Connecting diversity and process: eukaryotic phytoplankton community dynamics across the Yangtze River using environmental DNA

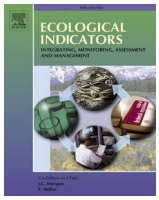
Shao, Y.; Stewart, K.A.; Gsell, A.S.; Wang, S.; Xie, B.; Lin, H.; ... ; Yan, Z.

Ecological Indicators 178 (2025) 114026

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Original Articles

Connecting diversity and process: Eukaryotic phytoplankton community dynamics across the Yangtze River using environmental DNA

Yun Shao [a,b,c], Kathryn A. Stewart [c], Alena S. Gsell [c,d], Shuping Wang [a], Boyuan Xie [e], Haojie Lin [a], Zhaomei Geng [f], Zhenguang Yan [a,g,*]

[a] State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, PR China
[b] Research Institute for Environmental Innovation (Tianjin Binhai), Tianjin 300450, PR China
[c] Institute of Environmental Science (CML), Leiden University, Leiden 2333 CC, the Netherlands
[d] Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO–KNAW), Wageningen 6708 PB, the Netherlands
[e] School of mathematics and statistics, Lanzhou University, Lanzhou 730107, PR China
[f] Herbert Wertheim School of Public Health & Human Longevity Science, University of California, San Diego, La Jolla 92093, USA
[g] Southern Marine Science and Engineering Guangdong Laboratory, Zhuhai 511458, PR China

ARTICLE INFO

Keywords:
eDNA
Eukaryotic phytoplankton
Multiple drivers
Community assembly

ABSTRACT

Rivers are collectors of both water and environmental changes in a catchment area, and act as dispersal pathways for biodiversity. But it remains unclear whether, and how strongly, environmental changes and dispersal dynamics shape the phytoplankton communities, especially within large river ecosystems. This study utilised environmental DNA (eDNA) to assess how water quality, land use, and geographical distance affect deterministic community assembly processes of eukaryotic phytoplankton across the entire Yangtze River—the world's third–largest river—in the dry and wet season. We found significant differences in phytoplankton community composition over time and space, with more phyla detected during the dry season, and α diversity increased from upstream to downstream. Generalist species' composition varied significantly across geographic regions, land use types, and environmental gradients during the wet season, whereas endemic species were primarily affected by local environmental conditions during the dry season. Stochastic processes dominated community assembly during both seasons, with dispersal limitation having a stronger influence than drift, particularly in the dry season. In contrast, deterministic processes had minimal effects, with α diversity showing weak responses to individual factors. By integrating eDNA–based biodiversity data with physicochemical and geospatial factors, we provide a comprehensive view of ecosystem conditions and highlight the need for region-specific conservation strategies in large, human-impacted rivers. It also indicates the predominant role of stochastic processes in shaping phytoplankton communities and suggests further investigation into the effects of hydrological factors.

1. Introduction

Environmental change is rapidly reducing biodiversity worldwide, with freshwater ecosystems among the most threatened (Albert et al., 2021; Arthington et al., 2024). Despite covering a small proportion of the Earth's surface, large rivers support high biodiversity, making their conservation critical (Alahuhta et al., 2019; Terui et al., 2021). However, the role of their geographical and environmental features in regulating biodiversity patterns remains largely unknown (Alahuhta et al., 2019). For example, rivers simultaneously act as collectors of water and environmental change (e.g. land use change, habitat fragment) within the entire catchment area (Allan, 2004), as well as act as dispersal pathways for biodiversity (Reynolds, 2006). Understanding how changes in habitat and water conditions affect aquatic biodiversity is crucial for guiding effective conservation and restoration efforts (Wang et al., 2021).

Eukaryotic phytoplankton are key primary producers in aquatic ecosystems, linking the physical environment (e.g., sunlight) and chemical conditions (e.g., water quality) to secondary consumers. They are essential for energy flow, nutrient cycling, and information transfer within these ecosystems (Agawin et al., 2000; Borics et al., 2021; Gao et al., 2023). Phytoplankton growth is influenced by various factors such

* Corresponding author at: State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, PR China.
E-mail address: zgyan@craes.org.cn (Z. Yan).

https://doi.org/10.1016/j.ecolind.2025.114026
Received 3 April 2025; Received in revised form 9 August 2025; Accepted 10 August 2025
Available online 21 August 2025

as nutrients, light availability, hydrodynamics, and grazing pressure from zooplankton grazing (Reynolds, 2006). Environmental changes and uneven nutrient distribution thus exert strong selective pressures on phytoplankton communities, driving compositional shifts (Naselli-Flores, 2014). Due to their microscopic size, wide distribution, short life cycles, and rapid response to environmental change, phytoplankton composition is widely recognized as an effective indicator of aquatic ecosystem health (Zhu et al., 2023). Studies show that environmental factors such as water temperature (Lian et al., 2023; Zhao et al., 2023), nutrient levels (McCarthy and Goldman, 1979), dissolved oxygen (Dhar and Baghel, 2015), and pH (Siegfried et al., 1989) significantly affect phytoplankton community composition. While previous studies have conducted relatively comprehensive research on the combined effects of water quality, land use, spatial isolation, and community dynamics, as well as their roles in phytoplanktonic community assembly processes (Chen et al., 2019; Isabwe et al., 2018; Wang et al., 2024; Zhang et al., 2019), such research remains limited in large–scale rivers.

One of the major challenges in using microscopic organisms as bio-indicators is the difficulty of species identification (Wang et al., 2024; Yan et al., 2023). Currently, microscopic observations are the primary method, but the reliance on distinct morphological features limits the accuracy and scope of using phytoplankton as indicators. This process is also often time–consuming and heavily reliant on advanced taxonomic expertise, highlighting the need to explore alternative approaches for biomonitoring. In recent years, with the widespread application of high–throughput sequencing technology in biological monitoring and biodiversity research (Didaskalou et al., 2022; Taberlet et al., 2018, 2012), environmental DNA (eDNA) offers a solution for studying microbial diversity and dynamics (Fan et al., 2020; Li et al., 2022; Sales et al., 2021).

The Yangtze River Basin, the world's third longest river, connects with eight major tributaries and has an annual average runoff exceeding 50 billion cubic meters. It supports rich aquatic biodiversity and provides vital ecosystem services for human survival and development (Chen et al., 2020; Xu et al., 2018). However, over the past two decades, intensive human activities have posed significant threats to these biological resources (Peng et al., 2023; Zhu et al., 2021). As a large river basin with pronounced seasonal water level changes and presence of multiple stressors, studying the phytoplankton community composition as indicator of human impacts across the Yangtze River can offer valuable insights for protecting large river ecosystems.

Our study employs an eDNA sampling approach to characterize eukaryotic phytoplankton communities across the entire Yangtze River Basin and aims to answer the following questions: What are the seasonal and spatial variations of the eukaryotic phytoplankton community composition across the Yangtze River Basin? How do stochastic and deterministic ecological processes drive these variations? And how do environmental factors affect the eukaryotic phytoplankton community? We hypothesize that eukaryotic phytoplankton community composition differs between wet and dry season and across three regions from up– to downstream, reflecting changing environmental conditions. Secondly, we hypothesize that community composition is largely driven by stochastic ecological processes, reflecting stronger influence of dispersal than selection effects. And thirdly, we hypothesize that community composition is weakly linked to environmental factors, reflecting the prevailing hydrology of a large riverine system.

2. Materials and methods

2.1. Research area

The Yangtze River Basin (90°33′E–122°19′E, 24°27′N–35°54′N) is the third longest river in the world and is incredibly ecologically diverse. It spans 6,396 km with an elevation drop exceeding 5,400 m from west to east and has a catchment area of 1.94×10^6 km^2 (Chen et al., 2014; Zheng et al., 2021). A large part of the Yangtze River Basin has a subtropical monsoon climate, the summer monsoon starts to influence the Basin in May and generally retreats in October. Precipitation is thus mostly concentrated in the summer season (June–August), accounting for nearly half of the annual total (Gemmer et al., 2008). This subsequently causes the water level of the Yangtze River to be low from November to April (dry season), and high from June to September (wet season). We divided the Yangtze River into the upstream, midstream, and downstream according to convention and threats to river ecology, as shown in Fig. 1. For details on each region, see the Study Area section of the Supporting Information.

2.2. Sampling

We collected water samples across the entire Yangtze River Basin, from the upstream to the downstream in both the wet (July–August 2022) and dry (March–April 2023) season. In total, we collected 81 samples from 38, 23 and 20 sites in the upstream, midstream and downstream sections of the Yangtze, respectively. To date, evidence suggests streams and rivers with turbulent flow are well–mixed with respect to eDNA (Shu et al., 2020; Strickland and Roberts, 2019). Following established protocols (e.g., Huo et al., 2020; Li et al., 2025), we collected all samples from the top 50 cm of the water surface using a sterile water sampler and Nalgene bottles, anticipating this approach garners us the eDNA of both pelagic and benthic community (Deiner et al., 2015; Lor et al., 2020). We stored all samples on ice and express–couriered 1 L of water to our laboratory for water physico-chemical analysis (< 4 days). Simultaneously, we filtered 5 L of water through a 0.22–μm Millipore polycarbonate membrane Sterivex filter (Millipore, USA) and stored tubes with membranes in liquid nitrogen until DNA extraction. Blank controls of each site were performed using autoclaved tap water (filtered 300 mL volume of water) to monitor possible contaminants. Details on eDNA sampling are available in the eDNA Collection and Preservation section of the Supporting Information.

