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Benthic Macroinvertebrate Community Characteristics, Environmental Drivers, and Ecological Health Assessment in the One Lake–Four Rivers Region, Hunan, China

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Abstract

Hunan Province, encompassing the "One Lake and Four Rivers" system, represents a core ecological barrier in the middle reaches of the Yangtze River. However, a systematic province-wide assessment of benthic macroinvertebrate communities is currently lacking. In this study, benthic macroinvertebrates were surveyed at 100 sampling sites across Hunan Province in spring and autumn 2025, together with measurements of 26 environmental factors. Community characteristics were assessed based on species richness, density and dominance. Multidimensional assessments of aquatic ecological health were performed using the Biotic Index (BI), Shannon–Wiener Index, and Benthic Quality Index (BQI). In addition, redundancy analysis (RDA) was employed to identify the key environmental factors driving macroinvertebrate community structure. A total of 253 taxa were identified, with Arthropoda accounting for 66.80% of the total taxa and *Sinotaia* sp. exhibiting the highest dominance. Community characteristics exhibited a spatial pattern, with higher species richness and density in rivers than in lakes and a gradient of higher values in the southwest/upstream reaches and lower values in the northeast/downstream reaches. Seasonally, species richness and density were higher in spring than in autumn. Overall, aquatic ecological health across Hunan Province ranged from moderate to good, with the Lishui River Basin exhibiting the best ecological condition and the lower Yuanjiang River Basin showing the most prominent degradation. Elevation, chemical oxygen demand (COD), and habitat score were identified as the key environmental factors driving variation in benthic macroinvertebrate community structure. These findings provide an important biological basis for aquatic ecosystem conservation, ecological restoration, and management in Hunan Province.

Keywords: benthic macroinvertebrates; community characteristics; aquatic ecological health; environmental factors; redundancy analysis; Hunan Province

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1. Introduction

Freshwater ecosystems are important reservoirs of global biodiversity and key ecosystem types supporting human socioeconomic development. As a central link in freshwater food webs, benthic macroinvertebrates perform vital ecological functions, including material cycling, energy transfer, and substrate disturbance. At the same time, owing to their relatively stable life cycles and sensitivity to environmental disturbances, they are widely used

as indicator groups for evaluating aquatic ecological health in watersheds [1–3]. Globally, under the combined pressures of human activities and climate change, freshwater biodiversity is declining at an accelerating rate, with community homogenization and increasing proportions of pollution-tolerant taxa becoming increasingly prominent [4]. Systematic surveys of macroinvertebrates at the watershed-scale, together with an analysis of their driving factors and an assessment of aquatic ecological health, have therefore become a core research direction in aquatic ecosystem conservation [5,6].

China's middle Yangtze River basin, with its dense network of rivers and lakes and well-developed waterways, is a hotspot for freshwater biodiversity. Within Hunan Province, the Xiangjiang River, Zijiang River, Yuanjiang River, and Lishui River converge into Dongting Lake, forming a complete Dongting Lake watershed. As China's second-largest freshwater lake, it serves as a core component of the middle Yangtze River's ecological barrier and fulfills multiple functions, including water supply, flood control, and navigation, as well as providing biological habitats [7,8]. In recent years, overall surface water quality in Hunan Province has remained stable and shown a general trend of improvement. However, rapid urbanization and industrialization, agricultural non-point-source pollution, urban domestic wastewater, and human disturbances such as water conservancy projects and river sand mining have continued to exert pressure on aquatic ecosystems. Localized habitat fragmentation, elevated pollutant loads in some tributary sections, and cumulative ecological stress have consequently emerged, affecting the composition and distribution of benthic communities to varying degrees [9–11].

China's aquatic ecological management is transitioning towards systematic and comprehensive governance. Benthic macroinvertebrates were established as a core assessment dimension in both the 2022 pilot aquatic ecological assessment of the Yangtze River Basin [12] and the 2025 national ecological evaluation of excellent surface water bodies. The "Four Rivers and One Lake" system comprising the Xiangjiang River, Zijiang River, Yuanjiang River, Lishui River, and Dongting Lake is a key priority area under these assessment programmes. However, existing studies of benthic macroinvertebrates in Hunan Province have largely focused on individual river basins or localized lake areas, and systematic province-wide surveys covering the major hydrological network remain scarce. Comprehensive analyses of the overall spatiotemporal patterns, ecological health status, and underlying drivers of macroinvertebrate assemblages across the province remain limited [11,13]. Accordingly, this study represents the first systematic province-wide survey of benthic macroinvertebrates in 2025 across Hunan Province's "One Lake and Four Rivers" region. The objectives were to: (1) fill the provincial-scale research gap and characterize community composition and distribution of dominant taxa across the province; (2) reveal the spatiotemporal patterns of species richness and density; (3) conduct a multidimensional assessment of aquatic ecological health across the river basins using biotic, diversity, and benthic quality indices; and (4) identify key environmental factors driving differences in community structure. The findings provide a scientific basis for aquatic ecological assessment, conservation and restoration in Hunan Province and offer foundational data support for biodiversity conservation in the middle reaches of the Yangtze River basin.

2. Materials and Methods

2.1. Overview of the Study Area

Hunan Province is located on the southern bank of the middle reaches of the Yangtze River (108°47′–114°15′ E, 24°38′–30°08′ N). With a total land area of 211,800 km^2, the province falls within the subtropical monsoon climate zone, where the average annual temperature ranges from 16 to 18 °C, with a multiannual average precipitation of 1200–1700 mm. Precipitation is unevenly distributed throughout the year, with the flood season concen-

trated from April to September [14]. This study covers the main stems and major first- and second-order tributaries of the Xiangjiang River, Zijiang River, Yuanjiang River, and Lishui River, as well as the three major lake areas of East Dongting Lake, South Dongting Lake, and West Dongting Lake. Sampling sites were selected to ensure spatial coverage and habitat representativeness, encompassing mountainous headwaters, hilly middle reaches, and lowland downstream areas. The sites represented diverse habitat types, including gravel-bed rapids, sandy slow-flowing streams, and muddy lake habitats, as well as water-quality gradients ranging from relatively clean to polluted conditions. A total of 100 sampling sites were established (Figure 1, Table S1).

