



OPEN Eukaryotic plankton community and assembly processes in a large-scale water diversion project in China

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The Middle Route of the South to North Water Diversion Project (MRP) and its water source, the Danjiangkou Reservoir (DJK), play a pivotal role in mitigating the chronic water scarcity challenges faced by northern China. Eukaryotic plankton are widespread in aquatic ecosystems, which are crucial for the water quality stability of DJK and MRP, yet comparative studies on their contemporaneous dynamics and assembly processes are scarce. In this study, amplicon sequencing was used to investigate the eukaryotic plankton communities. The results revealed that the similarity in community composition of DJK is significantly higher than that of MRP, exhibiting distance-decay patterns. Environmental heterogeneity exhibits significant differences between DJK and MRP, and it significantly influences community composition and alpha diversity. Additionally, the assembly processes of eukaryotic plankton in both DJK and MRP are predominantly influenced by stochastic processes. However, in comparison to DJK, deterministic processes have a more pronounced impact on MRP, accounting for 39.29% and 1.82%, respectively. The variations in total nitrogen (TN), chlorophyll *a* (Chl. *a*), and conductivity (Spc) have led to a transition in the assembly of eukaryotic phytoplankton communities in MRP from a stochastic process to a deterministic process. This study extends insights into the dynamics and assembly processes of eukaryotic plankton communities in the large, engineered drinking water diversion project and its water source, which is also useful for the management and regulation of the DJK and MRP.

Keywords Danjiangkou Reservoir, The middle route of the South-to-North Water Diversion Project, Eukaryotic plankton dynamics, Spatial patterns, Assembly processes

Microorganisms represent an essential component of ecosystems, exerting significant influence over material cycling processes and energy fluxes¹. These organisms are crucial determinants of water quality and safety, particularly in potable water sources. Microbial communities possess the capacity to influence water quality, which may result in the generation of unpleasant odors and potential public health risks². Research has shown that microorganisms are important producers of odor compounds and other metabolites, closely related to water quality³. Microorganisms present in potable water contribute significantly to the self-purification processes, which, in turn, have a profound impact on water quality and safety⁴. Furthermore, the succession of microbial communities could mirror alterations in water quality parameters, with their distribution intricately associated with the dynamic nature of local water quality attributes⁵. Microorganisms serve a crucial function as key indicators in assessing the condition of aquatic environments⁶. Accordingly, monitoring the dynamic fluctuations of microorganisms in drinking water is crucial for the early detection of microbial hazards and the effective management of drinking water safety.

Eukaryotic Plankton are passively floating organisms, broadly dispersed across oceans, lakes, rivers, and diverse aquatic ecosystems due to the influence of water currents. Eukaryotic plankton, which includes not only primary producers like algae but also consumers (e.g., protozoa) and decomposers (e.g., fungi), tend to

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be larger and more complex in structure compared to prokaryotic plankton. They often have more specific environmental requirements, such as particular temperature, nutrient, and light conditions, due to their higher metabolic demands and more intricate cellular structures^{7,8}. Investigating eukaryotic plankton's diversity and community structure offers an indirect assessment of the ecological environment water quality⁸. Presently, extensive researches have centered on the community composition, spatial distribution, and functional roles of phytoplankton, archaea, plants, bacteria, and animal taxa, as well as their associations with water quality^{9–12}. Comprehending the dynamic shifts and fundamental assembly mechanisms of eukaryotic communities poses a significant challenge¹³, particularly given the complexity of these communities within extensive engineered water diversion canals, which exhibit distinct characteristics compared to natural ecosystems. Moreover, contrasting the variations in community dynamics and assembly mechanisms between diversion channels and source water is crucial for regulating and predicting drinking water quality. Consequently, it is imperative to investigate eukaryotic plankton within water diversion channels and source waters.

In recent years, community assembly mechanisms have risen to prominence as a central theme in ecological research. Vellend (2010) proposed a conceptual synthesis, categorizing the processes of community formation into four distinct mechanisms: selection, dispersal, ecological drift, and diversification¹⁴. Moreover, Stegen (2013) constructed an analytical framework aimed at quantifying the relative contributions of dispersal (encompassing dispersal limitation and homogenizing dispersal), selection (including heterogeneous and homogenizing selection), and ecological drift¹⁵. Employing this mechanistic predictive framework, the respective contributions of these processes have been elucidated in both lentic water bodies^{16,17} and marine ecosystems¹⁸, utilizing a phylogenetic null model that facilitates the quantification of various ecological forces shaping microbial community assembly¹⁵. Moreover, clarifying the mechanisms of biotic community formation and maintenance is a fundamental element in understanding spatial distribution patterns, with distance-decay relationships of microbial communities frequently reported in freshwater lakes, rivers^{18–20}, oceans^{21,22}, and intertidal zones²³. While spatiotemporal distribution characteristics and assembly processes are critical for understanding eukaryotic plankton communities, information remains limited regarding the spatiotemporal dynamics and assembly processes of eukaryotic plankton in both water diversion channels and water sources.

The Danjiangkou Reservoir (DJK) is located on the Han River (the longest tributary of the Yangtze River in China), and it is the place where the Dan River flows into. Water in the DJK is mainly from the Han River and Dan River, and the total area is approximately 1023 km², with a water storage capacity of 29 billion m³.²⁴ Moreover, the DJK is the water source for the middle route of the South-to-North Water Diversion Project (MRP), which is the largest water transfer project in the world, spanning different watershed and different climatic zones, which has solved the water shortage problem in the northern four provinces and cities, namely Henan, Hebei, Beijing, and Tianjin²⁵. Eukaryotic plankton, a pivotal determinant in sustaining water quality stability within the DJK and MRP, remains insufficiently explored. Additionally, comprehensive comparative studies on the assembly processes of eukaryotic plankton communities between water diversion channels and their source waters have yet to be thoroughly investigated. The MRP consists of reinforced concrete, facilitating a robust hydrodynamic exchange and comprising multiple hydraulic structures and engineered disturbances (e.g., inverted siphons, aqueducts, and tunnels) along its main channel²⁶, thereby endowing its water characteristics with marked distinctions from those of natural water bodies²⁷. Following the application of MRP, the rapid proliferation and biomass accumulation of *Cladophora* at specific times suggested that eukaryotic phytoplankton exhibit swift responses to particular habitat conditions, this phenomenon represents a potential ecological issue that may compromise water quality stability. Consequently, examining the community dynamics of eukaryotic plankton is critical to elucidating community variability, nutrient cycling processes, and the prevailing water quality conditions. In addition to those, it is critical to explore the dynamics of eukaryotic plankton communities and their relationship to water environment, both in terms of ecological significance and as an important issue for the management and regulation of the DJK and MRP.

To be specific, our research is committed to elucidating the following issues: (1) What are the spatial patterns of eukaryotic plankton community in the DJK and MRP? (2) What are the factors that influence the distribution of eukaryotic plankton communities? (3) What is the relative importance of stochastic and deterministic assembly processes of eukaryotic plankton community? Which environmental factors mediate community assembly processes? Our results might add to the understanding of the dynamics of eukaryotic plankton communities and their assembly processes between water diversion channels and their source waters.

