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Networks and ordination analyses reveal the stream community structures of fish, macroinvertebrate and benthic algae, and their responses to nutrient enrichment

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ABSTRACT

Rivers are perhaps the most altered ecosystem due to the human disturbances of water contamination, habitat destruction, hydrological regime change, overexploitation and exotic species introduction. Knowledge about the integrated effects of both natural environmental gradients and anthropogenic disturbances on multiple aquatic organism at a watershed scale is limited. In this study, the physical, chemical, hydrological and biological data were collected in May 2009 and 2010 at 281 sampling sites spread out wide stress gradients in the Hun-Tai River. We conducted canonical correspondence analysis (CCA) and network analysis to reveal the aquatic assemblage structures of fish, macroinvertebrates and benthic algae under the steep gradients of nutrient concentration and multiple environmental parameters. The results showed that habitat quality, elevation, water depth, stream width, dissolved oxygen, conductivity, TN, TP, and N:P ratio were identified as the significant parameters on the community compositions of fish, macroinvertebrate and benthic algae. In contrast to the historical effects of organic pollution, the eutrophication became the currently dominant human influencing factor on aquatic organisms in the Hun-Tai River. The nutrient parameters of nitrogen and phosphorus, and stoichiometric of N:P (mainly influenced by TP) were all identified as the significant parameters in the CCA biplots of fish, macroinvertebrate and benthic algae. Fish community was closely related with stream hydromorphological and nutrients parameters, however, macroinvertebrate and periphyton communities were driven by morphometry, chemistry and nutrients parameters. Network analysis was used to reveal the taxa associations and their correlations with nutrient parameters. By calculating the topological parameters of the networks, both the benthic algae and macroinvertebrate assemblages showed higher values of network centralization, heterogeneity, and average numbers of neighbors than fish in the network, those which usually indicated the higher association among taxa and stability of community structures. *Huigobio chinssuensis*, *Cobitis granoei*, and *Ctenogobius brunneus* were the core fish taxa, the subfamily Tanypodiinae, *Epeorus* and *Ephemerella* were the core macroinvertebrate taxa, and genera of *Diatom*, *Fragilaria*, *Achnanthes*, *Navicula*, *Cymbella*, and *Nitzschia* were the core benthic algae taxa in the network. A few taxa of fish showed significantly negative correlations with enrichment of nutrients and increasing the N:P ratio, and significantly positive correlations only existed with N:P ratio. In contrast to the fish network, macroinvertebrate and benthic algae taxa showed stronger and broader negative correlations to that of enrichment and N:P ratios in the network. Meanwhile, the positive correlations also broadly existed in the benthic algae network. This finding offers new clues of specific taxa of macroinvertebrate and benthic algae could be used as the indicator of stream eutrophication. This study provided new insights into integrated application of ordination method and network analysis in structuring the assemblage composition. And the network analysis also improve our understanding of individual interactions in the community and their relations to environmental stressors at the species level.

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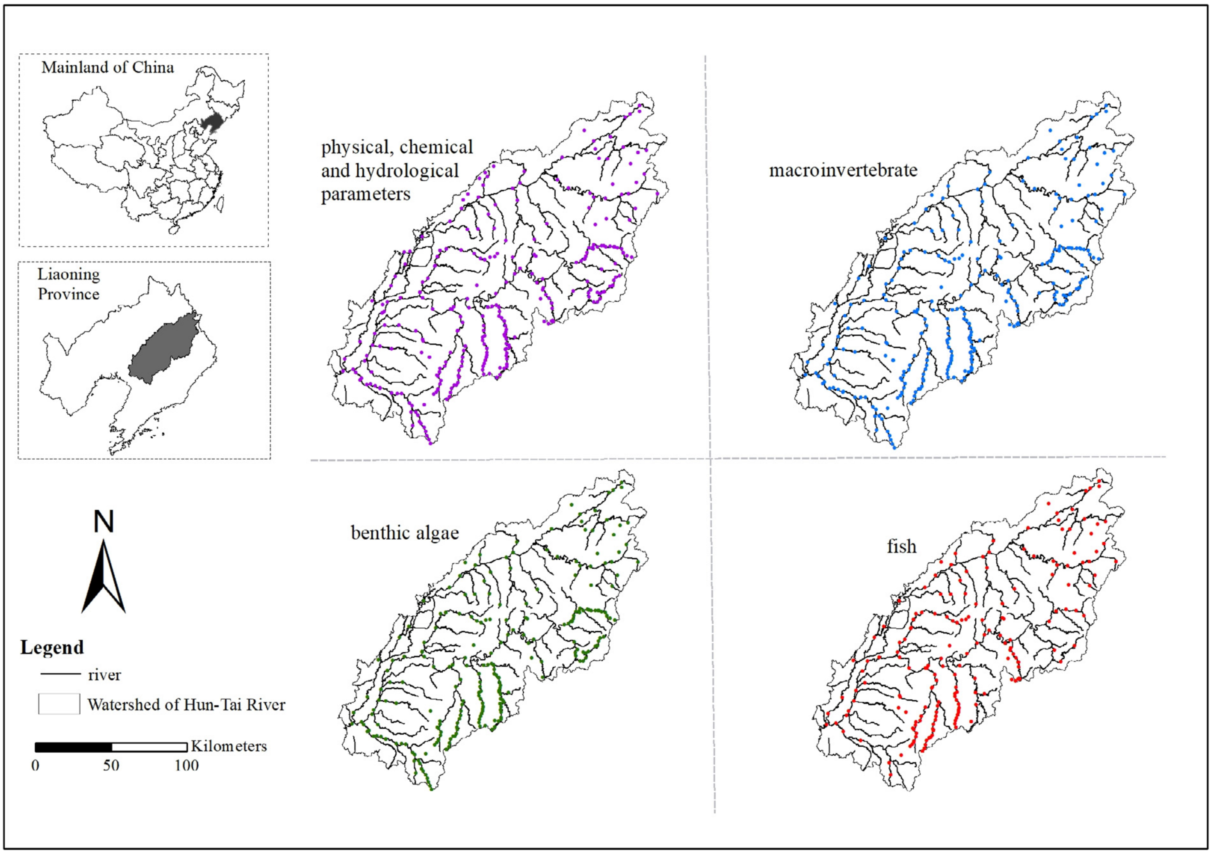

Fig. 1. Map of the study area and sampling sites. Four figures represented the sampling sites of physical, chemical and hydrological parameters (281 sites), macroinvertebrate (273 sites), benthic algae (246 sites), and fish (181 sites).

1. Introduction

In aquatic ecosystems, fish, macroinvertebrates, and benthic algae play important roles in maintaining ecosystem functions and structures, and have been shown to respond to environmental gradients, water chemistry and habitat variability on various scales (Sawyer et al., 2004; D'Ambrosio et al., 2014; Johnson and Ringler, 2014). Notwithstanding numerous studies on the response of individual taxonomic groups to environmental gradients and human-generated stress (Peters et al., 2014), few studies have simultaneously examined the integrated effects of various human impacts, such as nutrient enrichment, habitat disturbances and hydrological alteration, on aquatic assemblages of fish, macroinvertebrate, and benthic algae (Justus et al., 2010; Bae and Park, 2014; Mangadze et al., 2016). These groups of organisms have also been widely used to assess aquatic ecosystem health (Bachmann et al., 1996; Wallace and Webster, 1996; Oberdorff et al., 2002; Choi et al., 2011; Regina, 2012; Santos et al., 2017).