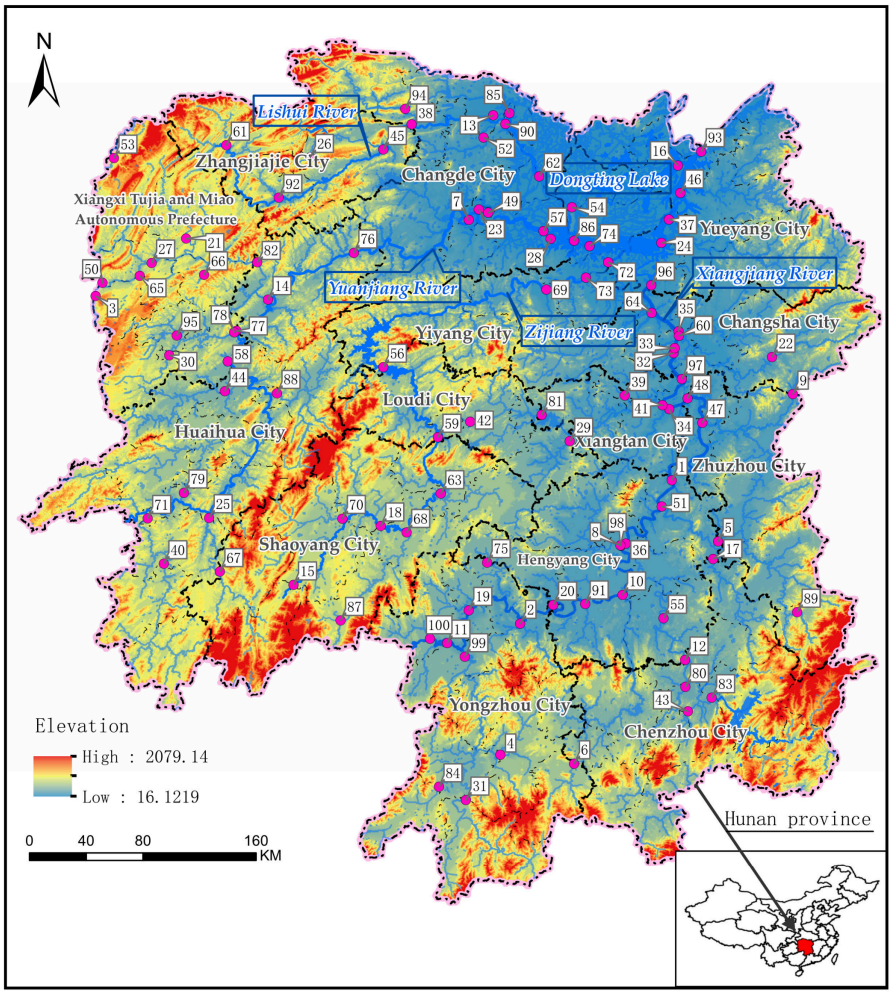


Figure 1. Distribution of benthic macroinvertebrate sampling sites across major river basins in Hunan Province. Note: Sampling points are numbered from 1 to 100.

2.2. Sample Collection and Analysis

2.2.1. Benthic Macroinvertebrates

Sampling was conducted once each in the spring (April–May) and fall (September–October) of 2025. Quantitative samples were collected using a 40-mesh Surber net (sampling area: 0.09 m^2, water depth < 30 cm) or a 1/16 m^2 Petersen mud sampler (water depth ≥ 30 cm); semi-quantitative samples were collected along a 10-m transect using a 0.09 m^2 Type D net [15].

After washing the collected samples on-site through a 40-mesh sieve to remove sediment, they were placed in polyethylene sample bottles and preserved with ethanol at a final

concentration of 75%. Upon return to the laboratory, the samples were classified, identified, and counted under a microscope [16].

2.2.2. Collection and Analysis of Environmental Samples

In conjunction with the sampling of benthic macroinvertebrates, on-site habitat surveys were conducted simultaneously, and water quality monitoring data were collected and compiled at each sampling site. The habitat surveys were conducted using a semi-quantitative field observation method, and on-site scoring records were completed; water quality monitoring parameters covered the 23 indicators specified in Table 1 of the "Surface Water Environmental Quality Standards" (GB 3838-2002) [17] (excluding fecal coliforms) as well as electrical conductivity. All sampling and analysis procedures strictly adhered to the "National Technical Specifications for Surface Water Environmental Quality Monitoring" [18]. Data collection and analysis methods were divided into two categories: four physicochemical parameters—water temperature, pH, dissolved oxygen, and conductivity—were measured on-site, while the remaining water quality parameters used the monthly average values from surface water environmental quality monitoring that were closest to the sampling time.

Table 1. Grading criteria of aquatic ecological health for each assessment index.

Index	Excellent	Good	Moderate	Poor	Very Poor
Biotic Index	BI ≤ 3.9	3.9 < BI ≤ 5.4	5.4 < BI ≤ 7.0	7.0 < BI ≤ 8.5	BI > 8.5
Shannon–Wiener index	H > 3.0	2.0 < H ≤ 3.0	1.0 < H ≤ 2.0	0 < H ≤ 1.0	H = 0
Benthic Quality Index	85 ≤ BQI ≤ 100	70 ≤ BQI ≤ 85	55 ≤ BQI ≤ 70	40 ≤ BQI ≤ 55	BQI < 40

2.3. Data Processing and Statistical Analysis

2.3.1. Calculation of Community Characteristic Indices

ArcGIS (version 10.7) was used to map the distribution of species richness and density at sampling sites, as well as the study area; river system data were obtained from the 1:200,000 vector data of the National Basic Geographic Information System. WPS 2026 was used to perform statistical analyses of the Biotic Index (BI), Benthic Quality Index (BQI), Shannon–Wiener index (H), Benthic Quality Index (BQI) and dominance (Y) [19,20], and Origin 2026 was used to generate the corresponding maps. The definitions and calculation methods of each index are described below.

Biotic Index (BI): The locally adapted Biotic Index (BI) used in this study was built upon the framework of the Hilsenhoff Biotic Index [21], with tolerance values revised based on the native benthic macroinvertebrate fauna of China. Its calculation formula is presented below:

$$\mathrm{BI} = \sum_{i=1}^{S} \frac{ni}{N} \times ti \quad (1)$$

where ti represents the pollution-tolerance value of species i, adopting locally revised tolerance values for Chinese benthic macroinvertebrate fauna; ni is the individual count of species i; N is the total number of benthic macroinvertebrate individuals; and S denotes the number of species.

Shannon–Wiener index [22]: The Shannon–Wiener index is a classical community diversity index, which characterizes the species diversity and individual distribution evenness of benthic macroinvertebrate communities. Its calculation formula is presented below:

$$H = \sum_{i=1}^{S} \frac{ni}{N} \times ln\frac{ni}{N} \quad (2)$$

where ni is the individual count of species; N is the total number of benthic macroinvertebrate individuals; and S denotes the number of species.

Benthic Quality Index (BQI): The Benthic Quality Index (BQI) is a core aquatic ecological-assessment indicator developed by the Ministry of Ecology and Environment of China to support the assessment of "excellent surface-water body proportion" for rivers [23]. Integrating two evaluation dimensions of pollution-tolerance trait and species richness, this index overcomes the limitations of single-metric indicators. Furthermore, it is simplified compared with the Index of Biological Integrity (IBI), enabling its operability for large-scale nationwide assessment. It is calculated as:

$$BQI = 50 \times \frac{10 - BI}{10 - BIref} + 50 \times \frac{S}{Sref} \quad (3)$$

where BI is the observed Biotic Index of benthic macroinvertebrates; BIref is the reference Biotic Index value of benthic macroinvertebrates, determined for each watershed by elevation zone; S is the observed number of benthic macroinvertebrate species; and Sref is the reference number of benthic macroinvertebrate species under the corresponding elevation-stratified reference condition.

