

# Effect of water stratification and mixing on phytoplankton functional groups: a case study of Xikeng Reservoir, China

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A shift in reservoir stratification and mixing significantly affects the water column ecosystem, which in turn leads to changes in phytoplankton abundance and community structure. To explore the effects of stratification and mixing on the phytoplankton community structure of a diversion reservoir, a 1-year survey was divided into a stratification period in 2020, a mixing period in 2020, and a stratification period in 2021, and redundancy analysis (RDA), variance partitioning analysis (VPA) and Pearson correlation analysis were used to analyse the key drivers affecting the phytoplankton functional groups, using Xikeng Reservoir as a case study. During the study period, 8 phyla, 69 genera and 9 major functional groups were observed in this reservoir. The dominant functional groups varied significantly, being X1 in the stratified period in 2020; P and D in the mixing period in 2020; and D, X1, and M in the stratified period in 2021. The phytoplankton diversity index was greater in the mixing period than in the stratification period, in agreement with the results of the aquatic ecological status evaluation (Q index, higher in the mixing period than in the stratification period). However, phytoplankton diversity of Xikeng Reservoir was of limited value in assessing the degree of water pollution, so should be considered in combination with the Q index. Water temperature (WT), mixing depth ( $Z_{mix}$ ), nitrogen–phosphorus ratio (N/P), and total nitrogen (TN) were important drivers of phytoplankton functional group dynamics in different periods. The study provides a valuable reference for assessing the relationship between environmental factors and phytoplankton communities, as well as for the evaluation and conservation of aquatic ecosystems in southern China's water diversion reservoirs.

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## INTRODUCTION

Phytoplankton plays a crucial role as a primary producer in aquatic ecosystems and is essential for maintaining the dynamic balance of food webs, energy flow, and nutrient cycling (Gogoi et al., 2021; Yuan et al., 2018; Zhang et al., 2020). The dominant species, diversity, and composition of phytoplankton communities are indicators of the aquatic environment, and these communities are also influenced by environmental factors (Rolland et al., 2009). The growth and reproduction of phytoplankton in aquatic ecosystems are mainly influenced by environmental factors such as light, nutrient concentrations, water temperature, pH, zooplankton predation, and hydrodynamics (Siegel et al., 2013; Deng et al., 2017; Jakobsen et al., 2015; Silva et al., 2014; Becker et al., 2010).

Traditional studies on phytoplankton communities have largely focused on systematic taxonomy, which has limitations in predicting the community structure and providing information about the biological and habitat characteristics of phytoplankton in aquatic ecosystems (Kim et al., 2020). To address this problem, Reynolds et al. (2002) introduced a functional group classification based on morphological traits and ecological adaptations of phytoplankton to better reflect changes in the aquatic environment (Sun and Wang, 2021; Reynolds et al., 2002). However, most research on phytoplankton functional groups has concentrated on natural or seasonal succession (Fu et al., 2020; Jia et al., 2020; Jiang et al., 2014). In contrast, the indistinct seasons of reservoirs in southern China, which are characterized by long summers and short or no winters, according to the Chinese national standard of division of climatic seasons, result in fewer samples in winter. Therefore, multi-year, high-frequency monitoring is often required to accurately capture the seasonal characteristics of phytoplankton.

To compensate for the shortcoming, the 1-year survey undertaken in this study was divided into different periods based on the water column's stratification and mixing status. As environmental conditions can vary between years, the study period was separated into the 2020 stratification period, the 2020 mixing period, and the 2021 stratification period. Previous studies on the effects of water stratification and mixing on phytoplankton functional groups are relatively scarce and outdated. Besides, water mixing regimes play a critical role in phytoplankton succession, and it is essential to study their effects on functional group structure and the driving factors. The objectives of this current study were to (i) investigate the effects of water mixing and stratification on the functional phytoplankton community, (ii) explore the response of phytoplankton diversity to water mixing and stratification, and (iii) to elucidate the relationship between functional communities and environmental factors or the driving factors. This study highlighted the effects of stratification and mixing on phytoplankton and provided a reference for the ecological protection of subtropical reservoirs in China.

## MATERIALS AND METHODS

### Study area

Xikeng Reservoir (113°59'30"–114°1'20" E, 22°41'20"–22°42'40" N) (Fig. 1) in Shenzhen, Guangdong Province, China, is a significant source of drinking water for the Pearl River Delta, and it is vital to the socioeconomic growth of the Guangdong–Hong Kong–Macao Greater Bay Area. It is situated in the South Subtropical Zone and is characterized as a warm and mono-mictic water body with a subtropical marine monsoon climate. The reservoir has a basin area of 5.0 km<sup>2</sup> and a water area of 2.0 km<sup>2</sup>, with a normal storage level of 75.0 m, maximum water depth of 22.0 m, and normal storage capacity of 1.8 × 10<sup>7</sup> m<sup>3</sup>. The climate is mild throughout the year, with an average annual temperature of 23.0°C. Rainfall is abundant but varies significantly from year to year, with more than 80% of the annual rainfall concentrated within the period from April to September.

### Sampling and sample analysis

In this study, Point S1, which is the deepest point, was chosen as the monitoring point (Noori et al., 2021) (Fig. 1). Field observations were conducted at the S1 site 3 times per month for a period of 13 months from May 2020 to May 2021. Table 1 shows the means and standard deviations of the environmental factors measured at a depth of 0.5 m below the surface layer, with the exception of the bottom water temperature.

### Phytoplankton collection and analysis

Surface phytoplankton samples were collected and processed for analysis. The qualitative phytoplankton samples for identifying algal species were collected with a 64 µm pore size net. One litre of water sample was treated with 15 mL of Lugol's iodine solution in situ, transported to the laboratory, and allowed to settle for 48 h. The sample was then concentrated to 30 mL and examined for phytoplankton identification and enumeration using a 400x light microscope with a 0.1 mL counting box containing

