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Research article

Growing status rather than temperature was more associated with phytoplankton stoichiometry

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ABSTRACT

Phytoplankton growth is regulated primarily by temperature and nutrient availability. Due to the increasing trend of global warming and eutrophication, it is important to unravel the responses of phytoplankton to varying temperatures and nutrients. This study investigated the interactive effects of temperature (15 °C vs 25 °C) and nitrogen/phosphorus availability (N/P ratios: 13–77) on phytoplankton stoichiometry and community assembly in subtropical reservoir communities. We assumed that (1) Temperature effect on stoichiometry would intensify under nutrient limitation due to altered metabolic demands. Phosphorus limitation would dominate at higher temperatures through growth rate-mediated utilization; (2) Stoichiometric homeostasis would primarily reflect growth phase rather than thermal regime. Results demonstrated that temperature-nutrient interactions shape cellular stoichiometry through growth dynamics. Biomass increased with warming and nutrient enrichment, particularly under P-repleted conditions. Alkaline phosphatase, acting as a strategy for P-limitation, showed temperature-dependent, phase-specific patterns. Cellular elemental contents exhibited greater thermal sensitivity during the exponential growth, aligning with ribosomal investment demands. The homeostasis of phytoplankton was growth-phase dependent, with stationary-phase communities showing plasticity at 25 °C and stability at 15 °C. Temperature affected the stoichiometry indirectly by adjusting the growth rate and metabolism which changes the nutrient demand and resource allocation within cells. Cyanobacteria dominated warmer treatments through enhanced P-use efficiency. This study highlighted temperature-mediated shifts in nutrient limitation thresholds and homeostasis strategies, which provides evidences for predicting bloom dynamic under eutrophication and climate change in this region.

1. Introduction

Nitrogen (N) and phosphorus (P) are the predominant limiting nutrients for phytoplankton growth. The N/P ratio has been recognized as a criterion to determine nutrient limitation, as proposed by the Redfield ratio (Redfield, 1958). The imbalance of these nutrients could hamper the algal growth and alter the community structure by decreasing production efficiency as nutrients ratio do not match the demand for biomass build-up (Cardinale et al., 2009; Elser et al., 2022). Phytoplankton, however, exhibit stoichiometric plasticity, adjusting their elemental ratios to the provided nutrients (Hessen et al., 2004). This plasticity bridges resource competition and ecological stoichiometry theories (Sterner and Elser, 2002). Ribosomes (P-rich) and proteins (N-rich) impose biochemical constraints on cellular elemental composition (Elser et al., 1996).

Temperature modulates stoichiometry via metabolic scaling (Brown et al., 2004). The temperature-dependent translation efficiency hypothesis proposes that temperature affects cellular stoichiometry by modulating ribosomal efficiency (Toseland et al., 2013; Raven and Geider, 1988). At low temperature, cells invest more P to produce more ribosomes. Conversely, higher temperature favors N-rich photosynthetic investment, reducing P demand and elevating optimal N/P ratios (Wood et al., 2003; Yvon-Durocher et al., 2015). Thomas et al. (2017) proposed that the optimum temperature for growth should be a saturating

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function of increasing nutrients, and they noticed a s-shaped relationship between optimal N/P and temperature, implying that there must be a lower and an upper limit to the optimal N/P ratio of a species.

Besides the direct effects on resource allocation mentioned above, phytoplankton responds to temperature changes at the community level, with changes in the structure of the assemblage (Siegel et al., 2020; De Senerpont Domis et al., 2007; Van de Waal and Litchman, 2020). It was previously thought that cyanobacteria were generally P-limited because they could easily obtain nitrogen through nitrogen fixation. Rising temperature have been reported to shift phytoplankton populations toward N-limitation (Van de Waal and Litchman, 2020). Thus, warming and N limitation could both lead to a cyanobacteria-dominated phytoplankton assemblage. In addition, different taxonomic lineages of phytoplankton exhibit different optimal stoichiometry patterns, which could alter bulk ecosystem C:N:P (Quigg et al., 2003).

The interactions between temperature and nutrient availability have been considered as the most critical factors shaping algal physiology (Raven and Geider, 1988) and modulating cellular stoichiometry (Schaum et al., 2018; Geider and La Roche, 2002). The concomitant effects of warming and eutrophication could increase the likelihood of algal blooms. Such cases would constitute a severe issue in the scenario of global warming with accelerating anthropogenic pressures. We pointed out that the absolute concentration of N and P, rather than their ratio, was the primary factor for the growth of phytoplankton in subtropical reservoirs in an earlier study (Yang et al., 2020). However, how temperature interacts with nutrients availability and affects the structure of phytoplankton assemblages remains unresolved. This study aims to investigate the interactive effects of temperature and different N or P levels on phytoplankton stoichiometry and assemblage structure. Our hypotheses are.

H1. The temperature effect on cellular stoichiometry would differ between nutrient-deplete and nutrient-replete conditions, as temperature would amplify the nutrient-limitation effect by affecting the growth rate and nutrient demand. Lower temperatures increase P-demand due to the greater requirement for ribosomes to compensate for reduced protein synthesis efficiency, while higher temperatures enhance growth rate and P-utilization, exacerbating the P-limitation;

H2. The degree of stoichiometry homeostasis depends more on the growth status than on temperature, with greater plasticity in the stationary phase under warming.

In the climate change scenario, the effect of temperature on algal stoichiometry could have important implications for biogeochemical cycles and for trophic dynamics in aquatic ecosystems. This study could provide insight into phytoplankton responses to eutrophication in the context of global temperature rise and accelerating environmental pollution.

2. Material and methods

2.1. Experimental setup and measurements

Surface water (0.5 m depth) from meso-oligotrophic Liuxihe reservoir (23°45′50″N; 113°46′52″E) was filtered (63 μm mesh) to exclude mesozooplankton. This reservoir, locates at the Tropic of Cancer, is a typical subtropical water body in Guangdong Province, China. Water after filtration through a 0.45 μm membrane was used to measure total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP). Nine treatments crossed three N (42, 52, 62 μmol/L) and three P levels (0.8, 2.2, and 3.3 μmol/L) were created by adding $NaNO_3$ and KH_2PO_4. The N/P ratios are shown in Table 1, which ranged from N-limitation (N/P < 20), co-limitation (20≤N/P ≤ 50), to P-limitation (N/P > 50) (Guildford and Hecky, 2000) with balanced and unbalanced ratios (Gerhard et al., 2019, 2022; Yang et al., 2020). The waterbodies in southern China often show quite low dissolved inorganic phosphorus concentrations (Wang et al., 2011). Thereby, the designed N/P ratios were P-biased with values less than 20. These temperatures were chosen based on the annual mean temperature varied between 19.6 and 21.5 °C in Liuxihe reservoir (Wang et al., 2012). A short-term temperature around the mean temperature (15 °C and 25 °C) were established for the experiment. Triplicate 500 mL flasks (Sarstedt T 175, Germany) were incubated at Low (15 °C) and High temperature (25 °C) in illuminating incubators from 28th June to 11th July 2019. The light intensity was 40 μmol/(m^2•s) with a 12 L: 12 D cycle.

