



Research article

Analysis of water quality and the response of phytoplankton in the low-temperature environment of Majiagou Urban River, China

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ABSTRACT

Majiagou River, a crucial urban river in Harbin, traverses densely populated areas including agricultural, suburban, and main urban areas, presenting highly intricate habitat characteristics. In recent years, urbanization has significantly intensified human interference, fundamentally reshaping the phytoplankton community. Understanding the response mechanism of phytoplankton to environmental factors is of paramount importance as they serve as primary producers in aquatic ecosystems. To investigate this, we established 25 sampling sites to analyze the phytoplankton community and 14 key physicochemical parameters, such as total phosphorus (TP) and total nitrogen (TN). Utilizing hierarchical clustering analysis (HCA) and One-way Analysis of Variance (ANOVA), we identified distinct river segments, revealing spatial distribution differences and environmental factor variations among phytoplankton species across segments. By adopting redundancy analysis (RDA), we pinpointed the primary environmental factors impacting phytoplankton communities and examined the correlation between phytoplankton and these factors to elucidate the driving mechanisms governing phytoplankton dynamics. The outcomes demonstrated that the phytoplankton community in Majiagou River was predominantly composed of Bacillariophyta and Chlorophyta, however, notable disparities in spatial distribution and species composition resulting from human interference were evident. Areas with intense human disturbance were dominated by diatoms and exhibited trends of homogenization and reduced biodiversity. RDA showed that pH, $\text{NH}_4^+\text{-N}$, $\text{NH}_3\text{-N}$, chemical oxygen demand (COD), and TP were key environmental factors influencing phytoplankton communities. We have confirmed that due to variations in environment conditions and different levels of human disturbance, there will be some differences in the critical limiting factors affecting phytoplankton. Our study offers valuable insights for governing urban rivers during the low-temperature period.

1. Introduction

Rivers play a crucial role by providing essential ecosystem services to humans and serving as unique habitats for a diverse range of plankton. Phytoplankton, as integral components for upholding the integrity of river ecosystems [1], offer valuable insights into water quality monitoring and comprehensive management strategies for various types of rivers [2,3]. However, in recent years, urban rivers have faced significant ecological pressures and heightened pollution risks due to human activities, resulting in a decline in the structure and functional levels of phytoplankton communities [4]. It is imperative, therefore, to conduct further research to investigate the

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driving mechanisms of phytoplankton under high pollution conditions, identify the key environmental factors that influence phytoplankton, and explore the correlations among them. These efforts are essential for the ecological conservation of urban rivers.

Numerous studies have investigated the response of phytoplankton in urban rivers to environmental factors. These studies have utilized various methods to examine the relationship between organisms and their surrounding environment. For instance, Toruan et al. found that the size structure of plankton communities in urban lakes and rivers was influenced by eutrophication parameters, particularly TP and chlorophyll-a [5]. Zhao et al. conducted an analysis on the main environmental factors affecting urban river phytoplankton communities and identified TP, TN, and chlorophyll-a as the primary driving factors [6], they also observed higher Cyanophyta abundance in urban areas compared to upstream areas. Qu et al. conducted a study on the functional groups of phytoplankton in northern rivers in Germany using redundancy analysis, which revealed that land use and physicochemical indicators were coupled and jointly influenced phytoplankton community composition, they demonstrated that physical and chemical factors, along with land use indicators, accounted for 68.4% of the variance, and Huang et al. emphasized that land use patterns are crucial elements in phytoplankton biodiversity [7]. Du et al. investigated the relationship between β -diversity and environmental factors, revealing a significant correlation between phytoplankton and nutrients such as TN, they also found that β -diversity decreased with increasing nutrient concentrations, explaining the variations in phytoplankton diversity, and Sun et al. researched the relationship between phytoplankton and nutrients in southeastern China and discovered that, apart from diatoms, other algal groups exhibited a significant negative correlation with nutrients [8]. Bao et al. analyzed the impact of artificial structures on phytoplankton and identified the construction of urban rubber dams as a contributor to increased phytoplankton biomass during the reservoir period, and they attributed phosphorus emissions as the primary cause of changes in the phytoplankton community [9]. Furthermore, studies have been conducted on the response of phytoplankton communities in the ecosystems of lakes and rivers to water management projects such as lake water diversion. Dai et al. quantitatively analyzed the differences in phytoplankton community before and after the implementation of a water diversion project in the Taihu Lake Basin, providing specific insights into the contribution of water diversion projects to the changes in phytoplankton community [10,11], these researches contributed to enhance the ecological benefits and management of water diversion projects. Chen et al. studied the driving mechanisms of phytoplankton in urban artificial lakes and identified dissolved oxygen (DO), chlorophyll, and TN as the main driving factors [12]. In summary, previous studies have demonstrated that the limiting factors for phytoplankton and the key factors influencing phytoplankton communities vary due to differences in environmental parameters and human disturbances in different rivers [13]. Therefore, it is necessary to conduct research on typical urban rivers of different periods to obtain more comprehensive conclusions and develop effective water quality management plans. However, research on water quality and phytoplankton during the approach of freezing remains limited Urban river environmental parameters are complex [14,15], involving interactions among various factors such as human pollution and stormwater runoff. Thus, it is essential to study the driving mechanisms of phytoplankton communities based on the coupling relationships among environmental factors [16].

