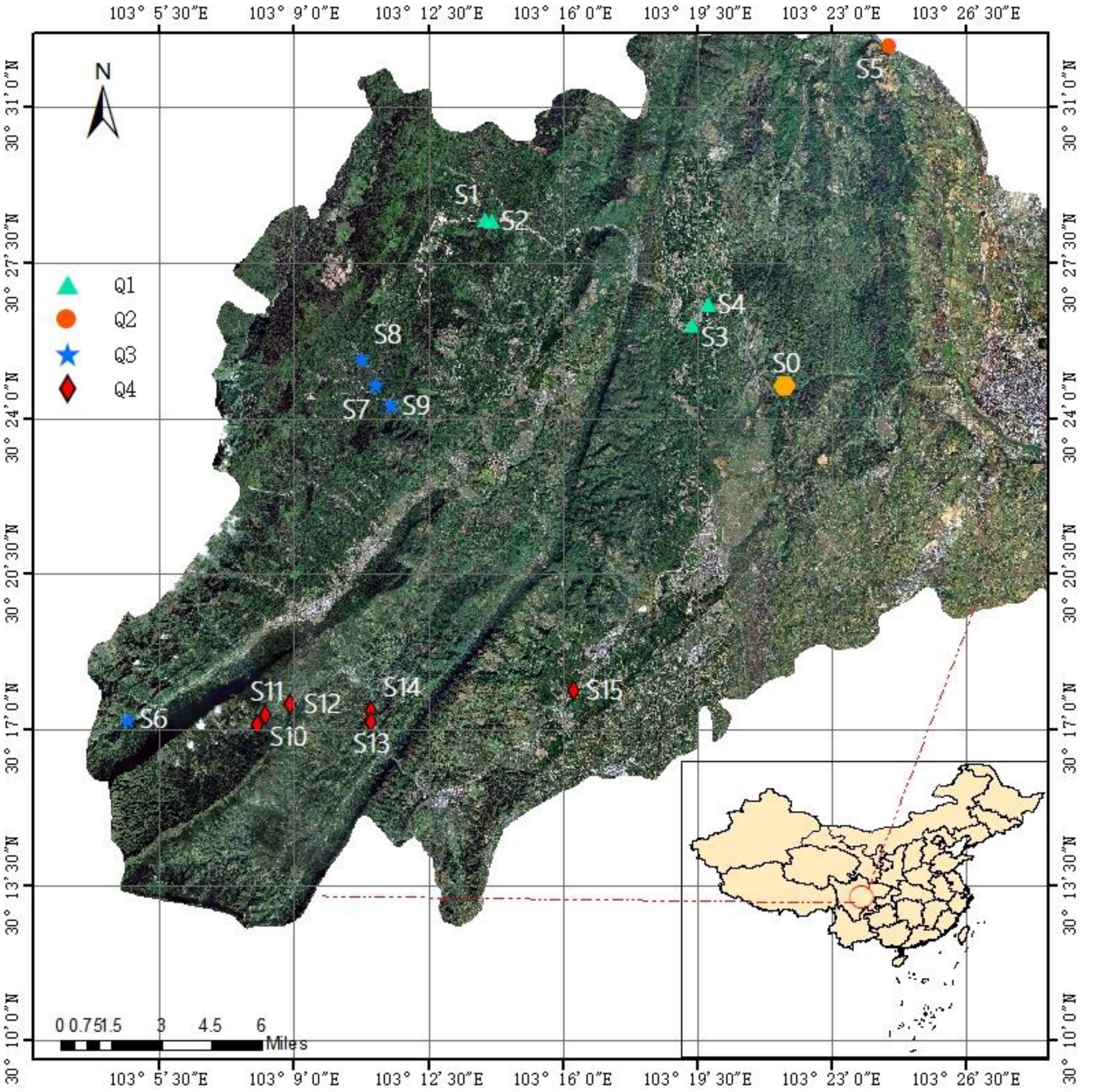

FIGURE 1 | Distribution of sampling points in Qionglai City.

Zooplankton Collection and Identification

Fifty liters of water sample were collected at the same place, and the mixed water samples were filtered through a No. 25 plankton net (pore size of 64 μm), and finally the concentrated solution was transferred to a polyethylene bottle, and the samples were stored in 5% formaldehyde solution, protected from light and low temperature. The shaken concentrated water samples were taken 0.1 mL on a plankton counting plate, covered with a coverslip, and the samples were identified and counted for species under a light microscope. Referring to the *Atlas of Freshwater Microorganisms* (Zhou, 2005) for classification and identification of species.

Benthic Animal Collection and Identification

Benthic animals were collected by a mud collector for 3-5 times, with the sampling area of 0.09 m^2. And the samples in the mud collector were passed through a 40 mesh sieve, washed and picked out as suspected benthic animals, and loaded into specimen bottles containing 30% alcohol. The samples were brought back to the laboratory and the benthic animals were then picked out on dissecting trays and stored in 75% alcohol at 4°C. The alcohol was replaced once a week pending identification. For quantitative benthic analysis, sample species identification and enumeration were carried out under a light microscope, mainly with reference to the *Atlas of Chinese Animals* (Tong, 1982), and identified to species.

Microorganism Collection and Analysis

The collected microbial water samples were filtered with a water body extractor (0.45 μm filter membrane) for microbial enrichment, and if the samples are turbid, centrifugation is used for enrichment. The enriched microbial water samples were sent to Shanghai Personalbio Technology Co., Ltd., for testing, and the microbial community-related data were obtained by high-throughput sequencing (HTS) and polymerase chain reaction (PCR) amplification of 16sRNA gene-specific fragments.

Data Analysis

Physical and chemical properties of water quality and spatial distribution of phytoplankton density and biomass use Personalbio for analysis. Distribution of each sampling point at different division levels uses Origin 2022 for analysis.

RESULTS AND DISCUSSION

Water Quality Conditions in the Study Basin

Water samples from S0, which is at the confluence of Baimo and Wenjing River, and 3 representative power stations (S4, S9, and S15) are selected to measure physical and chemical indexes, and the comparison maps of pH, water temperature (WT), conductivity (Cond), dissolved oxygen (DO) and transparency of each sampling site are drawn (**Figure 2**). As can be seen from **Figure 2**, the pH of water in the study basin varies between 8.5 and 8.8, belonging to weakly alkaline water quality; WT varies between 11.6°C and 14.3°C; Cond varies from 30.3 ms/m to 43.0 ms/m; DO varies between 12.99 ppm and 13.5 ppm; transparency varies between 0.4 m and 1.3 m. There are no significant differences in pH, WT, and DO among the sampling sites.

Fish Composition and Distribution Characteristics

The results of the fish survey are detailed in **Supplementary Table 1**. There are 30 species of fish in total. Among them, 6 species are obtained by on-site sampling, namely *Misgurnus anguillicaudatus*, *Monopterus albus*, *Pelteobagrus fulvidraco*, *Cyprinus carpio*, *Pseudorasbora parva* and *Carassius auratus*. A total of 10 species are found after checking the data, mainly including *Rhodeus sinensis*, *Rhodeus ocellatus*, *Abbottina rivularis* and *Hemibarbus maculatus*, etc. Through the interview survey, it is found that there are 14 kinds of fish, including *Silurus asotus*, *Hypophthalmichthys molitrix*, *Silurus meridionalis* and *Aristichthys nobilis*, etc.

A total of 6 species of fish are collected on site, among which *Misgurnus anguillicaudatus*, *Cyprinus carpio* and *Carassius auratus* are highly resistant to pollution and are medium pollution zone indicator fish, indicating that there may be a certain degree of pollution in the watershed. Zhu et al. (2021) collected 71 species of fish in the nearshore Jingjiang section of the Yangtze River near the mouth; Wang et al. (2020) collected 64 species of fish in the Xinzhou waters of Anqing of the Yangtze River; Zheng et al. (2019) collected 56 species of fish in the Wuhan section of the Yangtze River, the fish resources in the survey basin are relatively small compared to the aquatic resources in the upper reaches of the Yangtze River, it is possible that the construction of small hydropower stations has led to changes in river volume and flow rate, which has affected the survival and reproduction of fish (Sun and Liu, 2020), reducing the space in the water column and affecting the species composition of fish (Benejam et al., 2016). The construction of small hydropower stations has a greater impact on fish diversity than other aquatic organisms.

Phytoplankton Composition and Distribution Characteristics

The results of the phytoplankton survey in the study basin are shown in **Table 2**. The phytoplankton shows Q3 > Q4 > Q1 > Q2 in terms of species abundance and diversity. The proportion of phytoplankton in the three divisions of Bacillariophyta, Cyanophyta and Chlorophyta in the four watersheds is shown in **Figure 3**. The Bacillariophyta is mainly found in the basin Q1 and Q4, especially in the 4 sampling sites S1, S2, S10, and S11. And the Cyanobacteria and Chlorophyta are mainly distributed in the Q3 basin.

A total of 26 species of common phytoplankton are found in Q1, among which the Bacillariophyta is the most abundant with 22 species, mainly including *Diploneis elliptica* and *Navicula* sp., etc., followed by Chlorophyta, Cyanobacteria with 2 species each, namely, *Scenedesmus bijugatus*, *Ulothrix* sp. and *Pseudoanabaena* sp., *Aphanocapsa* sp. A total of 10 species of common phytoplankton are found in the basin Q2, among which Bacillariophyta are the most abundant with 9 species, mainly including *Cymbella affinis* and *C. cymbiformis*, etc., followed by Chlorophyta with only 1 species of *C. fracta*. A total of 32 species of common phytoplankton are found in Q3, among which 14 species of Chlorophyta, mainly including *Scenedesmus quadricauda*, etc., followed by the Bacillariophyta with 11 species, mainly including *Fragilaria capucina,* etc., there are 7 species of Cyanobacteria, mainly including *Phormidium faveolarum*, etc. A total of 28 species of common phytoplankton are found in Q4, among which Bacillariophyta is the most abundant with 22 species, mainly including *Diploneis elliptica* and *Navicula* sp., etc., followed by 3 species each of Cyanobacteria and Chlorophyta. In general, The largest relative abundance is in the Bacillariophyta, with 55.9%, followed by the Chlorophyta, with 28.8%, and the Cyanophyta, with 15.3% (**Supplementary Table 2**).

The distribution of phytoplankton population density and biomass at each sampling point is shown in **Figure 4**. Quantitative analysis statistics of phytoplankton from 15 sampling sites shows that the density of phytoplankton ranges from 8.0 pcs/L to 7869.1 pcs/L, with an average of 2967.1 pcs/L.

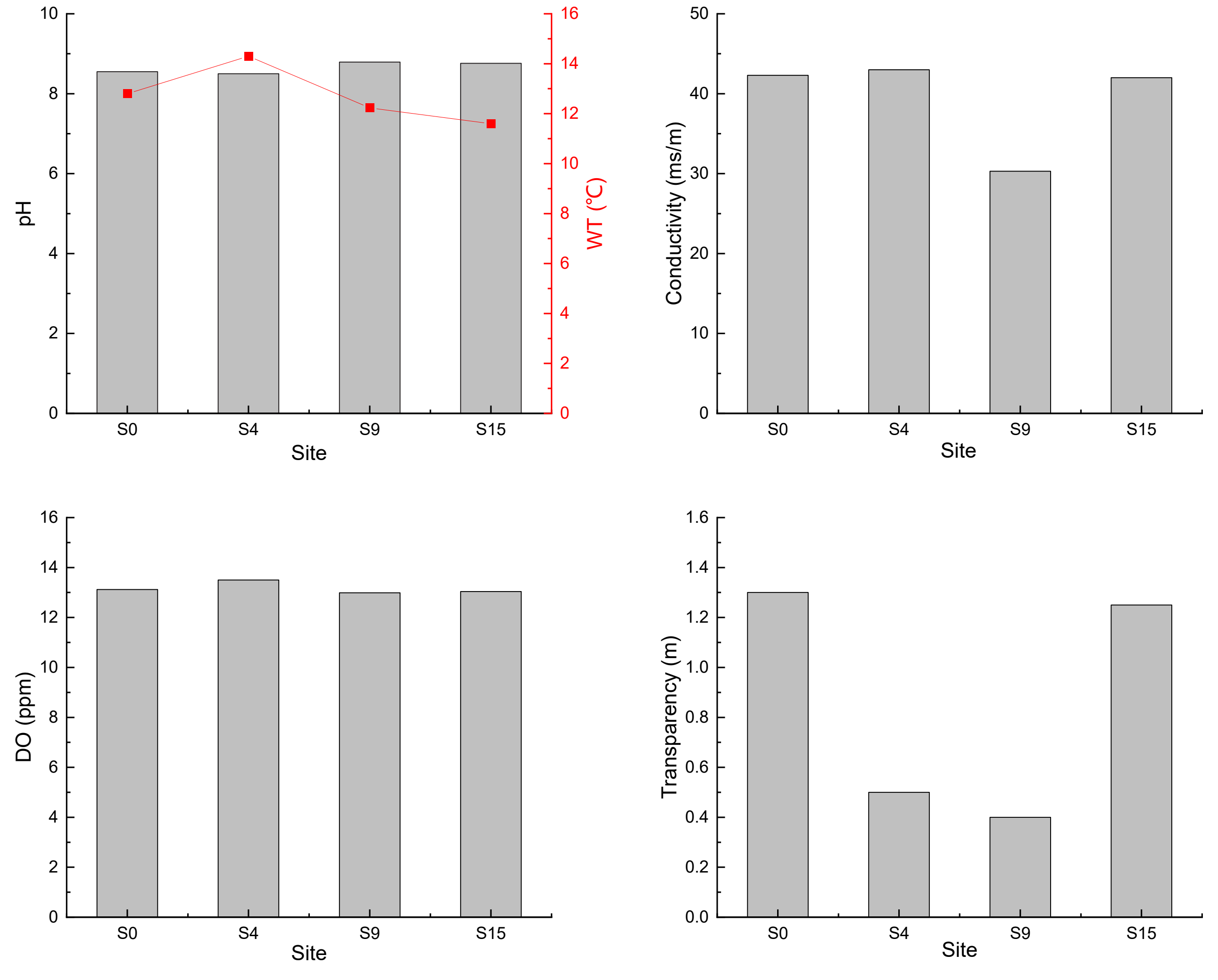

FIGURE 2 | Physical and chemical properties of water quality.

The highest sampling point is S6 with 7869.1 pcs/L, the second highest is S9 with 7396.8 pcs/L and the lowest is S5 with 8 pcs/L. The biomass of phytoplankton ranges from 0.0071 mg/L to 0.9908 mg/L, with an average of 0.3468 mg/L. The highest sampling point is S6 with 0.9908 mg/L, the second highest is S8 with 0.9227 mg/L, and the lowest is S5 with 0.0071 mg/L. The difference between sampling zones is significant ($p < 0.05$). Phytoplankton density in Q1 ranges from 11 pcs/L to 15 pcs/L, with an average of 12.8 pcs/L and biomass ranges from 0.0194 mg/L to 0.0234 mg/L, with an average of 0.0215 mg/L; phytoplankton density and biomass of Q2 are 8 pcs/L and 0.0071 mg/L, respectively; phytoplankton density in Q3 ranges from 7228.5 pcs/L to 7869.1 pcs/L, with an average of 7450.8 pcs/L and biomass ranges from 0.1759 mg/L to 0.9908 mg/L, with an average of 0.6838 mg/L; phytoplankton density in Q4 ranges from 2004.3 pcs/L to 2979.9 pcs/L, with an average of 2445.5 pcs/L and biomass ranges from 0.1759 mg/L to 0.2796 mg/L, with an average of 0.2507 mg/L.

A total of 59 species of 3 phytoplankton divisions are identified in the study basin. Tan et al. identified 95 phytoplankton species from 6 phyla in five sections from the upper reaches of the Yangtze River to Jiangjin (Tan et al., 2017); Min et al. found 79 phytoplankton species from 6 phyla in the Yangtze River source area (Min et al., 2020); Cui et al. recorded 175 phytoplankton species from 8 phyla in the main stream and its tributaries in the Yangtze River source area (Cui et al., 2020). In comparison, fewer phytoplankton species were found in this survey. The phytoplankton in the Q1, Q2, and Q4 basins are dominated by Bacillariophyta, occupying 84.6, 90.0, and 78.6%, respectively,

TABLE 2 | Phytoplankton diversity at different levels in the four basins.

Samples	Division	Class	Order	Family	Genus	Species
Q1	3	4	9	13	19	26
Q2	2	3	4	7	9	10
Q3	3	6	16	20	28	32
Q4	3	4	10	15	21	28

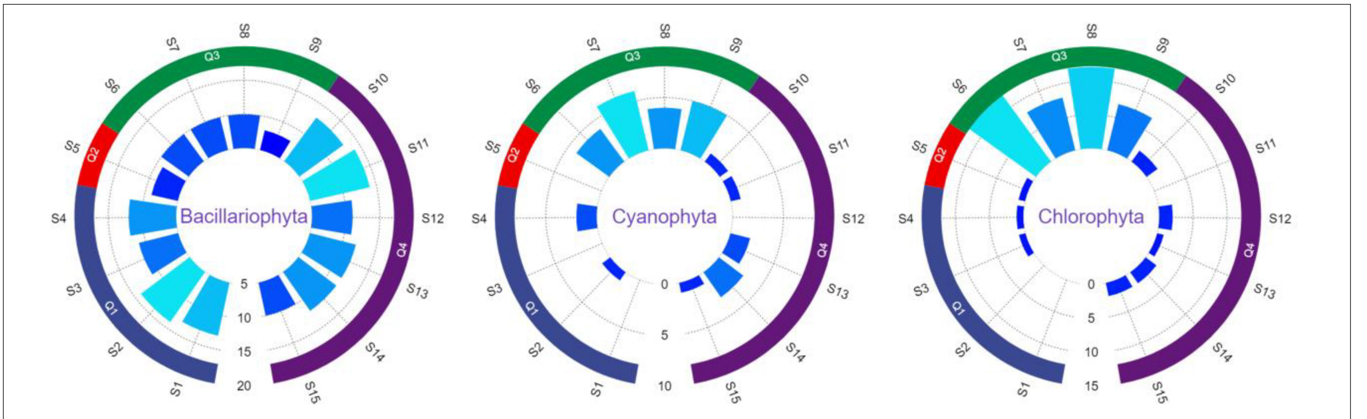

FIGURE 3 | The proportion of phytoplankton in the divisions of Bacillariophyta, Cyanophyta, and Chlorophyta in the four watersheds.

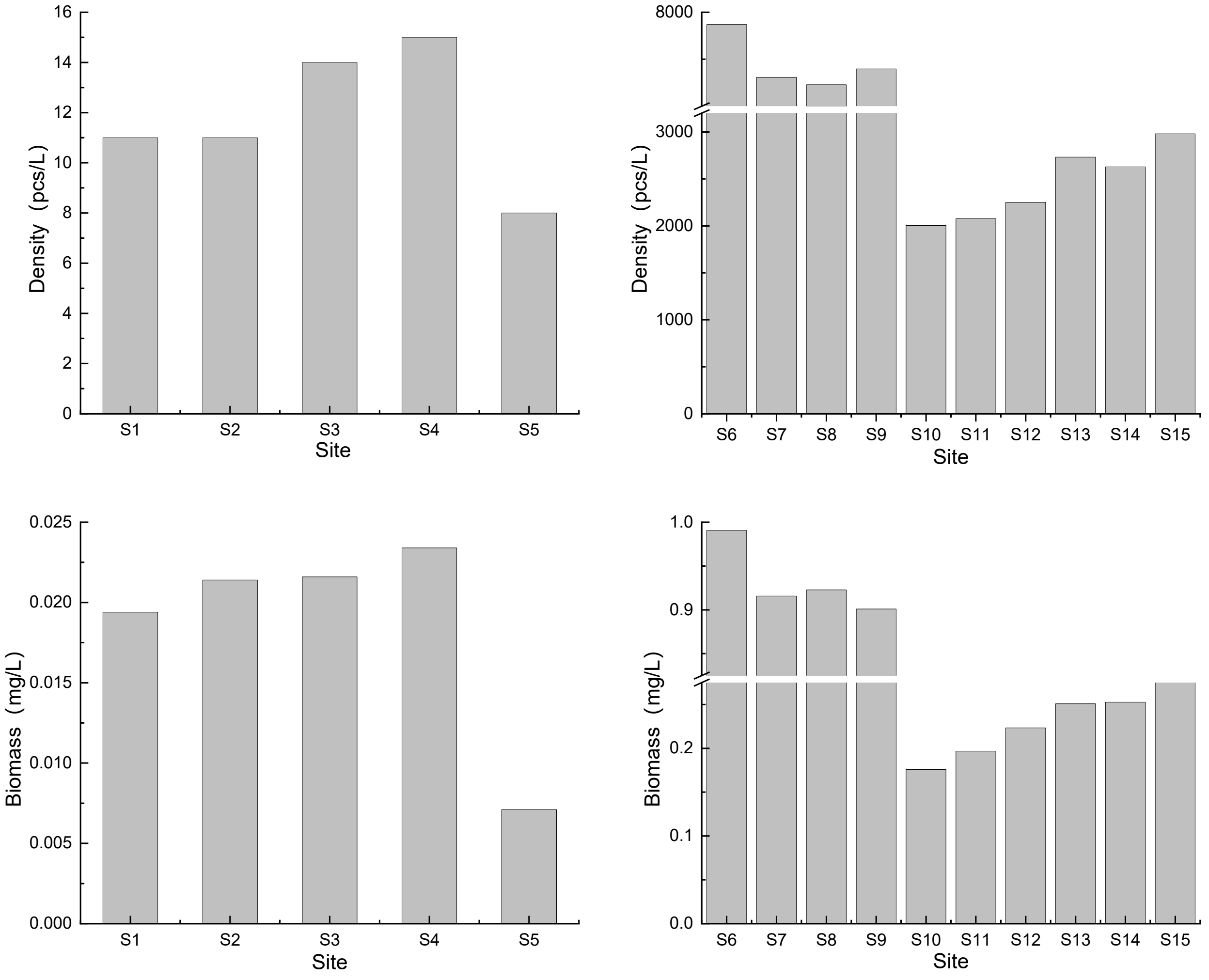

FIGURE 4 | Spatial distribution of phytoplankton density and biomass.

consistent with the theory that Bacillariophyta dominate the phytoplankton of many lowland rivers (Devercelli and O'Farrell, 2013). The Bacillariophyta has the largest biomass in winter (56.1%), followed by the Chlorophyta (29.8%), which is similar to the results of Tian et al. (2017) and Wu et al. (2014). Generally speaking, diatoms is the dominant species in clean water, green

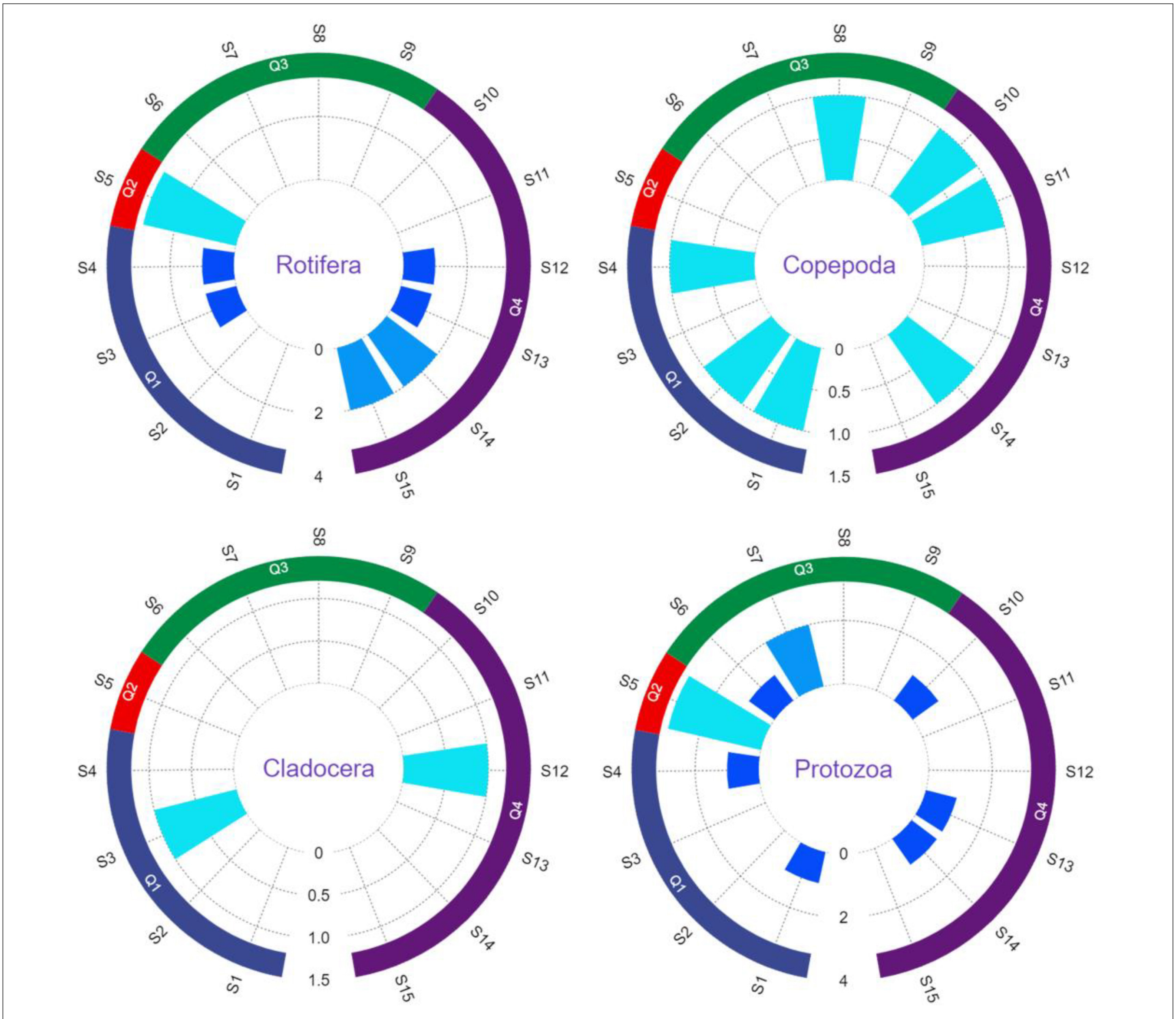

FIGURE 5 | The proportion of zooplankton in the phyla of Rotifera, Copepoda, Cladocera, and Protozoa in the four watersheds.

algae is the representative of medium-sized eutrophic water, and blue algae is the dominant species in eutrophic water (Zhen and Zhang, 2017). Green algae and blue algae accounted for 15.4% in Q1, 10.0% in Q2, 65.6% in Q3 and 21.4% in Q4 basin, which indicated that there is some pollution in this area.

Zooplankton Composition and Distribution Characteristics

Through identification, a total of 16 species of zooplankton in 4 categories are detected. And they are Protozoa, Cladocera, Copepoda and Rotifera, respectively. The largest relative abundance is Protozoa (43.8%), followed by Rotifera (31.3%), Copepoda (18.8%), and Cladocera (6.3%). The distribution of zooplankton species in the study basin in each phylum is shown in **Figure 5**. A total of 7 common zooplankton species in 4 categories are detected in Q1. The Protozoa includes *Arcella discoides* and *Epistylis* sp.; the Rotifera includes *Lepadella patella* and *Keratella cochlearis*; the Copepoda includes *Cyclopoid larva* and *Nauplius*; the Cladocera includes *Moina* sp. 6 common zooplankton species in 4 categories are detected in Q2. The Protozoa includes *Areclla vulgaris*, *Centropyxis aerophile* and *Paramecium caudatum*; the Rotifera includes *Keratella cochlearis*, *Notholca squamula* and *Brachionus calyciflorus*. 4 common zooplankton species in 2 categories are detected in Q3, among which the Protozoa is the dominant phylum with 3 species, including *Difflugia* sp., *Centropyxis aculeata* and *Arcella discoides*; 1 species of Copepoda is *Nitocra* sp. 8 common zooplankton species in 4 categories are detected in Q4, among which Rotifera is the dominant phylum with 3 species, including *Lepadella patella*, *Filinia longiseta* and *Keratella cochlearis*; the Protozoa

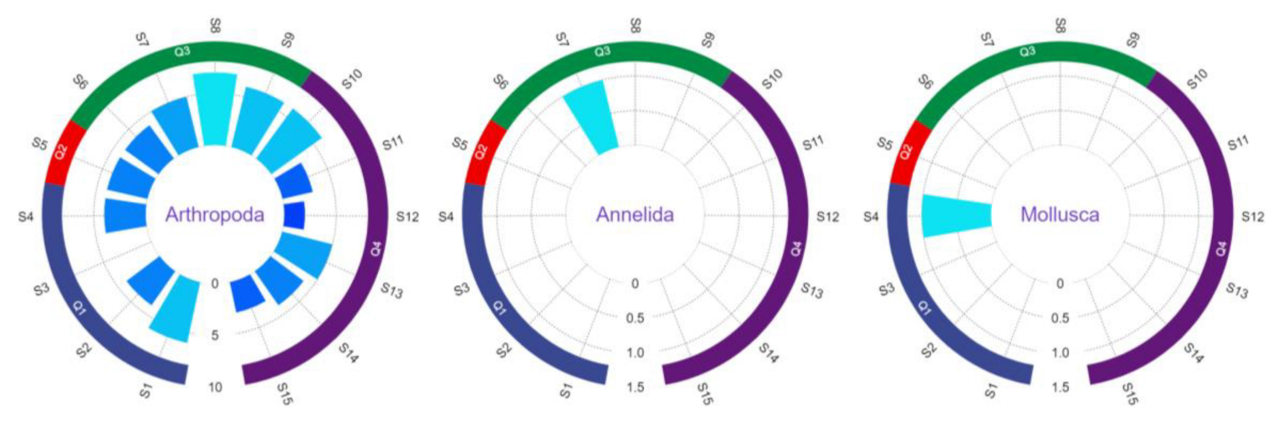

FIGURE 6 | Distribution of each sampling point at different phylum levels.

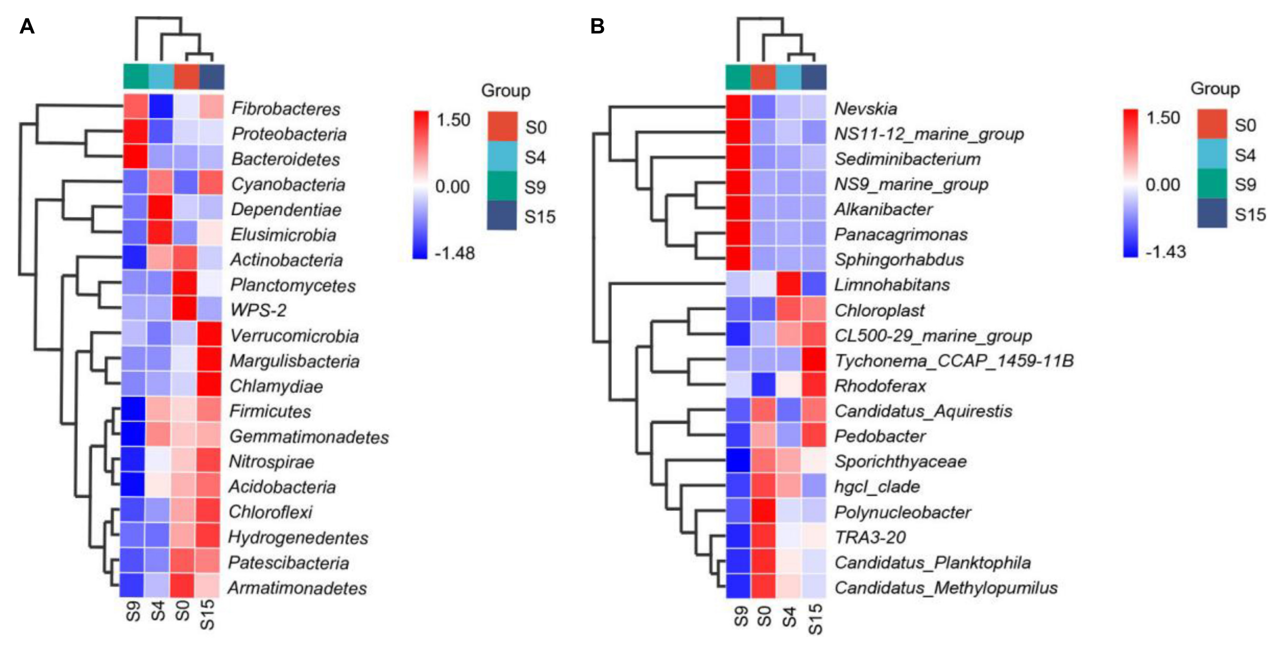

FIGURE 7 | Heat map of species composition at the phylum **(A)** and genus **(B)** level.

includes *Arcella discoides* and *Epistylis* sp.; the Copepoda includes *Cyclopoid larva* and *Nauplius;* the Cladocera includes *Moina* sp. (**Supplementary Table 3**).

The results show that Protozoa and Rotifera dominate the study basin, with *Arcella discoides* and *Keratella cochlearis* being the most widely distributed. Q3 has the lowest number of zooplankton species (4 species) and Q4 has the highest number of zooplankton species (8 species). Among other Yangtze River basins, Sun et al. (2021) collected 74 species of zooplankton in the middle reaches of the Yangtze River; Dai et al. collected 74 species of zooplankton in 4 categories in the Yangtze River Dolphin Protection Zone in Zhenjiang, Jiangsu (Dai et al., 2011), Dai et al. (2019) collected 46 species of zooplankton in 4 categories in the Xinzhou waters of Anqing, Yangtze River, in contrast, the zooplankton in the surveyed watersheds are less diverse and less abundant than those in the other basins of the Yangtze River, and species diversity is low, probably because the water temperature in the Qionglai area is low (11.2°C-14.3°C), which is not conducive to the growth and reproduction of zooplankton (Sun et al., 2021), while the steeper slope and faster current in the Qionglai area make it difficult for phytoplankton to survive, and the number of zooplankton feeding on phytoplankton is thus reduced (Liu et al., 2017).

Benthic Animal Composition and Distribution Characteristics

The species distribution of each phylum of benthic animals is shown in **Figure 6**. Among them, Arthropoda is widely distributed in all watersheds, with the most in watershed Q3, while only 1 species of Annelida is detected in Q3, and 1

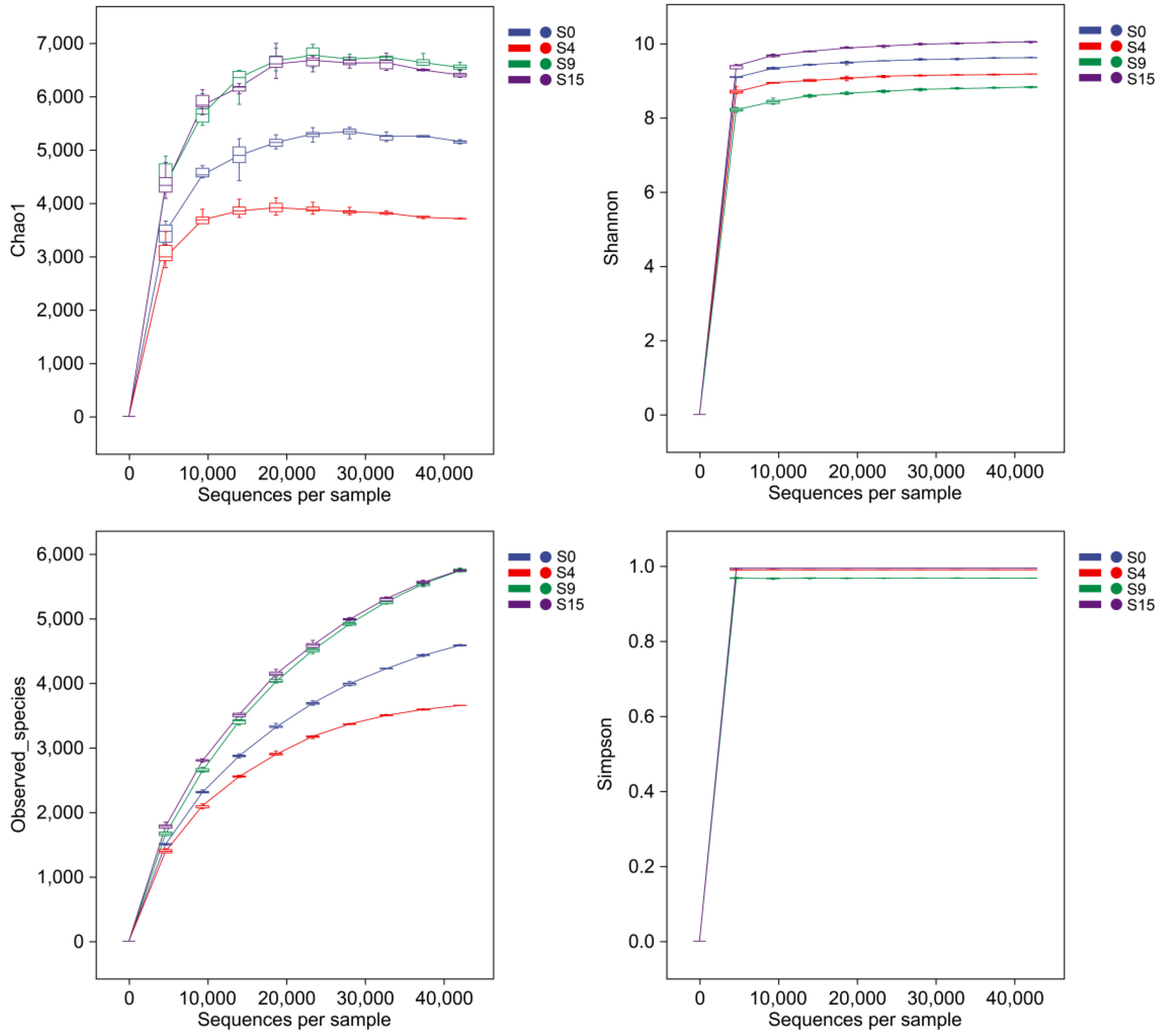

FIGURE 8 | Alpha diversity analysis.

species of Mollusca. 10 species of 2 common phyla are detected in the Q1 watershed, among which the Arthropoda is the dominant phylum with 9 species, mainly including the *Baetis* sp. and *Tipula* sp., etc.; followed by the Mollusca, with 1 species, which is *Limnoperna fortunei.* Q2 watershed detected common benthic animals 1 phylum 4 species, for the Arthropoda, including *stonefly*, *Rhyacophila* sp., etc. Q3 watershed detected common benthic animals 2 phylum 16 species, among which the Arthropoda is the dominant phylum with 15 species, including *Ephemera* sp. and *Baetis* sp., etc., followed by the Annelida with 1 species, which is *Limnodrilus hoffmeisteri*. 9 species of 1 common phylum were detected in the Q4 watershed, the Arthropoda, including *Corixidae* sp. and *Baetis* sp., etc. (**Supplementary Table 4**).

