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# Comparison of Fish, Macroinvertebrates and Diatom Communities in Response to Environmental Variation in the Wei River Basin, China

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Abstract: Land use changes usually lead to the deterioration of freshwater ecosystems and reduced biodiversity. Aquatic organisms are considered valuable indicators for reflecting the conditions of freshwater ecosystems. Understanding the relationship between organisms and land use type, as well as physiochemical conditions, is beneficial for the management, monitoring and restoration of aquatic ecosystems. In this study, fish, macroinvertebrates, and diatoms were investigated at 60 sampling sites in the Wei River basin from October 2012 to April 2013 to determine the relationships between the environment and aquatic organisms. The richness, abundance, Shannon diversity, evenness, Margalef diversity, and Simpson diversity were selected as biological indices for analyzing the correlation between these communities and environmental variables according to Pearson's coefficient. Canonical correspondence analysis (CCA) was used to analyze the relationship between the biotic communities and environmental variables. The results showed that three diatom indices were weakly correlated with chemical oxygen demand (COD), qualitative Habitat Evaluation Index (QH), and dissolved oxygen (DO). Four macroinvertebrate indices were associated with total phosphorus (TP) while total nitrogen (TN), and agricultural land (AL) had a significant influence on assemblages, suggesting that macroinvertebrates could respond to nutrient levels in the Wei River basin. All land use types had a strong effect on fish indices except AL, indicating that fish would be better used as indicators of spatial changes in the aquatic ecosystem. In conclusion, fish and macroinvertebrates have the potential for use in routine monitoring programs in the Wei River basin.

Keywords: land use; fish; macroinvertebrate; diatom; Wei River basin; correspondence analysis

## 1. Introduction

The effects of land use change on aquatic biotic communities have been widely demonstrated by ecologists throughout the world [1–4] Land use changes have resulted in strong disadvantages to the maintenance of the ecological integrity of river systems [5–7]. Urbanization, agriculturalization, industrialization, and commercialization has become more prevalent with the rapid development of society and economy and the rapid growth of the population [8–10] Much of the forest, grass, and other natural vegetation cover has been replaced with urban land. This may destroy the equilibrium of the primary ecosystem and alter the biotic community structure.



The demands of human development result in the exploitation of large amounts of natural land through processes such as deforestation. Soil erosion tends to increase with the decrease in forestland, which results in increases in sandy concentrations; nutrients such as nitrogen, phosphorus, and ammonia; heavy metal ions; organic contaminants and toxic pollutants, which flow into rivers via storm runoff [11,12]. Moreover, habitat diversity decreases and fine sediments replace cobblestone sediments, which adversely impact fish spawning and diatom or macroinvertebrate attachment [6]. Meanwhile, sewage from agriculture, industry, and domestic sources discharges into rivers with the increase in agriculturalization and urbanization, also reducing water quality [13]. Another effect of land use change on aquatic organisms is through hydraulic engineering; for example, the construction of dams and reservoirs and the channelization and realignment of rivers to satisfy agricultural irrigation demands, ensuring adequate water for industrial and domestic activities [14]. All of these changes have strong impacts on the original ecological environment.

Aquatic organisms play important roles in freshwater ecosystems and can indicate variations in ecosystem conditions through their richness, abundance, diversity, composition, or other biological indices [15–17]. Fish are relatively higher-order organisms and represent an important component of the aquatic ecosystem. Because of their strong mobilities and longer growing periods, fish can reflect the effects of land use change on aquatic organisms at large spatial and temporal scales. Almeida et al. [18] demonstrated that fish biotic integrity indices were positively correlated with forest cover and negatively correlated with agricultural and urban land cover percentages in a large Mediterranean river. Macroinvertebrates are also common in freshwater ecosystems and good indicators of changes in environmental conditions that are favored by many ecological researchers [19]. They are easily sampled and are a very biodiverse group that may inhabit waters contaminated to different extents from clean to highly polluted. Macroinvertebrates are also important for the cycling of organic matter and provide food resources for higher trophic levels. Wang et al. [20] showed that the diversity and community structure of macroinvertebrates exhibited obvious changes when forestland was converted to agricultural land because the proportion of Annelida taxa increased and the number of aquatic insects decreased. Diatoms are primary producers in the aquatic ecosystem and important food sources for higher trophic level organisms. Because diatoms are incapable of movement, they can be more sensitive to water quality changes that are caused by land use changes. Vázquez et al. [4] argued that diatom assemblages respond to micro-watershed conditions and can be used to monitor the effects of land use on streams in tropical regions. Moreover, their results indicated that forest coverage was positively correlated with acidophilus and oligo-eutraphentic diatom species, and coffee coverage was significantly positively correlated with motile species and significantly negatively correlated with pollution-sensitive diatom taxa. Li et al. [21] indicated that the biomass, abundance, richness, average density, and biological diatom index (IBD) of diatoms were higher in forestland than in any other land use. However, most of these studies used signal species to analyze the relationship between land use type and organism community. Different organisms will have different responses to the environment. Understanding the relationship between several organism communities and land use types will be beneficial for decision-making by governments or managers tasked with monitoring or restoring the aquatic ecosystem.

The Wei River basin is located in central China, where the development speed is limited and slower than that in the eastern part of China [22]. However, during the last decade, the local economy has grown very rapidly. Thus, developing methods of balancing social development and ecological security is becoming a crucial challenge for future development plans. Therefore, the objectives of this research were to (1) understand the distribution of land use types in the Wei River basin, (2) determine the relationship between the biological indices and land use types, and (3) determine which organism community would be a better indicator in routine monitoring. We hope that the results of this study will be valuable for urban planners and managers to make better decisions for future developments.

#### 2. Materials and Methods

#### 2.1. Study Area Description

The Wei River is the largest tributary of the Yellow River and is located in central China. Its elevation ranges from 227 to 3936 m (Figure 1). The mainstream length is approximately 818 km with a drainage area of  $1.34 \times 10^5$  km<sup>2</sup>. The Jing River is the largest tributary of the Wei River and flows across 455.1 km with a drainage area of  $4.54 \times 10^4$  km<sup>2</sup>. The Beiluo River is the second largest tributary of the Wei River and flows across 680.3 km with a drainage area of  $2.69 \times 10^4$  km<sup>2</sup> [23].

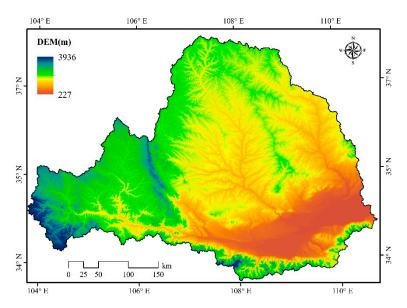


Figure 1. Elevation of the Wei River basin.

