



# Trophic homogeneity due to seasonal variation in nitrogen in shallow subtropical lakes

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## ABSTRACT

Lakes play a crucial role in the nitrogen (N) cycle, and eutrophication disrupts the balance of the nitrogen cycle within lakes, including both the N removal process and the N supplement process. However, the mechanisms by which different nutrient levels affect seasonal nitrogen variations in the water columns are not clear, especially for long-term and large-scale studies. In this study, we used 206 independent spatial samples from a total of 108 subtropical shallow lakes from four surveys in the middle and lower reaches of the Yangtze River, as well as time-series study data from Lake Taihu and Lake Donghu of up to 23 and 14 years, respectively, to analyze the changes in summer TN compared to spring (delta TN). Delta TN was significantly negatively correlated with initial spring TN concentrations, with similar trends observed in both space and time. Furthermore, the slopes of spring TN vs. delta TN varied little across lakes in both time and space, suggesting a consistent relationship between initial spring TN and summer TN changes. When initial TN or TN: TP ratio was low, N fixation by algae played a significant role in compensating for summer N removal, thus mitigating summer N reductions; when TN was high or TN: TP ratio was high, ammonia stress reduced the compensatory effect of algae and denitrification played a significant role in summer N removal, thus increasing summer N reductions. Our study suggested that no matter what the initial conditions are, lakes tend to evolve towards a common nutrient status through biological regulation.

## 1. Introduction

Lakes play a crucial role in the materials cycle, in which the cycling of nitrogen and phosphorus is greatly disturbed by human activities, and the cycling of nitrogen (N) in water bodies is much more complex than the cycling of phosphorus (P) (Rabalais 2002; Elser et al., 2007; Penuelas et al. 2020; Ji et al. 2024). One of the most complex biogeochemical cycles in aquatic ecosystems is the N cycle, with various types of N transformation, in which biological regulation plays an indispensable role (Naafs et al. 2019). And the contribution of the water column to nitrogen transformation cannot be ignored (Wang et al. 2022).

On the one hand, lakes participate in N exchange through inputs and outputs, and on the other hand, internal lake processes in the water column mediate N transformations (Yi et al. 2017). For the water column of lake ecosystems, N supplement mainly involves anthropogenic

and atmospheric deposition, sediment release, and biological N fixation (Foster et al. 2011; Kuypers et al. 2018). In contrast, biological N removal, sediment burial, effluents, and certain biochemical processes, such as typical denitrification, anaerobic ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA), contribute significantly to N loss from the water column (Burgin and Hamilton 2007; Ward et al. 2009). Compared to terrestrial ecosystems, biologically mediated denitrification rates are typically high in freshwater ecosystems, with most of the N lost in the form of N<sub>2</sub> (Seitzinger et al. 2006). Although N deficits due to anammox and DNRA processes have been identified in different ecosystems, their contribution to N losses in lakes is small compared to denitrification (Stueken et al. 2016). Moreover, macrophyte uptake also contributes to summer N loss, but N removal through denitrification is more important than sedimentation and macrophyte uptake (Saunders and Kalff 2001). Pina-Ochoa and

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Alvarez-Cobelas (2006) reviewed seasonal variations in denitrification rates and found that lakes had the greatest variation in denitrification rates, which peaked in summer. Although denitrification occurs predominantly at interfaces near the sediment, recent studies have shown that water quality, rather than sediment characteristics, is the main factors directly or indirectly affecting denitrification rates in lakes (Hasegawa and Okino 2004; McCrackin and Elser 2010; Liu et al. 2018).

Eutrophication caused by human activities disrupts the balance of nitrogen cycle in lakes, resulting in significant changes in ecosystems, including changes in water quality (nutrient contents and forms), species composition, and biodiversity (Barker et al. 2008; Conley et al. 2009). On the one hand, anthropogenic eutrophication leads to an increase in the initial nutrient levels of lakes (Shen et al. 2021). Low oxygen due to eutrophication further enhances the release of N from sediments (McCarthy et al. 2007; Taranu et al. 2015). In addition, N and P imbalance caused by eutrophication altered the phytoplankton community due to eutrophication altered the phytoplankton community, and N-fixing cyanobacteria increased exponentially when total N:P ratio was lower than 29:1, increased biological N fixation (Smith 1983; Paerl and Otten 2013). On the other hand, eutrophication can also lead to drastic changes in N loss rate. Many studies have found that factors that directly or indirectly affect denitrification in aquatic systems include nitrate ( $\text{NO}_3\text{-N}$ ) availability, oxygen concentration, organic carbon, P, temperature, light, water residence time (WRT), microbe activities and plant presence (Mitchell and Baldwin 1999; Pina-Ochoa and Alvarez-Cobelas 2006; Finlay et al. 2013). Adequate nitrate and low oxygen are two key factors in denitrification process (Wrage et al. 2001). N loss from lakes increases with increasing N loading (Finlay et al. 2013; Olsen et al. 2015). Moreover, ammonia nitrogen can provide substrate for denitrification through aerobic ammonia oxidation, while eutrophication enhances the release of ammonia nitrogen from sediments, further contributing to biological N removal (McCarthy et al. 2007; Roland et al. 2018).

As a limited factor, nitrogen cycling in aquatic ecosystem may affect phytoplankton abundance by altering N availability and stoichiometric ratios (Peñuelas et al. 2012). In addition to the abundance of nutrients, high temperature and low transparency also favoring cyanobacteria growth, the increase in N-fixing species in many freshwater ecosystems implies a relationship between changes in phytoplankton communities and summer N losses (Havens et al. 2003; Vrede et al. 2009). On the one hand, high nutrients in lakes promote phytoplankton growth (Conley et al. 2009; Jochimsen et al. 2012), and cyanobacterial biomass and dominance increase in eutrophic lakes during the summer (Chislock et al. 2014). On the other hand, processes mediating N loss reinforce N limitation to primary production (Anju et al. 2022). Algae may be limited by inadequate and sustained summer N supply (Anju et al. 2022). Moreover, P is released from the sediments into the water column during summer (Welch and Cooke 2005; Beutel and Horne 2018). The combined effect of summer N removal, N or P release and biological impacts makes the nutrient contents and variations more complicated in the water column. However, key factors that determine the change rates of TN in freshwater water column are poorly known. Does eutrophication tend to align nutrient status by regulating internal N cycling processes?

Under the influence of eutrophication, both N loss processes and compensation processes are affected, and the synergistic effect of the two ultimately has unpredictable results on the total nitrogen in the water column. We hypothesized that the weighting of the effects of nitrogen loss processes and compensation processes on overall N is different at different trophic levels. Eutrophication may accelerate the process of N loss, but its effect on the N:P ratio may compensate for the process of N loss by affecting the community structure of aquatic organisms, and these two processes contribute to different degrees in lakes with different initial trophic levels, making the trophic state of eutrophic lakes converge. To test this hypothesis, we carried out the study in the middle and lower reaches of the Yangtze River, which is highly

disturbed by human activities. Lakes in this region are mostly eutrophic and basically shallow, with a short water exchange cycle, which can reduce the influence of disturbances from physical factors, such as lake stratification, on the study results. We conducted a large-scale regional survey of 108 lakes over a period of four years, and time-series analyses of long-time continuous observations of two sentinel lakes to reveal the processes and mechanisms of N loss and N compensation in lakes with different trophic status.

## 2. Materials and methods

### 2.1. Study lakes and sampling point setting

Our studied lakes are in the middle and lower reaches of the Yangtze River, which has the richest lake group in China (Fig. 1a, Table S1). Most lakes were shallow and eutrophic (Table 1).