The survey results show that the benthic animals are mainly composed of Arthropoda, with the most widespread distribution of *Baetis* sp., *Tipula* sp., *Polypedilum* sp., *orthocladius* sp., and *Corixidae* sp. Studies have shown that *Ephemeroptera* are sensitive groups, mostly distributed in clean water, while *Chironomidae* and *Limnodrilus* are pollution-resistant groups, generally distributed in polluted areas (Wu et al., 2011). These three kinds of benthic animals are found in Qionglai watershed, of which *Ephemeroptera* account for 10.0% and *Chironomidae* account for 40.0% in Q1; *Ephemeroptera* account for 50.0% in Q2; *Ephemeroptera* account for 18.8%, *Chironomidae* and *Limnodrilus* account for 31.3% in Q3; *Ephemeroptera* account for 11.1% and *Chironomidae* accounted for 55.6% in Q4, therefore, there is a certain degree of pollution in the basin under investigation.

A total of 23 species from three phyla of benthic animals are identified in the study basin. Zhang et al. (2022a) collected 38 species of macrobenthic animals from 3 phyla in the Qiaobian River, a first-class tributary of the Yichang section of the Yangtze River; Zhang et al. (2022b) collected 31 species of benthic animals from 3 phyla in the Huangbai River basin, a first-class tributary on the left bank of the middle reaches of the Yangtze River;

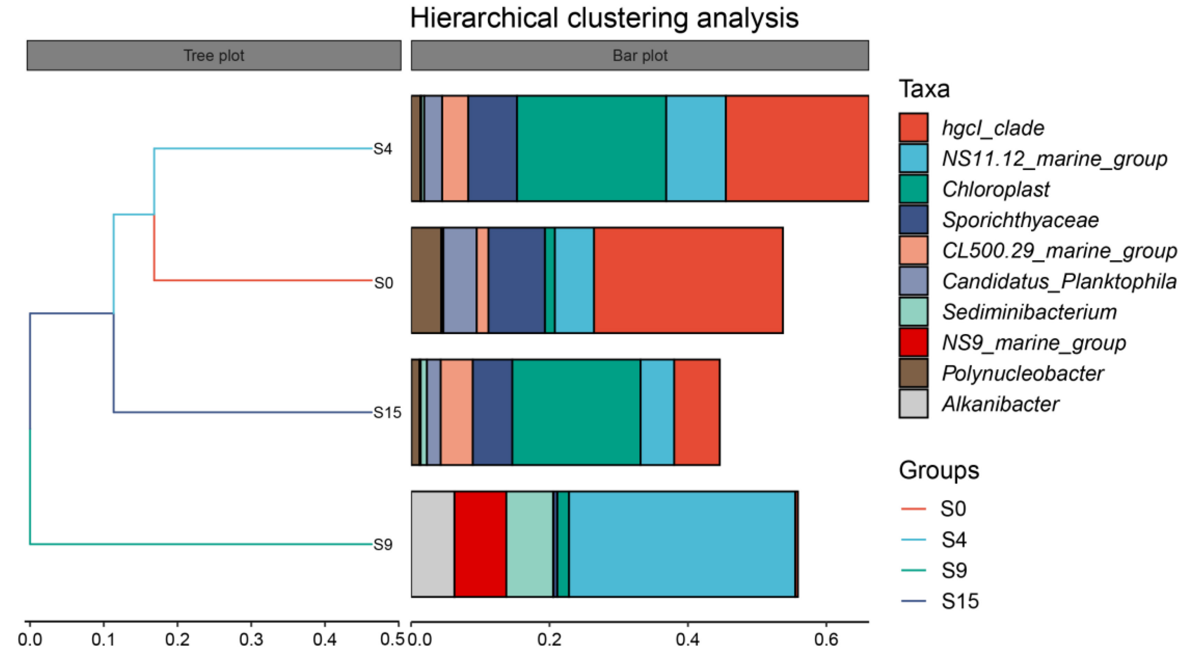

FIGURE 9 | Beta diversity hierarchical clustering map.

Li et al. (2020) collected 28 species of benthic animals from 3 phyla in the Qiaobian River, a tributary of the Yangtze River (Li et al., 2020). In contrast, the species abundance of benthic animals in the surveyed watershed is low and the overall resources are poor. It may be due to the construction of small hydropower stations that the water pollution is aggravated, the water depth and water flow velocity are changed (Premstaller et al., 2017), at the same times, the responses of different benthic animals to environmental changes are different (Ren et al., 2015), so the biomass and abundance of macrobenthos are decreased.

Microorganism

Microbial Community Diversity

The samples are clustered by euclidean distance of species composition data for UPGMA (default clustering algorithm), and the top 20 species are selected to draw species composition heat maps at the phylum level (**Figure 7A**) and genus level (**Figure 7B**). At the phylum level, Planctomycetes, WPS-2 and Armatimonadetes are highly expressed in S0; Dependentiae and Elusimicrobia are highly expressed in S4; Proteobacteria and Bacteroidetes are highly expressed in S9. Verrucomicrobia, Margulisbacteria, Chlamydiae, Nitrospirae, Chloroflexi and Hydrogenedentes are highly expressed in S15. At the genus level, *Polynucleobacter*, *TRA3-20*, *Candidatus_Planktophila* and *Candidatus_Methylopumilus* are highly expressed in S0; *Limnohabitans* is highly expressed in S4; *Nevskia*, *NS11-12_marine_group*, *Sediminibacterium*, *NS9_marine_group*, *Alkanibacter*, *Panacagrimonas* and *Sphingorhabdus* are highly expressed in S9; *Tychonema_CCAP_1459-11B*, *Rhodoferax* and *Pedobacter* are highly expressed in S15. Microbial diversity and species abundance are high at all sampling sites, and the construction of small hydropower stations had a low impact on microorganisms due to their small size and wide distribution, and because they are less affected by environmental factors than benthic invertebrates (Vilmi et al., 2020).

Alpha and Beta Diversity Analysis

Alpha diversity analysis of microorganisms in water samples from 4 sampling points at S0 which at the confluence of Wenjing and Baimo River and three representative power stations (S4, S9, and S15) are performed, and the results are shown in **Figure 8**. From the Chao1 index, the number of species is high at all sampling sites, of which S9 has the highest number of sampling sites. The Shannon index, Simpson index and Observed species index show that the species richness and diversity of each sampling site are high, and the species are evenly distributed, among which the species richness of S15 is higher than the other 3 sampling sites.

Beta diversity analysis shows (**Figure 9**) that S4 is more similar to the top 10 species in terms of the abundance to S0. The relative abundance of *hgcl_clade* (27.30%) and *Sporichthyaceae* (8.17%) are higher in S0; the relative abundance of *hgcl_clade* (21.55%) and *Chloroplast* (20.70%) are higher in S4; the relative abundance of *NS11.12_marine_group* (32.70%) is the highest in S9; the relative abundance of *Chloroplast* (18.53%) and *hgcl_clade* (6.59%) are higher in S15.

CONCLUSION

This study of the impact of small hydropower stations on aquatic organisms in the Qionglai watershed, which is located in the upper reaches of the Yangtze River, shows that the biodiversity of the Baimo and Wenjing River in Qionglai is at a low level, and the results of the fish diversity survey shows relatively few fish resources and poor species diversity, with only 6 species of fish found in the field sampling, mainly including Cyprinidae; 23 species of benthic animals are detected, and Arthropoda (91.3%) is dominant; the number of zooplankton species is less and the diversity is low, and only 16 species are detected in the study watershed; the biomass of Bacillariophyta accounts for the largest proportion of phytoplankton (56.1%), followed by Chlorophyta (29.8%) and Cyanobacteria (4%); both the diversity and species abundance of microorganisms are high, and the impact of small hydropower stations is the lowest. In summary, small hydropower stations have an impact on the biodiversity of the study watershed, showing an order of fish > benthic > plankton > microorganism.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PG, TW, and YX contributed to the conception and design of the study. YY and YZ organized the database. FL performed the statistical analysis. HM and JL wrote the first draft of the manuscript. ZZ, KY, and QL wrote sections of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Role of Impoundments Created by Low-Head Dams in Affecting Fish Assemblages in Subtropical Headwater Streams in China

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Low-head dams are ubiquitous human disturbances that degrade aquatic ecosystem function worldwide. The localized effects of low-head dams have been relatively well documented; however, most previous studies have ignored the concealed process caused by native-invasive species. Based on fish assemblage data from the first-order streams of four basins in the Wannan Mountains, we used a quantitative approach to assess the effects of low-head dams on fish assemblages by distinguishing between native and native-invasive species using occurrence- and abundance-based data, respectively. Low-head dams significantly decreased native fish alpha diversity while favoring native-invasive fish. The opposite pattern between the two fish types partly masked changes in the whole fish assemblage. Meanwhile, the establishment of widespread native-invasive species and the loss of native species driven by low-head dams influenced the interaction network structure. The degree to which local fish assemblages were altered by low-head dams, i.e., beta diversity (β-diversity) was significantly higher for abundance-based approaches than for occurrence-based ones, suggesting that the latter underestimated the effects of low-head dams. Furthermore, the species contribution to β-diversity of native species was significantly higher than that of native-invasive species in both impoundments and free-flowing segments for abundance-based data. In communities or regions where native fish species are predominant, our results suggest that understanding which species contribute to β-diversity will offer new insights into the development of effective conservation strategies by taking the identities of native and native-invasive species into account.

Keywords: low-head dams, stream fish, diversity patterns, interaction networks, native-invasive species

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INTRODUCTION

Habitat alteration and fragmentation resulting from anthropogenic disturbances greatly contribute to global freshwater biodiversity loss (Erős and Grant, 2015; Barbarossa et al., 2020; He et al., 2021). In a river network, headwaters have unique features and are extremely vulnerable to anthropogenic disturbances (Vannote et al., 1980; Meyer et al., 2007). Headwater streams not only export quantities of organic matter and nutrients to downstream ecosystems (Vannote et al., 1980; Eggert et al., 2012), but also provide heterogeneous habitats for a wide range of endemic species, which collectively contribute to the biodiversity of river networks (Meyer et al., 2007).

Low-head dams are ubiquitous human-made disturbances in mountainous headwater streams that are used for water supply, agricultural irrigation, and recreation (Yan et al., 2013; Jumani et al., 2020; Li et al., 2021). Dams can affect stream fish assemblages *via* multiple pathways, including blocking species dispersal between localities (Porto et al., 1999; Barbarossa et al., 2020) and changing the habitat and physicochemical conditions in streams (Smith et al., 2017; Hitchman et al., 2018; Turgeon et al., 2019). Specifically, the transformation from lotic to lentic conditions in impoundments can provide suitable conditions for potential invasive species to displace native species (Johnson et al., 2008; Liew et al., 2016; Turgeon et al., 2019). Further, habitat simplification can decrease resource and niche availability (Ruhí et al., 2016; Turgeon et al., 2019).

The localized effects of individual low-head dams have been relatively well documented, but the effects and conclusions differ among studies (see Fencl et al., 2017; Turgeon et al., 2019; Li et al., 2021). A potential reason for this inconsistency is that non-native or generalist native species can compensate for the difference in total local diversity in impoundments (Slawski et al., 2008; Liew et al., 2016; Turgeon et al., 2019). Invasions of non-native species caused by dams have a profound impact on global freshwater biodiversity and have drawn increasing attention from the public (Liew et al., 2016; Turgeon et al., 2019; Zhang et al., 2019). More importantly, in regions where native fish species are completely dominant, generalist native species that commonly occur in large streams would be potential invaders for upland streams and are usually termed as "native-invasive species" (Scott and Helfman, 2001; Dala-Corte et al., 2019). However, most previous studies on the ecological effects of low-head dams have ignored the invasion patterns of native-invasive species, as increased native diversity is generally viewed as a positive effect on ecosystem function.

Changes in species occurrences (i.e., local extinctions and colonizations) not only alter community α-diversity, but also affect community β-diversity and dynamics (Socolar et al., 2016; Erős et al., 2020), resulting in the formation of "novel communities" (Hobbs et al., 2006). A serious effect of native-invasive species invasion on native species is a change in trophic ecology through predation, competition, and the indirect effects in the context of environmental changes (Busst and Britton, 2017). Although the effects of low-head dams on habitat conditions and biodiversity have long been examined (Fencl et al., 2015, 2017; Turgeon et al., 2019), the responses in species interaction networks remain understudied. Recently, Momal et al. (2020) proposed a more robust method for inferring species interaction networks by incorporating environmental conditions (i.e., abiotic factors). Furthermore, native-invasive species typically have broad niches and environmental tolerances in headwater streams (Scott and Helfman, 2001). These native-invasive species with broad niches may contribute less to beta diversity than species with narrow or intermediate niches, because the latter species may occur in environmentally restricted conditions and contribute more to beta diversity (Heino and Grönroos, 2017). Therefore, exploring the contribution of each species to beta diversity may provide a platform for understanding the formation of community diversity patterns (Heino and Grönroos, 2017; Gavioli et al., 2019).

The Wannan Mountains of China, characterized by low-head dams becoming ubiquitous features of the landscape (Li et al., 2021), offer an excellent opportunity to evaluate the effects of low-head dams on fish assemblages. Empirical evidence has shown that spatial patterns in the ecological effects of low-head dams can be quantified effectively by selecting free-flowing reference segments (Fencl et al., 2017; Hitchman et al., 2018; Li et al., 2021). Based on fish assemblage data collected in the impoundments created by low-head dams (i.e., comparison sites) and free-flowing segments (i.e., reference sites) in the first-order streams of the Wannan Mountains, we used a quantitative approach to assess the patterns in low-head dams affecting fish assemblages by considering the distinction between native and native-invasive species. We aimed to: (1) determine the effects of low-head dams on within-community (i.e., α-diversity) and between-community (i.e., β-diversity) diversities, (2) quantify the contributions of each species to the β-diversity for each habitat type, and (3) assess the difference in species interaction networks between the habitat types.

MATERIALS AND METHODS

Study Area

The Wannan mountainous region is located in the southern part of Anhui Province, China, and is composed of the Jiuhua, Huangshan, and Tainmu Mountains, with elevations of 1,342, 1,841, and 1,787 m, respectively. The study region is influenced by a subtropical monsoon climate, where the average annual temperature is approximately 17.8°C and the average annual rainfall is approximately 2,000 mm, with precipitation occurring mostly from May to September. Streams are abundant and extremely complex in this region, with major examples including the Qingyi, Qiupu, Chang, and Xin'an streams (Li et al., 2021).

Our study included 61 low-head dams in the first-order headwater streams (stream order, Strahler, 1957) of the Wannan Mountains, of which 16, 9, 9, and 27 low-head dams were located in the Qingyi, Qiupu, Chang, and Xin'an streams, respectively, (**Figure 1**). To properly identify and understand the effects of low-head dams, two types of sampling sites were set for each dam surveyed, comprising the impoundments created by low-head dams (i.e., comparison sites) and free-flowing segments (i.e., reference sites) far away from the dams. In this study, our criteria for selecting reference sites were as follows: (1) all sites should be wadable and accessible (i.e., first-order stream); (2) since the geomorphological footprints of low-head dams are less than 2 km, the free-flowing reference segments were ensured to be at least 2 km from each dam (Fencl et al., 2015); and (3) all the reference sites were located below dams to avoid confusion; references selected above the dams would ignore the blocking effects of the dam (Li et al., 2021).

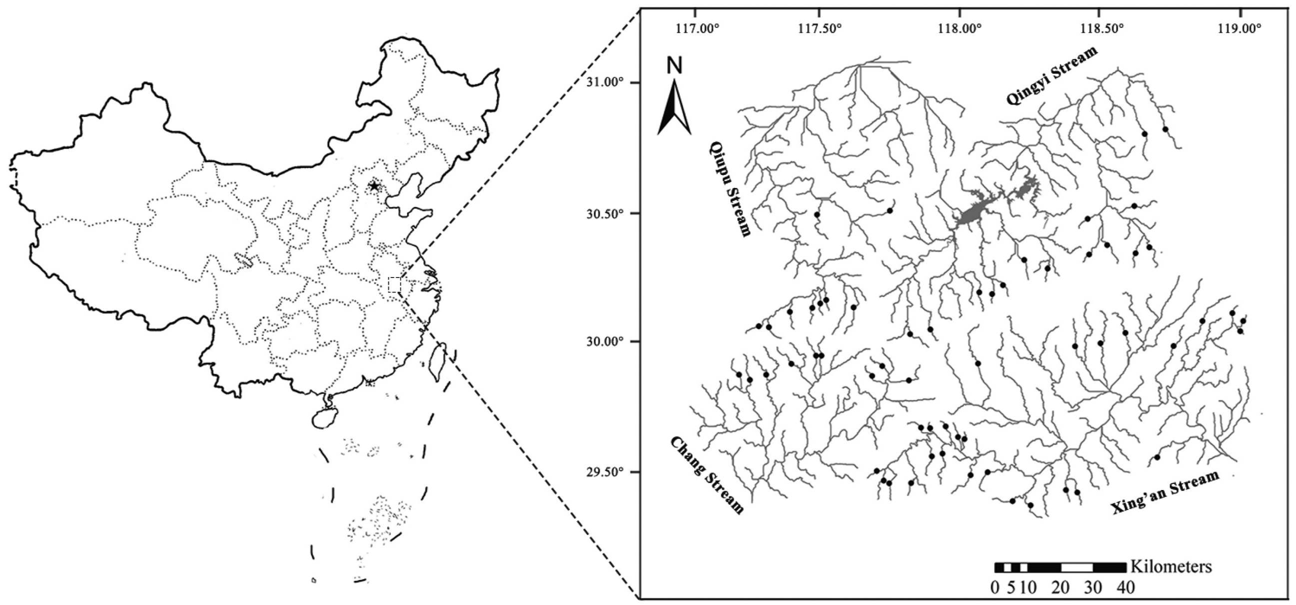

FIGURE 1 | Map showing the survey of low-head dams distributed among four basins in the Wannan Mountains, China. One impoundment area and one free-flowing segment were sampled for each dam.

Field Survey

Fish assemblages were collected from each site using a backpack electrofishing unit (CWB-2000 P, China; 12V import, and 250V export) by wading in two passes during August 2015. For more details on the fish sampling methods and sampling effects, see Li et al. (2021). The fish were identified to the species level using Nelson's (2006) classification method, counted, and released. Species were categorized as native and native-invasive species according to Scott and Helfman (2001), Chu et al. (2015); and Li et al. (2021).

The following local habitat conditions were measured at each site according to the methodology described by Li et al. (2021): (1) wetted width, (2) water depth, (3) current velocity, (4) dissolved oxygen, (5) substrate coarseness, and (6) heterogeneity. In addition, two dam metrics were used to quantify the size of each low-head dam: the dam height was estimated as the vertical height of the dam from the natural streambed to its lowest point on the dam crest, and the dam length was measured as the horizontal distance across the channel at the dam crest.

Data Statistics

The frequency of occurrence and relative abundance (*RA*) of each species were calculated for each habitat type. Species richness and abundance were regarded as the α-diversity. We used beta diversity to quantify the degree to which fish assemblages were altered by low-head dams. We calculated the temporal beta diversity (TBI) between the impoundment and free-flowing segment for each low-head dam for both occurrence and abundance data using the Sørensen dissimilarity index and its quantitative version, the Bray–Curtis dissimilarity index, respectively. The Sørensen indices, use occurrence-based data, which focus on species identities, i.e., abundant and rare species are treated equally (Koleff et al., 2003). However, abundance-based approaches give less weight to rare species, which means that they depend less on the sample size than occurrence-based approaches (Barwell et al., 2015).

For occurrence and abundance data, we obtained the fish community data (n site $\times$ p species) for two different habitat types, impoundments (*Im*) and free-flowing segments (*Fr*). We calculated the TBI between the impoundment and free-flowing reference segment for each dam. Specifically, we calculated the Sørensen ($\beta_{\text{Sør}}$) and Bray–Curtis dissimilarities (β_{BC}) for both occurrence- and abundance-based data (**Figure 2**). The TBI (i.e., beta diversity) was further partitioned into gain and loss components, based on the method of Legendre (2019). In addition, we first obtained the dissimilarity matrix ($n \times n$ site) for each habitat type separately using the Hellinger-transformed community data (n site $\times$ p species). Based on the dissimilarity matrix, we calculated the species contribution to beta diversity (SCBD) of individual species for each habitat type separately (**Figure 2**; Legendre and De Cáceres, 2013). All of the above diversity indices were calculated through the "*TBI*" function (Legendre, 2015) and "*adespatial*" package with the function "*beta.div*" (Legendre and De Cáceres, 2013) in R 4.0.2.

Using the standardized environmental variables, we employed principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA) to test the differences in habitat conditions between impoundments and free-flowing segments. The paired sample t-test was used to test the differences in species richness and abundance between the impoundments and free-flowing reference segments. Given that changes in the local diversities of whole species may not mean any change for native species (Turgeon et al., 2019), the

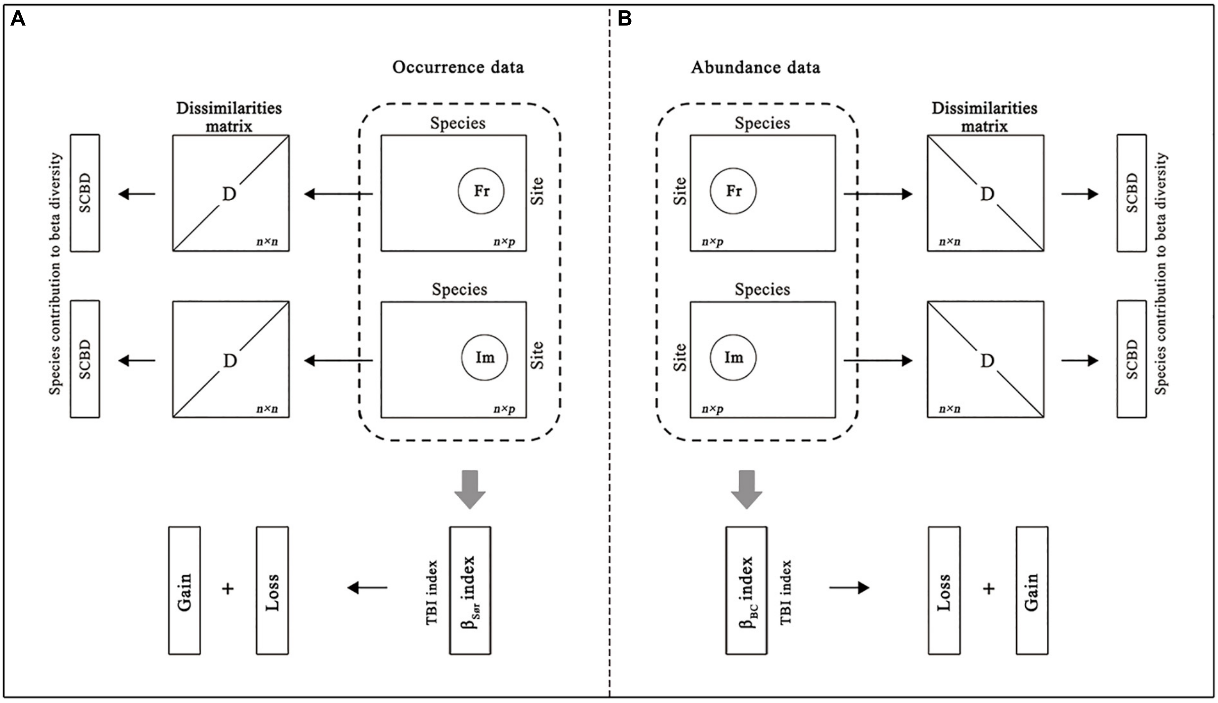

FIGURE 2 | Schematic diagram of the comparison of community data (*n* site × *p* species) for two different habitat types, impoundments (*Im*) and free-flowing segments (*Fr*). We calculated the temporal beta diversity (TBI) using the Sørensen dissimilarity index ($\beta_{Sør}$) for **(A)** occurrence data and the Bray-Curtis dissimilarity index (β_{BC}) for **(B)** abundance data. The TBI was further partitioned into gain and loss components, based on the method of Legendre (2019). In addition, we first obtained the dissimilarity matrix (*n* × *n* site) for each habitat type, and then we calculated the species contribution to beta diversity (SCBD) of individual species for each habitat type separately (adapted from Kuczynski et al., 2018).

paired samples *t*-test was conducted by distinguishing between native and native-invasive species. To determine whether the ecological effects of low-head dams depended on the data type, we used the paired samples *t*-test to test the differences in beta diversity for each dam between occurrence- and abundance-based measures. In addition, changes in local diversity associated with anthropogenic disturbances often depend on the relative number between the species lost and gained. As a complement to local diversity changes, we also used the paired samples *t*-test to test the differences between gain and loss components for each data type.

To quantify the contributions of native and native-invasive species to the β-diversity for each habitat type, we first obtained the SCBD of individual species that reflected the relative importance of species for a region (Legendre and De Cáceres, 2013; Heino and Grönroos, 2017). Analysis of variance was used to test the difference in SCBD values between the native and native-invasive species in impoundments and free-flowing segments. Statistical analyses were performed for occurrence- and abundance-based data separately.

Poisson log-normal (PLN) models are joint species distribution models that can be used to infer species interactions while controlling for environmental factors and sampling efforts (Chiquet et al., 2019). Following Chiquet et al. (2019), we constructed a suite of PLN models to infer the species interaction network of fish assemblages in each habitat type using environmental factors and species abundance data. Here, we built 13 PLN models that included different combinations for the free-flowing reference segments, and 17 PLN models were built for the impoundments by considering the dam height and length (**Supplementary Appendix 1**). Additionally, the sampling effort (i.e., the total number of fish caught at each site) was included in our models to control for differences in fish abundance at different sites (Chiquet et al., 2019; Brimacombe et al., 2021). The PLN models were evaluated to determine the best model using non-traditional Bayesian information criterion (BIC) and cumulative root-mean-square error. Generally, higher BIC scores indicate better-fitting models. If the PLN models showed the same BIC scores, the model with the lowest RMSE score was selected as the best model. Furthermore, we inferred the fish interaction networks for each habitat type using the EMtree method proposed by Momal et al. (2020). The EMtree method, which represents a single undirected connection between species nodes, can infer both potential direct and indirect interactions between species. A criterion for assessing the reliability of species connections is to use a higher threshold such that at least one node (i.e., species) has at least one connection to build the network (Bassett et al., 2006). We created networks with the highest threshold that remained connected. PLN models were performed using the "*PLN models*" package (Chiquet et al., 2019), and EMtree (EMtree version 1.1.0) was accomplished using the "*EMtree*" package (Momal et al., 2020).

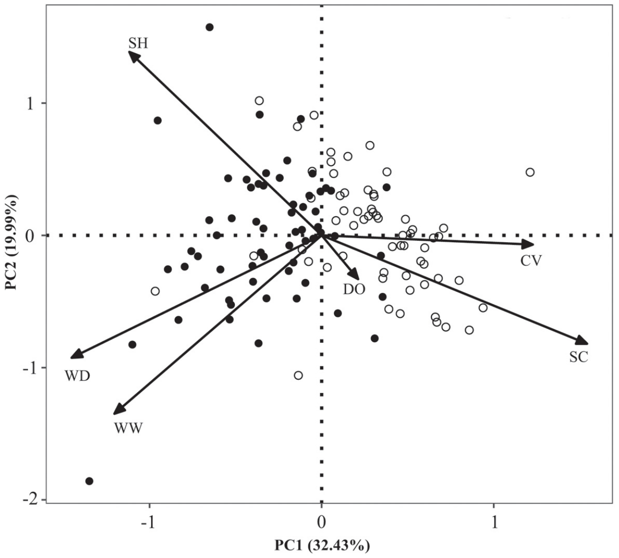

FIGURE 3 | Ordination plots of the principal component analysis (PCA) between impoundments and reference free-flowing segments. Solid and hollow circles represent the impoundments and free-flowing segments, respectively. WW, wetted width; WD, water depth; SH, substrate heterogeneity; SC, substrate coarseness; DO, dissolved oxygen; and CV, current velocity.

RESULTS

Habitat Conditions

The first and second axes of PCA explained 32.43 and 19.99% of the habitat variance, respectively. Sampling sites were scattered at the right section of the ordination plot for the impoundments and at the left section for the free-flowing segments (**Figure 3**). PERMANOVA revealed that habitat conditions significantly differed between impoundments and free-flowing segments ($R^2 = 0.15$, $P < 0.001$). Compared with free-flowing segments, conductivity, water width, and water depth were higher, and current velocity and substrate coarseness were lower in impoundments (**Figure 3**).

Community Composition

Thirty-two species belonging to 11 families and 5 orders were collected. Thirty and 29 species were collected in impoundments and free-flowing segments, respectively, of which the two habitat types shared 27 species, including 14 native and 13 native-invasive species. Additionally, two native-invasive species (*Micropercops swinhonis* and *Pelteobagrus fulvidraco*) were only collected in impoundments, whereas two native (*Leptobotia guilinensis* and *Parasinilabeo assimilis*) and one native-invasive (*Silurus asotus*) species only occurred in free-flowing segments (**Table 1**).

Changes in α- and β-Diversity

Regarding the whole assemblages, the paired samples t-test showed that the mean species abundance significantly decreased in impoundments compared to that in free-flowing segments ($P < 0.05$), whereas the species richness did not significantly vary between the two habitat types (**Table 1**). After distinguishing the two categories of fish assemblages, the species richness and abundance of native assemblages significantly varied between the two habitat types ($P < 0.05$), both of which were significantly lower in impoundments than in free-flowing segments. In addition, when only native-invasive assemblages were considered, the species richness was significantly higher in impoundments than in free-flowing segments ($P < 0.05$), whereas no significant difference was observed for species abundance (**Table 2**).

In addition, the mean β-diversity using occurrence-based approaches ($\beta_{\text{sør}}$) was significantly lower than that using abundance-based approaches (β_{BC}; $P < 0.05$). Meanwhile, the abundance-based approaches detected a higher loss component (0.34 ± 0.22) than the gain component (0.20 ± 0.19) for all assemblages ($P < 0.05$), but no significant difference was observed for occurrence-based approaches (**Table 3**).

Species Contribution to β-Diversity for Native and Native-Invasive Species

When using occurrence data, there was no significant difference in SCBD values between native and native-invasive species in either the impoundments or free-flowing segments ($P > 0.05$, **Figure 4A**). However, abundance data showed significant differences in SCBD values between native and native-invasive species in both habitat types ($P < 0.05$, **Figure 4B**). Specifically, the SCBD values were significantly higher for native fish species than for native-invasive ones in both the impoundments and free-flowing segments (**Figure 4B**).

Inferred Species Interaction Networks From EMtree

Within each habitat type, the best-fit PLN model was selected to infer the species interaction networks. Specifically, the PLN model with "Site name" was chosen for both habitat types, which had the highest BIC scores (free-flowing segments: -2,503.34; impoundments: -2,315.86; **Supplementary Appendix 1**). Meanwhile, native species, such as *Zacco platypus* and *Cobitis sinensis*, showed the highest betweenness centrality values (larger nodes in the figures) in the free-flowing segments (**Figure 5A**). However, native-invasive species, such as *Sarcocheilichthys parvus* and *Acheilognathus taenianalis*, achieved the highest betweenness centrality values in the impoundments (**Figure 5B**).

DISCUSSION

As a common anthropogenic disturbance in headwater streams, low-head dams may modify the local habitat in stream, including deeper water, slower flow, smaller substrate in impoundments upstream, and faster flow and larger substrate in the plunging areas downstream (Tiemann et al., 2004; Yan et al., 2013; Fencl et al., 2015). Due to the backwater effect of dams, the wetted width and water depth are greater in impoundments than that in free-flowing segments (Fencl et al., 2015). As

TABLE 1 | Species compositions, occurrence of frequency (*FO*), and relative abundance (*RA*) of fishes collected in impoundments (*Im*) and free-flowing segments (*Fr*).

Order/Family/Species	**Abbreviation**	**Abundance data**				**Occurrence data**			
		RA (%)		**SCBD (%)**		**FO (%)**		**SCBD (%)**	
		Im	*Fr*	*Im*	*Fr*	*Im*	*Fr*	*Im*	*Fr*
Cypriniformes									
Cobitidae									
Cobitis sinensis	*C. sinensis*	4.54	0.09	4.57	0.31	9.84	3.28	2.29	0.80
Cobitis rarus	*C. rarus*	6.49	4.55	9.63	9.31	39.34	29.51	9.14	6.69
*Misgurnus anguillicaudatus**	*M. anguillicaudatus*	4.04	1.94	8.03	5.03	45.90	31.15	9.67	7.04
Leptobotia guilinensis	*L. guilinensis*	0.00	0.12	0.00	0.20	0.00	4.92	0.00	1.23
Homalopteridae									
Vanmanenia stenosoma	*V. guilinensis*	2.04	9.52	3.41	8.71	31.15	72.13	6.38	8.18
Cyprinidae									
Zacco platypus	*Z. platypus*	30.50	35.54	15.30	19.56	83.61	80.33	7.20	7.75
Acrossocheilus fasciatus	*A. fasciatus*	10.74	9.07	12.81	12.02	62.30	55.74	10.84	9.58
Opasrrichthys bidens	*O. bidens*	0.42	0.30	0.78	0.45	9.84	9.84	2.10	2.18
Belligobio nummifer	*B. nummifer*	0.67	0.12	1.46	0.34	11.48	3.28	2.69	1.25
*Rhodeus ocellatus**	*R. ocellatus*	5.41	7.31	5.68	6.82	26.23	26.23	5.40	5.42
Acheilognathus chankaensis	*A. chankaensis*	0.58	0.97	0.41	1.53	1.64	3.28	0.24	0.68
Rhynchocypris oxycephalus	*R. oxycephalus*	4.95	4.91	2.48	7.18	6.56	18.03	2.34	6.12
*Carassius auratus**	*C. auratus*	2.70	0.64	3.27	0.69	16.39	11.48	4.02	2.66
*Abbottina rivulars**	*A. rivulars*	1.58	0.42	1.25	0.35	9.84	4.92	2.05	0.94
Aphyocypris chinensis	*A. chinensis*	0.42	0.85	0.83	1.64	8.20	4.92	1.57	0.94
*Sarcocheilichthys parvus**	*S. parvus*	0.54	0.61	1.29	1.09	4.92	4.92	1.00	1.17
*Squalidus argentatus**	*S. argentatus*	0.21	0.55	0.42	1.60	8.20	4.92	1.04	1.88
*Pseudorasbora parva**	*P. parva*	1.33	1.06	1.89	0.80	18.03	8.20	4.06	1.74
*Acheilognathus taenianalis**	*A. taenianalis*	0.04	0.06	0.11	0.12	1.64	1.64	0.57	0.51
Onychostoma barbatula	*O. barbatula*	0.08	0.18	0.79	0.51	1.64	6.56	0.41	2.10
Parasinilabeo assimilis	*P. assimilis*	0.00	0.09	0.00	0.10	0.00	3.28	0.00	1.03
Siluriformes									
Amblycipitidae									
Liobagrus styani	*L. styani*	0.29	1.15	0.45	1.82	4.92	16.39	0.98	4.43
Bagridae									
Pseudobagrus truncates	*P. truncates*	0.87	0.55	0.99	1.25	9.84	18.03	2.41	5.31
*Pelteobagrus fulvidraco**	*P. fulvidraco*	0.04	0.00	0.32	0.00	1.64	0.00	0.57	0.00
Siluridae									
*Silurus asotus**	*S. asotus*	0.00	0.03	0.00	0.14	0.00	1.64	0.00	0.61
Beloniformes									
Adrianichthyidae									
*Oryzias sinensis**	*O. sinensis*	0.37	0.06	0.29	0.06	3.28	1.64	0.51	0.28
Synbranchiformes									
Synbranchidae									
*Monopterus albus**	*M. albus*	0.54	0.09	0.79	0.13	11.48	4.92	2.91	1.44
Mastacembelidae									
*Sinobdella sinensis**	*S. sinensis*	0.17	0.24	0.33	0.40	6.56	9.84	1.51	2.43
Perciformes									
Odontobutidae									
*Odontobutis potamophila**	*O. potamophila*	3.54	1.46	6.20	3.29	29.51	22.95	6.40	6.28
*Micropercops swinhonis**	*M. swinhonis*	0.08	0.00	0.09	0.00	3.28	0.00	0.51	0.00
Gobiidae									
Ctenogobius sp.	*Ctenogobius* sp.	16.77	17.34	16.78	13.73	60.66	75.41	10.19	7.30
Percichthyidae									
*Siniperca chuatsi**	*S. chuatsi*	0.04	0.18	0.08	0.80	1.64	4.92	0.95	2.05

*SCBD, species contribution to beta diversity. *indicates native-invasive fish species based on* Scott and Helfman (2001); Chu et al. (2015); *and* Liu et al. (2019).

TABLE 2 | Spatial variations in species richness and fish abundance (mean ± standard deviation) between the impoundments (*Im*) and free-flowing segments (*Fr*) for whole, native, and native-invasive assemblages based on the paired samples *t*-test.

Assemblages types	Diversity	Habitat type		t	P
		Im	*Fr*		
Whole assemblages	Species richness	5.26 ± 2.51	5.48 ± 2.13	1.24	0.22
	Abundance	39.39 ± 30.43	54.07 ± 40.64	2.89	**0.005**
Native assemblages	Species richness	3.41 ± 1.81	4.05 ± 1.49	3.02	**0.004**
	Abundance	31.26 ± 28.75	46.15 ± 34.41	3.89	**0.000**
Native-invasive assemblages	Species richness	1.85 ± 1.92	1.43 ± 1.70	-2.73	**0.008**
	Abundance	8.13 ± 14.40	7.92 ± 16.45	-0.15	0.88

Values in bold indicate significant differences.

TABLE 3 | Variation in beta diversity (β-diversity) between the data types and the difference between losses and gains components for each data type based on paired samples *t*-test.

