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ORIGINAL RESEARCH ARTICLE

Spatiotemporal dynamics of benthic macrofaunal assemblages and environmental drivers in the Nan'ou River Basin, China

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Abstract

Macrobenthos, which are critical for ecological health through nutrient cycling, water quality maintenance, and food web dynamics, inhabit the bottoms of freshwater basins. This study investigated spatiotemporal dynamics of macrobenthos communities in the Nan'ou River Basin, covering the Nanxijiang and Oujiang Rivers in China, from 2021 to 2023, to assess river ecological health and resilience. A total of seven phyla, 12 classes, and 32 families of benthic macroinvertebrates were identified, with Arthropoda, Annelida, and Mollusca collectively dominating (97.38% of benthic composition), forming a typical riverine assemblage. Key findings revealed that spatial environmental gradients, particularly habitat heterogeneity (the gravel bed of the Nanxijiang River versus the tidal regions of both the Nanxijiang and Oujiang Rivers), exerted far stronger influence on community structure and biodiversity distribution than seasonal variation. Pielou's evenness index remained relatively high despite fluctuating species richness, indicating a comparatively balanced and equitable community. Redundancy analysis identified total nitrogen, electrical conductivity, and turbidity as the primary drivers shaping benthic distribution, highlighting the significant roles of nutrient enrichment and sediment conditions, largely linked to anthropogenic pressures. These results highlight that habitat-specific conditions and anthropogenic factors predominantly shape benthic biodiversity in this basin, providing a critical scientific baseline for understanding its ecological dynamics and underscoring the need for further investigation into sources of organic pollution through monitoring of bioaccumulation in benthic organisms and trophic transfer in food webs.

Keywords: Benthic invertebrate; Community structure; Seasonal variation; Environmental factors; River

1. Introduction

River basins, the land areas drained by a river and its tributaries, are vital ecological systems that support biodiversity and regulate environmental processes.[1,2] These dynamic landscapes encompass rivers, wetlands, and forests, and play a critical role in maintaining ecological balance and providing essential services to aquatic ecosystems.[3] A key function of river basins is their role in supporting biodiversity and serving as habitats for many species. River basins also regulate critical environmental processes, acting as natural water filters, with vegetation and soil trapping sediments and pollutants, improving water quality, and benefiting both aquatic ecosystems and human communities.[3,4] However, it is not as widely appreciated that nutrient-rich waters below and lateral to the channel support a complex food web composed of aquatic organisms that are hydrologically connected to the river and dependent on the surface water and groundwater exchange.[5] Anthropogenic activities threaten river basins through deforestation, urbanization, and pollution. These impacts reduce biodiversity and weaken ecosystem services, underscoring the need for conservation and sustainable management.[6]

Macrobenthos—including insects, crustaceans, worms, and other invertebrates inhabiting the bottom of river basins—are critical to the ecological health of river basins by supporting nutrient cycling, maintaining water quality, and shaping food web dynamics.[7,8] Macrobenthos constitute an important food source for numerous higher trophic levels, such as fish.[9] In river ecosystems, the presence of robust benthic communities often indicates favorable ecological conditions. However, benthic communities face threats from human activities, such as pollution, sedimentation, and habitat alteration. Agricultural runoff and industrial discharges introduce toxins that can threaten sensitive species, while damming and channelization disrupt natural sediment dynamics, smothering benthic habitats.[10] These impacts reduce biodiversity and weaken ecosystem services. Benthic invertebrates are diverse and abundant, but they are often patchily distributed and relatively difficult to sample, especially when they live in deep subsurface sediment. Protecting benthic animals through sustainable management and restoration is essential for maintaining the ecological integrity of river basins and ensuring the health of aquatic ecosystems.[10-12]

The Oujiang River, in the Nan'ou River Basin, is the second-largest river in Zhejiang Province, China, with a watershed area of 18,028 km^2 and a length of 388 km before flowing into Wenzhou Bay in the East China Sea. Nanxijiang River, located in the northern part of the Oujiang River, is the largest tributary of the Oujiang River, with a watershed area of 2,490 km^2 and a length of 140 km. The rapid economic development of Zhejiang Province has increased pollutant flux into Wenzhou Bay via the Oujiang River.[13] The discharge of industrial and agricultural wastewater and domestic sewage poses a serious threat to the ecological health of the Oujiang Estuary water environment.[13,14] Marine engineering construction and other human activities have also caused considerable damage and threaten the aquatic environment and ecological balance of the Oujiang Estuary.[15] Previous studies have primarily examined the responses of macrobenthos to environmental alterations, reporting that large, ecologically sensitive benthic taxa such as Ephemeroptera, Plecoptera, and Trichoptera have markedly declined or disappeared following the construction of dams on the Oujiang River.[16] These findings underscore the vulnerability of riverine benthic communities to anthropogenic disturbances. In contrast, the Nan'ou River Basin, which comprises the Oujiang River and Nanxijiang River and may represent less-impacted or understudied ecological environments, remains poorly understood. It is, therefore, essential to thoroughly assess the population dynamics of benthic animals in the Nan'ou River Basin to inform effective environmental conservation strategies and ensure the health of this river ecosystem.

In the current study, we investigated the seasonal changes in species composition, spatiotemporal variation characteristics, and population dynamics of dominant macrobenthos in the Nan'ou River Basin, including both Nanxijiang and Oujiang Rivers, through extensive surveys conducted at 12 locations from 2021 to 2023. The study aims to deepen understanding of ecosystem health and resilience in the Nan'ou River Basin by generating detailed data on the distribution, abundance, and ecological interactions of key indicator species, and to determine whether benthic communities are influenced by habitat region and seasonal change, as well as how they are associated with environmental parameters. Our findings provided evidence to support the development of conservation strategies, including the restoration of degraded habitats and the preservation of biodiversity, ultimately contributing to the sustainability of this large river basin.