At the same time, numerous models and methods have been adopted to study the driving mechanisms of phytoplankton in urban rivers. Wang et al. utilized RDA and stepwise multiple linear regression (SMLR) to analyze the relationship between water quality, land use, and phytoplankton communities at multiple scales. They found that land use significantly impacts water quality and phytoplankton, providing valuable insights for the protection of urban rivers [17]. Ma et al. conducted canonical correspondence analysis (CCA) to examine the relationship between phytoplankton and environmental factors. Their analysis revealed that water temperature and ammonium nitrogen are key indexes of phytoplankton communities [18]. Jia et al. developed ecological driving models for different phytoplankton types in urban water systems, shedding light on the succession mechanisms of dominant species and offering guidance for urban river management [19]. Zhang et al. quantitatively analyzed aquatic habitat suitability using principal component analysis (PCA), hierarchical cluster analysis (HCA), and a generalized additive model, providing reference for urban river management [20]. Mathematical models such as artificial neural networks (ANN) combined with clustering techniques have been utilized to explore the correlation between chlorophyll-a and other environmental factors [21]. Bayesian models have been employed to simulate ecological networks and predict population changes of aquatic organisms [22,23]. Maximum entropy models have been applied to predict suitable habitats for benthic organisms [24]. Our research focuses on the correlation between water quality factors and the driving mechanisms of phytoplankton communities during the approach of freezing of Mjiagou river. We conducted an analysis of the water quality and the response of phytoplankton to environmental factors using one-way analysis of variance (ANOVA), HCA, Pearson correlation coefficient, and RDA.

Harbin City, an important central city in Northeast China, is recognized for its significance in the region. Majiagou River, as a representative urban river in Northeast China, experiences freezing for approximately three months each year, from late November to the end of February. Spanning through major urban areas of Harbin City, studying the water quality and driving mechanisms of phytoplankton in this river holds great significance in assessing the ecological health of all types of rivers. Our research involves four main steps: (1) Establishing monitoring sample points across various sections of Majiagou River to examine the composition of phytoplankton communities and water quality indicators. (2) Adopting HCA to categorize the river sections of Majiagou River and utilizing ANOVA to demonstrate spatial differences in phytoplankton and water quality indicators among these sections. (3) Analyzing the relationships among water quality indicators using Pearson correlation coefficient. (4) Investigating the correlation between phytoplankton and environmental factors through the utilization of RDA. Our research aims to provide valuable insights into the comprehensive management of urban river ecosystems with freezing periods.

2. Materials and methods

2.1. Study sites

Majiagou River (126°41'–126°43'E, 45°32'–45°49'N) is a representative urban river in Northeast China's, spanning a total length of 44.3 km. It runs through all major urban areas of Harbin and joins the Songhua River [25,26], experiencing significant human interference. Due to the frigid climate conditions in the region, the river has an extended freezing period from November to February of the following year, showcasing typical characteristics associated with such conditions. The water source for Majiagou River primarily relies on natural rainwater recharge within the basin and upstream reservoir water storage. Harbin receives an average annual precipitation of 533 mm, predominantly concentrated during the summer. The annual runoff depth ranges from 40 mm to 95 mm, and the river's average width measures 34.8, and the river's cycle is highly distinct. The primary function of Majiagou River is to mitigate inland inundation in urban areas and it possesses 19 drainage outlets for rainwater and domestic sewage. As it traverses rural, suburban, and urban areas, each section of the river exhibits varying degrees of human activity interference and stress, leading to diverse environmental parameters. In consideration of the natural resources assessment conducted in inland waters fisheries and the unique hydrological characteristics of an urban river, a total of 25 phytoplankton monitoring sites were established (Fig. 1), these sites have been subject to varying degrees of pollution caused by human activities. The study sites encompass reservoirs (R1–R3, F4), suburban areas (S5–S8), villages (C9–C11), revegetation areas (V12–V14), urban areas (U15–U22), estuaries (I23–I24), and the Songhua River (R25).

2.2. Sampling

The Majiagou River was nearing freezing by the end of November, which allowed us to examine the response of phytoplankton communities to environmental factors in a low-temperature environment. Hence, we chose this time period to collect phytoplankton and water quality samples. The water temperatures at the sampling points ranged between 0.28 and 3.62 °C. Due to the large number of sampling points and the need to avoid the influence of light variation on phytoplankton communities, all our sampling was conducted during daylight hours. We divided the sampling into three days and conducted it during the same time period. On-site, a water quality analyzer (YSI 6600) was employed to measure a total of 11 data parameters immediately. Simultaneously, water samples (500 ml) were taken to the laboratory for further analysis. The determination of TN, TP, and chemical oxygen demand (COD) was carried out using the alkaline potassium persulfate digestion-UV spectrophotometric method, ammonium molybdate spectrophotometry, and