Diversity	Occurrence data	Abundance data	
β-diversity	0.37 ± 0.20	0.54 ± 0.21	*t* = 7.33, *P* = **0.000**
Loss	0.20 ± 0.19	0.34 ± 0.22	
Gain	0.16 ± 0.14	0.20 ± 0.19	
	t = -1.30, *P* = 0.20	*t* = -2.98, *P* = **0.004**	

Values in bold indicate significant differences.

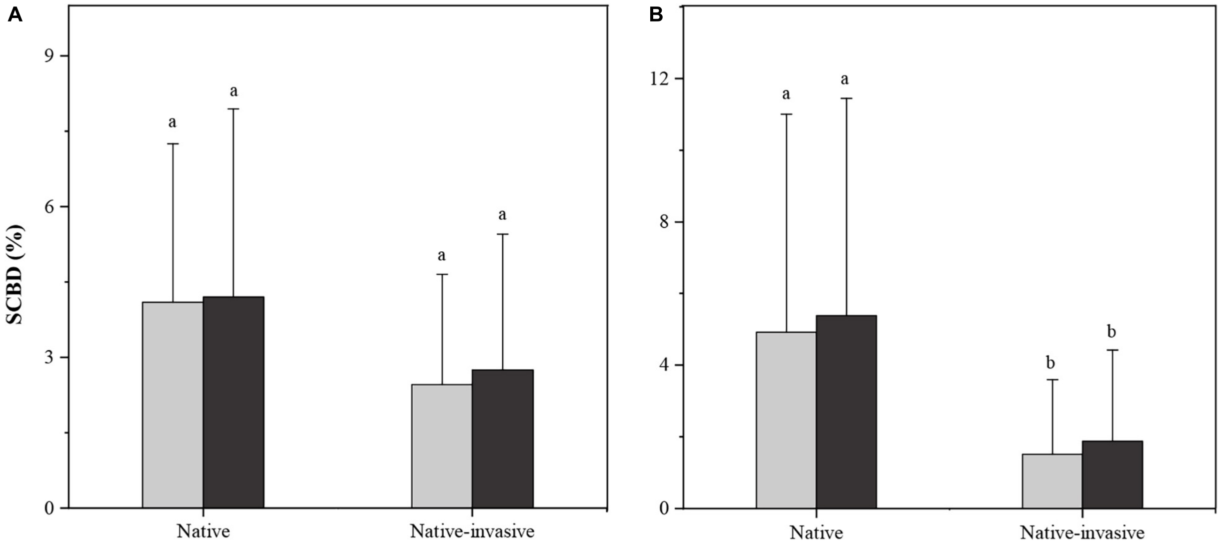

FIGURE 4 | Bar plots showing the mean ± SD of the species contribution to β-diversity (SCBD) of native species and native-invasive species in impoundments (black) and free-flowing segments (gray). Different letters represent significant differences ($P < 0.05$). **(A)** occurrence-based SCBD, **(B)** abundance-based SCBD.

the water depth increases in impoundments, current velocity is reduced and its sediment transport ability in the water column increases, generally resulting in an increase of sediment deposition (Tiemann et al., 2004; Yan et al., 2013). In this study, we found that low-head dams significantly decreased substrate coarseness, current velocity, and increased water depth, wetted width, and substrate heterogeneity in impoundments, which is consistent with the findings of other studies (Yan et al., 2013; Fencl et al., 2015).

Habitat modifications associated with anthropogenic activities not only cause the native species population to decline and even cause local extinctions, but also facilitate the invasion of alien/native-invasive species into upland streams (Slawski et al., 2008; Dala-Corte et al., 2019; Turgeon et al., 2019). A serious consequence associated with low-head dams is the addition of native-invasive species and exclusion of native species (Liew et al., 2016; Smith et al., 2017; Liu et al., 2019). Our study clearly documented that the species richness of native species significantly decreased in impoundments, while that of native-invasive species increased (**Table 2**). Changes in local diversities associated with anthropogenic activities often depend on the relative number of lost and gained species and their abundances

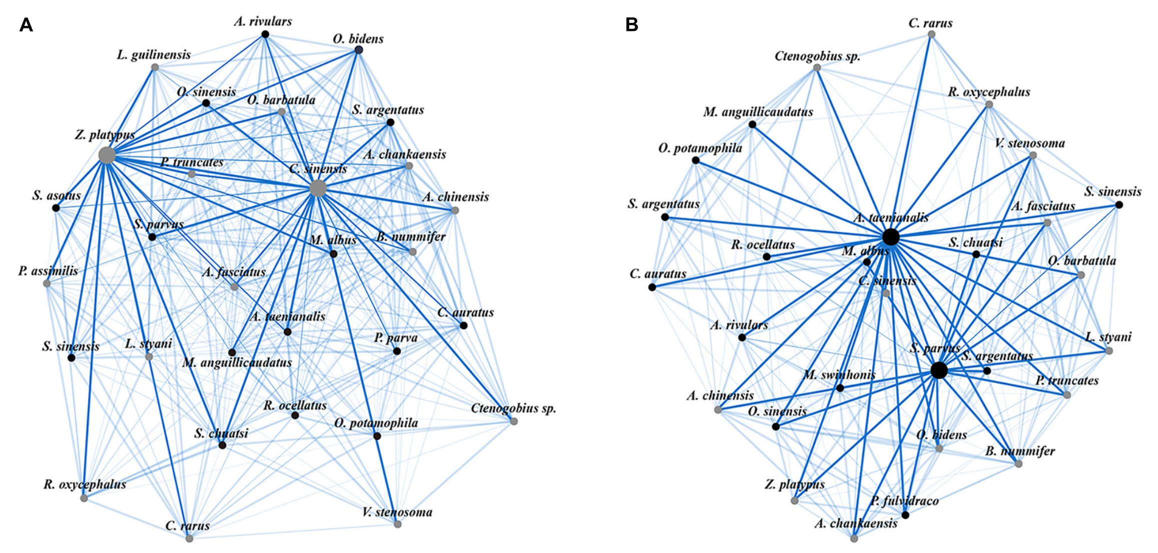

FIGURE 5 | Species interaction networks of stream fish assemblages in the free-flowing segments **(A)** and impoundments **(B)**. Each species is represented by a node, and each edge width is proportional to its conditional probability. The native (gray) and native-invasive species (black) are marked differently. For more details about species abbreviations, see **Table 1**.

(Turgeon et al., 2019). Unsurprisingly, increases in native-invasive species richness can largely compensate for the loss of native species, which could explain this pattern of changes in the total species richness in impoundments; this effect masked changes in the whole fish assemblage. When the loss of native species (abundance change: 14.89) outnumbers the gain of native-invasive species (abundance change: 0.21), changes in the species abundance patterns of whole assemblages may be driven by native species (**Table 2**). Overall, low-head dams favor native-invasive species, while negatively affecting native species with high sensitivity to environmental change (Slawski et al., 2008; Liu et al., 2019).

The variation in both the identity and abundance of species between different habitats can also affect the degree to which local fish community compositions differ (Barwell et al., 2015; Li et al., 2021). More importantly, our results showed that the local community changes in fish assemblages (i.e., the total β-diversity) associated with low-head dams were significantly higher for abundance-based approaches than for occurrence-based approaches (**Table 3**), suggesting that the latter could underestimate the localized effects of individual low-head dams. As low-head dams are less likely to result in local-scale species extinctions than large dams, most of the remaining species often experience changes in abundance (decreased, increased, or unchanged; Yan et al., 2013; Fencl et al., 2017). Previous studies have shown that changes in fish diversity patterns may be primarily driven by changes in the abundance of native species (Tiemann et al., 2004; Liu et al., 2019) in communities or regions where native-invasive species are still not predominant. If the number of shared species between communities does not change, but the abundance does, the dissimilarity index based on abundance approaches will show changes that depend on the magnitude of shifts in abundance (Cassey et al., 2008; Baselga, 2013; Legendre, 2014). In this study, 29 of the 32 species were found in both impoundments and free-flowing segments. However, besides the variations in the abundances of dominant native species, we also found that some native species (e.g., *Vanmanenia stenosoma* and *Aphyocypris chinensis*) drastically decreased and other native-invasive species (e.g., *Carassius auratus* and *Odontobutis potamophila*) markedly increased in abundance in the impoundments compared to in the free-flowing segments (**Table 1**). Therefore, the results of our study are not surprising when formerly rare species become dominant or *vice versa*, given that species abundances can change drastically enough to produce large differences relative to the changes in species composition from one community to another.

For occurrence- and abundance-based approaches, we also found that native species had higher SCBD values than native-invasive species in both habitat types, but significant variation was observed only in abundance-based approaches (**Figure 4**). Headwater streams with highly heterogeneous habitats nourish unique species assemblages (Meyer et al., 2007), where native species with small niche breadths occurring under environmentally restricted conditions may contribute more to β-diversity (Heino and Grönroos, 2017). As is generally observed in natural ecosystems, communities often exhibit heterogeneous species–abundant distributions (Magurran et al., 2011). In this study, the *RA* of most native species across sites was higher than that of native-invasive species, especially in regions where native fish species were completely predominant. Previous studies have shown that species with high total abundance across sites contribute most to the abundance-based β-diversity (Heino and Grönroos, 2017; Gavioli et al., 2019). When the identities of native and native-invasive species are considered, the high SCBD values can reveal which native-invasive species are the most abundant and widespread, whereas the low SCBD values can identify rare native species. Although some rare native species have a relatively lower contribution to β-diversity, such species may still need conservation actions owing to their local extinction risk (Gavioli et al., 2019). Meanwhile, the native-invasive species with high abundance should be strictly controlled to mitigate the ecological consequences of low-head dams.

Complex ecological networks of interacting species are crucial for maintaining biodiversity in local ecosystems and the effects of species interactions can differ either spatially or temporally (Tylianakis and Morris, 2017). Normally, a fraction of native species may primarily contribute to local community dynamics (Meyer et al., 2007), especially in headwater streams where native-invasive fish species are still not predominant. However, changes in species diversity and basal food resources resulting from habitat modifications can affect the species interaction networks (Brimacombe et al., 2021; Danet et al., 2021). Previous studies have shown that impoundments created by dams can act as powerful environmental filters that may select sets of species that are better adapted to particular habitat conditions (Liew et al., 2016). In this study, we found that the betweenness centrality of native-invasive species (i.e., omnivorous fish) was the highest in the impoundments (**Figure 5**), which reflected the importance of species for the interaction network structure. Indeed, betweenness centrality measures the ability of nodes (i.e., species) to act as a bridge in the interaction network, which can efficiently identify the "keystone species" in ecosystems (González et al., 2010; Momal et al., 2020). High betweenness centrality values of species represent high network connectance among species, which can strengthen the importance of species interaction networks (Thébault and Fontaine, 2010). In addition, the transformation of natural lotic habitats into artificial lentic habitats not only facilitate the establishment of native-invasive species (Chu et al., 2015; Liew et al., 2016), but also greatly change basal food resources (Ruhí et al., 2016). Feeding habits can play a crucial role in maintaining the stability of species interactions, and tropical streams are typically dominated by omnivorous mechanisms (Ruhí et al., 2016; Brimacombe et al., 2021). Therefore, native-invasive species (omnivorous fish) can take full advantage of niches specific to lentic habitats in impoundments, which are essential for maintaining the stability of interaction networks.

CONCLUSION

In this study, we used a quantitative approach to assess the patterns of low-head dams affecting stream fish assemblages by distinguishing between native and native-invasive species. Our results suggest that low-head dams affect stream fish diversity in two ways: by adding native-invasive species and by excluding native species typical of headwaters. However, changes in species richness and abundance not only alter the local community composition (Barwell et al., 2015; Li et al., 2021), but also affect the community stability (Brimacombe et al., 2021). Although species interactions were not directly observed in our system, we provide initial evidence that low-head dams influence the interaction network structure. Additionally, we found that occurrence-based approaches could underestimate the localized effects of low-head dams when fish assemblages differ primarily in abundance rather than species composition. SCBD values can determine which species contribute the most to β-diversity (Legendre and De Cáceres, 2013). We found that the abundance-based SCBD values were significantly higher for native fish species than for native-invasive ones in both habitat types. In communities or regions where native fish species are predominant, our results suggest that understanding which species contribute to β-diversity will offer new insights into the development of effective conservation strategies, by taking the identities of native and native-invasive species into account.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Committee of the Anhui Normal University.

AUTHOR CONTRIBUTIONS

YY and QL designed the study. QL, XL, KT, CZ, and YY conducted the field and/or laboratory work. QL, XL, and CZ analyzed data. LC, HF, and YG contributed materials, reagents, and analytical tools. QL drafted the manuscript with the assistance of CZ and YY. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.916873/full#supplementary-material

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Multifaceted fish diversities respond differently to impounding age and longitudinal location along a reservoir cascade

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Damming usually modifies riverine habitats, which affects various aspects of fish diversity, especially in a reservoir cascade. Their influence on fish assemblage has been studied widely, but a lack of data from the diversity perspective remains. The Gezhouba Reservoir and Three Gorges Reservoir are two of the largest cascaded reservoirs located on the upper Yangtze River. In this study, we investigated the current fish assemblages in 2020~2021 and retrieved 22 previous investigations in different sections of this cascade system to analyze how fish taxonomic, functional, and phylogenetic alpha- and beta-diversity change with the distance from the dams and the impounding age during 1998~2021, and all sampling sites are located in the upper section of the dams. The total species richness and phylogenetic diversity increased significantly with the distance from the dams, but the functional diversity did not change substantially. No significant difference was found in the influence of impounding age on the three aspects of fish diversity. We observed a noticeable increase in non-indigenous fish species richness, functional diversity, and phylogenetic diversity over time, these effects were similar in areas at different distances from the dams. The species richness and phylogenetic diversity of lotic fish decreased from the lotic to lentic zones, whereas the functional and phylogenetic diversities decreased significantly with impounding age. The taxonomic beta-diversity was remarkably higher than the functional and phylogenetic beta-diversities. The differences among the three facets of beta-diversity were driven by a lower functional turnover than the taxonomic and phylogenetic turnovers, and their nestedness components were low without exception. The present study suggests that trade-offs should be considered when designing policies to protect fish diversity based on different objectives.

KEYWORDS

reservoir cascade, species richness, functional diversity, phylogenetic diversity, conservation

Introduction

Freshwater ecosystems harbor some of the highest levels of fish diversity on earth but are also the most vulnerable (Tedesco et al., 2017). Dam construction is one of the major factors affecting the freshwater fish diversity worldwide, as it changes different aspects of the ecosystem, such as primary production, hydrological dynamics, and riverine connections (Loures and Pompeu, 2019). A new reservoir usually has the highest productivity and supports high fish abundance after its initial impounding due to the rich nutrient and plant material input from the surrounding areas. Subsequently, it enters a “decrease-stable” process with an increase in impounding age (Agostinho et al., 2008). Impoundment can alter the hydrological dynamics and generate a habitat gradient (comprising the lentic, transitional, and lotic habitats) in a reservoir; usually, these habitats have different physical and chemical characteristics and support different fish assemblage structures and diversity (Vašek et al., 2016). For example, the disruption of riverine habitats usually decreases lotic fish diversity due to destruction of their feeding and reproductive environment (Cheng et al., 2015). There is also the opinion that the lotic-lentic stretches are beneficial in maintaining species richness, as the lentic habitat may favor the establishment and dispersal of non-indigenous species (dos Santos et al., 2018). The interruption of longitudinal connectivity of natural rivers after damming often leads to considerable decline in migratory fish abundance due to loss of access to spawning and nursery habitats (Sá-Oliveira et al., 2015). Understanding the spatial and temporal changes in fish diversity after damming have always been a pressing concern for fish resource management, protection, and restoration.

Most previous studies on the effects of damming have considered fish taxonomic diversity (e.g., species richness) due to its simplicity and convenience; however, effective estimation and conservation efforts require a deeper understanding of other facets of fish diversity, such as functional and phylogenetic diversity (Wong et al., 2018; Su et al., 2021; Wang et al., 2021). Functional diversity quantifies the ranges of unique morphological, physiological, and ecological traits of fish community (Palacios-Salgado et al., 2019); phylogenetic diversity reflects the genetic variability within fish assemblage, which can influence the community’s adaptability in response to environmental variations (Lima-Junior et al., 2021). Previous studies revealed that one fish assemblage can exhibit similar variation patterns of taxonomic, functional, and phylogenetic diversities in response to environmental changes (e.g., Tuya et al., 2018), while further studies suggest that multiple approaches can furnish complementary information (e.g., Wong et al., 2018; Roa-Fuentes et al., 2019; Jiang et al., 2021). In addition, the three facets of diversity usually have different sensitivity to environmental variations, among which the functional diversity seems to be the most vulnerable one (Sanchez-Perez et al., 2020; Lin et al., 2021).

In addition to investigating multiple facets of fish diversity within different fish communities (alpha-diversity), quantifying the spatial and temporal variations across fish community compositions (beta-diversity) have become another important topic of investigation (Anderson et al., 2011). Beta-diversity evaluates dissimilarity among the communities directly, and it is being used more frequently in evaluating the geographic and temporal changes in fish diversity (Villéger et al., 2013; Li et al., 2018; Nakamura et al., 2020). Beta-diversity can be further divided into turnover and nestedness components (Baselga, 2010). The turnover component reflects the taxonomic, functional, and phylogenetic replacement among the communities, whereas the nestedness component implies the difference in taxonomic, functional, and phylogenetic quantity (Villéger et al., 2013). Quantifying the β-diversity of fish assemblages with different components can provide a comprehensive assessment for assembly mechanisms, if we consider the taxonomic, functional, and phylogenetic dimensions (Li et al., 2021). For example, fish communities with a high taxonomic beta-diversity may have low functional or phylogenetic beta-diversities if their respective species are functionally or phylogenetically similar (Jiang et al., 2021). High beta-diversity can result either from a low proportion of shared species (or function, phylogeny) among communities with similar species (or function, phylogeny) quantity, or from a different quantity of species (or function, phylogeny) among communities (Villéger et al., 2013). It is, therefore, crucial to understand the relationship between alpha- and beta-diversities as well as their different components to investigate the ecological processes structuring fish assemblages.

The Yangtze River is the world’s third largest river and its mainstem can be divided into the lower (Shanghai to Hukou, 938 km), middle (Hukou to Yichang, 955 km), and upper reaches (Yichang to headstream, 4,504 km; Xiong et al., 2021). The upper reach has an abundant level of hydropower resource and has the largest cascading reservoir system in China. The Three Gorges Reservoir (TGR) and Gezhouba Reservoir (GZB), both located in the Yichang, Hubei province, are two large reservoirs [i.e., height $>$ 15 m or height 5–15 m and impounding $>$ 3 million m^3; International Commission on Large Dams [ICOLD], 2019], forming the lowest part of the reservoir cascade. These reservoirs impound approximately 700 km long stretch of the Yangtze River, and their impact on fish has been extensively investigated (Xu et al., 2021). For example, several researchers evaluated the spatial and temporal changes of the fish assemblage structure (Wu et al., 2007; Gao et al., 2010; Liu et al., 2012; Yang et al., 2012; Zhao et al., 2015; Lin et al., 2019), biomass (Liao et al., 2018), life-history strategies (Perera et al., 2014;

Liao et al., 2019), and spawning conditions (Yu et al., 2019; Ma et al., 2020). In contrast, only a few studies have focused on these changes from a fish diversity perspective (e.g., Zhang et al., 2020). How facets of fish biodiversity change at different dimensions, the underlying causes and factors, and the spatial and temporal patterns of these alterations have not been studied in detail.

In this study, we mainly aim to assess the spatial and temporal variations of fish diversity in relation to the construction of the Three Gorges Dam and Gezhouba Dam and to provide scientific rationale for fish resource management and protection. To achieve these objectives, we investigated the current fish assemblages in 2020∼2021 and simultaneously retrieved previous investigations documenting species composition, and formed a total of 27 datasets to evaluate the taxonomic, functional, and phylogenetic alpha- and beta-diversities of fish assemblages across the GZB and TGR. We expected different change patterns over locations and impounding ages among different facets of fish diversity, and we hypothesized that taxonomic diversity decreased less with impounding age, compared to functional and phylogenetic diversities.

Materials and methods

Study area

The Three Gorges Reservoir is one of the largest reservoirs in the world. It was impounded through three stages, and its final impoundment formed a reservoir of 1,080 km^2 with a total length of 667 km in 2009. The Gezhouba Reservoir (GZB), located at about 60 km downstream of the Three Gorges Reservoir (TGR), was constructed in 1988 and formed a reservoir with a total length of 40 km. We based the current study at nine mainstem sites (GZB, Zigui, Wushan, Yunyang, Wanzhou, Zhongxian, Fuling, Banan, and Jiangjin) and three tributary sites (Xiakou, Shuanglong, and Gaoyang) along the two reservoirs. These sampling sites are located in the upper section of the Three Gorges Dam and Gezhouba Dam with different distance from the dam bodies, which represent diverse habitat characteristics (lentic, transitional, and lotic), and were affected by the impoundment of the TGR or GZB at different periods (**Table 1** and **Figure 1**). The upper reach of the Yangtze River runs from the "Three Gorges" (i.e., the TGR and GZB), which refers to reaches between Chongqing and Yichang (**Figure 1**). All 12 sampling sites were located in these areas, which showed riverine condition and have spatially similar fish assemblage structures before the dam construction. But the background fish assemblage structures were quite different between the upper and middle-lower reaches [Investigation Group of Fishery [IGF], 1975; Fan et al., 2012], so we only sampled fish assemblages above dams.

Data collection

From April 2020 to July 2021, we sampled fishes using experimental multi-panel gillnets and benthic fyke nets (12 m in length, 0.75 mm, knot to knot) at five sampling sites (Zigui, Xiakou, Wushan, Yunyang, and Zhongxian) of the TGR. To cover the benthic and pelagic water, we used two types of multi-panel gillnets, benthic (2 m in height) and pelagic (5 m in height), having the same length and mesh-size structures, i.e., the total length of each gillnet was 30 m and consisted of 12 different mesh-sizes (10, 16, 20, 25, 31, 39, 48, 58, 70, 86, 110, 125 mm, knot-to-knot). At each sampling site, we randomly selected three locations (≈ 500 m apart) and deployed three benthic gillnets, three pelagic gillnets, and three benthic fyke nets for 12 h (18:00–19:00 to 6:00–7:00) per location. We sampled each site for 2 days per season to increase our sample size.

We also retrieved historical fish presence/absence data from a variety of sources and as many as possible, including published articles, books, and scientific reports; and we recorded the present/absent fish as 1/0, respectively. To evaluate fish diversity in each period and section accurately, we discarded those sources that lack a detailed fish list. Given that the fish species documented in previous studies were based on different classified standards, we checked the Latin names of all species in FishBase dataset and updated them with the currently valid scientific names; and we adjusted the species composition data frame accordingly. We discarded those fish species that were recorded only once, and our final dataset included 27 datasets and 140 fish species.

Previous investigations along the cascade system of the TGR and GZB were conducted during different periods before and after their constructions. To evaluate the response of fish diversity to the filling process, we defined a pristine baseline related to the condition prior to the respective impoundment per sampling site (**Table 1**). The GZB was first impounded in 1988, followed by the first (2003), second (2006), and third impoundments (2009) of the TGR, which raised the water level to 145 m, 156 m, and 175 m, respectively. The three fillings of the TGR generated respective reservoir areas reaching Fuling, Changshou, and Jiangjin sections. Therefore, we considered 1988 as the baseline for the GZB site, 2003 for the sampling sites between the Three Gorges Dam and Fuling section, 2006 for the sampling sites between Fuling and Changshou sections, and 2009 for the sampling sites between Changshou and Jiangjin sections. These baselines should be appropriate to assess changes in fish assemblages with the impoundments of the TGR and GZB. The impounding age was calculated by the difference in sampling year from the baseline respectively. Simultaneously, aiming to estimate the temporal and spatial changes of beta-diversity, we defined the fish composition of the lotic Banan section, investigated in 1998, as a consistent baseline. This is mainly because Banan is located at the tail of

TABLE 1 Summary information of data sources, including their sampling sites, sampling period, and respective baselines.

Sites	Latitude/ longitude	Sampling period	Baseline	Habitat	Distance from the dam	Age	Species richness	Data source
GZB	30°44′40.5″N 111°16′44.5″E	2010	1988	Lentic	15	22	54	Liu et al., 2012
Zigui	30°51′08.1″N 110°59′37.2″E	2011	2003	Lentic	8	8	35	Perera et al., 2014
Zigui	30°51′08.1″N 110°59′37.2″E	2012	2003	Lentic	8	9	51	Yang et al., 2015
Zigui	30°51′08.1″N 110°59′37.2″E	2014	2003	Lentic	8	11	56	Lian, 2016
Zigui	30°51′08.1″N 110°59′37.2″E	2017	2003	Lentic	8	14	65	Sampling
Zigui	30°51′08.1″N 110°59′37.2″E	2021	2003	Lentic	8	18	52	Sampling
Xiakou	31°07′05.6″N 110°47′15.3″E	2013	2003	Lentic	50	10	45	Zhao et al., 2015
Xiakou	31°07′05.6″N 110°47′15.3″E	2021	2003	Lentic	50	18	40	Sampling
Shuanglong	31°08′29.1″N 109°54′27.8″E	2012	2003	Lentic	150	9	41	Yang et al., 2013
Wushan	31°03′51.2″N 109°53′16.3″E	2012	2003	Lentic	130	9	55	Yang et al., 2013
Wushan	31°03′51.2″N 109°53′16.3″E	2014	2003	Lentic	130	11	53	Lian, 2016
Wushan	31°03′51.2″N 109°53′16.3″E	2021	2003	Lentic	130	18	45	Sampling
Yunyang	30°56′36.6″N 108°39′43.5″E	2012	2003	Transitional	258	9	62	Yang et al., 2015
Yunyang	30°56′36.6″N 108°39′43.5″E	2014	2003	Transitional	258	11	64	Lian, 2016
Yunyang	30°56′36.6″N 108°39′43.5″E	2021	2003	Transitional	258	18	45	Sampling
Gaoyang	31°05′37.8″N 108°40′33.9″E	2010	2003	Transitional	278	7	56	Li et al., 2013
Wanzhou	30°48′55.1″N 108°23′46.0″E	2006	2003	Transitional	298	3	56	Gao et al., 2010
Wanzhou	30°48′55.1″N 108°23′46.0″E	2011	2003	Transitional	298	8	47	Perera et al., 2014
Wanzhou	30°48′55.1″N 108°23′46.0″E	2017	2003	Transitional	298	14	62	Sampling
Zhongxian	30°18′29.2″N 108°03′23.9″E	2014	2003	Transitional	378	11	62	Lian, 2016
Zhongxian	30°18′29.2″N 108°03′23.9″E	2021	2003	Transitional	378	18	51	Sampling
Fuling	29°43′03.1″N 107°24′7.0″E	2011	2006	Lotic	490	5	50	Perera et al., 2014; Gao et al., 2010
Fuling	29°43′03.1″N 107°24′7.0″E	2012	2006	Lotic	490	6	62	Yang et al., 2015
Banan	29°34′36.0″N 106°50′59.8″E	2014	2009	Lotic	600	5	88	Lian, 2016
Banan	29°34′36.0″N 106°50′59.8″E	2017	2009	Lotic	600	8	79	Sampling
Banan	29°34′36.0″N 106°50′59.8″E	1998	2009	Lotic	600	−11	68	Luo, 1999
Jiangjin	29°17′37.7″N 106°15′21.2″E	2012	2009	Lotic	680	3	65	Yang et al., 2015

the TGR with a relatively high species richness, which has not yet been affected by the TGR impoundments and maintained its natural state in 1998 (Luo, 1999; Yang et al., 2012; Liao et al., 2018). Lotic and non-indigenous fishes received much attention in previous studies, and we specially selected these fish faunas to analyze their diversity changes (Ba and Chen, 2012; Xiao et al., 2015).

Taxonomic, functional, and phylogenetic alpha diversity

The taxonomic diversity was assessed by species richness (SR), which was defined as the total number of fish species per sampling. We selected eleven functional traits representing the major trait categories of body size, feeding, migration, reproduction, and habitat preference (**Supplementary Table 1**). We collected the functional trait data from FishBase, published literatures, books, and our measurements. In the cases where a small number of species lacked published/unpublished data and preserved specimens, we replaced their functional data with that of their congeneric species. We used functional richness (FRic; Villéger et al., 2008) and functional dispersion (FDis; Laliberté and Legendre, 2010) to quantify functional diversity of each fish assemblage. We were also interested in and studied these diversity indices of non-indigenous, lotic, and lentic fish faunas.

We used the most common cytochrome b (Cytb) sequence to construct phylogenetic relationships among the 140 fish species that were studied. The FASTA-formatted sequence data were collected from the National Center for Biotechnology Information (NCBI). We imported the sequence data to MEGA software (version 7.0) and used Neighbor-Joining method to build a phylogenetic tree that was used to measure the phylogenetic diversities. We selected Faith's Phylogenetic Diversity (PD; Faith, 1992) and Phylogenetic Species Variability (PSV; Helmus et al., 2007) to quantify phylogenetic diversity of each fish assemblage. More specifically, the FRic and PD represent absolute diversities and are thought to be related to species richness, whereas the FDis and PSV represent diversity dispersion and are statistically independent of species

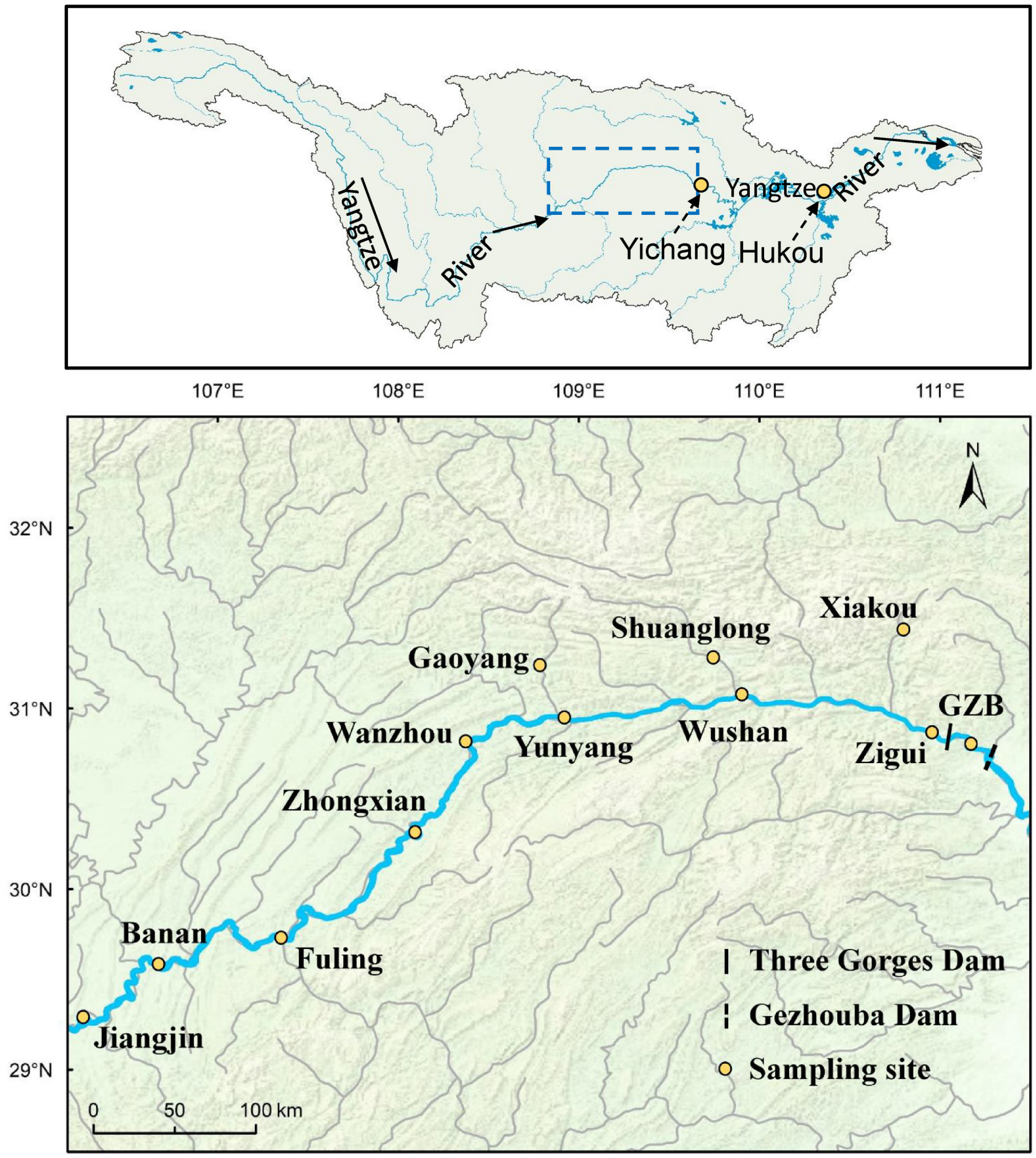

FIGURE 1
The map above shows the whole Yangtze River and its two demarcation points (Yichang and Hukou) and the study areas; the map below exhibits the Three Gorges Reservoir and Gezhouba Reservoir and all sampling sites. Solid arrows on the top panel represent the flow direction of the Yangtze River.

richness. These indexes are robust metrics reflecting the functional and phylogenetic diversities (Kellar et al., 2015; Rurangwa et al., 2021).

Taxonomic, functional, and phylogenetic beta-diversity

Based on the aforementioned "site × species," "species × traits," and "species × phylogenetic" datasets, we measured taxonomic, functional, and phylogenetic beta-diversities (β_{SPE}, β_{FUN}, and β_{PHY}; Villéger et al., 2013). These indices range from 0 to 1 and the higher values indicate greater dissimilarity between samplings. We measured the β_{SPE}, β_{FUN}, and β_{PHY} based on the Jaccard dissimilarity index, and divided each index into its respective turnover component and nestedness component (Baselga, 2010; Villéger et al., 2013). The three facets of beta-diversity and their components can be compared to each other, because their decomposition is based on the same metrics, namely shared and non-shared richness (Villéger et al., 2013).

Statistical analysis

The spatial and temporal comparisons in diversity indexes were assessed using linear mixed-effects models (LMM, Bates et al., 2015). The indexes of alpha- and beta-diversity were modeled as a function of impounding age, distance from the dam, and their interactions. As no significant interactions were found, we adjusted models by removing interactive terms. We included sampling time and sampling site as a random factor to account for the lack of independence between multiple sampling events conducted at the same site and during the same year (Bates et al., 2015). We also focused specifically on the taxonomic, functional, and phylogenetic diversities of non-indigenous and lotic fish faunas and followed the same LMM analysis process (model habitat and age) as we did for overall α-diversity. Model assumptions were verified and indicated no problems regarding the linearity, normality, and homogeneity of variances (Jacqmin-Gadda et al., 2007). We conducted all analyses in R (v.4.0.2, R Foundation for Statistical Computing, Vienna, Austria), using dbFD function in package FD (Laliberté et al., 2014), pd and psv functions in package picante (Kembel et al., 2020), beta.pair, functional.beta.pair, and phylo.beta.pair functions in package betapart (Baselga and Orme, 2012), check_heteroscedasticity function in package performance (Lüdecke et al., 2021), and lmer function in package lme4 (Bates et al., 2015). Figures were created using package ggplot2 (Wickham, 2016).

Results

A total of 140 species belonging to 10 orders, 23 families, and 88 genera were documented across the GZB and the TGR, from 1998 to 2021. Cypriniformes containing four families and 99 species was the most speciose order, followed by Siluriformes (five families, 19 species). The family Cyprinidae had the most species (77 species), followed by Cobitidae (14 species), and Bagridae (12 species). Among these species, a total of 11 species were recorded at each investigation: *Clenopharyngodon idellus*, *Aristichthys nobilis*, *Cyprinus carpio*, *Carassius auratus*, *Hemiculter leucisculus*, *Hemiculter bleekeri*, *Coreius heterodon*, *Squalidus argentatus*, *Saurogobio dabryi*, *Pelteobagrus nitidus*, and *Silurus asotus*; a total of 13 non-indigenous fish species and 56 lotic species were documented.

Temporal and spatial patterns of species, functional, and phylogenetic alpha-diversities

We found that values of the SR, FRic, and PD decreased with increasing impounding ages, and increased with distance from the dam, but only the influences of distance from the dam on the SR and PD were statistically significant (LMM, $p < 0.01$; **Figure 2** and **Table 2**). Values of the FDis and PSV changed in a different pattern, i.e., their values increased with impounding ages, but decreased with either decreased or increased distance from the dam, but only the influence of distance on the FDis was significant ($p < 0.05$; **Figure 2** and **Table 2**). The effects from the interaction of distance and impounding age were non-significant for any index ($p > 0.05$).

Spatial and temporal changes in alpha-diversities of non-indigenous, lotic, and lentic fish

The alpha-diversities of non-indigenous, lotic, and lentic fish were influenced by damming in different spatial and temporal patterns. Specifically, the SR, FRic, PD, and FDis of the non-indigenous fish increased significantly with impounding ages, except for the PSV, whereas these indexes were not significantly affected neither by the distance from the dam nor by the interactions between age and distance (**Table 3**). The average species number of non-indigenous fish investigated by 27 studies was 4.3.

While analyzing lotic species, we observed a significant increase in the SR and PD with increasing distance from the dam, where the FRic was not significantly affected by the distance but was negatively affected by the impounding age. We did not find a significant influence from impounding ages nor a statistical interaction between impounding age and distance for the FDis and PSV. As for lentic species, their SR values were significantly increased with impounding age but were spatially similar. From functional and phylogenetic perspectives, only FDis was positively influenced by distance significantly, and PSV was negatively affected by the impounding age (**Table 3**).

Temporal and spatial patterns of taxonomic, functional, and phylogenetic beta-diversities

Mean value of taxonomic beta-diversity (β_{SPE}: 0.59 ± 0.12) was significantly higher than those of functional beta-diversity (β_{FUN}: 0.16 ± 0.03) and phylogenetic beta-diversity (β_{PHY}: 0.38 ± 0.07; ANOVA, $F = 212$, $p < 0.01$); and the average β_{PHY} value was higher than that of the β_{Fun} (Tukey's HSD test, $p < 0.01$). Values of the β_{SPE}, β_{FUN}, and β_{PHY} increased with increasing impounding ages, but only the increase in β_{FUN} was significant (**Figure 3** and **Table 4**). As for spatial variations, over distance from the dam, the decreases in beta-diversities occur in a similar way for the β_{SPE}, β_{FUN}, and β_{PHY}, but the decrease in β_{FUN} was non-significant (LMM, main effect of distance, $X^2 = 0.05$, $p = 0.82$; **Figure 3** and **Table 4**).