Firstly, we conducted a regional survey in 4 separate years from 2002 to 2016 (Fig. 1b). To avoid the effects of drastic changes in the hydrological environment, we focused on lakes that have been separated from the Yangtze River by dikes. We surveyed 42 lakes, 52 lakes, 49 lakes, and 63 lakes in 2004, 2008, 2012, and 2016, respectively. For each surveyed lake, we set up a point in the center of the lake to avoid transient disturbances from exogenous pollution. Due to the large number of lakes surveyed in each sampling year, sampling spanned 30–40 days from the first to the last lake (spring, March–April; summer, July–August), but sampling times were kept within the same season. We sampled each lake twice per sampling year in two seasons, with each sampling year being studied in the spring and summer.

Secondly, we analyzed long-term continuous monitoring data (from Chinese Ecosystem Research Network, CERN) for Lake Donghu (Fig. 1c) from 2000 to 2013 and Lake Taihu (Fig. 1d) from 1992 to 2014. Lake Taihu is the third largest freshwater lake in China, with an area of 2388  $\text{km}^2$  and an average depth of 1.9 m. Lake Donghu covers an area of 33.9  $\text{km}^2$  with a mean depth of 2.6 m. For Lake Taihu, 3–6 points were sampled during 1992–2004, and 20–22 points were sampled during 2005–2014 (a total of 276 points); For Lake Donghu, 3 points were sampled (data from 2002 is missing, a total of 39 points). Sampling points in both Lake Taihu and Lake Donghu were evenly distributed in the open area of the lake, and the numbering of sampling points were fixed on the time series. Our sampling points were not located in the estuaries and littoral areas to avoid transient interference from exogenous pollution. We sampled Lake Taihu and Lake Donghu in the spring (May) and summer (August) of each sampling year.

### 2.2. Sampling and analysis

We measured water depth (WD) and transparency (SD) at each sampling site using a depth sounder (Speedtech, USA) and a black-white Secchi disk (with a diameter of 20 cm), respectively. Water temperature (WT), and chemical oxygen demand (COD) were surveyed in situ at each sampling point using a multiparameter water quality analyzer (YSI, USA). Mixed water samples were collected from the surface (0.5 m below water surface), the middle, and the bottom (0.5 m above sediment surface) of the water column at each site to assess total nitrogen (TN), nitrate ( $\text{NO}_3\text{-N}$ ), nitrite ( $\text{NO}_2\text{-N}$ ), ammonium ( $\text{NH}_4\text{-N}$ ), total phosphorus (TP), phosphate ( $\text{PO}_4\text{-P}$ ), and chlorophyll a (Chla) (Eaton et al. 1966). Chla was measured using the heat-ethanol method and the spectrophotometer by filtering depth-integrated water samples from each site through a 0.7  $\mu\text{m}$  GFF glass fiber filter membrane (Eaton et al. 1966).

Phytoplankton was only surveyed in 2012. 1-L depth-integrated water samples collected at each site were precipitated and concentrated in acetic Lugol's solution for phytoplankton analysis. Phytoplankton individuals were identified to genus and counted using a microscope at 40x magnification according to Hu (2006).

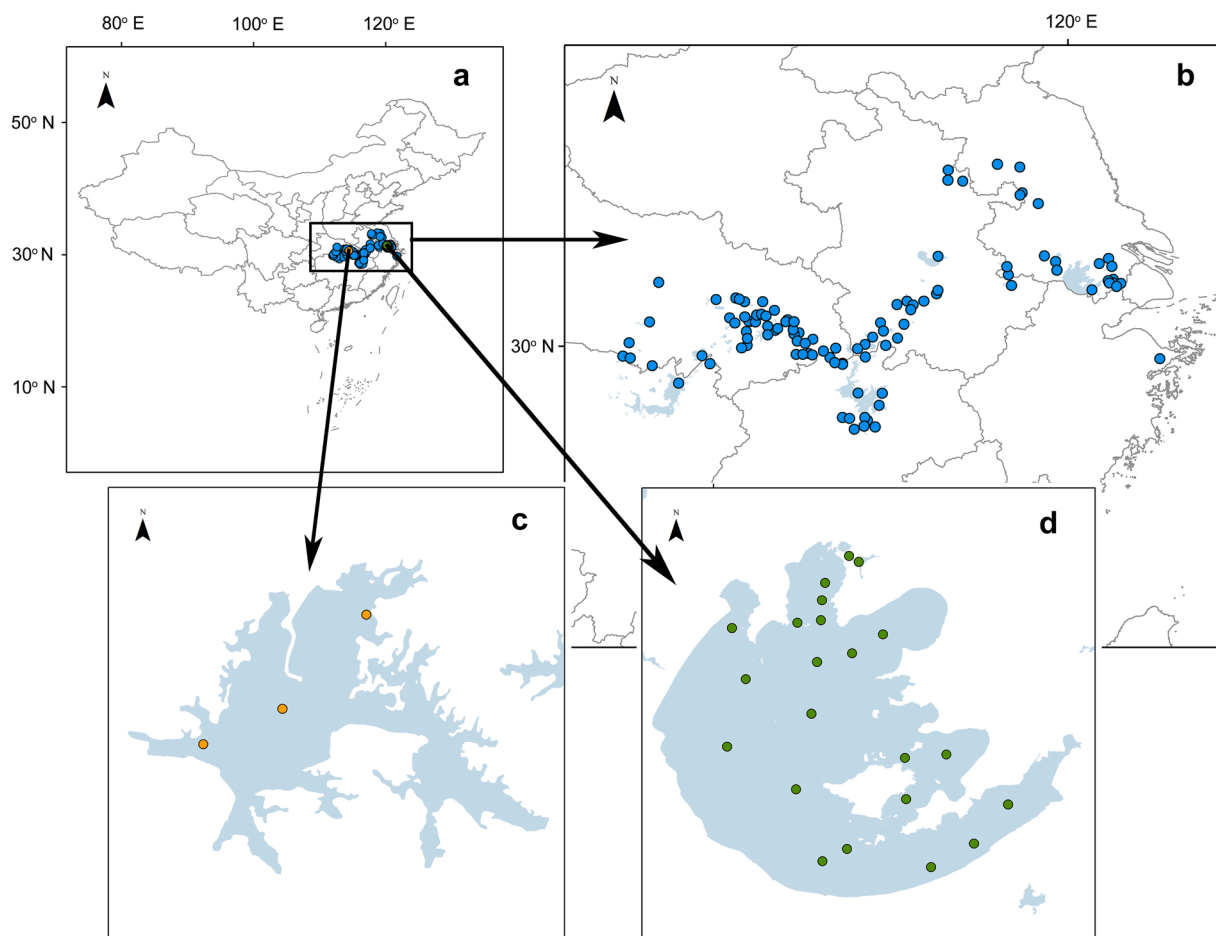


Fig. 1. a, Study area in China; b, sampling lakes of the middle-lower Yangtze River; c, sampling sites of Lake Donghu; d, sampling sites of Lake Taihu.

### 2.3. Statistics analysis

For the long-term continuous monitoring of Lake Taihu and Lake Donghu, the average value of all sampling sites (replications) each sampling time was used for analysis. In our study, we used delta to indicate the seasonal change of parameters in summer compared to spring, e.g.,  $\Delta \text{TN} = \text{summer TN} - \text{spring TN}$ .

