Relationships between characteristics of macrobenthic assemblages and environmental variables in the Heihe River Basin, China

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ABSTRACT

To clarify the characteristics of macrobenthic assemblages and their response to the aquatic environment in the upper and middle reaches of the Heihe River, water quality, sediments and macrobenthos measurements were conducted in the summers of 2018 and 2019. The results showed that 50 species of macrobenthos were identified, belonging to 3 phyla, 7 classes, 15 orders and 32 families, mainly arthropods (37 species) and mollusks (11 species). *Artyroneta aquatica*, *Chlazzioni* sp., dragonfly nymphs, *Palaemon modestus*, *Radix auricularia*, *Cyraulus albus* and *Suecinea* sp. were the dominant species in the whole study region; most of these are pollution-tolerant and moderately pollution-tolerant species. The macrobenthos density and biomass ranged from 10 to 577 ind./m² and from 0.0907 to 50.0562 g/m², respectively, showing high spatial heterogeneity. Predators were the main functional feeding group. One-way analysis of variance clarified that Margalef’s index and the Shannon–Wiener index differed significantly among the spatial areas (P < 0.05). Canonical correspondence analysis showed that the spatial heterogeneity of the macrobenthos was affected by the water temperature and the total nitrogen and total phosphorus in sediments (P < 0.05).

Key words: environmental parameters, Heihe River Basin, microbenthic organisms, spatial structure

HIGHLIGHTS

- The spatial variation community structures and species diversity of microbenthic in the Heihe River Basin (HRB) of China were investigated.
- Analyzed the intensive fluctuations along the way of macrobenthic assemblages and their response relationship with environmental parameters in the HRB.
- Natural environment factors and anthropogenic activities maintained the diversity and stability of macrobenthic communities of the HRB.

INTRODUCTION

The Heihe River Basin (HRB) is the largest inland river in the Hexi region of China and is located in the central part of the Hexi Corridor in the arid region of northwestern China. The competition for water between the economy and ecosystems is considered to be representative of all inland river basins around the world, including the Aral Sea Basin (Feng et al. 2001). The ecosystem health of the river is particularly relevant to economic growth and social progress at both regional and larger scales (Richard 2000; Pukšec et al. 2019). However, in recent years, with population growth, the expansion of industrial and agricultural activities, and global climate change (Li 2014; Wen et al. 2018), severe deterioration of the water and ecological environment of the HRB has occurred, especially in the upstream and midstream regions (Cheng et al. 2014). This deterioration in the upstream area has largely been caused by local anthropogenic activities, including grassland reclamation, sand mining activities and cascade hydropower development. As a result, the continuity of the river ecosystem has been damaged, and the physicochemical characteristics of the water body, medium transport patterns and cumulative effects along the river have been greatly changed. Because of the population density in the midstream region, the quantity and quality of the water have been primarily affected by industrial and agricultural sewage and excessive development of oases, which have had a series of impacts on the succession of the water environmental system and river ecological health (Feng et al. 2001; Chen et al. 2004; Hao et al. 2014). As a result, many natural oases have disappeared, and the amount of water entering the downstream area has significantly decreased. This has led to prominent ecological problems such as the incomplete development...
of the riverbed structure, a decrease in biodiversity and a decline in ecological function (Li et al. 2000; Burford et al. 2007; Feld & Hering 2007). Hence, a discussion of water ecological health in the upper and middle reaches of the HRB is very important. The critical factors of ecosystem health may be defined, and then suitable ecological restoration, management and regulation practices may be established to reduce the effects of deterioration. The physicochemical parameters of water bodies reflect the ecological health status of rivers instantaneously, while the impact of natural factors and human activities on water ecology is a long-term cumulative process (Chen et al. 2019). However, ecological indicators are an effective way to characterize river ecosystem health and can more comprehensively and objectively indicate the long-term changes in aquatic ecology (Peng et al. 2013).

Macrobenthos are major groups of organisms living in benthic river environments. Due to their position at the sediment–water interface and their relatively long and sedentary life, macrobenthos are sensitive to environmental perturbations and are easily collected (Peng et al. 2014). Macrobenthos have been considered to be effective ecological indicators for evaluating benthic environmental health (Tong et al. 2013; Keeley et al. 2014). Macrobenthos that inhabit the sediment–water interface are important components of river benthic systems, and their composition and dynamics play an important role in the biodiversity, energy flow and material cycling of river ecosystems (Covich et al. 2004). They exhibit heterogeneous spatial patterns that coincide with the heterogeneity of both biotic and abiotic factors (Chen et al. 2015). The structure and spatial patterns of macrobenthic communities are affected by many environmental factors, such as habitat characteristics, nutrient concentration, sediment quality and biological factors (Chen et al. 2006; Zbikowski & Kobak 2007; Lods-Croz et al. 2010). The spatial distribution of macrobenthos and their relationship with environmental variables have been extensively investigated. The study of the response relationship between the macrobenthic assemblages and the aquatic environment not only provides powerful information for the interpretation of cumulative effects but also has important guiding significance for maintaining the dynamic balance of the material cycle, energy flow and information transmission of aquatic ecosystems, as well as improving the understanding of watershed ecosystem management, protection and restoration.

As an important part of maintaining the structure and function of aquatic ecosystems, macrobenthos are an important medium to regulate or modify the physical, chemical and biological evolution of the whole aquatic ecosystem and have an important impact on the decomposition and circulation of nutrients in the aquatic ecosystem (Jiang et al. 2009; Gu et al. 2017). Their species composition, diversity, spatial distribution and seasonal dynamics can all reflect the changing laws of aquatic ecosystems and then characterize the health level of the water environment (Silvia et al. 2016). In recent years, research on the ecological effects of macrobenthos in monitoring the health of the ecological environment and its response to environmental factors has been widely considered by scholars.