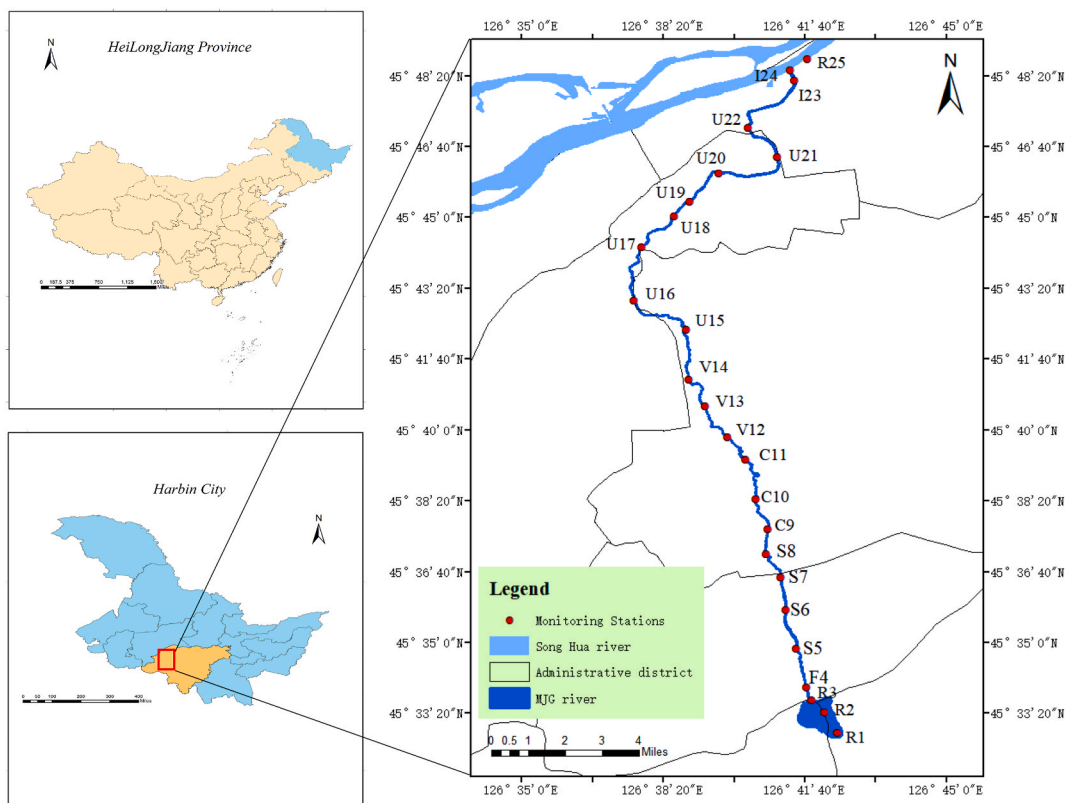


Fig. 1. Study area and location of environmental and biological monitoring samples.

USEPA digestion colorimetry method, respectively [27]. These parameters effectively reflect the water quality of the Majiagou River.

For the collection of water samples, a 1L Plexiglass water sampler was used at a depth of 0.5 m underwater. One liter of water was placed in the sampling bottle, and 1.5% Lugol’s solution was added on-site for fixation. After collecting the samples, they were allowed to stand in the laboratory for 48 h [28]. The supernatant was then concentrated and absorbed using a siphon method, while the remaining samples were transferred into a 50 ml polyethylene sample bottle. The volume was adjusted to 50 ml to maintain consistency. The algae specimens were identified and counted under a 10 × 40 optical microscope. As for diatoms, they underwent processing with strong acid and were subsequently identified and counted under a 10 × 100 optical microscope.

2.3. Data analysis

Given the complex hydrological characteristics of Majiagou River, HCA was employed to group the 25 monitoring samples based on water quality environmental factors. This approach aimed to enhance the reliability and robustness of subsequent models. To examine the significant differences among groups regarding phytoplankton community and environmental factors, ANOVA was conducted [29, 30]. Post hoc multiple comparisons were performed using the LSD test. In cases where the test of Homogeneity of Variances yielded a significance level below 0.05, the Kruskal-Wallis non-parametric test was employed as an alternative.

To elucidate the coupling relationship between water quality environmental factors and the phytoplankton community, RDA and Pearson correlation tests were employed [31,32]. Prior to conducting RDA, the Pearson correlation coefficient was used to robustly assess the strength and direction (positive or negative) of linear correlations between the response variables. In this study, the Pearson correlation analysis method was utilized to compute correlation coefficients between various environmental indicators and phytoplankton species. The strength of the linear relationship was determined based on the correlation coefficient. The data were transformed using the log10 (x + 1) transformation, followed by detrended correspondences analysis (DCA) [33]. The aforementioned analyses were conducted using CANOCO 4.5 software and R (version 4.2.1).

3. Result

3.1. Spatial pattern of Majiagou River based on HCA

To determine the optimal number of clusters, we initially utilized the sum of squared error (SSE) method. The Total Within Sum of Squares (WSS) value decreased as the number of clusters increased. We considered the point at which the gradient slowed down as the optimal number of clusters, as further increasing the number would not enhance robustness. In our study, the WSS gradient decreased after reaching 4 clusters (Fig. 2a). Therefore, the HCA results identified 4 clusters, aligning with the principle of sample setting and confirming the rationality of our sampling station arrangement, We will conduct further research based on the results of HCA.

The results indicated that the water quality characteristics of the Songhua River (R25) were similar to those of the Majiagou River Village section (C9–C11), leading both to be grouped in the same cluster. Near the Songhua River and village monitoring points, there were no industrial zones or sewage outlets, resulting in low levels of human interference for this cluster. The upstream reservoirs (RI-R3, F4) exhibited special water quality characteristics and formed a separate cluster. There was no human habitation near the reservoir, and it was subject to ecological management, resulting in extremely low levels of human interference. The third cluster included suburban areas (S5–S8) and U15. There was industrial and agricultural production activity in the suburban areas, resulting in a higher degree of human influence. Additionally, the urban sections of the river (V12–V14,U16–U22,I23–I24) were grouped together in one cluster. These monitoring points are located in the central urban area of Harbin City, where there is a significant level of human interference (Fig. 2b).

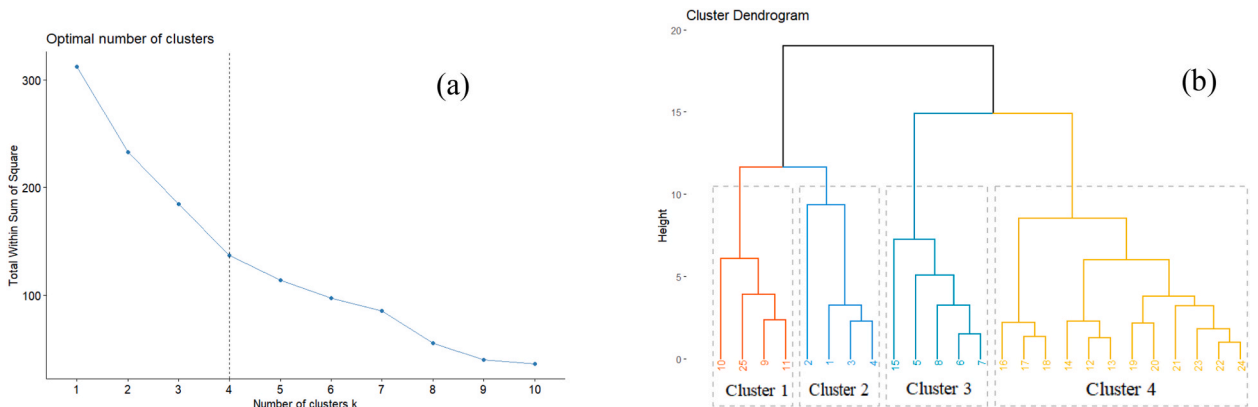


Fig. 2. (a) Sum of squared error (SSE) method used to determine the optimal number of clusters. (b) Dendrogram presenting the clusters of sampling stations.