2.3. Spatial and local environment variables

We measured spatial variables by calculating the Euclidean distance between sampling points and analyzing land use proportions. We assessed land use parameters within a 5 km upstream buffer around each site, categorizing them into five types: cropland, woodland, grassland, water cover, construction land, and unused land. We derived these classifications from a 1 km resolution land use raster map interpreted from Landsat TM/ETM imagery (National Earth System Science Data Center of China). Furthermore, we summed the percentages of cropland and construction land as the proxy of human activities.

We measured environmental physicochemical factors including water temperature (T, ℃), pH, dissolved oxygen (DO, mg/L), turbidity (Turb), electrical conductivity (EC), chemical oxygen demand (COD_{Cr}, mg/L), total phosphorus (TP, mg/L), total nitrogen (TN, mg/L), chlorophyll *a* (Chl a, mg/L), permanganate index (COD_{Mn}, mg/L), fluoride (F^-, mg/L), ammonium nitrogen (NH_3–N, mg/L), nitrite (NO^{2-}, mg/L), nitrate (NO^{3-}, mg/L), and total organic carbon (TOC, mg/L). T, pH, DO, Turb, and EC were measured on–site using the multi–parameter water quality detector Hydrolab HL4 (HACH, USA). We then measured all other environmental factors in the lab in accordance with standard protocols (NEPB, 2002).

2.4. eDNA experiments and bioinformatics analyses

We extracted DNA from our Sterivex filters using the DNeasy PowerWater Sterivex Kit (Qiagen, Germany). We then used two general primers, V8F (Bradley et al., 2016) and 1510R (Amaral-Zettler et al., 2009), to amplify the 18S V8 and V9 regions for eukaryotic phytoplankton. We amplified all eDNA samples and blank controls in three replicates to minimize potential polymerase chain reaction (PCR) bias,

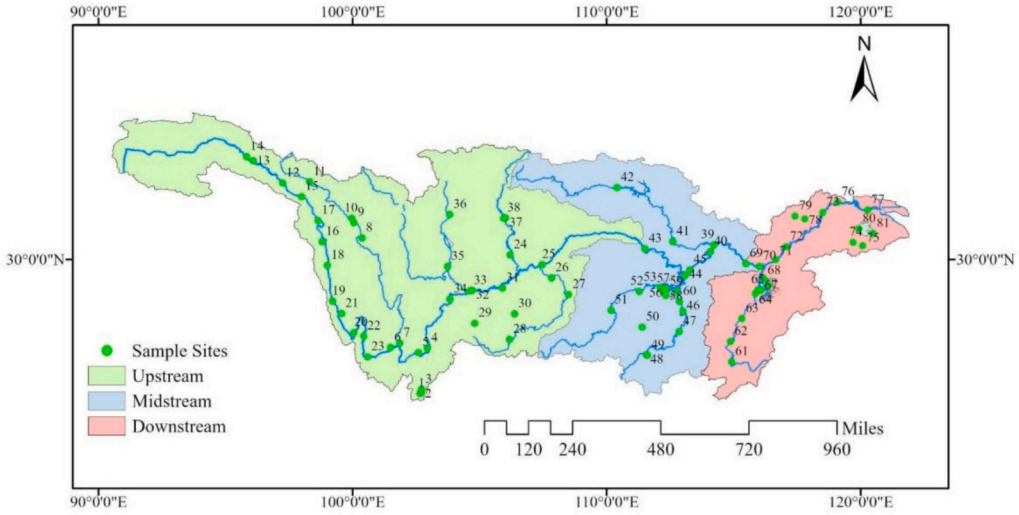


Fig. 1. Sampling map of the Yangtze River, with upstream, midstream, and downstream sections highlighted in distinct colors.

then pooled the purified PCR products in equimolar quantities for sequencing. We sent our DNA samples for high–throughput sequencing (Sangon Biotech, China). We obtained reads using second–generation sequencing that had undergone a first quality control via Fastp software (v0.12.4) (Chen et al., 2018) to remove chimeras and to filter out low–quality reads. We used FastQC (v0.11.9) to evaluate the quality of reads (Andrews et al., 2012). Then, we employed USEARCH (v11.0.667) to splice the filtered double–ended reads and selected 150,000 reads by default, and selected all if insufficient (Edgar, 2010). We then de–weighted the combined sequences using VSEARCH (v2.15.2) (Rognes et al., 2016). The OTU sequences obtained were compared by BLAST (v2.2.31) (Guillou et al., 2013). Details are available in the DNA Extraction, Amplification, Sequencing, and Bioinformatic Analyses section of the Supporting Information.

To prevent potential cross–contamination and mitigate limitations from incomplete reference databases, we implemented a curation step. As precise screening may lead to the loss of important taxonomic information due to the insufficient documentation of native species in public databases, we aimed to balance accuracy and data volume, by retaining 7 phyla that had been recorded in historical morphological surveys. We then assigned OTUs assigned to taxa at the phylum level and excluded OTUs that were absent in traditional surveys from subsequent analyses.

2.5. Statistical analysis

To assess the seasonal and spatial variation in eukaryotic phytoplankton communities of the Yangtze River, we visualized taxonomic composition with a percentage pile bar chart. Species overlap across upstream, midstream, and downstream were illustrated with Venn diagrams, and intergroup differences were analysed with t–tests. Nonmetric multidimensional scaling (NMDS) was applied to compare community structures, while Linear Discriminant Analysis Effect Size (LEfSe) analysis identified taxonomic differences. Generalized Additive Models (GAMs) were constructed to explore influencing environmental variables.

Community differences were evaluated using PERMANOVA, and environmental, spatial, and land use factors were analysed with variance inflation factor (VIF) screening. Correlation analysis and Mantel tests were used to assess environmental influences. Species were categorized as endemic species (species occurring in only one area) and generalist species (species occurring in more than one area) according to the Venn plot. The Infer Community Assembly Mechanisms by Phylogenetic–bin–based Null Model (iCAMP) was then applied to infer ecological processes shaping communities (Ning et al., 2020). Details are provided in the Supplementary Materials.

3. Results

In total, our 81 Yangtze River samples sequenced across both the wet season and dry season produced 7,080,244 sequencing reads (average of 87,410 per sample) in the wet season, and 19,346,836 sequencing reads (average of 238,849 per sample) in the dry season. According to the morphological identification in the historic record of the Yangtze River, only 7 observed phyla were retained, representing 2054 OTUs in the wet season and 1525 OTUs in the dry season.

3.1. Spatial and seasonal variation

In the wet season, Bacillariophyta and Chlorophyta alternated as the dominant groups across the basin, with Bacillariophyta being much more abundant in the midstream (Fig. 2 (a)). The dry season showed more diverse dominant taxa, including Bacillariophyta, Chlorophyta, Pyrrophyta, Chrysophyta, and Cryptophyta, with dominance patterns varying across upstream, midstream, and downstream sites instead of being evenly distributed throughout the basin (Fig. 2 (b)). Euglenophyta and Xanthophyta were not dominant in either season.

LEfSe analysis (Fig. S2) revealed significant regional taxonomic differences. In the wet season, *Bacillariophyceae* and *Naviculales* dominated upstream, *Sphaeropleales* and *Tetradesmus* were abundant midstream, and *Euglenida* and *Coscinodiscophyceae* were more prevalent downstream. In the dry season, *Discostella* was dominant upstream, while *Trebouxiophyceae* and *Chlorophyceae* were enriched midstream. Taxonomic structure varied most between upstream and downstream in the wet season, whereas in the dry season, significant differences were primarily observed in the midstream region.

Eukaryotic phytoplankton OTUs showed more overlap across the three river regions. The upstream region had the highest number of unique OTUs in both seasons (15.73 % in the wet season and 7.93 % in the dry season). In the wet season, more OTUs were found downstream than midstream, but in the dry season, the midstream region had more OTUs than the downstream. In total, 24.24 % of OTUs were common across all regions in the wet season, and 61.25 % were shared in the dry season (Fig. 3(a) (b)). The Shannon index was similar across seasons, with the downstream region having the highest index, followed by the midstream, and the lowest in the upstream. The difference in the Shannon index between regions was more pronounced in the wet season (Fig. 3 (c)).

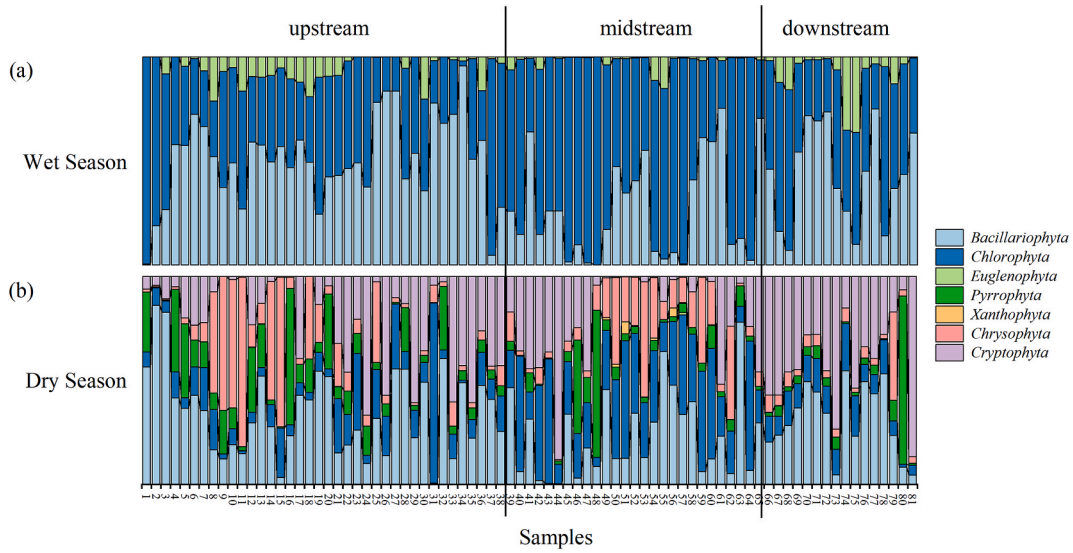


Fig. 2. Composition of micro–eukaryotic plankton at phylum level in the upstream, midstream and downstream of Yangtze River Basin (based on the normalized read count) in (a) wet and (b) dry season.