Firstly, Analysis of variance (ANOVA) (TN and TP of spring, WT and  $\text{NO}_3\text{-N}$  of summer) and Kruskal-Wallis test (other factors) were used to analyze the differences between years (2004, 2008, 2012, and 2016) in Table 1. Independent samples *t*-test was used to analyze the differences between Lake Taihu and Lake Donghu in Table 1.

Secondly, Paired-sampled T-test was used to test the value differences of TN, TP,  $\text{NO}_3\text{-N}$ , and  $\text{NH}_4\text{-N}$  between the spring and the summer in Fig. 2.

Thirdly, Covariance Analysis (ANCOVA) was used to analyze the differences among the slopes and intercepts of linear regression analysis (Fig. 3). The  $\Delta \text{TN}$  and  $\Delta \text{NO}_3\text{-N}$  were the response variables; Spring TN and  $\text{NO}_3\text{-N}$  were the covariance variables, respectively. For spatial regression coefficients, the group of 2004, 2008, 2012, and 2016 was a fixed factor. For temporal regression coefficients, the group of Lake Taihu and Lake Donghu was a fixed factor.

Fourthly, to test whether other environmental factors affect the relationship between  $\Delta \text{TN}$  and spring TN, we analyzed the effects of environmental factors on  $\Delta \text{TN}$  by using Generalized Mixed-Effects Models (GLMMs), and the sampling years were considered as random factor. To analyze what factors influence the change in weights of the nitrogen removal and nitrogen enrichment processes, we grouped the study lakes into 3 groups considering the initial trophic level (spring TN

was shown as  $A < B < C$ ) (details supported by supplementary information). For the spatial lakes, there are three groups, and the number of samples of Group A, B and C are 73, 75 and 58, respectively. Due to incomplete data for Lake Donghu, the analysis was not performed between Lake Taihu and Lake Donghu. In addition, differences of TP, COD, Chl<sub>a</sub>, and phytoplankton abundance among the three groups was compared using one-way ANOVA followed by LSD tests.

Finally, Structural Equation Model (SEM) was used to analyze the pathway of significant environmental factors on  $\Delta \text{TN}$  for all spatial lakes. Significant influencing factors of GLMMs were selected to conduct SEM. We used Comparative Fit Index (CFI, usually near 1), Standardized Root Mean Square Residual (SRMR, usually less than 0.08), Chi-square statistic and the significance to evaluate the model.

Normality and homogeneity were tested before analysis. Predictors were logarithmically transformed prior to analysis when necessary. Statistical analyses were conducted using SPSS 19 and R software with packages “lavaan” and “semPlot”.

## 3. Results

### 3.1. Changes of environmental variables among different lakes

From a comparison of the multi-year averages of the case study lakes, although the initial N concentrations were different between the two lakes in the spring, the summer N concentrations tended to be the same. In spring, the concentrations of TN and  $\text{NO}_3\text{-N}$  of Lake Taihu were significantly higher than those of Lake Donghu; in summer, the total nitrogen and nitrate nitrogen of Taihu Lake and Lake Donghu tended to be the same without significant difference (Table 1).

**Table 1**

Physiochemical parameters of lakes along the middle and lower reaches of the Yangtze River.

WT, water temperature; SD, secchi depth; WD, water depth of sampled sites; TN, total nitrogen; NO<sub>3</sub>-N, nitrate; NO<sub>2</sub>-N, nitrite; NH<sub>4</sub>-N, ammonium; TP, total phosphorus; PO<sub>4</sub>-P, phosphate; COD, chemical oxygen demand; Chl *a*, chlorophyll *a*.

The average and standard deviation of sampled lakes in each year were shown for 2004, 2008, 2012, and 2016, respectively. The average and standard deviation of sampled time (for each time, the average value of all sampling sites of each lake was used) was shown for Lake Taihu and Lake Donghu, respectively.

According to the results of Normality and homogeneity, ANOVA (TN and TP of spring, WT and NO<sub>3</sub>-N of summer) and Kruskal-Wallis test (other factors) were used to analyze the difference among different years (2004, 2008, 2012, and 2016). Independent samples *t*-test was used to analyze the difference between Lake Taihu and Lake Donghu.

Significant results (*p*) were indicated in bold.

Year/lake	Season		WT ( °C)	SD (cm)	WD (m)	TN (mg/L)	NO <sub>3</sub> -N (mg/L)	NO <sub>2</sub> -N (µg/L)	NH <sub>4</sub> -N (mg/L)	TP (µg/L)	COD (mg/L)	Chl <i>a</i> (µg/L)
2004	Spring	Average ± standard deviation	-	84 ± 62	2.08 ± 0.99	1.38 ± 1.02	0.37 ± 0.36	25 ± 43	0.07 ± 0.08	90 ± 76	3.49 ± 1.25	12.76 ± 21.75
2008	Spring	Average ± standard deviation	15.52 ± 2.67	67 ± 49	-	1.69 ± 1.52	0.53 ± 1.00	13 ± 31	0.27 ± 0.54	71 ± 64	5.87 ± 1.55	10.39 ± 11.18
2012	Spring	Average ± standard deviation	-	-	1.92 ± 1.07	1.77 ± 1.18	0.25 ± 0.20	-	0.42 ± 0.69	74 ± 59	4.34 ± 1.41	42.66 ± 41.87
2016	Spring	Average ± SD	-	-	2.51 ± 1.31	1.65 ± 0.99	0.41 ± 0.38	-	0.26 ± 0.38	60 ± 57	3.85 ± 1.48	20.91 ± 22.88
		<i>p</i>			<b>&lt; 0.001</b>	<b>0.452</b>	<b>&lt; 0.001</b>		<b>&lt; 0.001</b>	0.110	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
2004	Summer	Average ± SD	-	109 ± 81	3.33 ± 2.13	1.00 ± 0.59	0.18 ± 0.17	54 ± 64	0.08 ± 0.12	93 ± 76	5.06 ± 2.80	16.17 ± 20.22
2008	Summer	Average ± SD	29.90 ± 1.90	62 ± 41	-	1.14 ± 0.78	0.12 ± 0.27	25 ± 55	0.36 ± 0.36	103 ± 94	5.61 ± 1.21	11.22 ± 9.83
2012	Summer	Average ± SD	29.63 ± 2.15	43 ± 28	3.02 ± 1.25	1.56 ± 0.60	0.15 ± 0.27	-	0.10 ± 0.30	206 ± 181	5.88 ± 1.78	77.44 ± 49.06
2016	Summer	Average ± SD	30.05 ± 3.20	80 ± 54	4.49 ± 1.77	1.23 ± 0.57	0.21 ± 0.17	-	0.31 ± 0.26	79 ± 78	4.31 ± 1.53	35.45 ± 30.82
		<i>p</i>	0.684	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	0.120		<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
Lake Taihu	Spring	Average	21.84 ± 1.90	37 ± 12	2.32 ± 0.27	3.21 ± 0.77	1.30 ± 0.49	51 ± 79	0.41 ± 0.26	91 ± 39	4.48 ± 0.84	17.79 ± 13.06
Lake Donghu	Spring	Average	-	-	-	1.77 ± 1.04	0.49 ± 0.41	145 ± 420	0.34 ± 0.41	164 ± 82	-	-
		<i>p</i>				<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	0.437	0.548	<b>0.009</b>		
Lake Taihu	Summer	Average	28.96 ± 2.16	51 ± 22	2.73 ± 0.42	1.66 ± 0.29	0.35 ± 0.19	31 ± 22	0.38 ± 0.66	98 ± 33	5.11 ± 1.00	30.90 ± 13.63
Lake Donghu	Summer	Average	-	-	-	1.34 ± 0.54	0.32 ± 0.22	61 ± 85	0.15 ± 0.09	184 ± 93	-	-
		<i>p</i>				0.067	RD0.658	0.234	0.207	<b>0.006</b>		

From comparisons between years in regional lakes, although the initial NO<sub>3</sub>-N concentrations were different among the four periods in the spring, the summer NO<sub>3</sub>-N concentrations tended to be the same. In spring, there were significant differences in NO<sub>3</sub>-N, with the highest in 2008 and the lowest in 2012; in summer, NO<sub>3</sub>-N tended to be the same without significant differences between the years. There was no significant difference in TN in spring; in summer, there were significant differences in TN, with the highest in 2012 and the lowest in 2004 (Table 1).