Table 1
Setup of nitrogen (N) and phosphorus (P) levels mesocosm experiments.

Unit (μmol/L)	N/P ratio		
P3 (3.3)	13	16	19
P2 (2.2)	19	24	28
P1 (0.8)	52	65	77
	N1 (42)	N2 (52)	N3 (62)

The growth condition of phytoplankton in each treatment was evaluated by optical density at 680 nm (OD_{680}) kinetics. Samples were taken at the exponential phase (t1) and stationary phase (t2) based on the growth curves (Fig. S1). Alkaline phosphatase activity (APA) was estimated according to Hoppe (1983). Samples for cellular elemental measurements were filtered through 1.2 μm pore-size, pre-combusted (400 °C) glass filter membranes (Whatman GF/C). Cellular phosphorus (P) was measured using phosphorus-ammonium molybdate spectrophotometric method. Cellular carbon (C) and nitrogen (N) were measured using a CHN Elemental Analyzer (Vario EL). Phytoplankton organisms were enumerated to the finest taxonomic level under an inverted microscope (×40 magnification) after Lugol's fixation (Lund et al., 1958). Algal biomass of dominant species was estimated through cell size and morphology, and assuming a cell density of 1 g/cm^3 (Hillebrand et al., 1999).

2.2. Statistical analysis

The OD_{680} was used as a surrogate for phytoplankton abundance or biomass. To evaluate the effects of nutrient addition, log-response ratio (LRR) was calculated as ln (OD_f/OD_i), where OD_f is the OD_{680} value at the end of the bioassay, and OD_i is the initial OD_{680} value (Gerhard et al., 2019). Exponential phase was defined as the maximum increase of OD_{680} and stationary phase was the day with less than 5 % daily biomass change. Based on the OD_{680} values, samples were taken on the 7th and 14th day, which were regarded as the exponential phase and stationary phase, respectively (Fig. S1). Analysis of variance (ANOVA) tests were used to detect the differences in growth rates and cellular stoichiometry among treatments. A linear regression model was applied to analyze the relationship between cellular elemental contents and supplied nutrients. Permutational Multivariate Analysis of Variance (perMANOVA) was performed to test the significant differences among phytoplankton community structures using Bray-Curtis dissimilarities (Anderson, 2001). Non-metric multidimensional scaling (NMDS) was performed to visualize relationships between samples based on similarity/dissimilarity of phytoplankton communities along N or P levels. All statistical analyses were performed in R using package 'vegan' (Oksanen et al., 2024).

3. Results

3.1. Growth status of phytoplankton and APA

LRR increased with the enrichment of supplied N or P (Fig. 1) and differences in the LRR between temperature treatments were analyzed by ANOVA tests. Within the same temperature, there were significant differences among P (P1, P2 and P3) treatments ($p < 0.01$), and weakly significant differences among N treatments ($p < 0.05$). ANOVA tests