TABLE 2 Summary of linear mixed-effect models to explain variations in taxonomic, functional, and phylogenetic alpha-diversities as a function of impounding age and distance from the dam.

Variable	Factors	Estimates	*SE*	Wald's X^2	*P-value*
SR	Age	1.373	2.461	0.311	0.577
	Distance	8.045	2.107	14.584	**<0.001**
FRic	Age	−0.001	0.002	0.469	0.494
	Distance	0.002	0.002	0.960	0.327
FDis	Age	−0.001	0.002	0.473	0.491
	Distance	−0.004	0.002	5.669	**0.017**
PD	Age	0.049	0.200	0.060	0.806
	Distance	0.496	0.179	7.664	**0.006**
PSV	Age	0.002	0.004	0.319	0.573
	Distance	−0.001	0.006	0.050	0.823

Significant *p*-values are in bold.

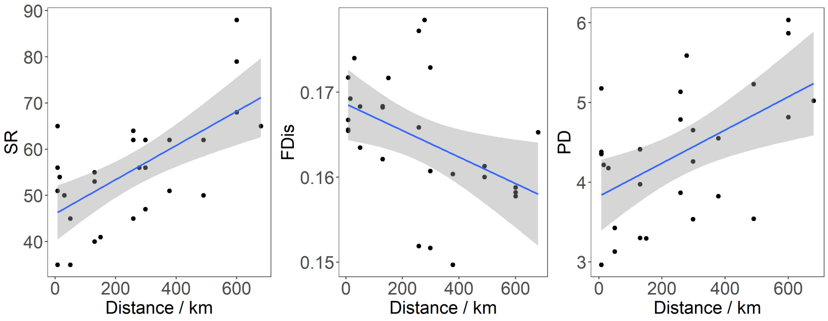

FIGURE 2
The effect of distance from the dam on species richness (SR), functional dispersion (FDis), and phylogenetic diversity (PD) of fish assemblages of the Three Gorges Reservoir and Gezhouba Reservoir. The gray band represents the 95% confidence interval.

The nestedness component values were similar among the β_{SPE} (0.09), β_{FUN} (0.10), and β_{PHY} (0.06; Kruskal–Wallis test, $X^2 = 2.68$, $p = 0.26$); whereas turnover component values of the β_{SPE} (0.50) were significantly higher than those of the β_{FUN} (0.06) and β_{PHY} (0.32; $X^2 = 66.80$, $P < 0.01$; and β_{FUN} vs. β_{PHY}: $p < 0.01$). The turnover component dominated the β_{SPE} and β_{PHY}, which accounted for 84.75% and 84.21%, respectively. The β_{FUN} was dominated by the nestedness component, which accounted for 62.50%.

Discussion

Dam construction, coupled with related hydrologic alteration and biological invasions, represents the greatest threat to fish diversities in rivers (Vega-Retter et al., 2020). In general, fish species richness usually decreases once dams are constructed and impounded (e.g., Lima et al., 2016), and this influence is the most serious in lentic habitats and can continue for years at some reservoirs (e.g., Araguari reservoir cascade system, Loures and Pompeu, 2018). Previous studies mainly focused on the response in fish assemblage structures in GZB and TGR and revealed both their rapid (e.g., Gao et al., 2010) and long-term variations (e.g., Liao et al., 2018) after dam construction. Most studies investigating fish alpha-diversity, conducted at different periods and sections, suggested that the fish species richness usually decreased after their constructions (Wu et al., 2007; Yang et al., 2012; Liao et al., 2018; Lin et al., 2019). Here, we pooled 27 investigations and found out that the spatial and temporal differences of the SR were driven mainly by the spatial factor (distance from the dam), and the SR increased from the lentic to lotic zones of the reservoir. This finding demonstrated that the degree of habitat alteration was the main factor affecting fish species richness (Lima et al., 2016).

Damming usually negatively affects species richness through disrupting migration routes and altering habitat, and such influence can last for years, either in the upper stream of the Yangtze River (Yang et al., 2012; Liao et al., 2018) or other reservoir ecosystems (e.g., Loures and Pompeu, 2018, 2019). In the present study, we found some differences, i.e., that the influence of impounding on the SR was non-significant over time. We added functional and phylogenetic diversities as supplements and found consistent results; like the SR, the FRic, PD, FDis, and PSV also did not significantly change with

TABLE 3 Summary of linear mixed-effect models to explain variations in taxonomic, functional, and phylogenetic alpha-diversities of non-indigenous, lotic, and lentic fish species, as a function of impounding age and distance from the dam.

Fish faunas	Variable	Coefficients	Estimates	*SE*	Wald's X^2	*P-value*
Non-indigenous species	SR	Age	1.062	0.322	10.857	<**0.001**
		Distance	0.082	0.286	0.082	0.774
	FRic	Age	0.036	0.015	1.217	**0.017**
		Distance	0.005	0.010	0.194	0.660
	FDis	Age	0.041	0.013	10.286	**0.001**
		Distance	0.015	0.009	2.399	0.121
	PD	Age	0.132	0.067	3.944	**0.047**
		Distance	0.021	0.044	0.232	0.629
	PSV	Age	0.028	0.035	0.667	0.414
		Distance	0.021	0.023	0.801	0.371
Lotic species	SR	Age	−1.569	1.171	1.795	0.180
		Distance	6.535	1.222	28.617	<**0.001**
	FRic	Age	−0.021	0.007	8.660	**0.003**
		Distance	0.018	0.006	1.973	0.160
	FDis	Age	−0.016	0.010	2.974	0.085
		Distance	−0.005	0.012	0.187	0.666
	PD	Age	−0.132	0.075	3.085	**0.049**
		Distance	0.381	0.075	25.920	<**0.001**
	PSV	Age	0.012	0.012	0.933	0.334
		Distance	0.009	0.011	0.647	0.421
Lentic species	SR	Age	0.213	0.195	0.521	**0.031**
		Distance	0.002	0.008	0.083	0.773
	FRic	Age	< −0.001	< 0.001	0.499	0.480
		Distance	< −0.001	< 0.001	1.104	0.293
	FDis	Age	0.001	< 0.001	1.694	0.193
		Distance	0.001	< 0.001	7.081	**0.008**
	PD	Age	−0.135	0.084	2.585	0.108
		Distance	−0.001	0.002	0.175	0.676
	PSV	Age	−0.003	< 0.001	5.235	**0.022**
		Distance	0.001	< 0.001	0.166	0.684

Significant *p*-values are in bold.

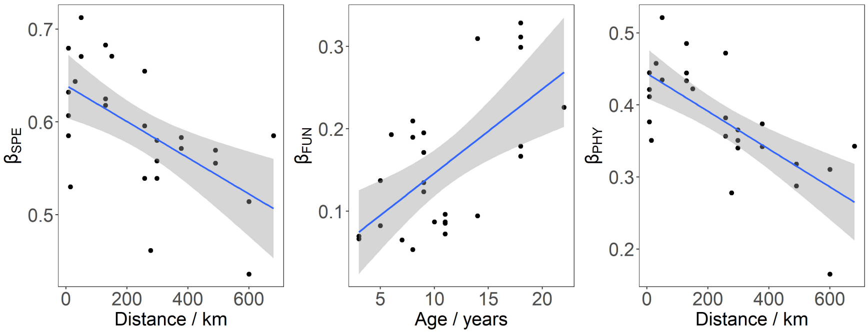

FIGURE 3
The effect of distance from the dam on taxonomic (β_{SPE}) and phylogenetic beta-diversities (β_{PHY}), and the effect of impounding age on functional beta-diversity (β_{FUN}) of the Three Gorges Reservoir and Gezhouba Reservoir. The gray band represents the 95% confidence interval.

TABLE 4 Summary of linear mixed-effect models to explain variations in taxonomic, functional, and phylogenetic beta-diversities as a function of impounding age and distance from the dam.

Variable	Coefficients	Estimates	*SE*	Wald's X^2	*P*-value
β_{SPE}	Age	−0.002	0.014	0.001	0.976
	Distance	−0.043	0.010	18.369	**<0.01**
β_{FUN}	Age	0.035	0.020	4.458	**0.033**
	Distance	−0.017	0.020	0.048	0.826
β_{PHY}	Age	−0.001	0.013	0.001	0.972
	Distance	−0.047	0.011	22.349	**<0.01**

Significant *p*-values are in bold.

the impounding age. This consistent pattern indicated that fish alpha-diversity gradually turned into a stable level after the dam construction and impoundment, especially at the scale of the entire reservoir. In addition, we found that all of the SR, FRic, and PD of the non-indigenous fish species increased with impounding years, suggesting that non-native fish species more or less offset the richness losses of other species (Liew et al., 2016; Turgeon et al., 2019).

The creation of reservoirs favors the establishment of non-indigenous fish populations, adding threats to native freshwater fish species (Johnson et al., 2008; Casimiro et al., 2017). Although some researchers proposed that dams can hinder fish spreading upward or downward into new water areas (Dana et al., 2011), the dam-driven hydrological alterations and catchments connection can often facilitate fish invasion (Casimiro et al., 2017; Kerr et al., 2021). The consistent increase in taxonomic, functional, and phylogenetic alpha-diversity indices of non-indigenous species with the impounding age confirmed that the construction of the GZB and TGR promoted fish invasion and increased non-indigenous fish alpha-diversity. A reservoir cascade along the Araguari River Basin exhibits a similar phenomenon; the non-native richness increased with reservoir age, and these effects were similar in both lotic and lentic habitats (Loures and Pompeu, 2019). Turgeon et al. (2019) concluded that non-indigenous fish species usually increased after damming in tropical and temperate river ecosystems. Artificial reservoirs provided "stepping-stone" habitats for the fish invasion (Johnson et al., 2008) and provided vacant niches that facilitated non-indigenous fishes colonizing and expanding their populations therein (Turgeon et al., 2019). In 2020∼2021, we sampled a total of ten non-indigenous fish species at five sampling sites, where we investigated five to seven indigenous species per sampling site, suggesting that dam provides an initial blockage and later facilitation of non-indigenous fish.

Damming usually modifies physical and chemical characteristics of rivers, such as flow dynamics, water velocity, water depth, water temperature, and channel geomorphology, and these variations form a longitudinal gradient based on distance from the dam (Vašek et al., 2016). In the present study, we found different responses in taxonomic, functional, and phylogenetic diversities to the longitudinal habitats among different fish faunas. The tail section of the TGR still exhibits lotic habitats and was reported to maintain a higher level of fish species richness (Liao et al., 2018). Our results demonstrated that the SR and PD of total fish assemblages or lotic fish faunas increased with distance from the dam, indicating that the remaining lotic habitats play a crucial role in maintaining species richness and genetic diversity (Loures and Pompeu, 2019). However, the functional diversity did not exhibit spatial gradient for fish assemblages or lotic fish faunas, which indicated that functional diversity seems to be more vulnerable to damming. Lin et al. (2021) also found that congruence is hard to achieve among the three facets of fish diversity, among which functional diversity was the most vulnerable to damming. Sanchez-Perez et al. (2020) also observed an increase in species richness but a decrease in functional richness in the Segura River, southern Spain. These patterns may be due to the functional traits being filtered more seriously by multiple abiotic and biotic factors (Arantes et al., 2019). However, the FRic of non-indigenous fish, consistent with the SR and PD, was non-significantly affected by the distance from the dam, indicating that the impoundment promoted non-indigenous species rapidly spreading to the whole reservoirs by connecting mainstem and distributaries (Júlio Júnior et al., 2009). Therefore, trade-offs should be considered when protecting fish diversity based on multiple facets (Doxa et al., 2020).

Understanding how fish taxonomic, functional, and phylogenetic composition varies spatially and temporally through beta-diversity is crucial (Frota et al., 2021). In the present study, we found that the three-facets of beta-diversity have different values and exhibited different spatial and temporal patterns. Firstly, the taxonomic and phylogenetic beta-diversities decreased significantly with distance from the dam and maintained stability with impounding ages, which further indicated that the taxonomic and phylogenetic structures were altered more severely in lentic habitats compared with the upper lotic habitats of the TGR and that the lotic habitats are not only crucial in conservations of both species and genetic diversities, but also in maintaining of their heterogeneity (Zhang et al., 2018). The functional beta-diversity was lower than taxonomic and phylogenetic beta-diversities, indicating that the current functional structure of fish assemblages is more similar to those during the pre-damming period compared to taxonomic and phylogenetic aspects. Moreover, unlike the taxonomic and phylogenetic beta-diversities, the functional beta-diversity was spatially similar but increased significantly with impounding ages. Some studies proposed that environmental filters and species invasion due to the habitat alteration and elimination of natural barriers contributed the most to the functional homogeneity (e.g., Vitule et al., 2012; Toussaint et al., 2018). Considering these patterns together, we deduced that species introduction and lotic/migratory function simplification should be considered as key factors explaining temporal functional homogeneity in these ecosystems (Santos and Araujo, 2015; Zhang et al., 2018).

Quantifying the multiple components of the three-faceted beta-diversity can further analyze this viewpoint (Villéger et al., 2013). In the present study, the differences among the three-facets of beta-diversity were driven by a lower functional turnover compared to the taxonomic and phylogenetic turnover, while their nestedness components were low without exception, indicating a higher species (phylogeny) replacement between fish assemblages of pre-damming and post-damming periods (Soininen et al., 2018). Damming caused a series of environmental alterations, such as water velocity, food availability, water level fluctuation, and flow dynamics; and these alterations usually caused species replacement, i.e., some sensitive fish species were replaced by eurytopic species and non-indigenous species (Frota et al., 2021). On the other hand, low functional beta-diversity revealed that the frequent species replacements in fish assemblages occurred mainly between fish species that were functionally redundant (Villéger et al., 2013), which also suggested that the GZB and TGR ecosystems may still retain some habitats that determined functional traits and diversities of fish assemblages. Ecologists advise that, for areas where turnover dominates beta diversity, we should protect a larger number of areas to conserve regional diversity; in contrast, we should specially protect one large area with high alpha diversity when nestedness dominates the overall beta diversity (Jiang et al., 2021). From these perspectives, we should protect not only lotic habitats in the tail section of this ecosystem, but also other small habitats in the mainstem and tributaries.

In this study, we analyzed the spatial and temporal changes of the taxonomic, functional, and phylogenetic facets of alpha- and beta-diversities of the GZB and TGR fish communities. We demonstrated that multiple diversity dimensions can add comprehensive information. The findings reveal that even three decades after GZB construction and 12 years after TGR construction, the longitudinal location still plays key role in variations of taxonomic and phylogenetic alpha diversities. With increasing impounding years, taxonomic and phylogenetic alpha diversities gradually became stable, due to the increased diversity of the non-indigenous species. We proposed that the impoundments of the reservoir cascades facilitated expansion of non-indigenous fish. In comparison, the functional diversity did not exhibit obvious spatial differences for overall fish assemblages or lotic fish faunas. Functional diversity seemed to be more vulnerable to damming. Our results regarding beta-diversity and its components also exhibited spatial and temporal patterns, reminding us that trade-offs should be considered when designing policies to protect fish diversity based on different objectives.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Ethics Committee of the Institute of Hydrobiology, Chinese Academy of Sciences. And all experimental methods were performed following the guidelines for the care and use of experimental animals of China (GB/T35892 2018).

Author contributions

CL: investigation, data analysis, writing original draft, and funding acquisition. JW: investigation, resources, and data curation. SY: methodology, resources, and writing—review and editing. WL: methodology and writing—review and editing. SC and TZ: writing—review and editing. JL: writing—review and editing and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.955053/full#supplementary-material

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Genetic sources and diversity of the paddy field carp in the Pearl River basin inferred from two mitochondrial loci

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Paddy field carp (PF-carp) is an economically important fish cocultured with rice in traditional agricultural systems. Several distinctive strains of PF-carp have been formed through years of artificial and cross breeding. There is a concern about the status of germplasm resources among PF-carp, since little is known about the genetic sources, diversity, or differentiation. In this study we collected 17 PF-carp populations covering Daotian carp (DTL), Ru Yuan No. 1 (RY), Jinbian carp (JBL), Shaijiang carp (SJL), and Wu carp (WL) along the Pearl River basin to explore their genetic sources and diversity using concatenated sequences of the mitochondrial cytochrome *b* gene and the D-Loop region. According to the haplotype network analyses, 1, 9, and 57 haplotypes originated from *Cyprinus carpio carpio*, *Cyprinus carpio haematopterus* and *Cyprinus carpio rubrofuscus*, respectively, confirming that genetic introgression has occurred in Pearl River PF-carp populations and *Cyprinus carpio carpio* was the most common species for genetic origin. The results showed that RY exhibited the lowest level of nucleotide diversity ($\pi = 0.0011$) due to high-intensity breeding and was significantly differentiated from the other four strains. PF-carp strains in these remote traditional systems tended to experience artificial selection and a lack of farmer connection that gradually increased genetic differentiation among strains. Notably, three populations of JBL exhibited significant high-level differentiation, since they originated from mountainous areas hindering farmers from fry exchange. In contrast, no significant differentiation was uncovered in the WL populations, since this strain is the most popular cultured strain and has undergone artificial exchange of parents and fry in many cultured regions. This study helps us to understand the status of germplasm resources among PF-carp and to trace their genetic origin before being introduced for local cultivation.

KEYWORDS

paddy field carp, genetic diversity, germplasm resource, farmer connectivity, introgression

Introduction

The common carp, *Cyprinus carpio*, is an economically important fish widely distributed in Eurasia (Kohlmann, 2015). It has adapted to various environments and has experienced long-term breeding to form numerous distinctive strains (Wu and Gui, 1999; Shen and Liu, 2000; Kohlmann, 2015). According to Monographs of Cyprinidae in China, four subspecies of common carp have been recorded in China, *Cyprinus carpio carpio* (CC), *Cyprinus carpio haematopterus* (CH), *Cyprinus carpio rubrofuscus* (CR), and *Cyprinus caprpio chilia* (CCH) (Wu, 1977a,b). However, Kottelat (2001) regarded CC and CR as two separate species and rejected CR as a valid species. Considering Zhou et al. (2004) demonstrated that CC, CH and CR formed three distinct clades using mitochondrial loci, we adopted the taxonomic classification in Wu (1977a,b). CC is distributed over vast areas of Central Asia and Europe, while being only found in Xinjiang in China. CH is distributed as far north as the Heilong River and as far south as the Min River. CR is distributed among the Pearl River, Ruan River, and Hainan Island, while CCH is found only in several lakes of Yunnan Province (Wu, 1977a; Yue, 2000).

In southern China, common carp is frequently co-cultured with rice and thus has been named the paddy field carp (PF-carp), which displays rapid growth, strong reproductive ability, and delicious meat with no muddy taste. This rice-fish system benefits both the agricultural environment and the economy. On the one hand, combining rice and fish farming can reduce the use of pesticides and fertilizers and ensure the health of the grain and fish (Berg, 2001; Ahmed and Garnett, 2011). On the other hand, the price of PF-carp is much higher compared to other freshwater fishes. The co-culture of rice and fish can maximize the use of paddy field resources, alleviate human conflict over land use, and ensure a bumper crop and increase farmers' income (Frei and Becker, 2005; Gan et al., 2019).

Notably, the 1,200-year-old rice–fish system of Qintian county, Zhejiang Province, was listed as one of the "Globally Important Agricultural Heritage Systems (GIAHS)" by the Food and Agriculture Organization of the United Nations in 2005[1] (Xie et al., 2011). PF-carp farming in the GIAHS rice-fish system named Oujiang carp has been practiced and passed down for generations (Wang, 1997). Within the Qintian GIAHS site, rice-fish farmer households exchange fry and parental carp for their own rice–fish production, thereby contributing to a high rate of gene flow and high genetic diversity of PF-carp (Ren et al., 2018).

In addition to the Oujiang carp in Zhejiang, various strains of PF-carp are bred and cultured in China, including Daotian carp (DTL), Jiangbian carp (JBL), Ruyuan NO. 1 (RY), Shaijiang carp (SJL), and Wu carp (Wl) (Zhu et al., 2018; Gan et al., 2019; Yan et al., 2020). Researchers have carried out genetic diversity and germplasm resources analyses of DTL and JBL in the Pearl River basin using mitochondrial genes (mtDNA) and microsatellites (Gan et al., 2019; Pan et al., 2019; Cheng et al., 2021). Cheng et al. (2021) examined the genetic structure of three DTL populations from Guangxi Province based on the mtDNA D-loop region and the mitochondrial Cytochrome *b* gene (Cyt *b*) sequences, indicating that all populations were significantly differentiated (Cheng et al., 2021). Meanwhile, researchers pointed out that certain frequencies of CC and CH were found in these populations, suggesting that PF-carp may suffer introgressive hybridization from CC or CH. Similarly, Pan et al. (2019) used mitochondrial D-loop region and cytochrome *c* oxidase I sequences to analyze the genetic diversity and phylogenetic relationships between PF-carp (JBL, WL) and wild carp. PF-carp showed lower haplotype diversity than wild carp, and exhibited clear differentiation from wild carp (Pan et al., 2019).

These studies preliminarily explained the genetic diversity of PF-carp in the Pearl River basin, although the sampled populations were rather small and covered few strains. In order to investigate the genetic diversity and structure of PF-carp more comprehensively, we analyzed Cyt *b* gene sequences and the D-loop region of 17 populations covering five strains of PF-carp. Our main goals were (i) to trace the germplasm resources of PF-carp populations in the Pearl River basin, (ii) to explore the impact of artificial selection or farmer connectivity on the difference of genetic diversity and differentiation for different populations or strains, and (iii) to provide an effective scientific basis for the protection of genetic resources, artificial breeding, and rational development of the resources of PF-carp.

Materials and methods

Sample collection

A total of 524 paddy field carp (PF-carp) specimens were sampled from 17 localities across Guang Xi Province and Guang Dong Province between 2020 and 2021 (**Figure 1** and **Supplementary Table 1**). Five morphologically different strains of PF-carp, "Daotian carp" (DTL, $n = 161$), "Ru Yuan No. 1" (RY, $n = 65$), "Jinbian carp" (JBL, $n = 64$), "Shaijiang carp" (SJL, $n = 42$), and "Wu carp" (WL, $n = 192$) were obtained (**Figure 2**). In addition, we collected both JBL and DTL at the same location of RS (**Supplementary Table 1**). DTL, JBL, and WL comprised five, three, and eight populations, respectively. RY specifically refers to the PF-carp that has been bred for multiple generations in Yao Autonomous County of Ruyuan. SJL is an indigenous PF-carp cultured in Shaijiang Village of Guangxi Province. A fin clip was taken from each specimen and kept in 99% ethanol. All specimens were stored at the Pearl River Fisheries Research Institute, Chinese Academic of Fishery Sciences.

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[1] www.fao.org/giahs/giahs-home/en/

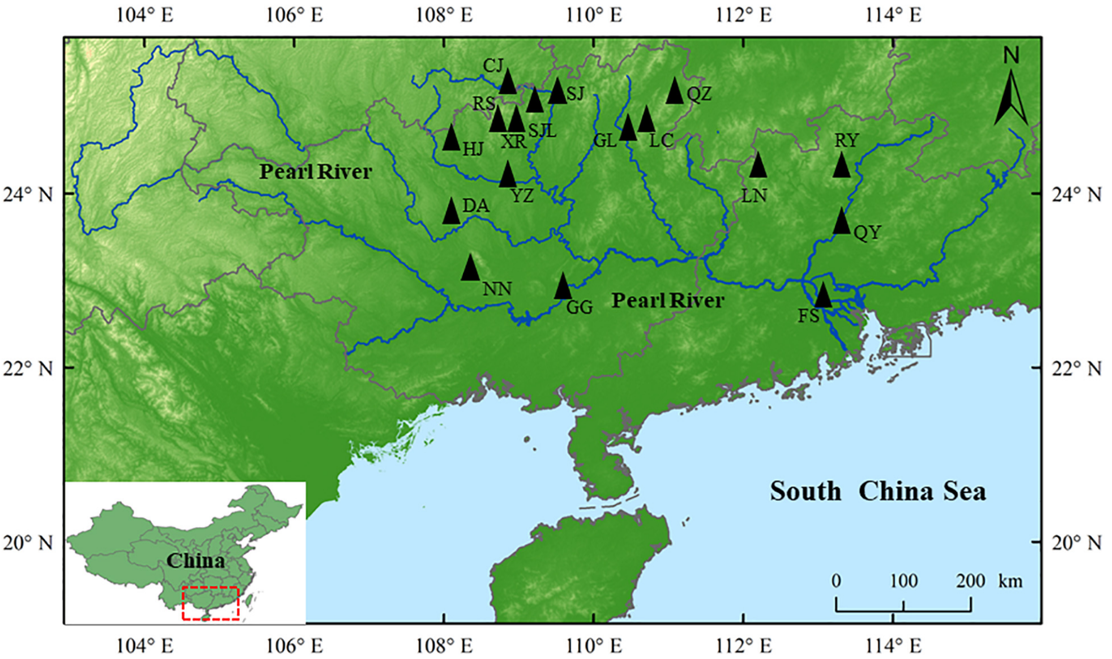

FIGURE 1
Map of sample sites of PF-carp specimens. Geographical distribution details and the number of samples collected per site are provided in **Supplementary Table 1**. CJ, Congjiang; DA, Du'an; FS, Foshan; GG, Guigang; GL, Guilin; HJ, Huanjiang; LC, Linchuan; LN, Liannan; NN, Nanning; QY, Qingyuan; QZ, Quanzhou; RS, Rongshui; RY, Ruyuan; SJ, Sanjiang; SJL, Shaijiang; XR, Xiaorong; YZ, Yizhou.

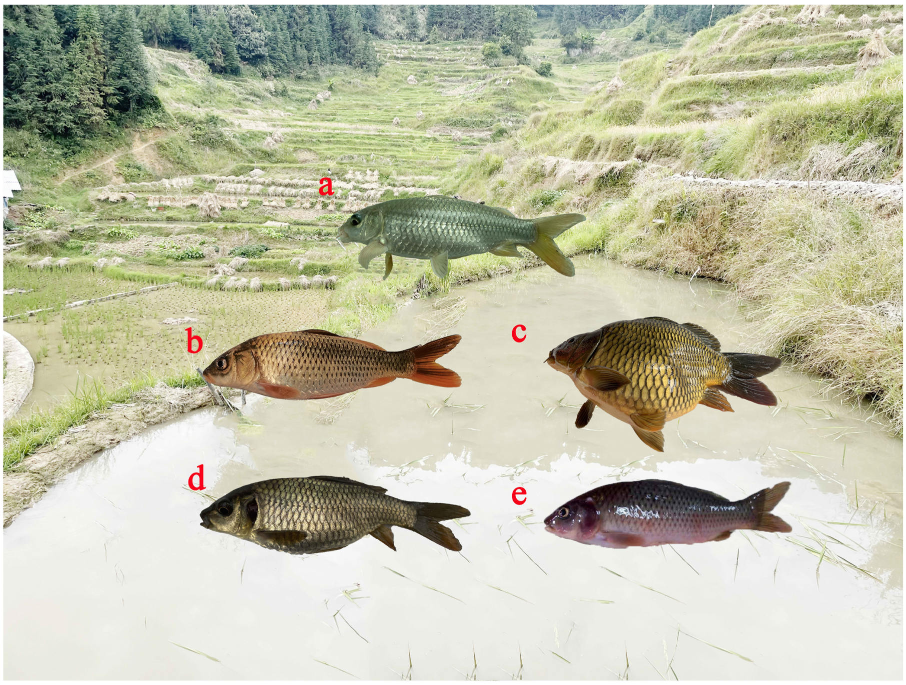

FIGURE 2
Morphology of five PF-carp strains of **(a)** Daotian carp, **(b)** Jinbian carp, **(c)** Ruyuan No. 1, **(d)** Shaijiang carp, and **(e)** Wu carp.

DNA extraction, sequencing, and data processing

Genomic DNA was extracted using a Genomic DNA Extraction Kit according to the manufacturer's instructions (Axygen, United States). DNA samples were kept at −20°C for further PCR amplification. The primers were designed for the Cyt *b* gene (Forward primer: 5′-GACTTGAAAAACCACCGTTG-3′, Reverse primer: 5′-CTCCGATCTCCGGATTACAAGAC-3′) and D-loop region (Forward primer: 5′-CATCTTAGCATCTTCAGTG-3′, Reverse primer: 5′- TCACCCCTGGCTCCCAAAGC-3′) based on the *Cyprinus carpio* mitochondrial genome sequence (GenBank: X61010.1). The amplification program was as follows: an initial denaturation at 94°C for 3 min and then 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min followed by final extension at 72°C for 5 min. The amplified products were checked and purified using 1.0% low-melting agarose gel electrophoresis and sequenced on 3,730 xl DNA Analyzer (Applied Biosystems, United States). The Cyt *b* gene and D-loop region were sequenced bi-directionally and unidirectionally, respectively. Cyt *b* contigs were assembled using SEQMAN (Swindell and Plasterer, 1997). All sequences were aligned, checked, and trimmed using MEGA X (Kumar et al., 2018). The Cyt *b* sequences and D-loop sequences were concatenated to form combined sequences.

Haplotype network analyses

PopART 1.7.2 was used to reconstruct phylogenetic relationships among haplotypes *via* a median-joining network method (Leigh and Bryant, 2015). To characterize the genetic resources of different strains and populations, Cyt *b* and D-loop sequences of *CR (n = 10), CH (n = 6), and CC (n = 4)* were downloaded from *Zhou et al. (2004) to* construct a haplotype network diagram. *Due to no corresponding Cyt b and D-loop sequences published for* CCH and the fact that most previous studies considered that the three abovementioned subspecies were sources for PF-carp selection breeding, our study did not include CCH.

Evaluation of genetic diversity and differentiation

The genetic diversity indices of the number of haplotypes (H), haplotype diversity (H_d) (Shohdy and West, 2020), private haplotype (ph) and nucleotide diversity (π) were assessed with Dansp 6.0 (Rozas et al., 2017) for 17 populations and five strains. Genetic differentiation among and within the five strains was estimated from the fixation index (F_{ST}) using Arlequin 3.5 (Excoffier and Lischer, 2010). Analysis of molecular variance (AMOVA) in Arlequin 3.5 was implemented to partition total genetic variation among and within strains and populations. Both F_{ST} calculation and AMOVA were based on the pairwise difference between haplotypes estimated according to a non-parametric permutation procedure (1,000 permutations/analysis). In order to assess the possible occurrence of an isolation-by-distance pattern, a Mantel test was carried out in Arlequin 3.5. Geographic distances among localities were estimated using Google Earth pro.

Results

Data information

For all the individuals analyzed (*n* = 524), we obtained sequences of 948 bp of the Cyt b gene and 708 bp of the D-loop after alignment and trimming. Sixty variable positions (48 parsimony-informative) and 41 variable positions (31 parsimony-informative) were found in the Cyt b and D-loop, respectively. A total of 66 haplotypes were obtained from the concatenated sequences (1,656 bp).

Haplotype network analysis

A haplotype network can reveal evolutionary relationships and frequency distributions of haplotypes among populations. Haplotype networks were obtained by the statistical parsimony procedure for all strains (**Figure 3**) and for three strains (WL, JBL, and SJL) containing more than two populations (**Figure 4**). Frequencies and distributions of each haplotype among the studied populations are presented in **Supplementary Table 2**. Overall, 1/66, 8/66, and 57/66 haplotypes originated from CC, CH, and CR, respectively (**Figure 3**). The genetic sources of DTL and SJL were derived from CC, CH, and CR, while the genetic resources of JBL, RY, and WL derived from CH and CR (**Figure 3**). According to the haplotype network of DTL (**Figure 4A**), CJ shared haplotypes with the other four populations, while Guigang (GG) and Liannan (LN) only shared haplotypes with Congjiang (CJ). In addition, Huanjiang (HJ) and Rongshui (RS) shared haplotypes. It was worth noting that only one shared haplotype was detected within three populations of JBL (**Figure 4B**). The pattern of shared haplotypes among eight populations within WL strain was frequently observed (**Figure 4C**).

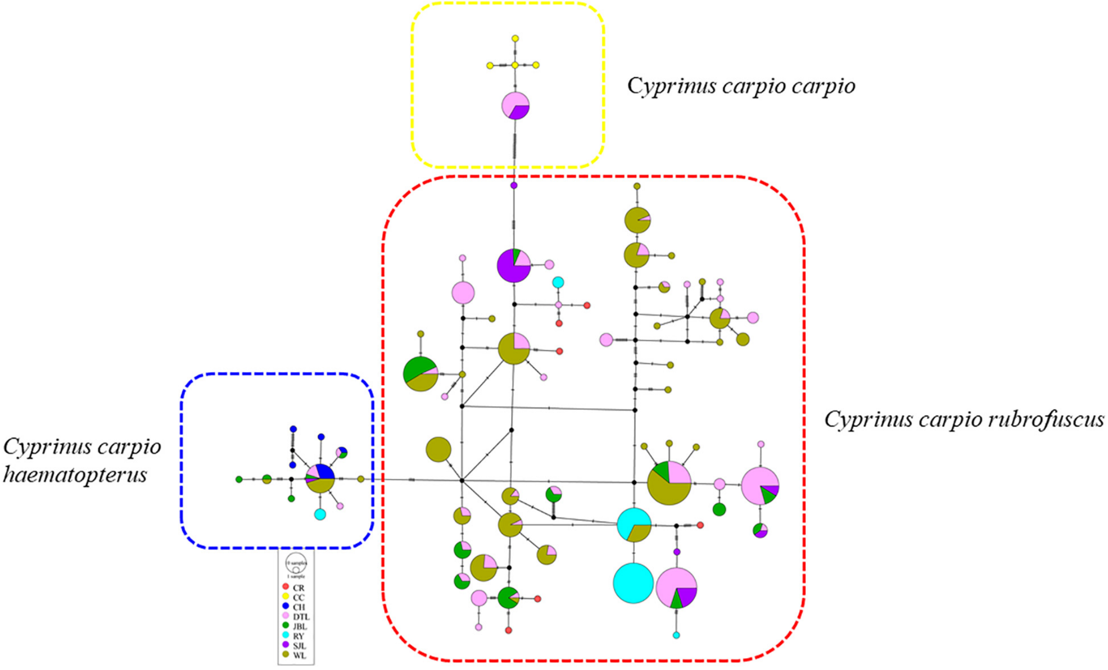

FIGURE 3
Haplotype network of five strains and three subspecies, including *Cyprinus carpio carpio* (CC), *Cyprinus carpio haematopterus* (CH) and *Cyprinus carpio rubrofuscus* (CR). Cycle size is roughly proportional to the haplotype frequency. DTL, Daotian carp; RY, Ru Yuan No. 1; JBL, Jinbian carp; SJL, Shaijiang carp; WL, Wu carp.

Genetic diversity of PF-carp

The indices of genetic diversity H, Hd, and π are summarized in **Table 1**. A total of 66 haplotypes were obtained from the 524 individual sequences, with 39, 16, 5, 8, and 32 haplotypes from the DTL, JBL, RY, SJL, and WL strains, respectively. There was a high overall haplotype genetic diversity (Hd = 0.959) while a relatively low nucleotide diversity (π = 0.0061) was observed in all strains. Hd of each population ranged from 0.559 (RY) to 0.953 (Du'an; DA), while π ranged from 0.0011 (RY) to 0.0079 (CJ). Meanwhile, the WL strain showed the highest number of unique haplotypes (ph = 16).

Inter-strain differentiation

In the pairwise comparisons of different strains, significant values of F_{ST} occurred in all pairs (**Table 2**), ranging from 0.051 to 0.365. It was noteworthy that RY exhibited high levels of differentiation from DTL, JBL, SJL, and WL (F_{ST} = 0.171, 0.248, 0.365, and 0.212, respectively). SJL showed moderate levels of differentiation from JBL and WL (F_{ST} = 0.148, 0.149, respectively). According to the results of the AMOVA, the majority (89.13%) of the genetic variation was attributed to variation within strains. Moderate levels of differentiation were detected among all the strains ($F_{ST} = 0.109$, $P < 0.001$, **Table 3**).

Intra-strain differentiation

To further explore the differentiation among populations within strains, average pairwise F_{ST} was calculated for DTL, JBL, and WL, strains that contained more than two populations. The F_{ST} values of DTL ranged from 0.072 to 0.359. Interestingly, a significantly high degree of differentiation was observed between RS and all other populations (**Supplementary Table 3**). Similarly, HJ also displayed a high level of differentiation from other populations except for a moderate level with CJ. Analysis of molecular variance showed that 76.63% of the genetic variation existed within populations (**Table 4**).

A particularly high level of differentiation was detected between populations of the JBL strain, including Sanjiang (SJ), Rongshuijinbian (RSJ), and Xiaorong (XR) (ranging from 0.217 to 0.282) (**Supplementary Table 4**). According to the results of AMOVA, the 73.81% of the genetic variation was attributed to variation within populations (**Table 4**).

With respect to the WL strain, the differentiation level was relatively low among eight populations, varying from −0.042 to 0.176 (**Supplementary Table 5**). F_{ST} values were significant for 7 of 28 combinations of pairs, within which a moderate level of differentiation was only observed in three pairs. The AMOVA results also showed a relatively high percentage of variation within populations (97.28%) (**Table 4**).