Understanding the biotic structures that control the nutrient-cycle capacities of rivers, especially nutrient-enriched lotic ecosystems, is of major interest for managing the health of riverine and coastal environments (Hoellein et al., 2011). Rivers are the primary receivers of nutrients and organic matter exported from terrestrial ecosystems (Vannote et al., 1980; Figueiredo et al., 2010; Deegan et al., 2011), thus these features crucially link upland watersheds with downstream aquatic ecosystems (Cronan, 2012). The nutrient enrichment delivered into aquatic ecosystems through human activities (Savage et al., 2010) exerts an extensive influence on riverine ecosystem structures and functions worldwide (Vitousek et al., 1997; Carpenter et al., 1998; Rabalais, 2002). Nutrient availability may affect aquatic biota and the ecological stoichiometry of organisms on multiple trophic levels, and is regarded as one of the most important regulating factors of lotic and lacustrine ecosystems (Sterner and Elser, 2002; Elser et al., 2007, 2009a; An et al., 2013; Taylor et al., 2014).

Fish assemblage plays an important role in stream ecosystems through top-down control on prey organisms as well as having a strong influence on nutrient dynamics through the uptake and release of nutrients (Vanni et al., 2002; McIntyre et al., 2007; Wilson and Xenopoulos, 2011). The nutrient regime (concentration and stoichiometry ratio) and available food can regulate fish taxonomic compositions and abundances in aquatic ecosystems (Jeppesen et al., 2000; Noble et al., 2007; Kim and An, 2015). At intermediate trophic levels, macroinvertebrates are influenced by both bottom-up and top-down forces in streams, and they have an important influence on nutrient cycle, primary productivity, decomposition, and translation of materials (Wallace and Webster, 1996). In turn, macroinvertebrate community structures and trophic dynamics are affected by nutrient availability and N:P ratios (Johnson et al., 2013; Mehler et al., 2013; Deans et al., 2015). Benthic algae provide primary productivity and play a key role in energy flow, nutrient cycling, and biogeochemical processes in stream ecosystems (Mayer and Likens, 1987; McCutchan and Lewis, 2002; Schiller et al., 2007; Godwin and Carrick, 2008). Numerous studies have demonstrated that nutrient concentrations and N:P ratios directly regulate the community structure, biomass, and the elemental composition of benthic algae (Stelzer and Lamberti, 2001; Dodds et al., 2002; Klausmeier et al., 2004). Variations in N:P stoichiometric ratios of the environment have been used to determine the abundance of specific taxa and the trophic levels of food webs (Stelzer and Lamberti, 2001; Volk and Kiffney, 2012; Deans et al., 2015).

Despite the importance of nutrient availability and N:P ratios in regulating community structures in lotic ecosystems, little is known about the interactions of different organisms within three assemblage and the integrated ecosystem structure under a wide gradient of nutrient concentrations and stoichiometry, especially under the effects of the other environmental gradients. In this study, we examined the ecosystem structure of the Hun-Tai River, a highly nutrient-enriched river with a steep gradient of N and P concentrations as well as stoichiometric ratios of N:P, in terms of the fish, macroinvertebrate and benthic algae assemblages, by using ordination and network analyses. Our main objectives were 1) to report the community compositions of three different aquatic organisms, 2) to reveal the effects of both the nutrient concentrations and the environmental gradients on the structure of the different assemblages, and 3) to identify interactions of different taxa within each assemblage and their associations with nutrients regime. The results are intended to provide basic and important information for ecosystem protection and management.

2. Material and methods

2.1. Study area

The Hun-Tai River (Fig. 1), located in northeastern China, is a section of the Liao River system formed by the confluence of two tributaries, the Hun River (415 km in length) and Taizi River (413 km in length). The watershed area is 25,360 km^2, the average annual temperature is 9 °C, and the average annual precipitation is 686.4 mm. Annual rainfall is 404–934 mm in the Hun River basin and 655–955 mm in the Taizi River basin. The total annual runoff is about 6.3 billion m^3, which provides the drinking water for upwards of 20 million people, as well as the water supply for domestic, industrial, and irrigation purposes. The Hun-Tai River drains across an obvious range of environmental gradients. The headwater of the Hun and Taizi rivers originate at the high elevations in the Changbai Mountains, where there is the least human disturbance and greatest riparian canopy cover. Areas of heavy industrial plants, urban development and agriculture are distributed along the middle and lower regions of the Hun-Tai River. River pollution was highly attributed to urbanization and financial development. Among the ten largest cities of Liaoning Province, five of them are located within the Hun-Tai River watershed (i.e. Shenyang, Anshan, Fushun, Benxi, and Liaoyang). Since the 1950s, the Hun-Taizi River basin had become an important region for coal, copper mining, iron manufacturing, and crude oil extraction. Following industrial development and urbanization, the Hun-Tai River suffered from industrial wastewater pollution as well as a high level of municipal sewage (Guo and He, 2013; Liu et al., 2015). During the past three decades, local government had made huge investments to control water contamination, such as through construction of wastewater treatment systems, and shutting down some coal mines and oil fields. Reports about restoring the water quality and aquatic ecosystems of the Hun-Tai River have gradually increased after 2000. However, nutrient enrichment originating from non-point pollution sources through agricultural development caused concerns among riverine scientists, local authorities and residents for the past few years (Zhang et al., 2013).

2.2. Sampling and analyzing

In total, 281 sampling sites along the Hun-Tai River were selected for measurement of physical, chemical and hydrological factors for all the sampling stations in the spring period of 2009 and 2010 (Fig. 1). At each sampling site, elevation was measured using a global position system receiver (Trimble-Juno SB). Water temperature (WT), pH, dissolved oxygen (DO), total dissolved solids (TDS) and conductivity (Cond) were measured using a handheld YSI Multiparameter instrument (professional plus) *in situ*. Suspended solids (SS) were measured by the weighting method (Chinese national standard for suspended substance) using 100 ml of river water filtered through a nitrocellulose and cellulose-diacetate blend membrane (pore size 0.45 μm). Stream surface-water samples were collected from each site to measure the chemical parameters. Total nitrogen (TN) and nitrate (NO_3^-) were analyzed by ion chromatography (EPA 300.0). Ammonium (NH_3-N) was analyzed using the indophenol colorimetric method (EPA 350.1). Total phosphorus (TP) and soluble reactive phosphorus (SRP) were analyzed using the ammonium molybdate method (EPA 365.3). Chemical oxygen demand (COD) was determined by the potassium dichromate method (EPA 410.1). At each site, stream surface-water samples were collected and acid-fixed and transported to the laboratory at 4 °C for chemical analyses. Mean stream width (W) was measured according to three random replicates in the sampling reach. Current velocity and water depth (WD) were measured using a digital velocity meter set in a sampling position appropriate to the macroinvertebrates and benthic algae, with three replicates (Global Water Flow Probe FP201). The qualitative habitat evaluation index (QHEI), designed to characterize the physical habitat of a stream, and was measured to reflect the habitat quality (Rankin, 1995). The QHEI covered ten key aspects: substrate composition, in-stream habitat complexity, range of combined water depth and velocity, bank stability, channel sinuosity, water quantity, visual inspection of water cleanliness, biodiversity of riparian plants, environmental stress from human activities, and land use types. Scoring for each aspect ranged from 0 to 20, representing low to high habitat quality. The scores were assigned based on the empirical experience of the monitors and then summarized as the final score for habitat quality.