For the case study lakes, there was no significant difference between the average NH<sub>4</sub>-N of Lake Taihu and Lake Donghu in both spring and summer (Table 1). For the regional lakes, there were significant differences in the average NH<sub>4</sub>-N among the different years in both spring and summer, with the highest spring NH<sub>4</sub>-N in 2012, the lowest spring NH<sub>4</sub>-N in 2004, the highest summer NH<sub>4</sub>-N in 2008, and the lowest summer NH<sub>4</sub>-N in 2004.

TP of Lake Taihu was significantly lower than that of Lake Donghu in spring, and remained significantly lower than that of Lake Donghu in summer; there was no significant difference in the mean TP values of the regional lakes between years in spring, and in summer, there was a significant difference in the mean TP values of the regional lakes between years, which was the highest in 2012 and the lowest in 2016 (Table 1).

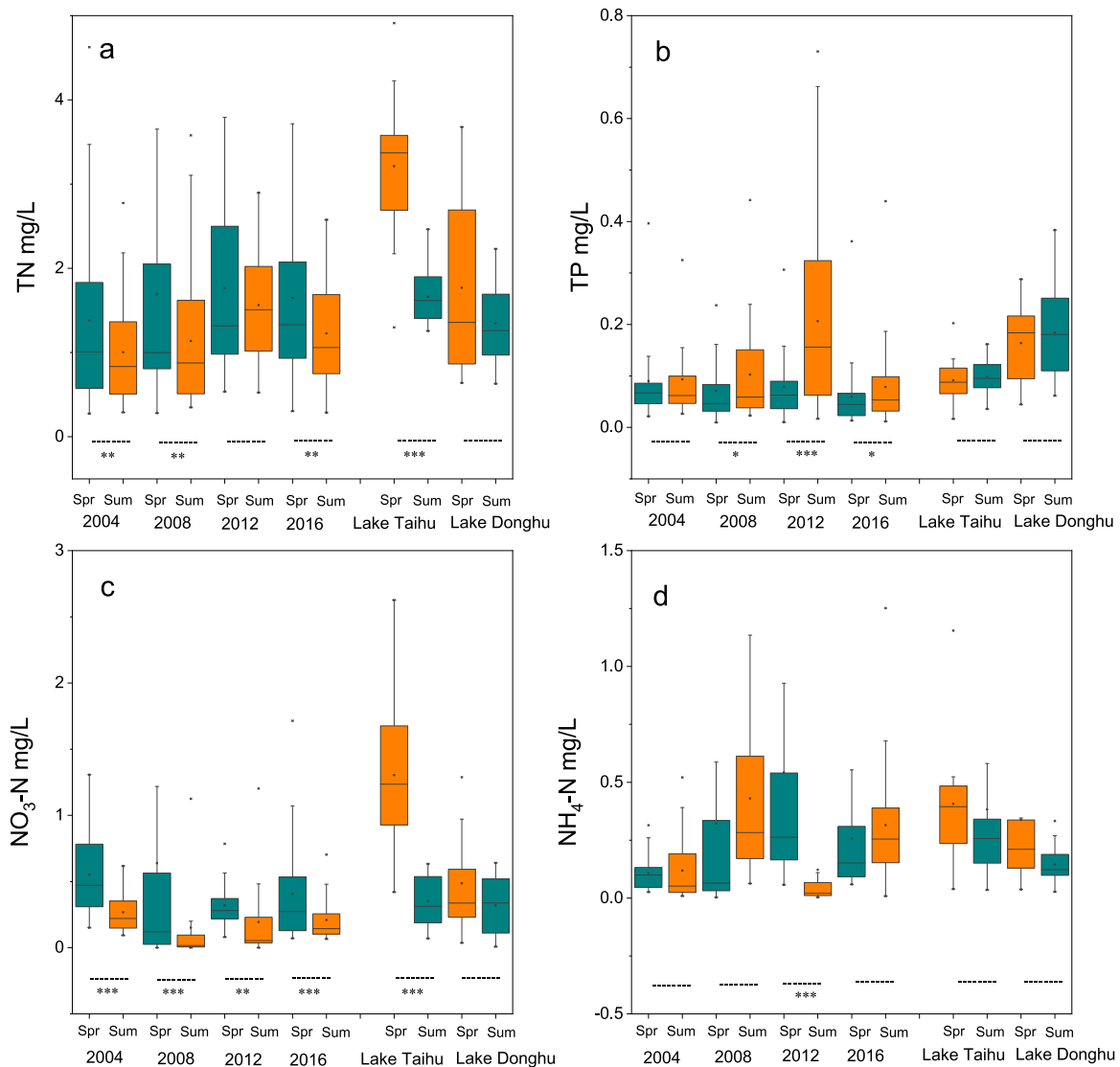
### 3.2. Changes of environmental variables between different seasons

From the comparison between spring and summer, TN and NO<sub>3</sub>-N decreased in both case study and regional lakes during the summer compared to the spring. The average summer TN of Lake Taihu was significantly lower than of spring; the average summer TN of regional lakes was significantly lower than of spring in 2004, 2008 and 2016 (Fig. 2a). The average summer NO<sub>3</sub>-N values of Lake Taihu were significantly lower than the spring values; the average summer NO<sub>3</sub>-N values of regional lakes were significantly lower than the spring values in 2004, 2008, 2012 and 2016 (Fig. 2c). There was no significant difference between spring and summer NO<sub>3</sub>-N and TN mean values in Lake Donghu. Furthermore, there was no significant difference between the mean NH<sub>4</sub>-N values in spring and summer in Lake Taihu and Lake Donghu. But NH<sub>4</sub>-N followed a different trend than TN and NO<sub>3</sub>-N, decreasing only in the summer compared to the spring of 2012 for the spatial lakes (Fig. 2d).

The spring-summer differences in total phosphorus were not significant for both Taihu and Donghu lakes; the mean summer total phosphorus was significantly higher than the spring total phosphorus for the regional lakes in 2008, 2012 and 2016 (Fig. 2b).

### 3.3. Relationships between delta N and initial spring N

Delta TN and delta NO<sub>3</sub>-N were significantly negatively correlated with initial spring TN and NO<sub>3</sub>-N values in both the case study and



**Fig. 2.** The concentrations of total nitrogen (TN), total phosphorus (TP), nitrate ( $\text{NO}_3\text{-N}$ ), and ammonium ( $\text{NH}_4\text{-N}$ ) in subtropical shallow lakes. Paired-sampled T-test was used to test the value difference between the spring and the summer. Significant results ( $p$ ) were indicated as follows:  $p < 0.05$ , \*;  $p < 0.01$ , \*\*;  $p < 0.001$ , \*\*\*. Spr, spring; Sum, summer.

spatial data (Fig. 3). Moreover, the slopes of linear regressions of delta TN and delta  $\text{NO}_3\text{-N}$  with initial values of spring TN and  $\text{NO}_3\text{-N}$  were consistent across the long time series of the comparison between Lake Taihu and Lake Donghu (Fig. 3c, d); the slopes of linear regressions of delta TN and delta  $\text{NO}_3\text{-N}$  with initial values of spring TN and  $\text{NO}_3\text{-N}$  in regional lakes were also consistent across the four time periods (Fig. 3a, b).