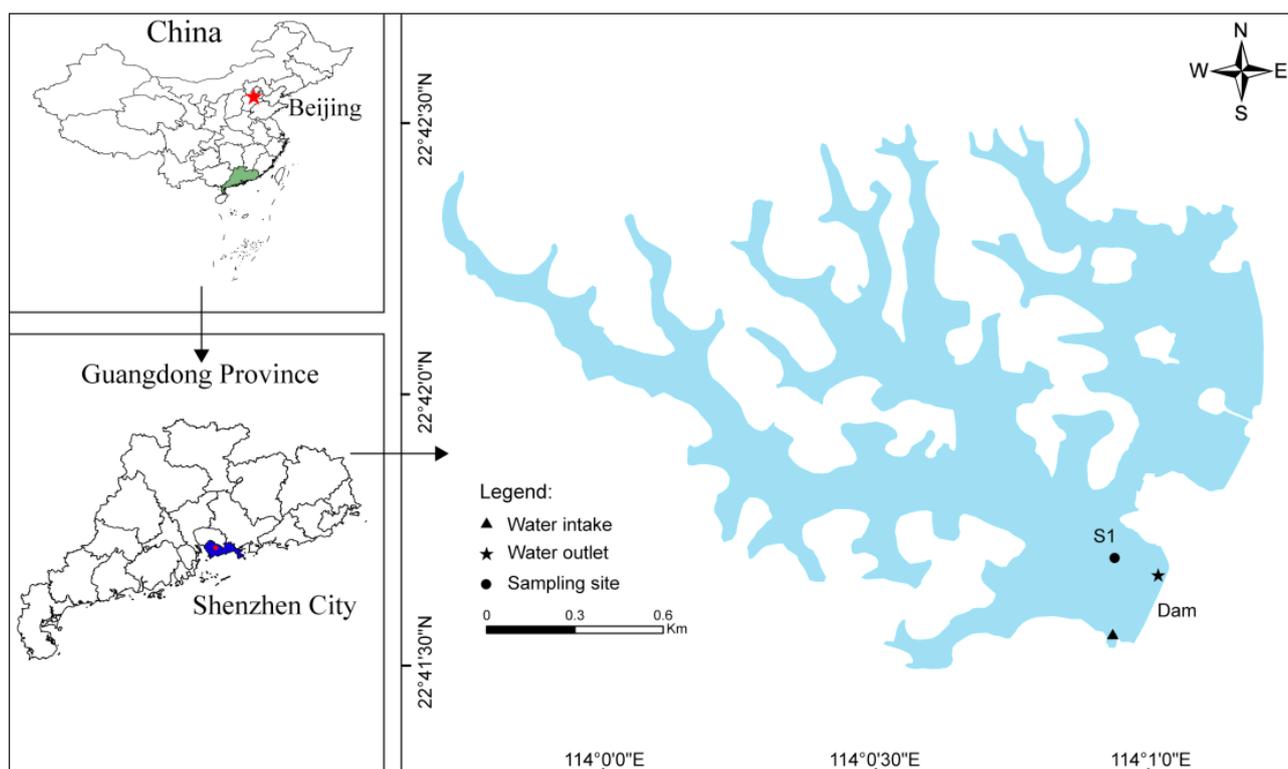
100 horizons based on the *Chinese Freshwater Algae–System, Classification and Ecology* (Wen et al., 2022). The functional grouping of phytoplankton communities was determined using the functional group classification method developed by Reynolds (Reynolds et al., 2002) and Padisák (Padisák et al., 2006).

### Environmental factors

In this study, surface water samples were collected and analysed for various water quality parameters. Unfiltered surface water samples were used to analyse total nitrogen (TN), total phosphorus (TP), and permanganate index (COD<sub>Mn</sub>), which were measured by the alkaline potassium persulfate digestion UV spectrophotometric method, ammonium molybdate spectrophotometric method, and acidic potassium permanganate method, respectively. Filtered samples, which passed through 0.45 µm glass microfiber filters, were utilized to analyse dissolved nitrate (NO<sub>3</sub>-N), ammonium (NH<sub>4</sub><sup>+</sup>-N), and dissolved total phosphorus (DTP) through the UV spectrophotometric method, Nessler's reagent spectrophotometry, and ammonium molybdate spectrophotometric method, respectively (Cao et al., 2018). Additionally, in-situ profiles of water temperature (WT), dissolved oxygen (DO), pH and chlorophyll *a* (Chl *a*) were collected using a Hydro-lab DS5 at 0.5–5 m intervals from the surface to the bottom. Transparency was measured using a standard Secchi disk (Kim et al., 2020). The stratification period was defined as the period when the temperature difference exceeds 1°C within 1 m depth at the metalimnion, and vice versa for the mixing period (Yu et al., 2014).

### Data analysis

In this study, the euphotic depth ( $Z_{eu}$ ) was determined as 2.7 times the transparency (Wen et al., 2022). The depth of the surface mixed layer ( $Z_{mix}$ ), defined by the gradient of the surface temperature of 0.2°C/m, was calculated according to Liu et al. (2012). The ratio of  $Z_{eu}/Z_{mix}$  was used as an indicator of light availability in the water column, following the methodology proposed by Jensen et al. (1994).



**Figure 1.** Map of Xikeng Reservoir with sampling sites in the northeast of Shenzhen City, Guangdong Province, China

Several indices were used to assess the ecological status of water bodies in this study. Specifically, the Shannon-Wiener diversity index ( $H'$ ), Pielou evenness index ( $J$ ), and Margalef richness index ( $D$ ) were employed (Ding et al., 2021). The formulas for these indices are as follows:

$$H' = -\sum_{i=1}^s p_i \ln p_i \quad (1)$$

$$J = \frac{H'}{\ln S} \quad (2)$$

$$D = \frac{S-1}{\ln N} \quad (3)$$

where  $p_i$  is the ratio of the density of the  $i$ -th phytoplankton functional group to the total density,  $S$  represents the number of functional groups, and  $N$  denotes the overall number of phytoplankton species. Based on the values obtained from these three diversity indicators, water quality was classified into four levels: no pollution ( $H' > 3.0$ ,  $0.8 < J < 1.0$  and  $D > 5.0$ ), light pollution ( $2.0 < H' < 3.0$ ,  $0.5 < J < 0.8$  and  $4.0 < D < 5.0$ ), moderate pollution ( $1.0 < H' < 2.0$ ,  $0.3 < J < 0.5$  and  $3.0 < D < 4.0$ ), and heavy pollution ( $0.0 < H' < 1.0$ ,  $0.0 < J < 0.3$  and  $0.0 < D < 3.0$ ).

The trophic status of the reservoir was evaluated using the commonly used trophic level index (TLI). The evaluation parameters included were Chl  $a$ , TN, TP, SD, and  $\text{COD}_{\text{Mn}}$  (Wang et al., 2019), which were calculated as follows:

$$\text{TLI}(\Sigma) = \sum_{j=1}^m W_j \times \text{TLI}(j) \quad (4)$$

$$W_j = r_{ij}^2 / \sum_{j=1}^m r_{ij}^2 \quad (5)$$

where  $\text{TLI}(j)$  is the composite index of  $j$  with the associated weight  $W_j$ ;  $r_{ij}$  is the coefficient of Chl  $a$  with each parameter  $j$  (Chl  $a$ , 1; TP, 0.84; TN, 0.82; SD, -0.83;  $\text{COD}_{\text{Mn}}$ , 0.83);  $m$  is the number of indices.  $\text{TLI}(\Sigma) \leq 30$  is considered poor nutrition;  $30 < \text{TLI}(\Sigma) \leq 50$  is moderate nutrition;  $50 < \text{TLI}(\Sigma) \leq 60$  is mild eutrophication.