Fish were collected from 181 sites using electrofishing techniques (Fig. 1). Each sampled reach was about 500 m long. Before sampling the site, two nylon monofilament gillnets (mesh size 10 mm) were set at the upper and lower bounds of the reach to block it off. The sampling duration was limited to 30 min. Fish were identified, enumerated, and weighed *in situ* according to the local standardized reference (Xie, 2007), except unnamed specimens were brought back to the laboratory for identification. Macroinvertebrate and benthic algae samples were collected using quantitative methods from 273 sites and 246 sites, respectively (Fig. 1). Nine stones were randomly picked up (diameter approximately 25 cm) within a 300 m section at the given site. Using a 3.5 cm diameter PVC pipe with a rubber core and a toothbrush, benthic algae were carefully brushed away and rinsed with distilled water. The rinse water were combined and preserved with 4% formalin in a 50 ml plastic bottle. Each sample of benthic algae was separated into two parts. One sample was used to identify the ‘soft’ algae, and the other was used to make the permanent diatom slides after oxidizing the organic material with acid disposal for identifying the benthic diatoms. In the laboratory, The ‘soft’ algae were identified directly using a 0.1-ml counting chamber at a magnification of ×400 with a minimum of 300 valves. For each diatom slide, a minimum of 300 valves were counted at ×1000 magnification under oil emersion by using the microscope (Nikon Ni-U). By using the classic manuals of Hu et al., (1980) and Zhu and Chen (2000), benthic algae was identified to the lowest taxonomic level possible (to species mostly). Benthic macroinvertebrates were collected using a Surber net (30 × 30 cm^2, 500 μm mesh) with three replicates. The substrate was kicked or disturbed using a shovel and directed into the net. The sample was then transferred from the net to a 10L plastic container. After rinsing each sample through a 40 mm mesh, all samples from each site were combined into a 500 ml jar and preserved with 70% alcohol. In the laboratory, macroinvertebrates were identified to the lowest taxonomic level (mainly to genus) referring to the manuals of Merritt and Cummins (1996) and Morse et al. (1999).

2.3. Statistical analyses

Stepwise linear regression was used to analyze the relationship between TN, TP, and N:P ratio (using SPSS 20.0, IBM, Armonk, NY, USA). Furthermore, the relationships between biodiversity indices (taxa richness and Shannon-Wiener index) and TN, TP, and N:P ratio were analyzed using Pearson correlation. The nutrient parameters of TN, TP and N:P ratio were logarithmically transformed before the linear regression and correlation analyses based on the formula of $\log_{10}(x + 1)$.

The data normality was checked by using the qq-plot method before the linear regression and correlation analysis, and they fit the normality distribution well.

Canonical correspondence analysis (CCA) was applied to analyze the spatial distribution of the aquatic organisms under the effect of environmental stresses using R (version 3.3.2 and 'vegan' package 2.4). Taxa data was square-root transformed and environmental data were logarithmically transformed before CCA based on the formula of $\log_{10}(x + 1)$ to eliminate influences of extreme values on the ordination. The pH was not transformed as it already fit the logarithmic scale. Monte Carlo permutations ($P < 0.05$) were used to select a set of environmental factors that had significant and independent effects on aquatic organism distribution. The environmental factors with high partial correlation coefficients ($P < 0.05$, $|r| > 0.5$) and variance inflation factors > 20 were omitted from the final CCA.

The Spearman correlations were calculated pairwise for each taxa using R (version 3.3.2). A Spearman correlation coefficient R score and *P*-value were calculated pairwise between the taxa using the Hmisc package (version 4.0–1) in R. Only significant ($P < 0.05$) associations were used to construct the network. Visualization of the significant associations was made using Cytoscape (version 3.4.0). An edge-weighted spring-embedded network was applied to reveal the arrangement of the variables. Each node represents the taxa in the network, and each edge represents a significant correlation. The positive correlations were represented as blue edges and negative correlations were represented as the red edges. Topological and node/edge metrics, including numbers of nodes, average clustering coefficient, network heterogeneity, average number of neighbors, and betweenness centrality were calculated using the Network Analyzer plugin within Cytoscape (Assenov et al., 2008). The clustering coefficient was calculated to represent tends of nodes clustering in the network. Network heterogeneity quantified the diversity of connections between nodes in networks even with different topologies. Average number of neighbors calculated the average connectivity of a node in the network (Jacob et al., 2017). Betweenness centrality is an indicator of a node's location in relation to other nodes, which measures the number of shortest paths passing through a given node. A high value of betweenness centrality indicates a core location of this node and reflects the potentially strong control that this node exerts over the interactions of other nodes in the network.

3. Results

3.1. Environmental gradients, nutrients status and aquatic biodiversity

Habitat quality varied considerably between the sampling regions. The QHEI habitat scores (HS) ranged from 35 to 192. Considering a maximum score of 200, the mean of 112 indicated a relatively high level of human disturbance (Table 1). Elevation ranged from 663 m at the headstream region to 4 m at the downstream region with a mean of 211 m. Water temperature in the study area ranged from 6.8 to 26.0 °C with a mean of 16.6 °C. Stream width ranged from 0.3 m at the upstream mountainous area to 420 m at the downstream floodplain with a mean of 38.3 m. The water depth range was 2–130 cm, with a mean of 25 cm. Minimum water velocity was 0 m/s as the result of sampling in the still water; maximum velocity was 1.14 m/s, indicating riffles and turbulent flow. The mean value of velocity is 0.41 m/s in the study area. The mean of pH was 8.4 with a maximum of 10.1 and minimum of 6.0. Conductivity ranged from 3.9 to 1431.0 μs/cm, with a mean of 296.6 μs/cm. The concentration of DO ranged from 0.1 to 15.6 mg/L, with a mean value of 9.5 mg/L. The concentration of SS ranged from 0.1 to 1110 mg/L, with a mean of 35.7 mg/L. The concentration of TDS ranged from 15.0 to 968.5 mg/L, with a mean of 203.6 mg/L. The concentration of COD ranged from 2.00 to 46.30 mg/L with a mean of 5.5 mg/L. Extremely low values of DO and high values of COD were located at the Nansha tributary owing to a high input of untreated contaminated water from the rural regions. The concentration of TN in the study area ranged from 0.68 to 22.6 mg/L, with a mean of 5.3 mg/L (Table 1). The concentration of TP ranged from 0.004 to 3.025 mg/L, with a mean of 0.159 mg/L (Table 1). The concentrations of TN were much higher than the TP at all sites. The N:P ratios ranged from 3 to 5234 with an mean of 360 (Table 1). When considering the mean values of the forms of TN, NO_3 (mean 2.83 mg/L) was more dominant than NH_3-N (mean 1.04 mg/L). The concentration of SRP ranged from 0.001 to 0.925 mg/L, with a mean of 0.093 mg/L. High N:P ratios indicated that the ecosystem was more P-limited. The high CV values of SS (2.45), NO_3 (2.17) and TP (1.96) reflected the high variation of soil erosion and nutrient enrichment in the study area. Regression analysis between log-transformed N:P ratios and the nutrient concentrations indicated that the N:P ratios were mainly affected by TP ($R^2 = 0.705$, $P < 0.001$; Fig. 2a) and weakly influenced by TN ($R^2 = 0.064$, $P < 0.001$; Fig. 2b). Concentrations of TN had a weak association with TP ($R^2 = 0.098$, $P < 0.001$; Fig. 2c).