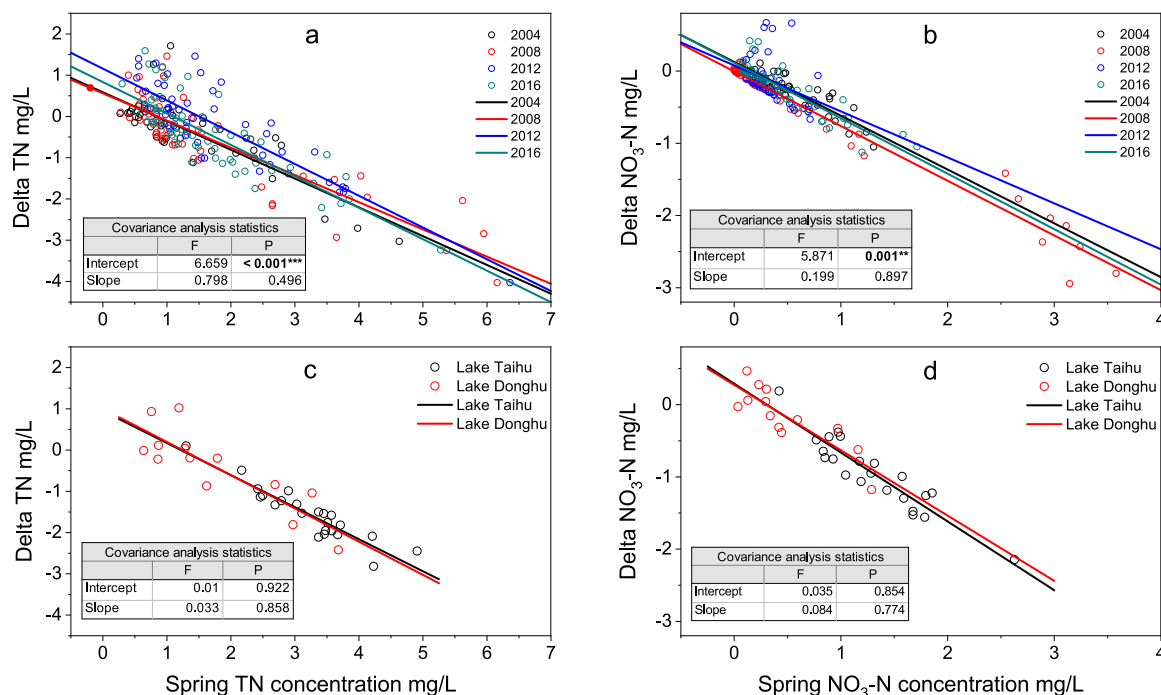
The linear regression intercepts of delta TN and delta  $\text{NO}_3\text{-N}$  versus the initial values of spring TN and  $\text{NO}_3\text{-N}$  in regional lakes were significantly different across the four time periods (Fig. 3a, b). In terms of changes in the long time series, delta TN and delta  $\text{NO}_3\text{-N}$  versus the initial values of spring TN and  $\text{NO}_3\text{-N}$  were consistent across the long time series of the comparison between Lake Taihu and Lake Donghu (Fig. 3c, d).

GLMMs showed the negative relationship between delta TN and spring TN was still significant when considering other predicted variables (Table 2). GLMMs results showed the model was fitted well ( $p < 0.001$ ) and delta COD, delta Chla and summer WT were also significantly related to delta TN when all samples were included.

#### 3.4. Changes of environmental variables and phytoplankton abundance among different groups

The mean concentrations of TP and COD in spring were presented as Groups  $C > A > B$  and  $C > B = A$ , respectively (Fig. 4b). Group C had the highest concentrations of spring TP and COD, with delta TN being consistently less than 0. Group B had similar spring COD concentrations as group A, but group B had lower summer COD concentrations than group A, which was accompanied by a higher decrease in summer TN in group B than in group A (Fig. 4b). TP and COD increased in summer compared to spring (Table 1; delta TP and delta COD were higher than 0, Fig. 4b). Both delta TP and delta COD were presented as Group  $C = B < A$ , which implies that group B and C consumed more TP and COD in summer. The average summer TN: TP ratios (calculated as TN divided by TP) were 10.80, 20.55, and 8.75 for the three intervals lakes in Groups A, B, and C, respectively, and the average TN: TP ratio for the summer decreased compared to the spring, with the largest reduction in Group B (Fig. 4c).

Chla increased in summer compared to spring (Table 1; Fig. 4b, delta Chla were higher than 0). The mean spring Chla concentrations of group A, B, and C were 19.26  $\mu\text{g/L}$ , 12.43  $\mu\text{g/L}$  and 38.17  $\mu\text{g/L}$ , respectively



**Fig. 3. Linear regressions between the spring concentrations of total nitrogen (TN) and ammonia nitrate (NO<sub>3</sub>-N) and Delta TN and delta NO<sub>3</sub>-N in subtropical shallow lakes.** Delta TN and delta NO<sub>3</sub>-N represent the difference between the parameters for summer and spring. Covariance analysis was used to analyze the difference among the slopes and intercepts of linear regression analysis. Spring TN and NO<sub>3</sub>-N were the covariance variables respectively. For spatial regression coefficients, the group of 2004, 2008, 2012, and 2016 was a fixed factor. For temporal regression coefficients, the group of Lake Taihu and Lake Donghu was a fixed factor. Significant results (*p*) were indicated in bold.

**Table 2**

Generalized Mixed-Effects Models (GLMMs) analyses changes in summer total nitrogen compared with spring (delta TN, ΔTN) and predicted parameters. For the spatial lakes, All, all data (the number of samples = 206); Group A: TN < 1.85 mg/L, ΔTN > 0 (the number of samples = 73); Group B: TN < 1.85 mg/L, ΔTN < 0 (the number of samples = 75); and Group C: TN > 1.85 mg/L, ΔTN < 0 (the number of samples = 58). Δ represents the difference between parameters for summer and spring, e.g., ΔTN=summer TN – spring TN. Response factor: ΔTN. Fixed variables: spring TN (spTN); initial values of spring of WD, TP, COD (spCOD), and Chl a and their changes in summer compared to spring (ΔWD, ΔTP, ΔCOD, and ΔChl a respectively); and summer values of SD and WT (suWT) (Missing data for spring). Random factor: the sampling years. WD: water depth of sampling site (m); TP: total phosphorus (mg l<sup>-1</sup>); COD: chemistry oxygen demand (mg l<sup>-1</sup>); Chl a: Chlorophyll a (μg l<sup>-1</sup>); SD: Secchi depth (m); and WT: surface water temperature (°C).