2. Materials and methods

2.1. Sampling sites

We conducted field investigations in 12 sampling sites (Figure 1) in the Nanxijiang and Oujiang Rivers in the Nan'ou River Basin in December 2021, July 2022, October 2022, and March–April 2023, covering all four seasons. The geographic coordinates of sampling sites are shown in Table S1. R1–R2 (both have two subsites, 1 and 2) is a gravel-bed region of the Nanxijiang River, located in the upper reaches of the Nan'ou River Basin. This section is 80–140 m wide, and the riverbed is mainly composed of sand and pebbles (Table S2). R4–R6 comprise a tidal region of the Nanxijiang River, 200–390 m wide, and the riverbed is mainly composed of sand and pebbles with mud (Table S2). R7–R11 cover a tidal region of the Oujiang River that is 780–1,600 m wide, while R10 and R11 are located at the river mouth. The riverbed is mainly composed of mud (Table S2). The sampling sites cover almost the entire watershed of the Nan'ou River Basin.

2.2. Environmental parameters and measurements

At each sampling site, the water profile properties, water temperature, dissolved oxygen (DO) concentration, pH, turbidity (Nephelometric turbidity unit, NTU), electrical conductivity (EC), oxidation–reduction potential (ORP), and chlorophyll a (Chl. a) concentration, were determined on-site using a multiparameter water quality meter (DS5X, Hach, USA) and a portable water quality meter (HQ40D, Hach, USA).[17] We collected 1 L of surface water using a water sampler (B-1000, ASIN, Japan) and transferred this to a sterile water sample bag, which was stored in a cool box and immediately brought back to the laboratory for chemical analysis within 48 h. Unfiltered water was used for measuring total nitrogen (TN) and total phosphorus (TP) concentrations. TN and TP were measured colorimetrically using a continuous-flow analyzer (San++, SKALAR, Netherlands) following the manufacturer's procedure. Water samples were filtered through a 0.45 μm cellulose membrane filter (diameter: 47 mm; HAWP04700, MF-Millipore, USA),[17] and the concentrations of dissolved heavy metals, including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn), were measured using inductively coupled plasma mass spectrometry (iCAP TQ ICP-MS, Thermo Fisher Scientific, USA). Water samples were filtered using a 0.7 μm glass fiber filter (diameter: 47 mm; Whatman, Kent, UK), and the residues were dried in

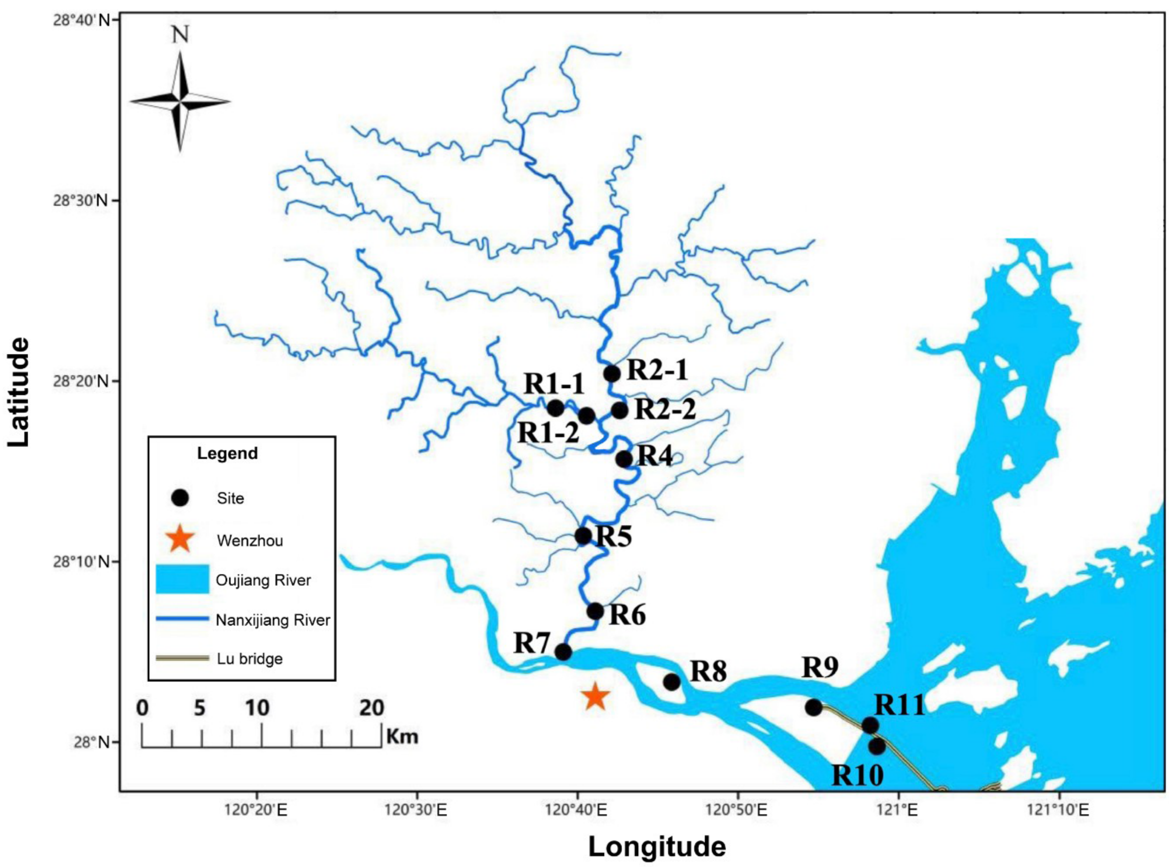

Figure 1. Location of the Nan'ou River Basin in Wenzhou, Zhejiang Province, and the 12 sampling sites in this study. R1–R6 are on the Nanxijiang River, and R7–R11 are on the Oujiang River.

an oven at 105 °C for 24 h and burned in a muffle furnace at 850 °C for 3 h. The weight loss was measured to calculate the particulate organic matter (POM). Suspended solid materials (SS) were measured after the drying procedure, as in the method for POM measurement, but without the final burning procedure.

2.3. Sample collection and species identification

In the gravel-bed region, benthic animals were collected using a Surber sampler (frame size: 30 cm × 30 cm, mesh size: 500 µm; 3-8968-01, ASONE, Japan). At the tidal region, the benthic animals were collected at the river bank and/or river channel, and sediments were collected using an Ekman–Berge grab sampler (0.023 m^2; No. 5141-A, RIGOSHA, Japan). Three replicates were collected for each sampling occasion. At each sampling site, collected samples were combined, thoroughly sieved, and cleaned using a 450 µm stainless steel standard sieve. All collected animals were transferred to 300 mL plastic bottles and fixed with 10% formalin solution prior to species identification and appropriate analysis. The benthic animals were identified and counted under a dissecting microscope in the laboratory.

