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## EDITED BY

Yujun Yi,  
Beijing Normal University, China

## REVIEWED BY

Yongjiu Cai,  
Chinese Academy of Sciences (CAS), China  
Atul Kabra,  
Chandigarh University, India

## \*CORRESPONDENCE

Long Yan  
✉ yanlong@iwhr.com  
Peng Hu  
✉ hp5426@126.com

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# Characteristics of the macroinvertebrate community structure and their habitat suitability conditions in the Chishui River

Xinyu Li<sup>1</sup>, Long Yan<sup>1\*</sup>, Xu Zhi<sup>2</sup>, Peng Hu<sup>1\*</sup>, Chongju Shang<sup>3</sup> and Baolong Zhao<sup>1</sup>

<sup>1</sup>State Key Laboratory of Simulation and Regulation of Water Cycle in River Basin, China Institute of Water Resource and Hydropower Research, Beijing, China, <sup>2</sup>China Three Gorges Corporation, Wuhan, Hubei, China, <sup>3</sup>Institute of Disaster Prevention and Water Conservation, Guizhou Institute of Water Conservancy Science, Guizhou, Guiyang, China

**Introduction:** As an important tributary of the upper Yangtze River without dams, the study of the macroinvertebrate community structure and habitat suitability conditions in the Chishui River holds significant implications for water ecological conservation and restoration.

**Methods:** In order to explore the species composition, community characteristics and their ecological needs of macroinvertebrates in the Chishui River, 26 sampling sites were deployed in the Chishui River in this study in March (dry season), July (wet season), and November (normal season) of 2023 and performed community structure analysis.

**Results:** A total of 153 macroinvertebrate taxa were identified, with 62 taxa recorded in the dry season, 46 in the wet season and 115 in the normal season. The assemblage was predominantly composed of aquatic insects from the EPT group (*Ephemeroptera*, *Plecoptera* and *Trichoptera*). The results of ANOSIM analysis indicate that the species composition of macroinvertebrates varies significantly across different water periods. The Shannon-Wiener diversity index and Margalef index were significantly higher during the normal season compared to the dry and wet seasons. In contrast, the Pielou index and Simpson index remained relatively stable, suggesting a higher level of evenness in community structure across the different water periods. Based on the habitat suitability curves, *Corbicula fluminea* and *Heptagenia* prefer environments with high flow velocities and substrate particle sizes (D50) smaller than 300 mm. *Hydropsyche* sp.1 and *Baetis* thrive in environments with medium to low water depths, high flow velocities, and D50 ranging from 100 to 300 mm. *Caridina* favor deeper waters, low flow velocities, and larger substrate particles. The GAM fitting results revealed that the number of macroinvertebrate taxa increased with rising pondus hydrogenii (pH) and electrical conductivity (EC) levels. Species richness initially declined and then rose within certain thresholds of total nitrogen (TN) and total phosphorus (TP) concentrations. The optimal conditions for macroinvertebrate communities in the Chishui River were determined to be a DO concentration of 9.8 mg/L and an NH<sub>3</sub>-N concentration of 0.12 mg/L.

**Discussion:** Overall, the macroinvertebrate community structure in the Chishui River is significantly influenced by factors such as water quality, flow velocity, and substrate particle size, and it demonstrates strong adaptability to seasonal variations.

#### KEYWORDS

undamed river, Chishui River, macroinvertebrates, community structure, habitat suitability

## 1 Introduction

Macroinvertebrates are primarily aquatic invertebrates that inhabit the riverbed for all or most of their life cycle and are visible to the naked eye (retained by a 500  $\mu\text{m}$  mesh sieve) (Chen et al., 2021). As one of the most widely distributed biological groups in river ecosystems, macroinvertebrates play a crucial role in the food chain by facilitating organic matter decomposition, promoting self-purification of water, and serving as a vital link in the material cycle and energy flow within river ecosystems (Barbour et al., 1999; Qu et al., 2007; Zhang K. et al., 2023). Characterized by their species richness, broad distribution, limited migratory capacity, large size, and ease of collection and identification, macroinvertebrates are effective indicators of river ecosystem health. They reflect the impacts of multiple stressors, including hydrological, physical, chemical, and biological factors (Zhang et al., 2017; Wang et al., 2018). With the intensification of climate change and the deepening of human activities, the hydrological processes of rivers worldwide have undergone significant changes. This has not only disrupted the stability of river ecosystems but has also led to the degradation of ecosystem services and a decline in biodiversity (Schmitt et al., 2018; Chen et al., 2020). In this context, studying the spatiotemporal distribution, biodiversity, and community structure of macroinvertebrates is of significant scientific value and practical importance for comprehensively understanding changes in riverine ecosystems (Fu et al., 2024). By establishing the relationship between macroinvertebrates and habitat factors, such research can provide a theoretical basis for the formulation and implementation of ecological restoration measures in river basins, thereby effectively promoting the recovery and maintenance of river ecosystem health (Hu et al., 2021).

In the Yangtze River Basin of China, although water quality has improved in recent years, issues such as the imbalance of aquatic ecosystems and the decline in aquatic biodiversity remain prominent challenges, hindering the high-quality development of the Yangtze River Economic Belt (Yu et al., 2022). The Chishui River, a vital first-order tributary of the upper Yangtze River, remains free from dam construction and sustains its natural flow regime. The Chishui River exhibits typical characteristics of a mountainous river, with numerous tributaries and a complex river network. The intricate hydrological conditions and high

habitat heterogeneity contribute to its uniqueness in terms of species diversity, evolutionary history among species, and ecosystem structure. This complex ecological environment not only provides rich habitats for aquatic organisms in the basin but also makes the Chishui River a region of significant scientific research value (Zhang D. et al., 2023). Therefore, conducting research on the biodiversity of the Chishui River basin is of significant importance. Understanding the ecological requirements and survival conditions of macroinvertebrates in this river can provide critical insights for basin authorities to develop and implement targeted ecological restoration strategies. Such efforts are essential for rehabilitating degraded river ecosystems, safeguarding aquatic biodiversity, and supporting the sustainable development and utilization of resources throughout the Yangtze River basin.

Previous studies on macroinvertebrates have often focused on the influence of water quality factors on their community structure (Jiang et al., 2024). For example, Liao et al. (2024) and Li et al. (2023) investigated the benthic macroinvertebrate community structure and its response to physico-chemical water properties in typical rivers of Xi'an and urban rivers in Dongguan, respectively. In recent years, with the deepening research on the community characteristics of large benthic invertebrates, an increasing number of scholars have recognized that, in addition to water quality factors, physical environmental factors of rivers, such as water depth ( $h$ ), flow velocity ( $v$ ), and the median particle size of the substrate ( $D_{50}$ ) also play a decisive role in shaping the community structure of large macroinvertebrates. Different macroinvertebrate taxa exhibit varying responses to these physical factors (Zhou et al., 2022). Therefore, understanding the impact of these environmental factors on benthic communities is of significant ecological importance, as different taxa respond differently to these environmental variables.

In response to the current state of research, this study aims to explore the primary factors influencing the habitat suitability of macroinvertebrates in the Chishui River by modeling the relationships between the macroinvertebrate community and environmental factors (including both water quality and physical environmental factors). Hypothesis 1: Water quality factors (such as dissolved oxygen and nutrient concentrations) have a significant seasonal impact on the macroinvertebrate community. Hypothesis 2: Physical environmental factors (such as flow velocity and

substrate particle size) affect the suitable habitats of different taxa in varying degrees, and taxa exhibit different response patterns. Through these analyses, this study seeks to provide theoretical support for ecological protection and water resource management in the Chishui River basin, as well as offer valuable insights and data to inform the ecological restoration and biodiversity conservation of similar rivers.