Table 1

Summary of environmental gradients and nutrient parameters analyzed from the study sites in the Hun-Tai River basin. The abbreviations used as followed: SD, standard deviation, CV, coefficient of variation (equals to the ratio of the standard deviation to the mean).

Types	Parameters	Max	Min	Mean ± SD	CV
Habitat score	HS	192	35	112 ± 28	0.25
Physical	Elevation (m)	663	4	211 ± 151	0.72
	WT (°C)	26.0	6.8	16.6 ± 4.2	0.25
	Stream width (m)	420.0	0.3	38.3 ± 67.0	1.75
Hydrological	WD (cm)	130	2	25 ± 14	0.55
	Velocity (m/s)	1.14	0.00	0.41 ± 0.24	0.57
Chemical	pH	10.1	6.0	8.4 ± 0.6	0.07
	Cond (μs/cm)	1431.0	3.9	296.6 ± 223.0	0.75
	DO (mg/L)	15.6	0.1	9.5 ± 3.1	0.33
	SS (mg/L)	1110.0	1.0	35.7 ± 87.5	2.45
	TDS (mg/L)	968.5	15.0	203.6 ± 138.4	0.68
Organic matter	COD (mg/L)	46.30	2.00	5.5 ± 6.4	1.16
Nutrients	TN (mg/L)	22.6	0.68	5.332 ± 4.225	0.79
	NH_3-N (mg/L)	14.50	0.03	1.041 ± 1.861	1.79
	NO_3 (mg/L)	15.90	0.006	2.834 ± 6.143	2.17
	TP (mg/L)	3.025	0.004	0.159 ± 0.311	1.96
	SRP (mg/L)	0.925	0.001	0.093 ± 0.134	1.44
	N:P (molar ratio)	5234	3	360 ± 649	1.80

We recorded 36 fish, 186 macroinvertebrate, and 540 benthic algae taxa, respectively, in the study area. The alpha diversity of taxa richness for fish, macroinvertebrates, and benthic algae was 6.45, 16.95, and 22.06, respectively. The averages of the Shannon index were 1.14, 1.45, and 1.96 for fish, macroinvertebrate, and benthic algae, respectively. Correlation analysis indicated that only TP had a significant negative correlation ($P < 0.05$) with fish taxa richness (Table 2). Whereas both TN and TP had a significant negative correlation ($P < 0.01$) with macroinvertebrate taxa richness and their Shannon index values (Table 2). For benthic algae, TP had a negative correlation with taxa richness, and N:P ratio had a positive correlation with them (Table 2).

3.2. Organism distribution along multiple environmental gradients

Canonical correspond analysis (CCA) was applied to elucidate the distributions of the aquatic organisms under the effects of environmental gradients and variable nutrients (Fig. 3). The results showed 12 environmental factors as the most significant variables affecting the distribution of fishes (Fig. 3a), these mainly included elevation, stream width, water depth, velocity, Cond, DO, TN, and TP (Monte Carlo test, $P < 0.05$). The first two axes accounted for 50.50% of the variance (axis 1: 35.57%; axis 2: 14.93%). The first axis was mainly defined by

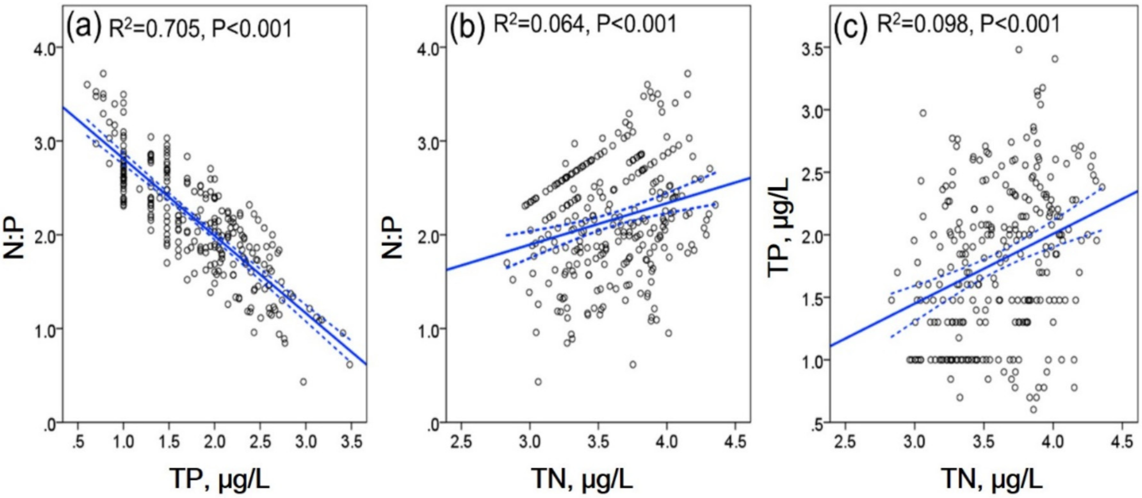

Fig. 2. Relationships between the log-transformed N:P ratios and the total nutrient concentrations. Solid lines correspond to the linear regression and dotted lines indicate 95% confidence intervals.

Table 2
Pearson correlations analysis between nutrients and biodiversity indices (*indicates the significance at $P < 0.05$; **indicates the significance at level $P < 0.01$).

	Fish		Macroinvertebrate		Benthic algae	
	Richness	Shannon	Richness	Shannon	Richness	Shannon
Log (TN, mg/L)	−0.111	−0.071	−0.354**	−0.339**	0.068	0.117
Log (TP, mg/L)	−0.169*	−0.093	−0.191**	−0.187**	−0.197**	−0.063
Log (N:P ratio)	0.102	0.052	−0.009	−0.003	0.237**	0.126

the hydrological variables and physical variables of water depth and stream width, chemical parameter of Cond and nutrients of TN and TP in a positive direction, and by elevation and velocity in a negative direction. For macroinvertebrates (Fig. 3b), 9 environmental factors were the significant variables affecting the community composition and spatial distributions (Monte Carlo test, $P < 0.05$), these mainly included elevation, TP, water depth, Cond, stream width, and N:P ratio. The first two axes accounted for 40.35% of the variance (axis 1: 23.01%; axis 2: 17.34%). The first axis was mainly defined by elevation in a positive direction and the TP, Cond and water depth in a negative direction. The second axis was defined by TP in a negative direction. For benthic algae (Fig. 3c), 11 environmental factors, mainly including DO, TN, Cond and the habitat-quality score, were the most significant variables to affect the taxa composition and spatial distributions (Monte Carlo test, $P < 0.001$). The first two axes accounted for 43.18% of the variance (axis 1: 28.37%; axis 2: 14.81%). The first axis was mainly defined by a combination of DO and elevation in a positive direction, and by TN in a negative direction. The second axis was mainly defined by Cond and TP in a positive direction, and habitat scores in a negative direction. In general, the hydromorphological and nutrient parameters were the factors most affecting the community compositions and spatial distributions of the fish community. The parameters of habitat quality, physical, chemical, hydrological and nutrients were the dominant factors affecting the community compositions and spatial distributions of benthic algae and macroinvertebrates. In addition, the nutrient variables of TN, TP, and the N:P ratio were also identified as significant parameters affected the fish, macroinvertebrate and benthic algae in the Hun-Tai River, except for TN at the macroinvertebrate CCA biplot. The results confirm the effects of nutrients enrichment and stoichiometry gradients on the community compositions of fish, macroinvertebrate and benthic algae in the Hun-Tai River.