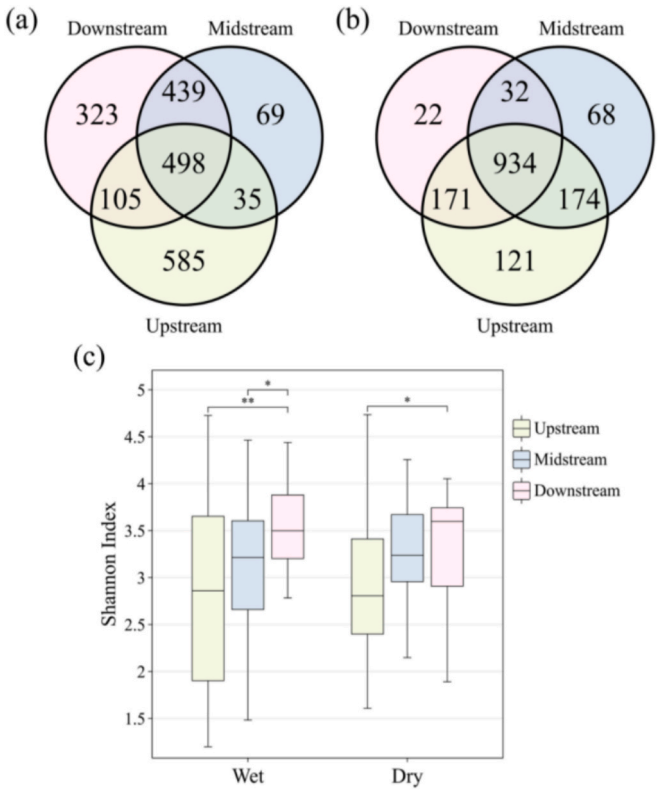


Fig. 3. Venn diagrams show the overlap in OTUs between the upstream, midstream, and downstream regions of the Yangtze River during (a) wet season and (b) dry season. (c) Box plot showing Shannon index per region in wet and dry seasons. Dissimilarity significance: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

NMDS analysis (Fig. 4) showed clear regional differentiation in both seasons, confirmed by the Adonis pairwise test (Table S1). In the wet season, the upstream community was highly variable and distinct, while midstream and downstream communities were similar. In the dry season, upstream diversity decreased, and midstream and downstream divergence increased.

3.2. Spatial and environmental drivers on eukaryotic planktonic community

.

3.3. Water quality influence

We found significant correlations among environmental factors, varying by season and region (Fig. 5, Fig. S3). For example, T and TN exhibited significant positive correlation with TN in the wet season, while T and pH exhibited significant negative correlation in dry season, potentially co–influencing the community composition (Fig. 5).

Correlation patterns also differed between species groups based on their generalist or endemic traits (Fig. S3). In the wet season, generalist species were more influenced than endemic species, with altitude, human activities, and TOC significantly affecting both. In the dry season, endemic species were more impacted, with pH being the only factor linked to generalists. Regional analysis showed stronger correlations with generalists upstream and midstream in the wet season, while downstream correlations were similar for both groups. In the dry season, no significant correlations were found upstream or midstream, but downstream, altitude and COD_{Cr} were significantly linked to both groups.

PERMANOVA analysis (Fig. 6) showed that environmental factors influenced community variation differently across seasons and river regions. In the wet season, several environmental variables, excluding TN, exhibited significant correlations with phytoplankton community composition. However, only a few factors, including pH, EC, and NH_3–N, showed significant associations in the dry season. Overall, the explanatory power of environmental variables was relatively low, as indicated by the R^2 values. This suggests that deterministic factors contributed limited effects to shaping community composition. Regional analyses revealed distinct but similarly weak associations: upstream communities were correlated with altitude, human activities, and a range of water quality factors; midstream communities were correlated with pH and TOC; and downstream communities were correlated with several environmental variables. In the dry season, human activities and water parameters (notably pH, EC, NH_3–N, and COD_{Cr}) showed stronger associations across regions, but still explained only a minor portion of community variation. These findings imply that other processes beyond environmental filtering, may play a more prominent role in shaping phytoplankton communities.

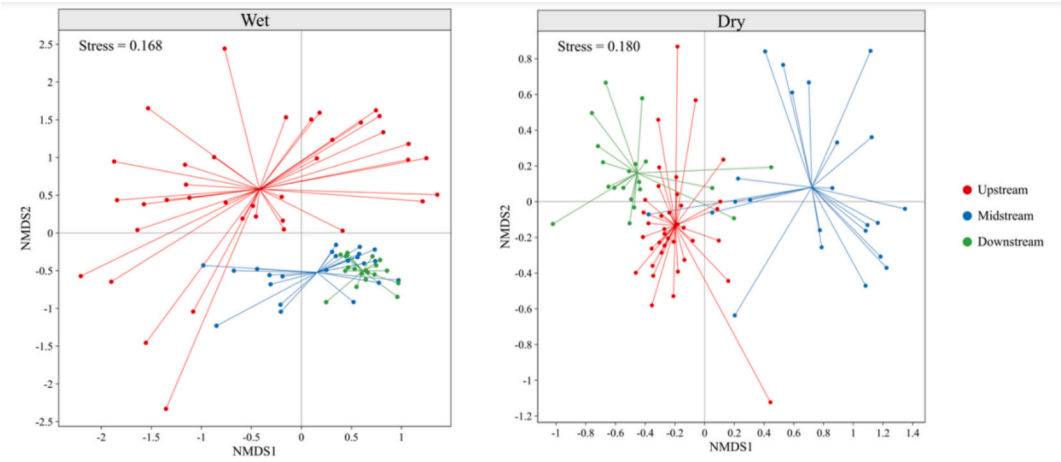


Fig. 4. NMDS plots of beta diversity in wet season and dry season with lines connecting data points with their respective seasonal means.

3.4. Geographical influence

Community similarity generally decreased with increasing geographical distance across regions of the Yangtze River in both seasons, except in the midstream region during the wet season where this trend was not significant (Fig. 7). During the wet season, distance–decay was more pronounced in the downstream regions than in the upstream, while no significant distance–decay was observed in the midstream. In the dry season, distance–decay was stronger downstream than in the midstream, and stronger in the midstream than in the upstream. Compared to the wet season, the distance–decay relationship in the upper reaches during the dry season was weaker, whereas it was stronger in the midstream and downstream regions.

3.5. Community assembly mechanism

Our null model showed the relative contribution of stochastic processes (dispersal limitation, homogenizing disposal, and undominated processes) and deterministic processes (homogenizing selection and heterogeneous selection) (Fig. 8). In the wet season, the upstream community assembly was mainly composed of undominated processes (drift, diversification, weak diffusion, weak selection, etc.; 73 %), midstream community variation was mainly driven by undominated processes (38 %) and dispersal limitation (34 %), whereas downstream community assembly was mainly driven by dispersal limitation (76 %), homogenizing selection (13 %–15 %), homogenizing disposal (0–10 %) and heterogeneous selection (1 %–6%). In the dry season, community assembly for the whole basin as well as the separated regions were mainly composed of undominated processes (36 %–62 %) and dispersal limitation (36 %–60 %).

4. Discussion

Utilising eDNA methods across the entire Yangtze River Basin, in combination with physicochemical and geospatial factors between hydrological seasons, we provide multiple insights into the eukaryotic phytoplankton community assembly processes and dynamics in large rivers. Our data suggest that in the Yangtze River, eukaryotic phytoplankton communities undergo turnover in response to environmental changes, while the diversity index remains relatively stable. Stochastic processes, such as drift and dispersal limitation, predominantly govern overall community assembly.

4.1. Seasonal and spatial phytoplankton community variations

Although our data span only a single annual cycle, we observed distinct shifts in community composition between the wet and dry season, which probably reflect phytoplankton responses to changes in hydrological conditions, nutrient availability, light penetration, and water physicochemistry. For example, Bacillariophyta and Chlorophyta dominated during the wet season, while Pyrrophyta, Chrysophyta, and Cryptophyta alternately dominated in the dry season. This may not indicate stable seasonal cycles but rather the result of short term environmental fluctuations typical of large river systems.