2.3.2. Aquatic Ecological Health Assessment Methods

The Biotic Index (BI) and Shannon–Wiener Index were used to assess species' pollution tolerance characteristics and community diversity, respectively. The BI reflects the biological responses of aquatic organisms to water pollution, whereas the Shannon–Wiener Index reflects community diversity and evenness. However, neither index alone can fully characterize the overall integrity and ecological functioning of benthic macroinvertebrate assemblages. Therefore, following the methodology for the Benthic Quality Index (BQI) described in the Technical Specifications for the Assessment of High-Quality Surface Water Bodies (Trial), the BQI was applied across the entire "One Lake and Four Rivers" system of Hunan Province. The BQI uses elevation-stratified reference conditions as the baseline and integrates species richness and pollution-tolerance characteristics. It thus complements the limitations of conventional single-index assessments by incorporating community structural integrity, adaptation to environmental stress, and responses to spatial gradients.

It should be noted that although BQI takes the localized BI as a core input parameter, its output is an integrated composite score. It cannot independently characterize the pollution tolerance level of benthic communities, nor can it directly identify the driving effect of pollution stress on community structure. Retaining BI allows for dimensional complementarity with BQI—the two indices reflect pollution stress response and overall community health, respectively, which is why both are retained in this study.

Collectively, the BI, Shannon–Wiener Index, and BQI constitute a multidimensional assessment framework encompassing pollution tolerance, species diversity, and community integrity. The grading criteria of each assessment index are shown in Table 1.

2.3.3. Correlation Analysis of Environmental Factors

Canoco 5.0 software was used to conduct correlation analyses between benthic macroinvertebrate communities and environmental factors. First, a Detrended Correspondence Analysis (DCA) [24] was performed on the species data, and the correspondence ordering model was selected based on gradient length: when the gradient length was <3, a linear model was applied using Redundancy Analysis (RDA); when the gradient length was >4, the unimodal model was applied using Canonical Correspondence Analysis (CCA); and when the gradient length was between 3 and 4, both RDA and CCA were applicable. Monte Carlo permutation tests (999 permutations) were used to test the significance of the explanatory effects of each environmental factor on community structure variation. Prior to

analysis, the species data underwent a Hellinger transformation, and the environmental factors (except pH) were transformed using the lg(x + 1) function [25].

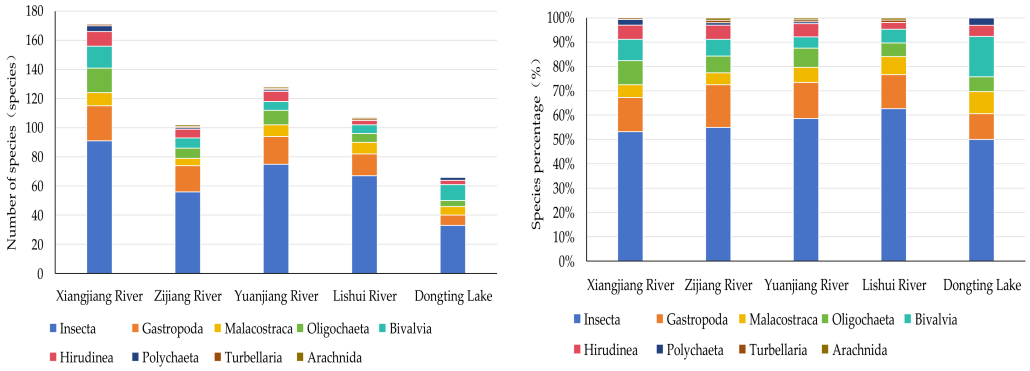
3. Results

3.1. Community Composition of Benthic Macroinvertebrates

3.1.1. Characteristics of Species Composition

A total of 253 taxa of benthic macroinvertebrates were identified across the province, belonging to 4 phyla, 9 classes, 35 orders, and 96 families. In terms of the proportion of species by phylum, the phylum Arthropoda was the dominant group, with 169 species, accounting for 66.80% of the total; of these, the class Insecta comprised 151 species, accounting for 89.35% of the phylum Arthropoda, the family Chironomidae, and the orders Ephemeroptera and Odonata were the major groups; the class Malacostraca comprised 16 species, while the class Arachnida comprised only 2 species. The phylum Mollusca comprised 47 species, accounting for 18.58%, including 27 species in the class Gastropoda and 20 species in the class Bivalvia. The phylum Annelida comprised 36 species, accounting for 14.23%, including 21 species of Oligochaeta, 11 species of Hirudinea, and 4 species of Polychaeta. The phylum Platyhelminthes had the fewest species, with only one species of Turbellaria, accounting for 0.39%, primarily distributed in the upper reaches of rivers.

According to the species composition characteristics of benthic macroinvertebrates in the river basins (Figure 2), there are significant spatial differences in species richness among the Xiangjiang River, the Zijiang River, Yuanjiang River, Lishui River, and Dongting Lake: the Xiangjiang River basin has the highest number of species, reaching 171; Dongting Lake has the fewest, with only 66; and the Yuanjiang River, Lishui River, and Zijiang River ranked in the middle in terms of species count, with an overall distribution pattern showing higher species richness in river systems than in lake areas. In terms of phylum composition, the taxonomic groups across the basins are generally similar but exhibit some differentiation, with the distribution of key groups showing distinct patterns: the class Insecta is a dominant group common to all basins; the Lishui River basin has the highest number and proportion of Insecta species, while Dongting Lake has the lowest number of Insecta species among all basins; Bivalves exhibit a marked enrichment in lake areas, with both the number and proportion of bivalve species in Dongting Lake being significantly higher than in the river basins; and Polychaetes are more prominently distributed in the Xiangjiang River basin and Dongting Lake, where the number of species is significantly higher than in the Zijiang River, Yuanjiang River, and Lishui River basins.



(**a**) (**b**)

Figure 2. Community structure of benthic macroinvertebrates in major river basins of Hunan Province. (**a**) Number of species in each taxonomic group; (**b**) species proportion of each taxonomic group.

3.1.2. Composition of Dominant Species and Spatial Variations

A total of eight dominant groups at the genus level were identified across the entire basin. Ranked in descending order of dominance (Y), they are: *Sinotaia* sp. (Y = 0.07), *Limnodrilus* sp. (Y = 0.05), *Caridina* sp. (Y = 0.05), *Polypedilum* sp. (Y = 0.04), *Radix* sp. (Y = 0.03), *Semisulcospira* sp. (Y = 0.03), *Chironomus* sp. (Y = 0.02), and *Macrobrachium* sp. (Y = 0.02). Among these, *Sinotaia* sp. has the widest distribution and the highest dominance across the entire basin.

There is significant spatial variation in the composition of dominant benthic macroinvertebrate species across different river basins (Table 2). In the Xiangjiang River basin, *Sinotaia* sp. and *Limnodrilus* sp. are the core dominant groups; in the Zijiang River basin, *Polypedilum* sp., *Limnodrilus* sp., and *Caridina* sp. exhibit high dominance with similar values; in the Yuanjiang River basin, mollusk groups are abundant, with high dominance of *Sinotaia* sp. and *Radix* sp. In the Lishui River basin, *Caridina* sp. is the dominant group; in the Dongting Lake area, lacustrine groups such as *Grandidierella* sp. and *Cryptochironomus* sp. dominate, accompanied by groups such as *Novaculina chinensis*.

Table 2. Dominant species and dominance of benthic macroinvertebrates in different river basins of Hunan Province.