3.3. Connectedness of aquatic organisms and their associations with nutrient parameters

Patterns of aquatic organism's connectedness and their associations with nutrient variables were explored by network analysis. The variables were 36 fish, 186 macroinvertebrate (mostly to species and genus level, except subfamily level for Chironomidae, and the subclass Oligochaete other than *Branchiura sowerbyi*), and 540 benthic algae taxa, and three nutrient components (TN, TP, and N:P ratio). The single networks are depicted for fish (Fig. 4a), macroinvertebrates (Fig. 4b), and benthic algae (Fig. 4c). Edge-weighted spring-embedded visualization of the interconnected networks revealed arrangement of the variables (Fig. 4).

Of the 987 tested correlations in the fish network, only 86 were considered significant ($P < 0.05$). This network has a clustering coefficient of 0.367 and average number of neighbors of 4.41. The value of network heterogeneity is 0.663. *Huigobio chinssuensis* had the highest betweenness centrality (BC) score (0.160), followed by *Cobitis granoei* (0.141), and *Ctenogobius brunneus* (0.130) (Fig. 4a). For the nutrient components, TN was associated with the maximum number of nodes (BC = 0.050), followed by N:P ratio (0.030). TP was associated with the minimum number of nodes (0.020). TN was negatively associated with nodes of *Gobio cynocephalus, Ctenogobius cliffordpopei, Phoxinus czekanowskii*, and *Leuciscus waleckii*. TP was negatively associated with nodes of *Cobitis granoei, Lefua costata*, and *Huigobio chinssuensis.* However, the N:P ratio was mostly positively associated with the nodes of *Lefua costata*, *Cobitis granoei* and *Leuciscus waleckii*, and negatively associated with the nodes of *Phoxinus czekanowskii.* For the purpose of summarizing the nutrient enrichment effects on fish communities, fish taxa that which showed significant associations with TN, TP and N:P ratio ($P < 0.05$), were combined according to the family levels of classification. The results showed that among all the fishes collected, the families of Cyprinidae and Cobitidae were the most negatively correlated with TN and TP; however, N:P ratio showed a positive correlation with those families (Table 4).

Of the 17,203 tested correlations in the macroinvertebrate network, 1649 were considered significant ($P < 0.05$). This network had a clustering coefficient of 0.549, an average number of neighbors of 17.913, and network heterogeneity of 0.807 (Table 3). The Tanypodiinae had the highest accumulated betweenness centrality score

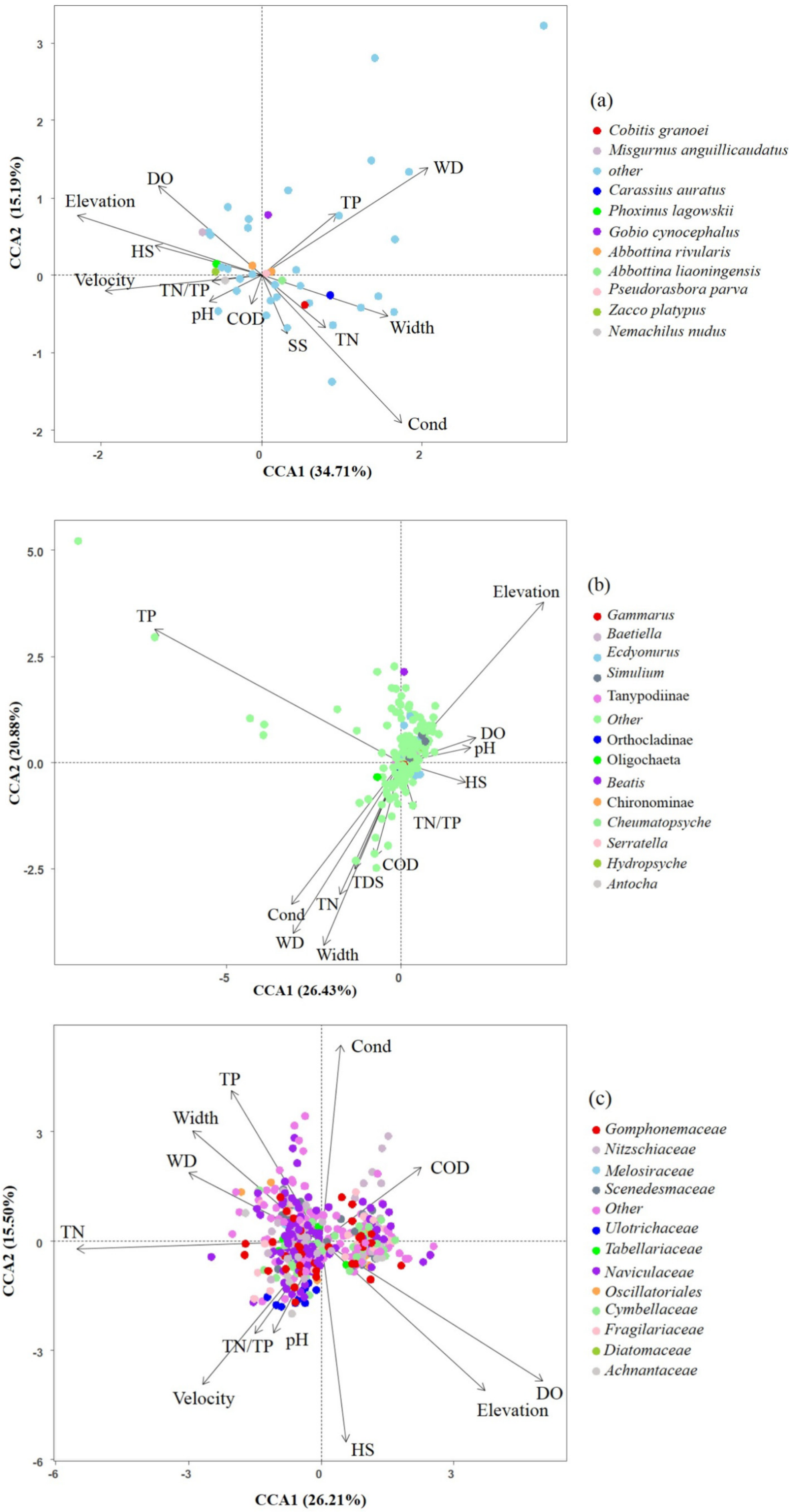

Fig. 3. Canonical Correspondence Analysis (CCA) revealing the distribution of aquatic organisms in relation to natural environmental gradients and nutrient components, for (a) fish, (b) macroinvertebrates, and (c) benthic algae. Taxa with relative abundance higher than 1% were selected as representative of each assemblage.