2.4. Data and statistical analyses

The dominance index of the taxa (D) at each sampling site was calculated using **Equation 1**:[18]

$$D = \left(\frac{N_i}{N}\right)^2 \quad (1)$$

where N_i was the density of taxon i, N was the total individual density. Taxa exhibiting a D greater than 0.02 were designated as dominant.

We quantified the biodiversity of the large benthic animals using the Shannon–Wiener index (H'), calculated using **Equation 2**:[19]

$$H' = -\sum_{i=1}^{R} p_i \times \ln p_i \quad (2)$$

where p_i was the proportion between the individual density of species i and the total density.

We quantified how evenly individuals in a community were distributed among the different sites using the Pielou evenness index (J'), calculated using **Equation 3**:[20]

$$J' = \frac{H'}{H'_{max}} \quad (3)$$

where H' is the Shannon–Wiener index, H'_{max} is the maximum H' which can be estimated from Ln(S), and S is the total species number.

Differences in each parameter among groups were tested using analysis of variance (ANOVA), and post hoc Tukey–Kramer tests were performed when ANOVA indicated a significant difference.[21] The distribution of benthic community structure and the relationship between environmental parameters were analyzed using redundancy analysis (RDA) with 999 permutations,[22] and the most influential environmental parameters were selected using stepwise analysis. These statistical analyses were performed using MATLAB software (vR2021bv, MathWorks, USA)[23] and R software (v4.2.3, R Foundation for Statistical Computing, Austria).[24] The significance level in each statistical analysis was set at $p < 0.05$.

3. Results

3.1. Environmental parameters

Abiotic and biotic environmental parameters among different sampling sites are shown in Figure S1. Water temperatures varied seasonally but were similar among sampling sites, averaging 19.1, 28.7, 21.0, and 16.2 °C in spring, summer, autumn, and winter, respectively. DO concentrations remained at approximately 8 mg L^{-1} throughout the investigation period, with no obvious changes. High pH values (~10) were observed in summer compared to other seasons and showed a slightly increasing trend in R4 in the Nanxijiang River. Both NTU and EC were stable at R1 and R2, but increased at R4 across all four seasons. ORP varied widely among the sites, but without a clear trend. TN, TP, POM, and SS concentrations varied substantially across sites and seasons, were relatively constant at R1 and R2, and increased at R4. TN concentrations reached their maximum, at approximately 10 mg L^{-1} in spring at R7–R11. TP concentrations were relatively high at R6–R11 in summer, and at R5–R9 in winter. Relatively high POM concentrations (~109.3 mg L^{-1}) were mostly observed at R4–R11. The highest SS concentrations reached 2,776 mg L^{-1} at R7 in winter. Chl. a concentrations largely varied among the different sites without clear trends, being on average 2.5, 3.0, 3.1, and 1.4 µg L^{-1} in spring, summer, autumn, and winter, respectively.

Heavy metal concentrations showed marked spatial and seasonal variation (Figure S2). Zn concentrations were the highest among the heavy metals, averaging 14.6, 30.5, 10.9, and 16.7 µg L^{-1} in spring, summer, autumn, and winter, respectively. The highest value (71.0 µg L^{-1}) was observed at R1 in summer. Cd exhibited the lowest concentrations, averaging 0.01–0.02 µg L^{-1} across seasons. Ni values were mostly < 4 µg L^{-1}, occasionally reaching 6.0–9.9 µg L^{-1} at R1 and R4 in summer. Cr showed a relatively constant trend across the different sites, averaging 1.7, 3.0, 2.5, and 1.2 µg

L^{-1} in spring, summer, autumn, and winter, respectively. Relatively high As concentrations (>0.5 μg L^{-1}) were observed in summer and autumn at R5–R10. No clear trends were found in Cu and Pb concentrations during the investigation period, with average concentrations of 1.0–1.8 μg L^{-1} and 0.2–1.6 μg L^{-1}, respectively, across seasons.

3.2. Spatiotemporal variation in benthos species composition

In this study, we confirmed a total of seven phyla and 12 classes of benthic macroinvertebrates in the Nan'ou River Basin (Figure S3). At the phylum level, Arthropoda (46.04%), Annelida (34.29%), and Mollusca (17.05%) comprised 97.38% of the total composition, followed by Nemertea (1.91%) and Platyhelminthes (0.68%); Cnidaria and Echinodermata together comprised 0.01% only (Figure S3). At the class level, Insecta (42.25%) was the most dominant group, followed by Oligotrichea (19.99%), Polychaeta (14.21%), and Gastropoda (11.45%); Bivalvia, Crustacea, and Enopla were 5.57%, 3.79%, and 1.91%, respectively; Actinozoa, Clitellata, Ophiuroidea, Turbellaria, and Sipunculida comprised < 1% of the total (Figure S3). Species compositions largely differed among regions between the Nanxijiang and Oujiang Rivers (Figure 2). At the phylum level, Arthropoda (74.89%) was the most dominant group in the gravel-bed region of the Nanxijiang River, whereas Annelida (64.55–89.02%) was the most dominant in the tidal region of both Nanxijiang and Oujiang Rivers. At the class level, animal compositions differed among the three regions: Insecta (74.89%) and Oligotrichea (85.57%) were most dominant in the gravel-bed and tidal regions of the Nanxijiang River, and Polychaeta (55.01%) was most dominant in the tidal region of the Oujiang River.

Seasonal variation in benthos compositions in the Nan'ou River Basin is shown in Figure 3. At the phylum level, Arthropoda (48.28%) was the most abundant, followed by Annelida (26.57%) and Mollusca (23.13%) in spring; Arthropoda (42.59–43.29%) and Annelida (44.75–47.69%) were the most abundant taxa in both summer and winter; and Arthropoda (52.20%) and Mollusca (35.92%) were dominant in autumn. At the class level, Insecta (39.67–48.31%) dominated throughout the year compared to the other groups. Oligotrichea (24.71–36.89%) and Polychaeta (10.77–19.90%) were the second- and third-most dominant groups in summer and winter, respectively. The second- and third-most dominant classes were Polychaeta (20.52%) and Gastropoda (18.09%), respectively, in spring; and Gastropoda (21.92%) and Bivalvia (14.00%), respectively, in autumn.