	All		Group A		Group B		Group C					
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>				
Fixed effects	Model	76.49	< 0.001	Model	4.36	< 0.001	Model	6.24	< 0.001	Model	14.59	< 0.001
	spTN	465.59	< 0.001	ΔCOD	13.52	0.001	spTN	47.53	< 0.001	spTN	101.70	< 0.001
	ΔCOD	26.94	< 0.001	ΔChl a	8.22	0.006	spCOD	5.90	0.018	suWT	6.84	0.012
	ΔChl a	20.50	< 0.001	suWT	5.59	0.021	ΔTP	13.46	0.001			
	suWT	17.00	< 0.001				ΔCOD	5.41	0.023			
							suWT	4.93	0.030			

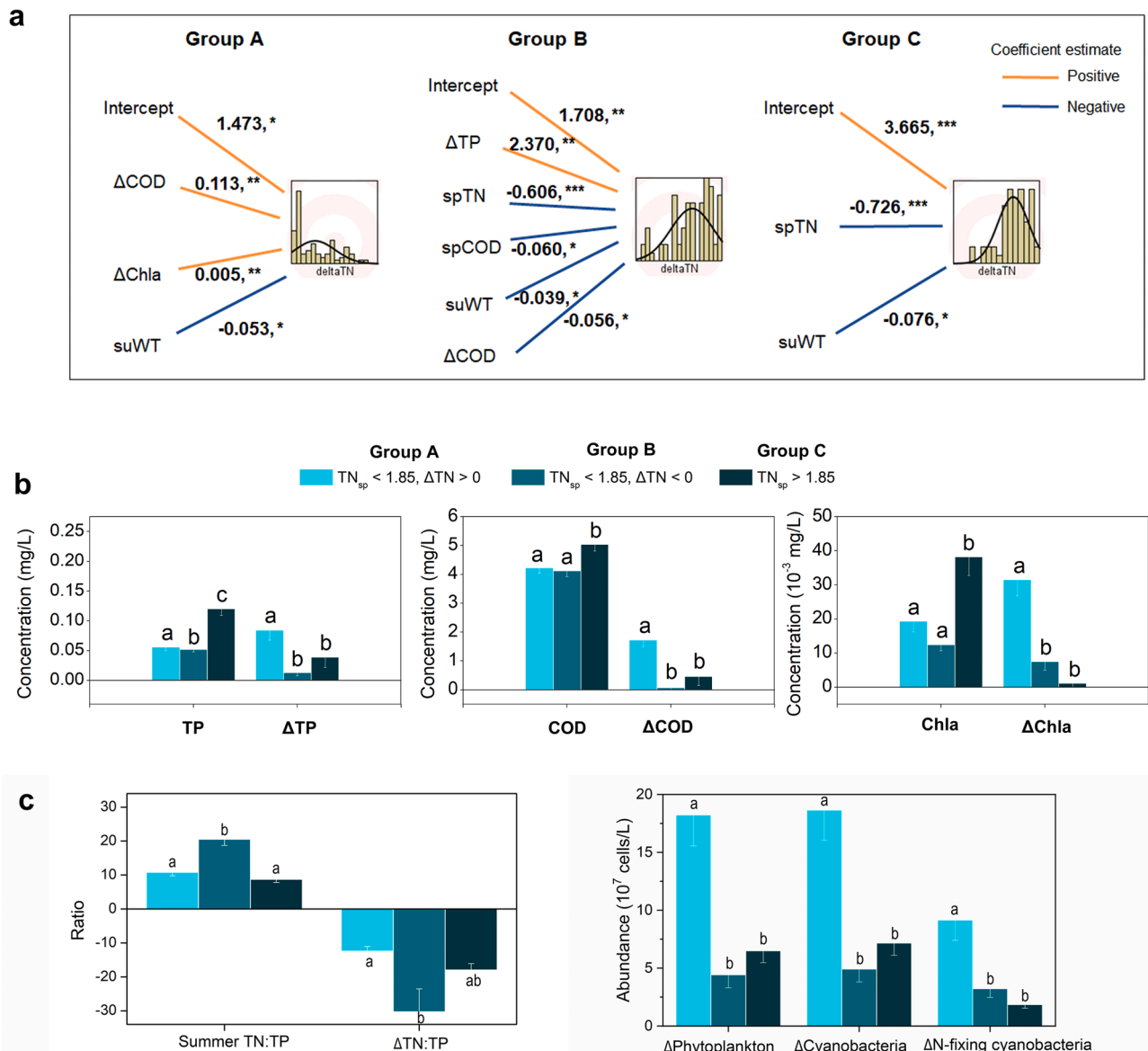
Significant results (*p* < 0.05) were indicated in bold. Only significant fixed variables were included.

(Group C > A = B), and delta Chl a was presented as Group C = B < A (Fig. 4b). Summer Chl a decreased with the increasing summer NH<sub>4</sub>-N: NO<sub>3</sub>-N ratios (Fig. 5).

The mean abundance of all phytoplankton, cyanobacteria, and N-fixing cyanobacteria increased in summer compared to spring (Fig. 4b). The mean spring abundance of total phytoplankton of group A, B, and C were 3.21 (± 2.37) × 10<sup>7</sup> cells/L, 1.77 (± 1.11) × 10<sup>7</sup> cells/L, and 3.48 (± 3.65) × 10<sup>7</sup> cells/L, respectively. Of these, the spring abundance of cyanobacteria of group A, B, and C accounted for 45.1 (± 22.3) %, 47.7 (± 24.0) %, and 28.5 (± 21.0) % of the total phytoplankton abundance, respectively; the spring abundance of N-fixing cyanobacteria of group A, B, and C accounted for 13.1 (± 19.7) %, 8.1 (± 10.2) %, and 8.7 (± 15.8) % of the total phytoplankton abundance, respectively. In summer,

phytoplankton increased in all groups and the increased abundance of phytoplankton was accounted by the increase of cyanobacteria that the abundance of cyanobacteria of group A, B, and C accounted for 88.7 (± 16.2) %, 83.1 (± 20.4) %, and 77.8 (± 18.8) % of the total phytoplankton abundance, respectively and the abundance of N-fixing cyanobacteria of group A, B, and C accounted for 38.5 (± 24.9) %, 40.7 (± 22.0) %, and 24.8 (± 15.0) % of the total phytoplankton abundance, respectively.

Group A showed the highest increase in cyanobacteria and N-fixing cyanobacteria (Fig. 4c). The increased abundance of cyanobacteria of group A, B, and C were 18.65 × 10<sup>7</sup> cells/L, 4.91 × 10<sup>7</sup> cells/L, and 7.16 × 10<sup>7</sup> cells/L, respectively; of these, the increased abundance of N-fixing cyanobacteria of group A, B, and C were 9.15 × 10<sup>7</sup> cells/L, 3.22 × 10<sup>7</sup>



**Fig. 4.** a Generalized Mixed-Effects Models (GLMMs) analyses for changes in summer total nitrogen compared to spring ( $\Delta$ TN) and predicted parameters. Response factor: delta TN; Fixed variables: spring TN (spTN); initial values of spring of WD, TP, COD (spCOD), and Chla and their changes in summer compared to spring ( $\Delta$ WD,  $\Delta$ TP,  $\Delta$ COD, and  $\Delta$ Chla respectively); and summer values of SD and WT (suWT) (Missing data for spring); Random factor: the sampling years. Only significant ( $p < 0.05$ , \*;  $p < 0.01$ , \*\*;  $p < 0.001$ , \*\*\*) fixed variables were included.

b Concentrations and changes in summer TP, COD and Chla compared to spring (average  $\pm$  SE). c Concentrations of summer TN: TP ratio and changes in summer TN: TP ratio, phytoplankton abundance, cyanobacteria abundance and N-fixing cyanobacteria abundance compared to spring ( $\Delta$ TN: TP,  $\Delta$ Phytoplankton,  $\Delta$ Cyanobacteria, and  $\Delta$ N-fixing cyanobacteria) (average  $\pm$  SE).