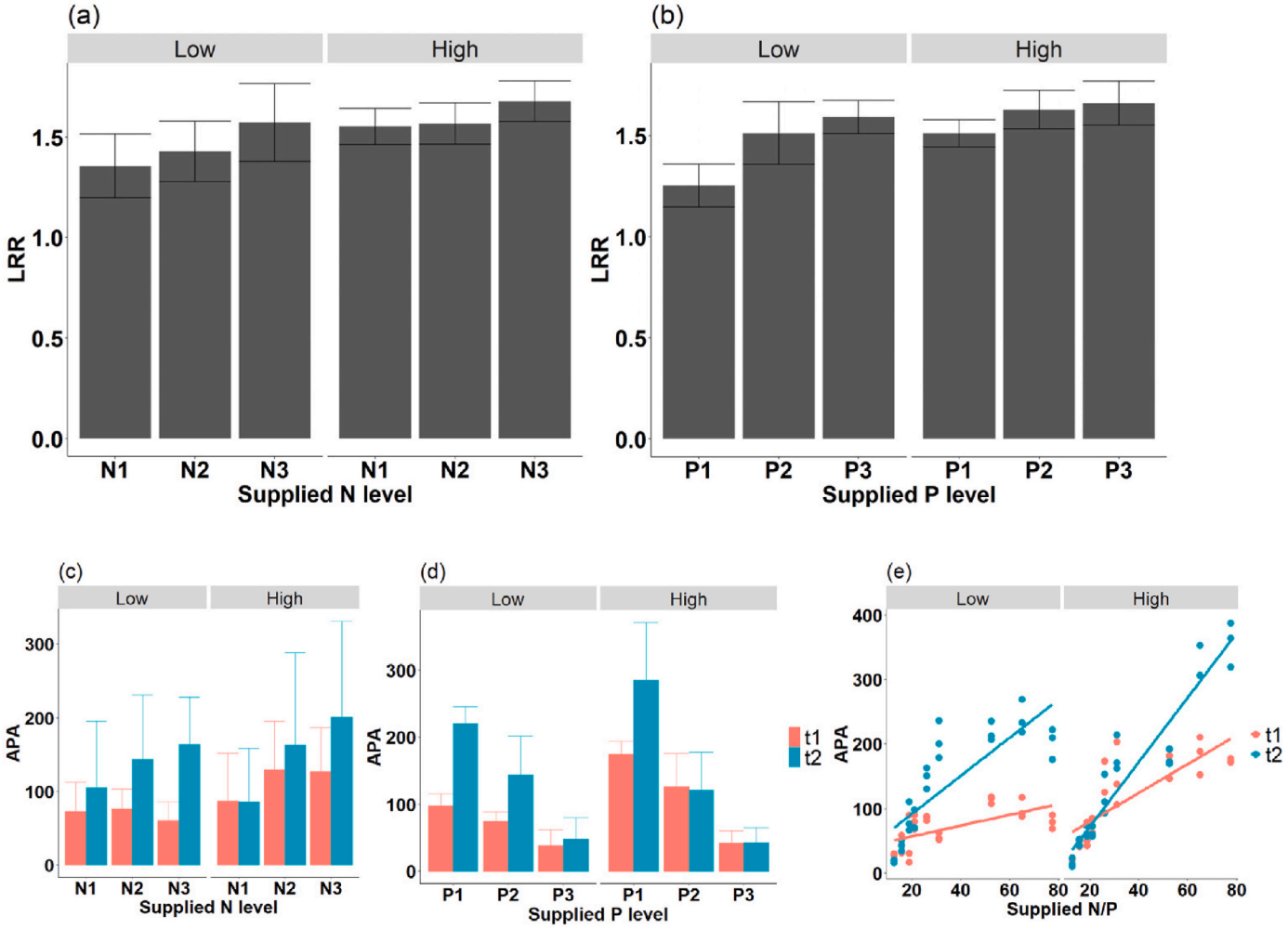

Fig. 1. Log-responseratio (LRR) for phytoplankton in treatments along levels of supplied N (a); and supplied P (b); Alkaline phosphatase activity (APA) in treatments with different levels of supplied N (c) and P (d), and N/P (e) ratio at two temperatures (Low:15 °C and High: 25 °C, red and blue lines refer to the linear regression model fitting values from t1: exponential phase and t2: stationary phase).

showed significant differences between low and high temperature when N level or P level was low (N1 and P1). At intermediate supplied N levels (N2), a weak significant difference was observed between temperatures ($p=0.03$). Moreover, the interactive effect of temperature and supplied P on LRR was significant ($p=0.03$), while no significant interactive effect of temperature and supplied N on LRR was observed ($p=0.61$).

Generally, APA was greater at high temperature than at low temperature in the exponential phase ($p<0.01$), and it was greater in the stationary phase than in the exponential phase at low temperature ($p<0.01$). Under both temperatures, APA showed significant variations with P levels. There was no significant difference in APA between two temperatures or phases among supplied N levels. Under low temperature, APA was significantly different between two phases within P1 and P2 treatments, whereas similar APA was detected in P3. Under high temperature, APA did not increase from the exponential phase to the stationary phase in P2 and P3 (Fig. 1).

3.2. Cellular elemental contents

Within each temperature, only cellular C showed significant difference ($p<0.01$) between the exponential phase and the stationary phase (Fig. 2). Cellular N and P, however, did not exhibit significant difference between the two phases ($p>0.01$). Within each phase, significant differences were noticed on cellular elemental contents between two temperatures (C: $p=0.01$; N: $p<0.001$; P: $p=0.08$) during the exponential phase. Only cellular C and N showed significant difference between two temperatures during the stationary phase ($p<0.001$). Within the same supplied N treatments during the exponential phase, cellular C, N and P were significantly higher at high temperature than at low temperature ($p<0.01$).

Within the same supplied P level, no significant differences were noted between the two temperatures during the exponential phase ($p>0.05$). Within each temperature and phase, the supplied N had no significant effects on cellular elemental concentration. Conversely, the supplied P showed significant effects ($p<0.001$) on cellular elemental contents during both phases at low temperature. At high temperature, significant effects were only observed during the exponential phase. During the stationary phase, supplied P only affected cellular P significantly ($p<0.001$). Pairwise comparison suggested that significant differences on cellular P were detected among supplied P levels ($p<0.01$). For cellular C and N, varying patterns were shown. At 15 °C, cellular C and N differed between P1 and P2, P1 and P3. At 25 °C, such patterns were only shown during the exponential phase.

3.3. Phytoplankton stoichiometry

Within each temperature, cellular stoichiometry exhibited larger variation in the stationary phase than in the exponential phase (Table 2). Cellular C/N and C/P differed significantly between the two phases ($p<0.001$), while cellular N/P only showed weak significant variations

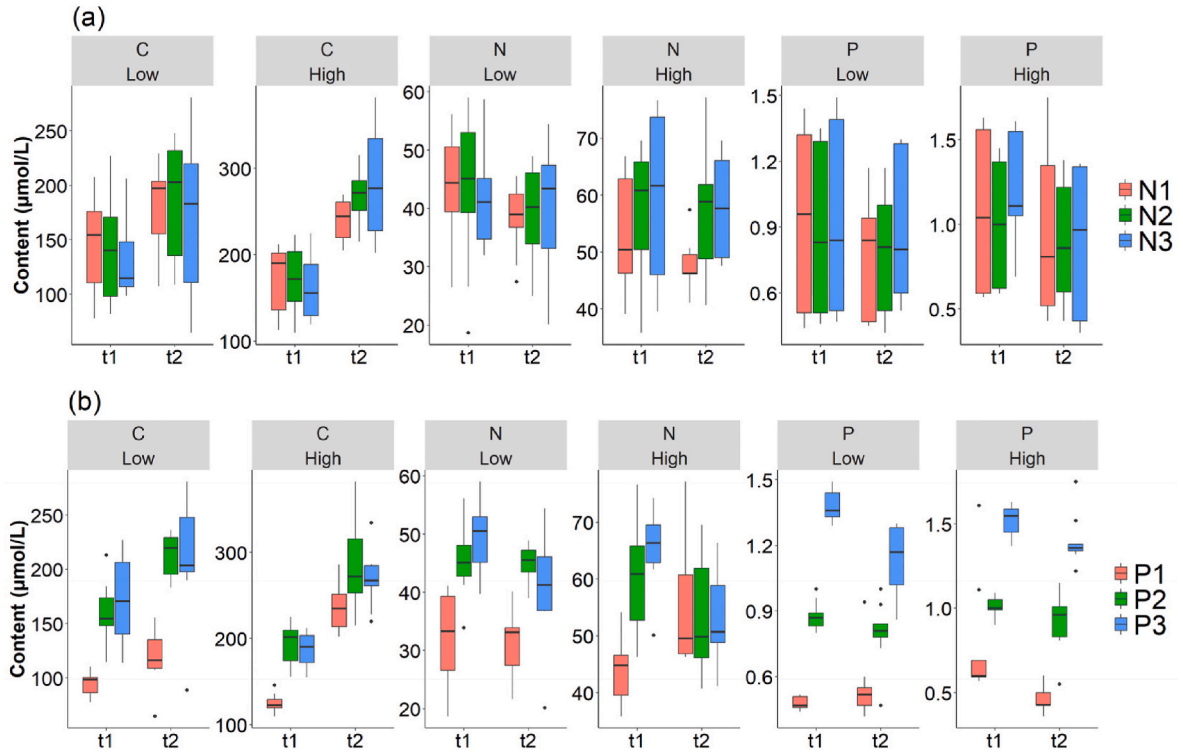

Fig. 2. Cellular C, N, P contents with different levels of supplied N (a) and P (b) during the exponential phase (t1) and the stationary phase (t2) at the two experimental temperature values (Low:15 °C and High: 25 °C).