We clarified the spatial and temporal variations of the dominance index (*D*) at the level of 32 families (Table 1). Naididae and Tubificinae were the most consistently dominant families throughout the year, although they showed distinct spatial and temporal distribution patterns. In the gravel-bed region of Nanxijiang River, Crambidae had the highest *D* value (0.15–0.16) in summer and autumn. *D* values were 0.14 for Baetidae, Chironomidae, and Orthocladiinae in spring, autumn, and winter, respectively, followed by Pleuroceridae (0.13) in autumn. In the tidal region of Nanxijiang River, Naididae had high *D* value (0.44–0.50) throughout the year at R5, and high Naididae *D* values (0.48–0.50) were also observed in autumn and winter at R6; a *D* value of 0.50 was also confirmed at R5 for Palaemonidae in autumn. Of all the test sampling sites, the highest *D* value (0.72) for Tubificinae was observed in spring at R7 in the tidal region of the Oujiang River; *D* values of 0.50 and 0.32 were observed for Corophiidae at R8 in summer and Dotillidae at R9 in autumn, respectively.

Although the ANOVA test showed no statistical differences in both population density and taxon richness among seasons ($n = 65$, $F_{3,61} = 2.3$, $p > 0.05$ for population density; $n = 65$, $F_{3,61} = 0.42$, $p > 0.05$ for taxon richness) (Figure S4), we found significantly different population density and taxon richness among different regions ($n = 65$, $F_{3,61} = 3.98$, $p < 0.05$ for population density; $n = 65$, $F_{3,61} = 30.17$, $p < 0.05$ for taxon richness) (Figure S5). Therefore, we evaluated population diversity and evenness among different regions. On average, values of the Shannon–Wiener index *H'* were 2.1 and 0.9 in the gravel-bed (R1–R2) and tidal regions (R4–R6) of the Nanxijiang River, and 1.0 and 2.0 in the tidal (R7–R9) and mouth (R10–R11) regions of the Oujiang River, respectively (Figure 4A). A post hoc Tukey–Kramer test showed significantly lower *H'* ($n = 9$, $p < 0.05$ for all) in the tidal regions of both Nanxijiang and Oujiang Rivers compared to the gravel-bed region of the Nanxijiang River and Oujiang River Estuary, whereas there was no significant difference ($n = 9$, $p > 0.05$) between the two tidal regions of the Nanxijiang and the Oujiang Rivers (Figure 4A). In contrast, the Pielou index *J'* varied within a similar range, averaging 0.64–0.75 across regions, with no statistically significant differences (ANOVA, $n = 9$, $F_{3,5} = 1.38$, $p > 0.05$) (Figure 4B).

3.3. Relationships among species, season, and habitat

The ordination biplots showed no clear pattern across seasons (Figure 5). RDA1 and RDA2 explained 40.23% and 21.78% of the total variance, respectively, cumulatively accounting for 62.01 % (Figure 5). Insecta, Oligochaeta, and Polychaeta exhibited the most varied distribution patterns compared to other taxa. Stepwise analysis selected four of 18 environmental parameters in the multiple model that