We detected Bacillariophyta, Chlorophyta, and Euglenophyta in the wet season, as well as Pyrrophyta, Xanthophyta, Chrysophyta, and Cryptophyta additionally detected in the dry season. Despite a lower number of detected phyla in the wet season, the total sequence reads (a proxy for species abundance (Muri et al., 2020; Tsuji et al., 2022)) were higher. Some lake sites followed the PEG (Plankton Ecology Group) model, in which Bacillariophyta blooms in spring (dry season) due to their ability to capitalize on high nutrient availability and mixing conditions, and Chlorophyta blooms in summer (wet season) due to increased temperature and light availability, leading to enhanced primary production (Cagle and Roelke, 2021; Sommer et al., 1986). Some studies on lakes along the Yangtze River have also demonstrated the PEG model (He et al., 2021; Meng et al., 2023; Zhang et al., 2020). However, large rivers may exhibit different dynamics due to continuous flow and stronger turbulence, patchy stratification, and hydrological connectivity (Meng et al., 2023; Rusanov et al., 2022). In the wet season for example, rising water levels and nutrient content are conducive to the propagation of phytoplankton with ensuant biomass and species richness increasing correspondingly (Calbet and Landry, 2004). Still stronger water flow and physical disturbances may hinder non–dominant taxa. Whereas in the dry season, reduced flow and stable sediments could create favorable conditions for the growth and dominance of mixotrophic and bloom–forming taxa, such as Pyrrophyta, Chrysophyta, and Cryptophyta, yet occurring in localized areas rather than system–wide (Li et al., 2018; Ruiz et al., 2004). Seasonal environmental changes, such as temperature, nutrient levels, and light availability, are further known to shape community composition. Higher water levels and turbidity in the wet season limit light penetration, restricting light–dependent algae. In contrast, lower water levels and improved transparency in the dry season promote their growth (Hewson and Fuhrman, 2004).

We also observed clear differentiation in microbial diversity and composition across regions. The Yangtze upstream serves as the source and species pool of biodiversity for the entire basin, as evidenced by the highest species richness observed during the dry season. In the wet season however, stronger hydrological flow from the upstream led to a greater diversity accumulation downstream, making it a collector of species from all regions (Ho et al., 2010; Lehman et al., 2008). The proportion of Chlorophyta increased significantly from the upstream to

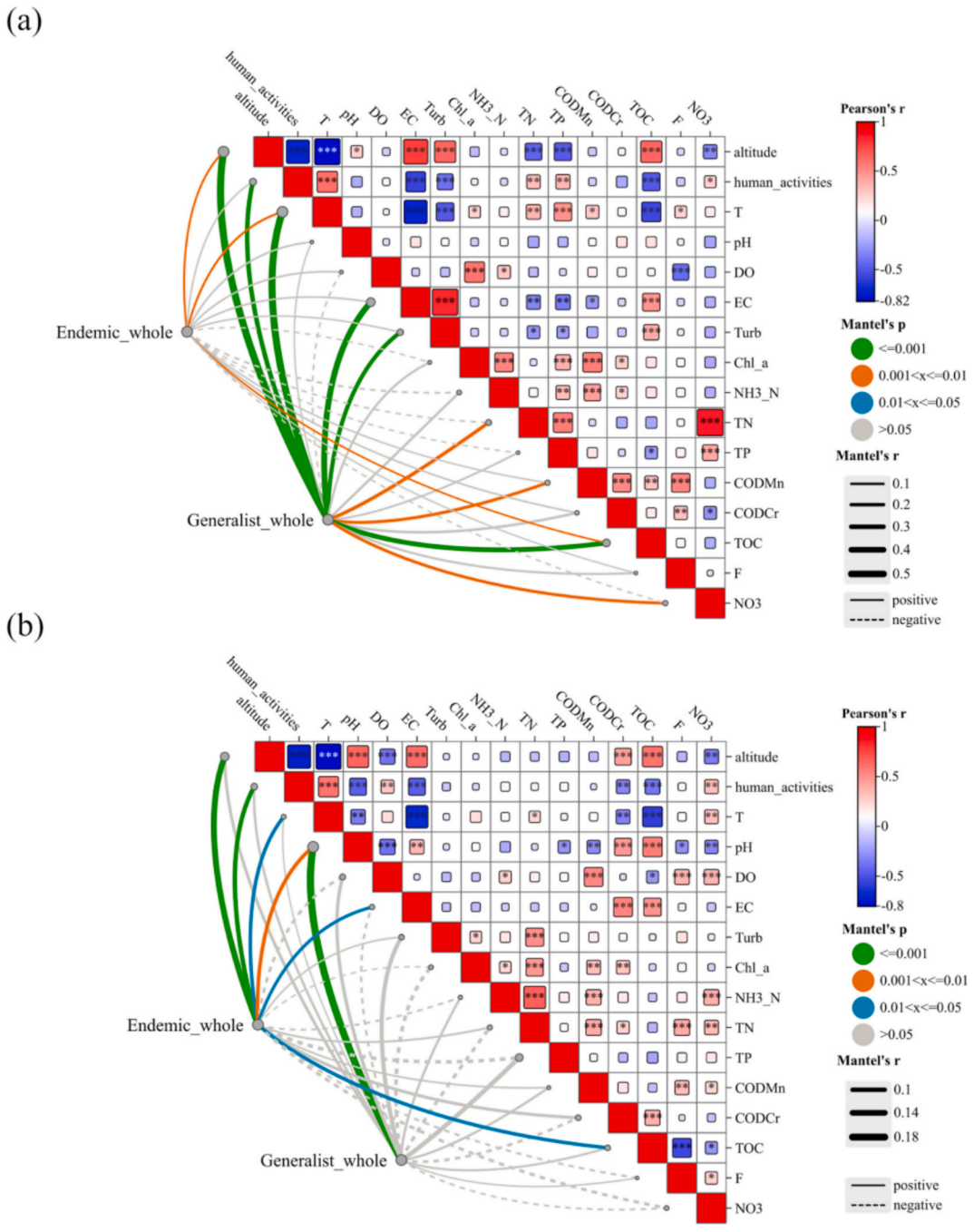


Fig. 5. Correlation analysis and mantel test between eukaryotic phytoplankton community and environmental factors in the whole basin of Yangtze River in (a) wet and (b) dry season. The significance of the correlation: *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

the midstream regions, which may also be attributed to diatoms (Bacillariophyta) being predominantly cold–water species, whereas green algae (Chlorophyta) generally thriving in warmer aquatic environments (Schlie and Karsten, 2017). Still, we acknowledge that our observations on community dynamics and stability are observed over a one–year period only, and a longer time series corroborating the effect size and direction of the observed patterns are warranted in future studies.

4.2. Effects of environmental factors on the eukaryotic phytoplankton community

Our study showed that environmental factors contributed to the spatial and seasonal variations of specific eukaryotic phytoplankton community composition in the Yangtze River, although their overall explanatory power was relatively low. During the wet season, the increased discharge seems to enhance the dispersal of phytoplankton, leading to reduced spatial isolation effects. Endemic species preferentially occupied specific ecological niches, resulting in stronger environmental filtering effects on generalist species. Generalist species were more responsive to environmental fluctuations, likely due to their broader ecological tolerance and adaptability to dynamic hydrological conditions. In contrast, during the dry season, endemic species exhibited stronger associations with environmental factors, particularly pH, suggesting that local habitat conditions play a more critical role in shaping their distribution under lower–flow conditions (Erős et al., 2024;

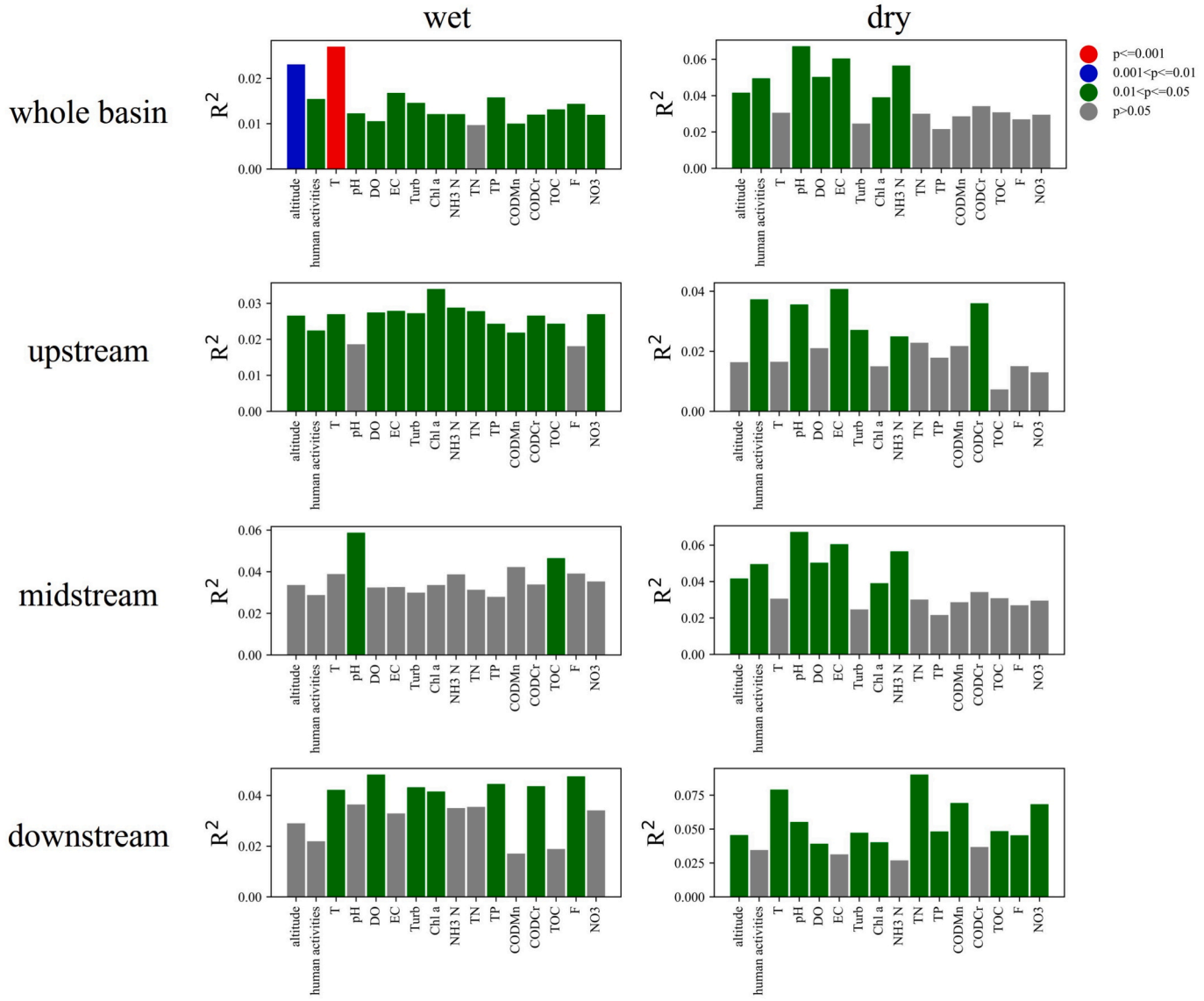


Fig. 6. R^2 values from PERMANOVA across wet and dry season, the columns are colored by p–value.