Materials and methods

Sample collection and environmental factors analysis

Surface water samples were collected in July 2022. Details of the sampling sites are shown in Fig. 1 and Table.S1. At each site, environmental factors, including water temperature (WT), pH, dissolved oxygen (DO), conductivity (SpC), salinity (Sal), and oxidation-reduction potential (ORP), were measured using a YSI Professional Plus meter (YSI, USA). Current water velocity (Vel) was recorded using an FL-KIT current meter. And mixed water samples of 2 L were collected at 0.5 m and 1 m depth below the water surface, which were divided into two parts for eukaryotic plankton community and chemical analyses, respectively. Eukaryotic plankton samples (1 L water samples) were filtered through 0.22 µm pore-size polycarbonate membrane filter (Whatman) to collect the eukaryotes and then frozen at -80 °C until DNA extraction. For measurements of chlorophyll *a* (Chl. *a*) contents, 500 ml water samples were filtered using GF/C filters, and Chl. *a* was then extracted with 90% acetone over 24 h and measured spectrophotometrically. 500 ml water samples were used for measuring total nitrogen (TN), ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), total phosphorus (TP), phosphate phosphorus (PO₄³⁻-P) and chemical oxygen demand (COD_{Mn}) according to the Monitoring and analysis methods of water and wastewater²⁸.

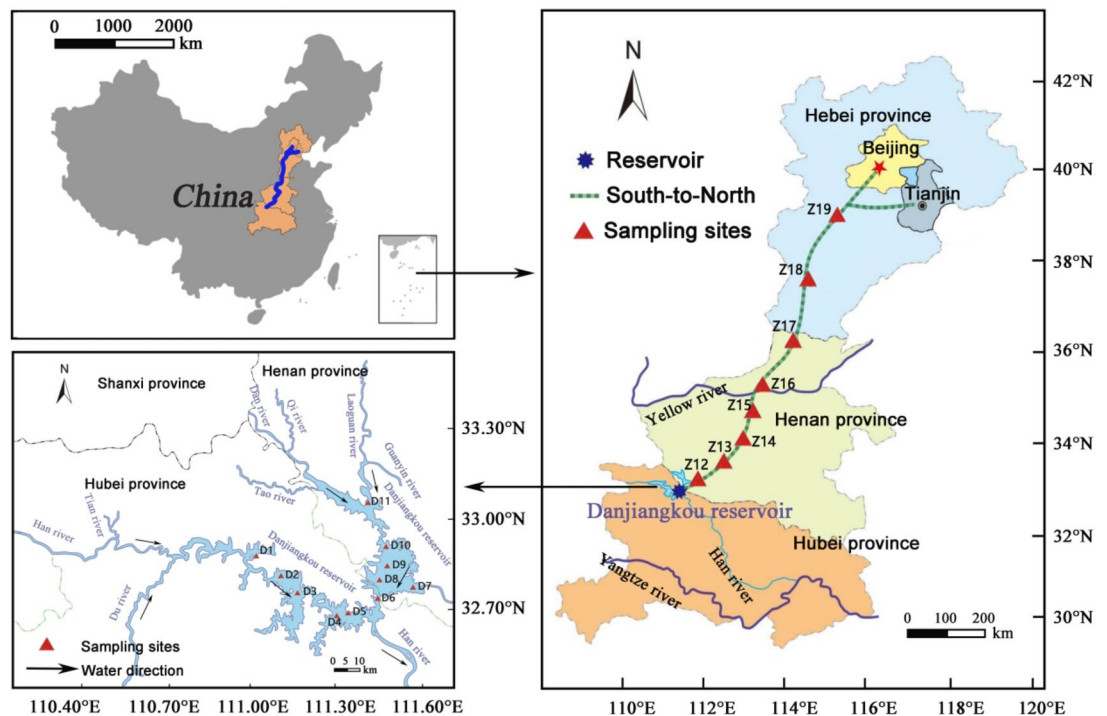


Fig. 1. Distribution of sampling sites in the Danjiangkou Reservoir and the channel of the middle route of South-to-North Water Diversion Project. (The base map was downloaded from the Standard Map Service System of the Ministry of Natural Resources of China (<http://bzdt.ch.mnr.gov.cn/>) and edited using Adobe Photoshop 2018 (<https://www.adobe.com/products/photoshop.html>)).

DNA extraction, PCR amplification, Illumina NovaSeq sequencing and data processing

Total genomic DNA was extracted from water samples using the TGuide S96 Magnetic Soil /Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.) according to manufacturer's instructions. The quality and quantity of the extracted DNA were examined using electrophoresis on a 1.8% agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, USA). DNA extracts were used as PCR templates, and barcoded primers targeting the V4 region of the 18S rRNA gene (TAREuk454FWD1: 5'-CCAGCASCYGC GGTAATTCC-3' and TAREukREV3: 5'-ACTTTTCGTTCTTGATYRA-3') were used to construct sequencing libraries for each sample tested^{29,30}. These primers have the Illumina sequencing primer sequences attached at their 5' ends. In the first amplification step, PCRs were carried out in a final volume of 12.5 μ L, containing 1.25 μ L of template DNA, 0.5 μ M of the primers, 3.13 μ L of Supreme NZYTaq 2x Green Master Mix (NZYTech, Portugal) and ultrapure water up to 12.5 μ L³¹. The reaction mixture was incubated as follows: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 48°C for 45 s, 72°C for 45 s and a final extension step at 72°C for 7 min. The oligonucleotide indices that are required for multiplexing different libraries in the same sequencing pool were attached in a second amplification step with identical conditions but only for 5 cycles and at 60°C annealing temperature³¹. A negative control that contained no DNA (BPCR) was included in every PCR round to check for contamination during library preparation¹¹. The amplified products were purified with Omega DNA purification kit (Omega Inc., Norcross, GA, USA) and quantified using Qsep-400 (BiOptic, Inc., New Taipei City, Taiwan, ROC). The amplicon library was paired-end sequenced (2 \times 250) on an Illumina novaseq 6000 (Beijing Biomarker Technologies Co., Ltd., Beijing, China).

The raw 18S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.23.1 and merged by FLASH version 1.2.11^{32–34}. Operational taxonomic units (OTUs) with a 97% similarity cutoff were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed³⁵. The taxonomy of each representative sequence was analyzed by RDP Classifier version 2.2 against the 18S rRNA database (Silva v138) using a confidence threshold of 0.7^{36,37}. Based on bioinformatics, Good's coverage values of all samples were above 99.9%, confirming that the libraries could represent most of the OTUs in the samples.

Statistical analysis

The alpha diversity indices (including Shannon Wiener Diversity Index, Simpson's Diversity Index, Chao1 Index and ACE Index) was calculated using QIIME version 1.9.1³⁹. One-way analysis of variance (ANOVA) was used to test the significance of the differences among the DJK and MRP based on the environmental factors and alpha diversity indices. The Fisher's Least Significant Difference (LSD) test was applied to test for the difference in the environmental data and the homogeneity of variances (Levene's test). Otherwise, the significance was tested

using Tamhane's in Post-hoc multiple comparisons. One-way ANOVA was completed by IBM SPSS Statistics for Windows (version 17, Chicago, IL, USA).