## 2 Materials and methods

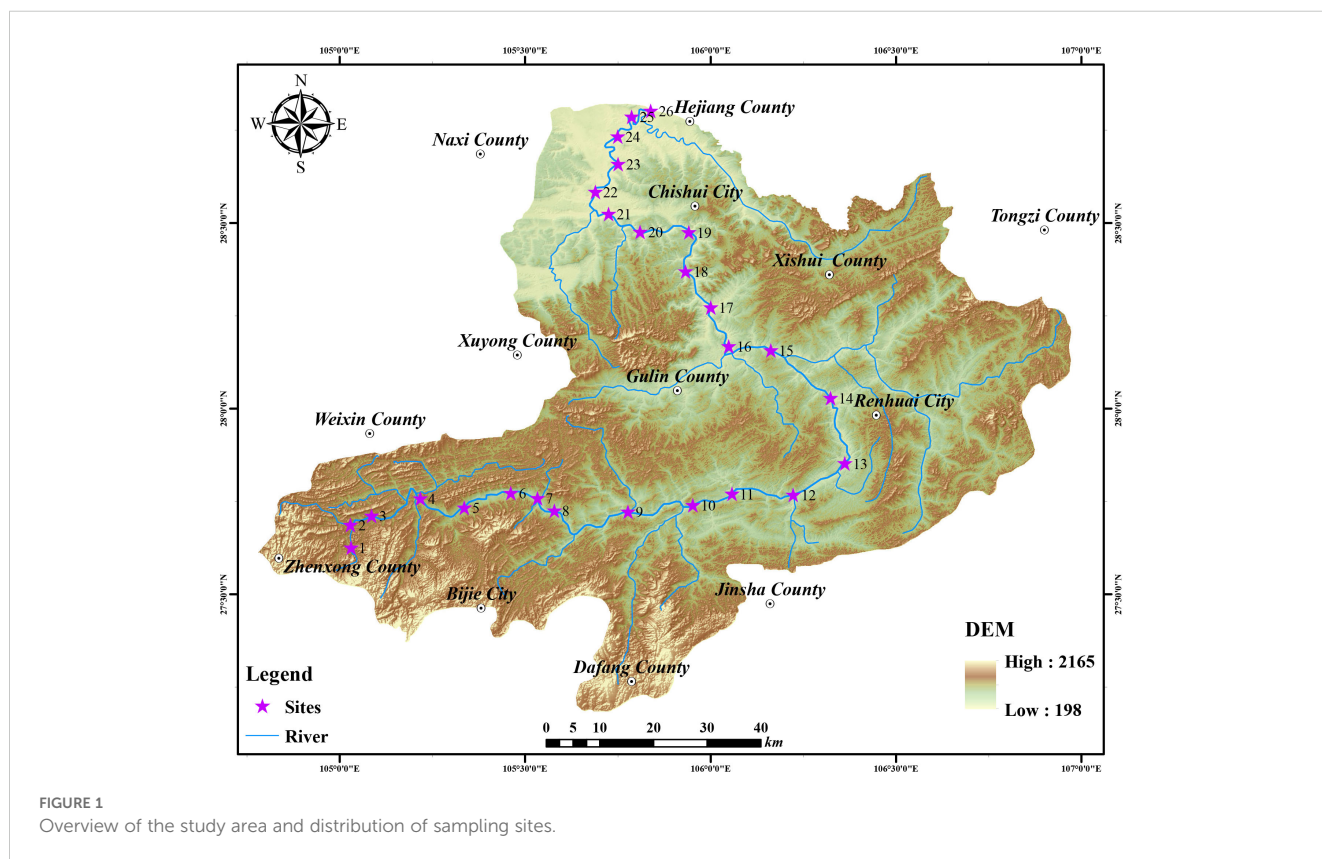
### 2.1 Overview of the study area and sampling sites

The Chishui River (E104°45′–106°51′, N27°20′–28°50′), historically known as the Chihui River, Anle Water, and Dashe Water, is now often referred to as the “River of Fine Liquor” and the “River of Heroes.” It is a significant first-order tributary on the right bank of the upper Yangtze River, the longest river in China. The Chishui River originates in Yinchuang Village, Chishuiyuan Town, Zhenxiang County, Yunnan Province. It flows eastward to Maotai Town in Renhuai City, where it turns northwest before continuing to Hejiang County, ultimately joining the Yangtze River. The main stream stretches 436.5 km, with a total elevation drop of 1,475 meters and an average gradient of 3.38‰. The Chishui River basin lies at the intersection of the Yunnan-Guizhou Plateau and the Sichuan Basin, featuring higher terrain in the south and lower

terrain in the north. It is flanked by the Wumeng Mountains to the southwest, the Dalou Mountains to the east, and the Sichuan Basin to the north, forming a distinct and varied topographical region. The basin is located in the monsoon region of the subtropical zone, with an average annual temperature ranging from 12.7 to 18.1°C and an average annual precipitation of 1020.6 mm. Forests and cultivated land are the primary land use types in the Chishui River basin. The region is rich in forest resources, characterized by typical subtropical evergreen broadleaf forests, and is an important area for the production of species such as Nan bamboo, pine, and fir. Cultivated land is scattered, with more concentrated areas primarily in the headwaters of the Chishui River, the river valley, and the gentle slopes along its tributaries.

### 2.2 Sample collection and identification

26 sampling sites were deployed for this study, as shown in Figure 1. The selection of these sites was informed by prior research on aquatic organisms in the Chishui River, supplemented by site visits to assess local conditions. Sampling sites were strategically chosen to reflect varying pollution levels, land use patterns along the riverbanks, and habitat complexity, ensuring comprehensive coverage of environmental conditions. Data collection was conducted in March 2023 (dry season), July 2023 (wet season), and November 2023 (normal season). However, during the wet season, a significant rise in water level at sampling site 14 within a



single day prevented the collection of benthic samples. Additionally, no dominant taxa characteristic of the wet season were recorded at sampling site 19.

In this study, a Surber net with a sampling area of 0.25 m<sup>2</sup> (0.5m×0.5m) and a mesh size of 40 meshes (The pore size is 0.425 millimeters) was used to quantitatively collect macroinvertebrates. At each sampling site, samples were collected four times across different habitat types. The collected specimens were sorted in white porcelain trays and preserved in 100 ml sample bottles containing 37% formaldehyde solution. The samples were then transported to the laboratory for identification to the lowest feasible taxonomic level, following deployed taxonomic references for macroinvertebrates. Specimens were weighed using an electronic balance with a precision of 0.0001 g. For mollusks, the weight was recorded with shells intact, after blotting excess water from their mantle cavities using absorbent paper, in accordance with the Technical Requirements for Freshwater Macroinvertebrate Monitoring (Trial) (China National Environmental Monitoring Centre, 2021) issued by the China National Environmental Monitoring Center. The results were subsequently converted into density (ind./m<sup>2</sup>) and biomass (g/m<sup>2</sup>).

At the sampling sites, water temperature (T), pH, dissolved oxygen (DO) and electrical conductivity (EC) were measured *in situ* using a HACHHQ30d multi-parameter water quality analyzer. Additionally, 500 ml water samples were collected with a water sampler, stored in plastic bottles, and refrigerated for laboratory analysis of ammonia nitrogen (NH<sub>3</sub>-N), total nitrogen (TN), and total phosphorus (TP).

All sample collection and measurement procedures followed the guidelines outlined in the Water and Wastewater Monitoring and Analysis Methods (State Environmental Protection Administration, 2002).

## 2.3 Data processing

### 2.3.1 Biodiversity

The biodiversity of macroinvertebrates was assessed using the Shannon-Wiener Diversity Index ( $H'$ ), the Pielou Index ( $J'$ ) and the Margalef Richness Index ( $Dm$ ). The specific formulas are as follows:

Shannon-Wiener Diversity Index:

$$H' = -\sum_{i=1}^S p_i \ln p_i \quad (1)$$

Where  $S$  is the total number of taxa,  $p_i = n_i/N$ ,  $p_i$  is the proportion of the  $i$ -th taxa in the total abundance,  $n_i$  is the abundance of the  $i$ -th taxa, and  $N$  is the total abundance.

Pielou Evenness Index:

$$J' = \frac{H'}{\log_2 S} \quad (2)$$

Where  $H'$  is the Shannon-Wiener Diversity Index, and  $S$  is the total number of taxa.

Margalef Richness Index:

$$Dm = \frac{(S-1)}{\ln N} \quad (3)$$

Where  $S$  is the total number of taxa, and  $N$  is the total abundance.

### 2.3.2 Species turnover rate

The species turnover rate (Du et al., 2011) ( $R$ ) is calculated using the following formula:

$$R = \frac{a+b-2c}{a+b-c} \times 100\% \quad (4)$$

Where  $a$  and  $b$  represent the number of taxa in two sampling events, and  $c$  is the number of taxa common to both samples.

### 2.3.3 ABC curve

The stability of the macroinvertebrate community in the Chishui River was assessed using the Abundance-Biomass Comparison (ABC) curve (Warwick, 1986). The  $W$ -value, representing the difference in area between the biomass and abundance curves and the coordinate axis, serves as the key statistic for the ABC analysis. When  $W > 0$ , the biomass cumulative percentage curve consistently lies above the abundance cumulative percentage curve, indicating a stable community dominated by large, slow-growing taxa. In contrast, when  $W < 0$  and the curves intersect, the community is considered disturbed, with an increase in small, fast-growing taxa. If  $W < 0$  and the abundance cumulative percentage curve is above the biomass curve, the community is severely disturbed, characterized by a dominance of small, fast-growing taxa (Yemane et al., 2005; Yang et al., 2022).

$$W = \sum_{i=1}^S \frac{(B_i - A_i)}{50(S-1)} \quad (5)$$

Where  $A_i$  is the cumulative percentage of the  $i$ -th taxon's abundance,  $B_i$  is the cumulative percentage of the  $i$ -th taxon's biomass, and  $S$  is the total number of taxa.

### 2.3.4 Index of relative importance

The Index of Relative Importance (IRI) (Cortes, 1997) was employed to identify the dominant taxa within the macroinvertebrate community of the Chishui River. This index incorporates the abundance, biomass, and distribution of each taxon. In this study, taxa with an IRI  $\geq 500$  were considered dominant, while those with an IRI  $\geq 1000$  were classified as absolutely dominant taxa (Dou et al., 2023).

$$IRI = (N + W) \times F \times 10^4 \quad (6)$$

Where  $N$  is the abundance percentage of a taxon,  $W$  is the biomass percentage of a taxon, and  $F$  is the frequency of occurrence of a taxon as a proportion of the total samples.