Gemmer et al., 2008; Lucas et al., 1999). Hydrologic and climatic factors largely controlled phytoplankton community structure during the wet season while anthropogenic related factors regulated phytoplankton during the dry season (Nwonumara and Okogwu, 2021).

We further found that spatial heterogeneity also significantly affected the relationship between environmental factors and phytoplankton communities. The Yangtze River has the largest elevation drop at its source leading to significant differences between environmental factors, which likely further resulted in significant differences in the structure of phytoplankton communities across elevations (Han et al., 2020). In the downstream area, intensive industrial activities had a prominent effect on community, reflected in the association between pollution factors and community structure (Feng et al., 2021). Temperature was the most explanatory factor in our study consistent with findings that water temperature is a key environmental variable influencing diatom community assemblages via size and growth rate (Canale and Vogel, 1974).

Previous investigations using traditional approaches to census eukaryotic phytoplankton in the Yangtze River indicated that water temperature, dissolved oxygen, biochemical oxygen demand, nitrite, and ammonia nitrogen were the most critical environmental factors explaining phytoplankton composition (Liu et al., 2019). Whereas another eDNA–based study on diatoms in the Yangtze River highlighted that photosynthetically active radiation, temperature, channel slope, and nutrient conditions are essential to diatom communities, alongside spatial dispersal (Wang et al., 2019). Although different studies focused on different types of factors, together with our study, evidence consistently indicates that water temperature, nutrient conditions, and river morphology are the primary factors explaining phytoplankton communities. Our study achieved only limited explanatory power from the perspectives of water quality, land use, and elevation. However, the seasonal pattern differences suggest the potential role of hydrology for future research.

4.3. *Ecological processes on phytoplankton community composition*

Within our study, stochastic processes, especially undominated processes and dispersal limitation, primarily drove the assembly of the eukaryotic phytoplankton community across the Yangtze River. Previous studies have demonstrated that stochastic and deterministic ecological processes exhibit scale dependency, whereas at local scales (including individual sites and neighboring locations), community assembly is primarily driven by deterministic processes (particularly homogeneous selection) (Gu et al., 2021; Pearman et al., 2022). In

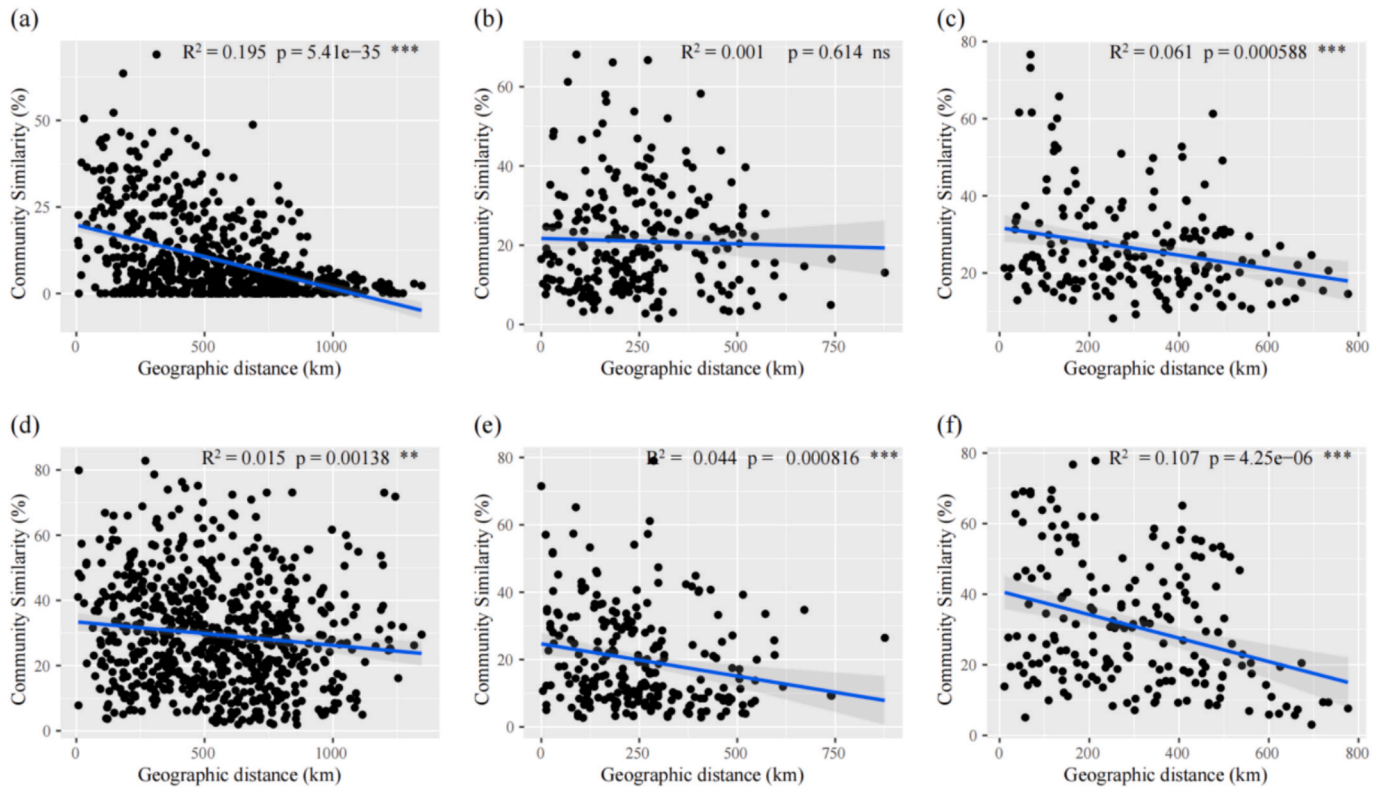


Fig. 7. Distance–decay analysis of eukaryotic phytoplankton communities in the Yangtze River reaches during wet (a–c) and dry (d–f) seasons: (a, d) upper, (b, e) middle, and (c, f) lower reaches. The correlation significance is indicated on the graph, ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

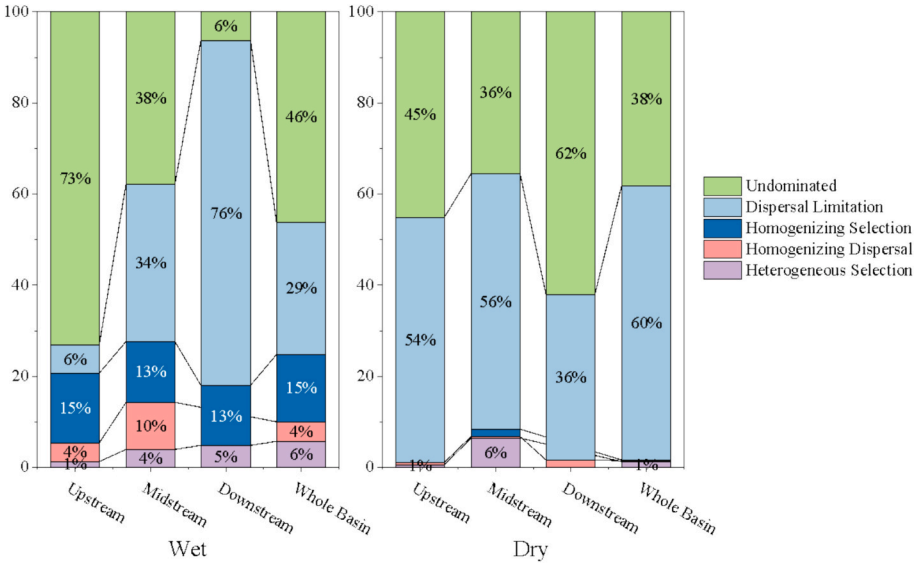


Fig. 8. The proportion of community assembly process in upstream, midstream, and downstream of Yangtze River Basin in wet and dry season.

contrast, at broader spatial scales, stochastic processes (mainly governed by dispersal limitation) play a more dominant role in structuring communities (Liu et al., 2023; Niño-García et al., 2016). Previous research on the assembly mechanisms of diatom communities within the Yangtze River Basin showed that the proportion of variation explained by pure environmental factors was higher than that explained by pure spatial factors, however, with most of the total variation remained unexplained (Wang et al., 2019). Our study speculates that in large river ecosystems like that of the Yangtze River, high environmental heterogeneity, complex hydrological conditions, and diverse biological interactions results in a more balanced influence of various ecological processes preventing any single process from completely dominating community assembly (Lu et al., 2020; Yao et al., 2024). As a result, the proportion of undominated processes is relatively high, leading to complex community structural dynamics. This being the case, our findings also suggest that future research should integrate finer spatial–scale analyses, multi–level ecological models, and long–term monitoring data to more accurately reveal the assembly mechanisms of phytoplankton communities and their ecological functions within the river basin ecosystem (Pearman et al., 2022).