Table 2
Cellular stoichiometry (C/N, C/P and N/P) features (t1: exponential phase; t2: stationary phase).

Temperature	Low (15 °C)		High (25 °C)	
Phase	t1	t2	t1	t2
C	141.08 ± 43.39	180.79 ± 58.10	168.87 ± 36.76	264.55 ± 47.08
N	42.82 ± 10.21	38.86 ± 8.61	56.73 ± 11.80	54.14 ± 9.76
P	0.91 ± 0.38	0.82 ± 0.28	1.10 ± 0.38	0.93 ± 0.41
C/N	3.33 ± 0.73	4.57 ± 0.79	3.02 ± 0.60	4.93 ± 0.60
C/P	167.53 ± 44.14	232.46 ± 77.53	165.91 ± 45.12	345.33 ± 150.27
N/P	51.99 ± 16.30	51.59 ± 16.64	55.96 ± 16.27	73.53 ± 40.33

between the two phases at high temperature ($p=0.04$). During the exponential phase, cellular C/N, C/P, and N/P did not vary significantly between the two temperatures. On the contrary, during the stationary phase, cellular C/P and N/P showed significant difference between the two temperatures (Fig. 3a).

The supplied N did not significantly affect the cellular stoichiometry (C/N, C/P, and N/P), regardless of temperature or phases (Fig. 3b). Supplied P, however, did not affect cellular C/N during the exponential phase. Cellular C/P and N/P were associated with supplied P (Fig. 3c).

The degree of phytoplankton stoichiometric homeostasis exhibited various patterns between the two temperatures and the two phases along different levels of supplied N or P (Fig. 3d). Cellular N/P exhibited different patterns in response to the different levels of supplied P along the N/P gradients between two temperatures, with relative stable values under low temperature and a greater variability under high temperature (Fig. 3f). The degree of homeostasis of cellular N/P was similar for the exponential phase with low variation at both temperatures (mean value around 52 and standard variation about 16). During the stationary phase, however, cellular N/P was plastic at high temperature, with the mean value of 73.53 and standard deviation of 40.33 (Table 2). When supplied P decreased or the supplied N and P were unbalanced (N/P getting higher), the cellular N/P varied with supplied N/P at high temperature, indicating plasticity. On the contrary, the homeostasis of cellular N/P was similar within different supplied N treatments (Fig. 3e).

3.4. Phytoplankton community diversity and structure

ANOVA tests showed significant differences in the Simpson index, Shannon index and evenness among the supplied P treatments ($p<0.01$), with values increasing along the supplied P levels, whereas no significant variations were detected among supplied N treatments (Fig. S2).

Phytoplankton abundance increased from the exponential phase to the stationary phase without significant variations. Significant differences were observed between the two temperatures on the abundance of phytoplankton and Cyanobacteria. Phytoplankton responded to nutrient amendments at different temperatures with variations in the relative abundance of species in the assemblage, dominated by Cyanobacteria, Chlorophyceae and Bacillariophyceae (Fig. 4a and b). Diatoms increased their proportions from the exponential phase to the stationary phase with relatively stable percentage in the assemblage. Green algae, decreased their share in the assemblage from the exponential phase to the stationary phase (Fig. 4c and d). The percentages of Cyanobacteria at high temperature were greater than at low temperature. The proportion of green algae and diatoms declined at high temperature, while the relative abundance of dominant groups varied significantly between two