The ecological status of water was assessed using the phytoplankton assemblage ( $Q$ ) index, developed by the EU Water Framework, taking into account the mutual response of functional groups and environmental characteristics. The index can classify the ecological status of a reservoir into 5 categories: poor (0–1), tolerant (1–2), medium (2–3), good (3–4), and excellent (4–5) (Padišák et al., 2006). The formula for calculating the  $Q$ -index is as follows:

$$Q = \sum_{i=1}^n p_i \cdot F_i \quad (6)$$

where  $n$  is the number of phytoplankton functional groups,  $p_i$  is the proportion of the total phytoplankton biomass accounted for by the  $i$ -th functional group, and  $F_i$  is the assignment value of the  $i$ -th functional group.

### Statistical analysis

One-way analysis of variance (ANOVA) was applied to test the differences in physical and chemical factors across months, while the Kruskal-Wallis rank sum test was used for factors that did not meet the ANOVA requirements. Post-hoc tests were conducted using the least significant difference (LSD). IBM SPSS Statistics 26 software was used to perform these statistical analyses (Cao et al., 2018; C. Yang et al., 2020).

Non-metric multidimensional scaling (NMDS) analysis was performed to assess the variability of phytoplankton communities in different periods (Kim et al., 2020).

The relationship between phytoplankton communities and environmental factors was analysed using redundancy analysis (RDA) after phytoplankton abundance was Hellinger-transformed. The RDA results were tested for replacement and variance expansion coefficients were calculated. To reduce covariance, environmental factors with expansion coefficients greater than 15 were excluded. The remaining environmental factors were ranked in a parsimonious model to identify their contribution to phytoplankton. Variance partitioning analysis (VPA) was used to further assess the environmental factors' contribution to the structure of phytoplankton functional groups during different periods.

A heatmap was created using Pearson correlation analysis to demonstrate the correlation between dominant functional groups of phytoplankton and the driving factors.

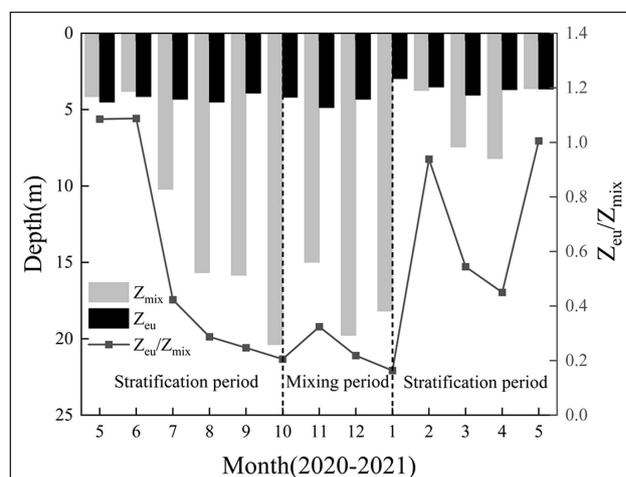
Sampling maps were drawn using ArcGIS10.8 software. The aforementioned analysis methods, excluding ANOVA, were conducted using the vegan, ggplot2, and heatmap packages of R 4.1.3 software.

## RESULTS

### Characteristics of changes in physicochemical factors during different periods

During the survey period, several environmental factors of the reservoir were significantly different, except for  $\text{NH}_4^+\text{-N}$ , DTP, and  $\text{COD}_{\text{Mn}}$  ( $p < 0.05$ , Table 1). WT gradually increased during the stratification periods in 2020, peaking in September. The concentration of Chl  $a$  was significantly higher in January than in other months. The transparency of the water varied significantly during the mixing period, reaching a minimum value of 1.0 m in January 2020. Influenced by the high nitrogen and phosphorus load of the diverted water, TN concentrations remained above 1.1 mg/L throughout the year, while TP concentrations were relatively high in August, September, and October 2020. In the other months, TP concentrations were generally around 0.030 mg/L. Additionally, the N/P (mass ratio) varied significantly among different months.

The water column of the reservoir can be divided into 3 periods: the stratification periods (May to September 2020; February to May 2021) and the mixing period (October 2020 to January 2021). As shown in Fig. 2, the mixing depth increased during the stratification period from July to September. The euphotic depth ( $Z_{\text{eu}}$ ) showed small fluctuations throughout the year, and the ratio of euphotic depth to mixing depth ( $Z_{\text{eu}}/Z_{\text{mix}}$ ) was primarily influenced by the variations generated by  $Z_{\text{mix}}$  fluctuations.