Compared to the wet season, the influence of dispersal limitation increased while homogenizing selection played a minimal role during the dry season. The balance between stochastic and deterministic ecological processes across geographic scales was primarily regulated by hydrological dynamics (Didaskalou et al., 2022; Isabwe et al., 2018). The reduced river discharge and lower hydrological connectivity likely create physical barriers to phytoplankton movement leading to greater regional differentiation, typically observed as distance–decay patterns (Segata et al., 2011). Here, we observed a more pronounced distance–decay effect with stronger dispersal limitations, consistent with findings from studies on phytoplankton in the Tibetan Plateau floodplain (Huang et al., 2023). While local environments during the dry season still influence the community structure of endemic species to some extent, high dispersal rates may overshadow or interact with local effects, making dispersal limitation the primary driver of local community structure (Amarasekare, 2004; Chase, 2005; Leibold et al., 2004). Therefore, a deeper understanding of the physical connectivity and hydrogeographic conditions of the Yangtze River Basin is crucial for elucidating the spatial distribution of plankton communities on a regional scale.

5. Limitations and scope

Our use of eDNA metabarcoding enabled the detection of over 1,000 OTUs per season, which far exceeded the diversity reported by traditional microscopy-based methods in the Yangtze River. Despite eDNA's apparent sensitivity, it is crucial to acknowledge some potential methodological limitations. The possibility of primer bias (Potvin and Lovejoy, 2009; Yeh et al., 2021), underrepresentation of rare taxa (Lohan, 2019; Zhan et al., 2013), and seasonal variation in DNA transport and degradation may affect detection outcomes. For example, dominant taxa in the wet season may have reduced the detectability of low-abundance OTUs, whereas decreased dilution in the dry season may have enhanced their identification. Due to cautious interpretation of the results presented in this study, we don't believe that these factors had an impact on the overall direction of our results. However, future studies that incorporate multiple molecular markers, improved spatial–temporal resolution, and integrative methodologies (e.g., combining eDNA with microscopy and RNA-based techniques) may further elucidate the understanding of phytoplankton community dynamics (Zhang et al., 2024).

6. Conclusions

Our eDNA study reveals the spatiotemporal dynamics of eukaryotic phytoplankton in the Yangtze River and their responses to varying hydrological conditions and pollutant influences. Stochastic processes, particularly dispersal limitation in the dry season, played a dominant role in community assembly, while seasonal hydrological changes shaped species interactions and biodiversity patterns. By distinguishing generalist and endemic species, our findings suggest potential early indicators of ecological disturbances from industrial pollution and hydrological alterations. These insights are crucial for ecosystem-based management, emphasizing the importance of hydrological connectivity and species dispersal dynamics. While our study identifies broad patterns, future research should integrate experimental approaches, multi-marker eDNA methods, and hydrodynamic modeling to refine species detection and enhance predictive frameworks for assessing phytoplankton responses to pollution and environmental change in large river systems.

Fundings

This study was financially supported by the National Key Research and Development Program of China (2021YFC3201005) and Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai) (SML2024SP004)

CRediT authorship contribution statement

Yun Shao: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Kathryn A. Stewart:** Writing – review & editing, Supervision. **Alena S. Gsell:** Writing – review & editing. **Shuping Wang:** Methodology, Funding acquisition. **Boyuan Xie:** Validation, Investigation. **Haojie Lin:** Visualization. **Zhaomei Geng:** Visualization. **Zhenguang Yan:** Supervision, Project administration, Funding acquisition.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2025.114026.

Data availability

The data that support the findings of this study are openly available at https://doi.org/10.5281/zenodo.15106198 (Yun, 2025).

The data presented in the study are deposited in the National Center for Biotechnology Information Sequence Read Archive repository, accession number PRJNA1242592.

References

Agawin, N.S., Duarte, C.M., Agustí, S., 2000. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol. Oceanogr. 45, 591–600.

Alahuhta, J., Erős, T., Kärnä, O.-M., Soininen, J., Wang, J., Heino, J., 2019. Understanding environmental change through the lens of trait-based, functional, and phylogenetic biodiversity in freshwater ecosystems. Environ. Rev. 27, 263–273. https://doi.org/10.1139/er-2018-0071.

Albert, J.S., Destouni, G., Duke-Sylvester, S.M., Magurran, A.E., Oberdorff, T., Reis, R.E., Winemiller, K.O., Ripple, W.J., 2021. Scientists' warning to humanity on the freshwater biodiversity crisis. Ambio 50, 85–94. https://doi.org/10.1007/s13280-020-01318-8.

Allan, J.D., 2004. Landscapes and Riverscapes: the Influence of Land Use on Stream Ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284. https://doi.org/10.1146/annurev.ecolsys.35.120202.110122.

Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W., Huse, S.M., 2009. A Method for Studying Protistan Diversity using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes. PLoS One 4, e6372.

Amarasekare, P., 2004. Spatial dynamics of mutualistic interactions. J. Anim. Ecol. 73, 128–142. https://doi.org/10.1046/j.0021-8790.2004.00788.x.

Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., Wingett, S., 2012. FastQC: a quality control tool for high throughput sequence data. [software]. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Arthington, A.H., Tickner, D., McClain, M.E., Acreman, M.C., Anderson, E.P., Babu, S., Dickens, C.W.S., Horne, A.C., Kaushal, N., Monk, W.A., O'Brien, G.C., Olden, J.D., Opperman, J.J., Owusu, A.G., LeRoy Poff, N., Richter, B.D., Salinas-Rodríguez, S.A., Shamboko Mbale, B., Tharme, R.E., Yarnell, S.M., 2024. Accelerating environmental flow implementation to bend the curve of global freshwater biodiversity loss. Environ. Rev. 32, 387–413. https://doi.org/10.1139/er-2022-0126.

Borics, G., Abonyi, A., Salmaso, N., Ptacnik, R., 2021. Freshwater phytoplankton diversity: models, drivers and implications for ecosystem properties. Hydrobiologia 848, 53–75. https://doi.org/10.1007/s10750-020-04332-9.

Bradley, I.M., Pinto, A.J., Guest, J.S., 2016. Design and Evaluation of Illumina MiSeq-Compatible, 18S rRNA Gene-Specific Primers for Improved Characterization of Mixed Phototrophic Communities. Appl. Environ. Microbiol. 82, 5878–5891. https://doi.org/10.1128/AEM.01630-16.

Cagle, S.E., Roelke, D.L., 2021. Relative roles of fundamental processes underpinning PEG dynamics in dimictic lakes as revealed by a self-organizing, multi-population plankton model. Ecol. Model. 462, 109793. https://doi.org/10.1016/j.ecolmodel.2021.109793.

Calbet, A., Landry, M.R., 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol. Oceanogr. 49, 51–57. https://doi.org/10.4319/lo.2004.49.1.0051.