(0.081), followed by *Epeorus* sp. (0.071) and *Ephemerella* sp. (0.060) (Table 3). For the nutrients component, TP was associated with the maximum number of nodes (BC = 0.037), followed by N:P ratio (0.032) and TN (0.028). TN was negatively associated with the nodes of *Ormosia* sp., Tanypodiinae, *Hydatophylax festivus*, *Setodes turbatus* and Limnephilidae spp. N:P ratio was negatively associated with nodes of *Clinocera* sp., *Drunella basalis*, *Arctopsyche* sp., and *Discocerina* sp. TP was negatively associated with nodes of *Dugesia* sp., *Epeorus* sp., and *Setodes* sp., and positively associated with nodes of *Lethocerus* sp., *Elophila* sp. and *Oyamia* sp. Those macroinvertebrate taxa mainly belonged to the Diptera of Chironomidae, Tipulidae, Tabanidae and Canacidae; the Ephemeroptera of Baetidae, Ephemerellidae, Heptageniidae and Leptophlebiidae; the Plecoptera of Perlidae, Chloroperlidae, Leuctuidae and Perlodidae; and the Trichoptera of Limnephilidae, Leptoceridae and Hydropsychidae, which all showed widely negative associations with TP, TN, and N:P ratio. The positive correlations, however, were significant between the TP and several taxa belonging to the Ephydridae, Leptophlebiidae, Crambidae, Belostomatidae, Perlidae, and Limnephilidae (Table 4).

Of the 149,328 tested correlations in the benthic algae network, 5723 were considered significant ($P < 0.05$). This network had a clustering coefficient of 0.610, an average number of neighbors of 21.076, and network heterogeneity of 0.749. Diatoms of the Surirellaceae had the highest betweenness centrality score (0.098), followed by the Nitzschiaceae (0.077) and Oscillatoriales (0.045) (Table 3). For the nutrients component, TN was associated with the maximum number of nodes (BC = 0.256), followed by N:P ratio (0.216), and TP (0.017). To reveal the effects of nutrient component on the taxa of benthic algae at the family level, the significant correlations ($P < 0.05$) were combined within the same family for each nutrient parameter. The results showed that the diatom of Cymbellaceae, Naviculaceae, and Fragilariaceae dominated the significant correlations with TN. The taxa belonging to the Cymbellaceae, Achnantaceae, and Gomphonemaceae were positively correlated with TP and N:P ratio. The taxa belonging to the Naviculaceae and Nitzschiaceae were negatively correlated with TP and N:P ratio (Table 4).

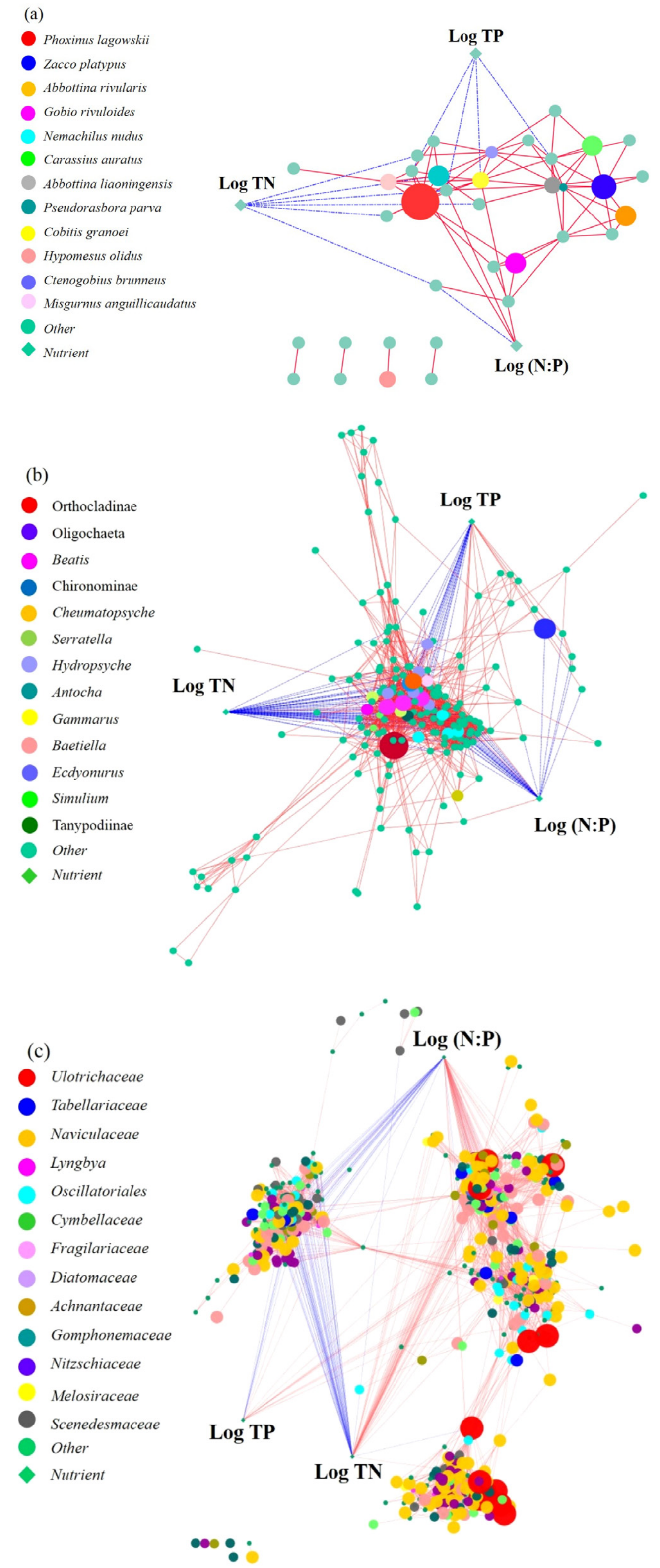

Fig. 4. Spearman correlation coefficient edge-weighted spring-embedded network visualizing significant correlations ($P < 0.05$) between different organisms and the nutrient variables, for (a) fish, (b) macroinvertebrates, (c) benthic algae. Node size is reflective of the betweenness centrality of the variable. Line types are indicative of the Spearman correlation coefficient (solid red = positive; dashed blue = negative). Taxa with a relative abundance higher than 1% were selected as the representative of each assemblage.

4. Discussion

CCA (Fig. 3) showed that the explanation rate by the first two CCA axes decreased slightly from 50.50% for fish to 40.35% for macroinvertebrate. The hydromorphological parameters of elevation, water velocity, stream width, and water depth commonly dominated the axes of three biplots in the CCA. However, the TN, TP and/or N:P ratio also played important roles in structuring the community structures of fish, macroinvertebrates, and benthic algae. Likewise, the nutrient parameters were also widely correlated with the dominant taxa of fish, macroinvertebrates, and benthic algae in the single network analyses (Fig. 4). Organic matter pollution in the study area was at one time quite severe (Fan et al., 2015), even so, COD was not identified as the dominant parameter affecting the distributions of the studied aquatic organisms in this research. In considering the relative high DO values, the organic contamination may be relative low for most sites. The study established that nutrient enrichment is presently the main threat to the aquatic communities since improvements to water treatment plants along the Hun-Tai River. Rivers influenced by a combination of non-point and point-source pollution have particularly high nutrient concentrations (Paul and Meyer, 2001; Mulholland et al., 2008; Schiller et al., 2007). According to the trophic state boundary for the stream (Dodds and Whiles, 2010), the Hun-Tai River is a nutrient-rich ecosystem, and especially nitrogen-rich. The molar N:P ratio is widely used as an index of nutrient limitations in various ecosystems (Smith, 1983; Ptacnik et al., 2010; Sterner and Elser, 2002). The community and diversity of producers are likely to be changed when nutrient ratios are skewed towards particular nutrients relative to others (Tilman, 1982; Elser et al., 2009b; Interlandi and Kilham, 2001). Species-specific

Table 3
Network topological parameters for fish, macroinvertebrates and benthic algae. The three most dominant values of betweenness centrality and its corresponding taxa of fish, macroinvertebrates and benthic algae were calculated. The values of betweenness centrality of TN, TP and N:P ratio in each network were also calculated.