**Figure 2.** Temporal variation of the mixing depth and euphotic depth in Xikeng Reservoir

**Table 1.** Changes in environmental factors for different months from 2020 to 2021

Indicators	Period												
	Stratification period					Mixing period				Stratification period			
	May	June	July	August	September	October	November	December	January	February	March	April	May
WT(°C) (Surface)	28.7 ± 0.34 abcd	30.62 ± 1.05 abc	31.59 ± 0.19 a	30.67 ± 0.46 abc	31.48 ± 0.93 ac	27.25 ± 1.86 abcd	24.9 ± 0.14 abcd	20.98 ± 1.1 bcd	17.57 ± 1.78 d	20.67 ± 2.11 bd	22.89 ± 1.90 bcd	25.12 ± 0.44 abcd	29.67 ± 2.31 bcd
WT(°C) (Bottom)	20.39 ± 0.19 cd	21.67 ± 1.41 cd	26.48 ± 2.67 ab	28.88 ± 0.70 a	29.45 ± 0.11 a	26.83 ± 2.26 ab	24.16 ± 0.62 bc	20.46 ± 1.73 cd	16.33 ± 1.49 e	16.04 ± 0.28 e	18.34 ± 1.93 de	21.50 ± 3.23 cd	20.39 ± 0.19 de
pH	8.75 ± 0.04 bc	8.61 ± 0.60 bc	8.64 ± 0.34 bc	8.20 ± 0.57 c	8.80 ± 0.85 bc	9.03 ± 1.12 bc	9.48 ± 0.41 b	9.39 ± 0.15 b	10.59 ± 0.18 a	8.81 ± 0.12 bc	8.47 ± 0.52 bc	8.85 ± 0.33 bc	9.21 ± 0.28 bc
DO (mg/L)	9.93 ± 0.53 b	9.82 ± 0.85 b	9.77 ± 0.34 b	8.83 ± 1.24 b	9.37 ± 2.88 b	8.61 ± 2.33 b	9.81 ± 0.85 b	10.09 ± 0.45 b	13.86 ± 0.07 a	11.00 ± 0.38 b	9.52 ± 0.72 b	9.32 ± 0.78 b	10.50 ± 0.59 b
Chl- <i>a</i> (µg/L)	7.54 ± 2.61 cdef	9.37 ± 0.98 cdef	5.10 ± 0.75 ef	5.52 ± 1.29 def	5.07 ± 1.10 f	9.13 ± 7.72 cdef	6.26 ± 0.38 def	10.00 ± 1.76 bcd	20.72 ± 5.33 a	17.57 ± 2.38 ab	12.87 ± 1.41 abc	13.41 ± 0.76 abc	9.58 ± 1.64 cde
SD (m)	1.67 ± 0.06 ab	1.53 ± 0.15 abc	1.60 ± 0.17 abc	1.67 ± 0.12 ab	1.45 ± 0.07 bc	1.55 ± 0.21 abc	1.80 ± 0.14 a	1.60 ± 0.14 abc	1.00 ± 0.00 d	1.30 ± 0.26 cd	1.50 ± 0.10 abc	1.37 ± 0.06 bcd	1.35 ± 0.07 bcd
TN (mg/L)	1.424 ± 0.206 bcd	1.1363 ± 0.200 bcd	1.296 ± 0.079 cd	1.660 ± 0.153 ab	1.944 ± 0.149 a	1.597 ± 0.117 bc	1.272 ± 0.040 d	1.222 ± 0.007 d	1.479 ± 0.037 bcd	1.277 ± 0.091 d	1.466 ± 0.128 bcd	1.491 ± 0.186 dbcd	1.361 ± 0.116 bcd
NO <sub>3</sub> <sup>-</sup> -N (mg/L)	1.016 ± 0.100 c	1.096 ± 0.145 abc	0.975 ± 0.133 c	1.293 ± 0.174 ab	1.362 ± 0.120 a	1.355 ± 0.177 a	1.165 ± 0.049 abc	1.070 ± 0.113 bc	1.040 ± 0.000 bc	1.027 ± 0.064 bc	1.143 ± 0.080 abc	1.195 ± 0.051 abc	1.180 ± 0.183 abc
NO <sub>2</sub> <sup>-</sup> -N (mg/L)	0.014 ± 0.004 ab	0.012 ± 0.002 bc	0.014 ± 0.004 ab	0.015 ± 0.006 ab	0.013 ± 0.001 b	0.017 ± 0.006 ab	0.011 ± 0.001 bc	0.004 ± 0.000 c	0.016 ± 0.006 ab	0.021 ± 0.002 a	0.022 ± 0.003 a	0.015 ± 0.006 ab	0.016 ± 0.003 ab
NH <sub>4</sub> <sup>+</sup> -N (mg/L)	0.051 ± 0.034 a	0.031 ± 0.018 a	0.069 ± 0.057 a	0.061 ± 0.064 a	0.079 ± 0.003 a	0.078 ± 0.004 a	0.050 ± 0.028 a	0.050 ± 0.032 a	0.089 ± 0.016 a	0.049 ± 0.052 a	0.038 ± 0.034 a	0.067 ± 0.028 a	0.035 ± 0.014 a
TP (mg/L)	0.023 ± 0.018 b	0.028 ± 0.005 ab	0.030 ± 0.007 ab	0.044 ± 0.13 ab	0.049 ± 0.015 a	0.042 ± 0.011 ab	0.028 ± 0.005 ab	0.032 ± 0.009 ab	0.035 ± 0.005 ab	0.026 ± 0.006 ab	0.033 ± 0.007 ab	0.029 ± 0.006 ab	0.030 ± 0.004 ab
DTP (mg/L)	0.010 ± 0.002 a	0.015 ± 0.013 a	0.021 ± 0.007 a	0.019 ± 0.004 a	0.023 ± 0.003 a	0.019 ± 0.011 a	0.009 ± 0.002 a	0.011 ± 0.000 a	0.011 ± 0.001 a	0.007 ± 0.001 a	0.017 ± 0.006 a	0.007 ± 0.001 a	0.009 ± 0.005 a
COD <sub>mn</sub> (mg/L)	2.90 ± 0.26 a	3.30 ± 0.26 a	2.73 ± 0.31 a	3.20 ± 0.85 a	3.10 ± 0.28 a	2.90 ± 0.85 a	2.30 ± 0.14 a	2.50 ± 0.57 a	2.90 ± 0.00 a	2.67 ± 0.31 a	2.80 ± 0.30 a	2.97 ± 0.42 a	2.90 ± 0.26 a
N/P (mass ratio)	106.34 ± 24.11 abc	138.65 ± 115.44 abc	52.60 ± 11.55 c	73.03 ± 18.58 abc	63.18 ± 13.08 bc	89.50 ± 43.86 abc	149.26 ± 45.16 ab	102.33 ± 7.41 abc	109.51 ± 8.41 abc	150.70 ± 16.56 ab	78.32 ± 36.02 abc	173.64 ± 8.83 a	175.60 ± 128.01 ab

Different lowercase letters indicate a significant difference ( $p < 0.05$ )

### Phytoplankton composition and dominant functional groups

During the study period, a total of 125 phytoplankton species belonging to 8 phyla and 69 genera were identified in the reservoir. The majority of the species were Chlorophyta (71 species, 56.8%), followed by Bacillariophyta (22 species, 17.6%), Cyanobacteria (19 species, 15.2%), and a few species from Dinophyta, Euglenophyta, Chrysophyta, Cryptophyta, and Xanthophyta. These species were classified into 22 functional groups (A, B, D, E, F, G, H1, J, K, LO, LM, M, MP, N, SN, P, S1, T, W1, X1, X2, and Y), with dominant groups being B, D, J, M, N, P, SN, S1, and X1 as shown in Table 2.

The study was performed to survey the effects of water mixing and stratification on the functional community structure of phytoplankton by analysing the changes in phytoplankton abundance and relative abundance at different periods. In Fig. 3, it is evident that during the stratification period of 2020, the functional group X1 of Chlorophyta had the highest relative abundance. Over the mixing period, however, the proportion of X1 gradually decreased, while that of functional groups P and D increased. Functional group D showed the highest relative abundance towards the end of the mixing period. During the following year's stratification period, the relative abundance of functional group D decreased significantly, while the abundances of functional groups M, X1, and SN gradually increased and reached their maximum in May. No significant changes were observed in the succession of functional groups B, J, and N during the study period.