Canale, R.P., Vogel, A.H., 1974. Effects of temperature on phytoplankton growth. J. Environ. Eng. Div. 100, 231–241.
Chase, J.M., 2005. Towards a really unified theory for metacommunities. Funct. Ecol. 19, 182–186. https://doi.org/10.1111/j.0269-8463.2005.00937.x.
Chen, J., Wu, X., Finlayson, B.L., Webber, M., Wei, T., Li, M., Chen, Z., 2014. Variability and trend in the hydrology of the Yangtze River, China: annual precipitation and runoff. J. Hydrol. 513, 403–412. https://doi.org/10.1016/j.jhydrol.2014.03.044.
Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinforma. Oxf. Engl. 34, i884–i890. https://doi.org/10.1093/bioinformatics/bty560.
Chen, T., Wang, Y., Gardner, C., Wu, F., 2020. Threats and protection policies of the aquatic biodiversity in the Yangtze River. J. Nat. Conserv. 58, 125931. https://doi.org/10.1016/j.jnc.2020.125931.
Chen, W., Ren, K., Isabwe, A., Chen, H., Liu, M., Yang, J., 2019. Stochastic processes shape microeukaryotic community assembly in a subtropical river across wet and dry seasons. Microbiome 7, 138. https://doi.org/10.1186/s40168-019-0749-8.
Deiner, K., Walser, J.-C., Mächler, E., Altermatt, F., 2015. Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. Biol. Conserv. 183, 53–63.
Dhar, J., Baghel, R.S., 2015. Role of dissolved oxygen on the plankton dynamics in spatio-temporal domain. Model. Earth Syst. Environ. 2, 6. https://doi.org/10.1007/s40808-015-0061-y.
Didaskalou, E.A., Trimbos, K.B., Stewart, K.A., 2022. Environmental DNA. Curr. Biol. 32, R1250–R1252. https://doi.org/10.1016/j.cub.2022.09.052.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinforma. Oxf. Engl. 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.
Erős, T., Funk, A., Pont, D., Hein, T., Meulenbroek, P., Preiszner, B., Valentini, A., Czeglédi, I., 2024. eDNA metabarcoding reveals the role of habitat specialization and spatial and environmental variability in shaping diversity patterns of fish metacommunities. PLoS One 19. https://doi.org/10.1371/journal.pone.0296310.
Fan, J., Wang, S., Li, H., Yan, Z., Zhang, Y., Zheng, X., Wang, P., 2020. Modeling the ecological status response of rivers to multiple stressors using machine learning: a comparison of environmental DNA metabarcoding and morphological data. Water Res. 183, 116004. https://doi.org/10.1016/j.watres.2020.116004.
Feng, B., Zhang, M., Chen, J., Xu, J., Xiao, B., Zhou, M., Zhang, M., 2021. Reduction in the phytoplankton index of biotic integrity in riverine ecosystems driven by industrial activities, dam construction and mining: a case study in the Ganjiang River. China. Ecol. Indic. 120, 106907. https://doi.org/10.1016/j.ecolind.2020.106907.
Gao, W., Xiong, F., Lu, Y., Qu, X., Xin, W., Chen, Y., 2023. Development of a phytoplankton-based index of biotic integrity for ecological health assessment in the Yangtze River. Ecol. Process. 12, 41. https://doi.org/10.1186/s13717-023-00456-7.
Gemmer, M., Jiang, T., Su, B., Kundzewicz, Z.W., 2008. Seasonal precipitation changes in the wet season and their influence on flood/drought hazards in the Yangtze River Basin, China. Quat. Int. Larger Asian Rivers and Their Interactions with Estuaries and Coasts 186, 12–21. https://doi.org/10.1016/j.quaint.2007.10.001.
Gu, Z., Liu, K., Pedersen, M.W., Wang, F., Chen, Y., Zeng, C., Liu, Y., 2021. Community assembly processes underlying the temporal dynamics of glacial stream and lake bacterial communities. Sci. Total Environ. 761, 143178. https://doi.org/10.1016/j.scitotenv.2020.143178.
Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604. https://doi.org/10.1093/nar/gks1160.
Han, X., Pan, B., Zhao, G., Li, D., Sun, H., Zhu, P., Lu, Y., 2020. Local and geographical factors jointly drive elevational patterns of phytoplankton in the source region of the Yangtze River. China. River Res. Appl. 37, 1145–1155. https://doi.org/10.1002/rra.3711.
He, Y., Jin, S., Shang, W., 2021. Water Quality Variability and Related Factors along the Yangtze River using Landsat-8. Remote Sens. 13, 2241. https://doi.org/10.3390/rs13122241.
Hewson, I., Fuhrman, J.A., 2004. Richness and Diversity of Bacterioplankton Species along an Estuarine Gradient in Moreton Bay. Australia. Appl. Environ. Microbiol. 70, 3425–3433. https://doi.org/10.1128/AEM.70.6.3425-3433.2004.
Ho, A.Y.T., Xu, J., Yin, K., Jiang, Y., Yuan, X., He, L., Anderson, D.M., Lee, J.H.W., Harrison, P.J., 2010. Phytoplankton Biomass and production in Subtropical Hong Kong Waters: Influence of the Pearl River Outflow. Estuar. Coasts 33, 170–181. https://doi.org/10.1007/s12237-009-9247-8.
Huang, Z., Pan, B., Soininen, J., Liu, X., Hou, Y., Liu, X., 2023. Seasonal variation of phytoplankton community assembly processes in Tibetan Plateau floodplain. Front. Microbiol. 14. https://doi.org/10.3389/fmicb.2023.1122838.
Huo, S., Li, X., Xi, B., Zhang, H., Ma, C., He, Z., 2020. Combining morphological and metabarcoding approaches reveals the freshwater eukaryotic phytoplankton community. Environ.Sci. Eur. 32, 37. https://doi.org/10.1186/s12302-020-00321-w.
Isabwe, A., Yang, J.R., Wang, Y., Liu, L., Chen, H., Yang, J., 2018. Community assembly processes underlying phytoplankton and bacterioplankton across a hydrologic change in a human-impacted river. Sci. Total Environ. 630, 658–667. https://doi.org/10.1016/j.scitotenv.2018.02.210.
Lehman, P.W., Sommer, T., Rivard, L., 2008. The influence of floodplain habitat on the quantity and quality of riverine phytoplankton carbon produced during the flood season in San Francisco Estuary. Aquat. Ecol. 42, 363–378. https://doi.org/10.1007/s10452-007-9102-6.
Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M., Gonzalez, A., 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecol. Lett. 7, 601–613. https://doi.org/10.1111/j.1461-0248.2004.00608.x.
Li, X., Chen, K., Wang, C., Zhuo, T., Li, H., Wu, Y., Lei, X., Li, M., Chen, B., Chai, B., 2025. Deciphering environmental factors influencing phytoplankton community structure in a polluted urban river. J. Environ. Sci. 148, 375–386. https://doi.org/10.1016/j.jes.2023.11.008.
Li, F., Guo, F., Gao, W., Cai, Y., Zhang, Y., Yang, Z., 2022. Environmental DNA Biomonitoring reveals the Interactive Effects of Dams and Nutrient Enrichment on Aquatic Multitrophic Communities. Environ. Sci. Technol. 56, 16952–16963. https://doi.org/10.1021/acs.est.2c06919.
Li, Q., Xiao, J., Ou, T., Han, M., Wang, J., Chen, J., Li, Y., Salmaso, N., 2018. Impact of water level fluctuations on the development of phytoplankton in a large subtropical reservoir: implications for the management of cyanobacteria. Environ. Sci. Pollut. Res. 25, 1306–1318. https://doi.org/10.1007/s11356-017-0502-4.
Lian, K., Liu, F., Li, Y., Wang, C., Zhang, C., McMinn, A., Wang, M., Wang, H., 2023. Environmental gradients shape microbiome assembly and stability in the East China sea. Environ. Res. 238, 117197. https://doi.org/10.1016/j.envres.2023.117197.
Liu, H., Lin, G., Gao, D., Chen, H., He, M., Lu, J., 2023. Geographic Scale Influences the Interactivities between Determinism and Stochasticity in the Assembly of Sedimentary Microbial Communities on the South China Sea Shelf. Microb. Ecol. 85, 121–136. https://doi.org/10.1007/s00248-021-01946-x.
Liu, Y., Xu, X., Wang, T., Ni, J., 2019. Microscopic view of phytoplankton along the Yangtze River. Sci. China Technol. Sci. 62, 1873–1884. https://doi.org/10.1007/s11431-019-9545-y.
Lohan, K.P., 2019. Intact vs. homogenized subsampling: testing impacts of pre-extraction processing of multi-species samples on invasive species detection. Manag. Biol. Invasions 10, 324–341. https://doi.org/10.3391/mbi.2019.10.2.08.
Lor, Y., Schreier, T.M., Waller, D.L., Merkes, C.M., 2020. Using environmental DNA (eDNA) to detect the endangered Spectaclecase Mussel (Margaritifera monodonta). Freshw. Sci. 39, 837–847. https://doi.org/10.1086/711673.
Lu, L., Zou, X., Yang, J., Xiao, Y., Wang, Y., Guo, J., Li, Z., 2020. Biogeography of eukaryotic plankton communities along the upper Yangtze River: the potential impact of cascade dams and reservoirs. J. Hydrol. 590, 125495. https://doi.org/10.1016/j.jhydrol.2020.125495.
Lucas, L.V., Koseff, J.R., Cloern, J.E., Monismith, S.G., Thompson, J.K., 1999. Processes governing phytoplankton blooms in estuaries. I: the local production-loss balance. Mar. Ecol. Prog. Ser. 187, 1–15. https://doi.org/10.3354/meps187001.
McCarthy, J.J., Goldman, J.C., 1979. Nitrogenous Nutrition of Marine Phytoplankton in Nutrient-Depleted Waters. Science 203, 670–672. https://doi.org/10.1126/science.203.4381.670.
Meng, Z., Chen, K., Hu, F., Liu, L., Yang, D., Li, X., 2023. Environmental and spatial factors play different roles in phytoplankton community assembly in different hydrological seasons in Lake Wuchang. China. Front. Ecol. Evol. 11. https://doi.org/10.3389/fevo.2023.1154695.
Muri, C.D., Handley, L.L., Bean, C.W., Li, J., Peirson, G., Sellers, G.S., Walsh, K., Watson, H.V., Winfield, I.J., Hänfling, B., 2020. Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. Metabarcoding Metagenomics 4, e56959. https://doi.org/10.3897/mbmg.4.56959.
Naselli-Flores, L., 2014. Morphological analysis of phytoplankton as a tool to assess ecological state of aquatic ecosystems: the case of Lake Arancio, Sicily, Italy. Inland Waters 4, 15–26. https://doi.org/10.5268/IW-4.1.686.
Niño-García, J.P., Ruiz-González, C., del Giorgio, P.A., 2016. Interactions between hydrology and water chemistry shape bacterioplankton biogeography across boreal freshwater networks. ISME J. 10, 1755–1766. https://doi.org/10.1038/ismej.2015.226.
Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., Yang, Y., Arkin, A.P., Firestone, M.K., Zhou, J., 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. Nat Commun 11, 4717. https://doi.org/10.1038/s41467-020-18560-zNEPB, 2002.
Nwonumara, G., Okogwu, O., 2021. Seasonal dynamics in water quality and phytoplankton of four tropical rivers in Ebonyi State, southeastern Nigeria. Afr. J. Aquat. Sci. 46, 402–413. https://doi.org/10.2989/16085914.2021.1924110.
Pearman, J.K., Thomson-Laing, G., Thompson, L., Waters, S., Vandergoes, M.J., Howarth, J.D., Duggan, I.C., Hogg, I.D., Wood, S.A., 2022. Human access and deterministic processes play a major role in structuring planktonic and sedimentary bacterial and eukaryotic communities in lakes. PeerJ 10, e14378. https://doi.org/10.7717/peerj.14378.
Peng, D., Chen, Y., Wang, W., 2023. Spatio-temporal analysis of the impact of land urbanization on the gross primary productivity of vegetation in the Middle Reaches of the Yangtze River Urban Agglomeration: new evidence from the township scale. Ecol. Evol Front. https://doi.org/10.3389/fevo.2023.1260641.
Potvin, M., Lovejoy, C., 2009. PCR-based diversity estimates of artificial and environmental 18S rRNA gene libraries. J. Eukaryot. Microbiol. 56, 174–181. https://doi.org/10.1111/j.1550-7408.2008.00386.x.
C.S. Reynolds The Ecology of Phytoplankton 2006 Cambridge University Press, Cambridge Ecology, Biodiversity and Conservation 10.1017/CBO9780511542145.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584.
Ruiz, J., Macias, D., Peters, F., 2004. Turbulence increases the average settling velocity of phytoplankton cells. PNAS 101 (51), 17720–17724. https://doi.org/10.1073/PNAS.0401539101.