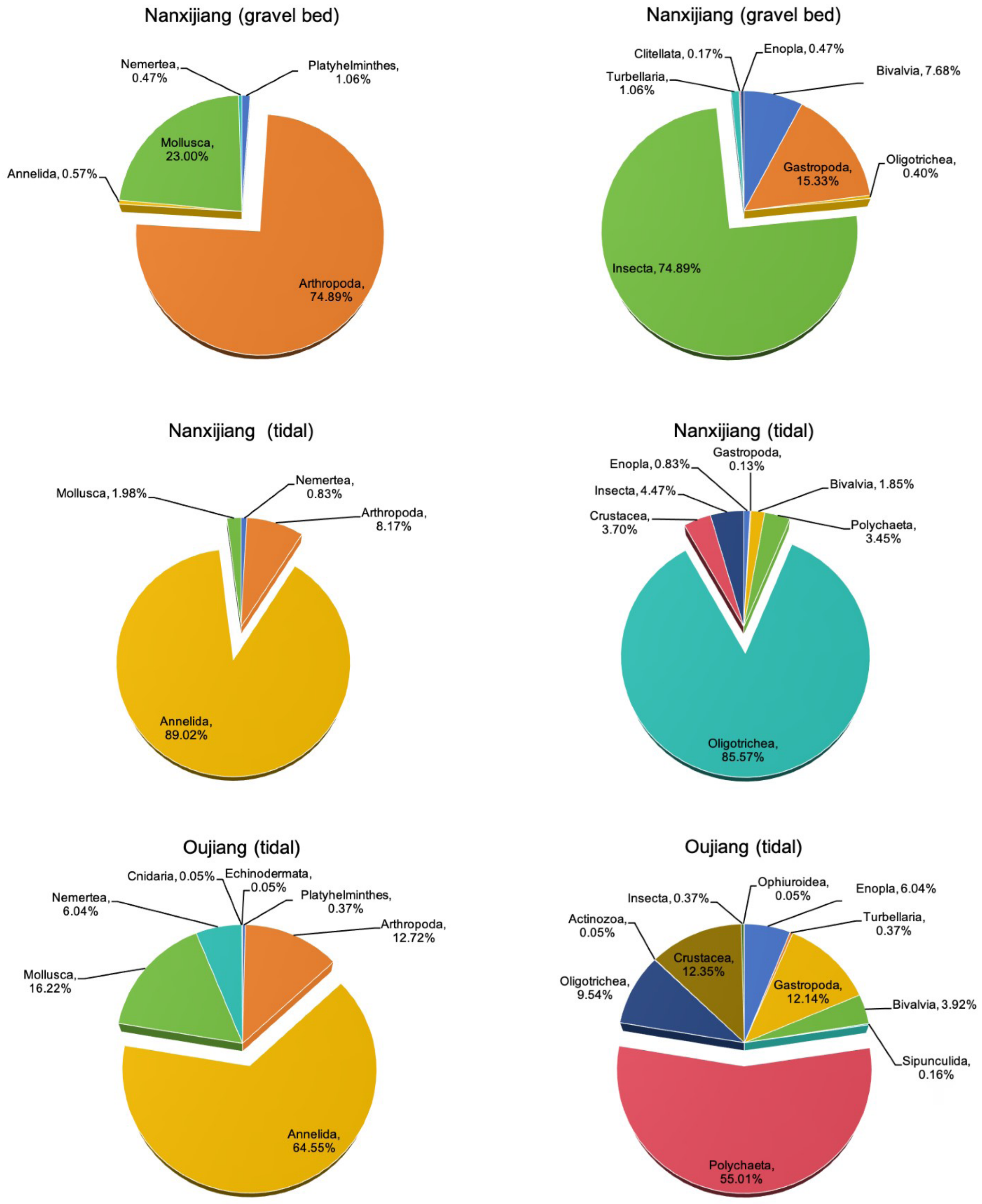

Figure 2. Composition of large benthic animals in the gravel-bed and tidal regions of the Nanxijiang River, and in the tidal region of the Oujiang River. Left panels represent the phylum level, and right panels represent the class level.

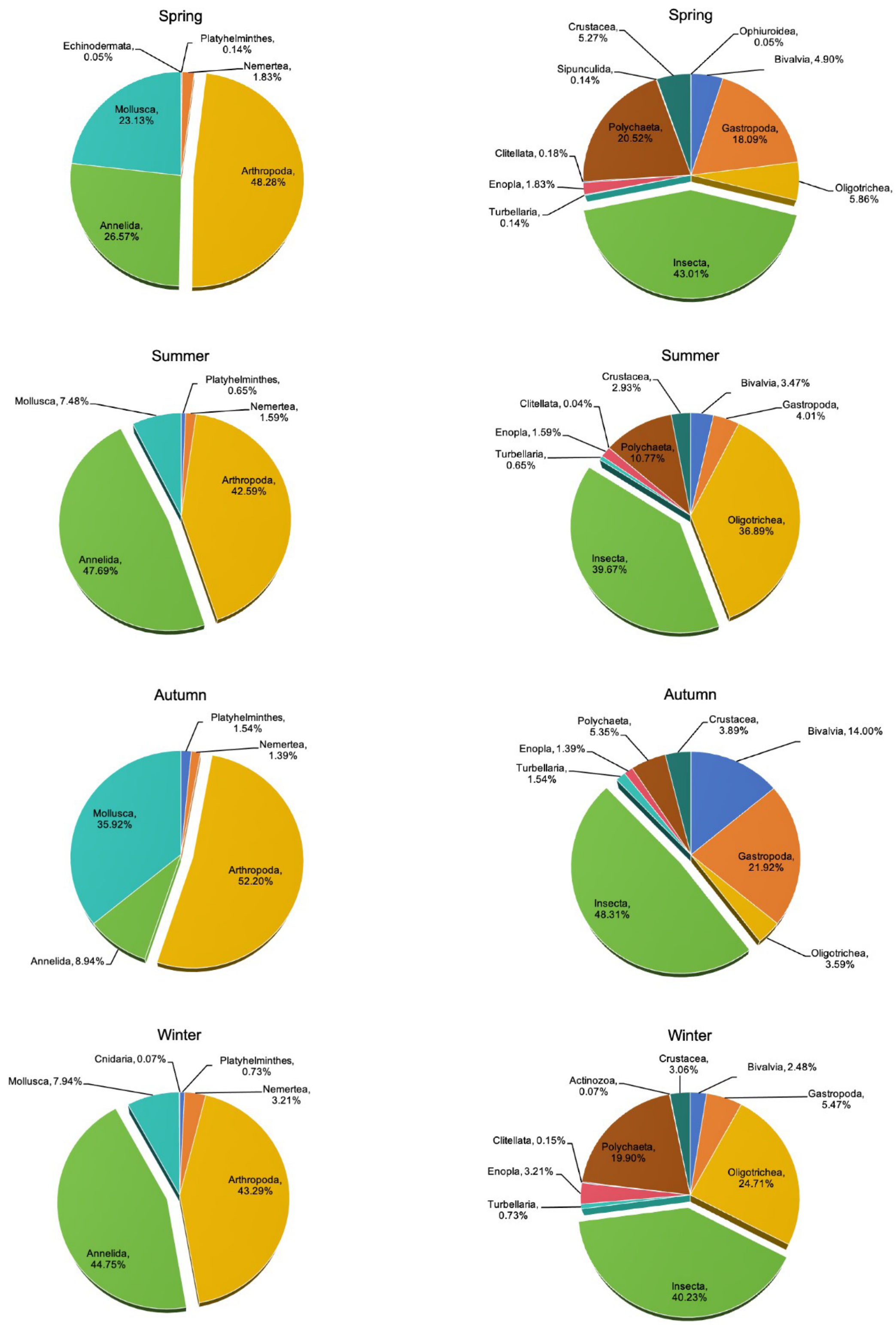

Figure 3. Seasonal changes in the composition of large benthic animals in the Nanxijiang and Oujiang Rivers. Left panels represent the phylum level, and right panels represent the class level.

Table 1. Dominance index (*D*) of the large benthic animals in the gravel-bed (R1–R2) and tidal regions (R4–R6) of Nanxijiang River, and tidal region (R7–R11) of Oujiang River