The geomorphology of the Wei River basin is complicated. The southern part of the basin is surrounded by the Qinling Mountains; however, the northern and western parts of the basin are on the Loess Plateau, where soil erosion is severe, which results in high turbidity and low transparency. The terrain comprises the Guanzhong Plain in the eastern part of the basin, where anthropogenic activities, such as high urbanization, industrialization, and commercialization, are common [24].

The Wei River basin is located in the arid to humid transition zone, and the climate is continental monsoon. The mean annual air temperature is approximately 3.7–13.9 °C, and the mean annual precipitation is approximately 290–910 mm. The wet season usually occurs from July to October when many rainstorms bring high amounts of precipitation. Runoff during the wet season accounts for 60% of the yearly total. The precipitation minimum usually occurs in January and December, when only 1.6–3.1% of the yearly runoff occurs [25].

### 2.2. Data Collection

#### 2.2.1. Sampling Sites

Sixty sampling sites were selected to investigate the characteristics of the fish, macroinvertebrate and diatom communities in October 2012 (wet season) and April 2013 (dry season). These sampling sites covered the entire basin. Most of the sampling stations were located in the fourth- or fifth-order streams because these streams were strongly affected by various human activities [26]. The sampling sites were divided into three groups: (1) W sites, which included 32 sampling sites located in the Wei River catchment; (2) J sites, which included 15 sampling sites located in the Jing River catchment; and (3) BL sites, which included 13 sampling sites in the Beiluo River catchment (Figure 2). A total of 120 samples were collected during these two periods for each biotic assemblage.

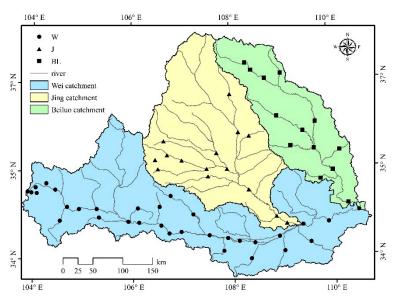


Figure 2. Sampling sites in the Wei River basin.

## 2.2.2. Fish Sampling

For the wadable streams, fish were collected across 200–300 m at each site using electrofishing for 30 min within. All types of habitat were included, such as pools, runs, and riffles. In the unwadable streams, fish collection was performed by boat using seines ( $30 \times 40$  mm). We identified the fish in situ by referring to the relevant reference books Chen (1998). Each fish species was counted and weighed using an electronic scale.

#### 2.2.3. Macroinvertebrate Sampling

Macroinvertebrates were collected using a Surber sampler ( $30 \times 30$  cm). For each sampling site, two parallel samples were collected from different randomly selected habitats, including stones, marginal areas, sand, mud, leaves, and vegetation (6–12 Surber samples for each site). All samples were mixed on a white tray, and the macroinvertebrates were collected and placed into a plastic bottle containing a 95% alcohol solution for preservation. The samples were identified in the laboratory by an anatomical lens or a microscope depending on the reference [27–29]. Each taxon was identified to the family or genus level.

#### 2.2.4. Epilithic Diatom Sampling

At each sampling station, three equal-sized pebbles were randomly selected and scraped by a toothbrush and bottle cap (11.34 cm<sup>2</sup>) to obtain the diatom samples from an equivalent size area. All samples were collected in a plastic bottle containing 4% formalin and transported to the laboratory, where they remained undisturbed for 48 h. Then, the supernatant liquids were extracted, and the remnant liquids were concentrated at 100 mL for future analysis. The diatom samples were corroded by concentrated nitric acid and sulfuric acid. Two replicate slides were taken for each sampling site, and 1000 valves per slide were identified under a microscope with a magnification of 1000× as described by Hu and Wei [30] and Zhu and Chen [31]. Each taxon was identified to the species level.

#### 2.2.5. Biodiversity Indices

Six biological indicators, i.e., richness, abundance, Shannon diversity (SD), Shannon evenness (SE), Margalef diversity (MD), and Simpson diversity (SP) were calculated for each community. Richness was calculated based on the taxonomic classification. Diversity and evenness were calculated as follows:

$$SD = -\sum_{i=1}^{S} p_i \log_2 p_i \tag{1}$$

$$SE = \frac{SD}{\log_2 S} \tag{2}$$

$$SP = 1 - \sum_{i=1}^{S} p_i^2$$
(3)

$$MD = \frac{(S-1)}{\ln N} \tag{4}$$

where  $p_i$  is the proportion of individuals found in the *i*th taxon; *S* is the total number of organisms in the sample; and *N* is the total number of individuals in the sample.

#### 2.2.6. Physiochemical Variable

Dissolved oxygen (DO) and electrical conductivity (EC) were measured in situ using a YSI Pro plus 85. Two-liter water samples were collected from each sampling station and sent to the laboratory within 48 h. Total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) were measured in the laboratory following the standards from the State Environmental Protection Administration of China (GB 3838-2002). The Qualitative Habitat Evaluation Index (QH) was used to evaluate the condition of the habitat according to Barbour et al. [32].

#### 2.2.7. Land Use Type

The land use types of the Wei River basin were obtained from the National Geomatics Center of China. The land use types were divided into the following eight categories according to the 30-m global land cover dataset from 2010 (Figure 3): agricultural land, forestland, grassland, shrubland, wetland, aquatic land, urban land, and bare land. Because forestland, grassland, agricultural land and urban land accounted for more than 99% of the Wei River basin, these four land use types were considered in the subsequent analysis.

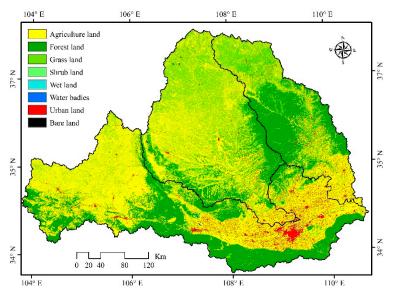


Figure 3. Land use types of the Wei River basin.

Each sampling site was selected as an outlet point, and the Wei River basin was delineated into 60 subbasins depending on the digital elevation model (DEM) at a  $30 \times 30$ -m resolution. Then, we obtained the land use composition of each sampling site at the sub-basin level.

#### 2.3. Data Analysis

The averages of each biological index, physiochemical variable, and land use type were calculated in the Wei River catchment, Jing River catchment and Beiluo River catchment, and the range was displayed by boxplots and violin figures to express the discrimination of the three catchments. The Indicator Species Analysis was used to define the indicator species for each catchment using PC-ORD 5.0 soft (https://www.pcord.com/pc5fixes.htm) [22].

The Kolmogorov–Smirnov test (K–S test) was used to examine whether all variables fit a normal distribution. In this study, values of p > 0.05 indicated that the variables fit a normal distribution. For such variables, Pearson's correlation analysis was used to analyze the relationships between the biological indices and land use types and physiochemical variables.