### 2.3.5 Community structure

One-way Similarity Analysis (ANOSIM) was employed to test for significant differences in macroinvertebrate communities across different hydrological periods of the Chishui River. The  $R$ -value, which ranges from -1 to 1, reflects the relative differences between within-group and between-group variations. An  $R$ -value close to 1 indicates substantial between-group differences, while a value close to 0 suggests that within-group and between-group differences are similar. A value approaching -1 signifies greater within-group variability and poor grouping effectiveness.  $P$ -values are used to assess whether observed differences are attributable to the grouping, with  $P < 0.05$  indicating statistically significant differences due to the grouping.

Cluster Analysis and SIMPER (Clarke et al., 2014) Analysis: After standardizing the abundance data of macroinvertebrates from the Chishui River, the Bray-Curtis dissimilarity matrix was employed to assess the similarity among sampling sites. Cluster analysis was conducted using the group average method. Similarity Percentage (SIMPER) analysis was then applied to identify the main contributing taxa within each group and their average contribution rates.

DCA and RDA Analysis: Ordination analysis methods were applied to examine the response relationships between dominant macroinvertebrate taxa and environmental factors. Detrended Correspondence Analysis (DCA) was conducted on the macroinvertebrate abundance data. If the first axis length in the DCA results exceeded 4.0, Canonical Correspondence Analysis (CCA) was used. If the length was between 3.0 and 4.0, either RDA or CCA could be applied. If the length was less than 3.0, Redundancy Analysis (RDA) was preferred over CCA.

### 2.3.6 Habitat suitability curve

The physical environmental factors were normalized to derive the corresponding Habitat Suitability Index (HSI). These factors were plotted on the x-axis, while the taxa' preference for each factor was represented on the y-axis, creating a continuous curve that illustrates the relationship between the target taxa' preferences and the physical environmental variables. This curve quantitatively describes the taxa' response to changes in various habitat conditions. A value between 0 and 1 is assigned to indicate the taxa' preference for a given physical environmental factor, where 0 denotes an unsuitable condition and 1 represents an optimal condition. The closer the value is to 1, the stronger the taxa' preference for that particular environmental factor.

### 2.3.7 Generalized additive models

Generalized Additive Models (GAM) were employed to model the relationship between macroinvertebrate community structure and environmental factors, with a 95% confidence interval. The  $R^2$  (coefficient of determination) and  $P$ -values are key statistics in the GAM model, used to assess the model's goodness of fit and the significance of predictor variables.  $R^2$  represents the proportion of the total variation in the response variable explained by the predictor variables, ranging from 0 to 1, with values closer to 1 indicating stronger explanatory power.  $P$ -values evaluate the

significance of predictor variables on the response variable, where  $P \geq 0.05$  suggests no significant effect,  $P < 0.05$  indicates a significant effect, and  $P < 0.01$  denotes a highly significant effect.

## 3 Results

### 3.1 Species composition

In 2023, a total of 153 taxa of macroinvertebrates were collected and identified from the main stream of the Chishui River, as shown in Figure 2. These taxa belong to 5 phyla, 8 classes, 21 orders, and 79 families. Aquatic insects were the most numerous group, with 120 taxa, accounting for 77.9% of the total taxa. Among these, EPT (*Ephemeroptera*, *Plecoptera*, and *Trichoptera*) aquatic insects were the dominant group, comprising 36.4% of the total taxa, indicating that the water quality of the Chishui River is relatively clean (Wang, 2003). The species composition of macroinvertebrates exhibited significant differences across different hydrological periods. The number of taxa in the dry, wet, and normal periods was 62, 46, and 115, respectively, with the highest species richness observed during the normal period. In this period, species diversity was most prominent across all sampling sites. The species turnover rate showed considerable fluctuations, with a turnover rate of 80% between the dry and wet periods, and a turnover rate of 70.2% between the wet and normal periods. A total of 17 taxa, accounting for 11% of the total taxa, were present in all three sampling periods, mainly consisting of aquatic insects. There were 102 taxa that appeared only in one sampling period, with 30 taxa in the dry period, 8 taxa in the wet period, and 64 taxa in the normal period. Overall, the total number of taxa of macroinvertebrates decreased gradually from upstream to downstream, with EPT taxa being dominant throughout. Spatial analysis revealed a decreasing trend in taxa numbers from upstream to downstream, with upstream areas predominantly inhabited by aquatic insects, indicating better water quality, whereas downstream areas had fewer taxa, lower diversity, and a higher abundance of mollusks. In general, the benthic macroinvertebrate community in the Chishui River exhibited significant temporal and spatial taxa variations, with notable taxa turnover, reflecting the seasonal and spatial changes in water quality and their influence on community structure.

In 2023, the abundance of macroinvertebrates in the main stream of the Chishui River ranged from 18 to 430 individuals per square meter (ind./m<sup>2</sup>) across different sampling sites, with an average abundance of 137.3 ind./m<sup>2</sup>. Among the various sampling sites, the EPT group of aquatic insects accounted for the highest proportion of macroinvertebrate abundance. The average abundance was 166.9 ind./m<sup>2</sup> during the dry season, 57.75 ind./m<sup>2</sup> during the wet season, and 184.31 ind./m<sup>2</sup> during the normal flow period. From a temporal perspective, the abundance composition of macroinvertebrates during both the dry and normal flow periods was dominated by EPT taxa, which accounted for 68.5% and 73.3%, respectively. During the wet season, EPT taxa still represented the highest abundance composition at 44.2%, followed by Malacostraca at 23.5% and Mollusca at 16.5%. The abundance of macroinvertebrates was significantly higher during the dry and normal flow periods

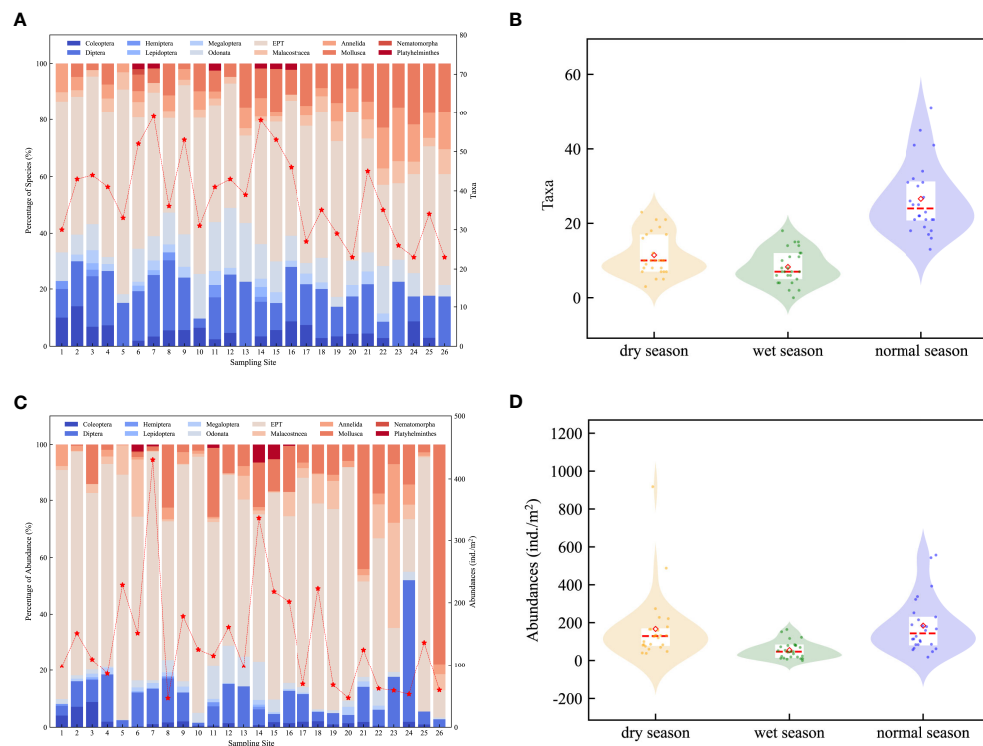


FIGURE 2  
Species composition of macrobenthos. (A, C) Taxa; (B, D) Abundances.

compared to the wet season. According to the ANOSIM results, with  $R = 0.48$  and  $P = 0.001$ , the seasonal differences in macroinvertebrate taxa abundance composition were significant. These results highlight the seasonal variability in macroinvertebrate abundance, with notable differences between dry, wet, and normal flow periods, emphasizing the influence of hydrological conditions on the composition of macroinvertebrate communities in the Chishui River. In the upstream areas, the abundance of macroinvertebrates was relatively high, with aquatic insect taxa being dominant and their abundance significantly greater than in the downstream areas. In contrast, the downstream regions exhibited lower species richness, with an increased proportion of mollusks, suggesting that water quality degradation may be affecting the community structure. Overall, the macroinvertebrate community structure in the Chishui River showed significant temporal and spatial variations, with species composition changing according to seasonal and spatial differences. Seasonal fluctuations and spatial heterogeneity to some extent determined the distribution patterns and species diversity of the benthic macroinvertebrates.