Some studies showed that the results of a water quality evaluation showed the same change trend according to the comprehensive water quality identification index method and the macrobenthos BI index method; however, due to the differences in the two assessment methods at the spatial–temporal scale, the evaluation of water bodies based on the physicochemical index was better than the biological assessment result of water quality (Gu et al. 2017). The trophic state index was significantly positively correlated with the density of macrobenthos and significantly negatively correlated with biomass, reflecting that the macrobenthos community was gradually dominated by pollution-tolerant species of small individuals as the trophic level increased (Cai et al. 2010). In addition, Pielou’s evenness index and Margalef’s species richness index were significantly negatively correlated with the trophic state index, indicating that the community structure of macrobenthos tended to be simplified with increasing trophic level (He et al. 2020).

Studying the response relationship between macrobenthic organisms and environmental parameters is of great significance not only to maintain the dynamic balance of the aquatic ecosystem but also to provide a critical scientific basis for the protection of the macrobenthos community and the management of watershed aquatic ecosystems, which is also the foundation of river ecology research and has always been a hot topic. A previous study determined the spatial variation in macrobenthic assemblages in the HRB by comparing historical survey results obtained from field investigations and concluded that microbenthic organisms changed greatly over time due to natural environmental variables and human disturbance (Li et al. 2001). However, research on long-term variations in the macrobenthic community structure of the HRB is lacking, as most research has focused on the response of zooplankton and phytoplankton community patterns to aquatic ecological health (Li et al. 2000; Chen et al. 2004; Hao et al. 2014; Ning et al. 2017).

Therefore, based on the needs of river ecosystem structure and function management in the HRB, in the present study, an ecological investigation of macrobenthos, the water body and sediment was conducted in a typical cross-section of the upper and middle reaches of the HRB. In addition, the community structure of macrobenthos and their response relationship with
environmental parameters were analyzed, the impact of ecological environmental differences in different regions on macro-benthic assemblages was clarified and the key driving environmental variables that affected the heterogeneity of macrobenthic organisms were revealed. To analyze and assess the river ecological health status more accurately, basic data and technical support for the protection and restoration of river ecological management should be provided in addition to a decision-making basis for aquatic ecology management.

MATERIALS AND METHODS

Study area and setting of sampling stations

The Heihe River (96°42′–102°04′E, 37°45′–42°40′N) is the second-largest inland river in the arid region of Northwest China, originating from the northern edge of the Qilian Mountains. It has a drainage area of $1.45 \times 10^4$ km$^2$, and the total length of the mainstream is approximately 821 km. The river consists of three parts, namely, the upper mountainous area (the source of the river), the middle oasis area (incorporating towns such as Zhangye and Jiuquan) and the lower terminal arid area near Ejina (Li et al. 2000). In this study, the upstream and midstream areas of the HRB were selected as the study area (Figure 1). The upstream area, with an elevation of 2,000–5,000 m, is the water conservation area in the Qilian Mountains and has a mean annual temperature of −5 to 4 °C. At elevations above 4,000 m, the vegetation is very sparse and is dominated by cushion plants. Meadows and shrubs occur below 3,300 m. The mean annual precipitation exceeds 350 mm, and the mean annual water resource availability is $1.6 \times 10^9$ m$^3$. Eight cascade hydropower stations were developed successively, creating the main runoff-producing area in the HRB. The cultivated oasis area in the midstream region is dominated by irrigated farmland and is rich in light and heat resources. The mean annual temperature in this subbasin is approximately 3–7 °C. The mean annual precipitation ranges from 50 to 150 mm, and the mean potential evaporation rate is approximately 1,400 mm, making this the main utilization area of Heihe River resources (Li et al. 2000).
As a typical inland river, the Heihe River is supplied by surface runoff, ice and snow meltwater, and groundwater formed by precipitation, among which atmospheric precipitation (90%) is the main source (Li et al. 2001; Yang et al. 2011; Ning et al. 2017). Changes in temperature have an important effect on the survival of macrobenthos and also affect the concentration of other physicochemical indicators in water. The survival rate of macrobenthos is low, and they are not easy to capture in the HRB due to the low temperature in spring and winter. In this study, the water, sediment and macrobenthos in the upstream and midstream areas of the HRB were investigated systematically in the summers of 2018 and 2019, and the relationship between the characteristics of the macrobenthos community and environmental variables was analyzed. We selected 19 stations to represent three zones in the upper-middle regions on the basis of physical and geographical characteristics, cascade hydropower development and the expansion of industrial and agricultural activities (Figure 1): six sampling points (stations G1–G6) were established in the upstream tributary area according to the distribution of animal husbandry and enterprise operating conditions, six sampling points (stations H7–H12) were established in the upstream area of the mainstream on the basis of cascade hydropower construction and seven sampling points (stations A13–A19) were established in the midstream area according to industrial and agricultural constructions and administrative division conditions. Among the three zones, sampling points with relatively little disturbance resulting from human activities were located in the upstream region of the HRB.

**Environmental variables**

Water samples were collected 0.5 m below the surface with 1-L prelabeled plastic containers at each station. Water temperature (WT) was measured in situ at each sampling station using a digital display thermometer (model XMD200; precision, 0.1 ° C), and the pH value, total dissolved solids (TDS), dissolved oxygen (DO) and salinity were measured directly in the field by means of a HACH multiparameter water quality analyzer (model DR300). Sediments were sampled using a modified Peterson grab sampler at all sampling stations. Each sediment sample was fully mixed and placed into sealed plastic bags. All samples were placed in iceboxes for immediate transport to the laboratory. In the laboratory, the samples were air-dried, powdered and passed through a 100-mesh sieve (Bao 2008). The total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) in the sediment were determined using the soil physical and chemical standard method introduced by Bao. Each sample was measured three times, and the average value was calculated.