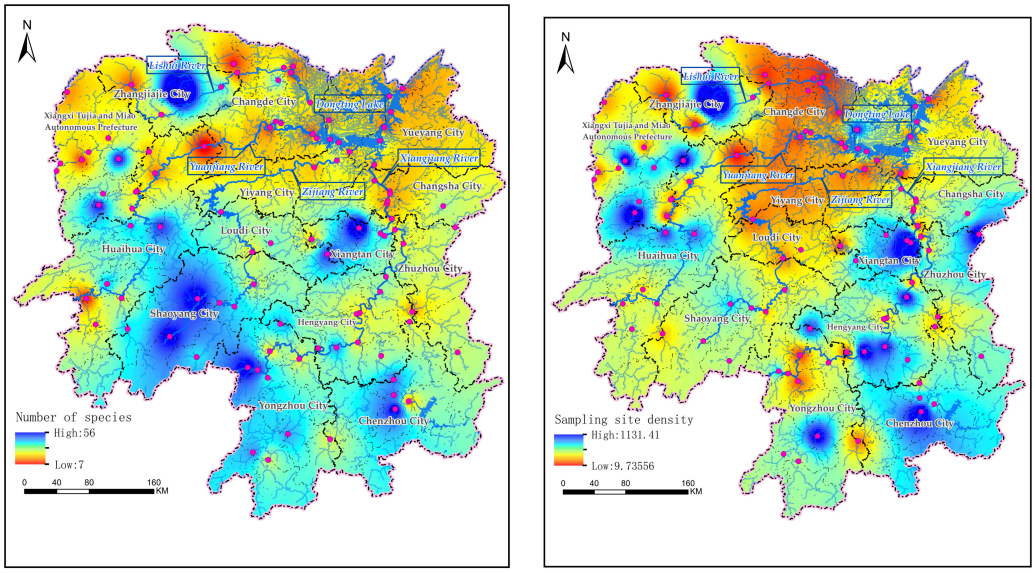
Phylum	Dominant Species	Xiangjiang River	Zijiang River	Yuanjiang River	Lishui River	Dongting Lake
Mollusca	*Sinotaia* sp.	0.11	0.04	0.07	0.03	—
	Semisulcospira sp.	0.05	—	—	—	—
	Radix sp.	0.02	—	0.07	0.03	—
	Limnoperna fortunei	—	—	0.03	—	—
	Novaculina chinensis	—	—	—	—	0.03
Arthropoda	*Caridina* sp.	0.06	0.07	—	0.11	—
	Neocaridina sp.	—	0.03	—	0.03	—
	Macrobrachium sp.	—	—	0.06	0.03	—
	Grandidierella sp.	—	—	—	—	0.16
	Chironomus sp.	0.03	—	—	—	—
	Polypedilum sp.	—	0.08	0.06	0.03	0.05
	Cryptochironomus sp.	—	—	—	—	0.08
	Harnischia sp.	—	—	—	—	0.03
	Microchironomus sp.	—	—	—	—	0.03
Annelida	*Limnodrilus* sp.	0.08	0.07	0.02	—	—

Note: Dashes ("—") indicate that the species did not meet the dominant species criteria.

3.2. Spatiotemporal Distribution Patterns of Communities

3.2.1. Spatial Distribution Characteristics

Both the species richness and density of benthic macroinvertebrates in Hunan Province exhibit significant spatial heterogeneity (Figure 3), generally following a distribution pattern characterized by "high in the southwest and low in the northeast, high in the upper reaches and low in the lower reaches." Areas of high species richness are primarily distributed in the upper reaches of the Xiangjiang River, the upper reaches of the Zijiang River, the middle and upper reaches of the Yuanjiang River, and the Zhangjiajie section of the upper Lishui River, with an average of more than 25 species per sampling site. Areas with low species richness are scattered in a patchy pattern across the Dongting Lake Plain and the middle and lower reaches of various river systems, with some sites hosting fewer than 10 species. In the eastern regions, such as the hilly plains of the middle and lower reaches of the Xiangjiang River and the Hengyang Basin, species richness is generally at a moderately low level.



(**a**) (**b**)

Figure 3. Spatial distribution of species number (**a**) and density (**b**) of benthic macroinvertebrates in major river basins of Hunan Province.

The spatial distribution of benthic macroinvertebrate density generally mirrors the overall pattern of species richness, but there are two notable differences in regional variation: First, although species richness is high in the mountainous areas of the upper Zijiang River, the density of benthic macroinvertebrates is significantly lower than in the upper reaches of other river basins—such as the Lishui, Yuanjiang, and Xiangjiang Rivers—and does not form a high-density zone. Second, the overall density level in the Xiangjiang River basin is higher than in other basins, with the Xiangtan and Hengyang sections of the middle Xiangjiang River and the Chenzhou section of the upper Xiangjiang River forming prominent high-density zones. Furthermore, in contrast to the patchy distribution of low species richness, the Dongting Lake Plain and the estuarine regions of the Lishui, Yuanjiang, and Zijiang Rivers constitute contiguous areas of low density, representing regions with the lowest density of benthic macroinvertebrates in the province.

3.2.2. Temporal Distribution Characteristics

Analysis of seasonal dynamics shows that both species richness and individual density of benthic macroinvertebrates in Hunan Province exhibit significant seasonal fluctuations and were generally higher in spring than in autumn; only the Xiangjiang River basin exhibits the opposite pattern (Figure 4). Seasonal variations in species richness differed across river basins: in the Zijiang, Yuanjiang, and Lishui Rivers, as well as in the Dongting Lake area, species richness was higher in spring than in autumn, with the Zijiang River having the highest average number of species in spring among all basins in the province; the Xiangjiang River was the only basin where species richness was higher in autumn than in spring. The seasonal patterns of individual density show greater consistency: in all five major river basins, spring density is higher than autumn density, with only the Lishui River showing a relatively small difference in density between spring and autumn; the Xiangjiang River has the highest spring density in the province, and densities in all river basins generally decline after the onset of autumn. The coefficient of variation (CV) reveals that the fluctuation magnitude of species richness between spring and autumn is significantly smaller than that of individual density across all river basins, with the Lishui River Basin showing the most intense fluctuations in both species richness and density.

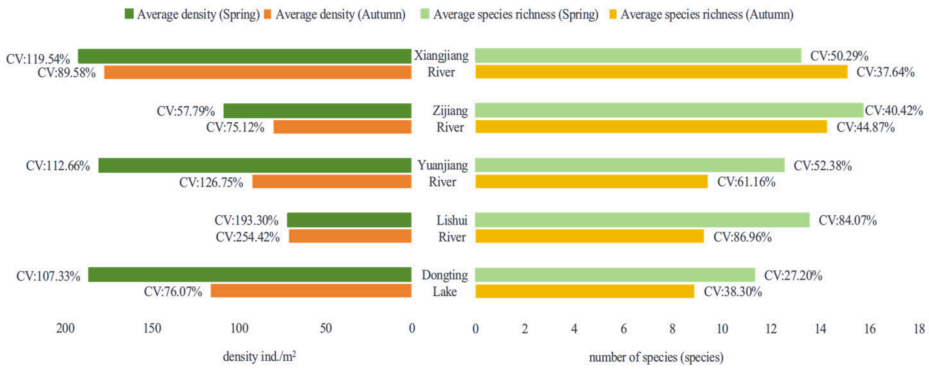


Figure 4. Temporal distribution of species number and density of benthic macroinvertebrates in major river basins of Hunan Province.