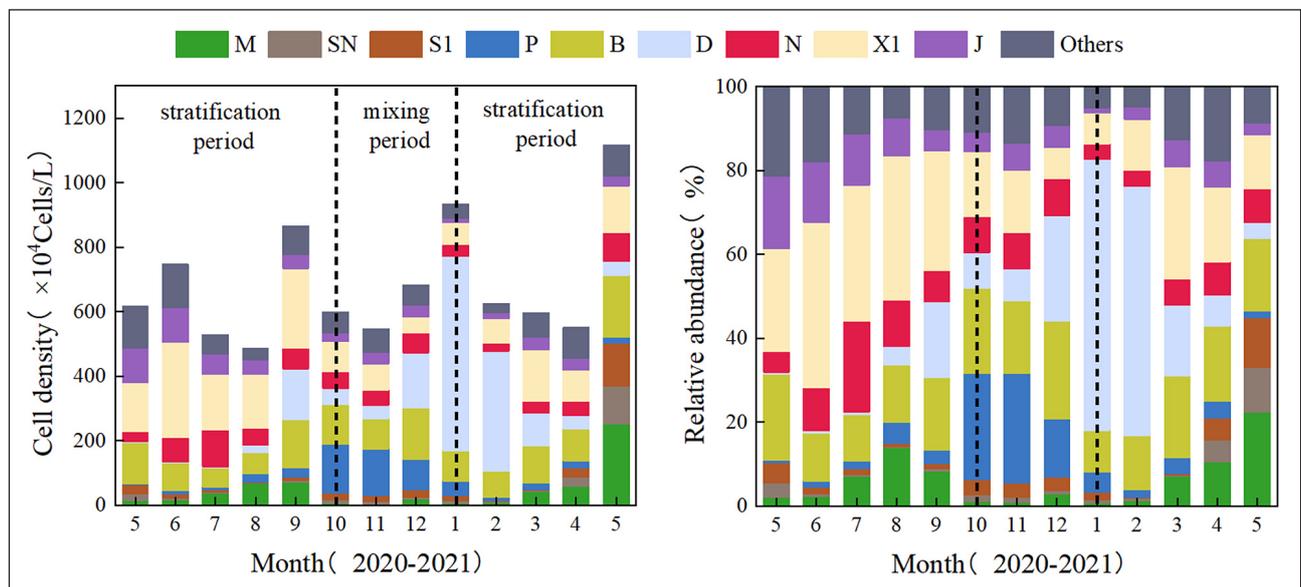
To investigate the impact of water mixing and stratification on the distribution characteristics of phytoplankton communities, the NMDS method based on Bray-Curtis similarity coefficients was used to analyse the phytoplankton community structure in different periods of the reservoir. Based on the NMDS analysis results depicted in Fig. 4, the phytoplankton functional groups exhibited marked differences across the three periods. The aggregation of samples during the stratification period was lower in comparison to the mixing period, and the aggregation of the two stratification periods also displayed considerable variation. These outcomes verified the soundness of the classification method strategy.

### Phytoplankton diversity and water trophic status

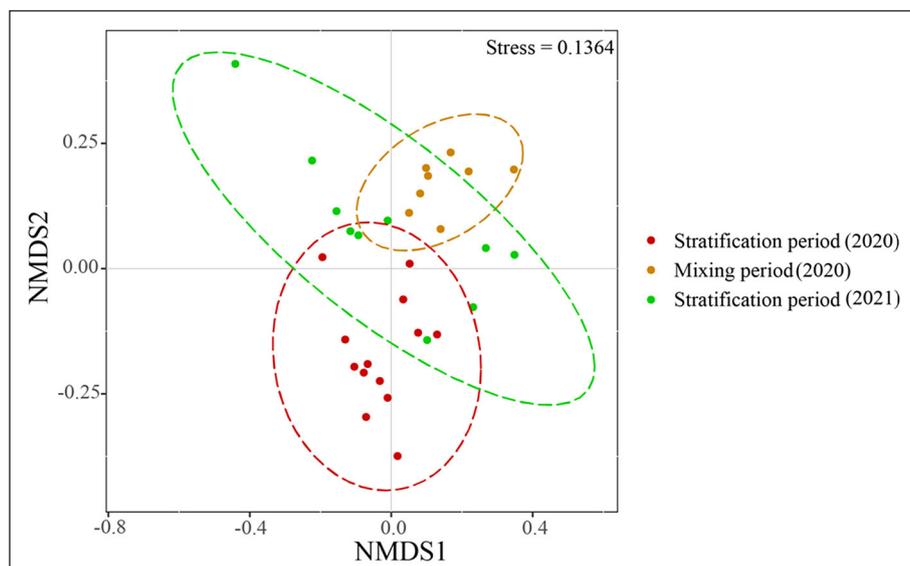
In this study, three commonly used evaluation indices were used to gain a more comprehensive understanding of the response characteristics of phytoplankton diversity to water stratification and mixing. The Shannon-Wiener diversity index revealed that the water body was slightly polluted, with no significant differences observed between the different periods (Fig. 5). Conversely, the Margalef richness index showed significant differences, indicating heavy water pollution. The Pielou evenness index suggested that the water body was slightly polluted, which was consistent with the Shannon-Wiener diversity index. The Q index, which gave similar results to the Shannon-Wiener diversity index and the Margalef richness index, revealed that the water ecological status was worse during the stratification period compared to the mixing period. Furthermore, the TLI index showed that the water bodies were mesotrophic, and there were no significant differences observed during the different periods (Table 1).