Beta diversity of eukaryotic plankton was assessed using the Bray-Curtis similarity coefficient and Nonmetric Multidimensional Scaling (NMDS)³⁹. The ANOSIM statistic (R) was used to compare mean ranks between groups, with values ranging from -1 to +1, where 0 indicates random grouping⁴⁰. Statistical significance ($\alpha=0.05$) was assessed with 999 permutations. Eukaryotic plankton data were clustered using Ward's Minimum Variance Method based on Euclidean distance⁴¹. Linear discriminant analysis (LDA) effect size (LefSe) was applied to identify significant differences among abundant modules, with a threshold of 4.0 on the logarithmic LDA score for discriminative functional biomarkers. LefSe analysis was performed using the Galaxy framework 2.0⁴².

All environmental factors, except pH, were standardized to improve normality and homoscedasticity ($X = (X - \bar{X})/SD$)⁴³. Principal component analysis (PCA) was conducted based on the methods outlined by Jolliffe and Cadima⁴⁴. Preliminary detrended correspondence analysis (DCA) of the eukaryotic data indicated that the longest gradient was less than 3. Therefore, redundancy analysis (RDA) was employed to explore the relationship between community structures and environmental factors. Variance inflation factors were kept below 10 to minimize multicollinearity among environmental factors. Before the RDA analysis, the 'ordiR2step' function in the R 'vegan' package was used for forward selection to filter out significantly affecting variables ($P < 0.05$)⁴⁵. Influential factors were selected using 999 Monte Carlo permutation tests at the $P < 0.05$ significance level. In addition, relative importance of environmental factors was assessed using variation partitioning analysis (VPA)⁴⁶. The Mantel test was also used to further investigate correlations between environmental factors and Bray-Curtis similarity of communities⁴⁷. Bray-Curtis similarity between samples and geographical distances were calculated using the R packages 'vegan' and 'geosphere'⁴⁸. Euclidean distances of environmental factors were also analyzed to detect whether they correlated with geographical distance. Regressions were generated with functions available in the 'ggplot2' package⁴⁹.

The Stegen null model was applied to quantify the relative importance of stochastic and deterministic processes⁵⁰. Phylogenetic and taxonomic diversity variations were assessed using β -diversity metrics based on null models (β NTI and RCbray). Specifically, $|\beta$ NTI| < 2 indicates the dominance of stochastic processes, while the relative contributions of variable and homogeneous selection were determined by the percentage of pairwise comparisons with β NTI > 2 and < -2, respectively⁵¹. The Bray-Curtis-based Raup Crick index (RCbray) was used to investigate pairwise comparisons not attributed to deterministic processes. By integrating |RCbray| values, we identified underlying community assembly processes such as homogenizing dispersal ($|\beta$ NTI| < 2 and RCbray < -0.95), dispersal limitation ($|\beta$ NTI| < 2 and RCbray > +0.95), and undominated processes (e.g., weak selection, weak dispersal, diversification, and drift), where $|\beta$ NTI| < 2 and |RCbray| < 0.95⁵¹. The null community for all samples was randomized 999 times to derive average null expectations.

Clustering, NMDS, PCA, DCA, RDA, Mantel test, and stochastic and deterministic processes were analyzed using the 'vegan', 'stats', 'ggcor', and 'picante' packages of R (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria).

Results

Community composition and diversity

A total of 2395 eukaryotic OTUs were identified from 1,493,024 valid sequences. The rarefaction curves for the OTUs detected and post-filtering sequencing data in this study are displayed in Supplementary Table. S2 and Fig. S1. Cluster analysis divided the 19 sampling sites into four groups (Fig. 2a). Overall, the clustering identified two large groups—the DJK (D1–D11) and the MRP (Z12–Z19). The former was further divided into two subgroups (Hanku: D1 to D5, Danku: D6 to D11), the latter was also further divided into two subgroups (Henan: Z12 to Z17, Hebei: Z18 and Z19) (Fig. 2a). The two-dimensional NMDS analysis showed a clear difference in species composition between the DJK and the MRP. Results with a stress of 0.07 are shown in Fig. 2b. ANOSIM analysis recorded a significance level of 0.001 of all the compared groups (Fig. S2), indicating significant differences between the eukaryotic plankton communities of the two groups.

A total of 185 orders of eukaryotic plankton were detected in the 19 samples. The taxa richness of the four subgroups (Danku, Hanku, Henan, Hebei) of eukaryotic planktons was 91, 90, 144, and 74, respectively. Thirty-four common species occurred in all four subgroups. Particular species (occurring only in Henan) had the highest ($n=45$) taxa richness, although Henan shared 67, 69, and 69 species with Hebei, Danku, and Hanku, constituting 36.22%, 37.29%, and 37.29% of their total order number, respectively (Fig. 3a).

The structures and relative abundances of the identified eukaryotic plankton at the phylum and order levels are shown in Fig. 3b and c, respectively. The most abundant phyla across all samples were Cryptophyta, Chlorophyta, Mollusca, Intramacronucleata, and Arthropoda, accounting for 28.97%, 11.06%, 10.37%, 10.16%, and 8.84% of all sequences, respectively (Fig. 3b). Cryptophyta was more abundant in the DJK (D1 to D11) than in the MRP. The relative abundance of Mollusca was higher in the Henan section compared to other sections, while Chytridiomycota was more abundant in the Hebei section (Fig. 3b). At the order level, Cryptomonadales, Sphaeropleales, and Calanoida were dominant in the DJK. Mytiloida was more abundant in the Henan section than in other sections (Fig. 3c).

The number of OTUs showed more significant differences in both the DJK and the MRP ($P < 0.001$) (Fig. 3d). For diversity, significant differences in the Chao1 index were found in both the DJK and the MRP (Fig. 3e, $P < 0.001$). In addition, the Simpson index and Shannon index of the MRP were significantly higher than those in the DJK ($P < 0.05$) (Fig. 3f–g).