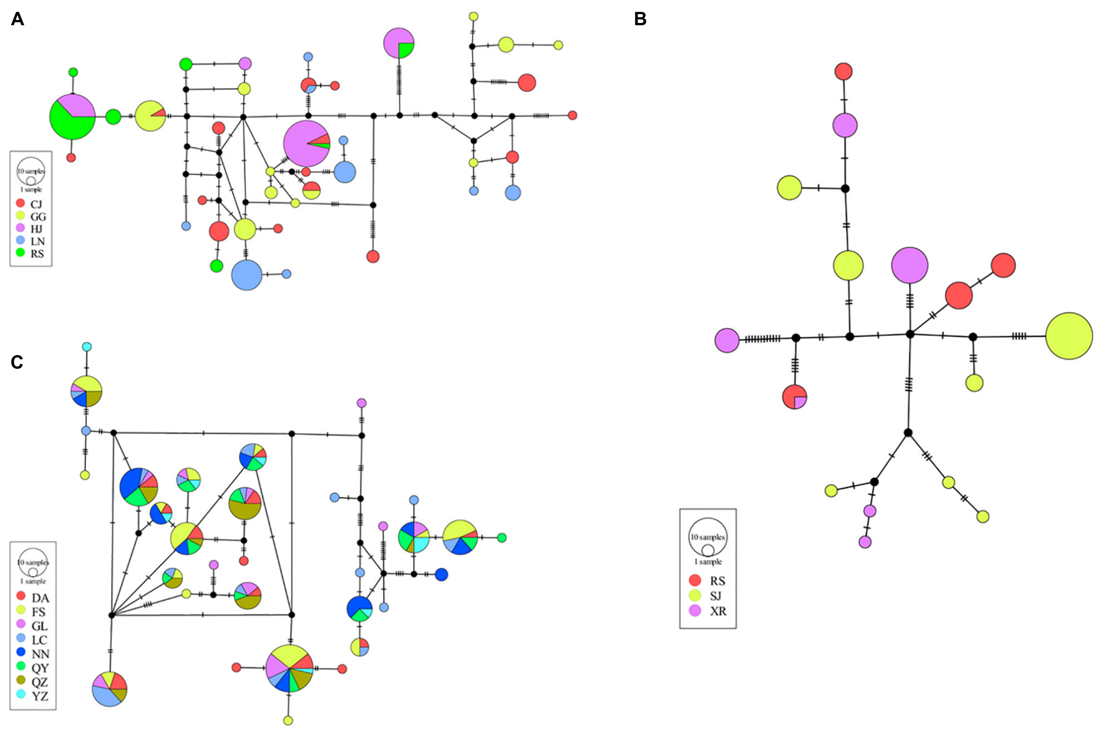

FIGURE 4
Haplotype network of PF-carp. Panels **(A–C)** represents Daotian carp (DTL), Jinbian carp (JBL), and Wu carp (WL), respectively. Cycle size is roughly proportional to the haplotype frequency. Location abbreviations refer to **Figure 1** and **Supplementary Table 1**.

The Mantel test revealed no significant correlation between geographic distance and F_{ST} for the three strains, (DTL: $P = 0.345$, JBL strain: $P = 0.188$, and WL: $P = 0.738$).

Discussion

Germplasm resources of PF-carp

It has been suggested that common carp primarily consists of three subspecies in European and Asian populations: CC, CH, and CR, an exception being CCH in lakes of the Yunnan–Guizhou plateau of China (Wu, 1977a; Zhou et al., 2003; Wang et al., 2010). In accordance with Cheng et al. (2021), the haplotype network in our study indicated that the majority haplotype (57/66) originated from CR, and thus this strain was deemed as the main matrilineal resource of the PF-carp. Moreover, certain frequencies of the mitochondrial haplotypes of CC (1/66) or CH (8/66) were also detected in these PF-carp. Considering that CC is distributed over vast areas of central Asia, Europe, and Xinjiang in China, one explanation for this observation is introgression. During its domestication, common carp has been intentionally introduced into different geographical locations, generating hybridization and genetic introgression during breeding (Wang et al., 2010; Dong et al., 2015). Moreover, it has been demonstrated that not only hybridization but also introgression was detected between European and Asian carp by mtDNA and allozyme markers (Kohlmann et al., 2003).

Effects of artificial selection on low genetic diversity of RY

In this study, we used one mitochondrial gene and the mitochondrial control region to estimate the genetic diversity and differentiation among and within strains. Notably, RY showed the lowest level of haplotype diversity (Hd = 0.559) and nucleotide diversity ($\pi = 0.0011$) while displaying a high degree of differentiation from the other four strains (**Table 2**). This result was consistent with our previous study that had revealed that the genetic diversity of selectively bred populations of RY was lower than in two landrace populations (Zhu et al., 2018). "RY NO. 1" is the first new variety of PF-carp approved in China, a strain that has been artificially selected for five generations based on desirable traits of rapid growth, a round body, and weak jumping ability. Researchers have revealed that consecutive breeding for many generations has led to decreased genetic diversity in golden mandarin fish (*Siniperca scherzeri* Steindachner) as revealed by microsatellite markers

TABLE 1 Estimates of genetic diversity for the 18 populations of PF-carp.

Population	*N*	*H*	Ph	Hd	π
DTL	161	39	15	0.922 + 0.011	0.0074 + 0.0003
CJ	28	15		0.942 + 0.240	0.0079 + 0.0006
GG	31	11		0.837 + 0.050	0.0042 + 0.0006
HJ	46	4		0.631 + 0.054	0.0063 + 0.0007
LN	27	9		0.761 + 0.068	0.0070 + 0.0008
RS	29	7		0.645 + 0.094	0.0042 + 0.0012
JBL	64	16	3	0.902 + 0.020	0.0058 + 0.0004
RSJ	14	4		0.780 + 0.061	0.0035 + 0.0008
SJ	30	7		0.708 + 0.072	0.0042 + 0.0005
XR	20	6		0.747 + 0.074	0.0064 + 0.0008
RY	65	5	4	0.559 + 0.050	0.0011 + 0.0003
SJL	42	8	2	0.725 + 0.057	0.0063 + 0.0008
WL	192	32	16	0.936 + 0.006	0.0054 + 0.0002
DA	20	13		0.953 + 0.028	0.0043 + 0.0007
FS	39	14		0.896 + 0.025	0.0053 + 0.0005
GL	18	11		0.915 + 0.050	0.0059 + 0.0009
LC	24	16		0.935 + 0.039	0.0055 + 0.0006
NN	31	10		0.903 + 0.027	0.0055 + 0.0004
QY	24	12		0.942 + 0.023	0.0057 + 0.0005
QZ	27	9		0.883 + 0.033	0.0045 + 0.0005
YZ	9	7		0.917 + 0.092	0.0061 + 0.0007
Overall	524	66	40	0.959 + 0.002	0.0061 + 0.0002

N, Number of each strain or population; H, Number of haplotypeph; ph, Private haplotype; Hd, Haplotype diversity; π, Nucleotide diversity. Location abbreviations refer to **Figure 1** and **Supplementary Table 1**.

(Luo et al., 2015). Likewise, artificial selection is likely to have imposed distinctive signatures on Atlantic salmon (*Salmo salar*) characterized by reduction in genetic diversity and high genetic differentiation from other populations (Lopez et al., 2019). The effects of artificial selection practices on loss of genetic diversity have been reflected in other aquaculture species such as the Pacific abalone (*Haliotis discus hannai*) (Chen et al., 2017), the eastern oyster (*Crassostrea virginica* Gmelin) (Yu and Guo, 2004) and clam (*Meretrix petechialis*). Long-term mass selection increases the levels of inbreeding and co-ancestry, thereby decreasing the pool of genetic diversity (Rodriguez-Ramilo et al., 2015). Therefore, it is reasonable to believe that genetic diversity of RY has been eroded by continuous intense selection practices.

Genetic differentiation and diversity of PF-carp cultured under traditional rich-fish systems

Researchers have pointed out that vicariance and geographic isolation contribute to genetic differentiation. In our case, it was noteworthy that the three populations of the JBL strain (SJ, RSJ, and XR) exhibited significantly high differentiation. Similarly, both HJ and RS were significantly differentiated from other populations of the DTL strain (**Supplementary Table 3**). The common point shared by these five populations was that they all originated from remote mountainous areas that hindered genetic exchange with other populations and thus intensified differentiation.

In this study, indigenous PF-carp in these regions are managed in traditional rice-fish co-culture systems, where farmers maintain small numbers of parental carp and produce fry for themselves. Unlike crop seeds that can be stored and transmitted from a long distance (Pautasso et al., 2013), parental PF-carp used in rice-fish co-culture systems have to be continuously cultivated by farmers. Notably, farmer connectivity (parental carp exchange and fry transmission) is indispensable to promote gene flow and maintain a high level of genetic diversity (Ren et al., 2018). Considering inbreeding, artificial selection or lack of farmer connection would gradually degrade the genetic diversity (Kincaid, 1983; Ren et al., 2018), and thus it was no wonder that the nucleotide diversity values of RS (π = 0.0042), RSJ (π = 0.0035) and SJ (π = 0.0042) were relatively low compared to other populations.

By contrast, the populations of the WL strain exhibited a low degree of differentiation (**Supplementary Table 5**). The WL strain is the most widely farmed strain in southwestern China, and parental carp and fry are frequently exchanged. In traditional farming systems, seed exchange can help maintain genetic diversity (Deletre et al., 2011; Labeyrie et al., 2016; Ren et al., 2018). Farmers dynamically manage agricultural biodiversity by continually collecting, testing, and selecting seeds with unusual and interesting traits through exchanging germplasm with other farmers (Deletre et al., 2011).

Implications for management and conservation

Research has revealed how the genetic diversity of crops and livestock can be maintained in traditional agricultural systems

TABLE 2 Pairwise F_{ST} (below diagonal) and significance probability estimates (above diagonal) among strains.

Strain	DTL	JBL	RY	SJL	WL
DTL	0	***	***	***	***
JBL	0.053	0	***	***	***
RY	0.171	0.248	0	***	***
SJL	0.053	0.148	0.365	0	***
WL	0.051	0.067	0.212	0.149	0

$^{***}P < 0.001$.

DTL, Daotian carp; RY, Ru Yuan No. 1; JBL, Jinbian carp; SJL, Shaijiang carp; WL, Wu carp.

TABLE 3 Analysis of molecular variance (AMOVA) of strains.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F-statistic
Among strains	4	236.741	0.567	10.87	0.109***
Within strains	519	2411.269	4.646	89.13	
Total	523	2648.01	5.212		

***$P < 0.001$.

TABLE 4 Analysis of molecular variance (AMOVA) of Daotian carp (DTL), Jinbian carp (JBL), and Wu carp (WL).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F-statistic
DTL					
Among populations	4	210.444	1.499	23.37	0.234***
Within populations	156	766.935	4.916	76.63	
Total	160	977.379	6.415		
JBL					
Among populations	2	64.371	1.391	26.19	0.262***
Within populations	61	239.176	3.920	73.81	
Total	63	303.547	5.312		
WL					
Among populations	7	50.914	0.122	2.72	0.027
Within populations	184	806.356	4.382	97.28	
Total	191	857.271	4.504		

***$P < 0.001$.

(Jarvis et al., 2008; Deletre et al., 2011; Labeyrie et al., 2016). For example, farmers are typically exchanging seed material with neighbors, relatives, and distant farmers, moving crop genetic diversity across farming units.

Moreover, farming practices including different cultivation methods and the use of multiple varieties shaped by culture, economics, and religion have been demonstrated to help conserve genetic diversity (Berthouly et al., 2009; Boettcher and Hoffmann, 2011). According to Ren's model, the genetic diversity (He) of PF-carp is positively related to the number of connected rice-fish farming farmer households and the frequency of exchange among these households (Ren et al., 2018). In this study, nucleotide diversity of RS, RSJ, and SJ was relatively low due to the lack of farmer connectivity. Therefore, it is necessary to encourage local farmers to share parental carp and fry to avoid genetic degradation and to maintain a dependable supply of genetically diverse fish. As for the geographically specific strains such as JBL and SJL, attention should be paid to preserve the native nucleotide diversity. On one hand, farmers should mind the intensity of selection and enhance communication with other RF-farmer households. On the other, farmers should watch out for introgression of exotic species. For example, we observed that CC genetic resources have invaded PF-carp in China, and this serves as a warning to trace the genetic origin of introduced common carp and to preserve native germplasm resources.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, OM933729—OM934252; https://www.ncbi.nlm.nih.gov/genbank/, OM934253—OM934776.

Ethics statement

The animal study was reviewed and approved by animal care and use committee of Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

Author contributions

ZZ and DM conceived this work and designed the methodology. ZZ, JF, HS, and YL collected and analyzed the data. ZZ, DM, and HZ created the original draft. DM supervised the analysis and writing process. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.896609/full#supplementary-material

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Spatial Variation in the Composition and Diversity of Fishes Inhabiting an Artificial Water Supply Lake, Eastern China

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With the intensification of eutrophication, many artificial water supply lakes that act as a biological filter for water diverted from rivers have been built to alleviate water scarcity in Eastern China. In this study, we selected Lake Yanlong, a representative artificial water supply lake in Yancheng City, as our experimental lake to explore how the community composition of fishes changed among different habitat types and assess potential consequences for effective water treatment. From October 2015 to October 2020, we conducted quarterly surveys of fish communities and environmental factors in the Mangshe River (MR; water for lake) compared to four different water treatment zones of Lake Yanlong (PZ, the pretreatment zone with inlet from the MR; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone with outlet to urban waterworks). A total of 16,339 individual fish belonging to 11 families and 49 species were collected. Six of the eight dominant species observed across zones were small-bodied fishes. Despite reduced fish species richness, the relative abundance and biomass of fishes tended to be higher in Lake Yanlong relative to the MR. The Shannon-Wiener diversity index, Pielou evenness index, Simpson's diversity index all decreased from the MR to the DZ in the following sequence: MR < PZ < EZ < SZ < DZ. Analysis of similarities and similarity percentage analysis confirmed that fish communities differed significantly among zones and *Coilia ectenes*, *Carassius auratus*, *Pseudobrama simony*, *Hemiculter leucisculus*, and *Hemiculter bleekeri* were the major differentiating species. Mantel's test and redundancy analysis revealed that water depth, aquatic vegetation coverage, and phytoplankton concentrations were the major factors determining the spatial distribution of fishes when moving from the MR to the DZ of Lake Yanlong. Stocking piscivorous fish can be used as an effective measure to control the abundance of prolific small-bodied fishes in Lake Yanlong. The details backing these findings are important for understanding how the community composition of fishes among habitat types in Lake Yanlong influence water quality, and to develop suitable biomanipulation strategies for the management of fish resources and maintaining proper function of these artificial water supply lakes.

Keywords: fish community, fish biodiversity, artificial water supply lake, Lake Yanlong, regulation strategies

INTRODUCTION

Eutrophication, caused by both natural and anthropogenic disturbance, is a pervasive problem facing surface water bodies (Smith and Schindler, 2009; Bhagowati and Ahamad, 2018) and has been an important topic for academic and social debate over the past few decades (Bonsdorff, 2021). The middle and lower reaches of the Yangtze River Basin (MLYRB) contains the majority of shallow lakes in China, with 651 lakes larger than 1 km^2 and 18 lakes larger than 100 km^2, accounting for about 1/3 of the total surface area of lakes in China (Zhang et al., 2019; Tang, 2020). With rapid socioeconomic development and the acceleration of urbanization since the 1980s, industrial and agricultural pollution as well as discharge of domestic sewage has resulted in the eutrophication of most lakes in the MLYRB (Zhu et al., 2019; Tang, 2020; Zou et al., 2020). Increasing levels of eutrophication pose a great threat to drinking water quality and human health (Qin et al., 2010; Moal et al., 2019), which has become an important ecological and socioeconomic problem in the MLYRB, particularly in the Eastern Plain of China (EPC) (Zou et al., 2020).

To alleviate this risk, the central and local governments of China have launched a series of water pollution control projects over the last decade (Guo et al., 2019), including the construction of artificial water supply lakes that act as a biological filter for water diverted from rivers within the EPC (Cai et al., 2019). Compared to natural lakes in the region, these artificial water supply lakes are often characterized by short construction time, small area, simple and unstable ecosystem structure, and are prone to degradation by human activities (e.g., improper fisheries management) (Cai et al., 2019; Li S. et al., 2021). Moreover, water quality and other ecosystem components (e.g., fish communities and extent of submerged aquatic vegetation) within these artificial lakes are affected by shifts in the environmental conditions of the rivers that feed them (Cai et al., 2019). There has been extensive research aimed toward developing restoration strategies for maintaining ecosystem function in natural lakes within the MLYRB [e.g., Taihu Lake (Wang et al., 2019) and Dongting Lake (Li B. et al., 2021)]. However, maintaining proper ecosystem function and identifying effective restoration strategies for artificial water supply lakes is much less common, despite their importance for protecting human health in the EPC. Therefore, it is necessary to understand the physical and biological characteristics of these artificial lakes, how different components work together to maintain core ecosystem functions and delivery of goods and services (e.g., purification of drinking water), and to establish effective restoration strategies that are suitable for these lakes given their unique properties when compared to natural lakes.

As the largest group of vertebrates and the primary mid- to upper-trophic level consumers in freshwater ecosystems, fish play important roles in the functioning of lakes by affecting the physical environment, trophic interactions, and community dynamics (Mello et al., 2009; Jeppesen et al., 2010). Under some circumstances, by consuming small zooplanktivorous fish, piscivorous fish can reduce grazing pressure on zooplankton communities, which in turn can increase grazing pressure on phytoplankton to an extent that improves water clarity—a more desirable state for many lakes that can also help support submerged macrophyte production (Mehner et al., 2004). Filter-feeding fish can also control undesirable cyanobacterial blooms by grazing phytoplankton directly (Xie and Liu, 2001). Alternatively, an unbalanced fish community can have a negative impact on water quality and lake ecosystem function (Cai, 2017; Guo et al., 2022). For example, the overabundance of herbivorous fish can precipitate submerged macrophyte loss in shallow lakes through over-grazing (Zhen et al., 2018). Often affected by overfishing and habitat loss, piscivorous fish in most MLYRB lakes and rivers have dramatically declined over the past decades, which has led to the proliferation of small-bodied fishes (Mao et al., 2011; Cai et al., 2019). Most of these small-bodied fish in the MLYRB feed on zooplankton (especially cladocerans and copepods), leading to increased phytoplankton biomass and reduced water clarity (Zhang, 2005). Furthermore, some studies demonstrated that declines in fish species diversity and the proportion of piscivorous fish present in aquatic ecosystems were accompanied by the deterioration of water quality (Karr, 1981, 1991; Karr et al., 1986). Given the strong roles fish play in aquatic ecosystem function, fish community structure has been regarded as an important ecological indicator and suitable for assessing the status of aquatic ecosystems (Oberdorff et al., 2001).

The physical environment can also have reciprocal effects on the composition and spatial distribution of fish communities through a variety of mechanisms (Geheber and Piller, 2012; Cote et al., 2013). Previous studies demonstrated that water velocity, macrophytes, water depth and trophic state were the primary factors mediating fish community dynamics (Brinsmead and Fox, 2002; Mehner et al., 2005; Li et al., 2010). For example, river fishes are generally more slender-bodied, and have smaller fins than fishes occupying lakes to reduce lotic drag (Brinsmead and Fox, 2002). In a survey of Xiaosihai Lake, China, areas with greater submerged macrophyte coverage supported a higher biomass and abundance of small fish (Li et al., 2010). Moreover, the coverage of submerged macrophytes is strongly correlated to water depth and trophic state, whereby deep or eutrophic lakes are often devoid of submerged macrophytes needed to support certain species of fish (Jeppesen et al., 1990). Thus, the composition and spatial distribution of fish communities are shaped by both environmental factors and biotic interactions, which could have consequences for ecosystem function (Mehner et al., 2005; Mehner and Brucet, 2021). Understanding these complexities may help inform proper restoration strategies in degraded lakes. However, the interdependencies among fish and the physical environment within artificial water supply lakes of the MLYRB are poorly studied.

The design and construction of artificial water supply lakes are tailored toward water treatment for human consumption. As a result, water quality and the physical habitat available for fish can vary from the inlet (where water is pumped from adjacent rivers) to the outlet as water travels through different purification zones of the lakes. Therefore, the community composition of fishes could vary along this gradient. Lake Yanlong (33°20′N, 120°1′E), built in 2012 and located at the intersection of the lower Yangtze River Basin and the lower Huaihe River Basin, is the first artificial water supply lake in the ECP and represented an ideal system for studying fish distribution and community composition (Zuo et al., 2015). The lake collects raw water from its waterhead river—the Mangshe River (MR)—through a pump station, and finally supplies water to the urban waterworks once traveling through four distinct purification zones: the pretreatment zone (PZ), the emergent macrophyte zone (EZ), the submerged macrophyte zone (SZ), and finally the deep purification zone (DZ). In this study, we conducted a quarterly survey of the fish community and environmental factors within each distinct purification zone of Lake Yanlong (PZ, EZ, SZ, DZ), as well as the adjacent Mangshe River (MR) from October 2015 to October 2020. We aimed to explore: (1) how fish species composition and diversity differed between Lake Yanlong and the Mangshe River; (2) the key environmental factors that could differentiate the fish community of the Mangshe River from Lake Yanlong as well as the distribution of fishes within the lake; and (3) suitable biomanipulation strategies for the management of fish resources and water quality in these artificial water supply lakes.

MATERIALS AND METHODS

Study Area

The Mangshe River is 43 km long and drains a basin area of 64,000 ha. It flows through the northern Lixiahe Plain of Jiangsu Province and used to be the primary drinking water source for residents of Yancheng City. The water quality of MR fluctuates throughout the year, and is typically classified between levels III to IV according to the environmental quality standards for surface water in China (GB3838-2002, Ministry of Environmental Protection of China, 2002). Additionally, the MR usually shows characteristics of organic pollution during summer (Zuo et al., 2015). In order to ensure the safety of drinking water for residents of Yancheng city, Lake Yanlong, an artificial water supply lake collecting raw water from the MR, was built in June 2012. After replacing the MR as the main source of drinking water in Yancheng City with Lake Yanlong, drinking water quality has improved and has become more stable (Zuo et al., 2015).

Lake Yanlong (210.9 ha) is a complex ecosystem exhibiting both wetland- and reservoir-type features and includes four distinct purification zones (**Figure 1**). Water enters the PZ first, which pretreats water from the MR mainly by means of precipitation, oxygenation and exposure to an established biofilm, and is characterized by relatively deep depths (mean ± SE; 3.25 ± 0.30 m) and relatively short water residence times as water moves through at measurable velocities (0.07 ± 0.04 m/s). Water enters the EZ second, which purifies water through assimilation, absorption and transformation of pollutants by emergent macrophytes (mainly *Phragmites australis* and *Typha orientalis*), and is characterized by relatively shallow depths (0.78 ± 0.19 m) and a high spatial coverage of emergent plants (86.3% ± 46.3%). Water enters the SZ third, which further purifies water through absorption and transformation of pollutants by submerged macrophytes (mainly *Myriophyllum verticillatum*, *Ceratophyllum demersum,* and *Vallisneria natans*). The DZ is the last purification zone before water exits the lake via a pumping station, which like the PZ, purifies water mainly through the settling of particulates, but is deeper (4.75 ± 0.22 m) and encompasses the largest surface area and storage capacity (4.2×10^6 m^3) of all zones. These four distinct purification zones are separated by sluices, which act as obstacles to fish movement among zones. The main characteristics of the five different zones (MR, PZ, EZ, SZ, and DZ) are shown in **Table 1** (Ren, 2021; Guo et al., 2022).

To help support a clear-water state in Lake Yanlong, which can bolster macrophyte production, biomanipulation was used. Different types of native piscivorous fish were stocked in each purification zone of Lake Yanlong from 2017 through 2020 to consume small-bodied fishes. By the end of June 2020, a total of 3.2×10^4 individual topmouth culter *Culter alburnus*, 3.3×10^4 individual mandarin fish *Siniperca chuatsi*, 2.0×10^4 individual catfish *Clarias gariepinus* and 1.87×10^4 individual snakehead fish *Channa argus* were stocked into the lake. Species and numbers stocked varied among zones to account for differences in surface area and habitat characteristics (**Table 2**).

Fish Sampling

A total of nine standard fish sampling sites (one site both for MR and PZ, two sites both for EZ and SZ, and three sites for DZ) were established across the MR and all purification zones of Lake Yanlong. The number of sites within each zone reflected the relative size of each region. Fishes were sampled quarterly from October 2015 to October 2020 using multi-mesh monofilament gill nets. The design of the gill nets was modified according to the standard method (Appelberg, 2000). Each net consisted of twelve 2.5 m long × 1.5 m tall mesh panels placed in random order (8.5, 4.0, 12.5, 2.0, 11.0, 1.6, 2.5, 4.8, 3.1, 1.0, 7.5, and 6.0 cm stretch measure). Three nets (two pelagic surface nets and one benthic net) were set in series at each sampling site overnight (12–15 h) for three consecutive nights each season (January, April, July, October). Fishes were separated and identified to the species level, and individually measured for total length to the nearest 1 mm and body weight to the nearest 0.01 g. We calculated the species richness, mean quarterly catch per unit effort in both numerical ($CPUE_N$; ind./m^2/12 h) and biomass ($CPUE_B$; g/m^2/12 h) terms. Small-bodied fishes were defined by having an age-at-first-maturity of less than 2 years (Cai et al., 2019). Each fish species was classified for feeding type (omnivorous, piscivorous, planktivorous, or herbivorous) and propensity for movement (migratory and resident) according to Ni and Wu (2006).

Environmental Variables

On each sampling occasion, eleven environmental variables were measured in conjunction with fish sampling at each sampling site (**Table 1**). Dissolved oxygen (DO; mg/L) and surface water temperature (WT;°C) were monitored using a YSI ProPlus meter (Thermo Fisher Scientific Company, Waltham, United States). Water depth (WD; m) was measured using a Speedtech SM-5 Portable Depth Sounder. Turbidity (NTU) was measured using a Hach 2100P portable turbidity meter. Chemical oxygen demand (COD_{Mn}; mg/L), total nitrogen (TN; mg/L) and total phosphorus (TP; mg/L) were determined according

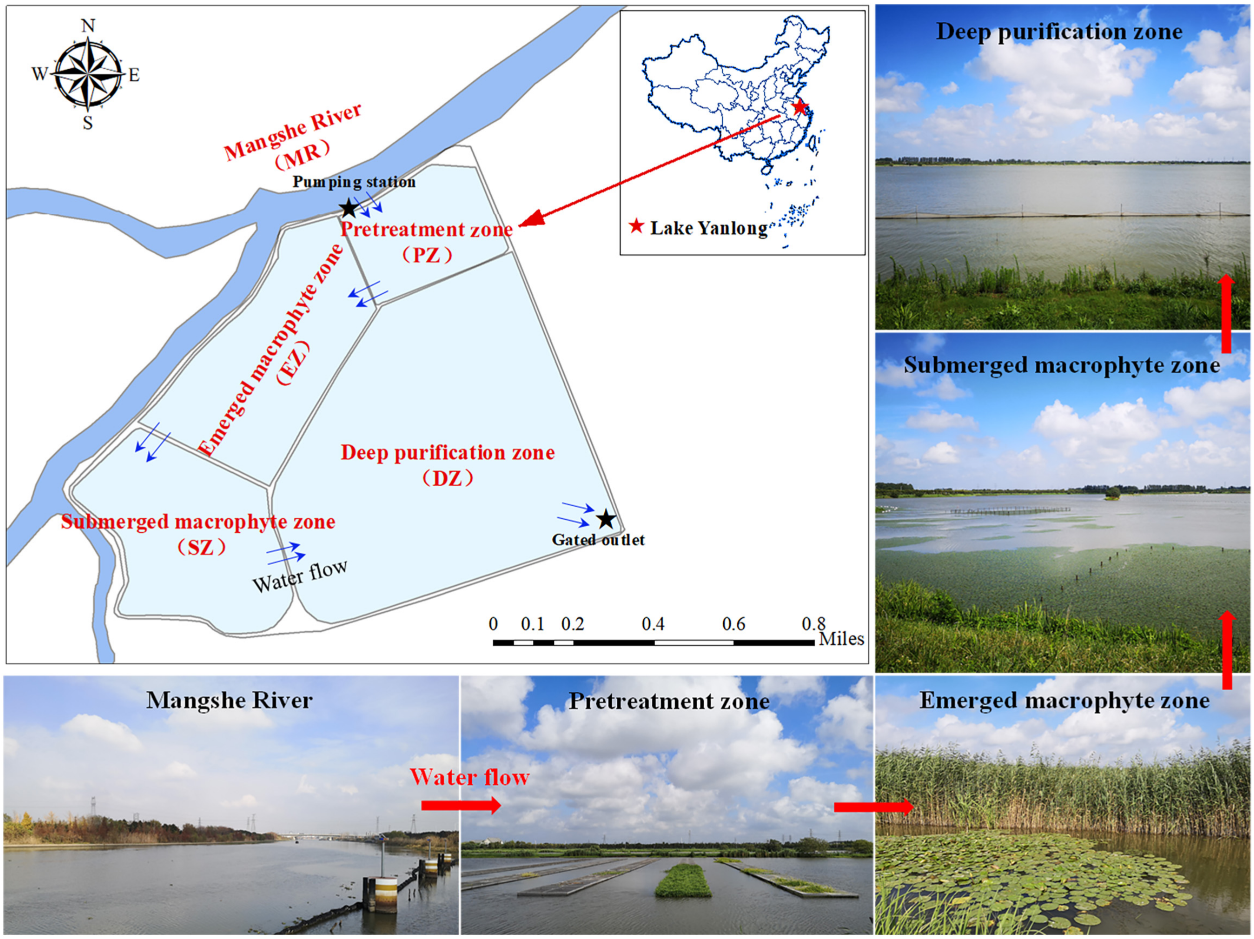

FIGURE 1 | Pictures and map of the Mangshe River (MR) and Lake Yanlong, showing the distribution of different purification zones (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone).

TABLE 1 | Annual means (±SE) of physicochemical parameters measured in the Mangshe River (MR) and different purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone).

Zones	MR	PZ	EZ	SZ	DZ
Area (ha)	–	20.3	41.3	40	109.3
Water depth (WD; m)	2.88 ± 1.50	3.25 ± 0.30	0.78 ± 0.19	1.43 ± 0.51	4.75 ± 0.22
Water temperature (WT; °C)	20.6 ± 8.9	21.0 ± 9.5	18.3 ± 10.7	20.6 ± 8.2	20.5 ± 8.2
Transparency (cm)	31.7 ± 10.0	27.6 ± 11.1	36.6 ± 10.8	39.1 ± 6.4	46.7 ± 16.1
Total phosphorus (TP; mg/L)	0.23 ± 0.08	0.25 ± 0.08	0.21 ± 0.05	0.20 ± 0.08	0.12 ± 0.04
Total nitrogen (TN; mg/L)	1.83 ± 0.57	2.05 ± 0.98	1.89 ± 0.69	1.46 ± 0.57	1.42 ± 0.85
Chemical oxygen demand (COD_{Mn}; mg/L)	5.74 ± 1.31	6.38 ± 1.38	6.25 ± 1.23	6.00 ± 0.98	5.31 ± 0.60
Dissolved oxygen (DO; mg/L)	5.54 ± 3.07	6.51 ± 2.59	7.67 ± 2.75	5.23 ± 3.78	8.62 ± 2.35
Chlorophyll-α (Chl-α; μg/L)	26.32 ± 15.34	36.56 ± 12.82	42.66 ± 15.32	38.46 ± 19.67	32.67 ± 22.00
Water velocity (m/s)	0.10 ± 0.07	0.07 ± 0.04	0.06 ± 0.04	0.05 ± 0.03	0.02 ± 0.02
Coverage of aquatic vegetation (%)	2.1 ± 1.2	5.6 ± 3.6	86.3 ± 46.3	12.1 ± 8.8	1.5 ± 0.8

to the standard methods described in American Public Health Association [APHA] (1992). Chlorophyll-α concentration (Chl-α; μg/L) was determined using a fluorimeter with methanol extraction of the filtrate (Holm-Hansen and Riemann, 1978). The biomass of aquatic vegetation (BAV; g/m^2) was recorded using a grass collection sampler (Guo et al., 2022). Family-specific zooplankton (NZ; ind./L) and phytoplankton density (NP; ind./L) were estimated following the standard methods described in Dumont (2002) and Paredes and Montecino (2011), respectively.

TABLE 2 | The total number and individual size of piscivorous fish species stocked annually into the four purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone) from 2017 to 2020.

Stocking		2017		2018			2019				2020			
		SZ	DZ	EZ	SZ	DZ	PZ	EZ	SZ	DZ	PZ	EZ	SZ	DZ
Topmouth culter	Number (10^3 ind.)	3.0	3.0	–	3.0	3.0	4.0	2.0	3.0	2.0	2.0	2.0	2.0	3.0
	Size (cm)	8~10	8~10	8~10	8~10	8~10	3~5	3~5	3~5	3~5	3~5	3~5	3~5	3~5
Mandarin fish	Number (10^3 ind.)	1.0	4.0	2.0	1.0	4.0	1.0	2.0	2.0	4.0	2.0	2.0	3.0	5.0
	Size (cm)	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15
Catfish	Number (10^3 ind.)	1.0	3.0	1.0	1.0	1.5	0.5	2.0	1.0	2.0	1.0	1.0	2.0	3.0
	Size (cm)	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15
Snakehead fish	Number (10^3 ind.)	1.0	1.5	1.0	1.0	1.5	0.5	1.0	1.0	2.0	1.0	1.0	2.0	3.0
	Size (cm)	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15	8~15

Species Diversity Indices

The following indices were calculated for each sampling occasion and used to quantify fish species diversity and rank the relative importance of each species among the MR and different purification zones of Lake Yanlong:

(i) Shannon–Wiener diversity index (H_N) (Shannon and Weaver, 1949)

$$H_N = -\Sigma[P_i \times lnP_i] \tag{1}$$

(ii) Pielou evenness index (J) (Pielou, 1975)

$$J = H_N/lnS \tag{2}$$

(iii) Simpson diversity index (D) (Whittaker, 1972)

$$D = 1 - \Sigma {P_i}^2 \tag{3}$$

(iv) Simpson dominance index (λ) (Simpson, 1949)

$$\lambda = \Sigma {P_i}^2 \tag{4}$$

(v) The index of relative importance (IRI) (Pianka, 1971)

$$IRI_i = (N_i\% + W_i\%) \times F_i \times 10000 \tag{5}$$

where Pi is the proportional abundance of species i referenced to the total number of fish individuals sampled; S is the total number of species; N_i is the numerical percentage of species i; W_i is the biomass percentage of species i; and F_i is the percent frequency of occurrence of species i. When IRI_i is larger than 1000, this species is defined as the dominant species (Yang et al., 2018).

Data Analysis

Differences in Fish Community Composition and Diversity

Potential differences in mean diversity indices (H_N, J, D, λ) and the relative abundance and biomass ($CPUE_N$ and $CPUE_B$) of different fishes observed from October 2015 to October 2020 among the four purification zones of Lake Yanlong and of the MR were tested using multiple non-parametric, one-way ANOVA's (Kruskal–Wallis tests) implemented in the R package "coin" (Hothorn et al., 2008). The different zones (averaging across sampling quarters) represented the fixed factor. Where applicable, Wilcoxon tests were used for *post hoc* comparisons and to determine which zones differed using the "rstatix" package (Kassambara, 2021). Moreover, we used one-way analysis of similarity (ANOSIM) to identify the significance of observed differences in fish community composition and then used similarity percentage analysis (SIMPER) to explore the discriminating species of fish within communities among zones using the "vegan" package (Oksanen et al., 2022).

Environmental Effects on Fish Community Composition

Spearman correlation analyses and Mantel's tests were used to explore the relationship between different environmental variables and the dominant fish species in each purification zone and the MR by using quarterly measurements, also implemented in R using package "linkET" (Huang, 2022). Redundancy analysis (RDA; provided within the "vegan" package) was then used to identify the key environmental factors that could explain differences in the abundance of dominant species among zones. Prior to these analyses, variance inflation factors (VIFs) were adopted to examine multicollinearity among environmental factors. Collinear environmental variables detected by forward-backward selection were excluded until all remaining variables had VIFs less than 5 (Gou et al., 2021). In addition, hierarchical partitioning (HP) and permutation analyses were performed to acquire the independent explanation power and variance of each environmental factor using the "rdacca.hp" package (Lai et al., 2022). Environmental variables were standardized to a mean of zero and unit variance by the "decostand" function in the "vegan" package. Lastly, $CPUE_N$ of dominant species were $\log_{10}(x + 1)$ transformed prior to analysis (James and McCulloch, 1990).

Effect of Stocking Piscivorous Fish on Small-Bodied Fishes and Species Diversity

To assess the effect of stocking of piscivorous fishes on small-bodied fishes and fish community diversity, we compared mean relative abundance and biomass values ($CPUE_N$ and $CPUE_B$) of small-bodied fishes and fish diversity indices (H_N, J, D) among the four purification zones of Lake Yanlong before (data from October of 2015 and 2016) and after (data from October of 2017, 2018, 2019, and 2020) stocking of piscivorous fishes occurred. For this analysis, we used Mann-Whitney tests implemented in the R package "stats" (R Core Team, 2021).

All statistical analyses and figures were made using R software (Version 4.1.2). Differences were considered significant at an α level of 0.05 (i.e., p-value < 0.05) for all tests.

RESULTS

Species Composition and Richness

In total, 16,339 individual fish belonging to 11 families and 49 species were collected from all purification zones of Lake Yanlong and the MR between October 2015 and October 2020 (**Supplementary Table 1**). Fish species richness was highest in the MR (39 species), followed by the SZ (31 species), the EZ (30 species), and richness was lowest in the DZ (22 species). The 49 fish species were divided into resident fish (39 species) and migratory fish (10 species) based on their propensity for movement. The species richness of migratory fish (e.g., *Elopichthys bambusa* and *Squaliobarbus curriculus*) in the MR was the highest (10 species), but decreased greatly after entering Lake Yanlong (five species in PZ; four species in EZ; six species in SZ; four species in DZ). Compared to migratory fish, resident fish (e.g., *Coilia ectenes* and *Pseudobrama simony*) were more prolific in the four purification zones of Lake Yanlong, especially in the EZ (26 species) and SZ (25 species), which reflected the transition from riverine to wetland- and reservoir-type habitats (**Figure 2**). Based on typical food items ingested, these 49 fish species were also classified into four trophic guilds: omnivores (23 species), piscivores (14 species), planktivores (7 species) and herbivores (5 species). For omnivorous fish, species richness differed between MR and the other four zones, being highest in MR (22 species), and lowest in DZ (8 species); For piscivorous fish, species richness was highest in EZ (12 species), followed by MR (7 species), and only 6 species each for PZ, SZ and DZ; For planktivorous fish, species richness was highest in SZ (6 species), followed by MR (5 species) and DZ (5 species), and only 1 species each for EZ; For herbivorous fish, species richness was highest in EZ, SZ and MR (each with 5 species), followed by PZ (4 species), and DZ (3 species) (**Figure 2**).