**Table 2.** Description of Reynolds functional groups (RFGs) of phytoplankton in Xikeng Reservoir

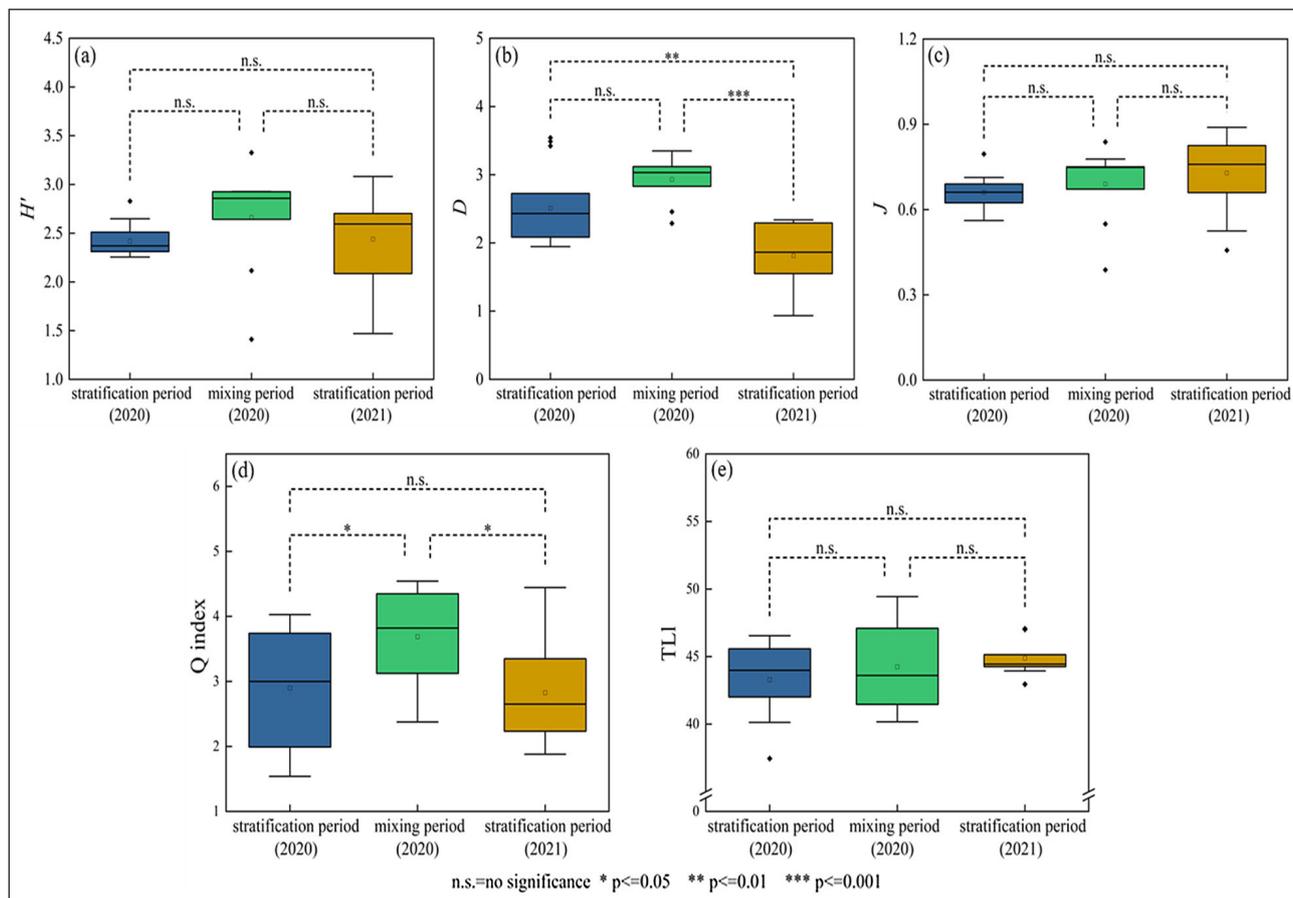
RFGs	Typical habitat	Representative species	Taxonomic group	F factor
B	Clear, deep water, sensitive to pH rise	<i>Cyclotella</i> sp.	Bacillariophyta	5
D	Turbid water, tolerant of flushing	<i>Synedra</i> sp.	Bacillariophyta	2
J	Eutrophic shallow water, sensitive to low light	<i>Scenedesum</i> sp. <i>Tetraedron</i> sp. <i>Crucigenia</i> sp. <i>Pediastrum</i> sp. <i>Coelastrum</i> sp.	Chlorophyta	1
M	Sun-tolerant, sensitive to flushing and low light	<i>Microcystis</i> sp.	Cyanobacteria	0
N	Mixed water layer, sensitive to pH rise and stratification	<i>Cosmarium</i> sp. <i>Staurastrum</i> sp.	Chlorophyta	5
P	Mixed water layer, sensitive to Si depletion and stratification	<i>Melosira</i> sp.	Bacillariophyta	5
SN	Warm and mixed water bodies, sensitive to flushing	<i>Cylindrospermum</i> sp. <i>Raphidiopsis</i> sp.	Cyanobacteria	0
S1	Medium to eutrophic, mixed, and low-transparency water bodies	<i>Pseudanabaena</i> sp.	Cyanobacteria	0
X1	Shallow mixed layers, tolerant of harsh environment	<i>Chlorella</i> sp.	Chlorophyta	5



**Figure 3.** Structure of phytoplankton community in Xikeng Reservoir



**Figure 4.** NMDS ordination plots of phytoplankton functional groups in different periods



**Figure 5.** (a) Shannon-Wiener diversity index ( $H'$ ); (b) Margalef richness index ( $D$ ); (c) Pielou evenness index ( $J$ ); (d) Q index; and (e) TLI index of the phytoplankton community during different periods in Xikeng Reservoir

### Relationships between functional phytoplankton communities and environmental factors

In this study, the RDA and VPA methods were used to screen the key environmental factors of the phytoplankton community in different periods. The RDA results (Fig. 6a) showed that WT and  $Z_{\text{mix}}$  were significantly correlated with phytoplankton functional groups during the stratification and mixing periods of 2020, respectively ( $p < 0.01$ ), and the dispersion of sample sites during the 2021 stratification period was consistent with NMDS analysis results. Additionally, the phytoplankton community structure was influenced by factors such as TN, WT, N/P, and TN. The first and second axes of the RDA analysis explained 29.18% and 5.82% of the variation, respectively. The VPA results (Fig. 6b) demonstrated that the contribution of WT was the highest (33.06%), followed by  $Z_{\text{mix}}$  (5.33%), N/P (3.69%), and TN (3.38%). The combined contribution of WT and  $Z_{\text{mix}}$  was 2.10%, and the total contribution of these four factors was 41.17%, suggesting their crucial role in the dynamics of phytoplankton functional groups in different periods.