**Macrobenthos sampling**

Macrobenthos were collected from the 19 stations, which were classified into three areas. Samples were collected in two to three replicates from each station using a modified Peterson grab sampler and hand-dip nets. The replicates were pooled and treated as a single sample. The collected samples were filtered and washed with a 60-mesh screen, and the retained materials were placed individually in labeled polyethylene bottles with 10% formalin. The sorted samples were preserved in 4–10% formalin and transported to the laboratory for further analysis. Sorting of the samples was performed in the laboratory, and 75% ethanol was used to fix the clean macrobenthos. All specimens were identified to the species or genus level as far as possible using the relevant identification guides, weighed individually on an electronic balance with 0.0001 g accuracy and then counted (Morse et al. 1994; Liu 1999; Peter 2010). The samples were divided into five functional feeding groups (FFGs): shredders (SH), collector filters (FC), collector-gatherers (GC), scrapers (SC) and predators (PR). The species were also classified into three categories on the basis of their pollution tolerance value (X) (Wang 2003): pollution-tolerant species (X≧7), moderately pollution-tolerant species (5 < X < 7) and sensitive species (X≦3).

**Data analyses**

For each sample, the Shannon–Wiener diversity index (H') (Shannon & Weaver 1963), Margalef's richness index (dM) (Margalef 1957), Pielou's evenness index (J) (Pielou 1966), dominance index (Y) (Yan et al. 2017), total biomass (g/m²), biomass of each taxonomic group, total abundance (ind./m²), abundance of each taxonomic group, total species number and species number of each taxonomic group were calculated. The three biodiversity indexes were calculated according to the following formulas (Equations (1)–(5)), and the dominance index was calculated on the basis of Equation (4). Data from the same
station collected during two different cruises were averaged for every period.

\[ H = - \sum_{i=1}^{s} \left( \frac{n_i}{N} \right) \log_2 \left( \frac{n_i}{N} \right) \]  

(1)

\[ d_M = \frac{(S - 1)}{\ln N} \]  

(2)

\[ I = \left( - \sum_{i=1}^{s} \left( \frac{n_i}{N} \right) \log_2 \left( \frac{n_i}{N} \right) \right) / \log_2 S \]  

(3)

\[ Y = \left( \frac{n_i}{N} \right) \times f_i \]  

(4)

where \( N \) is the total number of individuals, \( n_i \) is the number of individuals of the \( i \)th species, \( f_i \) is the frequency of occurrence of the \( i \)th species and \( S \) is the number of individuals of macrobenthos. When \( Y > 0.02 \), a species is considered a dominant species (Yan et al. 2017).

Microsoft Excel and OriginPro were used to complete the statistical analysis of all data and to draw relevant charts. The sampling plots in the HRB were drawn with ArcGIS. Significant variation was determined using IBM SPSS statistics. In addition, in order to improve the accuracy of the experimental data, the interference factors were taken into consideration during the experimental process, and the average value was obtained through multiple measurements. One-way analysis of variance (ANOVA) was used to test differences in the water environmental variables (i.e., WT, pH value, TDS, DO and salinity concentration), sediment variables (i.e., TOC, TP and TN contents) and characteristics of the macrobenthos community among zones.

Canonical ordination was used to assess the relative importance of environmental variables in determining the structural composition of the macrobenthic communities. Canonical correspondence analysis (CCA) and redundancy analysis (RDA) were used to investigate the biological–environmental relationships after detrended correspondence analysis (DCA) was used to determine whether to use CCA or RDA (Feld & Hering 2007). On the basis of the DCA, if the maximum gradient length of the axes was >3 SD, then CCA was determined to be more suitable; if the maximum gradient length of the axes was <3 SD, then RDA was deemed more suitable (Leps & Smilauer 2003). CCA was used to assess the correlations between the macrobenthic assemblages and environmental parameters because in the preliminary DCA, the maximum gradient length of the axes was 3.26. In the CCA, forward selection analyses and Monte Carlo permutation tests were used to identify the important environmental parameters influencing the abundance and distribution of the macrobenthos \( (P < 0.05) \). Before the statistical analyses, species abundance and environmental variables (all environmental parameters except pH) were log10. \( (x + 1) \), which transformed to reduce the heterogeneity of variance. Ordination was run with CANOCO V5.0 software.

**RESULTS**

**Environmental parameters**

During the investigations, the WT in the upper and middle reaches of the HRB ranged from 15.40 to 29.35 °C, with a mean value of 19.53 °C, and the law of spatial change was as follows: midstream > upper mainstream > upper tributary. The ANOVA results showed significant differences \( (P < 0.05) \). The pH value was mainly between 8.76 and 9.10, with a mean of 8.99. The water quality was weakly alkaline, with a smaller change along the way. The ANOVA results showed that the difference in pH was not significant \( (P > 0.05) \). TDS and salinity ranged from 232 to 428 mg/L and from 0.23 to 0.43‰, respectively, with mean values of 312.61 mg/L and 0.31‰, and the variation trends of the two indicators were consistent, all of which were the highest in the midstream, with significant differences between the upstream and midstream \( (P < 0.05) \). The concentration of DO changed greatly, and the variation ranged from 5.72 to 9.00 mg/L, with a mean value of 7.56 mg/L. According to the conventional water quality index evaluation standards of ‘Environmental Quality Standards for Surface Water (GB/T 3838-2002)’ (GB 3838-2002), the water quality of the river belonged to Class I or II, which basically met the standard limit of the functional zone (Figure 2).

