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The effects of absolute and relative nutrient concentrations (N/P) on phytoplankton in a subtropical reservoir

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ABSTRACT

The elemental composition of phytoplankton is a critical factor for primary production and nutrient recycling. The increase anthropogenic nutrient input into freshwater ecosystems is affecting phytoplankton assemblage structure and its stoichiometry. Reservoirs of South China generally show low level of phosphate and it is not clear how phytoplankton can grow and occasionally bloom in such conditions. Therefore, an indoor experiment was conducted to investigate the response of natural phytoplankton communities to 25 levels of supplied nitrogen to phosphorus ratios (N/P), arising from the combination of 5 levels of N and P. Our aim was to check the effects of absolute and relative N and P on phytoplankton growth and structure. We assumed that Alkaline Phosphatase (APA) provides a way to use alternative P resource. Our hypotheses include: (1) phytoplankton stoichiometry would be in homeostasis (*sensu* Sterner & Elser, 2002) under different N and P treatments; (2) absolute nutrient values rather than its ratio matters for phytoplankton assemblage; and (3) phytoplankton cell size declines to lower P requirements facing P limitation. Results showed that phytoplankton in this subtropical reservoir use alternative P sources via APA to support its growth. The absolute values of N and P instead and not their ratios were important for phytoplankton. The N/P ratio cannot be a reliable indicator for nutrient limitation or phytoplankton shift. During the fast-growing phase, their elemental contents were not sensitive to supplied nutrients, and their stoichiometry was more constrained compared to that in slow-growing phase. The plasticity of cellular stoichiometry was mainly due to the variations of cellular P. Phytoplankton stoichiometry was weakly homeostatic, and this mechanism provides a strategy to keep growth relatively stable in a variable nutrient environment.

1. Introduction

Several papers dealing with phytoplankton ecology consider not only the concentration of nutrients, but also their ratio as critical for phytoplankton development. Inland waters, such as lakes and reservoirs, are suffering from eutrophication (e.g. the rapid increase in their primary production) at an unprecedented rate. In spite of this, nitrogen (N) and phosphorus (P) are often considered limiting nutrients for primary production since they are essential for protein and nucleic acids synthesis and ATP production. Several attempts to explain phytoplankton composition based on water nutrient ratios are available in the literature since when Redfield (1958) noted that the chemical composition of phytoplankton was statistically stable and uniform according to the ratio 106C:16 N:1P. According to this author, the ratio reflected “the chemical of the water from which materials are withdrawn and to which they are returned”. The development of ecological stoichiometry (Sterner and Elser, 2002), further pointed out the importance of the Redfield ratio in the autotrophs, even though a high variability in the C:N:P composition has been found to occur at both inter- and intra-specific levels and through time (Reynolds, 1999; Elser et al., 2000). Phytoplankton, due to their small size and fast reproduction, can respond quickly to environmental changes and eventually alter their cellular C/P, N/P in response to large variations of P availability, a phenomenon called stoichiometry plasticity (Hessen et al., 2004; Persson et al., 2010). However, several organisms have been regarded as stoichiometrically homeostatic due to the composition of their biomolecules such as RNA and proteins, which show specific and constant elemental ratios (Elser et al., 1996). This homeostasis has been thought to cause mismatches between the environmental nutrient availability and the somatic nutrient contents.

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The different morphological and physiological traits of single phytoplankters, depending on the environmental template, can determine the competitive success of a species over another or impose fundamental constraints to growth. In particular, cell size, as the master trait, can reflect important differences in cellular physiology, such as the ability to uptake and store nutrients (Reynolds, 2006). Phytoplankton metabolism was found to be allometrically scaled with cell size and to decrease at increasing cell size (Marañón, 2015). If one nutrient limits growth, this might inhibit the uptake of other nutrients and thereby affect phytoplankton elemental composition.