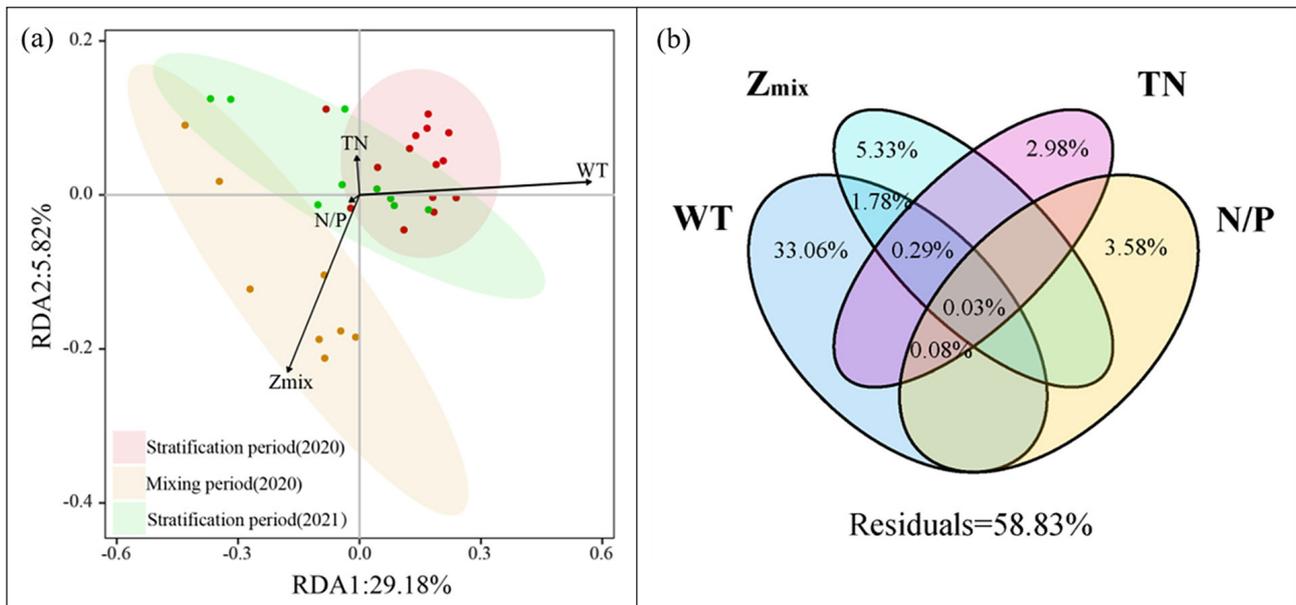
Pearson correlation analysis was used to investigate the relationships between major phytoplankton functional groups and key environmental factors. The results (Fig. 7) indicated that three of the above-mentioned four key factors, namely WT,  $Z_{\text{mix}}$ , and N/P, had a significant impact on the major phytoplankton functional groups. Specifically, WT was positively correlated with functional groups N, X1, and J ( $p < 0.05$ ) and negatively correlated with functional group D ( $p < 0.01$ ).  $Z_{\text{mix}}$  was positively correlated with functional group P ( $p < 0.01$ ) and negatively correlated with functional groups X1 and J ( $p < 0.05$ ). N/P was positively correlated with functional groups M, SN, and S1 ( $p < 0.05$ ).

### DISCUSSION

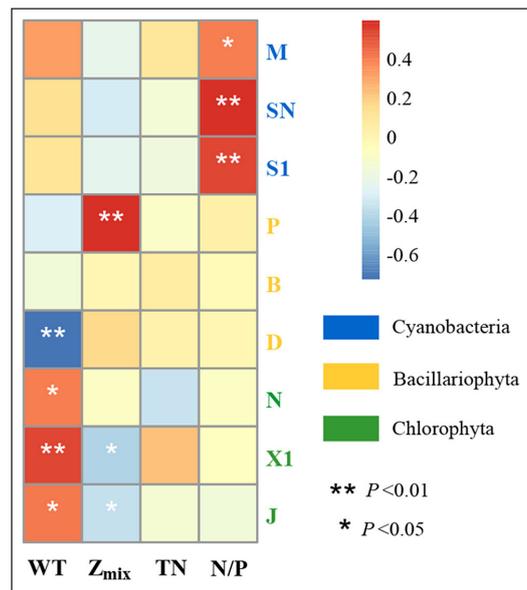
#### Key drivers affecting phytoplankton functional groups during different periods

Natural stratification and mixing are complex and important hydrological features of reservoirs that can directly affect the hydrodynamic conditions (e.g., mixing depth) and chemical parameters of the vertical water layers (Noori et al., 2021; Zhang et al., 2021). Variations in hydrological parameters and physicochemical elements often affect the functional group composition and structure of phytoplankton in aquatic ecosystems (Liu et al., 2012; Sun and Wang, 2021; Yao et al., 2020). In this study, screened environmental factors (WT,  $Z_{\text{mix}}$ , N/P, and TN) only explained approximately 40% of the phytoplankton community composition by VPA analysis (Fig. 6b). There could be several reasons for this. Firstly, environmental factors may have a time lag effect on phytoplankton communities. Secondly, water disturbances and changes in water levels can also impact the abundance and structure of the phytoplankton community due to water diversions.

Temperature can influence the physiological metabolism and enzyme activity of phytoplankton cells, which can affect their rates of reproduction and growth, and ultimately determine the composition of their communities (Su et al., 2017). WT is a complex and comprehensive factor, as both light and  $Z_{\text{mix}}$  are intimately linked to it (Liu et al., 2022). During the two stratification periods, phytoplankton was more greatly affected by WT, as depicted in Fig. 6a. Chlorophyta, particularly functional group X1 composed of *Chlorella* sp., showed a positive correlation with WT ( $p < 0.01$ ), indicating a high-temperature niche and thriving in high-temperature environments. Previous studies



**Figure 6.** (a) Redundancy and (b) variance partitioning analysis of phytoplankton dominant functional groups and the driving factors



**Figure 7.** Pearson analysis of phytoplankton dominant functional groups and the driving factors

have shown that as the temperature increased, the water column stratified to suit the growth of *Chlorella* sp. (Reynolds et al., 2002). Despite *Chlorella* sp. dominance during the 2020 stratification period, its abundance or relative abundance was not very high, due to its small surface area and low light availability. During the 2021 stratification period, WT reached its maximum in May, when cyanobacteria, consisting of *Microcystis* sp. (Group M), *Cylindrospermum* sp. (group SN), and *Pseudanabaena* sp. (Group S1), were dominant. Cyanobacteria, including *Microcystis* sp. and others, prefer high temperatures, and the positive impact of temperature on cyanobacteria has been observed in many aquatic ecosystems (Rao et al., 2021; Wang et al., 2021). The three species mentioned above are not only difficult to consume by filter-feeding zooplankton because of their large size or surface area (Paerl et al., 2011; Paerl and Huisman, 2008), but can gain a competitive advantage through the buoyancy regulation of gas vesicles. Besides, the functional groups of phytoplankton evolved from P to D during the mixing period, with functional group D

being more suitable for growth in cold conditions. Bracht et al. (2008) indicated that *Synedra* sp. of functional group D was one of the major Bacillariophyta during prolonged cold spring seasons, which aligns with our findings.

The effects of water mixing on phytoplankton mainly manifest in two ways: (i) the dynamic mixing process causes disturbance and flushing of the water column, which impacts the structure of the phytoplankton community; and (ii) mixing directly affects the availability of light and nutrients to phytoplankton (Becker et al., 2010, 2009; Zhong et al., 2022). In contrast to the sites of most studies (Liu et al., 2012; Wen et al., 2022), Xikeng Reservoir is relatively shallow (22 m) and experiences the highest temperature from July to September, when heat transferred from the water surface to the middle water column led to an increase in Z<sub>mix</sub>. Additionally, Z<sub>mix</sub> slightly increased in March and April, likely due to an increase in water diversion. The results showed that *Chlorella* sp. was suitable for growth in the mixed layer of

a semi-mixed or incompletely mixed water body, and Yu et al. (2018) also reported that *Chlorella* sp. was more dominant than *Microcystis* sp. in turbulent water. As the mixing depth continued to increase, the dominant species shifted from *Chlorella* sp. to *Melosira* sp., because the former is sensitive to deep mixing while the latter is suitable for complete mixing of the water body. Previous research has indicated that Bacillariophyta are capable of thriving in environments characterized by high turbulence, low temperatures, and high nutrient concentrations (Reynolds et al., 2002), particularly those with a high surface-to-volume ratio and weak light adaptation, such as functional group D. Furthermore, non-motile Bacillariophyta and Chlorophyta can remain in the euphotic layer for longer periods and receive light for photosynthesis, thanks to the carrying effect produced by vertical mixing (Li et al., 2019). The study confirmed this finding and was consistent with other studies (Wen et al., 2022). The results also demonstrate a decrease in  $Z_{eu}/Z_{mix}$  (light availability) in 2020, while there was an occasional increase in phytoplankton abundance, suggesting that light limitation was not a critical factor influencing the changes in phytoplankton, especially for non-motile algae.