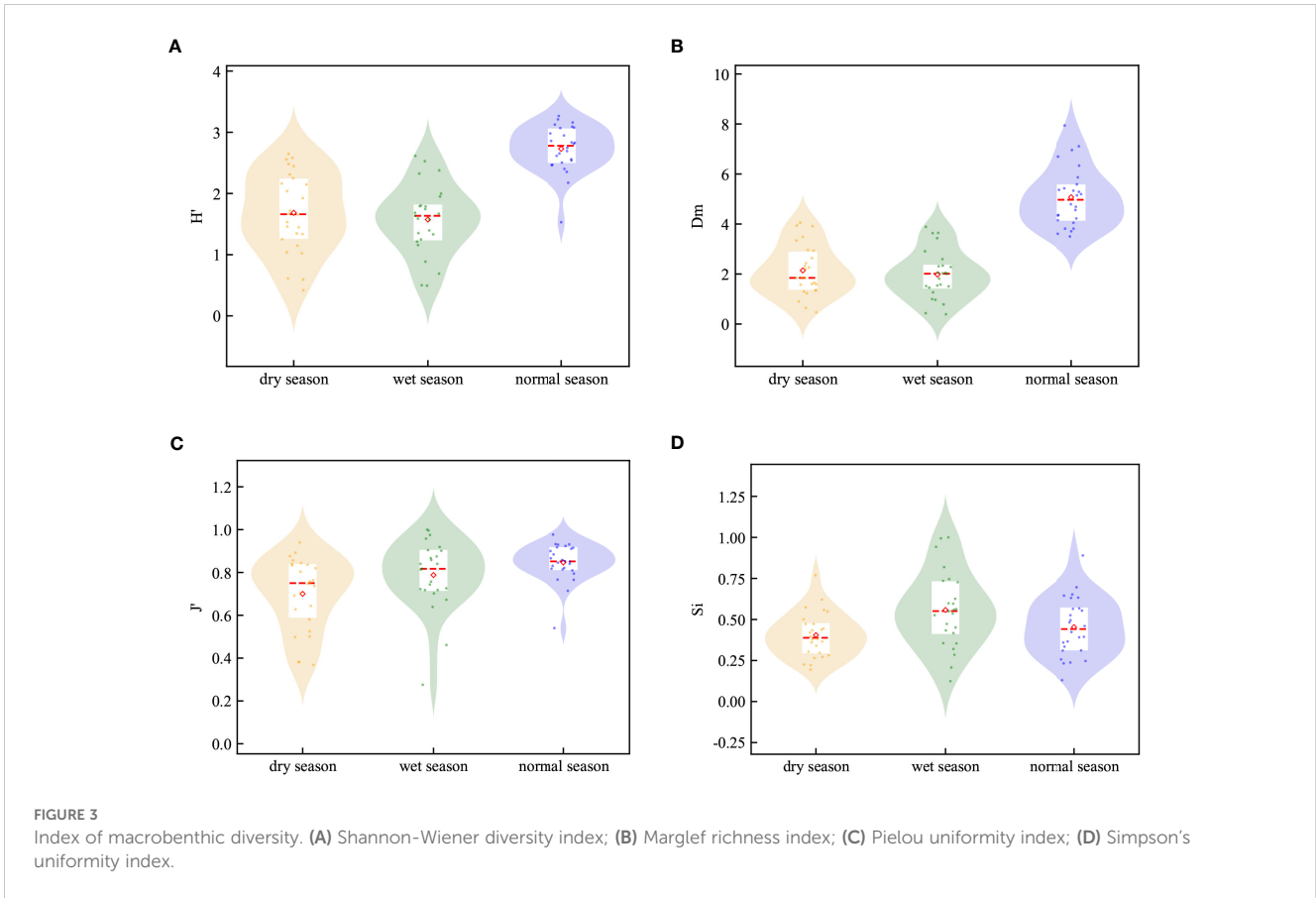
### 3.2 Biodiversity

The results for the Shannon-Wiener diversity index, Margalef richness index, Pielou evenness index, and Simpson evenness index for different water periods at each sampling site are shown in Figure 3. During the dry season, the indices ranged as follows: Shannon-Wiener (0.42-2.65), Margalef (0.47-4.05), Pielou (0.37-0.97), and Simpson (0.19-0.77). During the wet season, the indices

ranged: Shannon-Wiener (0.49-2.61), Margalef (0.39-3.89), Pielou (0.28-1), and Simpson (0.12-1). During the normal flow period, the indices ranged: Shannon-Wiener (1.53-3.27), Margalef (3.5-7.94), Pielou (0.54-0.98), and Simpson (0.13-0.89). Overall, the median and average values of the Shannon-Wiener diversity index and Margalef richness index during the normal flow period were significantly higher than those during the dry and wet periods, which is consistent with the distribution of macroinvertebrate taxa numbers. The differences in the Pielou evenness index and Simpson evenness index among different water periods were relatively small, with the Pielou evenness index being more concentrated during the normal flow period. According to ANOSIM results, the Shannon-Wiener diversity index had an  $R$  value of 0.29 and a  $p$ -value of 0.001; the Margalef richness index had an  $R$  value of 0.41 and a  $p$ -value of 0.001; the Pielou evenness index had an  $R$  value of 0.06 and a  $p$ -value of 0.004; and the Simpson evenness index had an  $R$  value of 0.03 and a  $p$ -value of 0.095. These results indicate that the differences between groups for the Shannon-Wiener diversity index and Margalef richness index were greater than the differences within groups, and the group differences were significant. For the Pielou evenness index and Simpson evenness index, the within-group and between-group differences were similar, and the seasonal differences in the Simpson evenness index were not significant.

### 3.3 ABC curves

The ABC (Abundance-Biomass Comparison) curves for macroinvertebrates in the Chishui River during the dry, wet, and

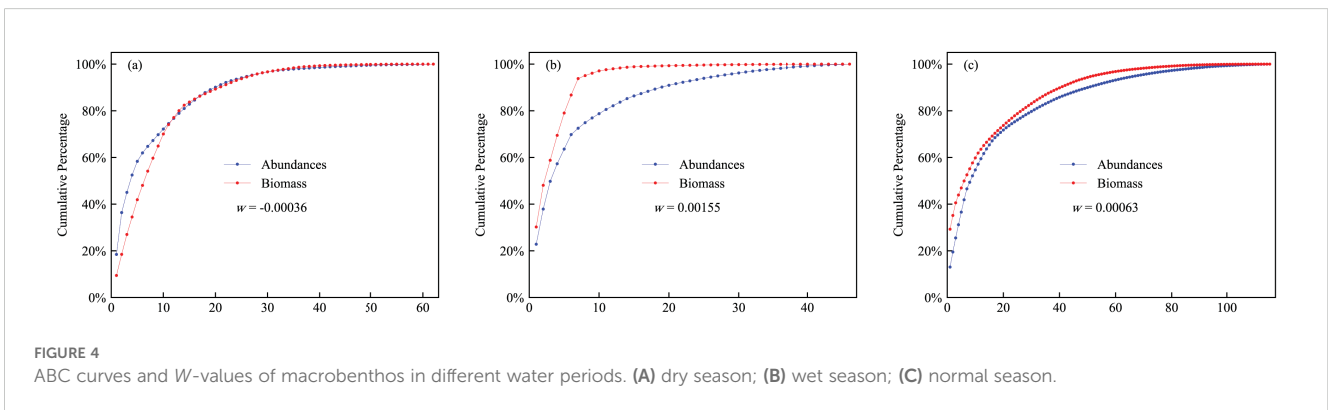


normal flow periods in 2023 are shown in **Figure 4**(a - dry season; b - wet season; c - normal season). These curves visually represent the relationship between the cumulative abundance and cumulative biomass of macroinvertebrates during each hydrological period. The analysis of these curves provides insights into the stability and community structure of macroinvertebrates under different flow conditions in the Chishui River.

The results indicate the following, in the dry Season, the top three taxa in terms of abundance were *Heptagenia* (18.46%), *Baetis* (17.92%), and *Hydropsyche sp.1* (8.65%). The top three taxa in terms of biomass were *Heptagenia* (9.43%), *Corbicula fluminea*

(9.05%), and *Semisulcospira cancellata* (8.52%). In the wet Season, the top three taxa in terms of abundance were *Caridina* (22.83%), *Hydropsyche sp.1*(15.06%), and *Semisulcospira cancellata* (11.88%). The top three taxa in terms of biomass were *Semisulcospira cancellata* (30.31%), *Radix ovata* (17.86%), and *Bellamyia* (10.71%). In the normal Season, the top three taxa in terms of abundance were *Hydropsyche sp.1* (13.04%), *Baetis* (6.45%), and *Cinygmmina* (6.03%). The top three taxa in terms of biomass were *Corbicula fluminea* (29.25%), *Limnoperna lacustris* (5.89%), and *Alainites* (5.41%).

Overall, during the dry season, the abundance curve initially exceeded the biomass curve, but eventually, the biomass curve



overtook it, causing both curves to intersect. With  $W < 0$ , this indicated that the macroinvertebrate community experienced a certain level of disturbance, leading to reduced stability. This period was characterized by smaller-bodied taxa. In contrast, during the wet and normal flow periods, the biomass curve consistently remained above the abundance curve, with  $W > 0$ . This suggested that the macroinvertebrate community structure was more stable and resilient during these periods.