Distribution of Dominant Species

A total of eight dominant species ($IRI > 1{,}000$) were identified among the MR and four purification zones. Six of the eight dominant species (*P. simony*, *Carassius auratus*, *Hemiculter leucisculus*, *C. ectenes*, *Hemiculter bleekeri* and *Toxabramis swinhonis*) were small-bodied fishes that feed primarily on zooplankton, algae and plants debris. In addition, the total number of dominant species identified varied among zones, being highest in the PZ (five dominant species), followed by EZ (four dominant species), DZ (three dominant species), SZ (two dominant species), and was lowest in the MR (one dominant species). In the MR, only *H. bleekeri* met conditions for dominance ($IRI = 1323.0$), and represented 10.0% of the total numerical catch across study years. In the PZ, *P. simony* ($IRI = 3168.3$) and *C. ectenes* ($IRI = 2217.5$) were the most dominant species, accounting for 16.5% and 12.5% of the total catch biomass across years, respectively. *C. auratus* ($IRI = 5243.3$), *P. simony* ($IRI = 6232.0$) and *C. ectenes* ($IRI = 5092.7$) were the most dominant species in the EZ, SZ and DZ, respectively. Lastly, *H. leucisculus* was relatively common in the PZ, EZ, SZ and DZ, while *T. swinhonis* and *Cyprinus carpio* only met conditions for dominance in the PZ and EZ, respectively (**Table 3**).

Diversity Indices and Relative Abundance and Biomass

There were significant differences in the diversity indices (H_N, J, D, λ) and relative abundance and biomass ($CPUE_N$ and $CPUE_B$) among the MR and four purification zones when averaging across study quarters (Kruskal–Wallis test, $p < 0.05$ for all cases) (**Table 4**). In terms of the diversity indices, H_N, J and D of the fish communities all showed a decreasing trend from the MR to DZ and followed the progression of: MR < PZ < EZ < SZ < DZ. Conversely, λ showed the opposite pattern (**Figure 3**). In terms of relative abundance and biomass, $CPUE_N$ and $CPUE_B$ both increased from the MR to Lake Yanlong. These values were lowest in the MR (9.6 ± 1.1 ind./m^2/12 h and 387.1 ± 60.5 g/m^2/12 h, respectively), and highest in the EZ (35.4 ± 5.1 ind./m^2/12 h and 1895.7 ± 318.2 g/m^2/12 h, respectively) (**Figure 4** and **Supplementary Table 2**).

Dissimilarity and Differentiating Species Among Fish Communities

The ANOSIM analysis confirmed that fish community composition differed significantly among zones ($R = 0.429$, $P < 0.001$). Dissimilarity of fish was greatest between the MR and SZ (averaged 83.9%), followed by MR and EZ (83.8%), MR and DZ (83.0%), EZ and DZ (81.7%), and was lowest between PZ and SZ (71.6%) (**Table 5**). According to the SIMPER analysis, *C. ectenes*, *C. auratus*, *P. simony*, *H. leucisculus,* and *H. bleekeri* were the major differentiating species among zones. Specifically, *C. ectenes* had a higher contribution than the other differentiating species in the MR vs. DZ, PZ vs. DZ, EZ vs. DZ, and SZ vs. DZ. Contributions of *C. ectenes* for these comparisons were 30.4, 25.2, 20.7, and 28.1%, respectively. The species *C. auratus* had a higher contribution than the other differentiating species in the MR vs. EZ, PZ vs. EZ and the EZ vs. SZ, with contributions of 22.0, 18.3, and 22.2%, respectively. Next, *H. bleekeri* had a higher contribution in the MR vs. PZ and the PZ vs. SZ, with corresponding contributions of 19.8 and 18.2%, respectively. Lastly, *P. simony* had a higher contribution in the MR vs. SZ (30.9%) (**Table 5**).

Environmental Effects on Fish Community Composition

The results from Mantel's test comparing environmental variables to the relative abundance of dominant species indicated that WD was significantly correlated with the $CPUE_N$ of *C. auratus* ($r = 0.08$), *C. carpio* ($r = 0.09$), *P. simony* ($r = 0.20$) and *C. ectenes* ($r = 0.25$). Further, BAV was significantly correlated with the $CPUE_N$ of *P. simony* ($r = 0.13$) and CCA ($r = 0.31$), while NP was significantly correlated with the $CPUE_N$ of *T. swinhonis* ($r = 0.20$) and *C. ectenes* ($r = 0.18$) ($p < 0.05$ for all

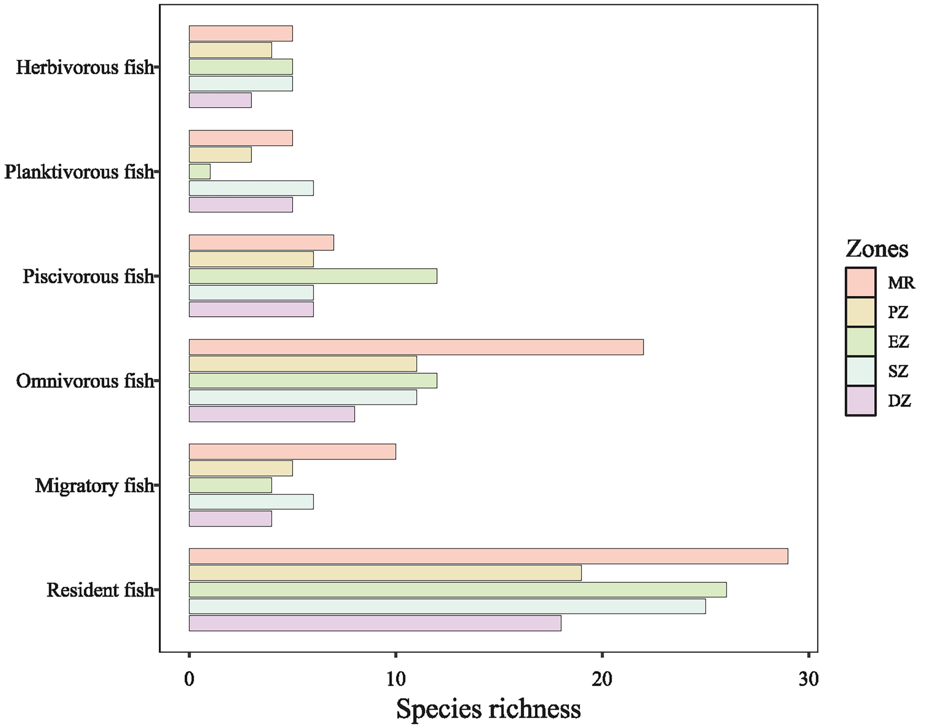

FIGURE 2 | The total number of fish species sampled (i.e., species richness) parsed into different different feeding types (omnivores, piscivores, planktivores, and herbivores) and propensity for movement (migratory or resident) among the Mangshe River (MR) and different purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone).

TABLE 3 | The percent relative abundance (*N*%) and biomass (*W*%) and the index of relative importance (*IRI*) of dominant fish species sampling from the Mangshe River (MR) and from different purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone).

Scientific name	Code	MR			PZ			EZ			SZ			DZ		
		N%	*W*%	*IRI*	*N*%	*W*%	*IRI*	*N*%	*W*%	*IRI*	*N*%	*W*%	*IRI*	*N*%	*W*%	*IRI*
P. simony	PSI	1.6	0.7	84.5	17.6	16.5	**3168.3**	15.2	5.1	**1754.7**	45.8	16.6	**6232.0**	8.8	5.0	765.3
C. auratus	CAU	3.3	2.3	275.7	1.1	9.4	374.6	24.3	28.1	**5243.3**	3.4	1.8	228.0	8.5	23.0	**2980.9**
H. leucisculus	HLE	5.7	1.4	442.9	16.7	8.9	**2012.3**	23.7	3.4	**2528.5**	16.6	4.7	**1999.7**	11.2	4.2	**1367.8**
C. ectenes	CEC	7.4	1.9	466.1	13.4	12.5	**2217.5**	0.0	0.0	–	3.5	0.7	259.4	43.9	10.1	**5092.7**
H. bleekeri	HBL	10.0	4.9	**1323.0**	19.6	12.2	**1816.0**	7.4	2.0	687.7	3.9	1.4	333.9	11.7	2.9	888.3
C. mongolicus	CMOO	4.3	3.0	450.0	7.5	3.8	892.5	4.6	5.7	755.5	1.8	2.5	241.6	5.6	2.4	802.8
T. swinhonis	TSW	1.6	0.3	91.7	13.8	4.2	**1416.9**	0.0	0.0	–	2.0	0.3	57.3	5.8	1.3	353.9
C. carpio	CCA	0.6	2.4	36.6	0.2	3.0	45.6	2.4	16.6	**1393.2**	0.2	0.4	8.3	0.0	0.9	5.0

N%, percentage based on total catch number of fish across study years; W%, percentage based on total catch biomass of fish across study years; IRI, index of relative importance; Code, fish code. Bold numbers indicate the dominant species.

cases). Spearman correlation analyses using the 11 environmental variables measured showed that WD was significantly negatively correlated with COD_{Mn} ($r = -0.24$, $p < 0.05$) and significantly negatively correlated with BAV ($r = -0.68$, $p < 0.001$). The variable BAV was also significantly negatively correlated with NP ($r = -0.23$, $p < 0.05$). Alternatively, we observed significant positive correlations among most of the nutrient indices (i.e., TN, TP, COD_{Mn}) and Chl-α (**Figure 5**).

The RDA model was significant ($p < 0.001$), indicating the existence of a relationship among environmental variables and the spatial distribution of dominant species. The model described 53.2 and 24.5% of variation in species distribution data along the first and second RDA axes (**Figure 6A**). All environmental variables (WT, WD, TN, TP, COD_{Mn}, Chl *a*, Turbidity, DO, BAV, NZ and NP) were selected for using in the RDA (VIFs were < 5 for all variables). According to the HP analysis and permutation tests, WD, NP and BAV explained 35.66, 19.08, and 15.13% of the total variation in fish community composition (all species) among zones, respectively. These were the same

TABLE 4 | Detailed information on results from the non-parametric, one-way ANOVA (i.e., Kruskal–Wallis test) models evaluating potential differences in the mean catch per unit effort of fishes [in both numerical ($CPUE_N$) and biomass ($CPUE_B$) terms] and the fish community diversity metrics (H_N, J, D, λ) among habitat zones.

Variables	Statistical parameters		
	χ^2	*df*	*P*
$CPUE_N$	18.495	4	< 0.001
$CPUE_B$	17.213	4	0.002
H_N	22.109	4	< 0.001
J	16.621	4	0.002
D	19.327	4	< 0.001
λ	18.818	4	< 0.001

environmental variables that explained the abundance of dominant fish species among zones (P_{HP} < 0.01 for WD, P_{HP} < 0.05 for NP and BAV) (**Figure 6B**). In addition, *C. ectenes* preferred habitats (principally the PZ and DZ) with deeper water depths and lower aquatic vegetation cover, while *H. bleekeri* preferred deep habitats within the MR that lacked phytoplankton. Alternatively, *P. simony*, *C. carpio,* and *C. auratus* were found in shallow habitats (principally the EZ and SZ) with abundant aquatic vegetation (**Figure 6A**).

Effects of Stocking Piscivorous Fish on Small-Bodied Fishes and Fish Community Diversity

There were significant differences in mean relative abundance and biomass of all small-bodied fishes combined (25 species) among the four purification zones of Lake Yanlong before ($CPUE_N$: 49.72 ± 1.01 ind./m^2/12 h; $CPUE_B$: 2342.40 ± 148.44 g/m^2/12 h) and after ($CPUE_N$: 17.65 ± 2.62 ind./m^2/12 h; $CPUE_B$: 381.74 ± 64.49 g/m^2/12 h) stocking of piscivorous fishes (Mann–Whitney test, P < 0.001 for all case) (**SupplementaryTable 3**). In addition, the fish community diversity indices (H_N, J, D) increased significantly among the four purification zones of Lake Yanlong before ($H_{N:}$ 1.32 ± 0.15, $J_{:}$ 0.64 ± 0.07, $D_{:}$ 0.61 ± 0.06) and after ($H_{N:}$ 1.66 ± 0.10, $J_{:}$ 0.72 ± 0.04, $D_{:}$ 0.76 ± 0.04) stocking of piscivorous fishes (Mann–Whitney test, P < 0.05 for all case) (**Supplementary Table 3**).

DISCUSSION

Spatial Patterns in Fish Community Composition

The species composition, diversity, relative abundance and biomass of fishes among shifting habitat types from the Mangshe

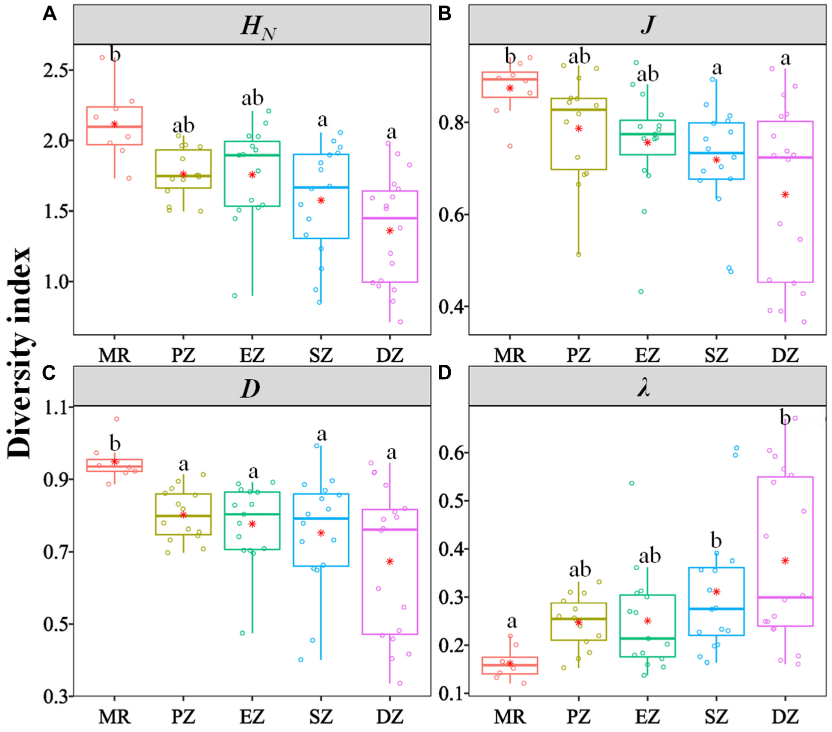

FIGURE 3 | Box and whisker plots characterizing the distribution of different indices [**(A)** Shannon–Wiener diversity index H_N, **(B)** Pielou evenness index J, **(C)** Simpson diversity index D, **(D)** Simpson dominance index λ] characterizing the biodiversity of fish communities inhabiting the Mangshe River (MR) and different purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone). Different letters above bars denote significant differences among zones. Asterisks represent mean values.

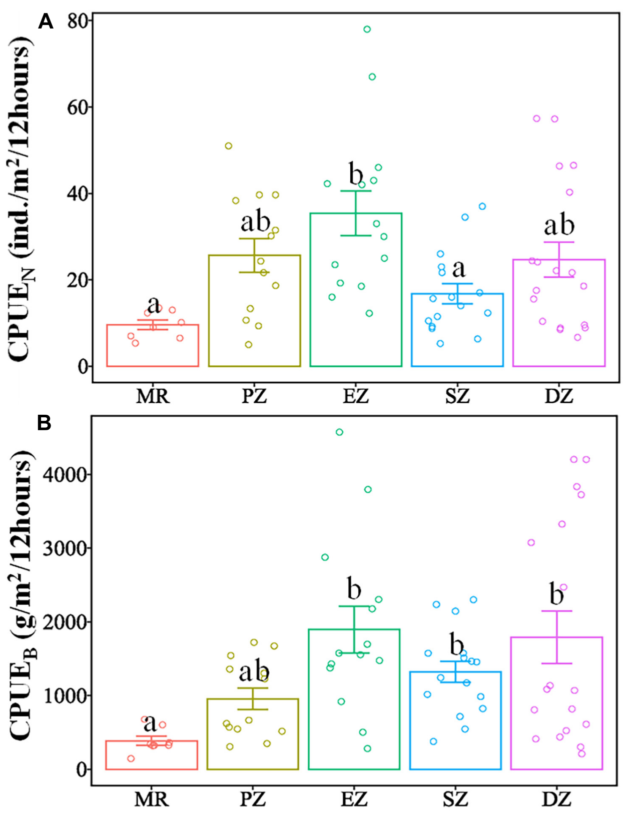

FIGURE 4 | Mean catch per unit effort (CPUE$_N$ and CPUE$_B$) of all fish species combined sampled from the Mangshe River (MR) and different purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone) **(A,B)**. Letters above bars denote significant differences among zones. Error bars represent 1 SE.

River to the outlet of Lake Yanlong were distinctly different. Compared to the Mangshe River, fish species richness in the four purification zones of Lake Yanlong was reduced, especially species of migratory fish (e.g., *E. bambusa* and *S. curriculus*). Migratory fish—those that spawn in rivers and migrate to lakes for feeding, growth and maturation—have been severely limited for key habitats for fulfilling their life history over the past several decades as a result of fragmentation imposed by the extensive construction of hydraulic engineering projects (Zhou et al., 2014; Liao et al., 2018). Similar to river-reservoir ecosystems created by dams, Lake Yanlong is connected to the Mangshe River by a large pumping station, which can block migratory channels and still negatively affect the reproduction and growth of migratory fish despite providing additional lake-type habitat that could otherwise be utilized by these fish if accessible (Bai et al., 2020).

Despite reduced species richness, the relative abundance and biomass of fishes tended to be higher in Lake Yanlong relative to the adjacent Mangshe River. Lake Yanlong is a newly constructed ecosystem formed through artificial excavation. It is still in the early stages of ecological development, and some potential niches remain vacant (Cai et al., 2019). Small-bodied fishes (e.g., *P. simony*, *H. leucisculus,* and *C. auratus*) introduced from the Mangshe River are considered r-strategists (or opportunistic strategists), which can quickly occupy vacant ecological niches. These fishes were able to increase in abundance quickly and became the dominant species after entering Lake Yanlong due to the lack of natural enemies (Cai et al., 2019; Jeschke et al., 2019). In this study, six of the eight dominant species observed across purification zones were small-bodied fishes, with *C. auratus*, *P. simony,* and *C. ectenes* accounting for 28.1, 45.8, and 43.9% of the CPUE$_N$ of fishes in the EZ, SZ, and DZ, respectively. High abundances of small-bodied fishes can reduce species diversity within fish communities and affect the complexity and stability of ecosystems (Lima et al., 2016; Liu et al., 2017). Some studies demonstrated that the overabundance of small-bodied fishes and declines in overall fish diversity were accompanied by the deterioration of water quality (Karr, 1981, 1991; Karr et al., 1986; Yu et al., 2021). Moreover, the present study revealed a significant reduction in the Shannon–Wiener diversity index, the Pielou evenness index and the Simpson's diversity index of fishes inhabiting the Mangshe River versus Lake Yanlong. Therefore, it is essential to seek appropriate strategies that help enhance the biodiversity of artificial water supply lakes in order to maintain proper ecosystem function.

Environmental Effects on Fish Community Composition

A wide range of studies have shown that the spatial distribution and composition of fish communities are influenced by both biotic (e.g., competition and predation) and abiotic factors (e.g., nutrient levels and habitat heterogeneity) (Li et al., 2010; Liu et al., 2017; Cai et al., 2019; Whiterod et al., 2021). Mantel's tests and the RDA model all revealed that water depth, aquatic vegetation coverage, and phytoplankton concentrations were the major factors determining the spatial distribution of fishes when moving from the Mangshe River to the DZ of Lake Yanlong. The present study also showed that water depth was the most important abiotic factor influencing fish distribution, as it influenced ecological conditions present within the water column (e.g., food availability and light penetration), which has been important in other related studies (Keast, 1978; Mehner et al., 2005; Fernandes et al., 2010; Li et al., 2010; Cai et al., 2019). Benthivorous fish (i.e., *C. carpio* and *C. auratus*) preferred to live in relatively shallow habitat, principally within the EZ and SZ, while pelagic fish (i.e., *C. ectenes*) generally inhabited deeper water, principally within the PZ and DZ. Phytoplankton is the primary food source for many planktivorous and omnivorous fish species, and can affect the spatial distribution of fishes by modifying the trophic state of and dissolved oxygen concentrations within the water column (McClatchie et al., 1997; Amarasinghe et al., 2012). In this study, the distribution of *H. bleekeri*, *C. ectenes*, and *T. swinhonis* was closely related to the abundance of phytoplankton. A previous study revealed that *H. bleekeri* preferred flowing water habitats that lacked phytoplankton (Li et al., 2020). Conversely, *C. ectenes* and *T. swinhonis* are very common zooplanktivorous fish within the MLYRB, and are better suited for living in habitats with abundant

TABLE 5 | Average dissimilarity and corresponding differentiating species of fish among the Mangshe River (MR) and different purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone).

Group	Average dissimilarity (%)	Discriminating species 1	Contribution (%)	Discriminating species 2	Contribution (%)	Discriminating species 3	Contribution (%)
MR-PZ	79.87	HBL	19.77	PSI	15.30	HLE	13.47
MR-EZ	83.82	CAU	22.00	PSI	14.78	HLE	14.58
MR-SZ	83.91	PSI	30.93	HBL	15.25	HLE	10.24
MR-DZ	82.98	CEC	30.37	HBL	15.41	HLE	7.85
PZ-EZ	76.78	CAU	18.29	HLE	13.80	HBL	12.52
PZ-SZ	71.62	HBL	18.17	PSI	17.71	HLE	14.60
PZ-DZ	73.03	CEC	25.20	HBL	17.41	PSI	13.39
EZ-SZ	76.25	CAU	22.19	PSI	17.23	HLE	14.48
EZ-DZ	81.70	CEC	20.74	CAU	17.04	PSI	12.55
SZ-DZ	80.58	CEC	28.05	PSI	21.61	HLE	9.35

Species codes can be referenced in ***Table 3****.*

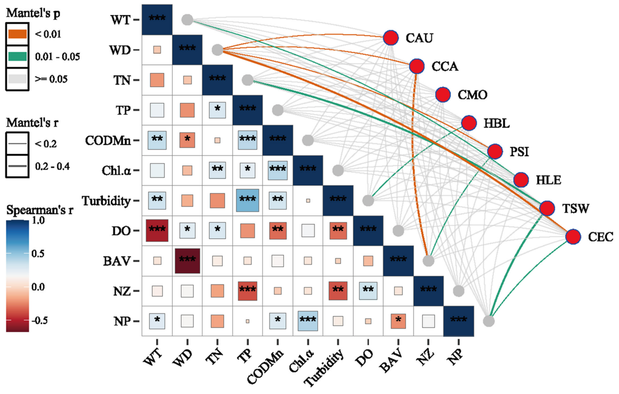

FIGURE 5 | Results from Spearman correlation analyses and Mantel's tests assessing associations among different environment variables and among environmental variables and the dominant species of fish sampled from the Mangshe River (MR) and different purification zones of Lake Yanlong (PZ, the pretreatment zone; EZ, the emergent macrophyte zone; SZ, the submerged macrophyte zone; DZ, the deep purification zone). WT, water temperature; WD, water depth; TN, total nitrogen; TP, total phosphorus; COD_{Mn}, chemical oxygen demand; Chl-α, Chlorophyll-α concentration; DO, dissolved oxygen; BAV, biomass of aquatic vegetation; NZ, density of zooplankton; NP, density of phytoplankton; CAU, *Carassius auratus*; CCA, *Cyprinus carpio*; CMO, *Culter mongolicus*; HBL, *Hemiculter bleekeri*; PSI, *Pseudobrama simony*; HLE, *Hemiculter leucisculus*; TSW, *Toxabramis swinhonis*; CEC, *Coilia ectenes*; * = $0.01 < p < 0.05$, ** = $0.001 < p < 0.01$, *** = $p < 0.001$.

plankton (Liu et al., 2008), so their association to phytoplankton in this study was not surprising.

The biomass of submerged aquatic vegetation was also a key factor affecting the distribution of fishes within Lake Yanlong, which is in accordance with other studies (Li et al., 2010; Massicotte et al., 2015; Cai et al., 2019). Generally, the density of fish, especially small-bodied fish, is positively correlated with the biomass of submerged aquatic vegetation (Cai et al., 2019). Extensive studies have shown that aquatic vegetation can affect the distribution of small-bodied fishes in the following ways: (1) providing abundant food resources for small fish (such as periphytic algae, invertebrates, and plant detritus) (Schneck et al., 2011; Massicotte et al., 2015); (2) the branches and leaves of different aquatic plants often interweave to form complex habitat, which can provide refuge for small fish (Clark et al., 2003; Kovalenko et al., 2012); and (3) the stems and leaves of aquatic plants can provide suitable spawning substrate for fish that produce either adhesive or semi- adhesive eggs (Su and Yao, 2002).

Strategies for Manipulating Fish Community Composition in Artificial Water Supply Lakes

Aforementioned studies and results from the present study indicate that the fish community in Lake Yanlong has undergone the process of miniaturization (i.e., over-abundance of small-bodied fishes), which has implications for ecosystem function, and is a common problem facing other artificial water supply lakes. Planktivorous small-bodied fishes (especially *T. swinhonis* and *C. ectenes*) in the MLYRB can exert heavy grazing pressure on the zooplankton community, leading to the release of phytoplankton, declines in water clarity and submerged macrophyte productivity, and loss of primary ecosystem function (i.e., purification of drinking water for humans) (Post and McQueen, 1987). Traditional biomanipulation, which aims to reduce the biomass of small-bodied fishes by stocking piscivorous fish, thus protecting large-bodied zooplankton capable of controlling phytoplankton, has been carried out extensively in temperate and subtropical lakes (Beklioglu et al., 2003; Mehner et al., 2004; Li et al., 2018). Based on the principle of traditional biomanipulation, we stocked native piscivorous fish (including *C. alburnus*, *S. chuatsi*, *C. gariepinus* and *C. argus*) into each purification zone of Lake Yanlong from 2017 through 2020. Our results revealed that the abundance and biomass of overabundant small-bodied fishes have declined significantly since biomanipulation started in 2017, indicating that the long-term stocking of piscivorous fish can effectively control small-bodied fishess. This in turn has supported environmental conditions more suitable for stimulating submerged macrophyte production and more efficient water purification. Some previous studies

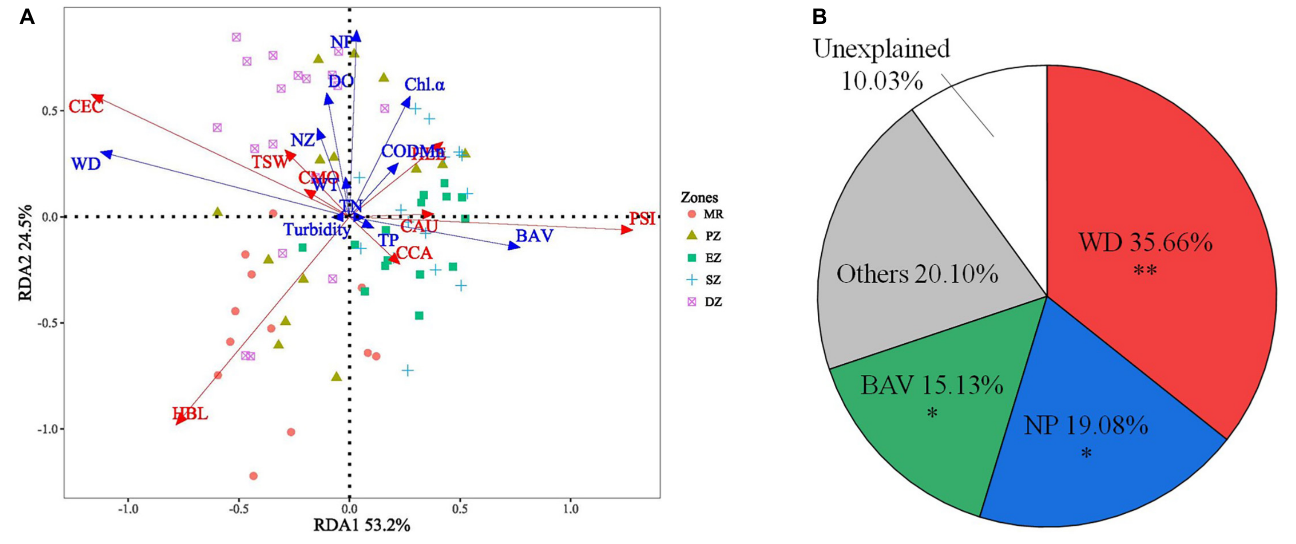

FIGURE 6 | Results from the redundancy analysis (RDA) identifying the key environment variables explaining differences in the relative abundance of dominant fish species among the Mangshe River (MR) and purification zones of Lake Yanlong **(A)**. The pie chart **(B)** describes results from the hierarchical portioning analysis (HP) characterizing the amount of independent explanatory power of each key environmental variable identified. WT, water temperature; WD, water depth; TN, total nitrogen; TP, total phosphorus; COD_{Mn}, chemical oxygen demand; Chl-α, Chlorophyll-α concentration; DO, dissolved oxygen; BAV, biomass of aquatic vegetation; NZ, density of zooplankton; NP, density of phytoplankton; CAU, *Carassius auratus*; CCA, *Cyprinus carpio*; CMO, *Culter mongolicus*; HBL, *Hemiculter bleekeri*; PSI, *Pseudobrama simony*; HLE, *Hemiculter leucisculus*; TSW, *Toxabramis swinhonis*; CEC, *Coilia ectenes*, * = $0.01 < p < 0.05$, ** = $0.001 < p < 0.01$.

arrived at the same conclusion (Jeppesen et al., 1997; Li et al., 2018).

Stocking of piscivorous fishes started 5 years after the construction of Lake Yanlong was completed (2012). During this period, small-bodied fishes occupied most of the ecological niches available in the lake. Because small-bodied fish were already well established before stocking was initiated, they could have limited the early growth and survival of piscivorous fish, necessitating the need for repeated stocking efforts. In addition, previous studies demonstrated that repeated biomanipulation (such as stocking piscivorous fish or removing benthivorous and zooplanktivorous fishes) was necessary for achieving and maintaining desired effects over the long-term in shallow eutrophic lakes (Søndergaard et al., 2008; Li et al., 2018; Setubal and Riccardi, 2020; Guo et al., 2022).

Although stocking of piscivorous fish positively influenced aquatic ecosystem function in the present study, stocking piscivores could have unintended consequences worth noting. For example, the unintentional introduction of non-native species and or genetic hybridization that could reduce overall biodiversity (Buoro et al., 2016; Cucherousset et al., 2020). Therefore, we propose three mitigation strategies for the management of fish resources and improving the water quality of artificial water supply lakes. First, the stocking of piscivorous fish should be carried out immediately after artificial lakes are built and subsequently maintained for the long-term. Second, different types of piscivorous fish should be selected according to the characteristics of different habitats present within the lakes. For example, shallow habitats were more suitable for benthic-oriented piscivorous fish (e.g., *S. chuatsi*, *C. gariepinus,* and *C. argus*), while deep water habitats were more suitable for pelagic-oriented piscivores (e.g., *C. alburnus*). Third, the piscivorous species stocked should be native to avoid potential ecological hazards caused by non-native species and genetic hybridization. Although piscivorous fish played a key role in controlling small-bodied fish in Lake Yanlong, the utility of stocking piscivorous fish for improving water quality should be further verified in other artificial water supply lakes.

CONCLUSION

This study provided basic information on the composition, relative abundance and biomass, and diversity characteristics of fish communities occupying distinct habitat zones extending from the Mangshe River through to the outlet of Lake Yanlong. We confirmed that water depth, the amount of submerged aquatic vegetation and phytoplankton concentrations were the primary factors determining the spatial distribution of fishes along this gradient of artificially constructed habitat types. Based on the effects of stocking piscivorous fish in Lake Yanlong from 2017 to 2020, we suggest that the long-term stocking of piscivorous fish can effectively control small-bodied fishes. However, the species of piscivorous stocked should be tailored to habitat conditions present within the different purification zones of Lake Yanlong.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CG: investigation, data analysis, and writing – original draft. SL: investigation, resources, and data curation. WL: methodology, resources, and writing – review and editing. CL: methodology and writing – review and editing. TZ: resources and funding acquisition. JL: resources, writing – review and editing, and supervision. LL: resources and data curation. JS: investigation and data curation. XC: investigation and writing – review and Editing. AH: resources and writing – review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.921082/full#supplementary-material

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Status, mechanism, suitable distribution areas and protection countermeasure of invasive species in the karst areas of Southwest China

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Biological invasion is one of the major threats to global biodiversity attracting a primary focus of scientific attention. During the past decades, due to the diversity and peculiarity of species, coupled with the vulnerable ecosystem, karst areas have received more and more attention. Numerous investigations and studies have confirmed that the karst areas in Southwest China are suffering from biological invasions under the intensified human activities and the climate change they caused. Despite some fundamental research on invasive species that has been conducted to understand the species and distribution in the karst areas, the mechanism of biological invasions and the response of karst ecosystem are still lack sufficient knowledge. In this paper, we summarized the habitat characteristics and invasion status of karst areas to biological invasions. This paper comprehensively analyzed the research results on biological invasions in karst areas to understand the status and development trends of biological invasions in the karst of China, so as to promote the relevant research on biological invasions in the karst areas. We found that the biological invasions in the karst areas were increasing with years. We also revealed the possible mechanism including competition, mutualism, allelopathy and phenotypic plasticity of biological invasion in karst by summarizing the relevant research results of in the karst areas. Moreover, the response of karst to biological invasion was described from the aspects of ecosystem, community, species and genetic levels, etc. By comparing the characteristics of invasive species that have been found in karst area, we analyzed the common characteristics including strong fecundity and rapid growth rate, strong environmental adaptability, strong phenotypic plasticity and high genetic diversity of the existing invasive species, we simulated and predicted the habitat of invasive species. Overall, we found three areas with high habitat suitability covering Chinese southwest Karst ecosystem, which include the southern Yunnan-Guizhou Plateau, foothill area on the Min-Yue-Gui and foothill area of southern Yunnan. It is also worth noting that the Sichuan Basin has a higher invasive risk compared to its surrounding Karst ecosystem, mainly because of the high habitat suitability of some invasive species. Therefore, we suggest that a general survey of alien invasive species in the karst areas of Southwest China should be carried out as soon as possible, focusing on the survey of the suitable areas of alien species for early warning. In addition, to establish a database of invasive alien species in the karst areas of southwest China, strengthen the monitoring of alien species, and evaluate the impact of invasive species in key areas on the biodiversity and ecosystem in the karst areas of Southwest China, so as to maintain the stability of cave biodiversity and the fragile ecosystem.

KEYWORDS

karst areas, invasive species, status quo, mechanism of biological invasions, potential distributions, countermeasures and proposals

Introduction

Biological invasion has been recognized as one of the main factors driving biodiversity declines, economic losses, and zoonotic disease emergences in the world (Pysek et al., 2020; Diagne et al., 2021; Zhang et al., 2022). By 2018, 667 invasive species had been recorded in China (Xu, 2018). Same as the "fully invasive species" in unified framework for biological invasions (Blackburn et al., 2011), these species with individuals reproducing, surviving and spreading at multiple sites have a serious impact. Biological invasion is a complex process that is influenced by various factors (Lake and Leishman, 2004; Geng et al., 2007). The invasiveness of alien species and the invasibility of the ecosystem being invaded are key factors for the successful invasion of alien species (Wang et al., 2015).

Southwest China's karst areas are the largest in the world and one of the hotspots of global biodiversity (Lu et al., 2013). The unique geological structure of karst areas has formed their unique habitats and provides rich ecological niches for the species in them (Xu, 2011). The characteristics of the karst ecosystem, such as shallow, calcium-rich soil and extensive caves, have led to the emergence of many unique and endemic species of plants and animals (Lu et al., 2013). Karst habitats in Southwest China are rich in rare, endangered and protected wildlife (Jiang, 2014; Shui, 2017). The specific habitat of karst not only promotes biodiversity but also determine the vulnerability of the ecosystem (Guo et al., 2011). For instance, the barren and easily erodible soil in the habitat is one of the major factors leading to the vulnerability of karst vegetation, which causes biodiversity decline and instability of the dynamic balance of the karst ecosystem (Jiang, 2014; Shui., 2017). Habitat disturbances in the karst area result from serious ecological disasters such as rocky desertification (Wang et al., 2004). Human activities also disturb habitat and will lead to biodiversity declines and biological invasions (Dukes and Mooney, 1999). For instance, land-use modifications significant affected biological invasion (Ficetola et al., 2010). Habitat loss facilitated the biological invasion of the American bullfrog (Rana catesbeiana) at Liuji Town, Jiangsu Province, China (Wang et al., 2022). At present, there are no relevant reports on the status and diffusion trend of biological invasion in the karst areas of Southwest China. The impact of biological invasion on the biodiversity of karst landforms has not attracted international attention. In this work, we collected, sorted and analyzed invasive species data in the karst areas of Southwest China, clarified the current status of biological invasion in those karst areas, and summarized the mechanism of biological invasion. We also put forward corresponding countermeasures and suggestions for the prevention and control of biological invasion in the karst areas of Southwest China.

Distribution and ecosystem characteristics of karst areas in southwest China

Distribution of karst areas in southwest China

The global extent of karst areas is approximately 2.2 million km^2 (Groves and Meiman, 2011), which accounts for approximately 12% of the world's total land area and provides drinking water to nearly 25% of the world's population. Karst ecosystems are an important part of the Earth's surface ecosystem (Goldscheider et al., 2020). Karst landforms in China are widely distributed and extensive. In particular, karst landforms in Southwest China are characterized by large area, rich biodiversity and a fragile ecosystem. (Lu et al., 2013). With the Yunnan-Guizhou Plateau as the core area, karst landforms in Southwest China are distributed in Guizhou, Yunnan, Sichuan and Chongqing (Jiang, 2014; Xu and Zhang, 2014). The Southwest karst region is the most well-known karst in the world, and its grassland ecosystem is vulnerable (Lu, 2012).