The TN content in sediments ranged between 0.08 and 1.08 mg/g, with a mean value of 0.41 mg/g, and the variation trend of the average content in each region was as follows: the midstream area > the upper mainstream > the upper tributary. The
Figure 2 | Distribution characteristics of environmental factors in different regions.
ANOVA results showed that there were significant differences in the spatial distribution ($P < 0.05$). According to the evaluation criteria for TN pollution in sediment formulated by the U.S. Environmental Protection Agency (EPA), the mean TN content in each region was less than 1.00 mg/g, indicating mild pollution. The TP content in sediments ranged from 0.03 to 0.54 mg/g, with a mean value of 0.20 mg/g, and the variation trend of the average content in each region was as follows: the midstream > the upper tributary > the upper mainstream. The ANOVA results showed that the spatial distribution of the TP content was significantly different ($P < 0.05$). According to the evaluation criteria for TP pollution in sediment formulated by the U.S. EPA (Scott et al. 2002), the mean value of the TP content in each region was lower than 0.45 mg/g, indicating mild pollution. The average TOC content in the sediments ranged from 3.48 to 22.08 mg/g, with a mean value of 8.77 mg/g, and the variation trend of the average content in each region was as follows: the upper tributary > the midstream > the upper mainstream. The maximum, minimum and average TOC contents in the tributary in the upper reaches were 20.04, 5.60 and 9.58 mg/g, respectively. The maximum, minimum and average TOC contents in the mainstream in the middle reaches were 20.08, 3.48 and 8.40 mg/g, respectively. The maximum, minimum and average TOC contents in the mainstream in the upper reaches were 13.27, 5.25 and 8.39 mg/g, respectively. The ANOVA results showed that the differences among the regions were not significant ($P > 0.05$) (Figure 2).

**Species composition and distribution of macrobenthos**

During the study period, a total of 50 macrobenthic species were collected from the upstream and midstream of the HRB and identified (Table 1). These species belonged to 3 phyla, 7 classes, 15 orders, 32 families, and 39 genera, including 37 species of arthropods (34 species of Insecta, 2 species of Crustacea, and 1 species of Arachnida), accounting for 74.51% of the total number of species; 11 species of mollusks (10 species of Gastropoda and 1 species of Lamellibranchia), accounting for 21.57%; and 2 species of annelids (1 species of Oligochaeta and 1 species of Hirudinea), accounting for 3.92%. The macrobenthic species statistics at the order level are shown in Table 1.

With respect to the composition of macrobenthic assemblages, arthropods, as the absolute dominant group of macrobenthos, appeared in the upper and middle reaches of the HRB (Figure 3). The number of species in the upstream tributary was 22, and arthropods accounted for 77.27% of the total species number; the number of species in the upper mainstream was 27, and arthropods accounted for 74.07%; and the number of species in the midstream area was 37, and arthropods accounted for 67.57%. The distribution characteristics of macrobenthos with different tolerance degrees are shown in Figure 4. The spatial variation of the pollution-tolerant species was as follows: the midstream (19 species) > the

**Table 1** Macrobenthic community structure of in the upstream and midstream of the Heihe River

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Families</th>
<th>Genera</th>
<th>Species</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthropoda</td>
<td>Insecta</td>
<td>Diptera</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Odonata</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichoptera</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plecoptera</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemiptera</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ephemeroptera</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coleoptera</td>
<td>5</td>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Crustacea</td>
<td>Decapoda</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ampipoda</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arachnida</td>
<td>Araneae</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mollusca</td>
<td>Gastropoda</td>
<td>Basommatophora</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesogastropoda</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lamellibranchia</td>
<td>Veneroida</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Annelida</td>
<td>Oligochaeta</td>
<td>Oligochaeta plesiopora</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hirudinea</td>
<td>Rhynchobdellida</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>7</td>
<td>15</td>
<td>39</td>
<td>50</td>
</tr>
</tbody>
</table>
upper mainstream (11 species) > the upper tributary (9 species). The variation trend of the moderately pollution-tolerant species along the way was as follows: the midstream (15 species) > the upper mainstream (12 species) > the upper tributary (8 species). The variation characteristics of the sensitive species were as follows: the upper tributary (5 species) > the upper mainstream (4 species) > the midstream (3 species).

The dominant species of macrobenthos in the upper and middle reaches of the HRB belonged to widely distributed species (Table 2). The results showed that *Argyoneta aquatica* was the dominant species in the upper and middle reaches of the HRB and the whole study area, with a pollution tolerance value of 3; *Tipulidae* and *Chlaznius* sp. were the dominant species in the tributary and the mainstream of the upper reaches, and their pollution tolerance values were 1.5 and 5, respectively; *Ischnura heterosticta*, dragonfly nymphs, *Palaemon modestus* and *Suecinea* sp. were the dominant species in the midstream area, with pollution tolerance values of 9, 9, 2 and 5, respectively; *Baetis* sp., *Rhantus suturalis* and *Ceraclea tsudai Akagi* were the
dominant species only in the upper mainstream, with pollution tolerance values of 4.5, 8 and 4, respectively; *Dolichus halensis* and *Anisogammarus* sp. were the dominant species only in the upstream tributaries, with pollution tolerance values of 5 and 4, respectively; *Radix Auricularia* and *Cyraulus albus* were the dominant species in the mainstreams of the upper and middle reaches, with pollution tolerance values of 8 and 5, respectively. In terms of the number of dominant species, the upper mainstream had the highest number of dominant species (eight species), followed by the midstream area (seven species), and the upstream tributary had five species. *A. aquatica* was absolutely dominant in all river sections, and the dominance values were between 0.074 and 0.141. The results showed that the complexity and stability of the biological community structure increased with an increasing number of dominant species and decreasing dominance (Chen *et al.* 2019).

**Density and biomass of different groups of macrobenthos**

During the investigation, the spatial variation characteristics of the macrobenthic standing stock in the upper and middle reaches of the HRB increased significantly from the upper reaches to the middle reaches. The total density and biomass of macrobenthos were 85 ind./m² and 1.937 g/m², respectively, and arthropods were the absolute dominant group, with a density and biomass of 76 ind./m² and 1.621 g/m², accounting for 89.21 and 83.70% of the total density and biomass, respectively. The total density and biomass of macrobenthos in the upper mainstream were 126 ind./m² and 4.019 g/m², respectively, and the arthropods accounted for 71.56 and 37.02% of the total density and biomass (density and biomass were 90 ind./m² and 1.488 g/m², respectively). The total density and biomass of macrobenthos in the midstream area were 247 ind./m² and 21.119 g/m², respectively, and the arthropods accounted for 50.81 and 43.25% of the total density and biomass (density and biomass were 125 ind./m² and 9.154 g/m², respectively) (Figure 5).