Group A: TN  $< 1.85$  mg/L,  $\Delta$ TN  $> 0$  (the number of samples = 73); Group B: TN  $< 1.85$  mg/L,  $\Delta$ TN  $< 0$  (the number of samples = 75); and Group C: TN  $> 1.85$  mg/L,  $\Delta$ TN  $< 0$  (the number of samples = 58). For b and c, the lowercase letters above the column indicate the significance of  $p < 0.05$  among the three groups based on ANOVA analysis.

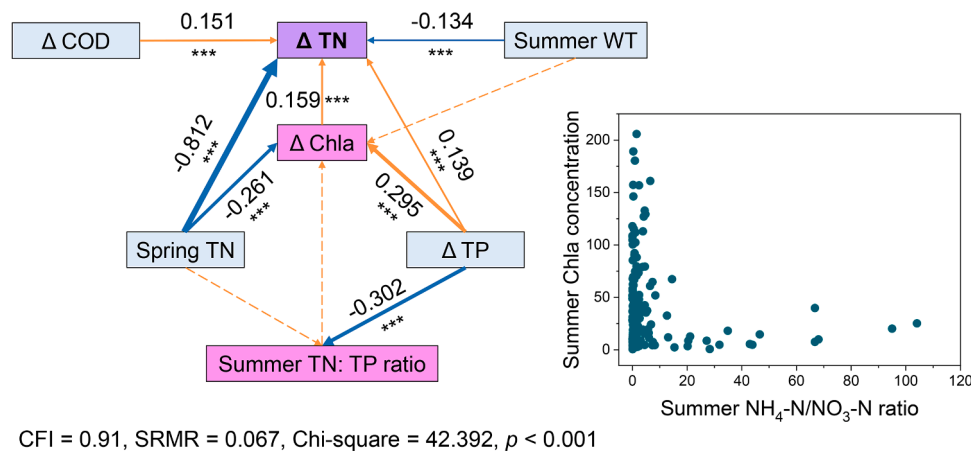
TN, total nitrogen (mg/L); TP, total phosphorus (mg/L); COD, chemistry oxygen demand (mg/L); Chla, Chlorophyll a ( $\mu$ g/L); WT, surface water temperature ( $^{\circ}$ C).

cells/L, and  $1.85 \times 10^7$  cells/L, respectively (Fig. 4c).

GLMMs results showed that the significant factors relating to delta TN differed among different groups (Table 2, Fig. 4a). For group A, Delta COD and delta Chla were positively related to delta TN, summer WT was negatively related to delta TN; for group B, spring TN, spring COD, delta COD and summer WT were negatively related to delta TN, only delta TP was positively related to delta TN; for group C, spring TN and summer WT were negatively related to delta TN (Fig. 4a). The results showed that an increase in summer WT accelerated the rate of summer nitrogen removal in all groups, an increase in spring TN accelerated the rate of summer nitrogen removal in both groups B and C, and a higher spring COD accelerated the rate of summer nitrogen removal in group B.

### 3.5. SEM results

SEM results showed the model fitted well (Fig. 5). Delta TN was directly negatively influenced by summer WT and spring TN for all spatial lakes. SEM results also showed summer TN: TP ratios were negatively influenced by delta TP. Moreover, delta Chla was positively influenced by delta TP and negatively influenced by spring TN, resulting in positive influence on delta TN (Fig. 5). SEM results indicated that higher summer WT and spring TN would directly increase summer N removal, but phytoplankton will compensate for N losses under the influence of lower summer TN: TP ratios.



**Fig. 5.** SEM of the effects of predicted parameters on changes in summer TN compared to spring ( $\Delta$ TN). The dotted line means the relationship was not significant. The orange line means positive effect and the blue line means negative effect. Model statistics: CFI (near 1), Comparative Fit Index; SRMR (less than 0.08), Standardized Root Mean Square Residual.

TN, total nitrogen (mg/L); TP, total phosphorus (mg/L); COD, chemistry oxygen demand (mg/L); Chla, Chlorophyll a ( $\mu$ g/L); WT, surface water temperature ( $^{\circ}$ C);  $\text{NO}_3\text{-N}$ , nitrate (mg/L);  $\text{NH}_4\text{-N}$ , ammonium (mg/L);  $\Delta$ TP,  $\Delta$ COD and  $\Delta$ Chla, changes in summer value compared to spring. Estimates of significant ( $p < 0.05$ , \*;  $p < 0.01$ , \*\*;  $p < 0.001$ , \*\*\*) relationships were indicated by solid line.

#### 4. Discussion

Our results showed that the declining quantity of nitrogen in summer was negatively related to spring TN, and the slopes were not significantly different at different spatial and temporal scales, suggesting that the summer nitrogen loss rate in subtropical shallow lakes was synchronous. The study suggests that no matter what the initial conditions are, lakes have a tendency to evolve towards a common nutrient status. This eutrophication-affected process works synergistically through lakes N loss mechanisms and N loss control mechanisms.

##### 4.1. Summer N loss

Our results showed that the reduction of TN in summer was mainly due to the reduction of  $\text{NO}_3\text{-N}$ , indicating that denitrification was the main mode of N removal.  $\text{NO}_3\text{-N}$ , as one of the proximal regulators, determines denitrification rates in lake ecosystems (Seitzinger et al. 2006; Bruesewitz et al. 2011; Saggari et al. 2013). Hasegawa and Okino (2004) and Liu et al. (2018) found that  $\text{NO}_3\text{-N}$  in the overlying water can control denitrification rates in lake sediments, thereby affecting N loss. High TP and high COD also increased the amount of N removal. Our results indicated that the higher concentrations of TP and COD in the spring implied higher consumption of TP and COD in the summer and higher N removal in the summer (Fig. 4).

The mechanism of the process can be summarized as follows. Firstly, in water bodies with high nutrient levels, abundant denitrifying substrates can increase the rate of N removal. Previous studies have shown that TN and  $\text{NO}_3\text{-N}$  concentrations in the water column were positively correlated with sediment denitrification rates (Liu et al. 2018). Study also showed that the addition of organic carbon stimulated denitrification (Hellman et al. 2019). Secondly, P promoted the removal rate of N and this situation occurred more frequently when TP was relatively low, for example, TP in the water column can significantly enhance denitrification rates when lakes changed from oligotrophic to mesotrophic state (Piña-Ochoa and Alvarez-Cobelas 2006; Finlay et al. 2013). Lower P addition promoted a decrease in  $\text{NO}_3\text{-N}$  as a result of competition between denitrification and nitrate assimilation, which was attenuated under low phosphorus conditions; therefore, nitrate was mostly denitrified (Kaste and Lyche-Solheim 2005). Thirdly, in high nutrients and high COD water bodies, microbial actions of both aerobic nitrifiers such as Nitrosomonas and Nitrososira and anaerobic ammonium oxidizers such as Planctomycete-like ANAMMOX bacteria will be enhanced in

summer, with the effect of high temperature and which was more likely to form an anoxic environment, accelerating the N removal process (Seidel et al. 2021; Chen et al. 2023). This is also consistent with our findings that an increase in summer WT would accelerate the rate of summer nitrogen removal (Fig. 4, 5). Lastly, the increase of excessive non-feedable algae in summer would lead to the blockage of nutrient transfer in the planktonic food chain, and the formation of blooms by algal aggregation, which would aggravate the anoxic environment in the water body after decay and decomposition, accelerating denitrification and the process of N removal process (Paerl and Otten 2013; Yao et al. 2024). Our results showed that cyanobacteria increased in all groups during summer (Fig. 4c), which may accelerate the rate of summer N removal. However, group A, which had the highest increase in cyanobacteria (Fig. 4c), did not show the highest summer N removal. SEM results further indicated that cyanobacteria, as the most dominant increasing phytoplankton in summer, would compensate for N losses (Fig. 5). These results suggested that the effect of increased cyanobacteria on summer N removal was less important than its effect on N compensation, and less important than the effect of initial nutrients on N removal.