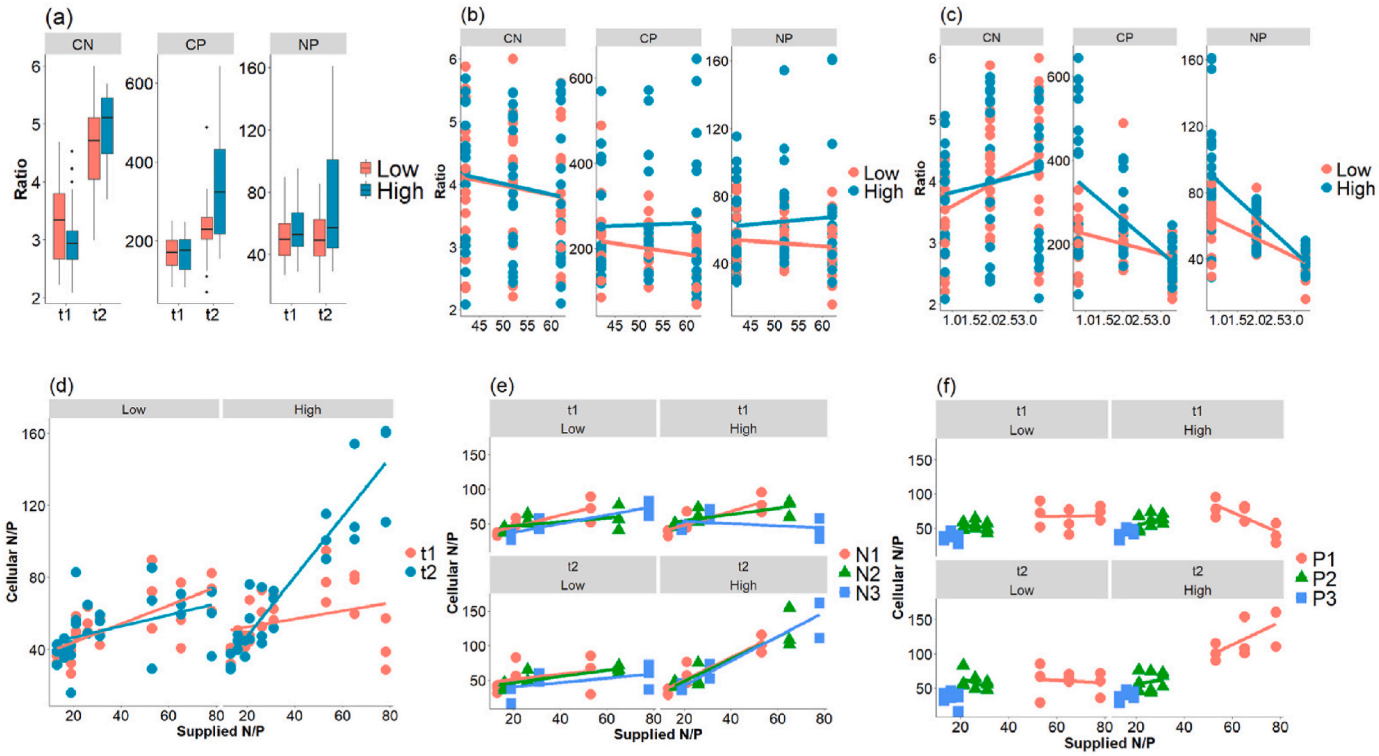

Fig. 3. Cellular C/N, C/P, and N/P variation at the two experimental temperatures during two growth phases (t1: exponential; t2: stationary) (a); along the supplied N (b); supplied P (c); and supplied N/P levels (d) at the two experimental temperature (red and blue lines refer to linear regression model fit values from t1 and t2, respectively); and stoichiometric homeostasis of algal N/P by supplied N levels (e); and supplied P (f) levels symbolizing by different shapes and colors, the corresponding lines are linear regression between cellular N/P and supplied N/P by supplied N and P (Low:15 °C and High: 25 °C, t1: exponential phase; t2: stationary phase).

temperatures during the stationary phase ($p < 0.05$). The points on the NMDS plots represent phytoplankton samples from different treatments. Points that are clustered closely together indicate more similar communities, while those farther apart reflect greater dissimilarity. Samples from different supplied N levels (N1, N2, and N3) remained closely grouped at both temperatures, suggesting no significant differences among N levels (Fig. 5a and c). In contrast, phytoplankton communities exhibited distinct shifts along the supplied P levels, with samples forming separated groups (P1, P2, and P3) under both low and high temperature (Fig. 5b and d).

The relative abundance of Cyanobacteria, Chlorophyceae and Bacillariophyceae among supplied P treatments were significantly different ($p < 0.01$), but such pattern was not present among supplied N levels. PerMANOVA results showed that significant differences were present among supplied P levels at low temperature ($p < 0.01$). At high temperature, weak significant differences were detected among supplied P levels during the exponential phase ($p = 0.03$). Besides, the structure of dominant groups was significantly different among the provided P levels under low temperature, except diatoms during the exponential phase ($p < 0.01$). Under high temperature, only green algae varied on the compositional level (Table 3).

Dominant taxa were *Raphidiopsis raciborskii*, belonging to Cyanobacteria, the green alga *Chlorella* sp., and the diatom *Achnanthes* sp. *R. raciboskii* and *Achananthes* sp. showed variation between the two temperatures ($p < 0.05$). *R. raciborskii* did not exhibit significant variations along provided N levels, while significant differences were detected along provided P levels at low temperature ($p < 0.01$) (Fig. 6a and b). Weak significant differences ($p < 0.05$) in the abundance of *Chlorella* sp. were found along the provided P levels (Fig. 6c and d). *Achnanthes* sp. did not respond to the provided N levels, whereas its abundance in P1 treatment was significantly lower than in the other two treatments (P2 and P3) in the stationary phase (Fig. 6e and f).

4. Discussion

4.1. Interactive effects of P and temperature

The results demonstrated complex interactions between nutrient and temperature in this typical P-deficient subtropical reservoir. P was the critical nutrient for phytoplankton growth in our study rather than N, as indicated by the stronger response to P enrichment. The interactive effect of temperature and P on LRR implies that warming amplifies P-limitation in this waterbody. This finding aligns with the P-limitation status observed in Liuxihe reservoir (Zou et al., 2014). At low temperature, P-demand increased due to more ribosome synthesis required to compensate for decreased translation efficiency (Elser et al., 1996). At high temperature, along with increasing P utilization efficiency, the rapid growth also consumed P, leading to a relatively stable degree of P limitation. P2 inducing a different response of APA (limitation at low temperature while less limitation at high temperature) implied the effect of temperature altering nutrient limitation thresholds. Thus, these observations supported our hypothesis (H1) that P limitation could amplify temperature sensitivity, particularly at low temperature. Furthermore, temperature modulated phytoplankton growth by affecting the threshold of nutrient limitation, which was consistent with findings by Mackay et al. (2020).

4.2. Effects of temperature and nutrient on cellular elemental

Cellular elemental contents and stoichiometry exhibited temperature-dependent plasticity. Temperature-driven acceleration of growth rate and metabolic processes was reflected by higher cellular elemental contents at high temperature. The significant increase in cellular C during the stationary phase may indicate enhanced carbon storage under stress with unbalanced nutrient supply (Klausmeier et al.,