The ANOVA results showed that the density and biomass of macrobenthos in different groups were significantly different in different river reaches. The total density of macrobenthos and the density of arthropods and mollusks were the highest in the midstream area, followed by those in the upper mainstream, and the lowest in the upstream tributary, while the density of annelids was the highest in the midstream area and the lowest in the upper mainstream. The total biomass and mollusk biomass were the highest in the midstream area, followed by those in the upper mainstream, and the lowest in the upstream tributary, while the biomass of arthropods and annelids was the highest in the midstream area and the lowest in the upper mainstream (Figure 5).
Figure 5 | Density and biomass of different macrobenthos groups (mean ± SD). The different letters in superscripts of the figure indicate relevant significant differences ($P < 0.05$). SD, standard deviation.
The ANOVA results of the density and biomass of macrobenthos with different pollution tolerances showed that the density and biomass of different groups were significantly different in different river reaches. The density and biomass of the pollution-tolerant species, moderately pollution-tolerant species and sensitive species were the highest in the midstream area, followed by those in the upper mainstream, and the lowest in the upstream tributaries. The density \( (F = 5.548, P = 0.015) \) and biomass \( (F = 71.324, P < 0.05) \) of the sensitive species and the biomass of the pollution-tolerant species \( (F = 5.148, P = 0.019) \) were significantly different among the different sections of the upper and middle reaches of the HRB \( (P < 0.05) \) (Table 3).

**Density and biomass of different FFGs of macrobenthos**

With respect to the composition of the FFGs of macrobenthos, there were significant differences in the distribution of the different functional groups in the upper and middle reaches of the HRB (Figures 6 and 7). The density of predators was the highest in each region; the upstream tributary reached 51.83 ind./m² (accounting for 60.98% of the total density), there were 43.83 ind./m² (accounting for 34.79%) in the upper mainstream and the midstream area had 96.71 ind./m² (accounting for 39.75%).

The predators in the upper tributary had the largest biomass (1.15 g/m², accounting for 58.46% of the total biomass), the scrapers in the upper mainstream had the largest biomass (2.10 g/m², accounting for 52.16%) and the biomass of scrapers in the middle mainstream was the highest, reaching 11.40 g/m² (accounting for 53.97%).

The ANOVA results of the different FFGs of macrobenthos showed that the density and biomass of each FFG were significantly different among the different regions \( (P < 0.05) \) of the sensitive species and the biomass of the pollution-tolerant species \( (P < 0.05) \). The density and biomass of each FFG, the predators reached the highest density in the midstream area, the second-highest density in the upper mainstream and the lowest density in the upper mainstream. The highest density of each FFG, the predators reached the highest density in the midstream area, the second-highest density in the upper mainstream and the lowest density in the upper mainstream. The highest number of scrapers was found in the midstream area, and the lowest was found in the upper tributary. The collector-gatherers and the shredders occurred in the highest numbers in the upper mainstream and in the lowest numbers in the upstream tributary. The collector-filterers occurred in the highest numbers in the upstream tributary and in the lowest numbers in the upper mainstream. The highest biomass of each FFG was recorded in the midstream area except for the shredders, which had the highest biomass in the upper mainstream, followed by the midstream area, and the lowest biomass was recorded in the upstream tributary. The scrapers and collector-filterers had the lowest biomass in the upstream tributaries, and the predators and collector-gatherers had the lowest biomass in the upper mainstream.

**Biodiversity characteristics of macrobenthos**

\( H', d_M \) and \( J \) ranged from 1.00 to 3.50, from 0.43 to 3.51 and from 0.62 to 1.00, respectively. The mean values of \( H' \) in the upper tributary, the upper mainstream and the midstream area were 2.03, 2.74 and 2.96, respectively; the mean values of \( d_M \) were 1.28, 2.20 and 2.80, respectively; and \( J \) was 0.84, 0.84 and 0.74, respectively. The ANOVA results for each diversity index showed that \( J \) was not significantly different among each region \( (P > 0.05) \); \( H' \) and \( d_M \) differed significantly among the different regions \( (F = 3.812, P = 0.044; F = 9.398, P = 0.002) \), and the variation trend along the way was basically the same.

### Table 3 | Density and biomass of species with different tolerance levels (mean ± SD)

<table>
<thead>
<tr>
<th>Index</th>
<th>Species</th>
<th>Upstream tributary ((n = 6))</th>
<th>Upper mainstream ((n = 6))</th>
<th>Middle stream ((n = 7))</th>
<th>(F)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (ind./m²)</td>
<td>Pollution-tolerant species</td>
<td>(29 ± 38.69^{b})</td>
<td>(33 ± 56.76^{ab})</td>
<td>(95 ± 58.89^{a})</td>
<td>3.085</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>Moderately pollution-tolerant species</td>
<td>(39 ± 44.72^{a})</td>
<td>(71 ± 107.00^{a})</td>
<td>(95 ± 85.40^{a})</td>
<td>0.749</td>
<td>0.489</td>
</tr>
<tr>
<td></td>
<td>Sensitive species</td>
<td>(17 ± 10.99^{b})</td>
<td>(23 ± 14.75^{b})</td>
<td>(58 ± 35.65^{a})</td>
<td>5.548</td>
<td>0.015</td>
</tr>
<tr>
<td>Biomass (g/m²)</td>
<td>Pollution-tolerant species</td>
<td>(0.471 ± 0.58^{b})</td>
<td>(2.462 ± 4.54^{b})</td>
<td>(7.508 ± 5.24^{a})</td>
<td>5.148</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Moderately pollution-tolerant species</td>
<td>(1.214 ± 1.27^{a})</td>
<td>(1.228 ± 1.85^{a})</td>
<td>(8.127 ± 11.06^{a})</td>
<td>2.223</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>Sensitive species</td>
<td>(0.252 ± 0.14^{b})</td>
<td>(0.330 ± 0.23^{b})</td>
<td>(5.048 ± 1.47^{a})</td>
<td>71.324</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*SD, standard deviation. The same line labeled by different letters in superscripts of the table indicated relevant significant differences \(P < 0.05\).*
The highest value was found in the midstream area, followed by the values in the upper mainstream and the lowest value in the upper tributary. The complexity and stability of the community structure of macrobenthos in the midstream area were higher than those in the mainstream and tributary of the upper reaches, while those in the upper mainstream were higher than those in the upper tributary (Table 4).