3.3. Aquatic Ecological Health Status of River Basins

Aquatic ecological health was assessed from three complementary dimensions using the Biotic Index (BI, indicating pollution tolerance), the Shannon–Wiener Index (indicating community diversity), and the Benthic Quality Index (BQI, indicating community integrity) [26,27]. The assessment results are presented in Figures 5 and 6. Species richness characteristics are not reiterated here, as they are detailed in Section 3.2.

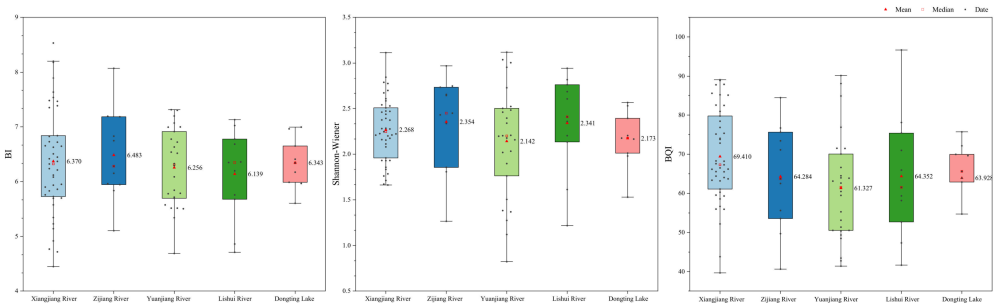


Figure 5. Distribution of BI, Shannon–Wiener Index and BQI for benthic macroinvertebrates across major river basins in Hunan Province.

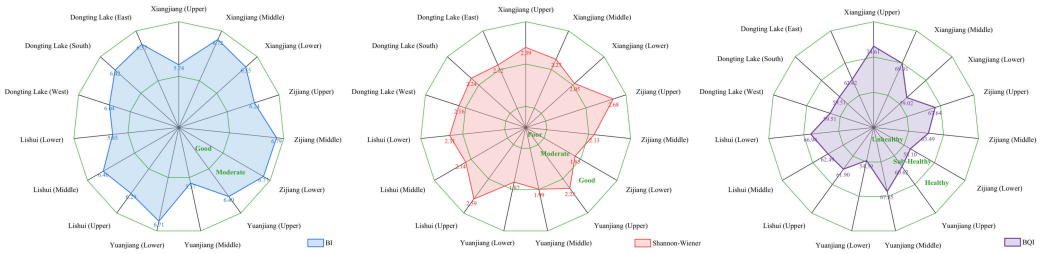


Figure 6. Spatial distribution of BI, Shannon–Wiener Index and BQI for benthic macroinvertebrates in different river reaches and Dongting Lake zones of Hunan Province.

3.3.1. BI Index Evaluation Results

Province-wide average BI values ranged from 6.14 to 6.48, corresponding to a "moderate" health level. No site reached "excellent", and only one site at the confluence of the Zhengshui River with the Xiangjiang River was rated "very poor."

In terms of basin averages, the Zijiang River had the highest BI value, followed by the Xiangjiang River and Dongting Lake, with the Yuanjiang River and Lishui River showing progressively lower values; the Lishui River basin had the lowest average. The Xiangjiang

River showed the widest grade coverage, spanning four assessment grades; other rivers were dominated by "moderate" sites, and Dongting Lake had the most concentrated BI distribution, with all sites rated "moderate".

Along the upstream–downstream gradient, BI values generally increased downstream (indicating higher pollution tolerance), most notably in the Xiangjiang and Zijiang rivers. West Dongting Lake had relatively lower BI values within the lake region.

3.3.2. Shannon–Wiener Index Evaluation Results

Province-wide average values ranged from 2.14 to 2.36, corresponding to a "good" diversity level, which was higher than the BI assessment results.

At the basin scale, the Zijiang River had the highest mean index value, followed by the Lishui River and Xiangjiang River, while the Yuanjiang River and Dongting Lake had relatively lower values. The Yuanjiang River exhibited the widest grade coverage, spanning four assessment grades, followed by the Xiangjiang River; the Zijiang and Lishui rivers were dominated by "Good" sites, and Dongting Lake had the most concentrated distribution.

Along the upstream–downstream gradient, Diversity was generally higher in upstream mountainous areas than in mid–downstream plains. The upper reaches of the Zijiang and Lishui rivers were diversity hotspots, while the lower reaches of the Yuanjiang and Zijiang rivers fell to "moderate" level. Spatial differentiation within Dongting Lake was limited.

3.3.3. Assessment Results of the Benthic Quality Index

Province-wide average BQI values ranged from 61.33 to 69.41, corresponding to an overall "sub-healthy" health status.

In terms of mean values, that of the Lishui River was the highest, followed by Zijiang River and Dongting Lake, with the Yuanjiang River ranking lowest. The Xiangjiang River also showed the largest spatial heterogeneity, while Dongting Lake had the most homogeneous distribution. The Xiangjiang River was dominated by "very healthy" to "sub-healthy" sites; the Yuanjiang and Lishui rivers covered four grades; Zijiang River and Dongting Lake covered three grades.

Along the upstream–downstream gradient, health status generally declined from upstream mountains to mid–downstream plains. The Lishui River exhibited minimal longitudinal differentiation. The upper Xiangjiang River attained "healthy" rating, while the lower Yuanjiang River was rated "unhealthy". Within Dongting Lake, East Dongting had slightly higher BQI values, and overall lake values were lower than river system values, consistent with species richness and Shannon–Wiener patterns.

3.4. Correlation Between Community Structure and Environmental Factors

A detrended correspondence analysis (DCA) was conducted using the densities of the dominant species of eight benthic macroinvertebrates, as well as the BI Index, Shannon–Wiener Index, and species richness—three aquatic ecological health assessment indicators—as response variables and the 26 screened environmental factors (including 24 water quality factors, habitat scores, and elevation) as explanatory variables. The maximum gradient length of the ordination axes was 0.7, which is less than 3 and thus meets the conditions for applying a linear model; therefore, Redundancy Analysis (RDA) was further employed. The results showed that the first two axes cumulatively explained 39.08% of the variation in community structure, reflecting to some extent the relationship between the benthic macroinvertebrate community and environmental factors.

The interpretation rate, contribution rate and significance test results of each environmental factor with regard to benthic community structure variation are summarized in Table 3. Monte Carlo permutation tests indicated that elevation made the highest contribu-

tion to community structure variation (contribution rate 23.9%, interpretation rate 9.4%), followed by chemical oxygen demand (COD) (contribution rate 20.8%, interpretation rate 8.1%) and habitat score (contribution rate 10.2%, interpretation rate 4.0%); these three factors reached a significance level of $p < 0.05$ and were identified as the key environmental factors driving the structural differentiation of benthic macroinvertebrate communities in Hunan Province. Sulfide, cadmium, and anionic surfactants also contributed to explaining community structure variation to some extent, but their effects did not reach statistical significance ($p > 0.05$).