Before analyzing the correlations between biotic abundance and environmental variables, a detrended correspondence analysis (DCA) was conducted to determine the model (linear model or unimodal model) that would be more appropriate for further analysis [22]. In this study, the gradient lengths of macroinvertebrate and fish abundance were greater than 3; therefore, a canonical correspondence analysis (CCA-unimodal model) was used to analyze the effects of land use type and physiochemical variables on the macroinvertebrate and fish communities. However, for gradient lengths of diatom abundance lower than 3, a redundancy analysis (RDA) was more appropriate for analyzing the association of diatom assemblages with environmental variables.

#### 3. Results

#### 3.1. Land Use Characteristics

The 30-m global land cover dataset in 2010 showed that agricultural land (AL) was the main land use type in the basin and accounted for 48.4% of the total area (Figure 4). At the reach scale, the proportion of AL was lowest in the BL catchment at nearly 43.3%, and it was 58.8% and 65.2% in the W and J catchments, respectively (Figure 5). The next most abundant land use types were forestland (FL) and grassland (GL), which were mainly distributed in the southern and northeastern parts of the Wei River basin, and they accounted for 28.9% and 19.0% of the total area, respectively (Figure 4). The proportion of FL and GL were both highest in the BL catchment at 10.7% and 42.9%, respectively, at the reach scale. The proportion of FL in the W catchment (9.5%) was higher than that in the J catchment (1.7%), whereas the results for GL showed an opposite trend, with proportions of 30.3% and 26.7% in the J and W catchments, respectively (Figure 5). Although the urban land area (UL) was relatively small, it was concentrated in the Guanzhong Plain in the eastern part of the basin, and the urban land area accounted for 3.0% of the total area (Figure 4). Most of the urban land area was distributed in the W catchment, and the proportion was 1.8%, which was twice the value in the J catchment. Urban land in the BL catchment was rather small at a proportion of only 0.2% (Figure 5).

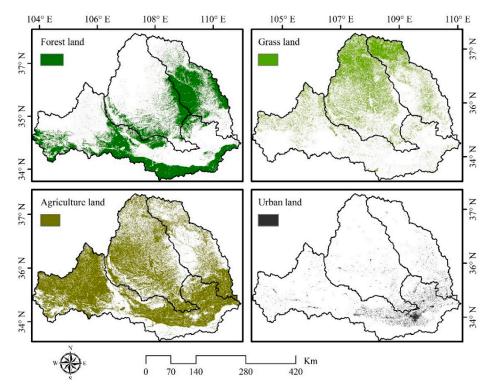
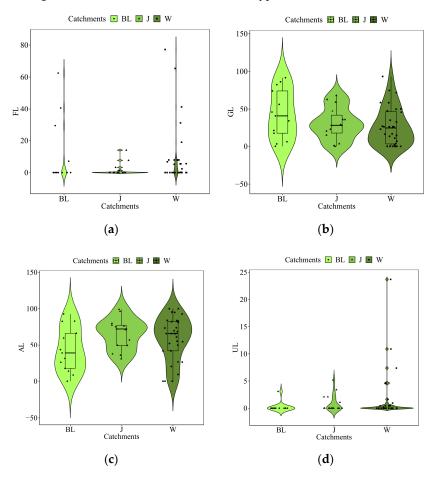


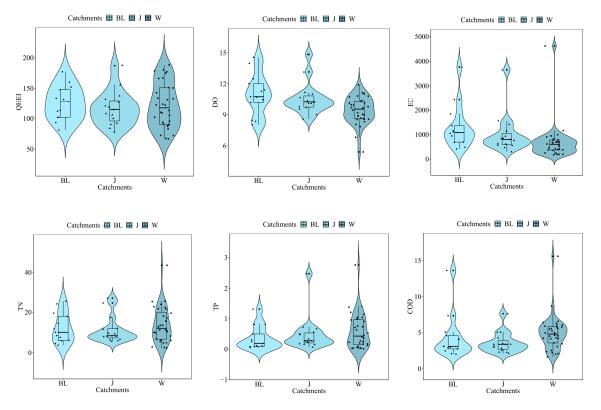
Figure 4. Distribution of the four land use types in the Wei River basin.



**Figure 5.** Distribution of the four land use types at three catchments in the Wei River basin. (a): forestland (FL), (b): grassland (GL), (c): agricultural land (AL), (d): urban land (UL).

#### 3.2. Physiochemical variables

Significant differences were observed for some variables in some catchments. QH, DO, and EC were slightly higher in the BL catchment than in the other catchments, and the average values were 125.7, 11.0 mg/L, and 1311.5 us/cm therein and 121.5, 9.4 mg/L, and 392.0 us/cm in the W catchment and 116.9, 10.6 mg/L, and 1006.6 us/cm in the J catchment, respectively. TN, TP, and COD were a slightly higher in the W catchment than in the other catchments, with average values of 13.9 mg/L, 0.6 mg/L, and 4.96 mg/L therein, and 12.3 mg/L, 0.36 mg/L, and 4.32 mg/L in the BL catchment, respectively. TN and COD were relatively lower in the J catchment than in the BL catchment, and the average values were 11.32 mg/L and 3.6 mg/L, respectively. TP was higher in the J catchment and had an average value of 0.49 mg/L (Figure 6).



**Figure 6.** Distribution of physiochemical variables (QHEI, DO, EC, TN, TP, COD) at each catchment in the Wei River basin.

#### 3.3. Community Structure and Biological Indices

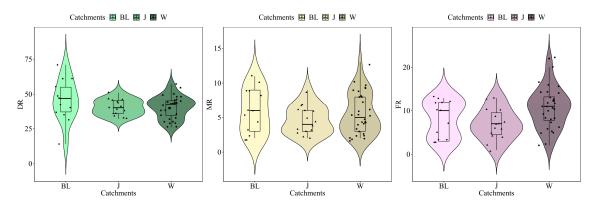
A total of 251 diatom species belonging to 31 genera were collected. *Navicula* was the most numerous genus and included 61 species. The species *Encyonema ventricosum* and *Achnanthidium minutissimum* were the indicator species for the W catchment, and *Pantocsekiella ocellata* was the indicator species for the J catchment. The number of indicator species of diatoms for the BL catchment was much higher than that of the above catchments, and the indicator species included *Diatoma elongata, Achnanthidium minutissimum var. cryptocephala, Chamaepinnularia begeri, Caloneis budensis,* and *Neidium kozlowi var. elliptica* (Table 1). In total, 73 macroinvertebrate species were identified, and they represented seven classes and 12 orders. *Diptera* was the dominant order and included 34 species in the Wei River basin. *Orthocladius makabensis, Rheocricotopus fuscipes, Polypylis hemisphaerula, Limnodrilus claparedianus,* and *Sinopotamidae* were the indicator species in the BL catchment; however, no macroinvertebrate indicator species were observed in the W and J catchments (Table 1). A total of 45 fish species were recorded in this study, and the most indicator fish species were observed in the W catchment, including *Triplophysa minxianensis, Cobitis granoei, Huigobio chinssuensis,* and *Gobio* 

*coriparoides*. Only one indicator fish species was found in the J catchment (*Triplophysa kungessana orientalis*) and BL catchment (*Gnathopogon imberbis*).