Relationship between macrobenthic assemblages and environmental parameters

The DCA results showed that the gradient length of the first ordination axis was the longest, and the maximum gradient length of the axes was 3.26. Therefore, the linear-model CCA was the most appropriate for analyzing the relationships between the macrobenthic assemblages and the environmental parameters. The quadrant containing an arrow in the figure indicates whether a positive or negative correlation exists between an environmental factor and the ordination axis. The degree of the correlation between an environmental factor and the community distribution is shown by the length of the arrow. The correlation between a certain environmental factor and the ordination axis is represented by the angle between the arrow and the sorting axis, with smaller angles indicating a stronger correlation (Leps & Smilauer 2003).

The CCA results showed that 50.3% of the community structure change information was explained by the selected environmental parameters. The eigenvalues of the first two ordination axes were 0.4806 and 0.3256; these axes accounted for 15.11 and 10.24%, respectively, of the variation in the macrobenthic community. The correlation coefficients between species and environmental factors were as high as 0.9729 and 0.9347, which explained 30.04 and 20.35% of the cumulative variation in species and environmental factors, respectively. The total cumulative percentage of the relationship between species and environmental variables for the first two axes was 50.39%, indicating that the relationships between species and environmental parameters could be better reflected by the ordination map (Table 5).
According to the Monte Carlo replacement test, the main environmental variables that could affect the community structure of the macrobenthos in the different zones in the upper and middle reaches of the HRB to the greatest extent were WT, TP and TN ($P < 0.05$), which explained 13.20, 12.50 and 8.60%, respectively, of the spatial variation in the macrobenthic community structure. Among these variables, WT had the largest marginal effects, indicating that this variable was the key environmental factor that affected the characteristics of the macrobenthic organisms ($F = 2.6, P = 0.002$), followed by TP ($F = 2.4, P = 0.002$) and TN ($F = 1.6, P = 0.036$). However, there were no significant correlations between the other environmental variables and the macrobenthic assemblages ($P > 0.05$).
The CCA results for the relationship between the distribution characteristics of macrobenthos and environmental factors at each sample site showed that there was a high correlation between WT, TP, TN and the first-order axis (Figures 8 and 9). As indicated by the order in which each environmental factor entered the CCA and the interpretation rate, the interpretation rates of WT and TP were relatively high, whereas the interpretation rate of TN was relatively low. The distribution of the monitoring points in the upstream tributary and the upper mainstream was opposite to the distributions of WT, TP and TN, while the monitoring points in the midstream were mainly distributed along WT, TP and TN. This indicated that WT, TP and TN were the main factors that affected the community structure of macrobenthos in the upper and middle reaches of the HRB.

CCA revealed the relationships between 50 species of macrobenthos and the environmental factors (Figure 9); the distributions of *I. heterosticta*, dragonfly nymphs, *P. modestus*, *R. auricularia*, *Suecinea* sp., *C. albus* and other species were positively correlated with WT and the TP and TN contents. Most of these species had higher dominance in the midstream.
area and were pollution-tolerant and low oxygen-tolerant species. *Baetis* sp., *R. suturalis* and *C. tsudai Akagi* were the dominant species only in the midstream area and were negatively correlated with WT and the TP and TN contents. *D. halensis* and *Anisogammarus* sp. were mainly found in the upper tributary and were negatively correlated with WT and the TP and TN contents. The pollution tolerance of *Tipulidae*, *Chlaznius* sp. and *A. aquatica* was low; these organisms are sensitive and
clean groups and were negatively correlated with WT and the TP and TN contents and were mainly distributed in the tributary and mainstream of the upper reaches. Species with relatively high pollution tolerance, such as members of Odonidae, Sphaeriidae and Pterodactylidae, were mainly distributed in the midstream area with high human interference intensity and were not found in the natural river section at the source of the HRB, that is, the tributary of the upper reaches.

**DISCUSSION**

The community structure and diversity of macrobenthos were affected by a variety of environmental factors and showed strong spatial divergence (Liu et al. 2016). Macrobenthos mainly inhabit river water–soil interface layers or sediments, and the physical, chemical and biological factors of the water and sediment environments jointly affect the community distribution (Tews et al. 2004; Yan et al. 2005; Cooper et al. 2007; Schneider & Sager 2007). A total of 50 species of macrobenthos were detected in this survey, and arthropods were absolutely dominant in terms of species composition, standing stock and diversity. However, the macrobenthic community structure, different degrees of tolerance and the composition of each FFG had their own distribution characteristics in the upper and middle reaches of the HRB. The number of species is one of the important indicators for measuring species diversity. A total of 22 species were identified in the upper tributary, and 27 species were identified in the upper mainstream. The highest number of species was recorded in the midstream area, i.e., 37 species. Density and biomass are important indicators of the standing stock. In this survey, the standing stock of macrobenthos in the upstream tributary with a simple habitat was significantly lower than that in the midstream area. $H'$ represents the impact of environmental variables on the distribution of macrobenthic communities, and the disappearance of environmentally sensitive groups will lead to a decline in the complexity and stability of the macrobenthic community structure (Gao & Song 2005). $d_M$ reflects the relationship between the macrobenthic community and environmental parameters, and a higher value indicates higher environmental stability (Margalef 1957). $J$ measures the uniformity of the individual distribution of macrobenthic species (Sun & Liu 2004). In the upper and middle reaches of the HRB, $H'$ ranged from 1.00 to 3.30, with a mean value of 2.60; $d_M$ fluctuated between 0.43 and 3.31, with a mean value of 2.13; and $J$ ranged between 0.62 and 1.00, with a mean value of 0.80. From the upper to the middle reaches of the HRB, $H'$ and $d_M$ increased with decreasing altitude, whereas the changing trend of $J$ was relatively stable. The species composition of macrobenthos in the upstream tributary was poor, the stability was poor and the diversity was low; the community structure and stability in the upper mainstream were improved compared with the upstream tributary, as was the diversity. The community structure of the midstream was relatively complex and stable, and the diversity was higher than the upper tributaries and mainstreams, which is consistent with the research results of Li et al. (2001) on the macrobenthic community in the HRB.