Ecosystem characteristics of karst areas in southwest China

The ecosystem of the karst areas in Southwest China are unique. The karst areas in Southwest China have a subtropical or tropical humid monsoon climate (Shui, 2017) and are warm in winter and hot in summer, with abundant annual rainfall but uneven seasonal distribution (Lu, 2012). In terms of habitat, the soil of the karst uplands is shallow and discontinuous, with a thick soil layer at the foot of the mountains and barren soil at the top, alkaline and calcium rich (Zhu, 2003). The different ecological niches such as stone crevices, caves, stone surfaces and soil layers have formed diversified small landforms in the karst areas in Southwest China (Jiang, 2014; Shui, 2017). For example, sinkholes provide refuge for many plants and caves combine with underground rivers to form a complex underground water system (Xiong, 2006). These isolated habitats provide rich ecological niches for organisms (Xu, 2011). In terms of plant species, the karst vegetation has evolved unique adaptive characteristics, including calcium and drought tolerance and strong roots, some of which can cling to rocks (Shui, 2017). These plant characteristics are due to strong water permeability and poor retention capacity of the shallow, calcium-rich, alkaline soil; fewer available water resources; and proclivity to drought of the karst landforms (Xiong, 2006; Shui, 2017). There are many unique cave animals and rock-dwelling animals in the karst areas due to the cave-rich ecosystems and special habitats, such as karst caves and underground water networks (Jiang, 2014). The uniqueness of karst plants also promotes the emergence of many unique and endemic species of animals in the ecosystem (Xu, 2011). Maolan Nature Reserve, for example, has more than 200 endemic species, including *Gekko liboensis*, *Nemacheilus liboensis*, *Sinocyclocheilus macrolepis* etc. (Jiang, 2014). Karst areas in Southwest China are rich in rare, endangered and protected wildlife. The unique karst habitats promote biodiversity and determine the vulnerability of their ecosystems. Once the karst landform is disturbed by human activities, serious ecological disasters such as rocky desertification occur easily (Wang et al., 2004; Guo et al., 2011; Xu and Zhang, 2014). Soil is one of the major factors among many leading to the vulnerability of karst ecosystems (Guo et al., 2011). For example, karst landforms in Southwest China have obvious seasonal droughts and weakly alkaline, high-calcium soil; the soil layer in many habitats is barren and easily erodible (Lu, 2012). These characteristics cause vulnerability of vegetation, which leads to the instability of the dynamic balance of the karst ecosystem.

Status quo of biological invasions in the karst areas of southwest China

Damage caused by biological invasion

In recent years, biological invasion caused by human activities have seriously affected the ecosystem in the karst areas of Southwest China. The species richness and diversity indexes of these areas are high. However, in recent years, these karst areas in Southwest China have suffered from serious biological invasion, which has decreased the biodiversity (Wang et al., 2004). Invasive species not only threaten the biodiversity of karst areas, but also may carry pathogens that threaten wildlife and human health (Zhang et al., 2022). Large numbers of invasive species were found in the karst areas of Guizhou, and malignant invasive species were found in more than 60% of survey sites (Yang et al., 2022). In the karst areas of Guizhou, the occurrence areas of invasive species *Ageratina adenophora*, *Alternanthera philoxeroides* and *Mikania micrantha* were 2,238, 79, and 1,467 km^2 respectively; and *Chromolaena odorata* has flooded the Nanpan River, Beipan River and Red River basins (Yang, 2020). After *Ageratina adenophora* and other invasive plants invaded the karst areas of Guizhou, the local biodiversity and the total number of soil animal groups decreased significantly, including a 41.3% decrease and 43.25% in grassland (Yang, 2020). As plant diversity decreases, herbivores lack food, and the food chain breaks down; thus, many karst endemic species have become endangered (Yang, 2020). In Caohai, Guizhou province, China, *Rana catesbiana* has occupied a niche and is proliferating (Liu and Li, 2009; Lv et al., 2020). The invasion of *Rana catesbiana* led to the extinction of *Cynops wolterstorffi* in Dianchi Lake, Yunnan Province (Li and Xie, 2004). Invasive species, such as *Pomacea canaliculata* and *Oreochromis nilotica*, are increasingly endangering the ecosystem in the karst areas of Guizhou, and in some areas invasive species are out of control (Qiu et al., 2019). The invasive species *Achating fulica* has endangered more than 200 species in Yunnan Province, posing a serious threat to local biodiversity (Li and Li, 2008). The impact of biological invasion on biodiversity in karst areas has not attracted broad international attention, but there are some case studies. For example, biological invasions have resulted in a significant reduction of biodiversity and total numbers of animal and plant groups and even endangered species (Jiang, 2014; Shui, 2017). The invasive plant *Aster subulatus* is one of the most harmful plants in nurseries and gardens in Southwest China, but the research on it is very limited (Pan et al., 2010). Therefore, biological invasions in the karst areas in Southwest China should receive more attention and research to prevent ecosystem degradation.

Invasive species

In recent years, the karst areas in Southwest China are suffering from serious biological invasion. To understand the status quo of biological invasion in these areas, we summarized the invasive species according to published literatures (Supplementary Table S1). A total of 172 invasive species were recorded, including 21 animal species (excluding insects) and 151 plants species. The 21 invasive animal species belonged to 16 families and six groups, of which fishes were the most abundant, with 12 species accounting for 57.14% of all species; mammals were the second abundant, with four species accounting for 19.05% of all species. Other groups had one or

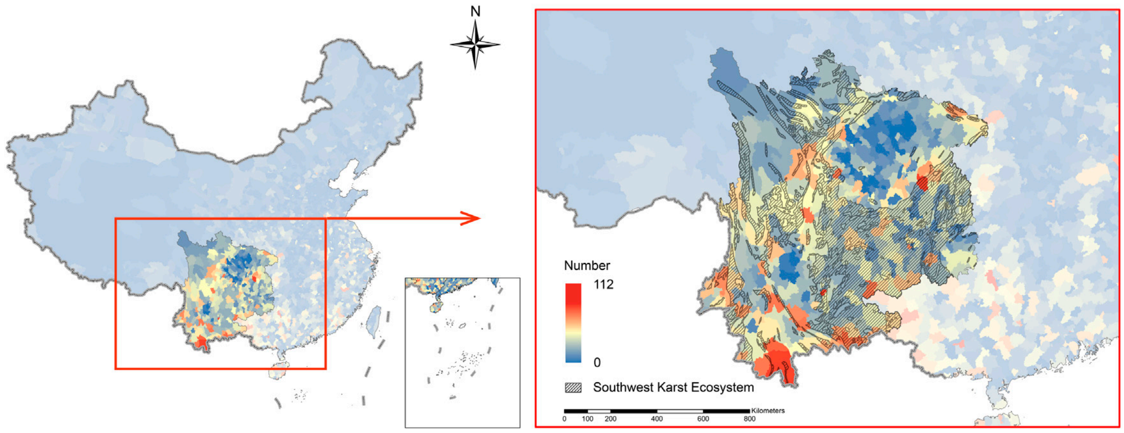


FIGURE 1
Richness of the typical invasive alien species among karst systems in southwest China. Blue represents counties with low richness, red represents counties with high richness. Crosshatch regions mean karst systems in southwest China.

two species. The 151 invasive plant species belonged to 36 families, of which Compositae was the most abundant, with 34 species accounting for 22.52% of all species; the second abundant was Leguminosae, with 19 species accounting for 12.58%. In addition, there are 14 species of Amaranthaceae accounting for 9.27% of all species and 13 species of Gramineae, accounting for 8.61% of all invasive species. Among the invasive plants, 32 species were malignant, 48 species were severe, 21 species were local, and 24 species were general. The malignant and severe invasive plants accounting for 52.98% of the total invasive plant species. Supplementary Table S1 showed that among the typical invasive animals in the karst areas of Southwest China, except for muroid, the other animal species were aquatic or amphibian. Among the invasive plants, *Ageratina Adenophora*, *Lantana camara*, *Alternanthera philoxeroides* and *Eichhornia crassipes* were hygrophytic species. These descriptions demonstrate that karst areas are being threatened by invasive species, especially some aquatic alien species, which have seriously affected the biodiversity of water ecosystems and the ecological safety in the karst areas. Effective measures are urgently needed to prevent and control invasive species.

Distribution of invasive species

We summarized the distribution of 151 invasive plant species and 21 invasive animal species with county-level resolution. For each animal species, we obtained information about their distribution from literatures. Because the data of most animal species' occurrence are only at the provincial level and some animal species, such as *Pomacea canaliculata*, *Pterygoplichthys pardalis* and *Rattus norvegicus* are widely distributed species, we believe that they are located in all counties of the recorded province. For plants, distribution data were extracted from the Invasive Plants module of the National Specimen Information Infrastructure (NSII) database. The Asteraceae family is widely distributed in all provinces including the karst areas, among which the most widely distributed species are *Bidens pilosa*, *Conyza canadensis*, *Crassocephalum crepidioides*, *Erigeron annuus* and *Galinsoga parviflora*. From Figure 1, it can be seen that there were more invasive species in the foothills area of the Min-Yue-Gui, Hengduan Mountain Area and Guizhou Plateau. The invasive species richness was low in Sichuan Basin and northwest Sichuan Province. We found that most species were not only distributed in the karst areas shown in Figure 1, but also distributed in other areas to varying degrees. For example, the adjacent Guangxi Province had a high invasive species richness, which might be related to the large karst area in this province.

Biological characteristics and mechanisms of invasive species in the karst areas of Southwest China

Biological invasion is a complex process and there are many factors affecting the spread of invasive species. The invasiveness of invasive species and the resistance to the invasion of the ecosystem are the key factors that determine the success of invasive species (Wang et al., 2015). Studies have shown that the invasiveness of invasive species is significantly related to its competitiveness, spatial growth ability, fecundity, resource utilization capacity and allelochemicals release etc. (Pyek and Richardson, 2007). The resistance to the invasion of the ecosystem is related to the species diversity and available resources of the local ecosystem (Lonsdale, 1999). During the process of biological invasion, alien species ensure their dominance in interspecific competition with local species especially for resources through the plasticity of their morphological characteristics, physiological characteristics and reproductive characteristics to enhance their invasiveness (Sodhi et al., 2019). In addition, alien species can increase their invasiveness by releasing allelochemicals that inhibit the growth of native species (Callaway and Ridenour, 2004). Generally, the biological invasion of alien species is the result of the combined action of different invasion methods.

Typical biological characteristics of invasive species

According to some studies, successful invasive species have several significant characteristics including high fecundity, strong environmental adaptability and high phenotypic plasticity (Mack et al., 2003). Among the 21 invasive animal species (insects excepted) in the karst areas of Southwest China, the successful invasive species had several significant characteristics in common. The first characteristic was omnivory (Table 1). Omnivorous alien species can find food and adapt to local environments rapidly. The American bullfrog (Rana catesbeiana) that invaded Yunnan, China, for instance, fed on more than 30 species from 10 taxonomic classes, including frogs native to Yunnan (Liu et al., 2015). *Procambarus clarkii*, which invaded Caohai, China, fed on plants, plankton, aquatic invertebrates, insects, etc. Its extensive diet contributed to maintaining and expanding the population (Tao, 2020). The second characteristic was strong fecundity (Table 1). Invasive animals showed strong fecundity. For example, each female American bullfrog laid 10,000 eggs at a time, with a survival rate of approximately 10% (Li and Xie, 2004). *Gambusia affinis* showed strong fecundity and high seasonal characteristics, which posed a serious threat to the survival of local fish in the areas invaded (Gao et al., 2019). The third characteristic was strong environmental tolerance with wide habitat adaptability (Cruz and Rebelo, 2007) (Table 1). *Procambarus clarkii* was highly adaptable to the hydrologic and temperature conditions of the new habitat (Gutiérrez-Yurrita et al., 1998), and it can reproduce in most water, even in the extreme environment of polluted water or the high-salinity water (Barbaresi and Gherardi, 2000). The last characteristic was high phenotypic plasticity (Table 1), which enables invasive animals to improve their adaptability to the environments. For example, *Gambusia affinis* had strong phenotypic plasticity, and environmental factors such as temperature, precipitation, altitude, salinity, electrical conductivity and dissolved oxygen all affected their life-history traits (Jourdan et al., 2016; Cheng et al., 2018). In addition, higher genetic diversity or genetic variation is beneficial to the adaptive evolution of invasive animals (Wang, 2015).

Among the 151 invasive, mostly herbaceous, plant species in the karst areas of Southwest China, the successful invasive species had several significant, common characteristics. The first characteristic was strong allelopathy (Table 1). For example, *Ageratum conyzoides*, *Parthenium hysterophorus* and *Lantana camara* have strong allelopathy (Kong et al., 2002). Studies have shown that *Lantana camara* and its extracts had allelopathic effects on seed germination and seedling growth of *Capsicum annuum*, *Brassica rapa* and *Brassica campestris*. The second characteristic was strong fecundity (Table 1). Table 1 shows that the invasive plants in the karst areas of Southwest China had strong fecundity, including vegetative propagation and sexual propagation. Some species had both propagation modes. For example, *Ageratum conyzoides* reproduced sexually in suitable conditions to ensure the variability of the population, and reproduced asexually under harsh conditions to ensure the fecundity of the species (Zhang et al., 2020). *Amaranthus spinosus* is a hermaphrodite angiosperms, with bisexual flowers and self-pollination. It can produce up to 10,000 seeds per plant and has strong fecundity (Li, 2016). The third characteristic was easy seed dispersal (Table 1). The reproductive strategy of annual invasive plants generally relies on the production of a large number of individuals to maintain the populations. This was usually manifested as a large number of seeds, while small seeds that spread easily by wind or river (Lu and Ma, 2005, 2006). For example, the seeds of *Bidens pilosa* and *Praxelis clematidea* were small, with long pappi and strong diffusibility in the wind (Zhong et al., 2016). The fourth characteristic was high phenotypic plasticity (Table 1). Phenotypic plasticity in plants plays an important role in the survival and maintenance of species under new environments and pressures by obtaining maximum fitness (Hendry, 2016). For example, *Alternanthera philoxeroides* showed high phenotypic plasticity in a variable environment (Geng et al., 2007). The last characteristic was higher genetic diversity or genetic variation. Most invasive plants in the karst areas of Southwest China have high genetic variation or diversity, such as *Praxelis clematidea*, *Amaranthus retroflexus*, *Amaranthus spinosus*, etc. (Chan and Sun, 1997; Li et al., 2007; Tang et al., 2009; Wang et al., 2015).

Ecosystem resistance to the invasive species

The ecosystem resistance to invasive species is related to available resources, species diversity, and human disturbance, etc. (Lonsdale, 1999). Biological invasions in the karst areas of Southwest China are related to available resources. If there are unused resources suitable for invasive species in the karst ecosystem, this is conducive to the early establishment of invasive species and to decreasing the ecosystem's resistance to invasion (Wan et al., 2015). For instance, in degraded karst sinkholes, invasive plants had fewer resources to use, which effectively reduced the invasion of *Ageratina adenophora*, resulting in different degrees of invasion in different karst habitats (Jiang et al., 2019). There were abundant aquatic plants and mosquitoes in the shallow lakes and wetlands in Caohai, Guizhou province, and the suitable conditions and available resources were conducive to the invasibility of the American bullfrog in this area (Lv et al., 2020). The species diversity of the karst ecosystem is also a factor affecting the degree of alien species invasion. For example, in western Panzhihua, Sichuan province, the higher species diversity of the shrub layer and the composition of the herb layer inhibited the invasion of *Ageratina adenophora*; the higher the species diversity of shrub layer, the lower the invasive degree of *Ageratina adenophora*, which was consistent with the diversity resistance hypothesis proposed by Albertson (1960).

TABLE 1 Main biological characteristics of invasive alien species in Karst areas of Southwest China.

	Group	Biological characteristics	Representative species	References
1	Invasive animals	Omnivorous feeding	*Rana catesbeiana, Procambarus clarkia, Pomacea canaliculata*	Liu et al. (2015); Marunouchi et al. (2003); Hofkin et al. (1992); Gutiérrez-Yurrita et al. (1998); Gherardi et al. (2001)
2		Strong fecundity and rapid growth rate	*Procambarus clarkia, Pomacea canaliculate, Gambusia affinis*	Barbaresi and Gherardi, (2000); Tanaka et al. (1999); Kai and Shi, (2017); Cheng et al. (2018)
3		Strong environmental adaptability	*Trachemys scripta elegans, Gambusia affinis*	Ramsay et al. (2007); Gao et al. (2019)
4		Strong phenotypic plasticity	*Pomacea canaliculate, Gambusia affinis*	Estebenet and Martín, (2002); Jourdan et al. (2016)
5		High genetic diversity	*Procambarus clarkia, Pomacea canaliculate*	Barbaresi et al., (2007); Xu et al., (2009); Yang et al. (2018)
6		Low genetic diversity	*Rana catesbeiana*	Austin et al., 2003; Bai et al. (2012)
7		Low genetic variation	*Rana catesbeiana*	(Austin et al., 2003; Bai et al. (2012)
8	Invasive plant	Strong allelopathy	*Amaranthus retroflexus, Amaranthus spinosus, Ageratum conyzoides, Aster subulatus, Bidens Pilosa, Erigeron canadensis, Conyza sumatrensis, Crassocephalum crepidioides, Chromolaena odorata, Dysphania ambrosioides, Avena fatua, Lantana camara*	Shahrokhi et al., (2011); Bakhshayeshan-Agdam et al., (2015); Macharia and Peffley, (1995); Kong et al., (1999); Kong et al., (2002); Sundufu and Shoushan, (2004); He et al., (2019); Zhang et al., (2016); Djurdjević et al., (2011); Wang et al., (2010); Wang et al., (2014); Zheng et al., (2015); Li et al., (2020); Liu et al., (2016)
9		Strong asexual reproduction ability and rapid growth rate	*Erigeron annuus, Parthenium hysterophorus, Praxelis clematidea, Oxalis corymbose, Lantana camara*	Fan et al., (2020); Adkins and Shabbir, (2014); Zhang et al., (2021); Sharma et al. (2008)
10		Strong sexual reproduction ability and rapid growth rate	*Conyza sumatrensis, Crassocephalum crepidioides*	Hao et al., 2009; Huang, (2008)
11		Strong ability of asexual and sexual reproduction	*Solidago canadensis, Phytolacca Americana, Eichhornia crassipes*	Xiao et al., (2019); Zhou et al., (2013); Huang and Ding, (2016)
12		Seeds spread easily	*Ageratum conyzoides, Ageratina Adenophora, Praxelis clematidea, Avena fatua*	Zhong et al., (2016); Lu and Ma, (2005); Lu and Ma, (2006); Intanon et al., (2020); Ņečajeva et al. (2021)
13		Strong environmental adaptability	*Erigeron canadensis, Ageratina Adenophora, Phytolacca Americana, Eichhornia crassipes*	Wang et al., (2021); Zhou et al., (2010); Dong et al., (2014); Teng et al. (2021)
14		strong phenotypic plasticity	*Alternanthera philoxeroides, Amaranthus retroflexus, Aster subulatus, Bidens Pilosa, Erigeron canadensis, Ageratina Adenophora, Parthenium hysterophorus*	Pan et al., (2006); Geng et al., (2007); Wang et al., (2015); Zhou et al., (2005); Pan Yumei et al., (2010); Pan et al., (2017); Mojzes et al., (2020); Zhao et al., (2012); Shi and Adkins, (2020)
15		High genetic diversity	*Amaranthus retroflexus, Amaranthus spinosus, Ageratum conyzoides, Erigeron annuus, Parthenium hysterophorus, Praxelis clematidea, Avena fatua*	(Suresh et al., 2013; Chan and Sun, 1997; Shukla et al., 2018; H. K. and N. T. H., 2017; Ni et al., 2009; Tang et al., 2009; Wang et al., 2015; Li et al. (2007)
16		Low genetic diversity	*Alternanthera philoxeroides, Chromolaena odorata, Eichhornia crassipes*	Wang et al., (2005); Ye et al., (2003); Yu et al., (2014); Li et al., (2006); Ren et al. (2005)
17		High genetic variation	*Erigeron canadensis, Erigeron annuus*	Marochio et al., (2017); Fernandes et al., (2015); Stratton, (1991)
18		Low genetic variation	*Praxelis clematidea, Eichhornia crassipes*	Wang et al., (2015); Zhang et al., (2010); Ren et al. (2005)

Ecosystem biodiversity is positively correlated with invasive species resistance. However, different conclusions were drawn about the impact of species diversity on ecosystem resistance to invasive species at different spatial scales. For example, at the scale of 25 m^2, the invasive degree of *Ageratina adenophora* was negatively correlated with local species diversity; at the scale of 400 m^2, there were both positive and negative correlations; and at the provincial scale, the invasive degree was positively correlated with the local biodiversity (Lu et al., 2008). In addition to the previously mentioned factors, human disturbance also affects the invasiveness of the karst ecosystem. With the intensification of human activities and global climate change, many natural ecosystems have been disturbed at various degrees. Excessive disturbance will lead to ecological imbalance and degradation, which will decrease the ecosystem resistance to invasion (Wan et al., 2015).

The mechanisms of alien species invasions

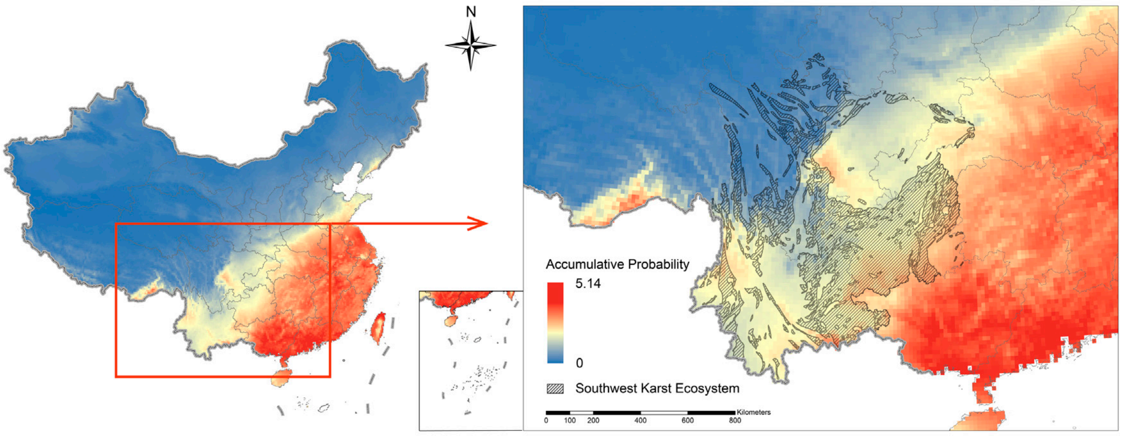
The main mechanisms of alien species invasion include competition, symbiosis, allelopathy and phenotypic plasticity. Compared with native species, invasive species have advantages in life-history traits, genetics and evolution (Callaway and Ridenour, 2004; Wan et al., 2015). The inherent superiority hypothesis holds that the successful invasion of some alien species is due to their morphological, physiological, ecological and other specific traits, which make them superior to other species in resource competition and environmental adaptation (Sax and Brown, 2000; Hufbauer and Torchin, 2008). Phenotypic plasticity in environmental adaptation is also an important strategy and mechanism for alien species invasion.

Species in the same ecological niche or trophic level compete for resources. Competition is an important way for native species to prevent biological invasion (Wan et al., 2015). For example, *Rana catesbeianus*can compete with native species in the same ecological niche of Caohai lake in Guizhou province for food and space resources because of their omnivory and large food intake (Liu and Li, 2009; Lv et al., 2020). Studies on the life-history traits of invasive plants showed that they had higher spatial growth capacity, resource utilization capacity, photosynthetic rate (Shen et al., 2011) as well as stronger fecundity (Qi et al., 2014). The height, specific leaf area, concentration of representative nutrients, and photosynthetic rate of invasive plants were significantly higher than those of native plants (Sodhi et al., 2019). These functional traits of invasive plants represent their efficient use of resources and contribute to their competitive ability and successful invasion (Lake and Leishman, 2004). Studies have shown that symbiosis is also a factor in plant invasion in the karst areas (Wan et al., 2015). The karst landforms have shallow and discontinuous soils that are calcium rich and nutrient poor, which determines their obvious spatial heterogeneity (Lu, 2012). These characteristics will significantly affect the development and distribution of plant populations (Qi et al., 2013). Most plants are symbiotic with arbuscular mycorrhizal (AM) fungi, which is highly adaptable in the heterogeneous habitat of karst landforms. Some research found that AM fungi can significantly improve the growth and nutrient use of the invasive plant *Bidens pilosa* in the karst areas and significantly improve its adaptability to the karst heterogeneous habitats (Xu, 2020). Some studies also found that mycorrhizal fungi can reduce the competitiveness of the native species *Kummerowia striata* and promote the invasion of *Solidago Canadensis* (Yang et al., 2014). Researchers have regarded invasive plants and AM fungi as a symbiotic relationship that facilitates invasion (Cui and He, 2009).

In addition to competition and symbiosis, invasive plants interact with native species by allelopathy. The allelopathy of invasive plants is mainly related to their rapid evolution and lack of coevolution with native plants. The new weapon hypothesis holds that an important reason for the success of alien plants is that they can release allelochemicals to inhibit the growth of native species (Callaway and Ridenour, 2004). A typical invasive weed in the Sichuan Basin, *Erigeron canadensis*, releases phenolic acid that can inhibit seed germination and seedling growth of species such as *Trifolium repens* (Djurdjević et al., 2011). The invasive weed *Parthenium hysterophorus* releases phenolic substances that inhibit the growth of *Brassica campestris*, *Brassica rapa* and *Brassica oleracea* seedlings (Singh et al., 2005). A tissue extract of the invasive weed *Amaranthus retroflexus* had allelopathic effects on the seed germination and growth of four important crops: cucumber (*Cucumis sativus*), alfalfa (*Medicago sativa*), kidney bean (*Phaseulus vulgaris*) and wheat (*Triticum aestivum*) (Bakhshayeshan-Agdam et al., 2015).

Phenotypic plasticity enables alien species to maintain high fitness in a variety of habitats, especially when the environment undergoes unsuitable changes. For example, the phenotypic plasticity of *Procambarus clarkii* is reflected in its feeding habits. An optimal feeding strategy can be achieved by adjusting feeding strategies at different times (Smart et al., 2002). Research found that in three different karst habitats, *Alternanthera philoxeroides* increased its adaptability to different habitats by changing a series of morphological characteristics and adjusting reproductive strategies (Zhang et al., 2017). The invasive species *Erigeron annuus* in Chongqing had strong phenotypic plasticity. In low-altitude areas, it adjusted a series of phenotypic traits to enhance ecological adaptability (Li, 2014). The most seriously invaded areas of *Ageratina adenophora* in China are in the Yunnan-Guizhou Plateau. Due to the large environmental differences between the invasion and origin, and the low genetic variation between and within populations in the invasion site, this species has

A 9 terrestrial invasive animals



B 80 typical invasive plants

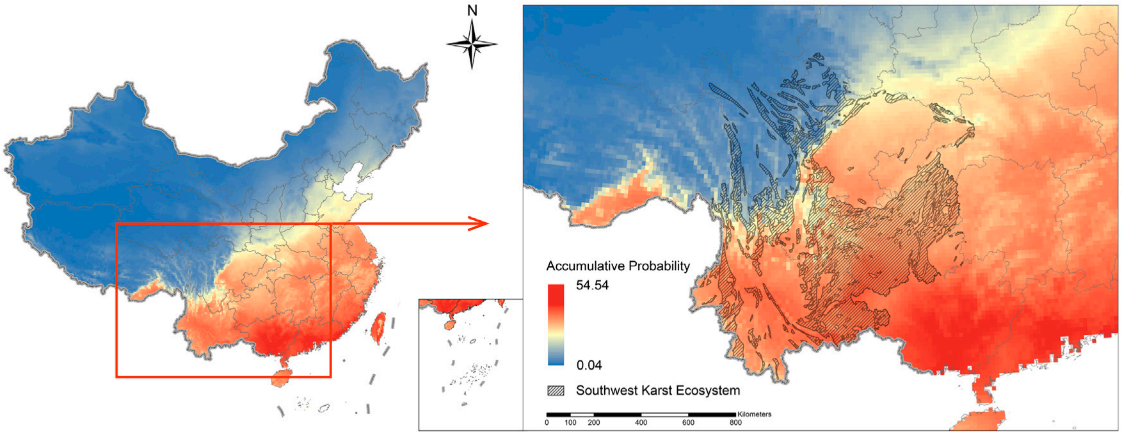


FIGURE 2
Accumulative potential distribution probability of typical invasive species in China predicted by the MaxEnt niche model. Blue represents grids with low probability which means low habitat suitability, red represents grids with high probability which means high habitat suitability. Crosshatch regions mean karst systems in southwest China.

great ecological adaptability (Sang et al., 2010), revealing the high phenotypic plasticity of its functional traits. That is the main strategy for its invasion of China (Zhao et al., 2012). This overview indicates that the main mechanisms of biological invasion in the karst ecosystems of Southwest China are competition, symbiosis, allelopathy and phenotypic plasticity.

Suitable habitats of invasive species in the karst areas of Southwest China

We collected global presence records for nine terrestrial invasive animal species and 80 malignant and severe invasive plant species (Supplementary Table S1) from the Global Biodiversity Information Facility (GBIF) and the citizen science database iNaturalist. Elevation data and 19 bioclimatic variables such as annual mean temperature are obtained from the WorldClim Climate Database (www.worldclim.org). The global potential habitat suitabilities for 89 invasive species were calculated by the maximum entropy niche model, using the software MaxEnt 3.4.4 and combined with these 20 environmental variables. For models of all species, the average training area under the curve (AUC) ranged from 0.656 to 0.99 (mean: 0.869; standard deviation: 0.076). Because of the need to use different model variables, aquatic invasive animals are not considered in this part. Habitat suitability in each grids were calculated as the accumulative distribution probabilities of these invasive species. A higher habitat suitability value for a given grid cell indicates a higher relative probability of invasion.

Except for two human-associated species, *Mus musculus* and *Rattus norvegicus*, the other seven invasive animal species had low habitat suitability in the karst area (Figure 2A.). For invasive plants, we found three areas with high habitat suitability, including the southern Yunnan-Guizhou Plateau, the Min-Yue-Gui foothills and the foothill of southern Yunnan. It can be observed from Figure 2 that, consistent with the current distribution pattern shown in Figure 1, the habitat suitability of 80 typical invasive species is highest in Guangxi Province adjacent to the study area and in Guangdong Province, which is at the same latitude. *Amaranthus lividus*, *Amaranthus viridis*, *Amaranthus spinosus*, *Conyza bonariensis* and *Pharbitis nil* have the largest suitable habitats areas in eastern and central China. In addition, the Sichuan Basin has a higher invasive risk compared to the surrounding karst areas, mainly because of the high habitat suitability of *Amaranthus lividus*, *Conyza bonariensis*, *Pharbitis nil*, *Pharbitis purpurea* and *Solanum khasianum*.

The Maxent model was used to predict the suitable distribution areas of *Alternanthera philoxeroides* in China, which were mainly distributed in tropical, subtropical and southeast warm temperate regions of China. In addition, high-suitability areas were mainly located in Guangxi, Shanghai, Jiangsu, Sichuan provinces and in Chongqing, where invasive species exhibit continuous expansion (Yan et al., 2020).

Countermeasures and suggestions

The plant communities in the karst areas of southwest China show complex and diverse species compositions, high species richness, and high diversity indexes and occupy important position in the global karst ecosystem. However, biological invasion caused by human activities has seriously affected the ecosystem of the karst areas in Southwest China in recent years. To effectively prevent and control biological invasion in this area, some countermeasures and suggestions are put forward as follows. It is necessary to conduct a survey of invasive species in the karst areas of Southwest China. A systematic survey of invasive species has not been carried out although there are many invasive species in the karst areas. We suggest that a survey of invasive species in the karst areas of Southwest China be conducted as soon as possible, especially in the important wetlands, plateaus, basins, and other suitable distribution areas. We must obtain the information of invasive species types, quantities, and key areas threatened to facilitate the development of an early warming and protection strategy. Moreover, for our models' results, the ROC analyses show fairly good performance by MaxEnt for training AUC values. But the high AUC values may result from the arbitrary selection of pseudo-absence data (Phillips et al., 2009; VanDerWal et al., 2009; Liu et al., 2011). The accurate presence-absence data is the key to further modeling.

It is necessary to establish a database of invasive species and a monitoring and early warning system for invasive species in the karst areas of Southwest China. Timely supplement and improvement of the database should be part of the follow-up investigation and research, to provide a basis for the establishment of the monitoring and early warning system. We also suggest increased monitoring of invasive species in the karst areas of Southwest China and evaluation of the impact of invasive species on local biodiversity and ecosystem in key areas. Concurrently, the key invasive species' habitat suitability should be simulated, so that species with a serious expansion trend can be monitored, warned against and controlled.

The last suggestion is to protect and improve the biodiversity of native species in the karst areas of Southwest China. We suggest strengthening the relevant research on the ecological characteristics of native species and restoring suitable ecosystem structures and functions to establish a benign successive ecological community and provide a basis for scientific replacement control of invasive species. In the prevention and control of alien plants, replacement control is a method that uses plants with ecological and economic value to replace invasive plants according to the succession rule of a plant community. We suggest strengthening the research on the ecological characteristics of native species, and giving full consideration to plant replacement restoration technologies in the prevention and control of alien species.

Author contributions

YL and JC designed the article; YL, TS, YL and LF collected the data and information; TS, YL and YH drew the figures; YL and JC wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.957216/full#supplementary-material

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Exploring the trophic niche characteristics of four carnivorous Cultrinae fish species in Lihu Lake, Taihu Basin, China

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This study found significant differences between the standard length distributions of humpback and redfin culter and between Mongolian culter and topmouth culter. A stable isotope analysis (δ^{13}C and δ^{15}N) was used to investigate the interspecies differences between the feeding habits and trophic niches of four carnivorous species of Cultrinae fish. The results showed that the δ^{13}C and δ^{15}N values were significantly correlated with the standard length and species. However, the δ^{13}C values of humpback and redfin culter were not significantly different, suggesting that these two fish species had similar food sources. The δ^{15}N values of Mongolian culter and topmouth culter were the highest, suggesting that they occupied a higher trophic level and that animal prey was more important in their diets. Moreover, variations in δ^{13}C and δ^{15}N indicated considerable niche overlap and interspecific competition among the four species. We also evaluated trophic niches, diversity, redundancy, and evenness utilizing isotopic niche metrics, and we estimated asymmetrical niche overlaps. The analysis revealed that four carnivorous species of Cultrinae displayed similar trophic niche sizes and trophic diversity. The trophic traits of topmouth culter clearly defined them as a trophic generalist in terms of the inter-individual variability in their isotopic niches. A significant finding was that the average niche overlap between them was as high as 64.02%; topmouth culter had the greatest trophic overlap with redfin culter (95.52%) and humpback (90.38%), followed by Mongolian culter onto redfin culter (85.32%), indicating that topmouth culter and Mongolian culter benefit the most from the food supply in the habitat, or they are more competitive in the presence of limited resources.

KEYWORDS

trophic niche, Cultrinae fish, interspecies relationship, Lihu Lake, stable isotope

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Introduction

"Niche" refers to the relationships between a species and all other species in a specific ecosystem (Elton, 1927). A niche includes the spatial and temporal positions occupied by a particular species, the status of environmental adaptation, and the utilization of resources by the species (Zhang and Xie, 1997; Li et al., 2006). Multidimensional hypervolume ecology not only emphasizes the habitat meaning of the niche but also includes the resource utilization by organisms and the relationship between them and the environment (Hutchinson, 1957). An occupied niche space implies resource utilization; moreover, understanding the factors that lead to changes in trophic niches is important in assessing food web structures (Yao et al., 2016), resource utilization (Ortega-Cisneros et al., 2017), and trophic interactions (Wallace et al., 2009). Different predators may occupy similar trophic niches, and the trophic overlaps indicate similar diets in freshwater ecosystems (Chen et al., 2011; Wang et al., 2019). Carbon and nitrogen stable isotopes have been proven to effectively quantify niches and niche overlaps of species (Post, 2002; Bearhop et al., 2004). The method allows for the estimation of the overlap among species' isotopic niches to suggest that feeding competition can occur if resources are to be limited and if niche partitioning occurs (Balzani et al., 2020). In conclusion, it is feasible and scientific to use stable isotopes to study isotopic niche overlap.

The topmouth culter (*Culter alburnus*), Mongolian culter (*Culter mongolicus*), humpback (*Culter dabryi*), and redfin culter (*Cultrichthys erythropterus*) are related carnivorous species in the genus Cultrinae (Cypriniformes and Cyprinidae) (Hu et al., 2011). The topmouth culter and Mongolian culter occupy higher trophic levels than the other two species because of their piscivorous feeding habits and larger sizes, although all four culter fish species are common predators in the aquatic food web. In addition, humpback and redfin culter are widely known as connecting links between forage living and top predators in some lake and reservoir ecosystems (Ye, 2006; Li et al., 2013). They are important commercial freshwater fishes and are widely distributed in China, inhabiting the middle and upper levels of large water bodies rich in aquatic plants (Chen, 1998; Zhang X. G. et al., 2008; Zhang X. L. et al., 2008). Mongolian culter and topmouth culter have the highest economic values because of their large size, rapid growth, and the desirable flavor of their meat. Redfin culter and humpback are smaller, more aggressive fish that are also economically valuable (Feng et al., 2007). Previous research has shown that culters, important top predators in lakes, primarily feed on lake anchovy (*Coilia nasus taihuensis*), followed by other small fish and shrimp. Hence, because they have obvious inhibitory effects on small fish and shrimp, they maintain the ecosystem's stability (Zhou et al., 2011).

In recent years, overfishing and the destruction of the spawning grounds of large fish such as Mongolian culter and topmouth culter have caused changes in the populations and distributions of humpback and redfin culter. Wild populations of four species are declining so quickly that have resulted in the domination of their communities by lake anchovy and other small fish (Liu et al., 2005, 2007; Xiong et al., 2022), and the overlap of their isotopic niches has become an extremely important issue (Wang et al., 2007). Ontogenetic niche shifts could change trophic relationships among species and would play diverse roles in food webs (Woodward and Hildrew, 2002). It has been established that culters experience significant increases in body size during their development, during which they utilize different resources, which results in different feeding habits and ontogenetic niche shifts (Zhou et al., 2011). Domestic and overseas scholars have conducted much research on the domestication and cultivation of culters (Lin et al., 2013; Wang F. et al., 2015), with the goals of protecting germplasm resources, increasing body sizes and economic benefits, and strengthening the ecological regulation of lakes. Restraining the biomass of small fish with culters could decrease the predation pressure on zooplankton. This would mitigate the competition for filter-feeding fish, such as silver carp and bighead carp, reinforcing the effects of control on algae growth. It would be extremely beneficial for populations of the culters to rebound (Liu et al., 2007; Hu et al., 2011). The improved ecological balance of waters and the prevention of fish resources being dominated by smaller individuals could be achieved by artificial propagation and release and by strengthening the management of lake eutrophication.