In subtropical reservoirs of South China, the concentration of dissolved inorganic phosphorus is generally low, leading to a high N:P ratio and to a supposed phosphorus limitation (Wang et al., 2011; Zhou et al., 2013; Zou et al., 2014). Phytoplankton in such aquatic ecosystems, however, could still grow and sometimes form a bloom. Therefore, either the nutrient ratio is not able to predict phytoplankton structure and growth or phytoplankton in such waterbodies should possess strategies to deal with such low phosphorus conditions.

As pointed out by Reynolds (1999), a frequent erroneous application of the resource-ratio hypothesis is to consider nitrogen as a limiting factor at low ratios of N:P and phosphorus as limiting in consequence of a high ratio, even when the absolute concentrations of each of these elements is well above any limiting threshold as set by the species-specific rates of uptake. Moreover, several strategies adopted by phytoplankton to cope with low phosphorus concentrations have been reported in the literature; these include 1) a high-affinity P uptake system (Scanlan et al., 2009); 2) luxury uptake of P, i.e. the ability to store P (Geider and Rocher, 2002); 3) low and variable species-specific P requirements (Dyhrman et al., 2012); and 4) the ability to use alternative P resources: Alkaline phosphatases (APA) are considered as the most important enzymes to catalyze the hydrolysis of dissolved organic phosphorus (DOP) and fulfill the inorganic phosphorus requirement of phytoplankton (Chrost, 1991). Moreover, due to the luxury uptake and storage of nutrients, cellular elemental contents might not mirror the environmental conditions. Although cytoplasmic structure and organization show a quite high conservatism which may reflect the Redfield ratio, it is not always clear to what extent phytoplankton stoichiometry is coupled to nutrient availability in the surrounding medium (Reynolds, 2006).

Sterner and Elser (2002) developed a method to test the homeostasis of organisms by comparing the slope of organism's stoichiometry to that of the available resource's stoichiometry. In particular, non-homeostasis refers to the elemental contents of organisms that strictly reflect their resource uptake; this content always matches available nutrients. Conversely, homeostasis occurs when organism's stoichiometry exhibited constant values. Fig. 1 shows conceptual model relating nutrient availability and cellular stoichiometry: the degree to which organism regulates their elemental contents reflects the strength of homeostasis and is represented by the slope of the line. A line between strict homeostasis (e.g. a constant cellular stoichiometry irrespective of nutrient availability) and non-homeostasis indicates adjustment in response to supplied nutrients. In the scenario of increasing anthropogenic-driven nutrients fluxes into aquatic ecosystems, concentration of nutrients and nutrient imbalance would affect the elemental contents of phytoplankton. This could cause changes in consumer community and nutrient cycling in the system as it determines the quantity and quality of food for zooplankton.

In this study, we conducted an indoor experiment using natural phytoplankton assemblages from a subtropical reservoir. By adjusting N and P concentrations, our aim was to investigate the effects of variable N and P concentrations and of their ratios on phytoplankton. We assumed that APA production provided an important strategy for P-limitation in the subtropical reservoir. Our hypotheses included: (1) the stoichiometry (N/P) of phytoplankton would be restricted within a certain range due to their homeostasis (Fig. 1); (2) the absolute value of N and P rather than their ratio (N/P) determine the phytoplankton structure as a consequence of the different competitive abilities of species; (3) cell size decreases in the condition of nutrient limitation.