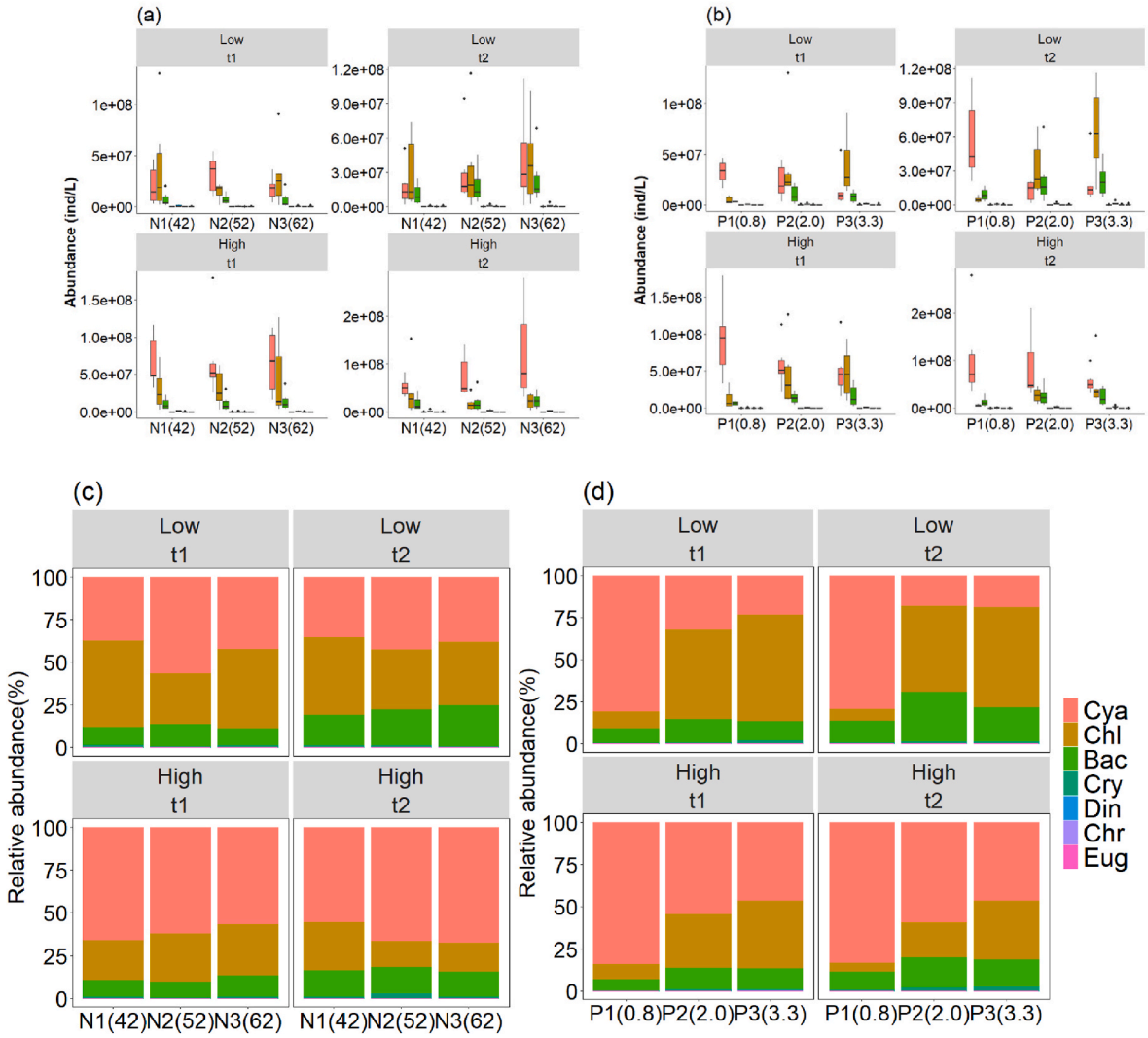

Fig. 4. Absolute abundance of phytoplankton at different supplied N (a) and P (b) levels, and relative abundance at different supplied N (c) and P (d) levels (Cya: Cyanobacteria; Chl: Chlorophyceae; Bac: Bacillariophyceae; Cry: Cryptophyceae; Din: Dinophyceae; Chr: Chrysophyceae; Eug: Euglenophyceae; Low:15 °C and High: 25 °C, t1: exponential phase, t2: stationary phase).

2004). The supplied P effect, however, was significant at low temperature due to feedback modulation. The higher P demand for metabolism at low temperature occurs when the low efficiency of ribosomes is compensated by a greater number of P-rich ribosomes. At high temperature, a significant P effect was only present in the exponential phase. This was because fast growth rate with large consumption still requires a large amount of P to support rapid growth (Fig. 7a). When phytoplankton entered the stationary phase, correlations were only showed between supplied P and cellular P, suggesting variation in the elemental allocation strategy. Even though the supplied P concentration was above the threshold of limitation, due to luxury uptake of P, and the element was rapidly consumed and P-limitation in the cultures appeared as indicated by the APA. Thus, supplied P was the primary critical factor for phytoplankton growth in this study due to unbalanced levels of supplied N and P.

Based on the thermal-dependent performance curve, the nutrient limitation would promote a lower optimal temperature for organisms (Baker et al., 2016; Bestion et al., 2018). Moreover, temperature could induce a shift in the optimal supply N/P (Thrane et al., 2017). The sensitivity to temperature increased when nutrients were deficient, implying that temperature effects were magnified under low nutrient conditions. On the contrary, when nutrients were sufficient, temperature effects declined as no significant difference was observed on cellular elemental structure. These findings were inconsistent with the study by Marañón et al. (2018), which reported a weakened temperature control on growth under low nutrient conditions in individual metabolic rates. Whereas our study measured the effects on community level, emphasizing that thermal modulation of cellular stoichiometry was nutrient-dependent. These results were consistent with our hypothesis (H1) (Fig. 7a).

4.3. *Growth rate-dependent stoichiometric plasticity*

Cellular N/P showed a greater extent of homeostasis, with a minor alteration only at high temperature. While cellular C/N and C/P exhibited plasticity in each phase, suggesting the variations mainly resulted from P dynamics. The non-significant temperature effect on cellular stoichiometry observed in the exponential phase, implies a stronger growth rate dependency of stoichiometry. Relative fast growth rate and metabolism highlighted the dominant role of biosynthesis processes in such conditions. The P allocation strategy was P-rich ribosome production (Sterner and Elser, 2002). When algae entered stationary phase, the growth rate declines due to consumption of P and increased photosynthesis. Therefore, the cellular N/P was constrained during the exponential phase, leading to a high degree of homeostasis. During the stationary phase, various extents of homeostasis occurred at