3.2. Spatial differences of phytoplankton communities

In our study, a total of 76 species from 52 genera and 7 phylum were identified through morphological identification in laboratory conditions. The most frequently occurring species belonged to the phylum Chlorophyta (44.7%), followed by Bacillariophyta (25%) and Cyanophyta (15.8%). Euglenophyta (6.6%), Cryptophyta (3.9%), Chrysophyta (2.6%), and Pyrroptata (1.3%) were less common. However, Bacillariophyta exhibited the highest number of species (49.1%), followed by Chlorophyta (19.3%) and Cyanophyta (17.8%). In terms of spatial distribution, Bacillariophyta were found to be widely distributed across various water bodies, with *C. meneghiniana* being particularly notable. Cyanophyta, Cryptophyta, Pyrroptata, and Chrysophyta were predominantly present in river sections that experienced minimal human disturbance, such as upstream reservoirs. Chlorophyta, on the other hand, were found in upstream reservoirs and urban suburbs, with lower densities observed in urban reaches. Euglenophyta species were slightly more abundant in river sections flowing through suburbs and villages. The main phytoplankton species in each reach are listed in Table 1.

Human interference has led to significant differences in species composition among all clusters. The bar chart reveals that the species composition of the first and second clusters share similarities, with a higher proportion of Chlorophyta compared to the third and fourth clusters. Conversely, the species composition of the third and fourth clusters is similar, with a higher proportion of Bacillariophyta (Fig. 3).

In our study, we conducted tests for Homogeneity of Variances and Shapiro-Wilk to ensure the suitability of using ANOVA. The results of both tests indicated that the phytoplankton biomass followed a normal distribution, enabling the use of ANOVA. The ANOVA results revealed that spatial variation had a significant impact on phytoplankton biomass ($F = 25.624$, $p < 0.001$). Furthermore, the results of multiple comparisons indicated that the biomass of the third and fourth clusters was significantly lower compared to the first and second clusters (Table 2).

3.3. Spatial distribution of water quality in Majiagou River

Utilizing box plots (Fig. 4a–h), we visually depict the spatial differences in phytoplankton biomass and various water quality factors based on the segmentation of the Majiagou River sections. For phytoplankton biomass, the first and second clusters exhibit significantly higher values compared to the third and fourth clusters, suggesting a greater biomass in suburban areas and upstream reservoirs. In terms of turbidity, the second cluster, representing the upstream reservoir, displays significantly lower values than the other sections. Considering conductivity, TP, and TN, the suburban areas and upstream reservoirs show significantly lower values compared to the urban section, indicating a pronounced human impact and higher nutrient concentrations in the urban area. With regards to

Table 1
Phytoplankton community composition in different clusters in the Majiagou River.

Clusters	Phylum	Genus	Species
Cluster 1	Bacillariophyta	<i>Cyclotella</i>	<i>C.meneghiniana</i>
		<i>Synedra</i>	<i>Synedra ulna</i>
		<i>Stauroneis</i>	<i>S.anceps</i>
		<i>Navicula</i>	<i>N.rhynchocephala</i>
		<i>Chlorella</i>	<i>C.vulgaris</i>
Cluster 2	Chlorophyta	<i>Euglena</i>	<i>Euglena acus</i>
	Euglenophyta	<i>Oscillaria</i>	<i>Oscillatoria tenuis</i>
	Cyanophyta	<i>Cyclotella</i>	<i>C.meneghiniana</i>
	Bacillariophyta	<i>Melosira</i>	<i>M.varians</i>
		<i>Chlamydomonas</i>	<i>C.ovalis</i>
Cluster 3	Chlorophyta	<i>C.globosa</i>	<i>C.ovalis</i>
		<i>Kirchneriella</i>	<i>C.globosa</i>
		<i>Ankistrodesmus</i>	<i>Kirchneriella lunaris</i>
		<i>Euglenastrum</i>	<i>A.falcatius</i>
		<i>Chroococcus</i>	<i>Phacus stokesii</i>
	Euglenophyta	<i>Chroococcus</i>	<i>Chroococcus minutus</i>
		<i>Raphidiopsis</i>	<i>Raphidiopsis sinensia</i>
	Cyanophyta	<i>Cryptophyta</i>	<i>Raphidiopsis sinensia</i>
		<i>Chroomonas</i>	<i>Cr.ovata</i>
		<i>Cyclotella</i>	<i>Chr.acuta</i>
Cluster 4	Bacillariophyta	<i>Cyclotella</i>	<i>C.meneghiniana</i>
		<i>Synedra</i>	<i>Synedra ulna</i>
	Chlorophyta	<i>Navicula</i>	<i>N.rhynchocephala</i>
		<i>Oscillaria</i>	<i>Oscillatoria tenuis</i>
	Euglenophyta	<i>Raphidiopsis</i>	<i>Raphidiopsis sinensia</i>
Cluster 4	Bacillariophyta	<i>Euglena</i>	<i>Euglena acus</i>
		<i>Cyclotella</i>	<i>C.meneghiniana</i>
	<i>Synedra</i>	<i>Synedra ulna</i>	
	<i>Stauroneis</i>	<i>S.anceps</i>	
	<i>Surirella</i>	<i>S.robusta</i>	
	Chlorophyta	<i>Scenedesmus</i>	<i>S.dimorphus</i>
		<i>Oscillaria</i>	<i>Oscillatoria tenuis</i>
Euglenophyta	<i>Raphidiopsis</i>	<i>Raphidiopsis sinensia</i>	
		<i>Euglena</i>	<i>Euglena acus</i>

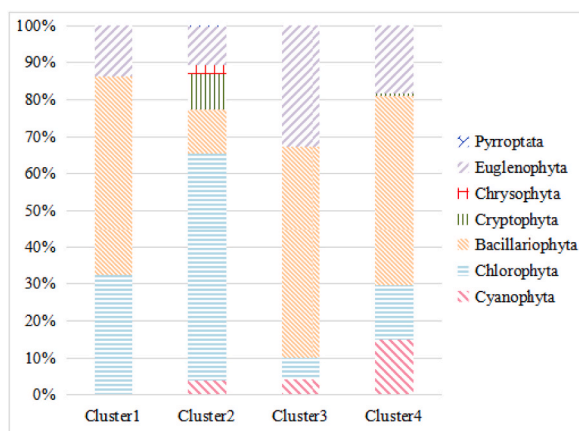


Fig. 3. Composition of phytoplankton community in different clusters.