The complex environmental conditions of the HRB were the main reason for the stair-wise increase in the community structure and diversity of macrobenthos in the upper tributary, upper mainstream and midstream area. In this study, the macrobenthos diversity in the upstream tributary was significantly lower than that in the upper mainstream and midstream areas. The macrobenthos community not only showed a simple structure and poor stability but also a weak ability to resist external environmental changes and internal population fluctuations. The main reasons for these observations were that this community was influenced by the upstream tributary located in the Qilian Mountains on the northeastern Qinghai-Tibetan Plateau, which has a high altitude (average altitude of 2,783 m) and low temperature, and ice and snow meltwater are the main water supplies. However, the annual accumulated temperature of the river water is affected by the incorporation of ice and snow meltwater, which has a relatively low temperature (the annual average temperature is less than 2 °C). The large slope of the riverbed in the upstream tributary also resulted in the flow velocity which was not conducive to the survival of macrobenthos. In addition, the flow rate and WT have a strong influence on the survival and reproduction of macrobenthos that are suitable for the survival of species that require flowing water and cold temperatures, providing habitats for predators such as Plecoptera and Trichoptera, which are adapted to riparian habitats (Allen et al. 2002; Peng et al. 2013; Chen et al. 2019). However, the spatial heterogeneity of a river habitat and the stability of sediment largely determine the spatial variation of macrobenthos, and the biodiversity of benthic organisms increases with habitat heterogeneity and riverbed stability (Li et al. 2015a). The substrate quality and the stability of gravel and sandstone in the upper tributary were poor, and the riverbed development was incomplete; thus, the distribution and diversity of macrobenthos were relatively poor. In addition, the upper tributary is not strongly influenced by anthropogenic activities, and the water quality is good, which provides living conditions for many clean species, such as members of Tipulidae and Cerambycidae. Other clean indicator species are mainly distributed.
in this river section, and the stability of the riverbed bottom and simple habitat heterogeneity (Li et al. 2000, 2015b) result in low biodiversity, which is similar to the characteristics of inland river systems. The water flow in the upper mainstream is slow, the sediment is easily deposited, the WT and transparency are good, which is conducive to the growth and reproduction of aquatic organisms, and the species number and quantity of zooplankton and aquatic vegetation greatly increased, providing a material basis and suitable habitat for macrobenthos with different living habits (Li et al. 2001). In this area, with a high intensity of human activities such as agricultural irrigation, industrial and domestic sewage is discharged into the river, increasing the organic matter (OM) and nutrient contents in the water body due to the continuous input and accumulation of exogenous substances, and rich nutritive salt promotes the proliferation of universal species (Schneider & Sager 2007); thus, the species composition and diversity of macrobenthos increased gradually. The construction of gates and dams has also disrupted the continuity of the river and hindered the natural migration of aquatic organisms, which is convenient for the accumulation of fine organic particles along the river course (Pringle et al. 2000; Dudgeon et al. 2006; Carlisle & Meador 2011). Additionally, the food sources of the macrobenthos were influenced by sand and gravel mining, which changed the physicochemical properties (the degree of water pollution and riverbed structures) of the water and substrate environments, and the subsequent turbid water quality affected photosynthesis by primary producers. Consequently, the heterogeneity of the habitat and the stability of the riverbed changed (Nairn et al. 2004; Erftemeijer & Lewis 2006), which were significantly improved compared with the upper tributary, and the standing stock and diversity of the macrobenthos were also directly affected, with more direct collectors eating organic debris (Peng et al. 2013).

The midstream area is located in the plain area of the Hexi Corridor, along which industrial, agricultural and domestic sewage is discharged into the river, increasing the OM and nutrient contents due to the continuous input and accumulation of exogenous substances and abundant food sources, which provide rich sources of food and diverse habitats for different groups of macrobenthos, among which the pollutant-sensitive groups gradually decreased in abundance, while the number of pollution-tolerant groups gradually increased (Chen et al. 2015). Additionally, the water flow slowed, and the WT was relatively high in the midstream region. The aquatic vascular plants occurring along the river course not only accumulate organic debris, stabilize riverbed sediments and resist the effect of erosion caused by hydrodynamics on aquatic organisms but also provide abundant nutrients and suitable living conditions for macrobenthos (Li et al. 2000; Chen et al. 2004, 2015). There were more collector-filterers and scrapers (Mollusca), which rely on water flow to obtain food (Thomson et al. 2005; Peng et al. 2013). The individual biomass of mollusks was relatively high, and these organisms were absolutely dominant compared with arthropods; they mainly inhabited the sediment in the shallow water area of the riverbed with a high OM content, and there were more species in the slow flow water area with lush aquatic plants (Liu 2006). An abundant material basis and complex habitat not only maintain the abundance of macrobenthos but also the stability of ecosystems.