LefSe analysis was performed to identify significant differences in the abundance of eukaryotic plankton taxa. The cladogram revealed distinct differences between the DJK and the MRP (Fig. 4a). The plot from LefSe analysis displays LDA scores of eukaryotic plankton taxa with significant differences between the DJK and the

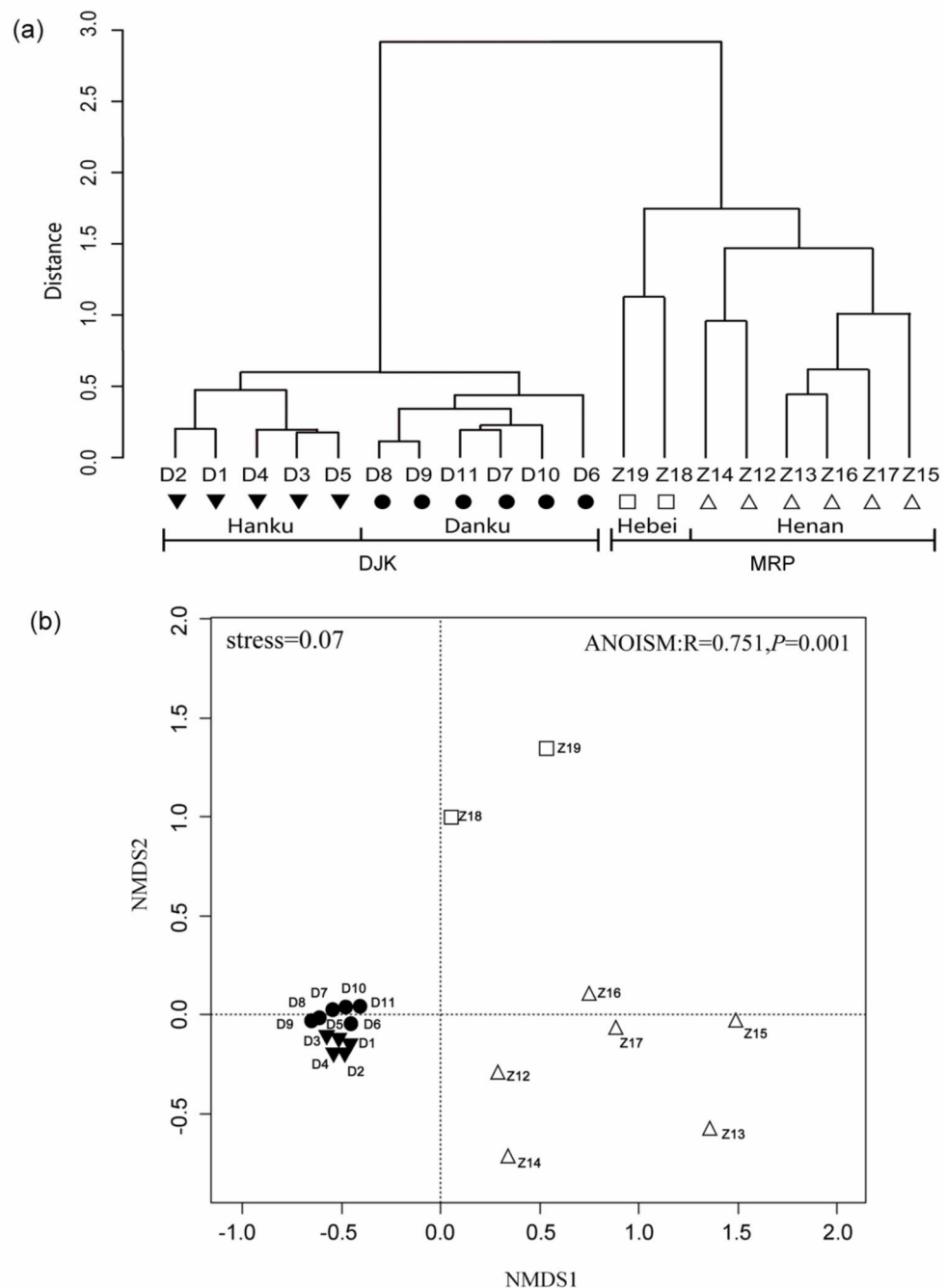


Fig. 2. (a) Cluster dendrogram of sampling sites based on eukaryotic plankton communities; (b) NMDS based on Bray-Curtis similarity analysis performed on the relative abundances of the eukaryotic plankton communities at each site.

MRP (Fig. 4b). At the order level, the biomarkers showing significant differences between the DJK and the MRP included Sphaeropleales, Chlorellales, Cryptomonadales, Ochromonadales, Pyrocystales, Chytridiales, and Mytiloida. The relative abundance of Chlorellales, Ochromonadales, Pyrocystales, Chytridiales, and Mytiloida was significantly higher in the MRP compared to the DJK.

Influencing factors of community variations

PCA demonstrated that samples showed a significant environmental heterogeneity between the DJK and the MRP ($P < 0.001$, Fig. S3). Fourteen measured environmental factors were included in the statistical analyses (Fig. 5). The nutrient-related measures of the MRP such as TN, NO_3^- -N, NH_4^+ -N and COD_{Mn} were significantly higher than the DJK ($P < 0.05$). Also, WT and pH differed, being higher in the DJK, respectively, than in the MRP ($P < 0.05$). The values of Spc, Sal, ORP, Chl.*a*, and Phosphorus (including TP and PO_4^{3-} -P) showed no

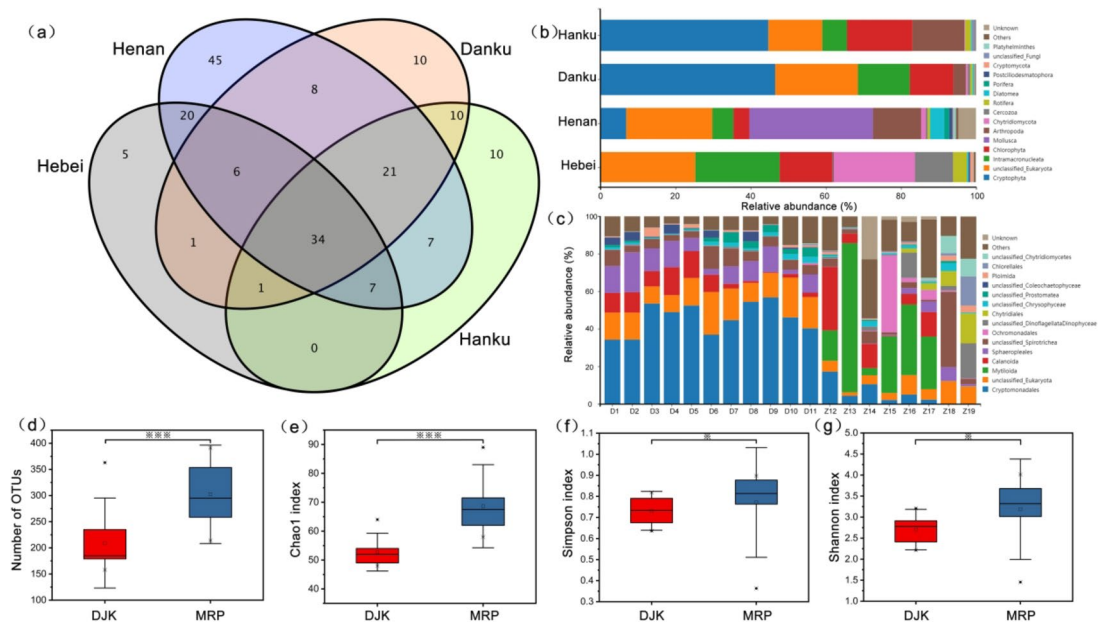


Fig. 3. (a) Venn diagram of the taxa richness of the eukaryotic plankton orders; (b–c) Composition of the major eukaryotic plankton phyla and order in the DJK and the MRP (Relative abundance of the top 15); Number of OTUs (d), Chao1 index (e), Simpson index (f) and Shannon index (g) in all samples. Significant differences (** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) between categories are indicated by asterisk, statistical analysis is ANOVA with Tamhane's with post hoc multiple comparisons.