### 3.4 Cluster analysis

Cluster analysis of the macroinvertebrate communities in the Chishui River was performed for different hydrological periods, resulting in three distinct clusters for each season, as illustrated in Figure 5 (a - dry season; b - wet season; c - normal season). During the dry season, Group 1 included sites 1, 2, 3, 4, 6, 7, 9, 11, 12, and 16; Group 2 included sites 5, 10, 13, 15, 17, 18, 19, 20, 21, 23, 24, 25, and 26; and Group 3 included sites 8, 14, and 22. During the wet season, Group 1 included sites 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19, 20, 22, 23, 25, and 26; Group 2 included site 17; and Group 3 included sites 21 and 24. During the normal season, Group 1 included sites 1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, and 25; Group 2 included sites 4 and 8; and Group 3 included sites 20, 23, 24, and 26. Subsequent SIMPER analysis was conducted to identify the main contributing taxa in each group, helping to understand the composition and the main factors driving the similarities within each cluster.

In the dry season, the main contributing taxa in Group 1 were *Hydropsyche sp.1* (8.15%), *Baetis* (6.64%), and *Ephemera* (6.54%); in Group 2, they were *Heptagenia* (19.16%), *Baetis* (16.17%), and *Leptophlebia* (11.7%); and in Group 3, they were *Dugesia japonica* (15.4%), *Ephemera* (10.63%), and *Heptagenia* (8.15%). In the wet season, the main contributing taxa in Group 1 were *Caridina*

(15.87%), *Hydropsyche sp.1* (11.8%), and *Ephemera* (5.83%); in Group 2, *Caridina* accounted for 53.85%; and in Group 3, *Semisulcospira cancellata* accounted for 88.89%. In the normal season, the main contributing taxa in Group 1 were *Hydropsyche sp.1* (7.75%), *Corbicula fluminea* (4.07%), and *Cinygmmina* (3.99%); in Group 2, they were *Hydropsyche sp.1* (36.54%), *Cinygmmina* (17.3%), and *Heptagenia* (13.46%); and in Group 3, they were *Bellamyia aeruginosa* (21.7%), *Palaemon* (9.59%), and *Caridina* (5.13%).

The results highlight clear distinctions in the macroinvertebrate community structure across different hydrological periods, with each group exhibiting unique characteristics. The taxa contributing significantly to the differentiation within each group provide insight into how the macroinvertebrate communities are influenced by environmental and ecological factors specific to each period. These groupings reflect the adaptability and dynamic nature of the macroinvertebrate communities, demonstrating their response to the seasonal changes in water flow and associated environmental conditions at the sampling sites.

### 3.5 Dominant taxa

The results of the relative importance index (IRI) are presented in Table 1. These results indicate that the dominant macroinvertebrate taxa in the Chishui River during the dry season are primarily *Heptagenia*, *Baetis*, and *Corbicula fluminea*, with *Baetis* and *Heptagenia* being the absolute dominant taxa during this period. During the wet season, the dominant taxa include *Caridina*, *Hydropsyche sp.1*, *Semisulcospira cancellata*, and *Ephemera*, with *Caridina*, *Hydropsyche sp.1* and *Semisulcospira cancellata* being the absolute dominant taxa. In the normal season, the dominant taxa are *Corbicula fluminea*, *Hydropsyche sp.1*, *Heptagenia*, *Cloeon*, and *Cinygmmina*, with *Corbicula fluminea* and *Hydropsyche sp.1* being the absolute dominant taxa. The dominant taxa throughout the year

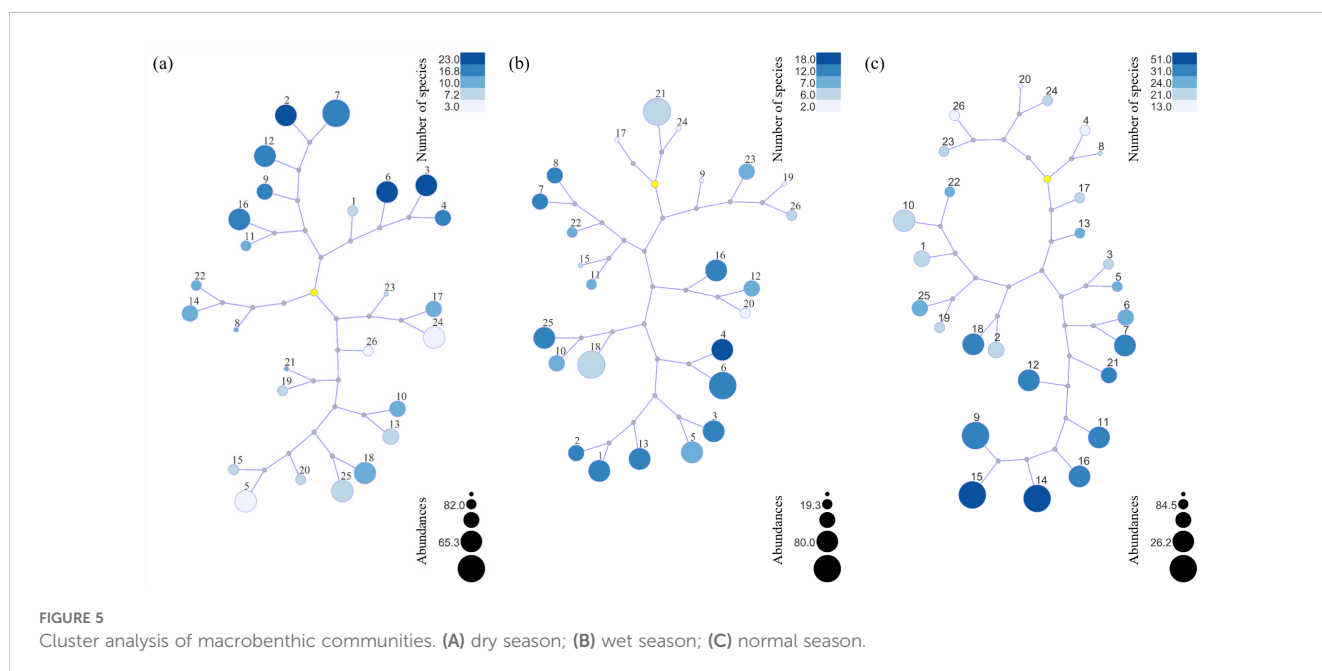




TABLE 1 Dominant taxa of macrobenthos in Chishui River.

Dry Season		Wet Season		Normal Season		All year	
Taxa	IRI	Taxa	IRI	Taxa	IRI	Taxa	IRI
<i>Heptagenia</i>	2575	<i>Caridina</i>	2681	<i>Corbicula fluminea</i>	2014	<i>Corbicula fluminea</i>	1756
<i>Baetis</i>	1524	<i>Hydropsyche sp.1</i>	1576	<i>Hydropsyche sp.1</i>	1419	<i>Hydropsyche sp.1</i>	1636
<i>Corbicula fluminea</i>	622	<i>Semisulcospira cancellata</i>	1010	<i>Heptagenia</i>	632	<i>Heptagenia</i>	1162
		<i>Ephemera</i>	613	<i>Cloeon</i>	553	<i>Baetis</i>	1097
				<i>Cinygmmina</i>	546	<i>Caridina</i>	713

include *Caridina*, *Hydropsyche sp.1*, *Heptagenia*, *Baetis*, and *Caridina*, with *Caridina*, *Hydropsyche sp.1*, *Heptagenia* and *Baetis* being the absolute dominant taxa.

### 3.6 Relationship between dominant taxa and water quality factors

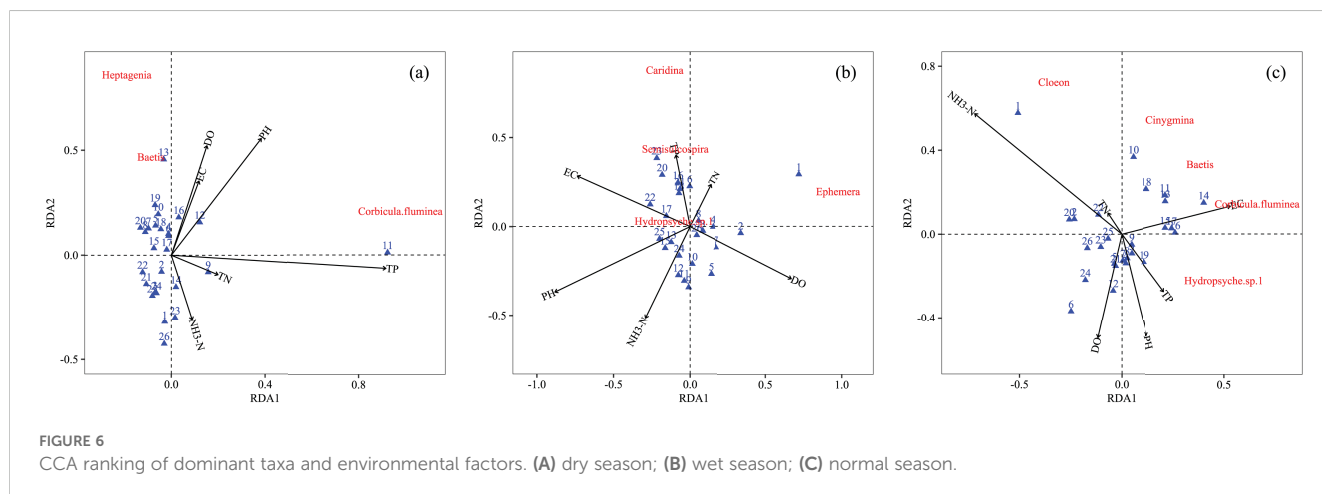
Detrended Correspondence Analysis (DCA) was conducted on the abundance of macroinvertebrates at each sampling site. The results revealed that the first axis lengths during the dry, wet, and normal seasons were 0.46, 0.36, and 0.52, respectively, all of which were less than 3. As a result, redundancy analysis (RDA) was employed to explore the relationship between the dominant taxa and environmental factors (Figure 6: a - dry season; b - wet season; c - normal season). The RDA ordination results indicated that the dominant macroinvertebrate taxa in the Chishui River exhibited varying responses to environmental factors across different seasons.