Table 3. Interpretation rate, contribution rate and significance test of each environmental factor in RDA analysis.

Environmental Factor	Interpretation Rate (%)	Contribution Rate (%)	*p* Value
Elevation	9.4	23.9	0.002
Chemical oxygen demand (COD)	8.1	20.8	0.008
Habitat score	4	10.2	0.032
Sulfide	3	7.6	0.066
Cadmium (Cd)	2.5	6.5	0.056
Anionic surfactants	2.9	7.4	0.056
Permanganate index	1.4	3.6	0.156
pH	1.3	3.4	0.192
5-day biochemical oxygen demand (BOD_5)	1.2	3.1	0.166
Electrical conductivity	1.5	3.7	0.164
Water temperature	0.6	1.5	0.36
Lead (Pb)	0.5	1.3	0.422
Total nitrogen (TN)	0.5	1.2	0.414
Cyanide	0.3	0.8	0.536
Selenium (Se)	0.4	1	0.456
Hexavalent chromium (Cr^{6+})	0.3	0.7	0.522
Ammonia nitrogen (NH_3-N)	0.5	1.2	0.462
Fluoride	0.3	0.8	0.544
Copper (Cu)	0.3	0.7	0.566
Zinc (Zn)	0.1	0.3	0.716
Volatile phenols	<0.1	0.1	0.82
Total phosphorus (TP)	<0.1	0.1	0.884
Petroleum hydrocarbons	<0.1	<0.1	0.898
Arsenic (As)	<0.1	<0.1	0.94
Dissolved oxygen (DO)	<0.1	<0.1	0.972

As shown in Figure 7, sampling sites for benthic macroinvertebrates across various river basins in Hunan Province exhibit distinct spatial differentiation patterns in the RDA

ordination plot. At sampling sites along the Zijiang River (orange), dominant species are predominantly concentrated along the positive direction of the first RDA axis and are positively correlated with the elevation gradient; at sampling sites along the Lishui River (pink), dominant species are relatively clustered along the negative direction of the second RDA axis, showing a positive correlation with habitat score and a negative correlation with chemical oxygen demand (COD), while a small number of sites exhibit a negative correlation with habitat score; dominant species at Dongting Lake (blue) sampling sites are primarily distributed along the negative direction of the first RDA axis, showing a negative correlation with both elevation and chemical oxygen demand; sampling sites along the Yuanjiang River (purple) and the Xiangjiang River (green) are relatively dispersed, and their dominant species show no significant correlation with any environmental factor.

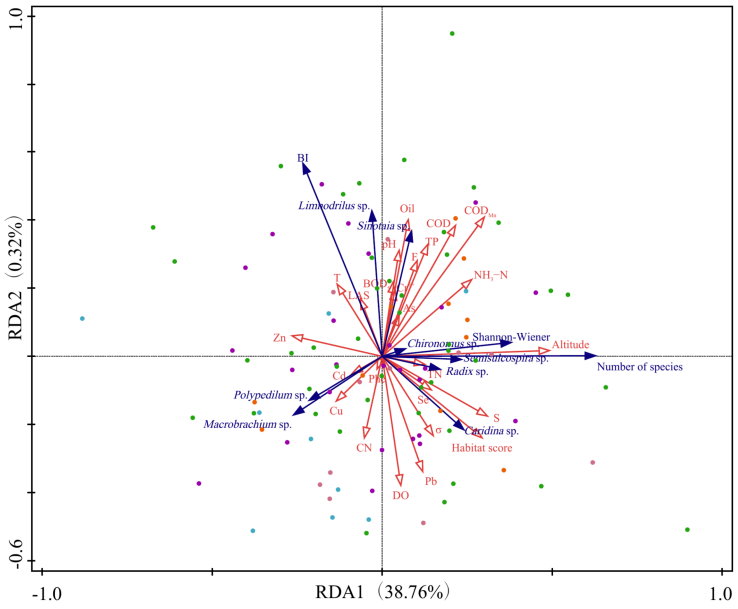


Figure 7. Redundancy analysis of macroinvertebrates and environmental factors in major river basins of Hunan Province. Note: Green indicates sampling sites in the Xiangjiang River basin; orange indicates sampling sites in the Zijiang River basin; purple indicates sampling sites in the Yuanjiang River basin; pink indicates sampling sites in the Lishui River basin; blue indicates sampling sites in Dongting Lake.

In terms of the response patterns between aquatic ecological health assessment indicators and environmental factors, the three health assessment indicators exhibit clear relationships with key environmental factors. Species richness and the Shannon–Wiener index are positively correlated with elevation and habitat scores; the BI index is positively correlated with chemical oxygen demand (COD). The distribution directions of the three health assessment indices in the ordination space align closely with those of the key environmental drivers.

An association analysis of dominant species density and environmental factors revealed that the eight dominant species exhibited differentiated response patterns to environmental gradients. Among pollution-tolerant groups, *Limnodrilus* sp. and *Sinotaia* sp. showed a positive correlation with COD; among clean-water groups, *Caridina* sp. and *Semisulcospira* sp. showed a positive correlation with elevation; *Radix* sp. and *Chironomus* sp. exhibited weak correlations with the three key environmental factors; and *Polypedilum* sp. and *Macrobrachium* sp. showed a negative correlation with elevation and COD.

4. Discussion

4.1. Community Composition and Spatiotemporal Distribution Characteristics

This study identified a total of 253 species of benthic macroinvertebrates in Hunan Province. The species composition was dominated by the class Insecta within the phylum Arthropoda, which is consistent with the patterns observed in benthic macroinvertebrate communities in most streams and rivers of the middle reaches of the Yangtze River in China [28]. Within the class Insecta, groups such as the family Chironomidae, the order Ephemeroptera, and the order Odonata were particularly prominent; overall, this indicates that water quality across the province ranges from clean to slightly polluted. The regional aquatic habitats exhibit high complexity, supporting the stable coexistence of benthic groups with different ecological habits [29]. In terms of differences across river basins, species richness in river systems was significantly higher than in the Dongting Lake areas. This pattern was primarily driven by habitat heterogeneity and differences in species ecological traits [30]: the upper reaches of the four major river basins are predominantly characterized by gravelly, fast-flowing habitats with complex habitat structures, providing diverse microhabitats for sensitive groups such as mayflies, stoneflies, and caddisflies. Meanwhile, most benthic macroinvertebrates are rheophilic taxa that depend on flowing water conditions to complete their life cycles. They are widely distributed in lotic habitats but cannot establish stable populations in lentic lake habitats [31]. In contrast, Dongting Lake is dominated by muddy, slow-flowing, and still-water habitats, with relatively monotonous habitat types; only groups adapted to still-water bottoms, such as Bivalvia, exhibit enrichment, which is consistent with the conclusions of previous studies on large lacustrine benthic macroinvertebrates [32]. Among the four major river basins, the Xiangjiang River basin exhibits a prominent dominance of pollution-tolerant groups, indicating high levels of anthropogenic disturbance; in contrast, the Lishui River basin shows the highest dominance of clean, sensitive groups, indicating the lowest level of anthropogenic disturbance.