CCA showed that WT and the TN and TP contents were the key environmental factors that affected the distribution of the macrobenthic community in the upper and middle reaches of the HRB. WT is considered to be the key natural variable affecting changes in the water environment, and the level of WT directly or indirectly interferes with the physical and chemical characteristics of water bodies and aquatic biological structures (Yuan et al. 2014). Cooper et al. (2007) found that WT was an important parameter affecting the community structure of macrobenthos. In the appropriate temperature range, the reproduction and growth rate of macrobenthos will accelerate with increasing temperature, and when the temperature is extremely low, the reproduction rate of some species will slow or even stop (Pringle et al. 2000; Dudgeon et al. 2006).

Species with a low-temperature tolerance (Tipulidae) and those that live in fixed habitats became the dominant groups, mainly distributed in the upstream tributaries, and other mollusk species, such as R. auricularia, Suceinea sp. and C. albus, with strong adaptability and wide ecological distributions, were mainly distributed in the midstream area with high temperature and slow flow. Yan et al. (2005) and Chen et al. (2015) showed that nutrients and OM were the key environmental factors affecting the distribution of macrobenthos. Xiong et al. (2003) found that excessive levels of phosphorus and nitrogen would lead to the gradual extinction of macrobenthos. High concentrations of nutrients will lead to a decrease in the DO concentration in the bottom environment and an increase in the sulfide content in the sediment and water body. The sensitive groups that are not adapted to anoxic environments tend to decrease or even disappear, while the pollution-tolerant species gradually increase. At the same time, smaller pollution-tolerant species will gradually replace larger species and will eventually become dominant species (Covich et al. 2004; Lods-Crozet et al. 2010; Chen et al. 2015). Predators with high pollution tolerance, such as I. heterosticta and dragonfly nymphs, became the dominant species in the midstream area. In addition, they inhabited sediment with a high OM content, and the biomass of mollusks feeding on organic debris, algae and microorganisms was absolutely dominant. A benthic environment with a low OM content cannot maintain a complex
and stable benthic food network structure and high macrobenthos biomass; thus, there was a single macrobenthos community structure in the upstream tributary, and the diversity was relatively low. Under the comprehensive effects of natural factors and human activities, the species composition and diversity of macrobenthos showed spatial heterogeneous patterns.

In view of the special geographical location, hydrological characteristics and the results obtained in this study for the upper and middle reaches of the HRB, the WT is generally low in this area, and the community distribution of macrobenthos is closely related to the WT and other natural factors. Meanwhile, it is tightly connected with the input of exogenous pollutants and nutrients, which affect the complexity and stability of macrobenthic fauna. This indicates that natural factors and human activities are the dual driving factors restricting the structure and diversity of the macrobenthos community; in turn, from the perspective of the influential factors, we discuss how to improve the ecological environment and comprehensively manipulate the environment of the basin to maintain the dynamic balance of the ecosystem in the HRB. In addition, there are some differences in the background values of water environment in different regions, and the diversity of macrobenthos also has their own suitable living conditions. When studying the characteristics of the macrobenthic community structure and its response to the water environment, we need to consider their consistency and mutual exclusion in both directions. Therefore, it is of great significance to strengthen long-term research on microbenthos and reveal the heterogeneity of microbenthos and their response to water environment.

**CONCLUSION**

This study explored the distribution characteristics of macrobenthic organisms in the upper and middle reaches of the HRB and their response to environmental variables based on a combination of indoor and outdoor experiments and theoretical analysis, which could provide a case study for other river health assessments. The main conclusions were as follows:

1. There were significant differences in the macrobenthos community structure. A total of 50 macrobenthos species were identified, i.e., 37 species of arthropods, 11 species of mollusks and 2 species of annelids. *A. aquatica, Chlaznius sp., dragonfly nymphs, P. modestus, R. auricularia, C. albus* and *Suecinea sp.* were the dominant species in the whole study area; most of these are pollution tolerant and moderately pollution-tolerant species, and few of them are sensitive species. The FFGs were mainly dominated by PR, SC, and GC.

2. The macrobenthos distribution characteristics showed significant spatial heterogeneity. Twenty-two macrobenthic species were present in the upstream tributary, 27 species were present in the upper mainstream and 37 species were present in the middle mainstream. The dominant species were the most abundant in the upper mainstream (8 species), followed by the middle mainstream (7 species), whereas the upstream tributary had the lowest number of species (5 species). A stepwise increase in the spatial divergence distribution pattern of macrobenthos density and biomass occurred from the upper stream to the midstream area, and arthropods were the dominant community. ANOVA showed that $d_M$ and $H'$ differed significantly among different spatial regions ($P < 0.05$), with the highest values in the middle mainstream, the second-highest values in the upper mainstream and the lowest values in the upstream tributary, reflecting the highest degree of complexity and stability of the macrobenthic community structure in the middle mainstream.

3. The distribution heterogeneity of macrobenthos was closely related to natural factors and human activities. The CCA method clarified that the key driving factors affecting the community structure and diversity of macrobenthic faunal assemblages were WT and the TN and TP contents in sediments, and the key to maintaining species diversity and stability was suitable habitat conditions.

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**AUTHORS CONTRIBUTIONS**

Y.W. was involved in the writing of original draft, methodology, formal analysis, conceptualization, supervision, project administration and funding acquisition. J.-j.L. was involved in the writing of review & editing, data curation, formal analysis, software and investigation. B.-l.L. performed the formal analysis and methodology. W.L. conceptualized the methodology,
formal analysis and conceptualization. Y.-f.Z. was involved in the formal analysis and software. D.-x.K. performed the investigation, data curation and formal analysis. J.-l.Z. was involved in the data curation and formal analysis.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
All studies did not involve human or animal ethics, and involving sampling investigations of water and sediment were approved by the Research Ethics Committee of the Lanzhou University of Technology and the Northwest Institute of Eco-Environment and Resources (Lanzhou, China).

DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

REFERENCES


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