Topological Parameters	Fish	Macroinvertebrates	Benthic algae
Number of nodes	39	189	543
Clustering coefficient	0.367	0.549	0.610
Network heterogeneity	0.663	0.807	0.749
Average number of neighbors	4.410	17.913	21.076
Betweenness centrality	*Huigobio chinssuensis* (0.160), *Cobitis granoei* (0.141), *Ctenogobius brunneus* (0.130), log TN (0.050), log (N:P ratio) (0.030), log TP (0.020)	Tanypodiinae (0.081), *Epeorus* (0.071), Orthocladinae (0.060), log TP (0.037), log (N:P ratio) (0.032), log TN (0.028)	Surirellaceae (0.098), Nitzschiaceae (0.077), Oscillatoriales (0.045), log TN (0.256), log (N:P ratio) (0.216), log TP (0.017)

Table 4
Significant correlations ($P < 0.05$) between nutrients and families of aquatic organisms of fish, macroinvertebrates and benthic algae within the network (superscripts represent the number of taxa having the significant associations belonging to the respective family).

Nutrient	Relationship	Category	Summary of aquatic families
TN	Positive	Macroinvertebrates	Phryganeidae
		Benthic algae	Fragilariaceae^{9}, Naviculaceae^{9}, Coscinodiscaceae, Volvocaceae, Thalassiosoraceae, Nitzschiaceae^{2}, Tabellariaceae^{2}, Cymbellaceae^{7}, Achnantaceae, Surirellaceae, Gomphonemaceae^{3}, Melosiraceae^{2}, Oscillatoriaceae, Scenedesmaceae, Ulotrichaceae
	Negative	Fish	Cyprinidae^{3}, Cobitidae, Gobiidae, Gasterosteidae
		Macroinvertebrates	Ephemerellidae, Baetidae, Heptageniidae^{4}, Leptophlebiidae^{2}, Ephemeridae, Perlidae^{3}, Chloroperlidae, Leuctuidae, Leptoceridae, Hydropsychidae^{5}, Stenopsychidae, Rhyacophilidae, Psychomyiidae, Limnephilidae^{3}, Odontoceridae, Phryganeidae, Psephenidae, Chironomidae^{2}, Tipulidae^{4}, Tabanidae, Empididae, Gomphidae^{3}, Galopterygidae, Calopterygidae, Planorbidae, Unionoidae, Ichneumonidae, Planariidae
		Benthic algae	Fragilariaceae^{7}, Bacillariaceae, Nitzschiaceae, Tabellariaceae^{2}, Cymbellaceae^{6}, Achnantaceae^{4}, Gomphonemaceae^{4}, Naviculaceae^{6}, Oscillatoriaceae^{3}, Phormidiaceae, Chaetophoraceae^{3}, Merismopediaceae, Entophysalidaceae, Desmidiaceae, Scenedesmaceae^{4}, Selenastraceae, Micractiniaceae, Closteriaceae, Ulotrichaceae, Tribonemataceae
TP	Positive	Macroinvertebrates	Perlidae, Leptophlebiidae, Limnephilidae, Ephydridae, Canacidae, Belostomatidae, Crambidae^{3},
		Benthic algae	Nitzschiaceae^{2}, Cymbellaceae, Melosiraceae, Naviculaceae^{2}, Nostocaceae, Volvocaceae, Euglenaceae^{2}, Closteriaceae, Microthamniales, Chaetophoraceae
	Negative	Fish	Cyprinidae^{2}, Cobitidae^{2}
		Macroinvertebrates	Baetidae, Heptageniidae, Ephemeridae, Hydropsychidae^{3}, Psychomyiidae, Leptoceridae, Chironomidae, Tipulidae, Tabanidae, Calopterygidae, Glossiphoniidae, Nematoda, Plannariidae
		Benthic algae	Cymbellaceae^{2}, Achnantaceae^{2}, Gomphonemaceae^{2},
N:P ratio	Positive	Fish	Cyprinidae, Cobitidae^{2}
		Macroinvertebrates	Carabidae, Tabanidae, Empididae
		Benthic algae	Fragilariaceae^{14}, Cymbellaceae^{14}, Naviculaceae^{10}, Nitzschiaceae^{3}, Tabellariaceae^{3}, Achnantaceae^{4}, Surirellaceae, Melosiraceae, Gomphonemaceae^{4}, Nostocaceae, Scenedesmaceae, Ulotrichaceae Selenastraceae,
	Negative	Fish	Cyprinidae
		Macroinvertebrates	Ephemerellidae^{2}, Heptageniidae^{2}, Leptophlebiidae, Ephemeridae, Perlidae^{3}, Perlodidae, Chloroperlidae, Hydropsychidae, Rhyacophilidae, Limnephilidae^{2}, Odontoceridae, Psephenidae, Ephydridae^{2}, Canacidae, Belostomatidae, Crambidae^{3}, Oligochaeta
		Benthic algae	Coscinodiscaceae, Melosiraceae, Achnantaceae, Fragilariaceae^{3}, Nitzschiaceae^{4}, Cymbellaceae^{2}, Gomphonemaceae, Naviculaceae^{8}, Tribonemataceae, Merismopediaceae, Nostocaceae, Oscillatoriaceae, Euglenaceae^{2}, Chaetophoraceae, Scenedesmaceae^{3}, Closteriaceae^{2}, Hydrodictyaceae

responses to nutrient enrichment through direct or indirect mechanisms likely culminate in shifts in community composition involving both producers and consumers (Taylor et al., 2014).

In the network analyses, our goal was to explore overall patterns of interactions to gain a holistic view of the three main aquatic assemblages and to improve our understanding of their correlations to the nutrient variables. The results suggested that the macroinvertebrate and benthic algae assemblages had the highest neighborhood connectivity and clustering coefficient, followed by the fish assemblage, indicating that the macroinvertebrates and benthic algae constitute a more complex associations among individuals than the fish community. The higher taxa richness of benthic diatom and macroinvertebrates mainly contributed to the network complexities, suggesting that species associations may negatively correlate with taxa richness within the same aquatic assemblages. Both the macroinvertebrate and benthic algae communities showed a high degree of interaction, through relatively high stability of communities controlled by dominant taxa (Fig. 4). Three patterns of co-occurrence for benthic algae reveals the community divisions and different ecological functions (Barberán et al., 2012; Fuhrman, 2009). A low value of betweenness centrality indicates a more peripheral location of the node and less influence on the other nodes. The wide variation in the betweenness values among the different aquatic assemblages reflects the situation of historically severe river pollution (Zhang et al., 2009), wherein the community of benthic algae and macroinvertebrates typically recovered sooner, but the fish community less quickly (Mangadze et al., 2016).