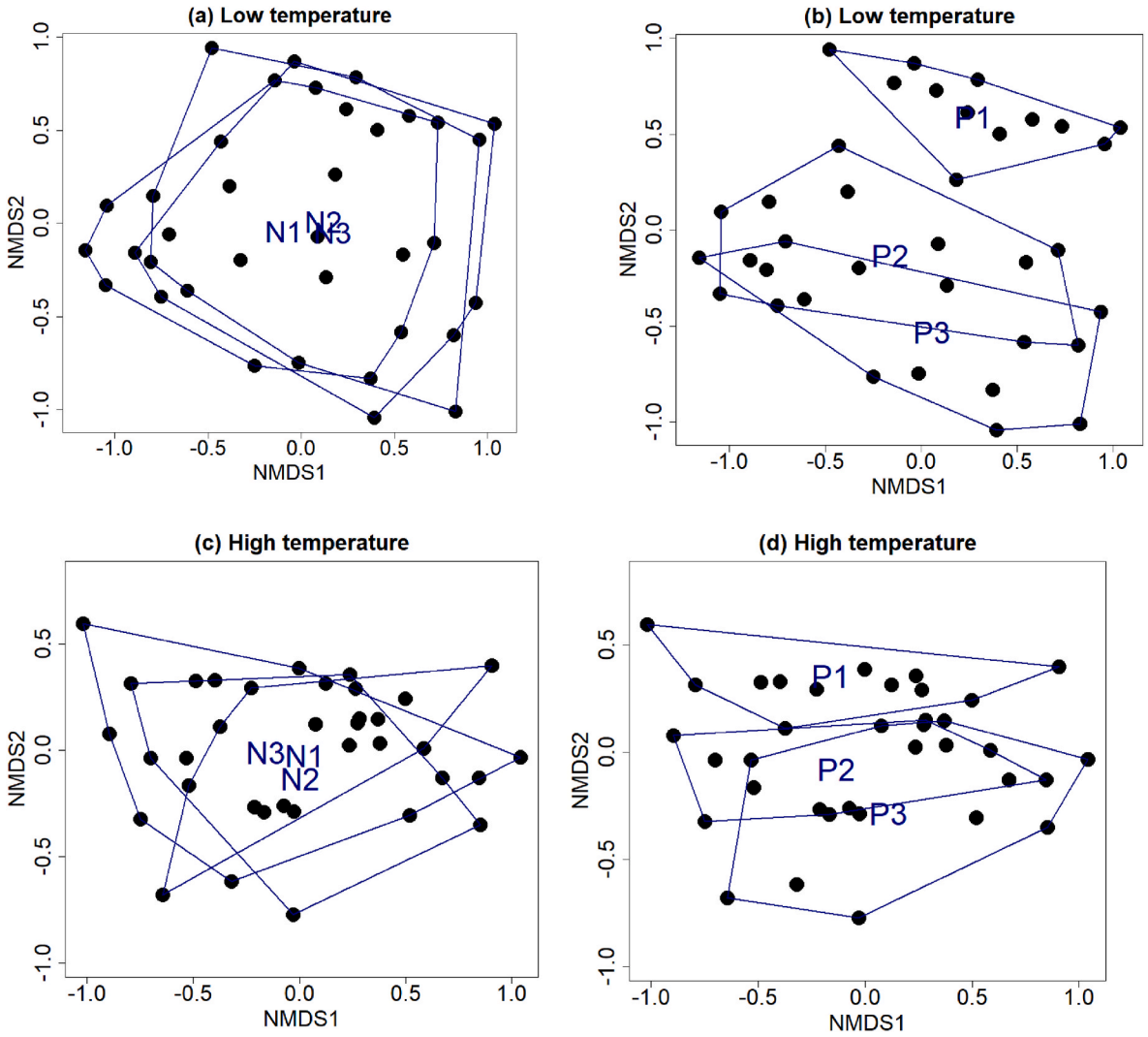

Fig. 5. NMDS plots of the phytoplankton community at low temperature along N (a) and P (b) levels at low temperature, and along N (c) and P (d) levels at high temperature (Low:15 °C and High: 25 °C).

Table 3
PerMANOVA on assemblage composition among supplied N and P levels (t1: exponential phase; t2: stationary phase; * refers to the significant difference with $p < 0.05$).

Temperature	15 °C		25 °C	
Phase	t1	t2	t1	t2
Phytoplankton	0.001*	0.001*	0.03*	0.09
Cyanobacteria	0.004*	0.001*	0.09	0.27
Chlorophyceae	0.001*	0.008*	0.02*	0.005*
Bacillariophyceae	0.10	0.006*	0.05	0.07

different temperatures. Temperature could affect growth and metabolic rate, causing changes in ribosomal translation rates and resulting in a variation of cellular stoichiometry. These results supported our hypothesis (H2), that stoichiometry was mainly driven by the growing status, which was constrained by P. Cellular stoichiometry was plastic in the stationary phase at high temperatures (Fig. 7b). High temperature induced metabolic kinetics with increasing efficiency and nutrient consumption, causing variations in stoichiometry. The homeostasis was primarily determined by growth status via modulating the cellular resource allocation, while temperature changed metabolism and indirectly affected nutrient allocation.

4.4. Synergistic effects of temperature and P on phytoplankton assemblages

Besides the effects on growth and biomass, a selective effect was observed on the phytoplankton assemblage structure and diversity. In our experiment, the dominance of Cyanobacteria (*Raphidiopsis raciborskii*) at high temperatures underscores their competitive advantages in warmer (e.g. Paerl and Huisman, 2009; Sukenik et al., 2012), consistent with the findings from the experiment by Schabhüttl et al. (2013). P-limited environments, consistent with their known thermal tolerance, and ability to regulate buoyancy for nutrient acquisition and produce AP (Wu et al., 2009, 2012; Bai et al., 2014). A meta-analysis suggested that its correlation with N/P was stronger in the meso-to eutrophic Lake Kinneret, and lower in the eutrophic Lake Paranoa (Recknagel et al., 2019). This study also highlighted that in subtropical and tropical lakes, *R. raciborskii* shows a weaker correlation with water temperature due to its all-year-round presence. The effect of temperature on the tolerance of nutrient deficiency provided *R. raciborskii* with competitive advantages in P-limited conditions (Galvanese et al., 2019). Conversely, the decline in Chlorophyceae and Bacillariophyceae at high temperature suggests their reduced fitness due to higher metabolic costs or inferior P-acquisition strategies. *Chlorella* sp. can exhibit mixotrophic behavior in the presence of organic carbon (Rincon et al., 2019). Algae belonging to this genus can accumulate and store nutrients within their cells for further