In the 1950s and 1960s, the water quality was good because of a high degree of abundant macrophytes (Chen et al., 2009). However, subsequently, due to increasing eutrophication and the resulting lack of macrophytes, Lihu Lake became the most highly polluted in Taihu Lake (Nian et al., 2006). The monitoring of Lihu Lake revealed that the water quality changed due to severe eutrophication as reported in 2001 (Gu and Lu, 2004; Bai et al., 2010). Since 2003, several environmental protection and ecological restoration projects have been implemented by the central government and local authorities. Technological measures, such as water purification fisheries, were conducted in Lihu Lake by releasing suspension-feeding animals between 2007 and 2010 (Chen et al., 2006; Huang et al., 2012; Xu et al., 2012). As a result of the program, the fish community structure in Lihu Lake has been adjusted to a certain extent, and the trophic level of the ecosystem has increased from 3.63 to 3.95 (Di, 2014). Furthermore, large-scale stock enhancement has been implemented in recent years by the Taihu Lake Fishery Management Committee Office. However, the phenomenon that lake anchovy has been the dominant species in Taihu Lake, while the four carnivorous Cultrinae fish resources are still extremely limited (Liu et al., 2005; Zhang et al., 2021). The main causes of this phenomenon were human overfishing, the destruction of spawning grounds (Mao et al., 2011), and considerable niche overlap. Now, following the comprehensive Yangtze River Ten-Year Fishing Ban in October 2020, a comprehensive fishing ban was also implemented in Taihu Lake. Regardless of whether the structure of the fish community in Taihu Lake and Lihu Lake, particularly the biomass of large culters, has improved since the ban's implementation, there is an urgent need for future research on the resources and trophic niche of the culters. This study focused on the effects of individual size on δ^{13}C and δ^{15}N values. Besides, the proportion of niche overlap was analyzed, showing considerable niche overlap among the four species examined in this study. The study aimed to provide new knowledge and sufficient data to support fishery management and biological resource conservation.

Materials and methods

Fish sampling sites

Lihu Lake is an extensive semi-enclosed bay toward to the northern Taihu Lake, located at 120°13′-120°27′E and 31°28′-31°35′N. It covers an area of 8.6 km^2 and has a perennial water level of 3.07 m and an average water depth of 1.60 m. In this study, we examine the overlap and competition among four fish species from the perspective of feeding ecology. During the fishing period in October 2013, samples from 46 topmouth culter, 35 Mongolian culter, 129 humpback, and 49 redfin culter were collected in Lihu Lake at three sampling sites, labeled S1, S2, and S3, based on previous research by our team (Figure 1).

Collection and pre-treatment of fish

The samples of redfin culter, topmouth culter, Mongolian culter, and humpback were collected in October 2013 by multi-mesh gillnets (standard length: 125 m; width: 1.5 m; mesh size: 1.2, 2, 4, 6, 8, 10, and 14 cm, average distribution in the entire net). Then the fish were preserved in ice, the standard length and body weight of each sample were measured at approximately 0.01 mm and 0.1 g, respectively, and the white dorsal muscle tissues were dissected in the laboratory (Pinnegar and Polunin, 1999). Samples of different ranges of standard lengths of the four species were selected for statistical comparison. The following groups were compared: 40–59, 60–79, 80–99, 100–119, 120–139, 140–159, 160–179, 180–199, 200–219, 220–239, and >280 mm. After 48 h of drying at 60°C, the muscle samples were ground into powder and stored in centrifuge tubes before measurement.

The biometrics including the standard length-weight relationship of 16 topmouth culter, 13 Mongolian culter, 101 humpback, and 30 redfin culter specimens were statistically analyzed. A one-way analysis of variance (ANOVA) was performed on 16 topmouth culter, 10 Mongolian culter, 14 humpback, and 8 redfin culter specimens. To determine the isotopic niche metrics and overlaps, 13 topmouth culter, 11 Mongolian culter, 14 humpback, and 11 redfin culter specimens were examined.

Stable isotope analysis

Carbon and nitrogen stable isotope ratios were determined using Vario EL cube-Iso Prime 100 (Elementar, GER) by isotope-ratio mass spectrometry and elemental analysis. The results were expressed in conventional delta (δ) notation as parts per thousand (‰) defined as follows:

$$\delta X(‰) = [(\frac{R_{sample}}{R_{standard}} - 1)] \times 1,000$$

where X is ^{13}C or ^{15}N, R is ^{13}C/^{12}C or ^{15}N/^{14}N, R_{standard} is the isotopic ratio in certified reference material, and R_{sample} is the isotopic ratio measured (Peterson and Fry, 1987). The isotopic ratio of C was expressed in relation to its abundance in Vienna Pee Dee Belemnite (VPDB); the isotopic ratio of N was expressed in relation to its abundance in standard atmospheric nitrogen, provided as a certified reference. We inserted one standard sample for every 10 sample measurements, and we randomly selected one or two samples for reanalysis. The standard errors of repeated measurements of δ^{13}C and δ^{15}N values were less than 0.2‰ and 0.3‰, respectively.

Statistical analysis

The data were analyzed using IBM SPSS Statistics. The relationships between the values of δ^{13}C and δ^{15}N of muscle and standard length were analyzed by partial correlation (Wang X. et al., 2015). Kruskal-Wallis test analysis was used to analyze whether there were significant differences in standard length distribution among the four fish species, which gives us a way to evaluate ordinal data in more depth and draw strict statistical interpretations from the results, including comparison of means (Gao and Chen, 2011). To identify whether there were any statistically significant differences between the standard length and δ^{13}C and δ^{15}N values in the four species, a multivariate analysis of variance (MANOVA) was performed, with δ^{13}C and δ^{15}N values as variables and standard length as covariates. *Post-hoc* multiple comparison tests using the least significant difference (LSD; $\alpha = 0.05$) were also performed to identify the significance of standard length and δ^{13}C and δ^{15}N values between the four species.

To assess the overlap between the isotopic niches, complementary approaches were used. The direct overlap of the maximum likelihood fitted standard ellipses (SEAc) was calculated using the R package SIBER (Jackson et al., 2011). To measure the total extent of spacing and trophic redundancy within a δ^{13}C and δ^{15}N biplot for fish communities or species,

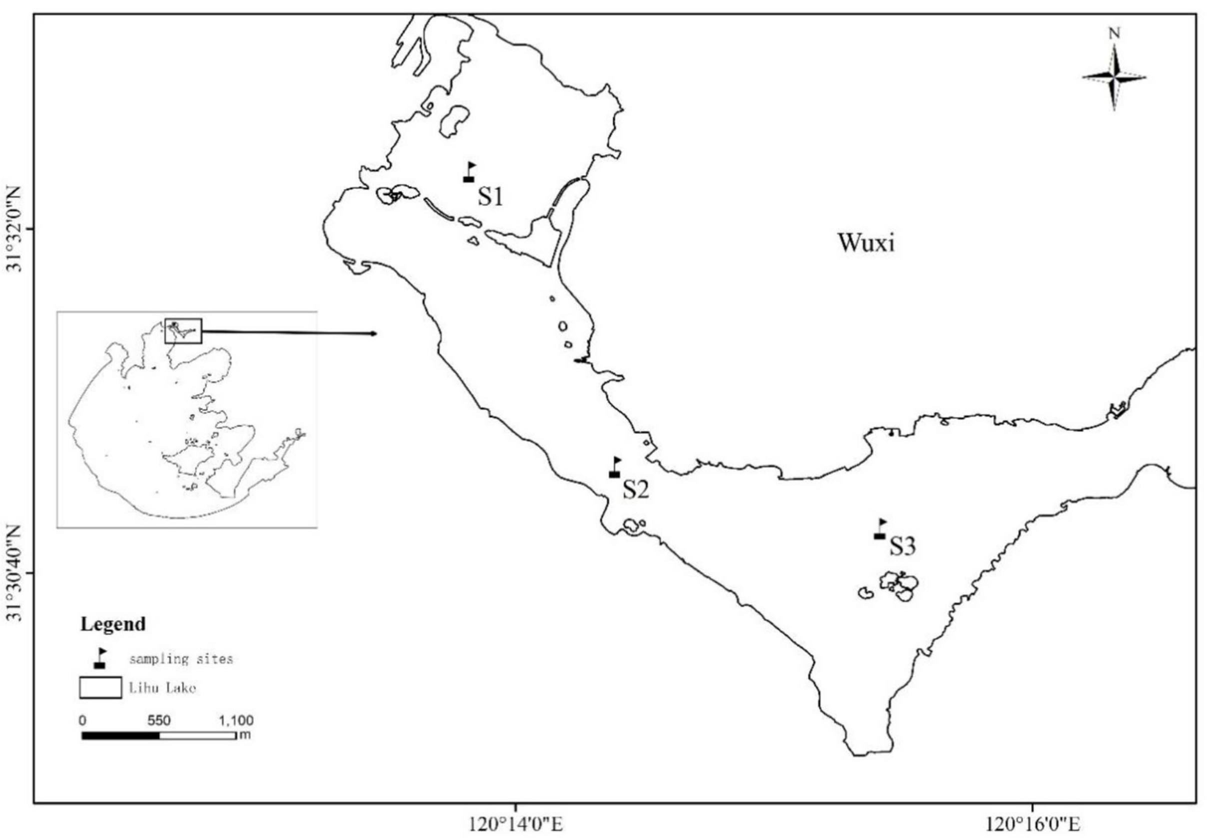

FIGURE 1
Sampling sites in Lihu Lake.

all data were calculated in the R package SIBER. These metrics were adapted from the community-wide metrics proposed by Layman (Layman et al., 2007, 2012; Jackson et al., 2012): carbon range (CR), nitrogen range (NR), mean distance to centroid (CD), total area (TA), standard ellipse corrected area (SEAc), mean nearest neighbor distance (MNND), and its standard deviation (SDNND). CR is the difference between individuals with the most enriched and the most depleted $\delta^{13}C$ and is a measure of basal resource diversity. NR is the difference between species with the most enriched and most depleted $\delta^{15}N$ values and is a measure of trophic length within a population. CD and TA are indices of species niche width. SEAc indicates the species' core isotopic niches, calculated from the variance and covariance of bivariate isotopic data by evaluating the trophic niche area of a species. MNND is the mean of the Euclidean distances to each specie's nearest neighbor in a biplot space and thus is a measure of the overall packing density of the species. SDNND is a measure of the evenness of species packing in a biplot space. Low SDNND values indicated an even distribution of trophic niches.

To measure the trophic niche size and to test whether trophic niche overlap was not equivalently weighted among species, we used the nicheROVER R package developed by Swanson et al. (2015). In this method, a Monte Carlo resampling routine (n = 10,000 draws) is applied to randomly draw from the sampled population and calculate a mean and 95% (or user-defined) credible interval of niche overlap probabilities. Swanson et al. (2015) defined the niche overlap of species A onto species B as the fraction of the intersection area between niche A and niche B over the total niche area of B and *vice versa*. We used a 95% probability for niche size and overlap.

Results

Relationship between standard length and weight

In this study, all fish samples were measured as shown in the table. Standard length and body–weight growth relationships were fitted to power functions (Figure 2) represented by $W = aL^b$: humpback $W = 3 \times 10^{-5} L^{2.8555}$ ($R^2 = 0.9859$, $P < 0.01$), redfin culter $W = 1 \times 10^{-5} L^{3.047}$ ($R^2 = 0.9666$, $P < 0.01$), Mongolian culter $W = 3 \times 10^{-5} L^{2.8627}$ ($R^2 = 0.9911$, $P <$

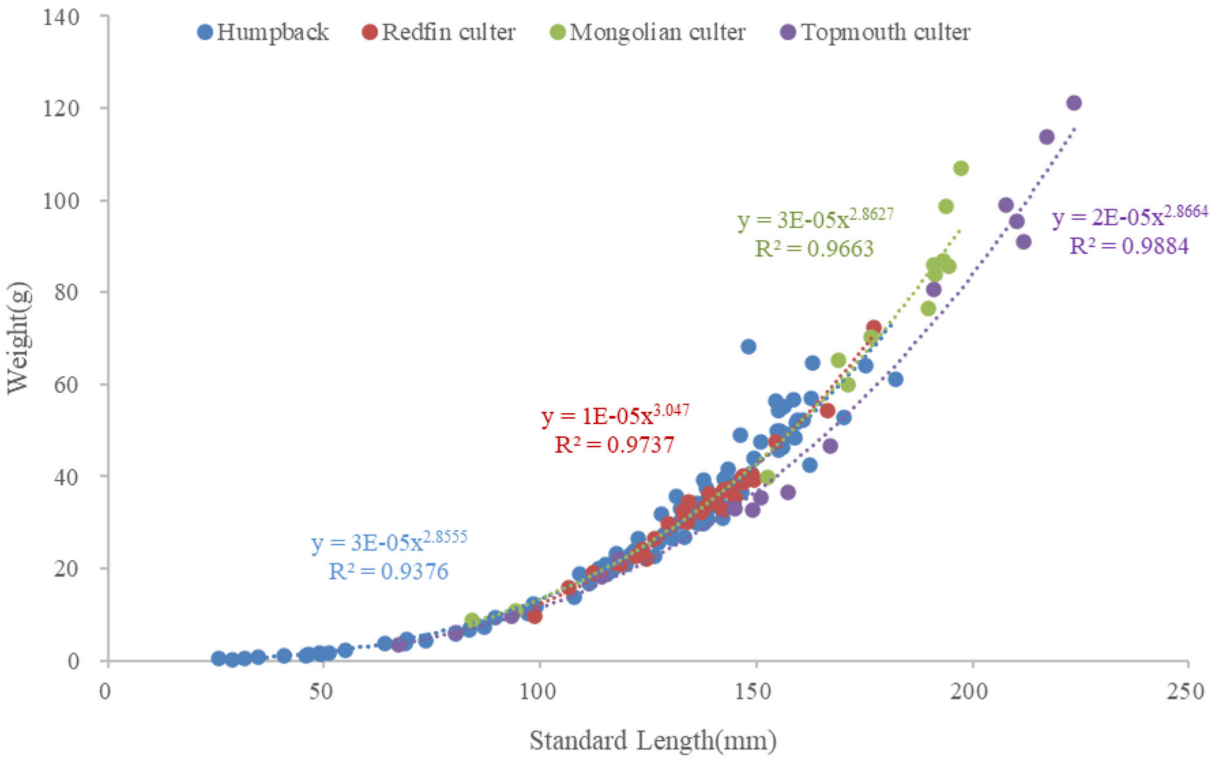

FIGURE 2
Relationships between the weights and standard lengths of humpback, redfin culter, Mongolian culter, and topmouth culter.

0.01), and topmouth culter $W = 2 \times 10^{-5} L^{2.8664}$ ($R^2 = 0.9941$, $P < 0.01$). The mean standard length of humpback (122.07 ± 36.62 mm) was similar to that of the redfin culter (136.81 ± 16.32 mm); however, both were much lower than that of the Mongolian culter (185.87 ± 72.46 mm) and topmouth culter (165.20 ± 61.22 mm).

The results of the Kruskal-Wallis test showed that there was a significant difference in the overall distribution of standard length among the four species ($\chi^2 = 22.99$, $P < 0.05$). There were significant differences between humpback to Mongolian culter and topmouth culter ($\chi^2 = 17.15$, $P < 0.05$; $\chi^2 = 6.61$, $P < 0.05$), and there was also a significant difference between redfin culter to Mongolian culter ($\chi^2 = 11.64$, $P < 0.05$), but no significant differences between humpback and topmouth culter ($\chi^2 = 2.39$, $P > 0.05$; $\chi^2 = 3.24$, $P > 0.05$), and there was also no significant difference between Mongolian culter and topmouth culter ($\chi^2 = 0.38$, $P > 0.05$) (Table 1).

δ^{13}C and δ^{15}N values and interspecies relationships

The results of the partial correlation analysis demonstrated a significant negative correlation in the δ^{13}C value of humpback with standard length ($P < 0.01$) and a significant positive correlation of the δ^{15}N value of humpback with standard length ($P < 0.01$). The δ^{13}C and δ^{15}N values of redfin culter, Mongolian culter, and topmouth culter all showed significant positive correlations with standard length ($P < 0.05$).

The mean δ^{13}C values for humpback, redfin culter, Mongolian culter, and topmouth culter were (−25.76 ± 0.65‰), (−25.93 ± 0.61‰), (−25.94 ± 0.69‰), and (−26.59 ± 0.90‰), respectively. The value of humpback was the highest, and that of the topmouth culter was the lowest within the same standard length group. The results of multivariate analysis indicated no significant variations in the distribution of δ^{13}C values ($P > 0.05$).

The mean δ^{15}N values for humpback, redfin culter, Mongolian culter, and topmouth culter were (12.08 ± 0.74‰), (12.80 ± 0.68‰), (13.60 ± 1.56‰), and (12.63 ± 1.21‰), respectively. The value of Mongolian culter was the highest and that of humpback was the lowest. The results of the multivariate analysis indicated significant variations in the distribution of δ^{15}N values ($P < 0.05$). The results showed that the δ^{15}N value of the redfin culter did not significantly differ from those of the others ($P > 0.05$) while that of the Mongolian culter was significantly different from that of the topmouth culter ($P < 0.05$) and humpback ($P < 0.01$).

TABLE 1 Standard length–weight relationships for four fish species in Lihu Lake.

Species	*n*	Parameter of standard length (mm)			*a*	*b*	R^2
		Mean ± SD	Min	Max			
Humpback	101	122.07 ± 36.62	25.56	182.12	3×10^{-5}	2.8555	0.9859
Redfin culter	30	136.81 ± 16.32	98.70	177.21	1×10^{-5}	3.0470	0.9666
Mongolian culter	13	169.12 ± 37.83	84.12	197.36	3×10^{-5}	2.8627	0.9911
Topmouth culter	16	156.47 ± 51.15	67.20	223.55	2×10^{-5}	2.8664	0.9941

a, intercept and b, power exponent in the expression $W = aL^b$, where W is weight in g and L is standard length in mm.

Isotopic niche metrics and overlaps

The results showed that the isotopic niches of the four species were confirmed by the considerable overlap of SEAc areas (2.95).

In addition, the results of the SIBER package analysis indicated that the topmouth culter had the highest CR, TA, SEAc, and CD values, followed by the Mongolian culter, but the NR, MNND, and SDNND values were higher in the Mongolian culter. Furthermore, the NR of the Mongolian culter (6.08‰) and topmouth culter (5.03‰) was greater than the magnitude of the average isotope fraction (3.4‰) per trophic level. In addition, the humpback displayed the lowest CR, TA, MNND, and SDNND values, and the redfin culter also possessed the lowest NR, SEAc, and CD values. CR, NR, TA, CD, MNND, SDNND, and SEAc showed that the topmouth culter and Mongolian culter were much higher than the humpback and redfin culter. Therefore, the topmouth culter, Mongolian culter, humpback, and redfin culter shared some parts of similar niche space sizes (Figure 3, Table 2), and the niche space of the former two species was wider than the others.

The results also showed various degrees of trophic overlap among the four species, with an overall average of 64.02% (nicheROVER). High trophic overlaps (>80%) were shown for topmouth culter onto redfin culter (95.52%) and topmouth culter onto humpback (90.38%) and Mongolian culter onto redfin culter (85.22%). The lowest trophic overlap was found between humpback and Mongolian culter (30.95%), while higher overlaps were found between redfin culter and Mongolian culter (33.70%), between redfin culter and topmouth culter (35.72%), and between humpback and topmouth culter (42.10%). The average trophic overlap between topmouth culter and other species was as high as 85.93%, followed by Mongolian culter (74.75%) onto the other three fish, which was greater than the trophic overlap between humpback (50.36%) and redfin culter (45.04%) (Figure 4).

Discussion

Body dimension relationships among the four species

In humpback and topmouth culter, the power exponent was $b < 3$, indicating that the two populations experienced positive allometric growth (i.e., the growth rate of standard length was higher than weight). The redfin culter and Mongolian culter showed negative allometric growth (Wang et al., 2020).

The growth rates of the four fish species have been shown to vary among water bodies, depending on geographical location, physical and chemical factors, food resources, and fishing intensity (Feng et al., 2007). Other factors that affect the standard length–weight relationship include habitat, seasons, gonad maturation state, and sex (Lv et al., 2018). In this study, we found significant differences in these relationships among the four fish species living in the same habitat.

Relationships between species and δ^{13}C and δ^{15}N values

Many species have been found to display similar patterns of increasing δ^{13}C and δ^{15}N values with size, presumably due to the ability of larger fish to capture larger and more diverse prey at a higher trophic level (Graham et al., 2007; Tsai et al., 2014). As shown in Figure 5, the values of δ^{13}C and δ^{15}N in the four species increased with standard length ($P < 0.05$), implying that their food source changed as they grew (Li et al., 2011; Wang F. et al., 2015). These observations are consistent with the results of a previous dietary analysis, which revealed that their food sources changed as they developed (Chen et al., 1994).

In this study, significantly larger bodies and higher values of the δ^{13}C were found in the topmouth culter than in the other three species. These results indicated that the carbon sources of the topmouth culter were more diverse and similar to previous studies; although they were in the same habitat, the

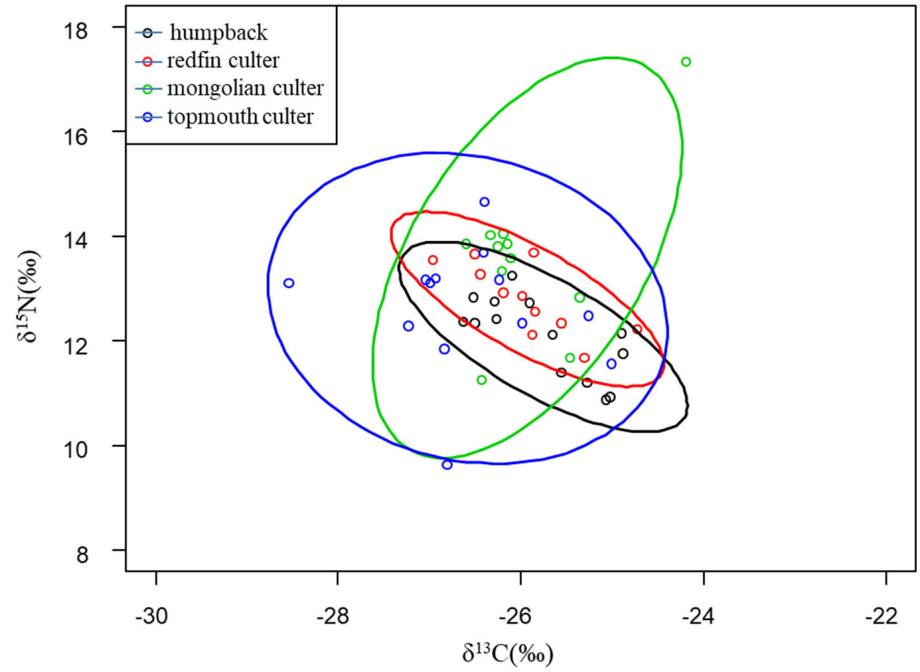

FIGURE 3
Isotopic biplot of four fish species in Lihu Lake.

humpback, redfin culter, and Mongolian culter had a smaller size, perhaps because of various individual sizes or different feeding habits, such as the topmouth culter could make better use of zooplankton, small prey fish, and shrimp, while the redfin culter can make better use of phytoplankton (Li, 2014).

Within the same standard length range, the $\delta^{15}N$ values of the Mongolian culter and topmouth culter were generally higher than those of the humpback and redfin culter (Figure 5), thus reflecting the former species' higher trophic level. The Mongolian culter and topmouth culter are more carnivorous than the other two carnivorous Cultrinae species, and animal prey accounts for a large proportion of their food resources (Wang et al., 2009).

Trophic niche of the four carnivorous Cultrinae fish species and overlaps

Individuals of the four fish species could be defined as more or less "generalist" by comparing either the inter-individual variability of their trophic niches or their intra-individual variability or both. In this study, we evaluated the inter-individual components of the realized trophic niches of different species by analyzing their isotopic niches and metrics. Several generalist species have low within-individual and high inter-individual diet variations, which indicates that the species includes specialists that utilize different resources. The overlap and breadth of generalist species' niches, such as those of topmouth culter and Mongolian culter, are much greater than those of the others. Moreover, the generalist species typically have a wider spectrum of trophic resources and feed at multiple trophic levels. They also have lower trophic redundancy, higher trophic evenness, higher trophic diversity, and larger trophic niches (Shea and Chesson, 2002; Ribeiro et al., 2007; Hayden et al., 2014; Cicala et al., 2020), indicating that these species take full advantage of the food supply in the habitat and could be more competitive in the presence of limited resources. In addition, previous studies have shown that adults of the Mongolian culter are carnivorous, mainly feeding on sharpbelly, while topmouth culters mainly feed on various fishes, such as fishes in Rhodeus, Culter, and so on (Xing and Yang, 1992; Ni and Wu, 2006; Zhang, 2008). Our study also showed that although both Mongolian culter and topmouth culter could be bigger and regarded as "generalist," the CR of Mongolian culter was lower than that of topmouth culter, indicating that the food sources of the former were narrower, and its viability would be more sensitive to environmental turbulence, which might be necessary to pay attention to by managers. Moreover, the researchers proposed that artificial release or formulating reasonable fishing ban periods, or prohibiting harmful fishing gear fishing laws were effective measures to proliferate large and medium-sized commercial fish, adjust community composition, and stabilize interspecies relationships in Taihu Lake (Yin and Miu, 1991). So as for the redfin culter and topmouth culter, the

TABLE 2 Layman's metrics and corrected standard ellipse area of topmouth culter, Mongolian culter, humpback, and redfin culter in Lihu Lake.

Species	Total length ± SD (mm)	Weight ± SD (g)	CR	NR	CD	TA	SEAc	MNND	SDNND
Topmouth culter	214.03 ± 75.92	74.53 ± 77.68	3.53	5.03	1.25	9.43	3.68	0.74	0.60
Mongolian culter	232.07 ± 94.84	146.95 ± 265.19	2.42	6.08	1.14	5.94	3.17	0.76	1.10
Humpback	150.64 ± 43.15	25.42 ± 17.76	1.76	2.36	0.89	2.08	1.13	0.30	0.17
Redfin culter	173.20 ± 22.29	36.55 ± 15.13	2.24	2.00	0.76	2.08	0.97	0.44	0.19

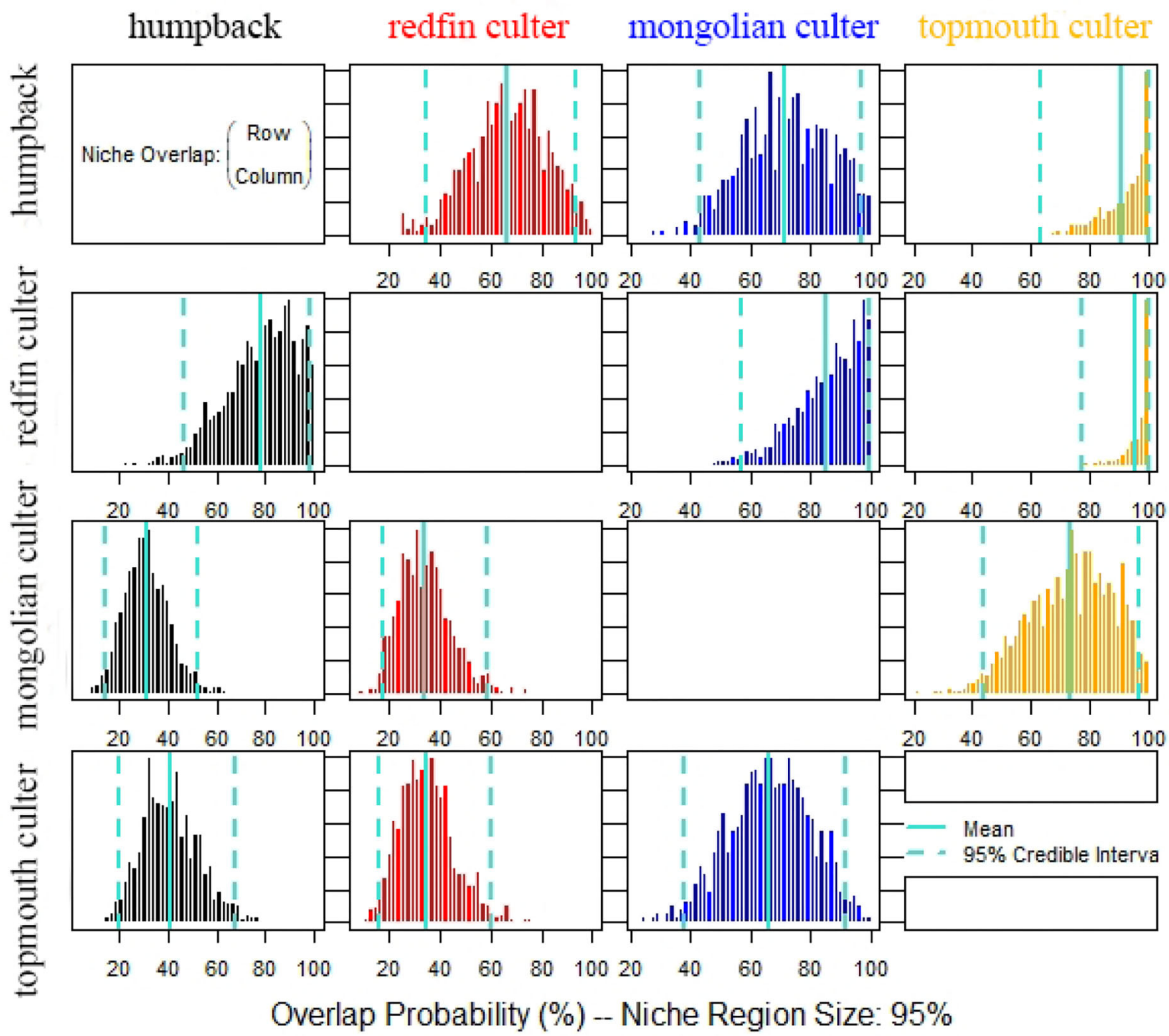

FIGURE 4
Posterior distribution of the probabilistic niche overlap metric (%) and niche region size (α) = 0.95, indicating the probability of one species (row) overlapping onto the niche of another species (column) among topmouth culter, Mongolian culter, humpback, and redfin culter in Lihu Lake.

competitive ability was weaker than the other two carnivorous Cultrinae fish species. We can take appropriate proliferation measures, such as setting a fishing ban period, fishing ban, or taking necessary artificial proliferation measures to maintain the stability of the Lihu Lake ecosystem.

Small topmouth culter and Mongolian culter are omnivorous, feeding on small zooplankton and small fish (e.g., Rhodeinae and *Pseudorasbora parva*) (Yang et al., 2002; Liu et al., 2007; Liu, 2008). However, large individuals are carnivorous, preferring high-energy lake anchovy and other

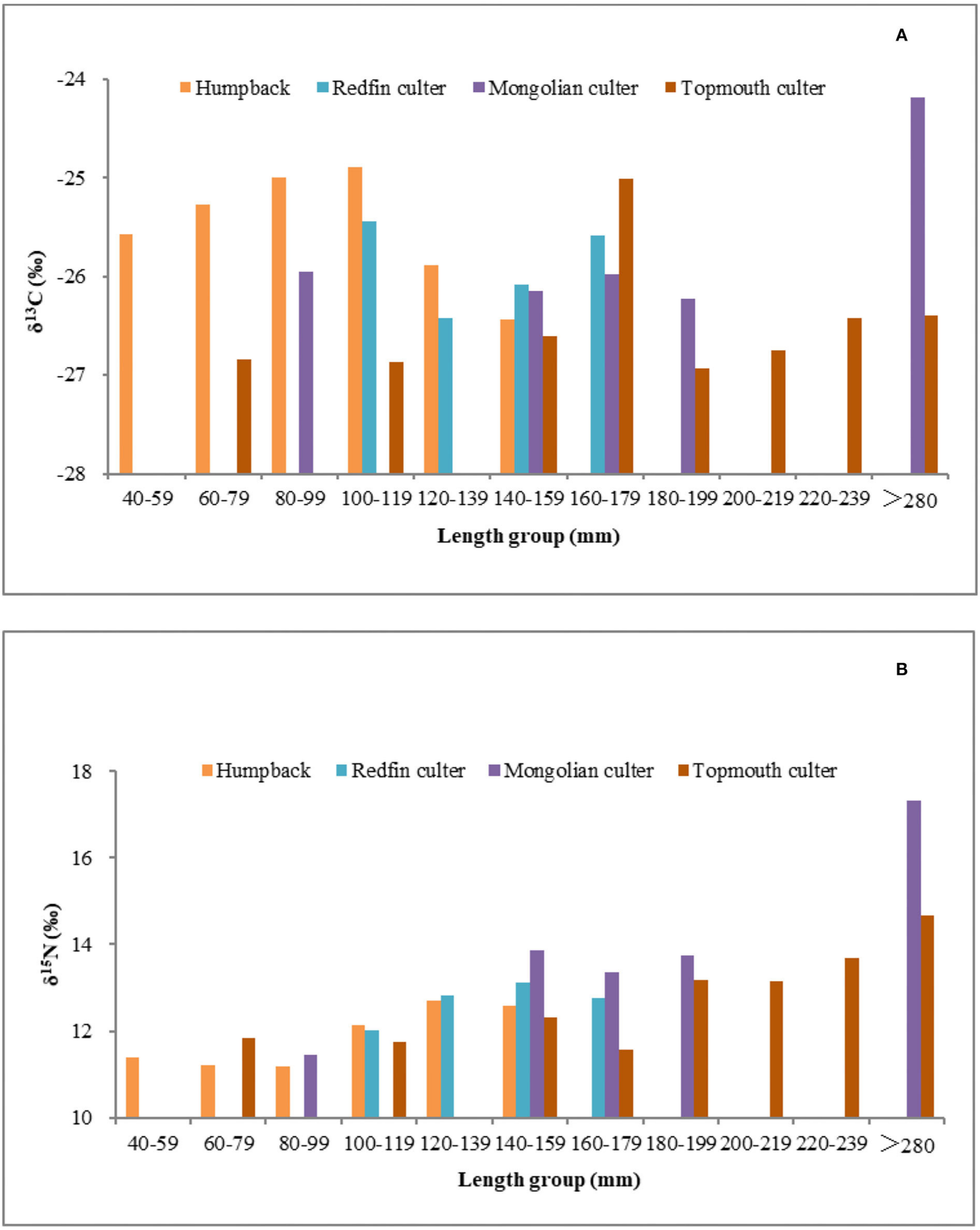

FIGURE 5
Relationships between $\delta^{13}C$ **(A)** and $\delta^{15}N$ **(B)** values and standard lengths of humpback, redfin culter, Mongolian culter, and topmouth culter.

small fish, such as redfin culter. Adult culters prefer open upper–middle water layers, whereas juveniles prefer slow-moving shoal water (Tan et al., 2009). Thus, their different food sources may reflect their different habitats. Food intake increases and the capacity for predation strengthens according to their body growth (Yu et al., 2012). Because of the dual effects of inter- and intraspecific competition, the culters tend to feed on high-energy food organisms that meet their growth requirements. While optimal foraging theories predict trophic niche broadening as a consequence of reduced food availability, where consumers relying on insufficient preferred food items are forced to add less profitable resources to their diet, hence widening their trophic niche. Other biological factors also influence the niche of a species, including food density and diversity, population size, and predator-prey relationships. In addition, seasonal changes in aquatic environmental factors also influence their distribution and food resources, thus affecting fish biomass in the trophic niche (Bearhop et al., 2004; Sha et al., 2015).

Conclusion

This study presents the trophic niche characteristics of four carnivorous Cultrinae fish species in Lihu Lake. The results support the preview hypothesis that the topmouth culter has a wide spectrum of trophic resources and feeds at multiple trophic levels compared with the Mongolian culter, humpback, and redfin culter. Based on comparisons of four similar species, we know that the competitive ability of redfin culter and humpback is weaker than the topmouth culter and Mongolian culter, and the Mongolian culter is more sensitive to environmental turbulence than the topmouth culter. All these findings show the influence of trophic interactions of culter and Cultrichthys in lake ecosystems and provide information that assists in resource protection with respect to nutritional ecology. Future research should be conducted to determine the mechanism of the differences in the nutrition acquisition of the four species examined in this study.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Regulations for the Administration of Affairs Concerning Experimental Animals and the State Council of the People's Republic of China.

Author contributions

YW and LR led the manuscript writing and analyzed the data. D-pX and D-aF contributed to the manuscript writing. YW designed the data analysis. LR determined and validated the species' determination. D-pX and YW designed the experiment and were the scientific supervisors the experiment. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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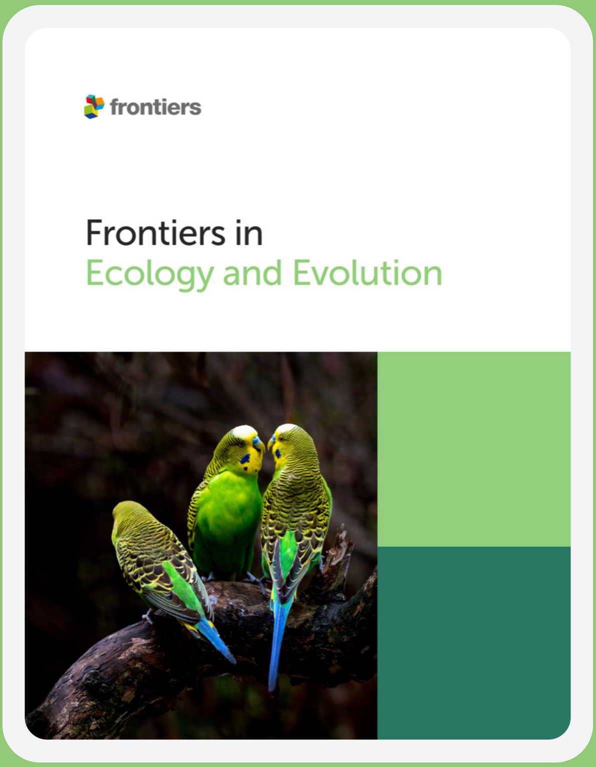

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