Cyprinids are one of the most diverse fish families occupying nearly every type of freshwater habitat and also play an important role in the biogeochemical processes (Rubio-Gracia et al., 2017). The Diptera, Ephemeroptera and Trichoptera dominated the macroinvertebrate assemblage, the Ephemeroptera and Trichoptera consisted largely of sensitive taxa in this study. However, the tolerant values of taxa of the Diptera ranged from the most sensitive to highly tolerant (Lecerf et al., 2006; Al-Shami et al., 2010; Arimoro et al., 2007). For example, in the upstream region of Hun-Tai River, *Potthastia* and *Pagastia* (Tanypodiinae, Chironomidae) were usually the most sensitive taxa, while *Tanypus* (Tanypodiinae, Chinomidae) usually dominated in the highly polluted downstream region. In our study, the subfamilies Tanypodiinae and Orthocladinae had relatively higher accumulative betweenness values, of 0.081 and 0.060, respectively, indicating that those taxa play an important role in structuring the macroinvertebrate assemblage in the Hun-Tai River. The results also prompted that classification of Chironomidae would be genus level at least in identifying the sensitive indictors to human disturbances. Owing to the strong effects of hydromorphological parameters (particularly elevation, water depth, and stream width) (Fig. 3b), relatively weak correlations were identified between the nutrient variables and the taxa of macroinvertebrate. This result also indicated weak betweenness centrality between the nutrients and the dominant taxa, namely the subfamilies Orthocladinae and Chironomidae, *Baetis* spp., and *Cheumatipsyche* sp. Those taxa usually show relatively high tolerance values to nutrient enrichment and organic-matter pollution (Rosenberg and Resh, 1993). Diatoms are one of the most abundant and successful microbial groups in freshwater ecosystems, both in the number of taxa richness and overall abundance (Kopalová et al., 2012; Vanormelingen et al., 2008). Diatoms have long been used to assess and monitor the human disturbances in streams and rivers (Hill et al., 2000) because of their rapid response to environmental changes (Potapova and Charles, 2002). Moreover, the diversity and community of epilithic diatoms may be demonstrably related to TN and TP (Jüttner et al., 2003). Diatoms can also be powerful indicators of eutrophication (Jüttner et al., 2003) and environmental changes associated with land use (Stewart et al., 1999). In our study, patterns of co-occurrence showed both negative and positive responses to nitrogen and the stoichiometry gradients. Taxa that correlated negatively with TN and N:P ratio reveal more complex community compositions and functions than those taxa with positive correlations. Among the diatom taxa, the Surirellaceae and Nitzschiaceae had higher scores of accumulative betweenness (0.098 and 0.077, respectively), signifying that these diatoms play a fundamental role in the Hun-Tai River. Diatoms are commonly dominant taxa of periphyton producer in rivers (Graham and Wilcox, 2000; Allan and Castillo, 2007). In streams, the nutrient enrichment could usually stimulate the explosion of benthic algae, especially for biomass and taxa richness of diatoms (Flecker et al., 2002). Even though the development of periphyton assemblage is also highly restricted by the other environmental parameters, such as temperature (Slavik et al., 2004) and forest shade (Greenwood and Rosemond, 2005). The CCA showed that diatoms mainly distributed along the first axis, which was mainly defined by a combination of TN and TP in a negative direction (Fig. 3c). The green algae Ulotrichaceae (Chlorophyta) mainly distributed along the second axis, which was defined partly by the N:P ratio in a negative direction (Fig. 3c). This result was comparable with the network analysis showing that TN and TP were negatively associated with the nodes belonging to diatoms, and positively associated with the nodes belonging to the Chlorophyta (Fig. 4c).

Only a few fish taxa of Cyprinidae, Cobitidae, Gobiidae, and Gasterosteidae showed negative correlations with TN, TP, and N:P ratio. These results confirm that fishes can be more tolerant to nutrient enrichment than benthic algae and macroinvertebrates (Mangadze et al., 2016; Marzin et al., 2012). Habitat condition, stream morphometry, and hydrological conditions may link closely with fish assemblages (Gratwicke et al., 2003; Jowett and Richardson, 2003). The macroinvertebrate assemblage showed widely negative correlations with increasing nutrient levels and the stoichiometry gradients. Unlike streams with nutrient input as the major stressor (Haase and Nolte, 2008), our results revealed that macroinvertebrates may be sensitive indicators in streams with wide variations in nutrient enrichment and multiple environmental gradients. Most of the macroinvertebrates identified as sensitive to nutrient enrichment belonged to EPT (Ephemeroptera, Plecoptera, and Trichoptera). It is comparable with large numbers of published literatures that confirmed the sensitivity of EPT indices (Barbour et al., 1999; Hering et al., 2004; Qu et al., 2016) and widely uses in the biological monitoring of river health assessment (Rosenberg and Resh, 1993). Therefore, the result of network could also be used to identify the sensitive taxa in the species level. Benthic algae assemblages are widely acknowledged as some of the most sensitive to nutrient gradients (Lavoie et al., 2004; Porter, 2008; Potapova and Charles, 2002); our results additionally revealed significant influences on community structures. However, the network analysis identified both negative and positive correlations between the benthic algae assemblage and nutrient gradients.

Although Cytoscape software is commonly developed for molecular interaction, gene expression profiles and other biological pathways networks, its utilities in ecology and stream ecology are increasing. For example the networks has been applied to reveal the predator-prey associations in the food webs (Ings et al., 2009; Bell et al., 2010), and species competition or coexistences in the community (Ren et al., 2017; McGarvey and Veech, 2018; Tulloch et al., 2018). In our study, the network analysis provides a method to understand the associations of different taxa within the assemblage, and their correlations with targeted environmental parameters, such as the nitrogen and phosphorus. Compared to the traditional analysis, the integrated applications of network analysis and CCA can improve our understanding of among taxa interactions within an aquatic assemblage in relation to environmental stressors. Meanwhile, the network analysis could also promote our understanding of the constructive species in dominance of community composition, assemblage stabilities, and ecological functions.

5. Conclusions

This study examined the ecosystem structures of aquatic organisms (fish, macroinvertebrates, and benthic algae assemblages) in the Hun-Tai River, a polluted river spanning a steep gradient of N and P concentrations and N:P stoichiometric ratios. Using CCA and network analysis, the results revealed that stream hydromorphometric and nutrient parameters were closely linked with the fish assemblages. Macroinvertebrates and benthic algae were strongly influenced by stream morphology, chemistry, nutrient concentrations and stoichiometric ratios. Furthermore, those groups would be useful indicators of habitat degradation and nutrient enrichment. Fishes of Cyprinidae, the macroinvertebrate orders Diptera and Ephemeroptera, and benthic algae in the form of diatoms play fundamental role in the stability of the local aquatic ecosystem structure. The simplicity of the network confirmed degradation of the community and relative unconsolidated connections in the fish assemblage. Yet, the complex associations among individuals of macroinvertebrates and benthic algae revealed consolidated correlations among the different taxa, and relatively greater stability and resistance to human impacts. The results also suggested faster community recovery of benthic algae and macroinvertebrate than fish in response to the stream restoration projects. Network analysis revealed a range of correlations between the taxa of aquatic assemblages and the nutrient parameters, which provided a useful and possible list of sensitive taxa of macroinvertebrates and benthic algae to be considered as early indicators of eutrophication. Broad positive correlations occurred especially between the benthic algae assemblage, the nutrient concentrations and N:P ratios, revealing complex relationships and tolerant taxa of benthic algae at the family level. We concluded that wide variations may exist in the nutrient sensitivities of benthic algae within the same family. The study try to use the integrated analyses to narrow the knowledge gap of the cumulative or multiplicative effects of multiple environmental stresses, both natural and artificial, on aquatic biotic assemblages in rivers. Meanwhile, this study also provides a holistic understanding of the ecosystem structure of the Hun-Tai River, with information that could contribute to ecosystem protection and management.

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