Variations in nutrient concentrations of the reservoir were influenced more by water diversion than the mixing process. These changes can affect phytoplankton abundance dynamics and species turnover (Diatta et al., 2020; Lie et al., 2011). This study demonstrated that DTP concentrations in May 2021 were generally lower than in the 2020 stratification period, with the former dominated by *Microcystis* sp. and the latter by *Chlorella* sp.. Consistent with previous studies, *Chlorella* sp., a small unicellular Chlorophyta with rapid nutrient uptake, was favoured more than *Microcystis* sp. by high phosphorus water (Cui et al., 2021; Yu et al., 2018; Zhong et al., 2022). Moreover, some studies have considered N/P as a factor that affects phytoplankton structure rather than absolute N and P concentrations (Yang et al., 2020). The results showed a predominance of Cyanobacteria in the functional groups M, SN, and S1 in April and May 2021, associated with a higher N/P at this time (Fig. 7). Numerous studies have indicated that Bacillariophyta and Chlorophyta will be replaced by Cyanobacteria as N/P increases (De Tezanos Pinto and Litchman, 2010a; b; Liu et al., 2021; Teubner and Dokulil, 2002). In May 2021, the results showed that a higher proportion of non-nitrogen-fixing cyanobacteria (Groups M and S1) were present than nitrogen-fixing Cyanobacteria (group SN) when N/P was higher, as found in other studies (Carey et al., 2012; Chislock et al., 2014; Liu et al., 2021; Scott et al., 2019). There was no significant correlation between TN and phytoplankton functional groups and the contribution rate was also the lowest, indicating that TN concentration in phosphorus-limited water had no significant effect on phytoplankton community structure.

In summary, the dynamic changes in the phytoplankton functional group structure during different periods in Xikeng Reservoir were jointly determined by WT,  $Z_{mix}$ , N/P, and TN. Additionally, the dynamics of the phytoplankton functional groups were found to be more complex in the 2021 stratification period compared to the 2020 stratification period, as WT,  $Z_{mix}$ , and N/P underwent significant changes simultaneously.

### Evaluation of ecological state

Previous studies (Isabwe et al., 2018; Wu et al., 2019; Yan et al., 2014) have shown that phytoplankton diversity was a valid indicator of aquatic ecosystem status. However, the study revealed that the Margalef richness index yielded different results to the Shannon-Wiener diversity index and the Pielou evenness index, due to the proximity of the sampling site to the diversion area and the

dynamic water diversion process (short hydraulic residence times) that may have reduced the phytoplankton species richness and abundance. Among the three indices, the decrease in the number of species (S) had the greatest impact on the Margalef richness index, indicating the limitation of this index in evaluating the water body's ecological state. The study did not detect any significant changes in the diversity index in different periods (Fig. 5), which supports the findings of other studies (Ochieng et al., 2022). On the one hand, the reservoir is situated in the warm subtropics, and WT was around 20°C during the mixing period, which provided favourable conditions for a considerable proportion of phytoplankton to survive. On the other hand, stratification is often accompanied by hypoxia in the hypolimnetic, leading to the release of nutrients (mainly N and P) from the sediment; then the reservoir mixing process causes an increase in the nutrient concentrations in the surface water column, promoting phytoplankton blooms (Chen et al., 2018). Consequently, the diversity index during the mixing period was not significantly lower than that during the stratification period and was even slightly higher. Overall, using phytoplankton diversity indices as indicators of ecosystem state in complex water bodies under changing environmental conditions may have some limitations, and a similar conclusion was reached for the study by Ding et al. (2021).

Given the limitation of the phytoplankton diversity index, the TLI index and Q index were used to assess the trophic and ecological status of the water bodies, respectively. The results showed that the two assessments were consistent, indicating that the trophic status of the water bodies was either medium or good, and the ecological status was moderate. Besides, the ecological condition of the water column during the mixing period was better than that of the stratification period. Similar observations have been reported for other studies (Dembowska et al., 2015; Wen et al., 2022). Therefore, it is crucial to extend the mixing period to enhance the ecological status of the reservoir. A practical application of this principle is for reservoir managers to perform artificial mixing through water-lifting aerators (WLAs) at an appropriate time to extend the mixing period (Li et al., 2020; Wen et al., 2022).

### CONCLUSION

The study explored the effects of stratification and mixing on phytoplankton community structure and diversity. The main findings were as follows:

- The stratification and mixing of the reservoir caused the phytoplankton community to display obvious differences: Chlorophyta or Cyanobacteria were dominant during the stratification period, corresponding to phytoplankton functional groups X1 or M; Bacillariophyta composed of functional groups P and D were the main functional groups in the mixing period.
- Consistent with the Q index, the phytoplankton diversity index was higher in the mixing period compared to the stratification period, indicating better ecological conditions in the mixing period. However, the Margalef richness index may be inadequate and unconvincing as an indicator of the ecological state of water bodies in Xikeng Reservoir, and it is necessary to combine it with the Q index to better evaluate ecological status.
- Under lower N/P mass ratio and higher mixing depth conditions, phytoplankton functional group X1 was favoured more than M, while functional group D was advantaged relative to P in low-temperature conditions. Moreover, the dynamic changes of phytoplankton functional groups were mainly influenced by WT,  $Z_{mix}$ , N/P, and TN, as indicated by the RDA results.

- In addition, future research should investigate the complex effects of changing hydrodynamic conditions (e.g., flow velocity and Reynolds number) on phytoplankton communities resulting from dynamic water diversion processes. For the stratification periods with poor ecological conditions, especially in the southern subtropics where water stratification lasts for a longer time, reservoir managers should consider vertical monitoring and enhance the frequency of monitoring to prevent phytoplankton blooms.

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## AUTHOR CONTRIBUTIONS

Yunhao Bai: conceptualization; methodology; writing – original draft; writing-review and editing. Tinglin Huang: conceptualization; visualization; investigation. Pengcheng Yang: data curation; investigation.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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