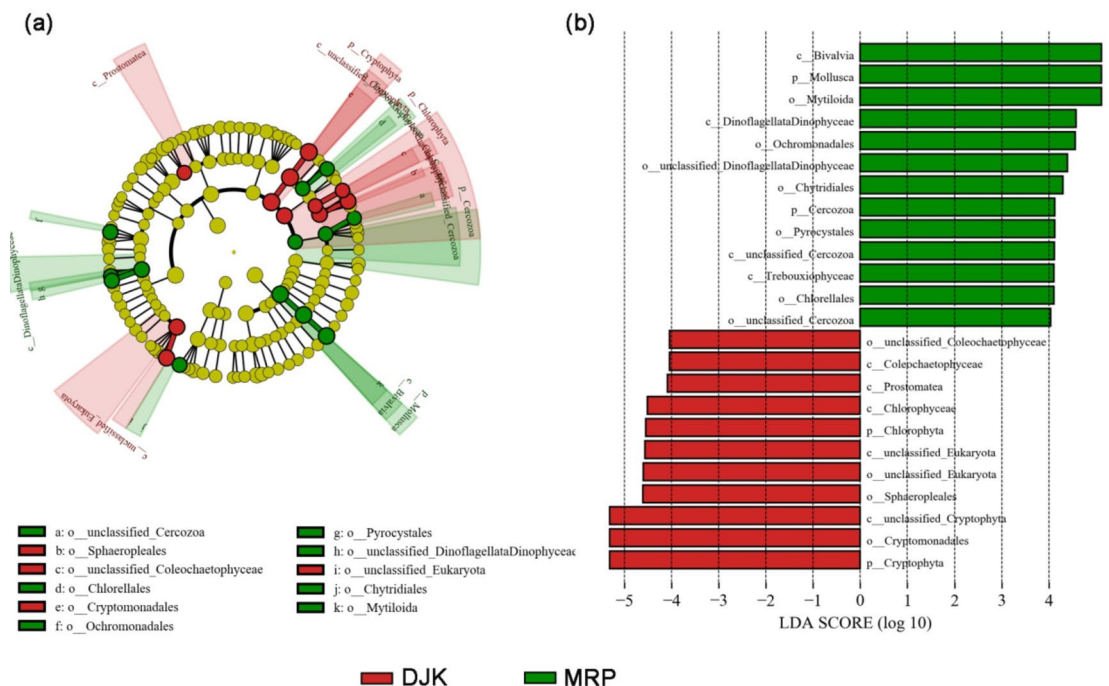


Fig. 4. (a) The cladogram diagram shows the eukaryotic plankton order displaying significant differences in the two groups. Red and green indicate different groups, species classification being shown at the level of phylum, class, order from the inside to the outside. (b) Species exhibiting significant difference with an LDA score greater than the estimated value; the default score is 4.0. The length of the histogram represents the LDA score; i.e., the degree of influence of species with significant differences between groups.

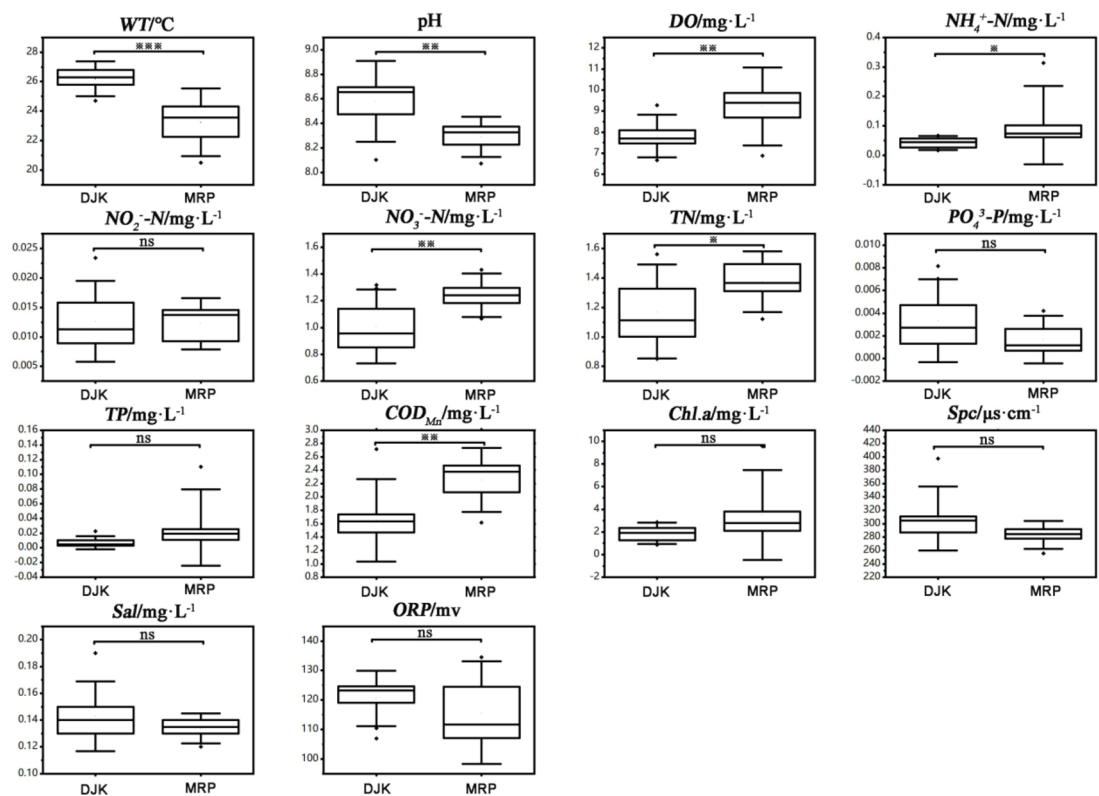


Fig. 5. Environmental factors in the Danjiangkou Reservoir and the channel of the middle route of South-to-North Water Diversion Project. Significant differences (** $P < 0.001$, * $P < 0.01$, $P < 0.05$) between categories are indicated by asterisk, statistical analysis is ANOVA with LSD with post hoc multiple comparisons.

differences between the two groups ($P > 0.05$). The F and P values determined by one-way ANOVA are shown in Table. S3. Vel of the MRP are shown in Table. S4. There was fluctuating variation in the spatial variation of the environmental factors, the Euclidean distances calculated for studied environmental factors showed significant positive correlation with geographical distances (Fig. S4).