Sampling site	Dominant species		Spring	Summer	Autumn	Winter
	Phylum	Family				
R1-1	Arthropoda	Baetidae	0.07	–	–	–
		Chironomidae	0.02	–	0.12	–
		Orthocladiinae	0.06	–	–	–
		Philopotamidae	–	0.10	–	–
		Tanipodinae	–	0.02	–	–
	Mollusca	Cyrenidae	–	0.02	0.02	–
		Lymnaeidae	–	–	0.03	–
		Pleuroceridae	–	–	0.04	–
R1-2	Arthropoda	Baetidae	0.14	–	–	0.02
		Chironomidae	0.07	–	0.14	0.03
		Heptageniidae	–	0.02	–	–
		Orthocladiinae	0.02	–	–	0.14
		Psephenidae	–	0.09	–	–
		Simulidae	–	–	–	0.02
	Mollusca	Lymnaeidae	–	–	0.06	–
		Pleuroceridae	0.02	–	0.13	–
R2-1	Arthropoda	Crambidae	–	0.15	–	0.09
		Elmidae	–	–	0.02	–
		Psephenidae	–	0.07	–	–
	Mollusca	Cyrenidae	–	–	0.08	–
		Pleuroceridae	0.08	–	–	–
R2-2	Arthropoda	Crambidae	–	–	0.16	–
		Elmidae	0.05	–	0.04	–
		Heptageniidae	–	0.02	–	–
		Psephenidae	0.05	–	–	–
		Tipulidae	–	0.05	–	–
R4	Annelida	Nereididae	0.54	–	–	0.11
	Arthropoda	Dolichopodidae	–	0.22	–	–
		Dotillidae	0.03	–	–	–
	Mollusca	Corbiculidae	–	0.03	–	0.18
R5	Annelida	Naididae	0.45	0.44	0.50	0.45
	Arthropoda	Dotillidae	0.33	0.07	–	–
		Dolichopodidae	–	0.02	–	–
		Palaemonidae	–	–	0.50	–

(*cont'd...*)

Table 1. (*Continued*)

Sampling site	Dominant species		Spring	Summer	Autumn	Winter
	Phylum	Family				
R6	Annelida	Capitellidae	–	0.30	–	–
		Naididae	0.32	0.21	0.48	0.50
	Arthropoda	Ocypodidae	–	0.02	–	–
		Dolichopodidae	0.14	0.02	–	–
		Dotillidae	–	–	0.04	–
R7	Annelida	Capitellidae	0.02	0.04	–	–
		Phyllodocidae	–	–	0.06	–
		Spionidae	–	–	0.06	–
		Tubificinae	0.72	0.22	0.50	0.25
R8	Annelida	Capitellidae	0.03	0.04	0.07	–
		Tubificinae	0.11	0.14	0.14	–
	Arthropoda	Corophiidae	–	0.50	–	–
		Leuconidae	–	–	0.04	–
		Mysidae	0.15	–	–	–
R9	Annelida	Capitellidae	–	0.13	0.06	0.18
		Leuconidae	0.02	–	–	–
		Nephtyidae	0.08	0.28	–	–
		Pilargidae	0.03	–	–	–
	Arthropoda	Corophiidae	–	0.04	–	–
		Dotillidae	–	–	0.32	–
	Mollusca	Assimineidae	0.11	–	–	–
R10	Annelida	Capitellidae	–	–	0.18	0.36
		Spionidae	0.16	–	–	0.03
	Mollusca	Amphibolidae	–	0.12	–	–
R11	Annelida	Capitellidae	0.09	–	–	–
		Nephtyidae	0.03	0.03	0.14	0.04
		Spionidae	–	0.03	–	–
	Arthropoda	Corophiidae	–	–	0.06	–

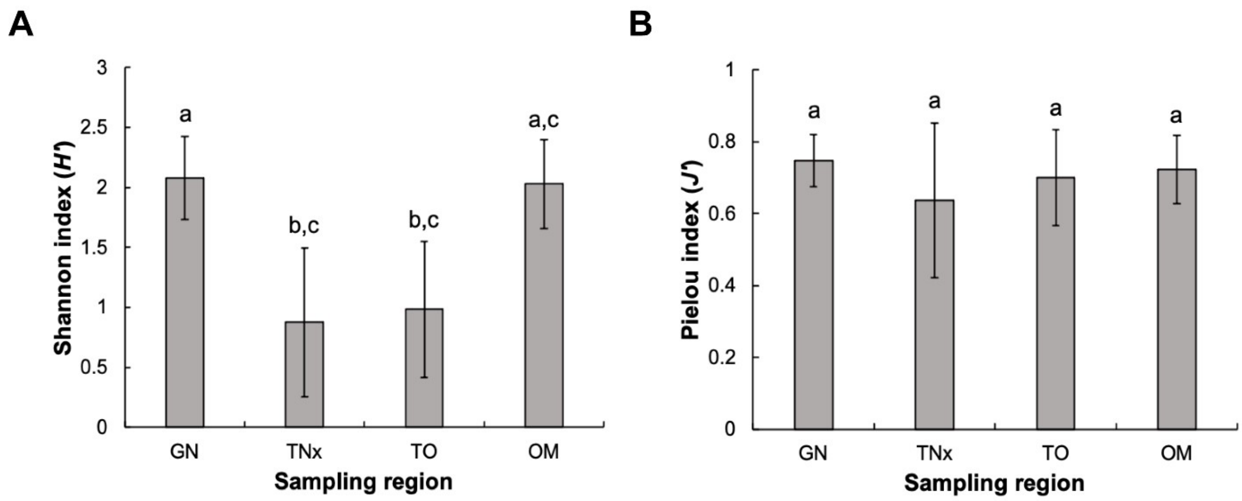

Figure 4. Shannon–Wiener index (*H'*) (A) and Pielou evenness index (*J'*) (B) of large benthic animals among four investigated regions in the Nanxijiang and Oujiang Rivers. Notes: The same letters above boxes indicate no significant differences among groups at the significance level of $p < 0.05$.
Abbreviations: GN: Gravel-bed region of Nanxijiang River; OM: Oujiang River Mouth; TNx: Tidal region of Nanxijiang River; TO: Tidal region of Oujiang River.