In terms of spatial distribution, the macroinvertebrate communities in Hunan Province as a whole exhibit a pattern of "higher in the southwest and lower in the northeast, higher in the upper reaches and lower in the lower reaches," which is highly correlated with the spatial coupling of regional human activity intensity and elevation gradients. The mountainous areas of western and southern Hunan, as well as the upper reaches of each river system, have higher elevations, lower levels of urbanization and industrial and agricultural activity, mild water pollution, and well-preserved substrate habitats, which support high species richness; in contrast, the mid- and lower-reaches plain areas are densely populated and subject to significant agricultural non-point source pollution and urban wastewater discharge, resulting in severe habitat fragmentation. This leads to the disappearance of sensitive groups and a decline in species richness—a pattern consistent with research findings from other provinces in the Yangtze River Basin [33–35]. It is worth noting that species richness in the upper reaches of the Zijiang River is high, but density is relatively low; this is presumed to be related to the predominance of large, sensitive insect groups in this region and the low number of individuals per sampling site. In contrast, the middle reaches of the Xiangjiang River form a high-density zone, primarily due to the massive proliferation of pollution-tolerant groups such as *Sinotaia* and *Limnodrilus*, which indirectly reflects the relatively high level of organic pollution in this river section [36].

In terms of seasonal dynamics, the number of species and density of benthic macroinvertebrates across the province generally exhibit a pattern of high levels in spring and low levels in autumn, primarily regulated by temperature and life history cycles. As water temperatures gradually rise in spring, benthic macroinvertebrates enter a phase of reproduction and rapid growth, with both larvae and adults present simultaneously, resulting in high levels of both species richness and density within the community. In autumn, as

water temperatures drop, most short-generation insect groups complete their life cycles, and population sizes decline, leading to a decrease in the overall community levels [37,38]. Species richness in the Xiangjiang River basin exhibits a reverse pattern, with higher levels in autumn than in spring, which may be related to this factor: spring sampling in the Xiangjiang River occurs relatively early, concentrated in April, when most benthic macroinvertebrates are still in the egg or early larval stages, and their tiny size makes them prone to being missed during sorting [39].

4.2. Assessment of Aquatic Ecological Health in River Basins

In this study, the BI, Shannon–Wiener Index, and Benthic Quality Index (BQI) were used to assess aquatic ecological health from three complementary dimensions. The BI index adopts only the calculation framework of the Hilsenhoff Biotic Index, with pollution tolerance thresholds calibrated based on native Chinese benthic species. It is applicable to Chinese freshwater ecosystems [40] and focuses on characterizing responses to pollution stress. The Shannon–Wiener index, an internationally recognized diversity metric, is used to reflect the species richness and evenness of benthic communities. The BQI is a domestically developed comprehensive assessment index in China, focusing on the structural integrity of benthic communities. The three indices complement one another across dimensions and jointly support a multi-dimensional assessment of aquatic ecological health.

The results indicated that the overall ecological health status of Hunan Province was characterized as "moderate" based on the BI, "good" based on the Shannon–Wiener Index, and "sub-healthy" based on the BQI. Although the three indices exhibited generally consistent spatial patterns, they differed to some extent in their assessment dimensions. The BI, which is based on the pollution-tolerance characteristics of macroinvertebrate taxa, primarily reflects the degree of pollution stress in aquatic systems. The Shannon–Wiener Index mainly characterizes species diversity and evenness, whereas the BQI places greater emphasis on the structural integrity and ecological functioning of benthic macroinvertebrate assemblages. The differences among these indices suggest that macroinvertebrate assemblages in the river basins of Hunan Province have retained relatively high species diversity, but the proportion of pollution-tolerant taxa has increased to some extent, accompanied by a certain degree of deterioration in community structural integrity. Consequently, the aquatic ecosystems of the province as a whole exhibit characteristics of a sub-healthy condition.

Among the river basins, the mean values of the three indices showed different ranking patterns, reflecting differences in the predominant types of environmental stressors among basins. The Lishui River Basin exhibited relatively favorable assessment levels across all three indices, indicating comparatively low pollution stress and high-water-quality conditions. This pattern is directly associated with the relatively limited anthropogenic disturbance and high habitat quality in the Zhangjiajie section of the upper reaches. The Zijiang River Basin had the highest mean BI and Shannon–Wiener Index values, while its mean BQI was intermediate. This indicates that the basin supported the highest levels of macroinvertebrate species diversity and evenness in the province, although the relatively high proportion of pollution-tolerant taxa suggests that pollution stress has begun to emerge. The Xiangjiang River Basin exhibited the widest range of assessment grades for both the BQI and BI, with pronounced differences among sampling sites. This pattern closely corresponded to the socioeconomic development gradient across the basin: the mountainous upper reaches were characterized by high habitat heterogeneity and relatively intact community structure, whereas substantial degradation occurred in industrially intensive sections of the middle and lower reaches, consistent with previous studies [41]. In the Dongting Lake region, the Shannon–Wiener index and BQI were relatively low, whereas the BI remained at an intermediate level. Their assessment grades were comparatively concentrated, which may

be jointly attributable to high dominance of certain taxa, pollutant loading entering the lake, sediment homogenization, and fluctuations in hydrological regimes [42,43]. In the Yuanjiang River Basin, the relatively low Shannon–Wiener Index and BQI, combined with an intermediate mean BI, suggest that organic pollution was not the predominant limiting factor. Instead, insufficient macroinvertebrate diversity, impaired community structural integrity, and pronounced ecological functional degradation appear to be major ecological concerns. These conditions may be associated with intensive cascade hydropower development along the mainstem of the Yuanjiang River and frequent sand-mining activities in the middle and lower reaches and near the lake inflow area [13,44]. The assessment results further highlight the differences in the dimensions represented by the three indices: neither a single diversity index nor a single pollution-tolerance index can comprehensively characterize the structural integrity and ecological functioning of benthic macroinvertebrate assemblages. The combined application of these indices therefore provides a more comprehensive framework for evaluating aquatic ecological health.

Along the upstream–downstream gradient, all four major river systems generally exhibited better ecological health conditions in the upper reaches than in the middle and lower reaches. The deterioration of ecological health was particularly pronounced in the lower reaches of the Yuanjiang River Basin, consistent with the spatial pattern of species richness and further supporting the inference that anthropogenic disturbance is a major driver of aquatic ecological degradation across the river basins [45,46]. Within the Dongting Lake region, spatial variation in ecological health was smaller than that observed across the river systems. Pollution stress exhibited a spatial gradient, with lower levels in western Dongting Lake than in eastern and southern Dongting Lake. This pattern may be related to the relatively lower intensity of pollutant inputs from inflowing rivers and the comparatively well-preserved wetland habitats in western Dongting Lake.