Catchments	ID	Species	Value	Р
	D126	Encyonema ventricosum	41.4	0.003
	D182	Achnanthidium minutissimum	41.1	0.004
W Catchment	F6	Triplophysa minxianensis	37.9	0.008
w Catchinent	F14	Cobitis granoei	28.1	0.016
	F27	Huigobio chinssuensis	25.0	0.019
	F33	Gobio coriparoides	24.3	0.042
I Catchment	D225	Pantocsekiella ocellata	41.0	0.002
JCatchinent	F10	Triplophysa kungessana orientalis	37.3	0.004
	D159	Diatoma elongata	53.8	0.001
	D183	Achnanthidium minutissimum var. cryptocephala	43.6	0.001
	D14	Chamaepinnularia begeri	42.6	0.003
	D65	Caloneis budensis	29.2	0.005
	D236	Neidium kozlowi var. elliptica	30.8	0.002
BL Catchment	B25	Orthocladius makabensis Sasa	42.8	0.001
	B33	Rheocricotopus fuscipes	27.5	0.005
	B61	Polypylis hemisphaerula	17.7	0.038
	B66	Limnodrilus.claparedianus	31.3	0.012
	B71	Sinopotamidae	13.3	0.042
	F26	Gnathopogon imberbis	30.9	0.035

**Table 1.** Indicator species at each catchment in the Wei River basin. See Appendices A–C for abbreviations of the diatom, macroinvertebrate and fish species.

For the BL catchment, the richness of diatoms and macroinvertebrate was the highest among the three catchments, and the average values were nearly 45.4 and 5.9, respectively. The richness of fish in the BL catchment was lower than that of the W catchment but higher than that of the J catchment, reaching a mean value of 8.2. For the J catchment, the richness of fish and macroinvertebrates was the lowest among the three catchments, and the average values were 6.9 and 4.7, respectively. The mean richness value of diatoms was 40.5. For the W catchment, the richness of fish was the highest among the three catchments, with a mean value of 10.6. The average richness of diatom and macroinvertebrates was 5.5 and 40.3, respectively (Figure 7).

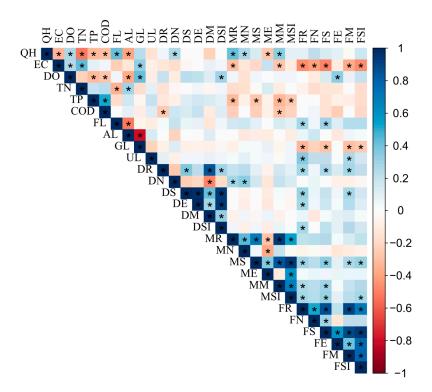


**Figure 7.** Distribution of three biotic indicators of diatom, macroinvertebrate and fish assemblages at each catchment in the Wei River basin. DR: diatom richness, MR: macroinvertebrate richness, FR: fish richness.

#### 3.4. Correlations between Biological Indices and Environmental Variables

For the diatom indices, only richness, abundance, and Simpson diversity were significantly associated with COD, QH, and DO, respectively. For the macroinvertebrate biotic indices, QH and TP

both had a great effect on the four biological indices. Macroinvertebrate richness and Margalef diversity were both statistically correlated with QH and TP, while macroinvertebrate abundance was associated with QH and macroinvertebrate Simpson diversity was associated with TP. Macroinvertebrate richness and macroinvertebrate Margalef diversity were also affected by EC. In addition, macroinvertebrate Margalef diversity was associated with COD as well. For the fish, all of the biotic indices had a significant correlation with EC except evenness. GL had a strong association with fish richness, Shannon diversity, Margalef diversity and Simpson diversity. FL was correlated with fish richness and Shannon diversity, while UL was associated with fish richness and Margalef diversity. Only DO had a strong correlation with fish evenness (Figure 8).



**Figure 8.** Correlation matrix between environmental variables and biotic indicators in the Wei River basin (\* p < 0.05). D: diatom, M: macroinvertebrate, F: fish, R: richness, N: abundance, S: Shannon diversity, E: evenness, M: Margalef diversity, and SI: Simpson diversity.

#### 3.5. Relationships between Biological Assemblages and Environmental Variables

In the RDA model for the diatom assemblage, axes 1 and 2 explained 5.4% and 3.3% of the variation, respectively (Table 2). The Monte Carlo permutation test indicated that QH, FL, and GL had a significant influence on diatom assemblages (*p* < 0.05) (Table 3). *Encyonema ventricosum* and *Achnanthidium minutissimum*, which were the indicator species, were positively correlated with QH and FL (Figure 9). *Pantocsekiella ocellata* was the indicator species for the J catchment, and it was positively associated with COD. *Diatoma elongata* and *Caloneis budensis* had a great positive association with COD, and *Achnanthidium minutissimum var. cryptocephala* and *Chamaepinnularia begeri* were strongly correlated with GL and FL, respectively. In the CCA for the macroinvertebrates, TN and AL were selected as the main factors influencing assemblage structure. Axes 1 and 2 explained 20.1% and 18.3% of the variation, respectively. *Limnodrilus claparedianus* was associated with TN, and *Sinopotamidae* was correlated with AL. In the CCA model for fish, QH, DO, TP and GL had significant associations with the assemblage structure. Axes 1 and 2 explained 29.8% and 14.6% of the variation, respectively. *Huigobio chinssuensis, Gobio coriparoides, and Triplophysa kungessana orientalis* were strongly associated with TP; meanwhile, *Cobitis granoei* and *Gnathopogon imberbis* were not. *Triplophysa minxianensis* had a high correlation with QH and DO.

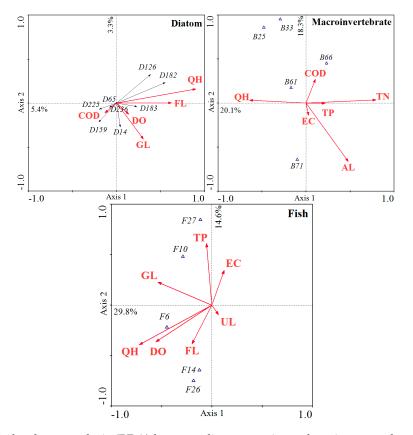
Axis	Dia	tom	Macroinv	vertebrate	Fi	sh
AXIS	Eigenvalues	Correlations	Eigenvalues	Correlations	Eigenvalues	Correlations
Axis 1	0.054	0.816	0.201	0.751	0.298	0.763
Axis 2	0.033	0.786	0.183	0.748	0.146	0.698
Axis 3	0.026	0.753	0.124	0.757	0.092	0.660
Axis 4	0.022	0.842	0.105	0.724	0.061	0.674

Table 2. Eigenvalues and species-environment correlations of each canonical axis.