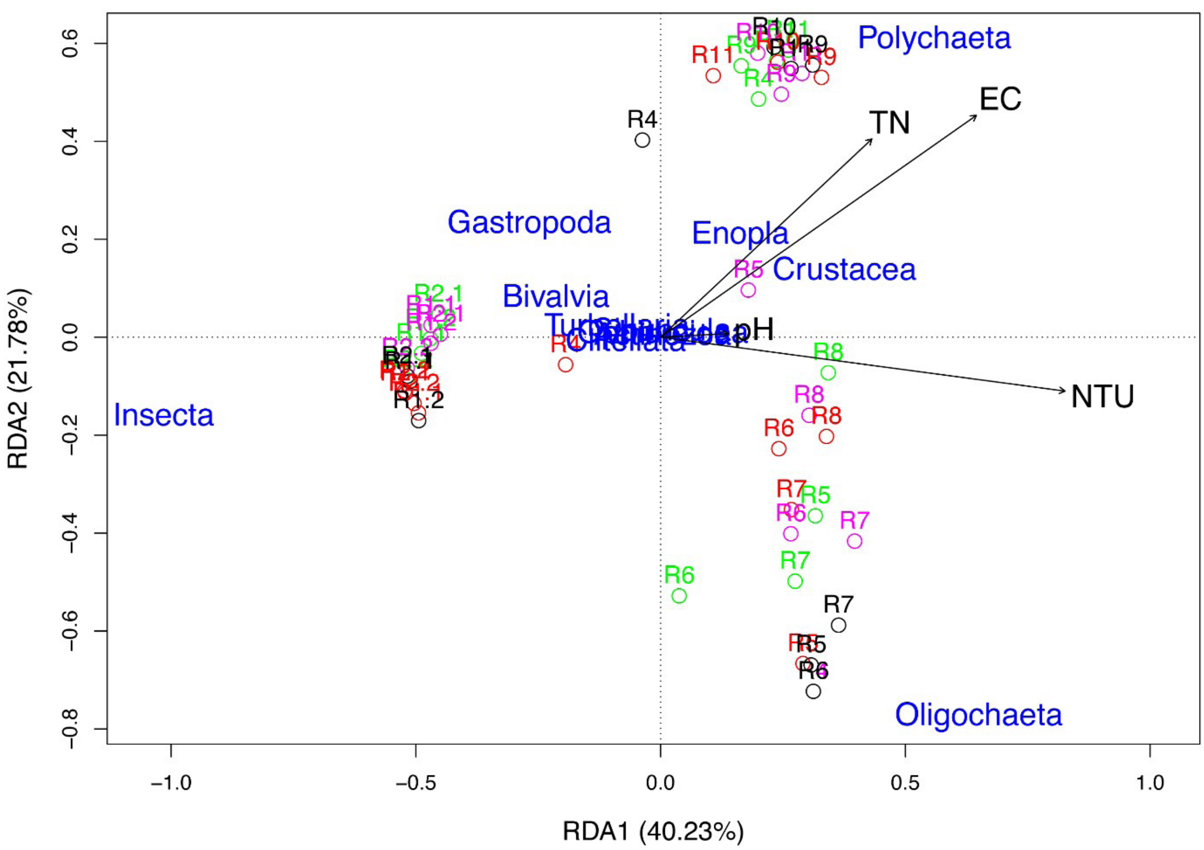

Figure 5. Ordination biplots showing the relationships among species, season, and habitat. The color coding indicates the seasons: green, spring; red, summer; magenta, autumn; and black, winter. The annotations for the sampling site are shown in Table S1. Arrows indicate the selected environmental parameters that influence taxa distributions in the multivariate linear model, identified through stepwise analysis.
Abbreviations: EC: Electrical conductivity; NTU: Nephelometric turbidity unit; RDA: Redundancy analysis; TN: Total nitrogen.

effectively influence species distribution patterns (Figure 5). Among the tested parameters, TN, EC, and NTU were much more influential compared to pH, whereas no heavy metals showed regulatory effects.

4. Discussion

4.1. Benthic community composition and biodiversity

In the Nan'ou River Basin, seasonal variations in water temperature are consistent with typical temperate river systems, and the stability of DO concentrations indicates well-oxygenated conditions, which are critical for supporting diverse aquatic organisms. The abrupt changes in biochemical and nutritional conditions, including NTU, EC, TN, TP, POM, and SS, from the R4 region suggest a shift in environmental conditions from the tidal region of the Nanxijiang River. In the investigated regions, we found seven phyla, 12 classes, and 32 families of benthic macroinvertebrates. These were dominated by Arthropoda, Annelida, and Mollusca, which collectively accounted for 97.38% of the benthic composition, reflecting a typical riverine macroinvertebrate assemblage adapted to diverse habitats.[25,26] Previous studies have shown that Arthropoda, Annelida, and Mollusca, followed by Cnidaria and Echinodermata, are the five most dominant taxonomic groups in both shallow and deep waters.[25,27] The spatial differences in community composition between the gravel-bed and tidal regions of the Nanxijiang and Oujiang Rivers underscore the role of habitat type in shaping benthic assemblages. One of the central findings of this study is the predominant role of spatial factors over temporal ones in structuring benthic communities in the Nan'ou River Basin. Significant differences in population density and taxon richness were evident among regions but not across seasons, as consistently supported by both ANOVA and RDA analyses. This pattern indicates that spatial drivers, particularly habitat heterogeneity and anthropogenic pressures, may be the primary determinants of the distribution of benthic biodiversity in this system. In contrast, seasonal fluctuations appear to have comparatively limited influence on overall community structure and diversity metrics. The higher biodiversity in the gravel-bed region of the Nanxijiang River, compared to tidal regions in both the Nanxijiang and Oujiang Rivers, indicates greater species diversity and ecosystem stability in upstream habitats, likely due to lower pollution levels and more diverse microhabitats.[28-30] Benthic environments were strongly associated with significant increases in taxonomic diversity, abundance, and biological cover, enhancing the overall diversity and biomass in aquatic ecosystems.[31]