Rusanov, A.G., Bíró, T., Kiss, K.T., Buczkó, K., Grigorszky, I., Hidas, A., Duleba, M., Trábert, Z., Földi, A., Ács, É., 2022. Relative importance of climate and spatial processes in shaping species composition, functional structure and beta diversity of phytoplankton in a large river. Sci. Total Environ. 807, 150891. https://doi.org/10.1016/j.scitotenv.2021.150891.

Sales, N.G., Wangensteen, O.S., Carvalho, D.C., Deiner, K., Præbel, K., Coscia, I., McDevitt, A.D., Mariani, S., 2021. Space-time dynamics in monitoring neotropical fish communities using eDNA metabarcoding. Sci. Total Environ. 754, 142096. https://doi.org/10.1016/j.scitotenv.2020.142096.

Schlie, C., Karsten, U., 2017. Microphytobenthic diatoms isolated from sediments of the Adventfjorden (Svalbard): growth as function of temperature. Polar Biol. 40, 1043–1051. https://doi.org/10.1007/s00300-016-2030-y.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60. https://doi.org/10.1186/gb-2011-12-6-r60.

Shu, L., Ludwig, A., Peng, Z., 2020. Standards for Methods Utilizing Environmental DNA for Detection of fish Species. Genes 11, 296. https://doi.org/10.3390/genes11030296.

Siegfried, C.A., Bloomfield, J.A., Sutherland, J.W., 1989. Acidity status and phytoplankton species richness, standing crop, and community composition in Adirondack, New York, U.S.A. lakes. Hydrobiologia 175, 13–32. https://doi.org/10.1007/BF00008472.

Sommer, U., Gliwicz, Z.M., Lampert, W., Duncan, A., 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. Arch. Für Hydrobiol. 106, 433–471. https://doi.org/10.1127/archiv-hydrobiol/106/1986/433.

Strickland, G.J., Roberts, J.H., 2019. Utility of eDNA and occupancy models for monitoring an endangered fish across diverse riverine habitats. Hydrobiologia 826, 129–144. https://doi.org/10.1007/s10750-018-3723-8.

Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. Environmental DNA: for Biodiversity Research and monitoring. Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001.

Taberlet, P., Coissac, E., Hajibabaei, M., Rieseberg, L.H., 2012. Environmental DNA: ENVIRONMENTAL DNA. Mol. Ecol. 21, 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x.

Terui, A., Kim, S., Dolph, C.L., Kadoya, T., Miyazaki, Y., 2021. Emergent dual scaling of riverine biodiversity. Proc. Natl. Acad. Sci. 118. https://doi.org/10.1073/pnas.2105574118.

Tsuji, S., Inui, R., Nakao, R., Miyazono, S., Saito, M., Kono, T., Akamatsu, Y., 2022. Quantitative environmental DNA metabarcoding shows high potential as a novel approach to quantitatively assess fish community. Sci. Rep. 12, 21524. https://doi.org/10.1038/s41598-022-25274-3.

Wang, J., Liu, Q., Zhao, X., Borthwick, A.G.L., Liu, Y., Chen, Q., Ni, J., 2019. Molecular biogeography of planktonic and benthic diatoms in the Yangtze River. Microbiome 7, 153. https://doi.org/10.1186/s40168-019-0771-x.

Wang, J., Soininen, J., Heino, J., 2021. Ecological indicators for aquatic biodiversity, ecosystem functions, human activities and climate change. Ecol. Ind. 132, 108250. https://doi.org/10.1016/j.ecolind.2021.108250.

Wang, S., Gu, S., Zhang, Y., Deng, Y., Qiu, W., Sun, Q., Zhang, T., Wang, P., Yan, Z., 2024. Microeukaryotic plankton community dynamics under ecological water replenishment: Insights from eDNA metabarcoding. Environ. Sci. Ecotechnology 20, 100409. https://doi.org/10.1016/j.ese.2024.100409.

Xu, X., Yang, G., Tan, Y., Liu, J., Hu, H., 2018. Ecosystem services trade-offs and determinants in China's Yangtze River Economic Belt from 2000 to 2015. Sci. Total Environ. 634, 1601–1614. https://doi.org/10.1016/j.scitotenv.2018.04.046.

Yan, Z., Zhu, X., Zhang, S., Jiang, H., Wang, S.-P., Wei, C., Wang, J., Shao, Y., Liu, C., Wang, H., 2023. Environmental DNA sequencing reveals the regional difference in diversity and community assembly mechanisms of eukaryotic plankton in coastal waters. Front. Microbiol. 14. https://doi.org/10.3389/fmicb.2023.1132925.

Yao, L., Wu, J., Liu, S., Xing, H., Wang, P., Gao, W., Wu, Z., Zhou, Q., 2024. Distinct drivers of bacterial community assembly processes in riverine islands in the middle and lower reaches of the Yangtze River. Microbiol. Spectr. 12, e00818. https://doi.org/10.1128/spectrum.00818-24.

Yeh, Y.-C., Mcnichol, J., Needham, D., Fichot, E., Berdjeb, L., Fuhrman, J., 2021. Comprehensive single-PCR 16S and 18S rRNA community analysis validated with mock communities, and estimation of sequencing bias against 18S. Environ. Microbiol. https://doi.org/10.1111/1462-2920.15553.

Zhan, A., Hulák, M., Sylvester, F., Huang, X., Adebayo, A.A., Abbott, C., Adamowicz, S., Heath, D., Cristescu, M., MacIsaac, H., 2013. High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. Methods Ecol. Evol. 4. https://doi.org/10.1111/2041-210X.12037.

Zhang, Y., Peng, C., Huang, S., Wang, J., Xiong, X., Li, D., 2019. The relative role of spatial and environmental processes on seasonal variations of phytoplankton beta diversity along different anthropogenic disturbances of subtropical rivers in China. Environ. Sci. Pollut. Res. 26, 1422–1434. https://doi.org/10.1007/s11356-018-3632-4.

Zhang, Y., Qiu, Y., Liu, K., Zhong, W., Yang, J., Altermatt, F., Zhang, X., 2024. Evaluating eDNA and eRNA metabarcoding for aquatic biodiversity assessment: from bacteria to vertebrates. Environ. Sci. Ecotechnology 21, 100441. https://doi.org/10.1016/j.ese.2024.100441.

Zhang, Y., Zuo, J., Salimova, A., Li, A., Li, L., Li, D., 2020. Phytoplankton distribution characteristics and its relationship with bacterioplankton in Dianchi Lake. Environ. Sci. Pollut. Res. 27, 40592–40603. https://doi.org/10.1007/s11356-020-10033-6.

Zhao, Q., Van den Brink, P.J., Xu, C., Wang, S., Clark, A.T., Karakoç, C., Sugihara, G., Widdicombe, C.E., Atkinson, A., Matsuzaki, S.S., Shinohara, R., He, S., Wang, Y.X.G., De Laender, F., 2023. Relationships of temperature and biodiversity with stability of natural aquatic food webs. Nat. Commun. 14, 3507. https://doi.org/10.1038/s41467-023-38977-6.

Zheng, S., Zhong, Z., Zou, Q., Ding, Y., Yang, L., Luo, X., 2021. Overall Situation of Yangtze River Basin. In: Zheng, S., Zhong, Z., Zou, Q., Ding, Y., Yang, L., Luo, X. (Eds.), Flood Resources Utilization in the Yangtze River Basin. Springer, Singapore, pp. 19–69. https://doi.org/10.1007/978-981-15-8108-3_2.

Zhu, H., Hu, X.-D., Wu, P.-P., Chen, W.-M., Wu, S.-S., Li, Z.-Q., Zhu, L., Xi, Y.-L., Huang, R., 2021. Development and testing of the phytoplankton biological integrity index (P-IBI) in dry and wet seasons for Lake Gehu. Ecol. Ind. 129, 107882. https://doi.org/10.1016/j.ecolind.2021.107882.

Zhu, Y., Qi, Q., Lu, X., Fan, Y., Liu, Y., Tan, X., 2023. Local environmental variables outperform spatial and land use pattern in the maintenance and assembly of phytoplankton communities in the wetland cluster. J. Clean. Prod. 419, 138275. https://doi.org/10.1016/j.jclepro.2023.138275.