During the dry season, TN and TP was strongly positively correlated with the first axis, while DO exhibited a strong positive correlation with the second axis. The taxa *Baetis* and *Heptagenia* was strongly positively correlated with DO, but strongly negatively correlated with NH<sub>3</sub>-N and *Corbicula fluminea* was strongly positively correlated with TN and TP. Overall, the upstream sites showed a positive correlation with EC and DO, and a negative correlation with NH<sub>3</sub>-N, whereas the downstream sites exhibited the opposite trend.

During the wet season, DO was strongly positively correlated with the first axis, while TP、TN was strongly negatively correlated with the second axis. *Caridina* and *Semisulcospira cancellata* was strongly positively correlated with TN and TP and strongly negatively correlated with NH<sub>3</sub>-N. *Ephemera* was strongly positively correlated with DO and strongly negatively correlated with EC and *Hydropsyche sp.1* had weak correlations with various environmental factors. Overall, the upstream sites showed a positive correlation with DO and a negative correlation with EC, whereas the downstream sites exhibited the opposite trend.

During the normal season, EC was strongly positively correlated with the first axis, while pH and DO was strongly negatively correlated with the second axis. *Corbicula fluminea*, *Hydropsyche sp.1* and *Baetis* were strongly positively correlated with EC, *Cinygmmina* was strongly negatively correlated with DO and *Cloeon* was strongly positively correlated with NH<sub>3</sub>-N. Overall, the upstream sites showed a positive correlation with pH and DO, and a negative correlation with NH<sub>3</sub>-N, whereas the downstream sites exhibited the opposite trend.

The comprehensive results showed that *Corbicula fluminea*, *Semisulcospira cancellata* and *Caridina* preferred environments with high phosphorus and nitrogen levels, while *Ephemera*, *Heptagenia*, *Baetis* and *Cinygmmina* exhibited a strong correlation with DO. Overall, upstream sites were positively correlated with and DO, and negatively correlated with NH<sub>3</sub>-N, whereas downstream sites showed the opposite trend. Upstream areas are typically shallower, which allows for greater exposure to sunlight, promoting photosynthesis by algae and



phytoplankton. This process leads to an increase in oxygen production, directly raising DO levels in the water. Furthermore, upstream sites generally experience fewer human activities and pollution sources, resulting in lower organic matter content and minimal oxygen consumption by microorganisms. As a result, DO levels remain high, favoring aerobic taxa like mayflies in these areas. Therefore, upstream sites are positively correlated with DO levels. In contrast, downstream areas are more affected by pollution and eutrophication, leading to shifts in the benthic community composition. These areas tend to favor taxa that feed on organic detritus, such as mollusks and oligochaetes, as well as pollution-tolerant taxa like chironomids and tubificid worms. The abundance of these taxa is positively correlated with NH<sub>3</sub>-N and TP levels, reflecting the environmental stress and altered conditions typical of downstream regions.

### 3.7 Relationship between dominant taxa and physical environmental factors

During the benthic macroinvertebrate sampling in the Chishui River basin, water depth, flow velocity, and the median particle size of the substrate (D50) were measured at each sampling site. A frequency histogram was constructed to illustrate the distribution of the absolute dominant taxa throughout the year in the Chishui River basin. The ratio of the abundance of dominant taxa within each interval to the total abundance of the samples was calculated.

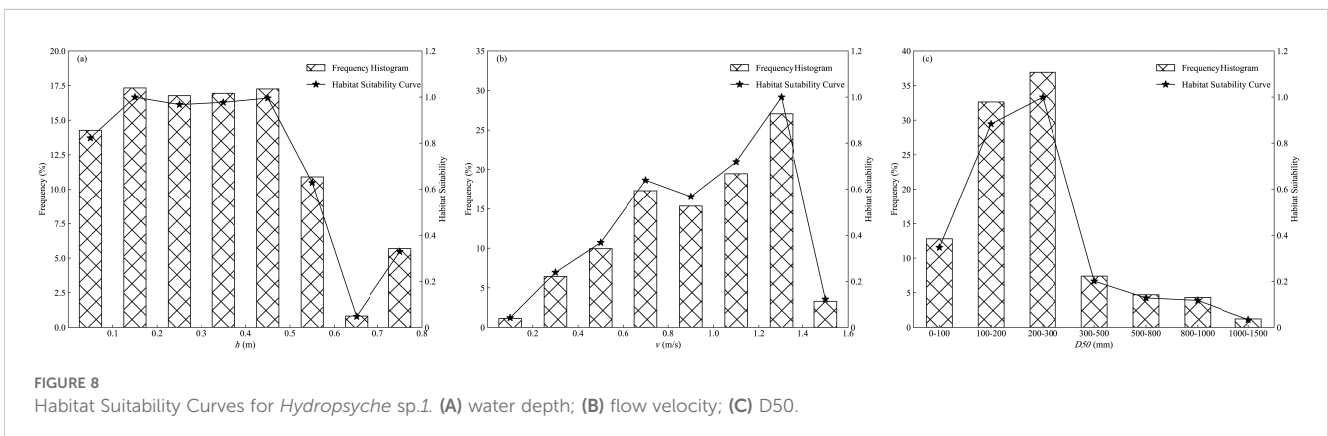
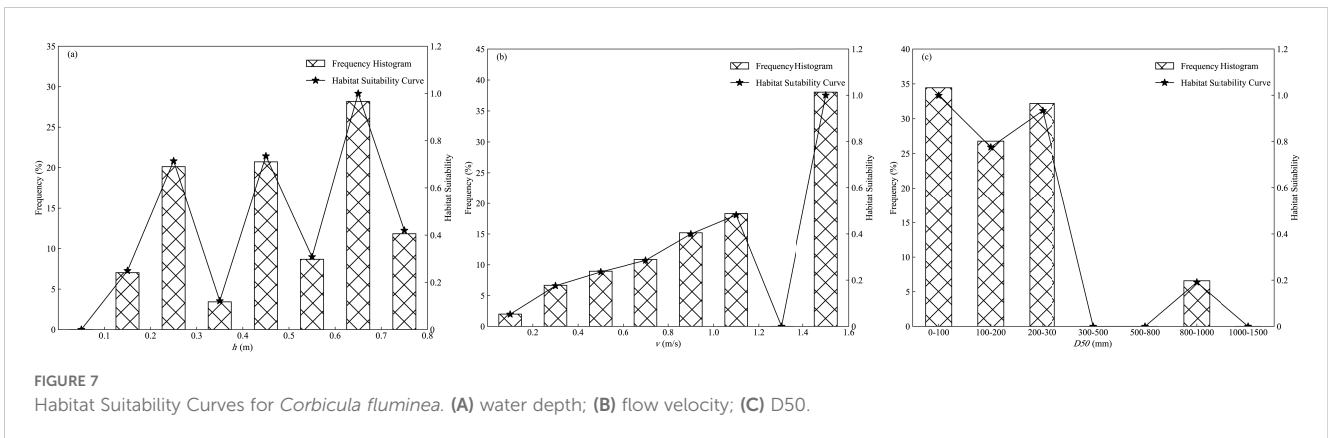
Using the suitability univariate method, the relative abundance values corresponding to each physical environmental factor were normalized. Habitat suitability for each range was then established, with the habitat suitability for each dominant taxon defined as the ratio of the relative abundance value corresponding to each variable range to the maximum relative abundance value.