2. Materials and methods

Water samples were collected from Liuxihe (LXH) reservoir, a subtropical reservoir at the Tropic of Cancer, in Guangdong province, China. After filtration through 63 μm mesh to remove large zooplankton, water was distributed into 500 mL cell culture flasks (Sarstedt T175, Germany). Based on the original dissolved nitrogen and phosphorus concentration, additional nitrate and phosphate were added to adjust the N/P gradients. Five levels of supplied N (ranging from 41.1 μmol/L to 82.1 μmol/L) and five levels of supplied P (ranging from 0.8 μmol/L to 3.3 μmol/L) were established to create 25 levels of N/P ratio (Supplementary material). Cultures started on Feb 20th and lasted till Mar 13th 2019. The temperature was 25 °C and light intensity was 40 μmol/ (m^2-s) with a 12 L: 12D cycle. Phytoplankton growth was evaluated by measuring optical density (OD) at 680 nm. Samples for cellular content of C, N and P, APA, phytoplankton composition, and cell size of dominant species were taken once at exponential phase and once at stationary phase. Samples for cellular elemental concentrations were filtered through pre-combusted Whatman GF/C glass filter membranes with pore size of 1.2 μm. Cellular P concentration was measured using phosphorus-ammonium molybdate spectrophotometric method. Cellular C and N were measured using an Elemental Analyzer (Vario EL). APA activity was estimated using fluorogenic substrate analogues according to Hoppe (1983). Phytoplankton was enumerated according to Lund et al. (1958) in Utermöhl chambers after Lugol's fixation using an inverted microscope. Simultaneously, cell size of dominant species was measured. Biomass was estimated based on the morphology of cells and assuming a cell density of 1 g cm^{-3} (Hillebrand et al., 1999).

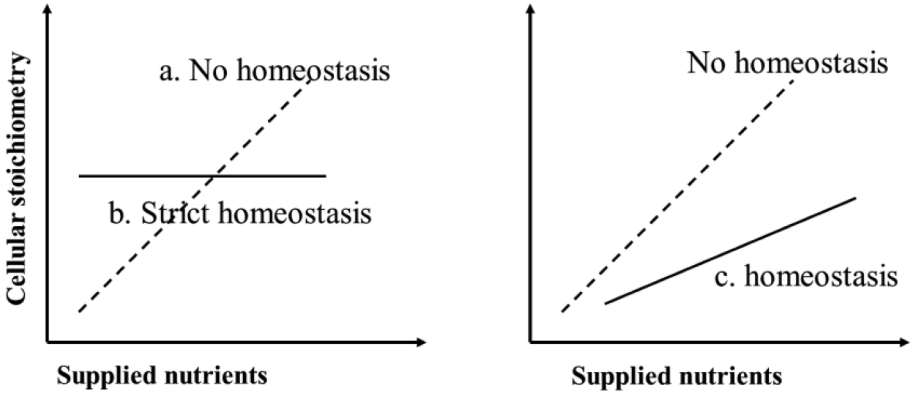

Fig. 1. Generalized stoichiometric patterns about phytoplankton stoichiometry to supplied nutrients (revised from Sterner & Elser, 2002). a. points on the 1:1 line (dashed line) represent no homeostasis, e.g. elemental composition of organisms match the supplied nutrients; b. organisms show a constant stoichiometry irrespective of supplied nutrients, which indicates strict homeostasis (any horizontal line); c. a line with a slope between 0 and 1 represents the degree of homeostasis.

2.1. Statistical analysis

Based on the OD values, samples from day 13 and day 21 were chosen to represent the exponential (t1) and stationary (t2) phases of the assemblages (Fig. S1). Analysis of variance (ANOVA) tests were used to detect the differences of cellular stoichiometry among treatments. Permutational multivariate analysis of variance (PerMANOVA) was applied to investigate the community compositional differences (Anderson, 2001). Redundancy analysis (RDA) was performed to assess how much of the variation in phytoplankton community can be explained by the variation of environmental variables, in this study, the supplied N or P. It provides the information about how community changes with selected environmental gradients (Rao, 1964). The degree of homeostasis was calculated as the slope of the log–log relationship between algal cellular N/P and supplied N/P ratios. This calculated value is the inverse of the regulatory coefficient (1/H), where the regulatory coefficient is denoted as 'H'. therefore, 1/H can be used as an indicator of homeostasis, with values close to 0 as non-homeostasis and 1 as strict homeostasis (Sterner & Elser, 2002). All statistical analyses were performed in R (R core team, 2013).