**Table 3.** Results of the Monte Carlo permutation tests in redundancy analysis (RDA) and canonical correspondence analysis (CCA).

Variable –	Dia	tom	Macroin	vertebrate	Fi	sh
variable –	F	Р	F	Р	F	Р
QH	2.88	0.001	1.15	0.251	3.84	0.001
DO	0.99	0.438	1.34	0.091	2.14	0.006
EC	0.93	0.596	0.91	0.596	1.03	0.405
TN	1.04	0.416	1.85	0.007	1.20	0.261
TP	0.67	0.886	1.05	0.401	2.05	0.028
COD	1.23	0.168	0.93	0.550	0.62	0.870
FL	1.51	0.024	1.08	0.366	1.17	0.240
GL	1.38	0.039	0.75	0.807	1.63	0.032
AL	1.20	0.172	1.51	0.044	1.10	0.326
UL	0.88	0.593	1.02	0.393	0.79	0.660

The bold numbers indicate that the variables had a significant influence on the biological assemblages.



**Figure 9.** Redundancy analysis (RDA) between diatom metrics and environmental variables and canonical correspondence analysis (CCA) of macroinvertebrate and fish metrics and environmental variables in the Wei River basin. See Appendices A–C for abbreviations of the diatom, macroinvertebrate and fish species.

#### 4. Discussion

#### 4.1. Characteristic of Aquatic Ecosystems

Anthropogenic influences and land use are most likely responsible for the variations in water guality [33]. In our study, QH and DO were the highest in the BL catchment and TN and TP were the lowest. Meanwhile, the percentage of agricultural land and urban land were obviously lower in the BL catchment than in the other catchments while the percentages of forestland and grassland were higher. Moreover, the richness of diatoms and macroinvertebrates was the highest in the BL catchment. These findings suggest that land use may affect the water quality and biological community structure, which is consistent with a number of previous studies [18,34,35]. Ding [12] found that water quality was most strongly affected by the configuration metrics of land use. Agricultural land was the main land use type in the Wei River basin at both the large scale and the reach scale, suggesting that aquatic ecosystems were severely affected by the agricultural activity. Urban land, which accounted for 3% of the area, was a minor land use type in the Wei River basin, indicating that economic development was relatively slower than that observed in the eastern parts of China, such as in Shanghai or Hangzhou. Therefore, non-point pollution was considered the main source of contamination in the Wei River basin because of the higher proportion of agricultural land and lower proportion of urban land [23]. Other studies, such as Longyang [36], reported that runoff would carry agrochemicals into rivers and cause non-point source pollution. Forest land and grassland are often considered filter strips that could decrease the nutrient content of water resources caused by non-point pollution, reinforce bank stability and provide aquatic habitats [26].

#### 4.2. Influence of Environmental Variables on Biological Indices

The indices of macroinvertebrates and fish were more sensitive to the environmental parameters than the indices of diatoms in Wei River basin, and the macroinvertebrate indices were more strongly correlated with physicochemical variables while the fish indices were more strongly correlated with the land use type. The weak correlations observed for the indices based on diatom were primarily related to the degraded habitat and high amounts of silt sediment. The Loess Plateau is located in the Wei River basin, and considerable amounts of runoff with silt or sand enter the river and lead to finer sediment, which decreases the survival of diatoms. Many studies have indicated that diatom indices are sensitive to the nitrogen or phosphorous content and are beneficial indicators for evaluating the eutrophication conditions of freshwater ecosystems [37-40]. In our study, diatom richness was correlated with organic pollution, such as COD, indicating that the nutrient content was not sufficient to cause eutrophication; rather, organic pollution was the major limiting factor for diatom growth. Although many studies have demonstrated that diatom assemblages represent the "first choice" for detecting nutrient enrichment levels in water quality [26,37,39], several studies have confirmed that diatom indices could be a useful indicator for predicting organic pollution as well [41]. Hence, diatom indices could be used as an indicator for organic pollution in the Wei River basin. All of the macroinvertebrate indices had a strong correlation with environmental variables, especially the macroinvertebrate richness and Margalef diversity. QH and TP were the major environmental parameters that influenced the four macroinvertebrate indices. Zhang et al. [42] demonstrated that the concentration of nitrogen had a great effect on the distribution of the macroinvertebrate community in basins where agricultural area was the main land use type, which is consistent with our results. The fish indices were also strongly associated with environmental variables in our study, especially EC. Maceda-Veiga et al. [43] showed that high water conductivity was negatively correlated with migratory, pelagic, invertivorous and native fish in Spain and suggested that the current condition of riparian zones was sufficient to decrease the pollution effects on fish, with high conductivity presenting a significant inverse association with the length of the food chain [44]. In conclusion, diatom and macroinvertebrate indices represent better indicators for organic pollution and eutrophication, respectively, and fish indices represent better indicators for conductivity in the Wei River basin.

#### 4.3. Response of Biological Assemblages to Environmental Variables

The results of the RDA showed that QH, FL, and GL were significantly correlated with the diatom assemblages. Forest land and grassland were strongly correlated with the water quality and indirectly affected the biological assemblages [26]. We found that Encyonema and Achnanthidium preferred habitat with a higher percentage of forest or grassland use, consistent with several studies indicating that these genera are indicators of good water quality. For instance, some studies showed that Achnathidium *minutissimum* was so sensitive to water quality that it was rarely observed in impaired sites, especially when the phosphorus content was over 0.3 mg/L [37,45]. Pantocsekiella has been defined as a tolerant species that can indicate polluted areas. Shen et al. [39] divided the Ying River into three regions based on nutrient status and found that Achnathidium minutissimum was the dominant species in the region with the lowest nutrient level, whereas *Pantocsekiella meneghiniana* was the dominant species in the region with the highest nutrient level. These findings are consistent with our results. TN and AL were the significant variables for the macroinvertebrate assemblage in the Wei River basin. The richness and diversity indices and assemblage structure were correlated with nutrient variables, suggesting that the macroinvertebrates could be indicators of nutrition status in the Wei River basin. Limnodrilus was extensively adaptable to the environment and often acted as the dominant species in impaired stations. Because of the extreme tolerance of this species, it generally indicated poor water quality [46–49]. In this study, *Limnodrilus* was strongly positively associated with TP, TN and COD, consistent with previous studies [48,49]. In addition, QH, TP, DO and GL were all significantly correlated with the fish assemblages in the Wei River basin. Wu et al. [50] demonstrated that Triplophysa was the dominant species at altitudes over 800 m, corresponding to locations at the origin of the river in the Wei River basin, which generally present good water quality. Our results showed that Triplophysa minxianensis was associated with QH and DO, which was in agreement with the results of Wu et al. [50]. Moreover, the fish indices and assemblage structure were strongly correlated with physiochemical variables and land use types, suggesting that fish could be considered the "best" organism for indicating the degree of pollution in the Wei River basin. Uncertainty was inevitable in the sampling process. We investigated only twice at different hydrological periods. The physiochemical parameters were easily affected by discharge and anthropogenic activities as well as biological assemblages, and this likely affects the relationships between biological indices and environmental variables. More investigation events would be required for future research.