The negative correlations between community similarity and both geographic distance and environmental distance were significant in eukaryotic plankton communities (Fig. 6a–b). The eigenvalues of the first two RDA axes ($\gamma_1 = 0.1632$, $\gamma_2 = 0.0589$) explained 29.44% and 10.63% of the variation in the species–environment relationships, respectively (Fig. 6c). The RDA scores showed strong relationships between the environmental factors (i.e., TN, TP, WT, ORP, SPC, COD_{Mn} , Chl.*a* and Vel) and community variation. The DJK was strongly correlated with ORP, SPC and WT, while the MRP had strong correlation with TN, TP, Vel, COD_{Mn} and Chl.*a*. The Henan section had a high content of TN and TP, while the content of COD_{Mn} and Chl.*a* in Hebei section were higher than other sections. The relationship between dominant biological communities of the eukaryotic plankton communities and environment factors were analyzed by the Mantel test (Fig. S5). The results showed that WT, COD_{Mn} , Chl.*a* and ORP were the main environmental factors affecting the eukaryotic plankton community.

Community assembly processes

The range of variation in βNTI of the two groups (The DJK and The MRP) was mainly concentrated in $|\beta\text{NTI}| < 2$ and $\beta\text{NTI} < 2$, respectively (Fig. 7a). In the DJK and the MRP, the stochastic processes explained a higher proportion of community variation than deterministic processes (60.72–98.19% vs. 1.82–39.29%). As shown in Fig. 7b, the communities at the DJK were mainly controlled by undominated processes and homogenizing dispersal. By contrast, the communities at the MRP were mainly driven by homogeneous selection, undominated processes, homogenizing dispersal and dispersal limitation. Homogeneous selection played a more important role in the assembly of the MRP (39.29%) than that in the DJK (1.82%).

The relationships between βNTI and the variation of environmental factors were also examined (Fig. S6 and Fig. 8). The βNTI values in the communities at the DJK were not significantly and positively related to environmental factors, but the βNTI values were still in the region of the stochastic process ($-2 < \beta\text{NTI} < 2$) (Fig. S6f–i). Pairwise comparisons of βNTI for the communities at the MRP were significantly and negatively correlated with Chl.*a*, SPC and TN (Fig. 8), indicating increasing variations in Chl.*a*, SPC and TN led to a shift from primarily stochastic community assembly ($-2 < \beta\text{NTI} < 2$) to homogeneous selection ($\beta\text{NTI} < -2$).

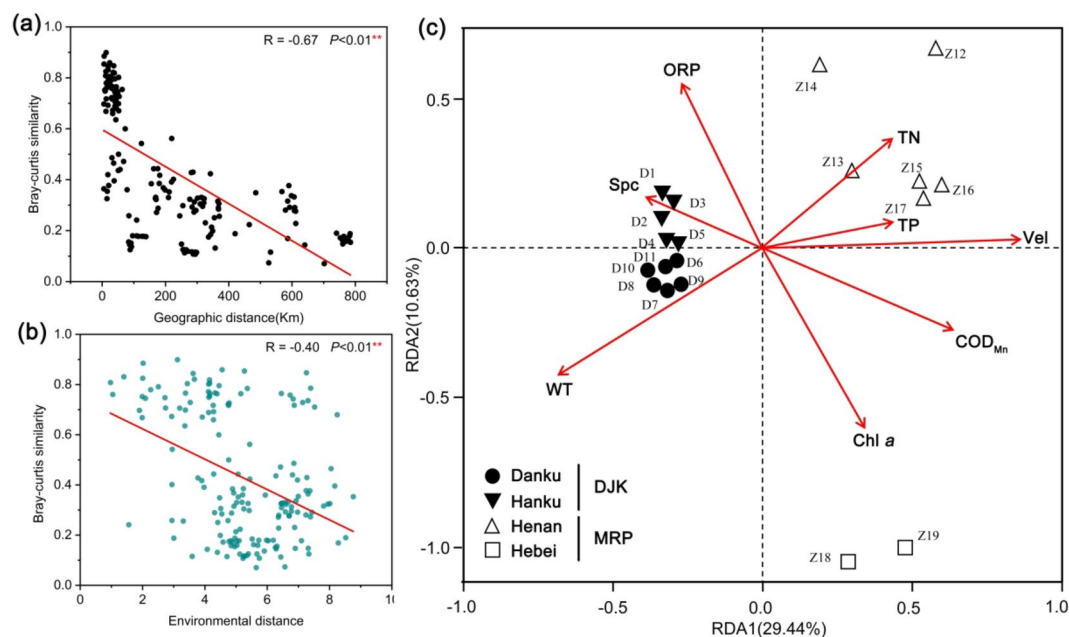


Fig. 6. Bray-Curtis similarity of the eukaryotic plankton community against geographical distance (a) and environmental distance (b), RDA ordinations of sampling sites based on environmental factors (c). Danku, Hanku, Henan and Hebei are indicated by different symbols as mentioned in Fig. 2(a).

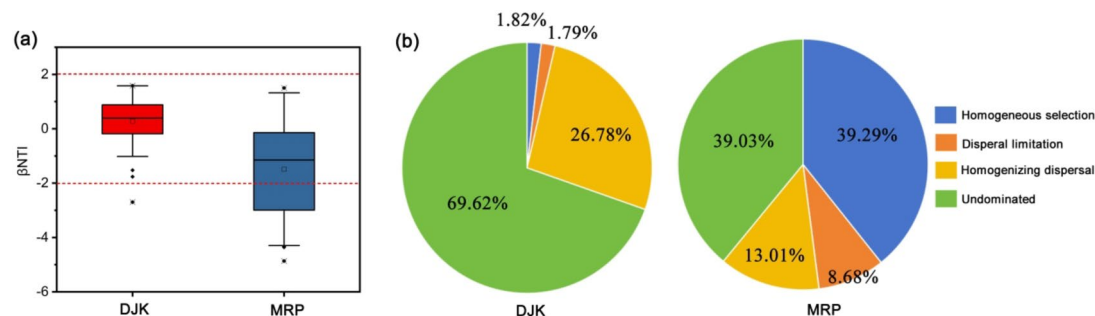


Fig. 7. Comparison of β -nearest taxon index (β NTI) in different groups (a) and Null model analysis for the assembly mechanisms of eukaryotic plankton communities (b).

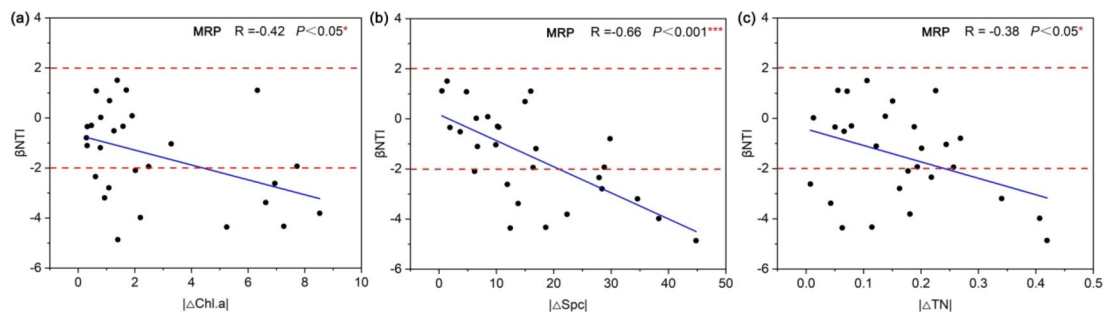


Fig. 8. Correlations between β -nearest taxon index (β NTI) of communities and changes in environmental factors. horizontal-dashed lines indicate the β NTI significance thresholds of +2 and -2; Δ : Changes in environmental factors between samples.