3. Results

3.1. Growth of phytoplankton

ANOVA tests showed significant differences on the OD values not only among treatments with different N/P ratios, but also among different N or P treatments ($p < 0.01$). However, at the same supplied N concentration, the increase of P in some treatments did not affect the OD values, and vice versa (e.g. at the N1 and P5 concentrations).

APA activity was greater in t2 phase than in t1 phase, indicating a stronger P limitation in the stationary phase. In t2, the values exhibited a positive correlation with cellular C and N and a negative correlation with supplied P and cellular P ($p < 0.001$). Moreover, large variations of APA activity were observed when supplied P was low (Fig. 2).

3.2. Phytoplankton composition and cell size

The major groups in phytoplankton assemblage were Bacillariophyceae, Chlorophyceae and Cyanobacteria. Diatoms represented the dominant group in all the treatments during the whole experimental process. The abundance of total phytoplankton and major groups exhibited increasing trend as supplied N with some minor differences. In t1 phase, abundance increases with supplied P levels, but in t2 phase, the abundance did not change with P levels. Different groups of phytoplankton showed various patterns to different supplied P levels (Fig. 3). The original assemblage was dominated by diatoms, whose abundance accounted for 46% of total phytoplankton biomass. The proportions of Cyanobacteria, Chlorophyceae, Chrysophyceae, Dinophyceae and Cryptophyceae were 29%, 7%, 15%, 3% and 1% of total abundance, respectively. (Fig. S3).

In the exponential phase, among supplied N levels, total abundance, abundance of Chlorophyceae and Bacillariophyceae exhibited significant difference ($p = 0.05$, 0.04, 0.05 respectively). In the stationary phase, among supplied N levels, total abundance and Chlorophyceae abundance showed significant difference ($p = 0.02$ and 0.04 respectively). Among supplied P levels, only Chlorophyceae abundance showed significant difference during the two phases ($p = 0.05$ and 0.04) (Table 1). With increasing supplied N levels, total abundance exhibited increasing trend. Whereas total abundance did not change with supplied P in t2 phase. For cellular contents, there was no specific pattern between total abundance and cellular N, and positive correlation between total abundance and cellular P (Fig. S2).

In the exponential phase, weak significant differences in the phytoplankton and Chlorophyceae composition were detected among supplied N levels ($p = 0.05$). Weak significant differences were observed on phytoplankton assemblage among supplied N and P levels ($p = 0.05$). This was reflected in the RDA biplots of phytoplankton assemblage distribution in different N and P treatments during the exponential phase (Fig. 4a-b). Assemblages separated to some extent in different treatments in t1, whereas they overlapped in the treatments in t2 (Fig. 4c-d). During the stationary phase, there were significant difference on Chlorophyceae and Bacillariophyceae assemblage ($p = 0.01$ and 0.02, respectively). However, significant differences were observed on phytoplankton structure among supplied N/P levels ($p < 0.05$) (Table 2). Indicator species in exponential phase were *Achnanthes* sp., and in the stationary phase were *Scenesesmus bijuga* and *Pseudoanabaena* sp.

Among Cyanobacteria, *Pseudanabaena* sp. and *Aphanocapsa* sp. were the dominant species, with increasing abundance in the treatments with higher supplied N. Both their abundances were greatest at P3 level. *Scenedesmus bijuga,* the most abundant chlorophycean alga in the experiment, showed a lower abundance at decreasing P concentrations and highest abundance at N4 level. *Achnanthes* sp. was the dominant species in all treatments. It showed hump-shaped response to supplied N and U-shaped response to supplied P. *Cyclotella* sp. exhibited increasing abundance with increasing N levels whereas it showed a decreasing trend at declining levels of supplied P in the exponential phase, but no variations in relation to supplied P were recorded in the stationary phase (Fig. 5). No specific pattern was observed on dominant cell size among supplied N and P levels. *Pseudoanabaena* sp. cell size exhibited a decreasing trend with supplied N/P ratio (Fig. 6).