#### 5. Conclusions

This study demonstrated the response of fish, macroinvertebrate, and diatom assemblages to four land use types and six physiochemical variables in the Wei River basin. According to our results, diatoms were weakly associated with nutrient variables compared with macroinvertebrates and fish; however, macroinvertebrate indices and assemblages were significantly correlated with TP, TN, and AL, suggesting that they represented powerful indicators of the nutrient level in the Wei River basin. The fish indices and assemblage structure were strongly correlated with all variables but AL, TN, and COD, indicating that fish could adequately reflect spatial changes, such as the changes in land use type, in the Wei River basin. In conclusion, diatoms are not a good indicator in routine monitoring programs in the Wei River basin, macroinvertebrates could be beneficial for indicating the nutrient level, and fish represent the best indicator of spatial changes in the Wei River basin.

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## **Conflicts of Interest:** The authors declare no conflict of interest.

# Appendix A. The Distribution of Diatom Assemblage in the W, J, and BL Catchments

ID	Species	W	J	BL
D1	Melosira varians	+		+
D2	Melosira granulata	+	+	+
D3	Melosira granulata var. angustissima	+		
D4	Navicula lanceolata	+	+	+
D5	Navicula exigua Krasske		+	+
D6	Navicula confervacea	+		+
D7	Navicula cryptocephala	+	+	+
D8	Navicula cryptocephala var. intermedia	+	+	+
D9	Navicula cryptocephala var. venta	+	+	+
D10	Navicula cincta	+	+	+
D11	Navicula cincta var. leptocephala	+	+	+
D12	Navicula cincta var. heufleri			+
D13	Navicula pusilla	+		+
D14	Chamaepinnularia begeri	+	+	+
D15	Navicula pupula	+	+	+
D16	Navicula pupula var. capitata	+	+	+
D17	Navicula cuspidata	+		
D18	Navicula cuspidata var. heribaudii	+	+	+
D19	Navicula radiosq	+	+	+
D20	Navicula cari	+	+	+
D21	Navicula cari var. angusta	+		
D22	Navicula salinarum			+
D23	Navicula viridula	+	+	+
D24	Navicula viridula var. capitata	+	+	+
D25	Navicula viridula var. pamirensis	+	+	
D26	Navicula simplex	+	+	+
D27	Navicula gothlandica	+	+	+
D28	Navicula accommoda	+	+	+
D29	Navicula rhynchocephala	+		+
D30	Navicula virihensis	+	+	+
D31	Navicula menisculus	+	+	+
D32	Naviclua anglica	+		
D33	Navicula exigua Ehr	+		
D34	Navicula gracilis	+	+	+
D35	Navicula gracilis var. neglecta	+		+
D36	Navicula rostellata		+	+
D37	Navicula seminulum		+	
D38	Navicula seminuloides	+	+	+
D39	Navicula	+	+	+
D40	Navicula muralis	+	+	+
D41	Navicula notha	+	+	+
D42	Navicula halophilioides	+	+	+
D43	Navicula scabellum	·	+	·
D44	Navicula omissa		+	
D45	Navicula perrostrata	+		
D46	Navicula disjuncta	+		
D40 D47	Navicula disjuncta f. anglica	+		
D47 D48	Navicula minuscula	+	+	
D40 D49	Navicula placentula	+		
D50	Navicula asellus	+		
D50 D51	Navicula dicephala	+		
D51 D52	Navicula lenzii	+		

## **Table A1.** The list of diatom species in Wei River basin.

ID	Species	W	J	BL
D53	Navicula rotaenea	+		
D54	Navicula virihensis	+	+	
D55	Navicula hasta Pantocsek	+		
D56	Navicula protracta	+		
D57	Navicula protracta var. elliptica	+		
D58	Navicula adversa	+		
D59	Navicula tuscula	+	+	+
D60	Navicula atomus		+	+
D61	Navicula permitis	+	+	+
D62	Navicula nivaloides		+	
D63	Navicula virihensis		+	
D64	Navicula virihensis	+		
D65	Pinnularia appendiculata var. budensis	+	+	+
D65 D66	Pinnularia molaris	Т	+	+
D60 D67			т	т
	Pinnularia bogotensis	+		
D68	Hantzschia amphioxys	+		+
D69	Hantzschia amphioxys var. aequalis	+		
D70	Meridion circulare	+		+
D71	Nitzschia obtusa var. scalpelliformis			+
D72	Nitzschia palea	+	+	+
D73	Nitzschia acicularis	+	+	+
D74	Nitzschia hantzschiana	+		
D75	Nitzschia frustulum	+	+	
D76	Nitzschia frustulum var. perpusilla			+
D77	Nitzschia frustulum var. perminuta	+		
D78	Nitzschia frustulum var. subsalina	+	+	+
D79	Nitzschia recta	+	+	+
D80	Nitzschia dissipata	+	+	+
D81	Nitischia constricta	+	+	+
D82	Nitzschia hungarica	+		+
D83	Nitzschia sigmoides	+	+	
D84	Nitzschia linearis	+	+	+
D85	Nitzschia microcephala	+	+	+
D86	Nitzschia heuflerana	+		
D87	Nitzschia stagnorum	+		+
D88	Nitzschia fonticola	+	+	+
D89	Nitzschia ovalis	I	+	+
-			т	
D90 D91	Nitzschia paleacea Nitzschia	+	+	++
D91 D92	Nitzschia thermalis	т	т	+
D93	Nitzschia tryblionella var. victorise	+	+	+
D94	Nitzschia tryblionella var. levidensis	+		
D95	Nitzschia commutata		+	
D96	Nitzschia acula	+	+	
D97	Nitzschia debilis	+		+
D98	Nitzschia actinastroides	+		
D99	Nitzschia communis var. abbreviata	+	+	
D100	Nitzschia heidenii	+	+	
D101	Nitzschia angustata var. acuta	+		
D102	Nitzschia kuetzingiana	+		
D103	Nitzschia sinuata var. tabellaria	+	+	+
D104	Nitzschia gracilis	+	+	+
D105	Nitzschia romana	+		
D106	Nitzschia amphbia	+	+	
D107	Nitzschia clausi		+	
D108	Nitzschia sublinearis		+	
D109	Stauroneis anceps			+
D110	Stauroneis anceps var. linearis			+