Table 2

Effects of spatial variation in water quality factors and phytoplankton biomass; the significant effect of seasonal variation on the index ($p < 0.05$) is in bold.

	Clusters				Effects of Cluster	
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Statistics	P-value
Phytoplankton Biomass	8.03 ± 2.72	10.02 ± 2.40	2.25 ± 0.77	2.81 ± 1.34	F = 25.624	<0.001
DO	13.2 ± 0.76	12.81 ± 3.02	10.58 ± 1.81	13.3 ± 2.68	F = 1.616	0.016
BP	750.3 ± 0.91	748.9 ± 0.52	750.2 ± 0.3	751.05 ± 0.86	F = 8.425	0.001
Turbidity	20.3 ± 1.26	4.375 ± 3.16	16.54 ± 4.71	10.1 ± 3.36	F = 18.541	<0.001
Conductivity	0.54 ± 0.21	0.64 ± 0.15	1.22 ± 0.19	0.98 ± 0.06	F = 25.828	<0.001
CL-	505.6 ± 129.2	787.1 ± 179.4	760.3 ± 93.1	876.8 ± 117.7	F = 8.721	0.001
COD	15.2 ± 4.57	27.4 ± 3.45	23.24 ± 14	16.06 ± 5.19	F = 3.149	0.046

chloride (Cl^-), its concentration in the upstream area is lower than that in the urban section, although the difference is not statistically significant. This suggests that pollution in the urban section of the Majiagou River primarily stems from domestic wastewater discharge. COD is higher in the second and third clusters compared to the first and fourth clusters, resulting in lower DO levels in the former, consequently, this scenario can foster the proliferation of anaerobic microorganisms.

Based on the results of the homogeneity of variance test and normality test, six environmental factors satisfy the assumptions for conducting the analysis of variance. Table 2 displays the observed spatial distribution differences in turbidity and conductivity, which are statistically significant ($F = 18.541$, $F = 25.828$, $p < 0.001$). Post-hoc comparisons elucidate that the second cluster exhibits significantly lower turbidity compared to the other sections, which can be attributed to the presence of vegetation coverage along the riverbanks. Conversely, the third cluster demonstrates significantly higher conductivity in comparison to the other sections.

3.4. Coupling relationships between phytoplankton and environmental factors

Based on the Pearson correlation coefficient, our study reveals the correlations between phytoplankton and various environmental factors. Fig. 5a illustrates a highly significant positive correlation between Cyanophyta and Cryptophyta, as well as a moderate positive correlation between Chrysophyta and Cryptophyta. Additionally, Fig. 5b demonstrates a positive correlation between chlorophyll-a and Cl^- . Other correlations include a positive relationship between salinity and conductivity, a positive correlation between pH and DO, and positive correlations between TN and conductivity, TP, and salinity. However, TN exhibits a negative correlation with water temperature.

Based on the lengths of the gradient axis, it is evident that RDA yields superior results compared to CCA. Therefore, we have chosen RDA as the basis for our study. In Fig. 6, the length of the arrow indicates the strength of the correlation between environmental variables and phytoplankton. A longer arrow represents a stronger correlation. The vertical distance between different categories of phytoplankton and the axis of environmental variables reflects their correlation, with a greater distance indicating a stronger correlation. The eigenvalues of the four axes are 0.6472, 0.1611, 0.0605, and 0.0193, respectively. The correlations between phytoplankton and the environment for Axis 1 and Axis 2 are 0.9853 and 0.905, respectively. Axis 1 (accounting for 64.72% of the correlation) and Axis 2 (accounting for 16.1% of the correlation) together account for 80.82% of the phytoplankton-environment correlation. These two axes explain 90.27% of the variance in the phytoplankton-environment correlation (Table 3).

Specifically, Pyrroptata, Cryptophyta, and Chrysophyta exhibit strong positive correlations with chlorophyll-a, pH, $\text{NH}_4^+\text{-N}$, and $\text{NH}_3\text{-N}$. Cyanophyta and Chlorophyta show strong positive correlations with COD and chlorophyll-a. Euglenophyta demonstrates a