3.3. Cellular elemental composition

Cellular elemental concentrations exhibited different responses to supplied N and P. In t2, internal P was lower than that in t1, corresponding to the lower growth rate. Absolute concentration of supplied N or P did not show significant effects on the cellular contents of P or N in t1. Supplied N did not show significant effects on cellular P, but supplied P had significant effects on cellular N in t2. The absolute concentrations of supplied N and P had significant effects on the phytoplankton cellular N/P ($p < 0.001$). Among cellular elemental contents, only C and N showed a significant positive correlation ($p < 0.01$). Cellular C and N did not change with supplied P in the exponential phase, but decreased with increasing supplied P levels in the stationary phase (Fig. 7).

Compared to cellular C/P, cellular C/N was more constrained and ranged from 4 to 18, close to the Redfield value. Cellular C/P varied as a linear function of supplied P and exhibited large variations. Cellular C/N had insignificant response to supplied P, and showed decreasing trend in t1, but increasing trend in t2 to the supplied N ($p < 0.05$). Cellular N/P was more constrained in t1 [6.8, 69.49] than in t2 [4.46, 81.94]. Homeostasis degree showed that with the supplied N/P ranging between 12 and 103 phytoplankton assemblages were weakly homeostatic (coefficient was about 0.60). The degree of homeostasis varied as the various level of supplied N, but it increased with the supplied P levels, with the greatest homeostasis at lowest P (Fig. 8).

4. Discussions

4.1. Phytoplankton growth and APA activity

In our study, the lowest supplied N concentration was 570 μg/L and P was 20 μg/L. The nutrient concentrations (and their ratios N/P) did not show any limiting constraint to phytoplankton growth. Moreover, different combinations of N and P can result in the same value of N/P and if the supplied nutrients are above the limiting threshold, the N/P cannot be considered as a predictor for phytoplankton growth. Therefore, as already discussed by Reynolds (1999), the absolute value of nutrients, especially when their concentrations are close to or below the limiting threshold, matter for phytoplankton growth, not their ratio.