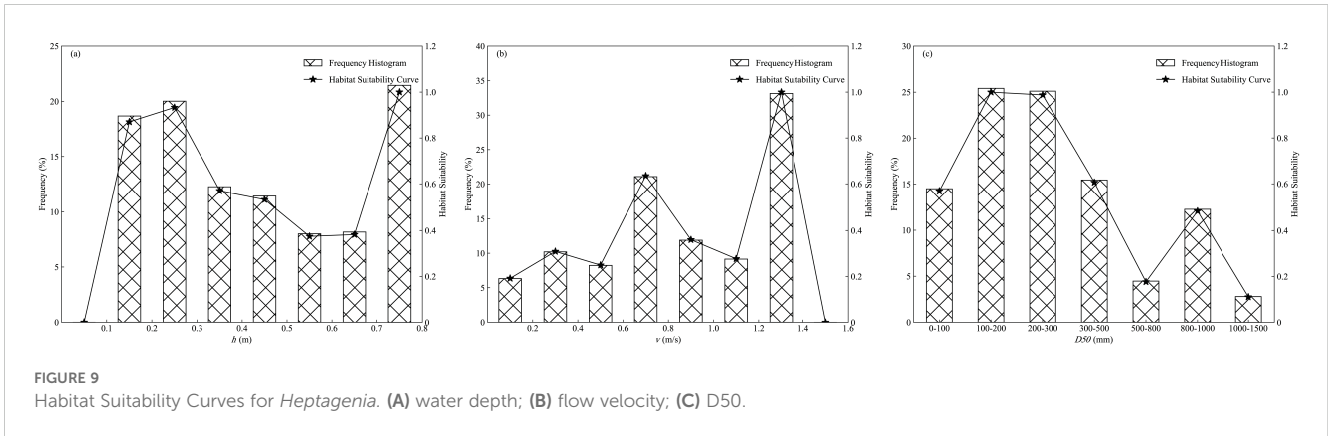
The suitability curves for water depth, flow velocity, and substrate composition for each of the absolute dominant taxa are shown in Figures 7–11 (Figure 7: *Corbicula fluminea*; Figure 8: *Hydropsyche sp.1*; Figure 9: *Heptagenia*; Figure 10: *Baetis*; Figure 11: *Caridina*). From these figures, it is evident that *Corbicula fluminea* and *Ephemera* prefer environments with high flow velocities and substrates with a D50 smaller than 300 mm. *Hydropsyche sp.1* and *Baetis* taxa prefer environments with medium to low water depth, high flow velocity, and substrates with a D50 between 100–300 mm. Macrobrachium taxa favor environments with deeper water, low flow velocity, and larger substrate particle sizes.

## 4 Discussion

### 4.1 Macroinvertebrate community structure in the Chishui River

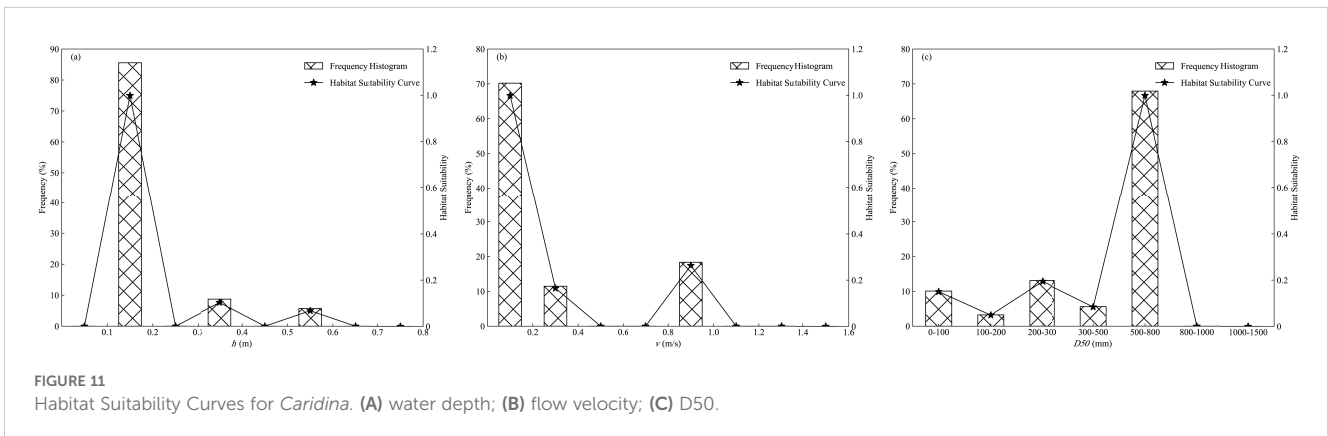
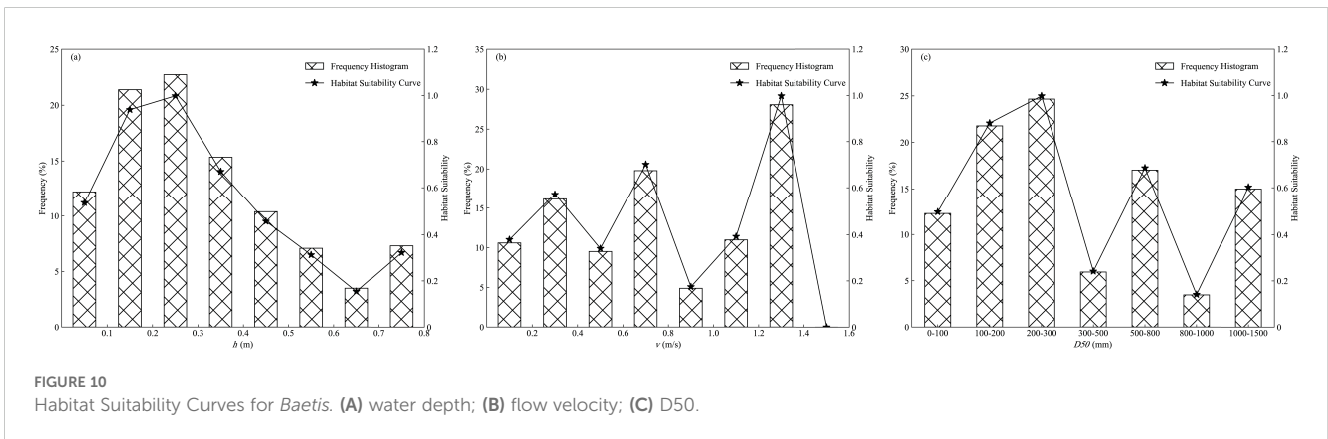
The survey results indicate that in 2023, a total of 153 taxa of macroinvertebrates were collected from the Chishui River, with 62





taxa identified in the dry season, 46 taxa in the wet season, and 115 taxa in the normal season. The community was predominantly composed of aquatic insect EPT groups. Compared to other rivers of similar scale (Sun et al., 2023; Zhang K. et al., 2023; Wang et al., 2024), the Chishui River basin exhibits relatively high macroinvertebrate diversity. This richness is linked to its significant role in the Yangtze River basin. The Chishui River, as a major undammed tributary of the upper Yangtze and a core area of the national nature reserve for rare and endemic fish species in the upper Yangtze, maintains natural river flow, lush vegetation, and low pollution levels. The absence of hydraulic engineering projects and near-natural ecosystem conditions provide a solid

foundation for the biodiversity of aquatic organisms within the basin (Cao, 2000; Ja et al., 2020; Min et al., 2021). Additionally, the Chishui River displays typical characteristics of a mountainous river, with numerous tributaries and a complex river network. The distinct environmental variations along different river sections and the high habitat heterogeneity created by the complex hydrological conditions contribute to the rich diversity of macroinvertebrates in the Chishui River (Zhai and Qiu, 2011; Zhou and Gao, 2023). However, compared to past studies (Jiang, 2012; Wang, 2018; Zhang D. et al., 2023), the species richness of macroinvertebrates in the Chishui River has been gradually declined. This decline is primarily attributed to human activities



and changes in the natural environment, which have destroyed suitable habitats and altered the conditions of habitats for benthic taxa.

The macroinvertebrate community in the Chishui River is significantly shaped by different hydrological periods, with temporal changes influencing hydrological characteristics, hydrodynamic conditions, physicochemical factors, and riparian vegetation. In the dry season, *Ephemeroptera* (*Heptagenia* and *Baetis*) and *Mollusca* (*Corbicula fluminea*) are relatively abundant. In the wet season, *Decapoda* (*Caridina*), *Trichoptera* (*Hydropsyche* sp.1), *Mollusca* (*Semisulcospira cancellata*) and *Ephemeroptera* (*Ephemera*) dominate. During the normal season, *Hydropsyche* sp.1, *Corbicula fluminea* and *Ephemeroptera* (*Baetis*, *Cloeon*, *Cinygmmina*) are more prevalent. The dominance of more sensitive taxa such as *Ephemeroptera* and *Trichoptera* in the dry and normal seasons contrasts with the prevalence of more pollution-tolerant taxa like *Caridina* and mollusks during the wet season. Through the combined analysis with water quality factors, we hypothesize that in the wet season, the concentrations of water pollutants such as  $\text{NH}_3\text{-N}$ , TN, and TP are higher, while DO levels are lower. These water conditions may lead to the disappearance of sensitive taxa, such as mayflies (*Ephemeroptera*), and be replaced by more pollution-tolerant taxa, such as taxa of *Caridina* and mollusks. Furthermore, the number of taxa observed during the normal season surpasses that of the dry season, which in turn exceeds the wet season. This trend may be attributed to the fact that during the normal season, aquatic insects like mayflies are in the juvenile stage of their life cycle, making them easier to collect and leading to higher diversity. In contrast, the wet season coincides with frequent rainfall and high water flow, which results in greater disturbance to benthic communities. Floods and the influx of pollutants, including

agricultural fertilizers, often reduce or eliminate some macroinvertebrate taxa, leading to decreased diversity.

Additionally, spatial analysis results revealed significant differences in species abundance and diversity along the main stem of the Chishui River. The upstream areas exhibited higher abundance, with aquatic insects predominating, indicating relatively clean water quality and a diverse macroinvertebrate community. In contrast, the downstream areas had lower species richness, with a notable increase in the abundance and diversity of mollusks, suggesting possible water quality degradation and environmental changes. These spatial differences may be closely related to variations in pollution levels, flow velocity, substrate composition, and physicochemical factors in the water.