## Table A1. Cont.

ID	Species	W	J	BL
D111	Stauroneis schroederi		+	+
D112	Stauroneis dubitabilis	+		
D113	Stauroneis kriegeri	+		
D114	Stauroneis palustris	+		
D115	Rhoicosphenia curvata	+	+	
D116	Amphora ovalis	+	+	+
D117	Amphora ovalis var. gracilis	+	+	+
D118	Amphora perpusilla	+	+	·
D119	Cymbella ehrenbergii	+	+	+
D120	Cymbella sinnata	+	+	+
D120	Cymbella microcephala	+	+	+
D121	Cymbella pusilla	+	+	+
D122 D123	Cymbella cistula	+	+	+
D123 D124	Cymbella cistula var. maculata	+	Т	+
D124 D125				Ŧ
D125 D126	Cymbella cistula var. caldostagnensis	+ +		
	Encyonema ventricosum		+	+
D127	Cymbella ventricosa var. simicircularis	+		+
D128	Cymbella amphicephala	+	+	+
D129	Cymbella amphicephala var. intermedia	+		
D130	Cymbella aequalis	+	+	+
D131	Cymbella tumidula	+		+
D132	Cymbella turgida	+	+	+
D133	Cymbella turgidula			+
D134	Cymbella aequalis	+		
D135	Cymbella aequalis var. pisciculus	+	+	+
D136	Cymbella prostrata	+	+	+
D137	Cymbella gaeumanni	+		+
D138	Cymbella sphaerophora	+	+	+
D139	Cymbella cymbiformis	+	+	+
D140	Cymbella perpusilla	+	+	
D141	Cymbella bremii	+	+	
D142	Cymbella lata	+	+	
D143	Cymbella gracilis	+		
D144	Cymbella lunata	+		
D145	Cymbella excisa	+		
D146	Cymbella alpina var. minuta	+		
D147	Cymbella lapponica	+		
D148	Cymbella aspera	+		
D140 D149	Cymbella hustedtii	+	+	
D14)	Cymbella jolmolungnensis	+	Т	
D150	Cymbella hauckii	+		
D151 D152				
D152 D153	Cymbella parva	+		
	Cymbella hybrida	+	+	+
D154	Cymbella helvatica	+		
D155	Diatoma vulgare	+	+	+
D156	Diatoma vulgare var. lineare	+	+	+
D157	Diatoma vulgare var. producta	+	+	+
D158	Diatoma anceps	+		
D159	Diatoma elongata			+
D160	Diatoma elongata var. tenuis	+	+	+
D161	Diatoma hiemale			+
D162	Gomphonema parvulum	+	+	+
D163	Gomphonema parvulum var. subellipticum	+	+	+
D164	Gomphonema parvulum var. exilissima	+		+
D165	Gomphonema angustatum	+	+	+
D166	Gomphonema angustatum var. aequalis	+		
D167	Gomphonema intricatum	+	+	+
	Gomphonema intricatum var.			
D168	dichotomiformis	+	+	+

Table A1. Cont.

ID	Species	W	J	BL
D169	Gomphonema olivaceum	+	+	+
D170	Gomphonema olivaceum var. minutissima			+
D171	Gomphonema turris	+		
D172	Gomphonema gracile	+	+	+
D173	Gomphonema gracile var. intricatiformis		+	+
D174	Gomphonema constrictum	+	+	+
D175	Gomphonema montanum	+	+	
D176	Gomphonema sphaerophorum	+	+	+
D177	Gomphonema	+		
D178	Gomphonema tergestium	+		
D179	Achnanthes lanceolata	+		
D180	Achnanthes lanceolata f. ventricosa	+	+	+
D181	Achnanthes linearis	+		+
D182	Achnanthidium minutissimum	+	+	+
	Achnanthidium minutissimum var.			
D183	cryptocephala	+	+	+
D184	Achnanthes amphicephala	+		+
D185	Achnanthes hauckiana	+	+	+
D185	Achnanthes tibetica	Т	Т	+
D180 D187	Achnanthes affinis			
	Achnanthes affinis Achnanthes delicatula	+	+	+
D188	Achnanthes montana	+		+
D189		+	+	+
D190	Achnanthes crassa	+	+	+
D191	Achnanthes exilis			+
D192	Achnanthes subhudsonis	+		
D193	Achnanthes conspicua	+		
D194	Achnanthes clevei	+		
D195	Achnanthes nodosa	+		
D196	Achnanthes microcephala	+	+	
D197	Cocconeis pediculus	+	+	+
D198	Cocconeis placentula	+	+	+
D199	Fragilaria capucina		+	+
D200	Fragilaria capucina var. mesolepta		+	
D201	Fragilaria var. subsalina	+	+	+
D202	Fragilaria ungeriana			+
D203	Fragilaria intermedia	+		
D204	Fragilaria vaucheriae var. capitellata	+		
D205	Fragilaria virescens var. mesolepta	+		
D206	Synedra acus	+	+	
D207	Synedra acus var. radians			+
D208	Synedra ulna	+	+	+
D209	Synedra ulna var. danica	+	+	+
D210	Synedra ulna var. contracta	+	+	+
D210	Synedra ulna var. oxyrhnchus	+	+	
D211 D212	Synedra amphicephala	+	Т	
D212	Syntaria ampriloophala Surirella subsalsa	+		
D213 D214	Surirella tibetica	+	+	
D214 D215	Surirella ovalis	т	т	+
D215 D216	Surirella ovalis var. salina			+
		+	+	+
D217	S.brebissonii	+	+	+
D218	Surirella	+		
D219	Surirella robusta	+		
D220	Surirella capronii	+		
D221	Surirella angusta	+	+	
D222	Cyclotella stelligera	+	+	+
D223	Cyclotella meneghiniana	+	+	+
D224	Cyclotella kuetzingiana	+	+	+
D225	Pantocsekiella ocellata	+	+	+