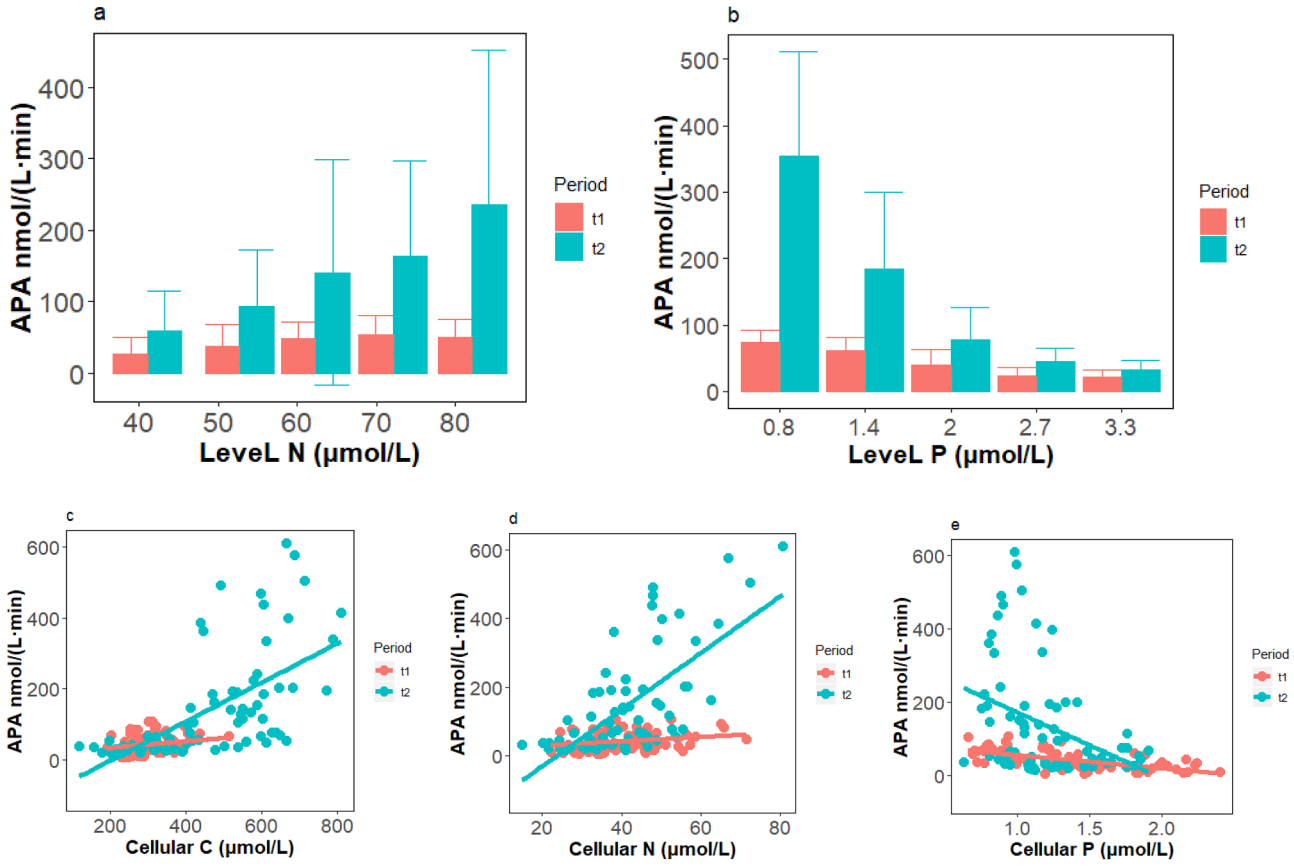

Fig. 2. APA activity in different N and P treatments (a-b) and its relationship with cellular C, N and P contents (c-e) (t1: exponential phase; t2: stationary phase).

In this reservoir (and probably in the majority of cases when nutrients are above the limiting threshold), N/P could not indicate any limitation for phytoplankton growth. Phosphorus concentrations above 3 μg/L (≈0.01 μmol/L) cannot actually be regarded as limiting for many phytoplankton species (Reynolds, 2006).

APA production provided to be an efficient tool to use alternative P source and to support growth when P concentrations in the surrounding medium were low. APA activity indicated P limitation, which resulted from the consumption of P used for phytoplankton biomass accumulation, indicated by cellular C and N contents. The ability to produce APA varied among species, which led to the variability of APA activity at low P. Once the inorganic P becomes available, algae take up P quickly, which leads to constant low P values in the water. As indicated by APA activity, it could be regarded as no N limitation and a certain level of P limitation in the stationary phase, which mainly resulted from P luxury uptake in the exponential phase (Fig. S2). Thus, the high N/P observed in Liuxihe reservoir should be regarded as the result of phytoplankton development, rather than as a driver for a shift in phytoplankton composition. The N/P could be important based on the absolute concentration of P in the waterbody, because it can indicate the balance of nutrients. The capacity of luxury uptake and the P storage of phytoplankton attenuated the effects of different levels of supplied P in this study.