Discussion

Eukaryotic plankton community dynamics

Our analyses indicated a significant environmental heterogeneity between the DJK and the MRP, the spatial heterogeneity among various sampling sites within the MRP was higher than that in the DJK (Fig. S3). The MRP passes through subtropical monsoon climate zones and warm temperate monsoon climate zones. The long-distance water conveyance project has obvious differences in water temperature, climate, and environment, resulting in significant regional differences in water quality along the entire route⁵². In this study, we found that the concentrations of COD_{Mn} and $\text{Chl.}a$ in the water of the MRP in Hebei section are higher than those in Henan section, while the concentrations of $\text{NH}_3\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN are lower than those in Henan section (Fig. 5). Previous research has shown that the density of algae cells in the MRP shows an increasing trend from south to north, and is significantly higher after the Yellow River crossing project than before⁵³. COD_{Mn} represents the concentration of organic and inorganic substances in water that can be oxidized by permanganate⁵⁴. The release of proteins and microbial residues into the water during autogenous processes such as algae and microbial activities in the water body increases the COD_{Mn} concentration of the water body⁵⁵. Some studies have shown that nutrients are necessary for the growth and reproduction of plankton, and the reason for the massive proliferation of algae in early spring is the rapid absorption and utilization of nitrogen⁵⁶. In our study, the lower concentrations of dissolved nitrogen ($\text{NO}_2^-\text{-N}$, $\text{NH}_3\text{-N}$, $\text{NO}_3^-\text{-N}$) in the water of the MRP in Hebei section may be caused by the massive reproduction of plankton. The complex environmental and hydrological hydrodynamic conditions along the MRP indirectly affect the migration and transformation laws of substances, leading to changes in the growth rate and distribution pattern of plankton, and ultimately affecting the changes in the physicochemical characteristics of the water body, resulting in higher spatial heterogeneity of the water environment in the MRP.

In this study, we found that Chlorophyta and Cryptophyta play an important role in the DJK, and Cryptophyta, Mollusca, Diatomea and Arthropoda were the dominant taxa in the Henan section, while the Hebei section were dominated by Intramacronucleata, Chlorophyta, Chytridiomycota and Cercozoa, and the relative abundance of Cryptophyta decreased over long distances of water diversion in the MRP (Figs. 3 and 4). These results were highly in accordance with the previous analysis of planktonic eukaryotic in the DJK^{8,57} and the MRP^{53,58}. Certain Cryptomycota species are recognized as primary parasites of Chlorophyta⁵⁹, utilizing phagocytosis to absorb nutrients, thereby directly inhibiting phytoplankton growth⁶⁰. Previous research has indicated that when the relative abundance of Cryptomycota was highest, the abundance of phytoplankton, particularly Chlorophyta, decreased significantly, which was similar to that of our study (Fig. 3b)⁶¹. Furthermore, it has been reported that Cryptophyta often grow in low flow velocity, eutrophic environments⁶² and that reducing nutrients and increasing flow velocity could control the proliferation of Cryptophyta⁶³. Consequently, the observed decline in Cryptophyta could be attributed to adverse environmental shifts, including diminished nutrient availability and elevated flow velocities in the channel relative to the source water reservoir, conditions that may ultimately undermine their competitive dominance^{64,65}. Generally, Chlorophyta and Cryptophyta are better adapted for growth in quiescent or low-disturbance aquatic environments, while Diatomea have a competitive advantage in terms of proliferation in flowing or turbulent aquatic environments⁶⁶. An unusually high species richness of Cryptomycota and Chlorophyta was noted in this study. Chlorophyta exhibited dominance across diverse habitats, likely attributable to their autotrophic mode of nutrition, capacity for mixed acid fermentation⁶⁷, and resilience in the face of environmental stressors⁶⁸. Cryptomycota also demonstrated considerable genetic diversity and exhibited efficient mechanisms for transferring carbon and energy through trophic levels⁶⁹. Hence, the still or low-disturbance water bodies in the DJK allowed Cryptomonas and Chlorophyta to be the important dominant species. Additionally, the higher COD_{Mn} concentration in the Hebei section of the MRP indicates that this water body has a large amount of organic matter, which can provide sufficient food sources for Chytridiomycota and Arthropoda. In addition, the appearance of Mollusca also indicates that the MRP has some attributes of the natural river channel.

Our results indicated that there are significant differences in the composition of eukaryotic plankton communities between the DJK and the MRP. Moreover, the similarity of community compositions among sampling sites in the MRP was relatively low, while the similarity among sampling sites in the DJK was relatively high (Fig. 2). Notably, environmental heterogeneity was significantly different between the DJK and the MRP ($P < 0.001$, Fig. S3), and significantly influenced communities and alpha diversity ($P < 0.05$, Fig. 3d–g). It is clear that local diffusion will make the microbial community structure tend to be homogenized in a connected water habitat without a preferred water flow direction⁷⁰. The previous study has demonstrated that disordered hydraulic forces can increase the similarity of phytoplankton communities between interconnected water bodies⁷¹, and indeed, similar results have also been found in planktonic bacterial communities⁷². It has been reported that the wind-induced turbulence in the DJK may play a role in enhancing water column mixing, thereby exerting a certain influence on the distribution and community structure of plankton⁵⁷. Furthermore, the previous investigation into the spatial patterns of the bacterioplankton in lake Taihu revealed that the disordered hydraulic mixing effect can promote the spatial diffusion of planktonic bacteria, thereby weakening the spatial difference of the planktonic bacterial community structure⁷³. Thereby, the high similarity of the eukaryotic plankton communities in the DJK may be due to the disordered wind and wave disturbance. In contrast, it has been reported that the community structure of prokaryotic plankton generally exhibited significant spatial differences in a large spatial span^{72,74} or between regions with geographical isolation^{75,76}. The Yellow River Crossing Project of the MRP (dark conditions and high flow velocity) leads to significant differences in the community structure of prokaryotic microorganisms⁷⁷ and the density of phytoplankton cells⁵³ before and after the Yellow River Crossing Project of the main canal. In addition, it has been reported that the physical and chemical factors also have an important impact on the community structure and distribution of plankton⁷⁸. In the present study, the

large geographical span of the sampling sites and the higher spatial heterogeneity of the water environment in the MRP may have led to the low similarity of the community structure of eukaryotic plankton.