## Table A1. Cont.

ID	Species	W	J	BL
D226	Cyclotella catenata	+	+	+
D227	Cyclotella asterocostata	+		
D228	Gyrosigma scalproides	+	+	+
D229	Gyrosigma acuminatum		+	+
D230	Gyrosigma attenuatum			+
D231	Gyrosigma kuetzingii	+	+	+
D232	Cymatopleuta solea	+	+	
D233	Diploneis elliptica			+
D234	Diploneis ovalis	+		+
D235	Diploneis pseudovalis	+		
D236	Neidium kozlowi var. elliptica			+
D237	Neidium iridis var. ampliatum			+
D238	Stephanodiscus minutulus			+
D239	Ceratoneis arcus	+	+	+
D240	Ceratoneis arcus var. linearis	+	+	+
D241	Ceratoneis arcus var. linearis f.recta			+
D242	Ceratoneis arcus var. amphioxys		+	
D243	Caloneis alpestris var. lanceolata		+	
D244	Caloneis bacilaria	+		
D245	Caloneis amphisbaena		+	
D246	Amphiraphia xizangensis	+		
D247	Rhopalodia gibba	+		
D248	Amphipleura pellucida		+	+
D249	Didymosphenia geminata	+		
D250	Denticula elegans	+		
D251	Frustulia vulgaris	+		

## Appendix B. The Distribution of Macroinvertebrate Assemblage in the W, J, and BL Catchments

ID	Species	W	J	BL
B1	Baetidae Analetridae	+	+	+
B2	Baetis vaillanti	+	+	+
B3	Serratellasp.	+	+	+
B4	Leptophlebiasp.	+	+	+
B5	Cinygmasp.	+	+	
B6	Polymitarcyidae	+		
B7	Epeorsu curvispinosa	+		
B8	Chromarcyssp.	+		
B9	Ephemera nigroptera	+		
B10	Osobenussp.	+	+	
B11	Hydropsychesp.	+	+	+
B12	Brunnea larva			
B13	Dolophilodes sp.	+		
B14	Austrotinodessp.	+		
B15	Tipulasp.	+		
B16	Antochasp.	+		
B17	Tabanussp.	+	+	
B18	Natarsia punctata			
B19	Ablabesmyia phatta			+
B20	Procladius choreus	+	+	+
B21	Conchapelopia sp.	+	+	+
B22	Polypedilum scalaenum	+		

**Table A2.** The list of macroinvertebrate species in Wei River basin.

ID	Species	W	J	BL
B23	Procladius paradouxus	+		
B24	Orthocaladius mixtus	+		
B25	Orthocladius makabensis Sasa	+		+
B26	Cricotopus albiforceps			+
B27	Cricotopus trifasciatus	+	+	+
B28	Cricotopus triannulatus	+	+	+
B29	Cricotopus bicinctus	+	+	·
B30	Paracricotopus sp.	+	+	
B31	Cricotopus anulator Goetghebuer	+		
B32	Diplocladius Kieffer	+		
B33	Rheocricotopus fuscipes	•	+	+
B34	Rheocricotopus effuses	+	·	
B35	Paratrichocladius rufivertris	I.		+
B36	Rheotanytarsus sp.	+		1
B37	Chironomus riparius Meigen	+	+	+
B38	Chironomus salinarius Kiffer	+	т	+
B39	Thienmanniola sp.	+	+	י +
B40	Chironomus sp.	+	+	+
B40 B41	Polypedilum paraviceps Niitsuma	+	т	+ +
B41 B42	Micropesectra atrofasciata			+ +
B42 B43	Cyphomella cornea	+		Ŧ
B43 B44		+		
B44 B45	Antocha bifida Alexander			+
B43 B46	Sympotthastia takatensis	+		
	Lappodiamesa sp.	+	+	
B47	Simuliumsp.	+	+	+
B48	Sciomyzidae sp.	+	+	
B49	Psychodasp.	+	+	+
B50	Liodessussp.	+		
B51	Hydrous sp.	+		
B52	Stenelmis sp adult	+	+	+
B53	Gomphussp.	+	+	
B54	Gomphidae sp.	+		+
B55	Aeschna sp.			+
B56	Pontamalota sp.	+		
B57	Epitheca.marginata			
B58	Radix clessini	+	+	
B59	Radix ovata	+	+	+
B60	physa acuta cf.	+		+
B61	Polypylis hemisphaerula		+	+
B62	Bellamya aeruginosa	+		
B63	Schistodesmus lampreyanus	+		+
B64	Limnodrilus hoffmeisteri	+	+	+
B65	Branchiura sowerbyi	+		
B66	Limnodrilus claparedianus	+		+
B67	Tubifex sinicus	+		+
B68	Whitmania pigra	+		
B69	Barbronia weberi	+		
B70	Gammarussp.	+	+	
B71	Sinopotamidae.sp	+		+
B72	Exopalaemon modestus	+	+	
B73	, Macrobrachium nipponense de Haan	+		

Table A2. Cont.

ID	Species	W	J	BL
F1	Protosalanx hyalocranius	+		
F2	Paracobitis variegates	+		
F3	Triplophysa dalaica	+	+	+
F4	Triplophysa sellaefer	+	+	+
F5	Triplophysa shaanxiensis	+	+	+
F6	Triphysa stoliczkae	+	+	+
F7	Triplopphysa bleekeri	+	+	+
F8	Triplophysa robusta	+	+	+
F9	Triplophysa stoliczkae dorsonotata	+	+	+
F10	Triplophysa kungessana orientalis	+	+	
F11	Triplophysa pappenheimi	+	+	+
F12	Triplophysa sp.	+		
F13	Botia superciliaris	+		
F14	Cobitis granoei	+		
F15	Misgurnus anguillicaudatus	+	+	+
F16	Paramisgurnus dabryyanus		+	+
F17	Opasariichthys bidens	+	+	+
F18	Brachymystax lenok	+	·	·
F19	Phoxinus lagowskii	+	+	+
F20	Rhodeus sinensis	+	I	+
F21	Rhoaeus lighti	+	+	+
F21	Hemiculter leucisculus	+	т	+
F22 F23	Belligobio nummifer			
F23 F24		+		+
F24 F25	Oryzias latipes	+		+
	Pseudorasbora parva	+	+	+
F26	Gnathopogon imberbis	+		+
F27	Huigobio chinssuensis	+		
F28	Gobio rivuloides	+		
F29	Abbottina rivularis	+	+	+
F30	Sarcocheilichthys nigripinnis	+		
F31	Huigobio chinssuensis	+	+	+
F32	Scaphesthes macrolepis	+		
F33	Gymnodiptychus pachycheilus weiheensis	+		
F34	Schizopygopsis pylzovi	+		
F35	Cyprinus carpio	+		
F36	Carassius auratus	+	+	+
F37	Silurus asotus	+	+	+
F38	Pelteobagrus nitidus	+		
F39	Hypseleotris swinhonis	+		+
F40	Ctenogobius cliffordpopei	+	+	+
F41	Ctenogobius brunneus	+	+	+
F42	Ctenogobius gymnauchen	+		+
F43	Ctenogobius shennongensis	+		+
F44	Ctenogobius giurinus	+		+
F45	Channa argus	+		

## Appendix C. The Distribution of Fish Assemblage in the W, J, and BL Catchments

Table A3. The list of fish species in Wei River basin.

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