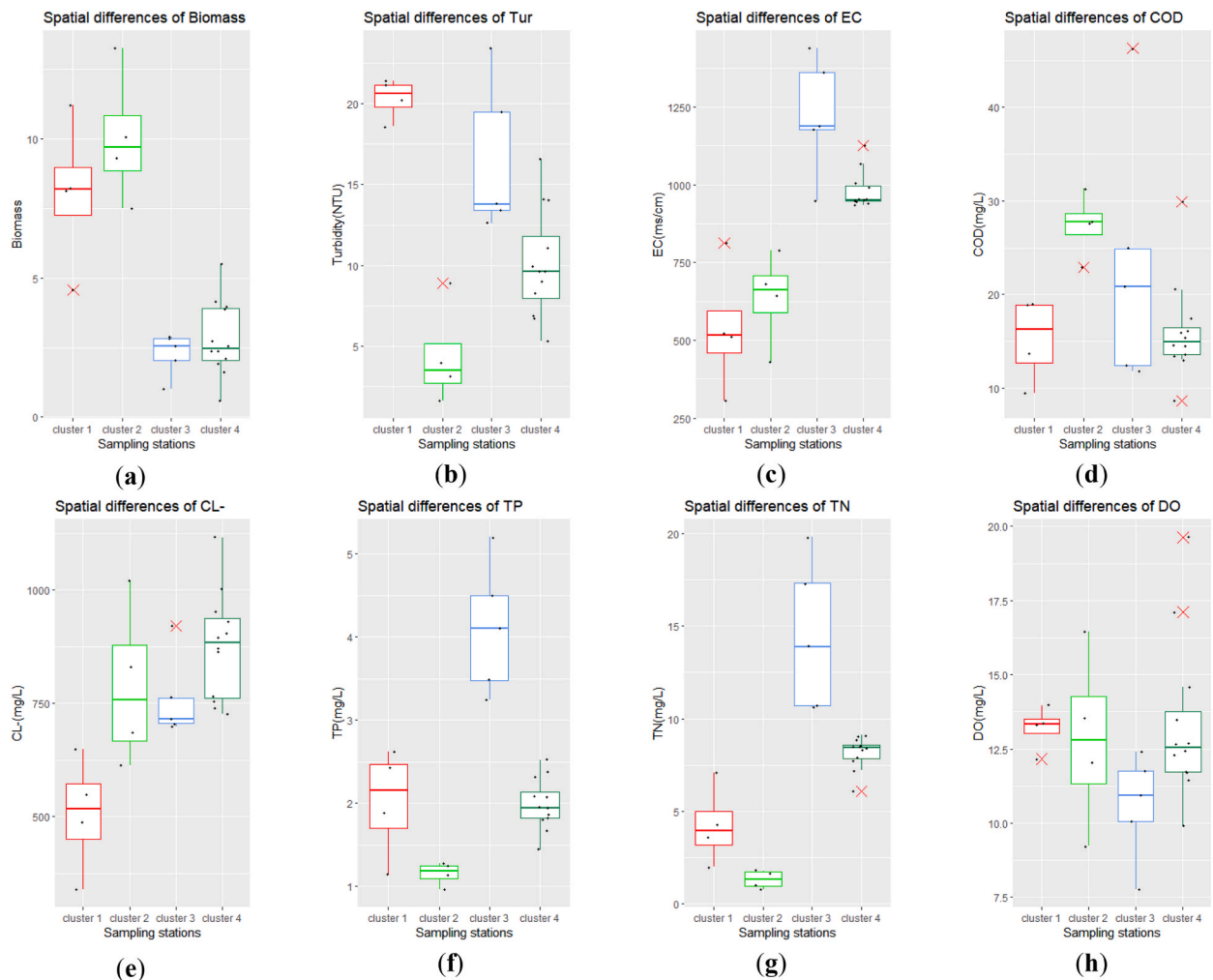


Fig. 4. Boxplots of water quality indexes in different clusters: (a) phytoplankton biomass; (b) Turbidity; (c) Conductivity; (d) COD; (e) CL^- ; (f) TP; (g) TN; (h) DO.

strong positive correlation with DO, while Bacillariophyta shows certain positive correlations with DO and turbidity.

4. Discussion

4.1. Responses of phytoplankton to environmental factors

In our study, we focused on the spatial variations of environmental factors in urban rivers under the influence of human activities. We conducted HCA to identify different clusters and analyzed the differences in phytoplankton biomass among these clusters. By establishing a response model between phytoplankton and environmental factors, we were able to determine the correlations between different phytoplankton phylum and environmental factors. This study provides valuable insights for the conservation of riverine phytoplankton diversity in various habitats and the management of urban rivers. The River Continuum Concept (RCC) suggests that nutrient salts and organic matter in water exhibit specific spatial patterns along the river's course, resulting from the river's continuity, typically, species diversity is lower in the source and downstream reaches. However, the spatial distribution of environmental indicators in urban river water shows significant variations, primarily due to human interference. Consequently, phytoplankton communities are indirectly reshaped as a consequence of human disturbance. We observe that Bacillariophyta dominate in heavily disturbed areas with high nutrient levels, while the opposite trend is observed for Chlorophyta. Nevertheless, Bacillariophyta and Chlorophyta remain the primary phytoplankton communities in urban rivers, which aligns with the findings of Koekemoer' study [34, 35].

Phytoplankton plays a crucial ecological role in urban rivers and is highly sensitive to changes in water quality. Therefore, assessing phytoplankton biomass and diversity is an important aspect of urban river water quality evaluation. Previous studies on phytoplankton

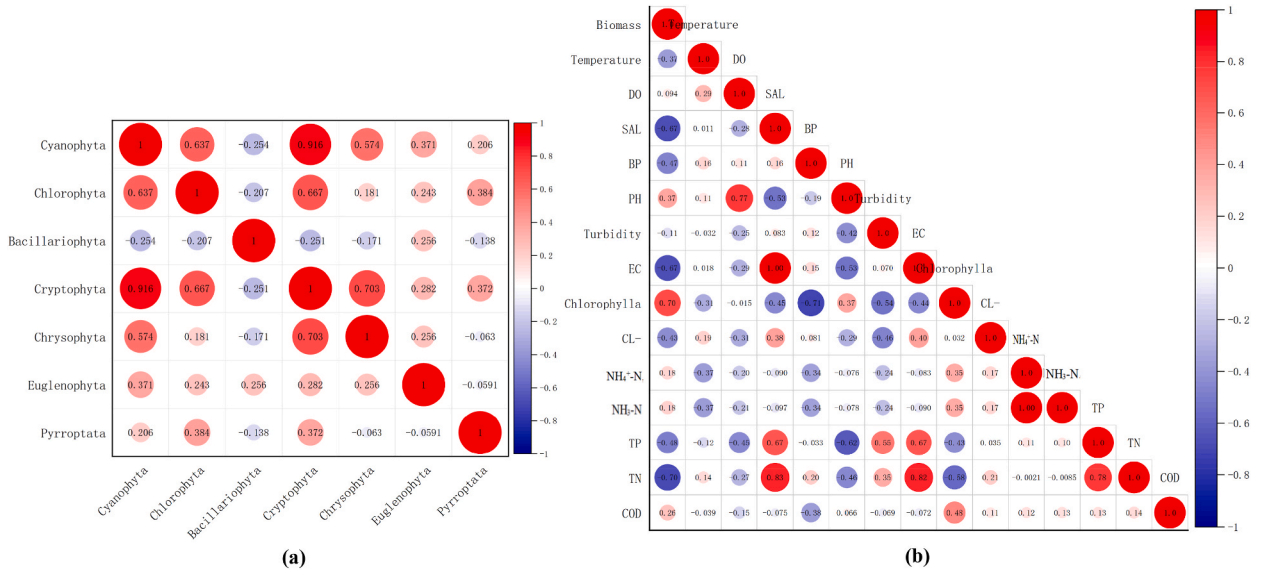


Fig. 5. Correlation coefficients of phytoplankton(a) and water environmental factors(b).