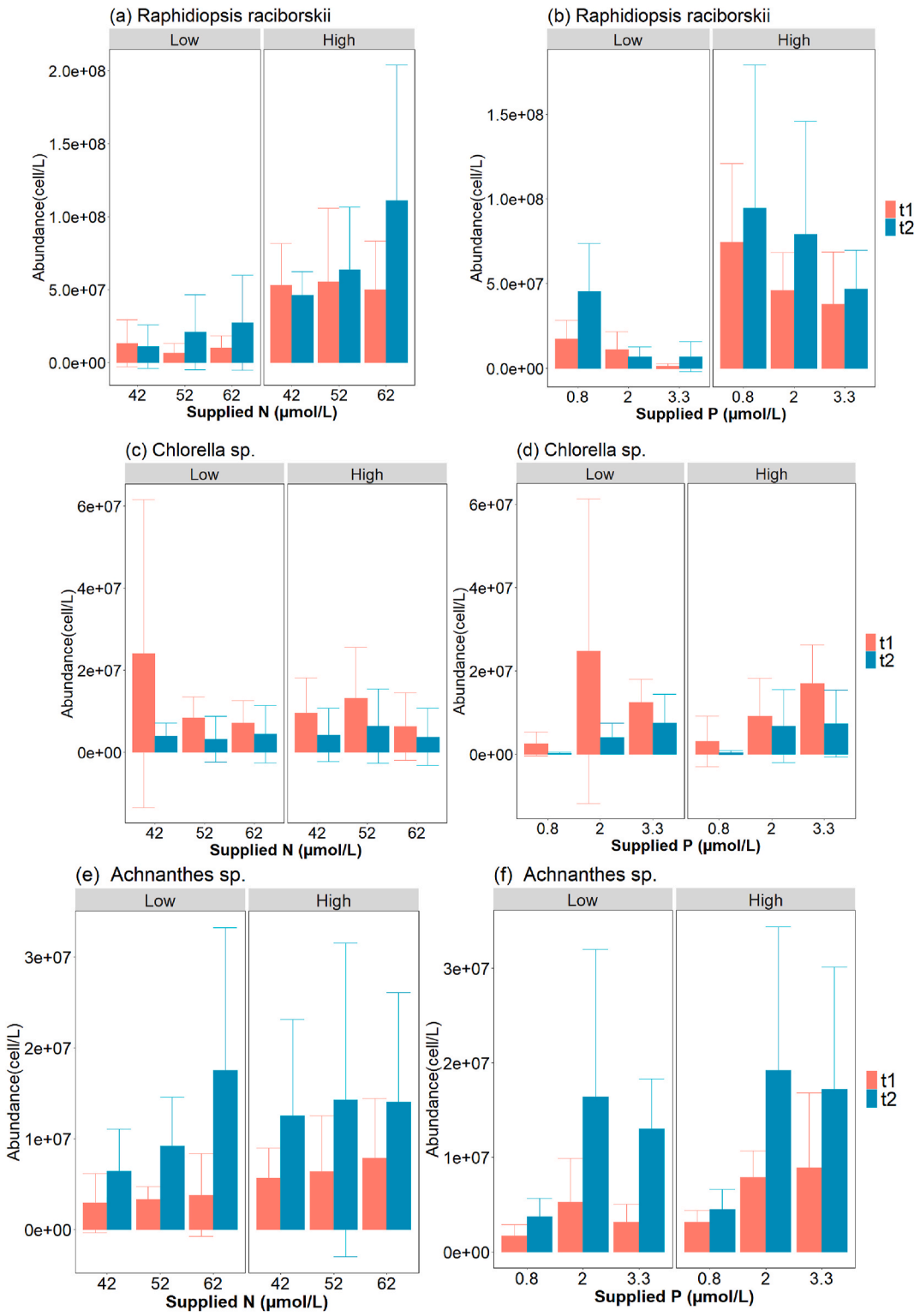

Fig. 6. Abundance of dominant taxa in Cyanobacteria (a and b), Chlorophyceae (c and d), and Bacillariophyceae (e and f) along different levels of supplied N and P (Low:15 °C and High: 25 °C, t1: exponential phase; t2: stationary phase).

use. This can explain their declining abundance along the decreasing nutrient levels. *Achnanthes* sp. did not increase in the high temperature treatments due to a relatively lower optimal temperature. Besides, the significant differences among P treatments further emphasize P as a key factor of assemblage composition, particularly under low temperature. The increased diversity indices with P enrichment imply the role of P in structuring subtropical phytoplankton communities via niche differentiation.

5. Conclusions

This study demonstrated that warming amplifies P limitation in subtropical reservoirs, driving shifts in phytoplankton stoichiometry, enzymatic activity, and community structure. Phosphorus was the

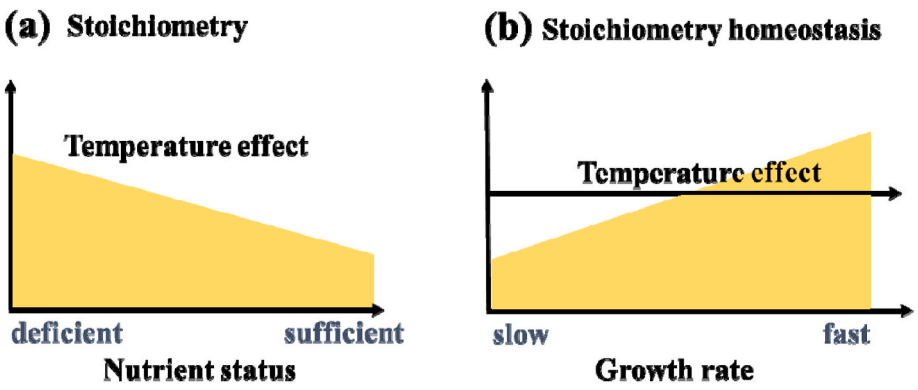

Fig. 7. Illustration of two hypotheses: temperature-dependent hypothesis: Temperature effect on stoichiometry is stronger under nutrient-deficient conditions than nutrient-sufficient conditions (a); and Growth-status-dependent hypothesis: stoichiometry homeostasis is more affected by growth rate rather than temperature (b).

primary factor for phytoplankton community growth and structure in these reservoirs. The plasticity under high temperature highlights adaptive strategies to nutrient imbalance, favoring Cyanobacteria dominance. Stochiometric homeostasis was mainly associated with growth status. Temperature involves in stoichiometric plasticity through affecting the growth rate and indirectly altering nutrient requirement and allocation dynamics. This study underscores the vulnerability of subtropical reservoirs to synergistic effects of eutrophication and climate change. The long-term acclimation of phytoplankton in this region should be considered for water quality management in the future.

CRediT authorship contribution statement

Yang Yang: Writing – original draft, Visualization, Supervision, Conceptualization. **Qinglan Chen:** Writing – original draft, Software, Methodology, Data curation. **Jingyun Pan:** Validation, Methodology, Data curation. **Yingliang Liu:** Writing – review & editing, Supervision, Resources, Project administration. **Luigi Naselli-Flores:** Writing – review & editing, Methodology.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.125175.

Data availability

Data will be made available on request.

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