Overall, the macroinvertebrate community structure in the Chishui River exhibited significant temporal and spatial variations. Seasonal changes and spatial heterogeneity jointly determined the distribution patterns and species diversity of benthic macroinvertebrates, reflecting the impact of environmental changes on the community. Therefore, protecting the water quality and ecological environment of the Chishui River, particularly across different seasons and spatial regions, is of crucial ecological significance.

## 4.2 Relationship between macroinvertebrate communities and water quality factors

Using GAMs, we explored the relationship between macroinvertebrate communities and environmental factors. The model fitting results are illustrated in Figure 12. The  $R^2$  values for pH, DO, EC,  $\text{NH}_3\text{-N}$ , TN and TP were 0.449, 0.182, 0.144, 0.148, 0.222,

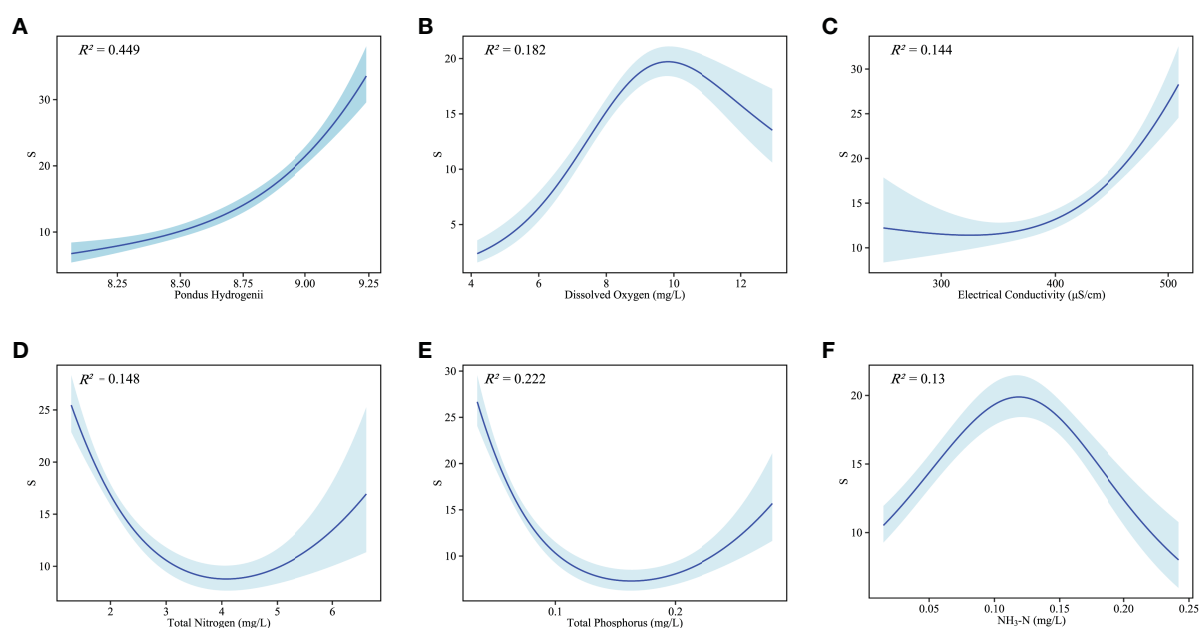


FIGURE 12 Response curve of macrobenthic community to environmental factors. (A) pH; (B) DO; (C) EC; (D) TN; (E) TP; (F)  $\text{NH}_3\text{-N}$ .

and 0.13, respectively, with all  $p$ -values being less than 0.01. Macroinvertebrates predominantly thrive in water environments with appropriate pH levels. In our study, we observed that as pH and EC increased, the diversity of macroinvertebrate species in the Chishui River also increased, until a threshold was reached (pH = 9.24).

Previous studies (Zhu et al., 2019; Su et al., 2020) have shown that DO levels significantly influence the feeding, reproduction, and overall life processes of aquatic organisms, with varying tolerances to DO among different taxa. Based on the GAM results, the optimal DO concentration for macroinvertebrate survival in the Chishui River was found to be 9.8 mg/L. According to the “Environmental Quality Standards for Surface Water” in China, Class I water quality is defined as having DO levels > 7.5 mg/L (State Environmental Protection Administration and General Administration of Quality Supervision, 2002). In our study, 77.9% of the sampling sites met this Class I standard, further reinforcing the conclusion that the Chishui River maintains relatively low pollution levels and high water quality.

TN and TP are critical factors in evaluating river eutrophication and act as reliable indicators of macroinvertebrate biodiversity and taxa richness (Yu et al., 2019; Zhang et al., 2021). The GAM analysis indicates a sharp decline in macroinvertebrate taxa richness as TN and TP concentrations rise. Interestingly, once TN reaches 0.41 mg/L and TP reaches 0.163 mg/L, a slight increase in taxa richness is observed. This trend reflects the varying tolerances of macroinvertebrate taxa to nutrient levels. In nutrient-rich environments, taxa such as *Corbicula fluminea*, *Semisulcospira cancellata*, and *Caridina* were identified as dominant, supporting findings by Zhang Y. et al. (2023). In contrast, taxa associated with cleaner waters, such as *Ephemeroptera*, declined significantly or even disappeared under high-nitrogen and high-phosphorus conditions, consistent with observations by Gong et al. (2001). These results highlight the differential responses of pollution-tolerant and sensitive taxa to changes in nutrient levels, emphasizing the ecological impact of eutrophication on macroinvertebrate communities.

Research indicates that  $\text{NH}_3\text{-N}$  serves as a vital nutrient for the growth of aquatic plants and algae, which can indirectly enhance macroinvertebrate productivity by supporting the base of the food web (Johnson et al., 2013). However,  $\text{NH}_3\text{-N}$  also has direct physiological effects on macroinvertebrates, influencing their immune responses and damaging tissues, with excessive concentrations proving lethal. The GAM fitting results reveal a nonlinear relationship between  $\text{NH}_3\text{-N}$  concentration and macroinvertebrate taxa richness in the Chishui River. As  $\text{NH}_3\text{-N}$  levels increase, taxa richness initially rises but subsequently declines. The analysis identifies 0.12 mg/L as the optimal  $\text{NH}_3\text{-N}$  concentration for macroinvertebrate survival, aligning with findings from Hong et al. (2007). This highlights the dual role of  $\text{NH}_3\text{-N}$  as both a nutrient and a potential stressor, emphasizing the need for balanced nutrient levels to support macroinvertebrate biodiversity.

## 5 Conclusions

Based on the analysis of macroinvertebrate community structure in the Chishui River and its relationship with environmental factors, the following conclusions were drawn:

1. A total of 153 taxa of benthic macroinvertebrates were identified in the Chishui River basin during this survey, predominantly aquatic insects from the EPT group. The composition and diversity of macroinvertebrates varied significantly across different hydrological periods. Compared to other rivers of similar scale, the Chishui River exhibits relatively high macroinvertebrate diversity. However, the species richness of macroinvertebrates is gradually declining due to anthropogenic activities and changes in the natural environment.
2. Macroinvertebrate communities at upstream sampling sites were positively correlated with DO and negatively correlated with  $\text{NH}_3\text{-N}$ , while downstream sites exhibited the opposite trends. These spatial differences reflect the influence of hydrological and environmental gradients along the river.
3. According to the GAM fitting results, the number of macroinvertebrate taxa in the Chishui River increased with increasing pH and EC; decreased initially and then increased to some extent with increasing TN and TP concentrations; and increased initially and then decreased with increasing DO and  $\text{NH}_3\text{-N}$  concentrations.
4. Based on this study, we suggest implementing pollution control measures targeting  $\text{NH}_3\text{-N}$  to protect sensitive taxa such as mayflies. Firstly, the efficiency of nitrogen removal in sewage treatment plants should be enhanced to reduce  $\text{NH}_3\text{-N}$  emissions from domestic and industrial wastewater. Secondly, given the large amount of farmland along the Chishui River, measures such as planting buffer strips and optimizing fertilization timing and dosage should be implemented to reduce agricultural nitrogen runoff.
5. This study relies on short-term monitoring data, which may not fully capture the long-term responses of benthic communities to environmental changes. Future research should include long-term monitoring and interannual variability analysis to evaluate the time-scale effects of community dynamics and identify delayed or cumulative impacts of environmental changes.
6. Given the multivariate nature of the relationships between taxa and environmental factors, causal relationships remain difficult to establish based on observational data alone. Future studies could incorporate experimental approaches, such as substrate preference or pollution simulation experiments, to directly assess the effects of key environmental variables on community structure, thereby advancing our understanding of these interactions.

## 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

XL: Data curation, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. LY: Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. XZ: Funding acquisition, Supervision, Writing – review & editing. PH: Funding acquisition, Investigation, Resources, Supervision, Writing – review & editing. CS: Funding acquisition, Investigation, Supervision, Writing – review & editing. BZ: Investigation, Software, Writing – review & editing.

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## Conflict of interest

Author XZ was employed by China Three Gorges Corporation. 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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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2024.1459468/full#supplementary-material>

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