4.3. Environmental Drivers of Community Structure

In this study, RDA was conducted following DCA; the results showed that elevation, chemical oxygen demand (COD), and habitat scores were the top three environmental factors driving the structural differentiation of benthic macroinvertebrate communities in Hunan Province, with a cumulative contribution rate of 54.9%, among which elevation was the primary driving factor with the highest contribution rate (23.9%). This finding is consistent with the conclusions of studies in most river basins in China [47–49]. As a comprehensive environmental factor, elevation does not act directly on benthic macroinvertebrates but indirectly influences community composition by regulating habitat elements such as water temperature, dissolved oxygen, substrate type, and flow velocity: streams in high-elevation mountainous areas have fast flow velocities, high dissolved oxygen levels, and a high proportion of gravel substrates, which are suitable for the survival of clean-water-sensitive taxa; low-elevation plain river sections, with slow flow and sediment accumulation, are more suitable for the proliferation of pollution-tolerant groups [50]. COD, as the second largest contributing factor (20.8%), is a core indicator of organic pollution in water bodies; its elevated concentration directly leads to the disappearance of sensitive groups and an increase in the dominance of pollution-tolerant groups, making it a key polluting factor driving the degradation of community structure [51,52]. Habitat scores, ranking third with a contribution rate of 10.2%, comprehensively reflect the physical structural complexity of a habitat; the higher the habitat heterogeneity, the more niches it can accommodate, and the higher the species diversity—which is consistent with the core tenets of the habitat template theory [53–55]. In addition, sulfide, cadmium and anionic surfactants also had certain explanatory power for community structure variation, but their effects were not statistically significant ($p > 0.05$), indicating that heavy metal pollution and toxic pollutants

have not yet become the dominant driving forces of benthic community variation in the study area.

From the perspective of spatial differentiation within river basins, sampling sites in the Zijiang River basin showed a positive correlation with elevation, corresponding to its geographical characteristics of a high proportion of upstream mountainous river sections and a significant elevation gradient; in the Lishui River basin, sampling sites showed a positive correlation with habitat scores and a negative correlation with chemical oxygen demand (COD), confirming the current status of the Lishui River as having high habitat quality and low pollution levels; sampling sites in the Dongting Lake area showed negative correlations with both elevation and chemical oxygen demand (COD), while a small number of sampling sites exhibited a negative correlation with habitat scores. This reflects the unique characteristics of the lake—which is connected to rivers—featuring low elevation and slow-flow to still-water habitats. The ecological requirements of its dominant groups differ fundamentally from those of riverine species, resulting in the formation of an independent distribution zone within the ordination space [56]. Sampling sites in the Xiangjiang River and Yuanjiang River basins are relatively scattered, and their overall correlation with environmental factors is not significant. This is presumed to be related to the vast extent of these two major river basins, their diverse habitat types, and complex pollution sources; a single environmental gradient cannot fully explain their community differentiation.

The differentiated responses of dominant species further validate the driving role of key environmental factors. Typical pollution-tolerant groups, such as *Sinotaia* and *Limnodrilus*, show a positive correlation with chemical oxygen demand (COD); among these, *Limnodrilus* is an internationally recognized indicator of organic pollution [57], and an increase in its density directly reflects heightened organic pollution in the water body. Clean-water groups such as *Caridina* and *Semisulcospira* showed a positive correlation with elevation [58]; they are predominantly distributed in clean upstream streams and can serve as indicator groups for the health status of high-elevation streams. The distribution patterns of the three health assessment indices in the ordination space align closely with the key driving factors, indicating that the BI index, the Shannon–Wiener index, and species richness can effectively respond to changes in environmental gradients and are suitable for the rapid assessment of aquatic ecological health in river basins in Hunan Province.

4.4. Limitations and Future Directions

For the first time, this study conducts a comprehensive survey of benthic macroinvertebrates across the entire "One Lake and Four Rivers" region of Hunan Province; however, it still has certain limitations: First, sampling was conducted only in the spring and autumn, excluding the summer and winter seasons, making it difficult to fully reflect the annual dynamic changes in the community [59]. Second, some water quality indicators used monthly average values from the same period, resulting in a certain degree of temporal mismatch with the sampling of benthic macroinvertebrates, which may have had a subtle impact on the results of correlation analyses. Third, environmental factors were limited to water quality, habitat, and elevation, without considering important driving factors such as flow rate, hydrological rhythms, and land use within the watershed, leaving the analysis of driving mechanisms to be further explored. Fourth, the benthic assessment indices (BI and BQI) used in this study are calibrated based on Chinese specifications and indigenous faunal characteristics. There are systematic differences between these indices and the mainstream systems in Europe and the United States, making direct quantitative comparison of the results difficult [59]. Future research could be improved in three ways: (1) Conduct long-term, fixed-site monitoring and increase sampling frequency to reveal

the interannual and seasonal dynamics of community structure. (2) Incorporate multidimensional environmental factors such as hydrology and land use, and combine these with methods such as path analysis to quantify the relative contributions of different driving factors. (3) Integrate molecular biology techniques such as DNA barcoding to improve species identification accuracy [60], conduct an in-depth analysis of the assembly mechanisms of benthic macroinvertebrate communities, and provide a more robust theoretical foundation for the precise conservation of watershed aquatic ecosystems. Conduct comparative studies with international mainstream benthic ecological assessment systems to improve the international comparability of regional aquatic ecological health assessment results.

5. Conclusions

(1) Community Composition Characteristics: In 2025, a total of 253 taxa of benthic macroinvertebrates were identified in the major river basins of Hunan Province, belonging to 4 phyla, 9 classes, 35 orders, and 96 families, with the phylum Arthropoda being the absolutely dominant group; species richness in river systems was generally higher than in the Dongting Lake area, and the composition of dominant species differed significantly across river basins, exhibiting distinct riverine and lacustrine community differentiation.

(2) Spatiotemporal Distribution Patterns: Community species richness and density generally exhibited a spatial distribution characterized by "higher values in the southwest and lower in the northeast, and higher in the upper reaches and lower in the lower reaches." High-value areas were concentrated in the mountainous upper reaches of each river system, while low-value areas were primarily distributed in the Dongting Lake Plain and the middle and lower reaches of the main streams; seasonal dynamics generally followed a pattern of higher values in spring and lower in autumn, with the exception of the Xiangjiang River basin, where species richness was higher in autumn than in spring.

(3) Aquatic Ecological Health Status: The overall aquatic ecological health of Hunan Province was characterized as ranging from "sub-healthy (moderate)" to "good", indicating that no single conventional index can comprehensively characterize the overall health status of benthic macroinvertebrate assemblages. The predominant types of environmental stressors varied among river basins. The Lishui River Basin exhibited a relatively optimal ecological health status, whereas the Xiangjiang River Basin showed the greatest among-site variation in ecological health. The Zijiang River Basin had the highest community diversity but also a relatively high proportion of pollution-tolerant taxa. All four major river systems generally exhibited a longitudinal gradient, with better ecological health conditions in the upper reaches than in the middle and lower reaches, and pronounced deterioration in the lower reaches of the Yuanjiang River Basin. Within the Dongting Lake region, western Dongting Lake exhibited a slightly better ecological health status.

(4) Environmental Driving Mechanisms: Elevation, chemical oxygen demand (COD), and habitat scores are the key environmental factors driving the structural differentiation of benthic macroinvertebrate communities in Hunan Province; sampling sites across the river basins exhibit distinct spatial differentiation in the RDA ordination space, and the eight dominant species as well as the three health assessment indices all demonstrate differentiated ecological responses to environmental gradients.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18110709/s1, Table S1. Detailed information of sampling sites. Table S2. Taxonomic list of benthic macroinvertebrates. Table S3. Aquatic ecological health assessment indices at sampling sites.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author due to the fact that the data have not yet been made publicly available.

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