In addition, we already know that anoxic plays an important role in the process of N removal process, and denitrification occurs primarily in sediments and suspended particulate matter, both habitats with anoxic (oxygen-deficient within particulate matter) environments (Liu et al. 2018). The lakes we studied were basically shallow lakes where hydrodynamics affected dissolved oxygen a lot in the water column, resulting in hypoxia to be less important as a limiting factor than initial nutrients. Although certain studies have found that the light regime and submerged macrophytes can affect the denitrification rate (Piña-Ochoa and Álvarez-Cobelas 2006). A recent study showed that macrophytes have less effect on denitrification rates in lakes along the Yangtze River in China (Liu et al. 2018). Our results implied that when initial nutrients are high, lakes may tend to reduce nutrient level by the above processes.

##### 4.2. Control of summer N loss

Our results implied that the weights of nitrogen compensation and removal were different under different nutrient conditions. Through inter-annual analysis of TN in China, Tong et al. (2019) found that summer TN significantly decreased in water bodies (lakes and reservoirs) with high TN values ( $> 1.5$  mg/L) but increased in those with low TN values ( $< 1.0$  mg/L).



On the one hand, our results showed that under low N and low TN: TP ratio conditions, total N even increased in the summer, and cyanobacteria, especially nitrogen-fixing cyanobacteria, grew dramatically in summer. Low N and high P caused a decrease in the TN: TP ratio, which favored the predominance of N-fixing cyanobacteria (i.e., *Anabaena* and *Aphanizomenon*) (Noges et al. 2008; Chen et al. 2013; Liu et al. 2021), which may increase N compensation. Disproportionate inputs of nitrogen and phosphorus, as well as processes such as summer N deficiency and P release, all can breakfast eutrophication lead to a decrease in lake TN: TP stoichiometry and an increase in cyanobacteria (Zhou et al. 2022). The effects of low TN: TP ratios on algal communities were also reflected in the lake restoration process. After the restoration of damaged lakes, nutrient reduction, especially the imbalance reduction of N and P after a rapid reduction of N, instead promoted a large growth of algae, which in turn led to the outbreak of cyanobacterial blooms (Li et al. 2022b). Azotification by N-fixing algae can provide as much as 6%–82% of the nitrogen load in lakes (Howarth et al. 1988). Nevertheless, N inputs from N-fixing algae cannot compensate for N losses through denitrification and burial (Shatwell and Kohler 2019). Furthermore, our results showed delta COD was only positively related to delta TN when the initial N is low (Fig. 4a), which meant when the initial N was low, summer input from external source may also contribute the compensatory of N.

On the other hand, high TN: TP ratio or high TN was unfavorable to the growth of nitrogen-fixing cyanobacteria and reduced the amount of N fixing. First, high TN: TP ratios or high TN promoted the growth of non-nitrogen-fixing cyanobacteria such as *Microcystis aeruginosa* (Yang and Jin 2008; Liu et al. 2011). These effects on algal community structure reduced the control of N loss by algal nitrogen fixation. Second, the mechanism by which N loss affected algae was not only through changes in community structure by changes in the N: P ratio, but also through the effects of amine stress. Our results showed that summer phytoplankton decreased with increasing ammonia: nitrate ratios. The high ammonia: nitrate ratio produced amine stress for phytoplankton (Liu et al. 2022; Liu et al. 2023). The loss of oxygen in summer increased  $\text{NH}_3/\text{NH}_4^+$  release from sediments in addition to P release (McCarthy et al. 2007), which made predictions of the effect on net N content and N:P ratios much more difficult, but may contribute to the increased of ammonia: nitrate ratios. Furthermore, the rate of nitrate loss was greater than that of ammonia in summer, resulting in higher ammonia: nitrate values which inhibited algal growth, including N-fixing cyanobacteria. This may explain why Group A in this study possessed the highest algal abundance. Therefore, the higher the initial value, the greater the N loss in summer, the greater the change in ammonia: nitrate values, the stronger the amine stress on algae, and thus limit the algae's ability to compensate for the N loss. This may also explain why only a few lakes in the middle and lower reaches of the Yangtze River showed cyanobacterial blooms although most of them were eutrophic (Li et al. 2022b; Li et al. 2022c).

When initial nutrients were low, lakes tend to increase nutrient level by biological N-fixing process of algae to control summer N loss; but the biological N-fixing process of algae would be limited when initial nutrients are high. This process suggests that biological regulation plays a crucial role in summer N variations, and that in addition to the TN: TP ratios, the different reduction rate of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  may be important factors affecting summer phytoplankton, and that these processes, in turn, regulate the summer N.

#### 4.3. Human activities

The lakes in the middle and lower reaches of the Yangtze River are disturbed by anthropogenic activities in a relatively homogenous way, making the eutrophication process and water retention time and other changes relatively consistent. For example, only the degree of pollutants deepened, but the types of nutrients-pollutants remained consistent in a region (Strokal et al. 2021; Quadra and Brovini 2023); the climatic

conditions changes in a same region shares the same trend, and the input pollution brought by surface runoff in summer occurred more similar (Ning et al. 2024).

WRT is an important physical process that can play a significant role in N loss either directly by downstream outputs or indirectly by promoting denitrification, sedimentation, or plant assimilation (Saunders and Kalff 2001; Seitzinger et al. 2006). Lake features, such as area, depth, and catchment area, can determine the WRT and further influence nutrient changes in different ways. Tong et al. (2019) found that the effect of WRT is also related to variations in N concentrations in lakes and reservoirs. The N removal efficiency in ecosystems increases as WRT increases (Saunders and Kalff 2001; Finlay et al. 2013). Due to the construction of dams are blocked from the river, the regulation of the water level and water exchange is mainly through artificial control, the consistency of these anthropogenic disturbances has also led to the reduction of heterogeneity of the Yangtze River lakes in the middle and lower reaches of the Yangtze River (Li et al. 2021; Li et al. 2022a).

## 5. Conclusion

In summary, eutrophic lakes under anthropogenic disturbance converge through biological regulation within the system, despite differences in initial nutrient status. At low initial nutrient levels, nitrogen fixation by algae compensated for summer lake N losses, whereas at high initial nutrient levels, biological-mediated denitrification rates increased. When the initial nutrient levels were high, the differences in the rates of different forms of N loss led to an increase in the ammonia: nitrate ratios, resulting in the ammonia pressure on algal growth and limiting the N compensation by algae. Moreover, the rich denitrifying substrates and high temperature in summer accelerated the process of denitrification-dominated N loss. Through the above processes, the overall effect of eutrophication on lakes in the same region was a tendency of trophic levels to move in the direction of a seasonally similar state.

### CRedit authorship contribution statement

**Yun Li:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Min Zhang:** Methodology. **Xia Liu:** Resources, Data curation. **Lu Zhang:** Investigation. **Feizhou Chen:** Writing – review & editing, Project administration, Investigation, Conceptualization.

### 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.

### Data availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2024.122321](https://doi.org/10.1016/j.watres.2024.122321).

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