4.2. Ecological stability and community dynamics

In the Nan'ou River Basin, the Pielou evenness index *J'* remained consistently close to 0.7 among all regions, with no statistically significant differences. These moderate values indicate a relatively stable and balanced ecological community throughout the investigated area. Despite variations in species richness, the distribution of individuals among species was generally even, reflecting a comparatively equitable community structure. In particular, Insecta accounted for the largest proportion, followed by Oligochaeta, similar to results from a previous study.[16] The similarity to previous studies in the Nan'ou River Basin suggests relatively stable ecological conditions or consistent environmental pressures shaping benthic communities over time. Since aquatic insects are hydrologically connected to the river and dependent on the water exchange,[5] the persistence of Insecta across seasons reflects their ecological versatility. Insecta are highly adaptable to a wide range of environmental conditions, and the Nan'ou River Basin often supports heterogeneous habitats, providing ideal niches for aquatic insect larvae, which thrive in well-oxygenated waters and exploit diverse food resources, including detritus, algae, and other organisms. The prevalence of Insecta in the gravel-bed region of the Nanxijiang River, which is characterized by coarser substrates, aligns with the preference of members of this class for such stable habitats. Although various insect taxa are sensitive to water quality, others can tolerate moderate disturbances, making them prevalent in rivers with good to moderate physicochemical conditions. Numerous large aquatic insects spend their lives in the nutrient-rich subsurface gravels throughout the gravel-bed river floodplain.[5,32] Both Insecta and Oligochaeta are often found in freshwater ecosystems, and their dominance reflects a typical benthic community structure in temperate rivers.[33] Most oligochaetes generally prefer standing water or slow-flowing conditions.[34] Oligochaeta are primarily associated with organic pollution and are well known for their tolerance to a broader range of environmental conditions, including high levels of organic pollution, because they use readily degradable organic matter as food.[33,35] Elevated dominance indices of Tubificinae in the tidal region of the Oujiang River, coinciding with relatively high levels of POM and SS values, strongly suggest high environmental tolerance within this family. Members of Tubificinae are well-documented opportunistic indicators of organic pollution.[36,37] In Lake Taihu (China), Oligochaeta and Mollusca have been found to be the most dominant groups due to their strong tolerance to pollution.[38]

In this study, Polychaeta, Gastropoda, and Bivalvia were less dominant in the Nan'ou River Basin. Similarly, the relatively low diversity of Polychaeta, Bivalvia, and Gastropoda was observed in the Pahang Estuary, Malaysia.[39] The dominance of Polychaeta in tidal regions suggests tolerance to fine sediments, higher organic content, and fluctuating salinity, which are characteristic of these zones.[7] In this study, the presence of members of the class Bivalvia has been confirmed in all investigated sections along the Nan'ou River Basin, indicating natural clearance ability in the investigated region. Bivalvia play an important role in aquatic ecosystems as biofilters and bioindicators of environmental quality.[40] Generally, as the water depth increases, a significant presence of Bivalvia species is found; these are well-suited to the middle and lower reaches of the river.[41] Seasonal shifts in other members of the benthic composition, such as Mollusca (peaking in spring and autumn) and Annelida (peaking in summer and winter), can be considered as driven by life cycle patterns and environmental conditions. The relatively high dominance of Gastropoda and Bivalvia in autumn may be linked to favorable food availability. Food availability appears to drive Gastropoda growth more strongly than temperature.[42] In Chongming Dongtan Nature Reserve (China), the density of Bivalvia in autumn was significantly higher than that in spring.[43] Seasonal variation in growth in Bivalvia from sub-Arctic Greenland is linked to food availability.[44] Shell and tissue growth in Bivalvia were significant, with differences occurring only among food levels.[45]

4.3. Environmental parameters shaping the benthic community

The RDA results highlight differences in nutrient composition among the investigated regions. RDA identified TN, EC, and NTU as the primary variables related to benthic species distribution, consistent with their typical interpretation as indicators of nutrient status and sediment dynamics. The relationship between benthic composition and environmental parameters may differ among ecosystems owing to complex causality.[46,47] In the Hun-Tai River (China), TN has been identified as a significant parameter influencing macroinvertebrate community composition.[48] In Lake Baiyangdian (China), a study of the spatial and temporal distribution of macroinvertebrates identified TN and transparency as the main factors affecting spatial distribution.[49] Water temperature, water depth, and TN concentrations influence benthic macroinvertebrates, with each factor contributing approximately 20%.[16] Since water temperatures were similar among different sites in the Nan'ou River Basin (Figure S1), water temperature appeared to have no influence on benthic diversity. The absence of a clear seasonal pattern in ordination biplots further supports the finding that spatial environmental gradients are more critical than seasonal changes in shaping community structure. The RDA also indicated that heavy metals did not influence species distribution in the Nan'ou River Basin. Spatial and temporal variability in heavy metal concentrations, along with the absence of clear trends, highlights the complex dynamics of pollutant distribution. This suggests that, while heavy metals are present, their concentrations may be too low to alter benthic community structure in Nan'ou River Basin. Generally, benthic animals can tolerate high concentrations of heavy metals. For example, the ranges of heavy metal concentrations in benthic macroinvertebrates (e.g., Unionidae, Baetidae, Gerridae) collected from River Isiukhu (Kenya) were 21.93–46.64 $\mu g\ g^{-1}$ for Zn, 1.11–6.54 $\mu g\ g^{-1}$ for Cu, 0.22–1.61 $\mu g\ g^{-1}$ for Cr.[50] Additionally, Mollusca in the Tigris River (Turkey) can survive at Cu and Ni concentrations of 58 and 200 $\mu g\ L^{-1}$, respectively.[51] These heavy metal concentrations were one to two orders of magnitude higher than those in the Nan'ou River Basin. The high dominance of pollution-tolerant taxa, such as Naididae, in tidal regions suggests ongoing environmental stress,[52,53] warranting further investigation into the sources of organic pollution. Future research should focus on long-term monitoring of bioaccumulation in benthic organisms and their transfer through food webs. Integrating molecular techniques, such as metabarcoding, may provide finer resolution of species composition and detect cryptic taxa, enhancing our understanding of biodiversity patterns.

5. Conclusion

This study examined spatiotemporal variations in macrobenthos communities in the Nanxijiang and Oujiang Rivers (Nan'ou River Basin) from 2021 to 2023, analyzing species composition, richness, and ecological interactions to evaluate ecosystem health. We found that spatial environmental gradients shaped benthic community structure more than seasonal changes, with distinct assemblages in the gravel bed of the Nanxijiang River compared to the tidal regions of the Nanxijiang and Oujiang Rivers, underscoring the role of habitat type. We observed relatively consistent species evenness across regions, despite differences in species richness. Environmental parameters, such as TN, EC, and NTU, were highly associated with patterns in benthic community distribution and might be linked to nutrient and sediment conditions. These novel findings highlight the overriding role of spatial habitat heterogeneity in structuring benthic communities in subtropical tidal-influenced rivers. They point to opportunities for targeted biomonitoring of bioaccumulation in benthic organisms to trace sources of organic pollution, as well as for detailed investigations into trophic transfer within food webs to assess risks of pollution propagation. Such efforts can ultimately support enhanced long-term ecological health and biodiversity conservation in the basin.

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Conflict of interest

The authors declare they have no competing interests.

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Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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