The RDA showed that WT, SPC, and ORP were the significant water environment factors affecting the community distribution of planktonic eukaryotes in the DJK, while the MRP was mainly significantly influenced by Vel, TN, TP, COD_{Mn} , and Chl.*a* (Fig. 6c). Water quality, water temperature, and hydrological factors affected the phytoplankton community structure⁷⁹. Some studies have shown that temperature can directly affect the growth of phytoplankton by influencing its metabolic process⁸⁰, and it will also affect the growth of zooplankton and fish as well as other physical and chemical conditions, resulting in changes in the density and community structure of phytoplankton⁸¹. The flow velocity of the MRP ranges from 0.1 to 2.1 m·s⁻¹, which is relatively faster than that of other water bodies⁸². Previous research has indicated that the cell density of cyanobacteria and Chlorophyta in the MRP is significantly negatively correlated with the flow velocity, and the relative abundance of Diatomea is positively correlated with the flow velocity⁵³. The high flow velocity of the MRP enhances the dominant position of Diatomea, which was similar to that of our study. Moreover, previous research on phytoplankton in the lower reaches of the Xijiang River showed that increased flow rate and flow velocity are beneficial to the increase of species richness⁸³. Consistent with these previous results, we found that the number of OTUs and alpha diversity index of eukaryotic plankton in the MRP were higher than those in the DJK (Fig. 3). Additionally, studies have shown that the plankton community is affected by the water body type and its physical and chemical properties such as pH, temperature, electrical conductivity, and nitrogen, phosphorus and organic matter^{84–86}.

Moreover, eukaryotic plankton communities demonstrated analogous spatial variability, with community similarities displaying a pronounced distance-decay pattern (Fig. 6a–b). Prior research has revealed that spatial variation within small eukaryotic communities is predominantly regulated by dispersal constraints and/or the breadth of their ecological niches⁸⁷. On one hand, expanding geographical distance may amplify community heterogeneity by intensifying dispersal limitation effects⁸⁸. Comparable findings have been reported in studies of loosely connected soil ecosystems as well as certain interconnected lake systems^{72,89}. Conversely, greater geographical distances can also foster habitat variability, as indicated by the Euclidean distances between environmental factors and geographical separation (Fig. S4), ultimately restricting the likelihood of microbial dispersal across multiple sites⁹⁰. Consequently, habitat heterogeneity arising from increased geographical distances, coupled with dispersal limitation, may collectively shape the spatial distribution patterns of eukaryotic plankton communities within the DJK and MRP.

Influencing factors of community assembly processes

In our study, the null model supported that the stochastic processes played a relatively more critical role than the deterministic processes (Fig. 7), which might be influenced by the connectivity of water bodies and the frequent hydrodynamic exchange within the DJK and the MRP. On one hand, the enhanced connectivity facilitated the homogenizing dispersal of eukaryotic plankton within the channel, aligning with findings from prior studies on groundwater bacteria⁹¹ and testate amoebae in reservoir systems⁹². The stochastic hydrodynamic disturbances can lead to the random aggregation of planktonic bacteria in hydrologically connected aquatic habitats⁹³. Furthermore, the disordered hydraulic mixing effect within the DJK also contributed to a reduction in the spatial heterogeneity of environmental variables (Fig. S3), which may have attenuated the selective pressure exerted by deterministic processes. In ecosystems characterized by diminished environmental variability, stochastic processes are likely to supersede deterministic mechanisms⁹⁴. Previous researches have elucidated that the biogeographical distribution of microorganisms is modulated by a complex array of factors. In addition to localized environmental conditions, historical and evolutionary determinants—encompassing geographic distance and dispersal history—also play a crucial role in shaping microbial spatial patterns^{95,96}. Consequently, within interconnected habitats characterized by elevated hydrodynamic exchange, the assembly processes of eukaryotic plankton communities are likely to exhibit a greater propensity toward stochasticity.

Our results indicate that although stochastic processes predominantly govern the spatial distribution of eukaryotic plankton in both the DJK and the MRP, deterministic processes exert a more pronounced influence in the MRP compared to the DJK, accounting for 39.29% and 1.82%, respectively (Fig. 7b). For instance, the dominant influence of stochastic processes in community assembly observed in some lakes with low environmental gradients was similar to our study⁷². Conversely, in aquatic ecosystems where environmental gradients are more pronounced, deterministic processes have been shown to predominate in shaping community structure^{75,97}. The previous study has identified that the MRP, in its efforts to optimize operational management by regulating flow velocity, discharge, and other hydrodynamic conditions, has been equipped with numerous hydraulic structures along its course, these interventions significantly influence the spatiotemporal distribution of nutrients within the water body, ultimately leading to the formation of diverse aquatic microhabitats⁵³. Additional studies have revealed that more heterogeneous spaces, characterized by a multitude of microhabitat structures, can provide diverse survival conditions for bacteria, thereby enhancing the influence of deterministic processes on bacterial community assembly⁹⁸. Thereby, we speculated that the increased environmental complexity along the MRP favors the predominance of deterministic processes in shaping the spatial patterns of eukaryotic plankton communities.

In addition, we found that the dominant effect on the eukaryotic plankton community assembly changed from primarily stochastic community assembly to homogeneous selection with increasing variations in TN, Chl.*a* and Spc (Fig. 8). The high concentration of nitrogen nutrient salts may affect the composition and diversity of bacterial communities⁹⁹. Moreover, the difference in salinity and nutrient condition played a critical role in governing the composition, connectivity, and assembly process of prokaryotic communities¹⁰⁰, the elevated TN and Spc concentrations in aquatic environments facilitate selective pressures that favor specific species, thereby diminishing the influence of stochastic processes on community assembly. The previous study has demonstrated

that high concentrations of chlorophyll represent increased primary productivity, leading to a rise in organic carbon resources and intensified microbial competition, which in turn promotes deterministic processes¹⁰¹. In our study, we found that the concentrations of Chl.*a* in the water of the MRP in Hebei section are higher than those in Henan section, while the concentrations of Spc and TN are lower than those in Henan section (Fig. 5). The significant correlation between β NTI values and variations in TN, Spc and Chl.*a* further suggests that these are primary factors mediating the balance between stochastic and deterministic assembly processes in the eukaryotic plankton communities (Fig. 8). Collectively, these findings underscore the critical link between environmental variables and community assembly processes, which may, in turn, influence diversity and community stability.

Conclusion

The present study revealed spatial heterogeneity of eukaryotic plankton communities. The similarity in community composition of DJK is significantly higher than that of MRP, exhibiting distance-decay patterns. Environmental heterogeneity exhibits significant differences between DJK and MRP, and it significantly influences community composition and alpha diversity. Additionally, the assembly processes of eukaryotic plankton in both DJK and MRP are predominantly influenced by stochastic processes. However, in comparison to DJK, deterministic processes have a more pronounced impact on MRP. The variations in TN, Chl.*a*, and Spc have led to a transition in the assembly of eukaryotic phytoplankton communities in MRP from a stochastic process to a deterministic process. These findings provide the basis for further insights into the important role of eukaryotic plankton communities in maintaining ecological function and water quality stability in a long-distance water diversion channel and its water source.

Data availability

All sequencing data are available through the NCBI Sequence Read Archive under the accession number PRJ-NA1177785.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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