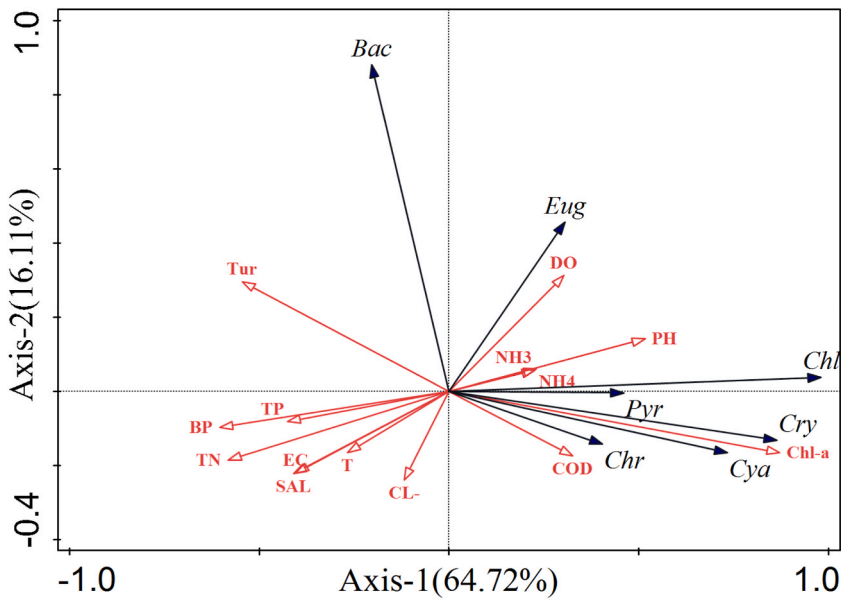


Fig. 6. RDA results of phytoplankton biomass and environment factors. Pyrroptata (*Pyr*), Euglenophyta (*Eug*), Chrysophyta (*Chr*), Cryptophyta (*Cry*), Bacillariophyta (*Bac*), Chlorophyta (*Chl*), Cyanophyta (*Cya*).

Table 3
Summary of RDA between phytoplankton species and environmental variables.

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.6472	0.1611	0.0605	0.0193
phytoplankton-environment correlation	0.9853	0.9050	0.8015	0.7660
Explained variation (cumulative)	64.72	80.82	86.88	88.81
Explained fitted variation (cumulative)	72.28	90.27	97.03	99.19

in urban rivers generally agree that environmental factors, especially the increased concentrations of TP and TN caused by human interference, have a significant impact on phytoplankton [36,37]. These changes can reshape the food web and disrupt the ecological niche of phytoplankton. However, it is important to note that the key limiting factors for phytoplankton can vary significantly due to variations in environmental parameters and human disturbances across different rivers. Lin et al. utilized environmental DNA technology to study the phytoplankton community in urban lakes and found that the relationship between phytoplankton and nutrient substances (TP, TN) is not consistent across different sampling points [28]. Similarly, Ma et al. concluded through canonical correspondence analysis that DO, TP, and COD are the key environmental factors influencing phytoplankton [18]. However, Xia's study indicated that temperature, TN, and TP are the key factors affecting phytoplankton, suggesting variations due to different environmental parameters at the study sites [38]. Therefore, in our study of the Majiagou River, we recognize the need to address this issue. By segmenting the river into sections and analyzing the water quality and phytoplankton in each section, we determined that the first and second clusters exhibit similar levels of human disturbance and environmental parameters, while the third and fourth clusters share similar characteristics. However, there are differences between these two groups, thus, we will employ RDA to separately explore the correlation between phytoplankton and environmental factors for each group.

After thorough verification, it was determined that RDA yielded better results compared to CCA for both groups. Consequently, RDA was selected for further investigation, based on Fig. 7a, it can be observed that in areas with lower levels of human disturbance, there is a significant positive correlation between Chlorophyta and COD as well as CL^- . However, in areas with higher levels of human disturbance, Chlorophyta exhibits a significant positive correlation with pH (Fig. 7b). Conversely, Bacillariophyta displays a significant positive correlation with TP in areas with lower levels of human disturbance, while in areas with higher levels of human disturbance, it demonstrates a significant negative correlation. This phenomenon may be attributed to the non-linear relationship between Bacillariophyta biomass and TP concentration [39]. In the Bacillariophyta response curve to TP, a peak value of Bacillariophyta biomass occurs at a specific concentration of TP. Below this specific value, a positive correlation is observed, whereas above this value, a negative correlation is observed. However, regardless of the situation, Cyanobacteria consistently exhibits a positive correlation with chlorophyll-a.

4.2. RDA based on HCA and Pearson correlation coefficient

HCA is a valuable tool for categorizing monitoring points based on similarities in environmental parameters and other factors, aiding in the analysis of spatial differences in water quality and phytoplankton across different sections of urban rivers. Additionally, conducting ANOVA helps us understand the variations in these factors. In our study, we utilized RDA to analyze the relationship between environmental factors and various phytoplankton species. The data analysis revealed that chlorophyll-a, TN, pH, NH_4^+ -N and NH_3 -N, COD, and TP significantly influenced phytoplankton communities. Based on the RDA sequencing diagram, it was determined that TN, turbidity, and chlorophyll-a were the primary regulatory factors impacting phytoplankton at different sampling sites. The robustness of RDA in simulating the correlation between phytoplankton and environmental indicators is of great importance in understanding the underlying principles governing phytoplankton responses. Our study employed a comprehensive RDA method combined with HCA and Pearson correlation coefficient, providing valuable insights for effective urban river management.

4.3. Limitations and prospects of our study

Our study provides detailed data on the phytoplankton community in urban rivers with a freezing period, but due to the limitations of the study area, our research doesn't represent all rivers with a freezing period. This is because different rivers have different levels of anthropogenic disturbances and environmental parameters, resulting in varied limiting factors for phytoplankton communities. Due to the high level of anthropogenic disturbance in the Majiagou River, our focus was on monitoring nutrient substances in water quality indicators, while the monitoring of environmental parameters had limitations and requires further expansion in the next step.

When studying urban rivers, it is crucial to consider not only the individual impact of environmental factors on phytoplankton communities but also the coupling relationship between these factors. Previous research has highlighted the non-linear nature of the relationship between phytoplankton and environmental factors, necessitating the development of more models [40]. While our study adopted a non-quantitative analysis method, it is important to acknowledge its limitations. For instance, the use of the GAM model enables accurate analysis of the response mechanism of plankton to various environmental factors, facilitating quantitative analysis [41–43]. Nonetheless, our research holds promising prospects. Firstly, a combination of qualitative and quantitative approaches can be employed when studying the influencing factors of phytoplankton to ensure more robust results. Secondly, in addition to focusing solely on phytoplankton biomass, further exploration of diversity is warranted. Lastly, for rivers with a freezing period, research can be conducted even during the freezing period to examine the composition of sub-ice phytoplankton communities and water quality factors. This can provide valuable insights into analyzing the probability of algal blooms in the subsequent spring season.

5. Conclusion

In our study, we utilized HCA and ANOVA to analyze the spatial distribution differences of environmental factors in urban rivers. RDA helped to identify the key environmental factors that affect the phytoplankton community, and we thoroughly examined the correlation between different phytoplankton species and these environmental factors. Here are the main conclusions:

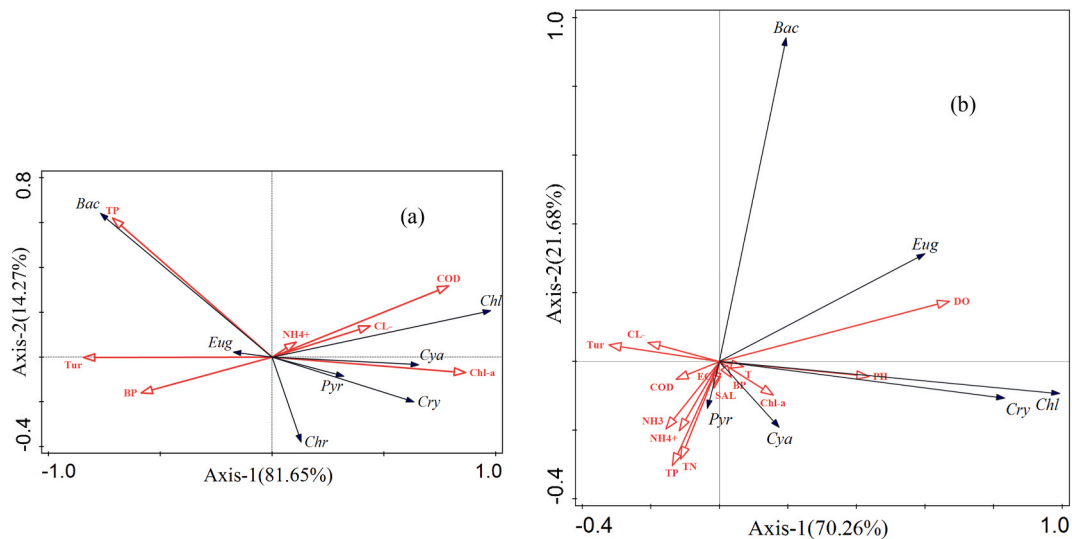


Fig. 7. RDA results of phytoplankton biomass and environment factors in two groups. (a) Group1: Cluster 1, Cluster 2 (b) Group2: Cluster 3, Cluster 4.

- (1) The spatial distribution of phytoplankton communities in the Majiagou River varied significantly. Areas with intense human disturbance were dominated by diatoms and exhibited trends of homogenization and reduced biodiversity. Conversely, the upstream area was dominated by chlorophyta, but displayed a higher biodiversity.
- (2) Results from ANOVA indicated significant spatial differences ($p < 0.001$) in turbidity and conductivity. Box plots visually demonstrated higher levels of nutrients in urban river sections, which can be attributed to domestic sewage and runoff, including TP, TN, and COD. Additionally, certain correlations were observed among environmental factors as well as among phytoplankton groups from different phyla.
- (3) We conducted a small-scale analysis of the response mechanism of phytoplankton to environmental factors prior to the freezing period, pH, $\text{NH}_4^+\text{-N}$ and $\text{NH}_3\text{-N}$, COD, and TP exhibited high correlations with the phytoplankton community. And we have confirmed that due to variations in environment conditions and different levels of human disturbance, there will be some differences in the critical limiting factors affecting phytoplankton. In the study on the Majiagou River, Chlorophyta showed different correlations with the same environmental factor under different levels of human disturbance, and the same phenomenon also occurred with Bacillariophyta.

Ethics approval

The submitted manuscript is original and have not been published elsewhere in any form or language.

Consent to publish

All authors agreed with the content and that all gave explicit consent to submit.

Data availability statement

The authors do not have permission to share data.

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CRedit authorship contribution statement

Yongxin Zhang: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Hongxian Yu:** Writing – review & editing, Writing – original draft, Validation, Resources, Investigation, Funding acquisition, Data curation, Conceptualization. **Jiamin Liu:** Writing – review & editing, Methodology, Investigation, Data curation. **Yao Guo:** Writing – review & editing, Software, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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