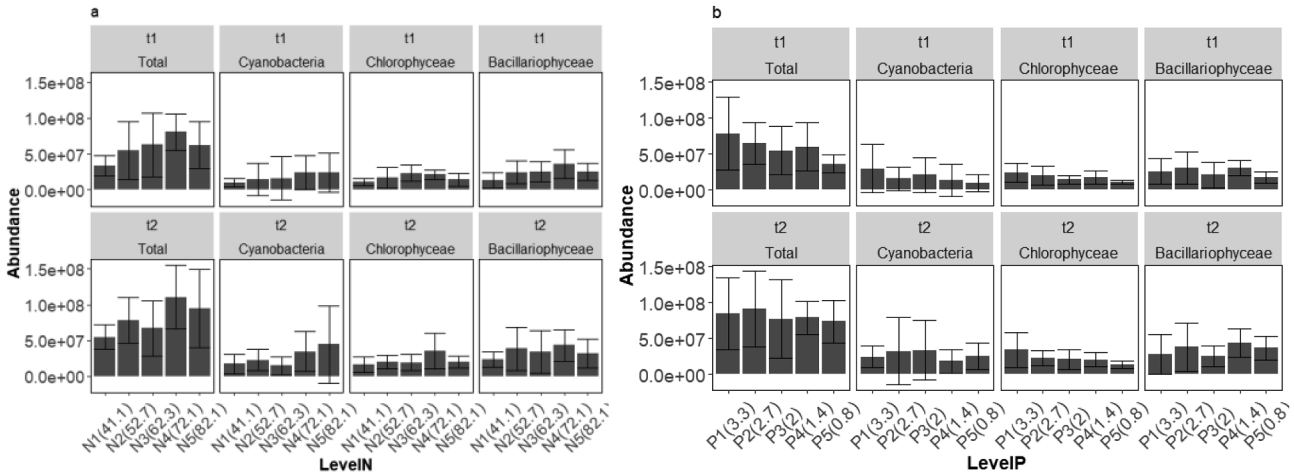

Fig. 3. Abundance of total phytoplankton assemblage and major groups among supplied N (a) and P (b) levels at the exponential (t1) and stationary phase (t2) phases of the experiment.

Table 1
ANOVA tests on the abundance of phytoplankton among different supplied N, P and N/P levels.

Period	Group	Level N	Level P	N/P ratio
T1	Total abundance	0.05	0.11	0.12
	Cyanobacteria	0.59	0.33	0.31
	Chlorophyceae	0.05	0.05	*0.04*
	Bacillariophyceae	0.04	0.28	0.48
T2	Total abundance	*0.02*	0.91	0.85
	Cyanobacteria	0.14	0.80	0.71
	Chlorophyceae	*0.04*	*0.04*	0.06
	Bacillariophyceae	0.42	0.37	0.28

Table 2
PerMANOVA tests on phytoplankton composition (T1: exponential phase; T2: stationary phase).

Phase	Group	N/P	N supplied	P supplied
T1	Phytoplankton	*0.04*	0.06	0.06
	Chlorophyceae	*0.03*	0.05	0.16
	Bacillariophyceae	0.50	0.10	0.23
T2	Phytoplankton	*0.01*	0.44	0.10
	Chlorophyceae	*0.01*	0.48	0.06
	Bacillariophyceae	*0.02*	0.42	0.08

4.2. Cellular elemental contents

During fast growing period, no significant variations were observed for cellular elemental contents, suggesting that phytoplankton was able to take up nutrients quickly. Factors other than nutrient concentrations determine the dominance of different phytoplankton assemblages. During slow growing period, cellular C and N changed with supplied N or P, indicating some extent of limitation and a re-arrangement in the cellular metabolic pathways and gene expression (Reynolds, 2006; Xu et al., 2020). In accordance to Hillebrand et al. (2013), in our study, the internal nutrient concentrations appeared to be reliable indicators for algal growth. This was shown by the more stable and higher cellular P recorded in the exponential phase compared to the stationary phase. Similar results were shown by Ågren (2004) who proposed a model predicting that cellular N/P ratio increases at relatively low growth rates, passes a maximum and then decreases at relatively high growth rates.

Supplied N only affected cellular C/N in the exponential phase, as shown by the variation of cellular N. Low P concentrations had weak influence on cellular C/N ($p = 0.05$) and C/P changed with supplied P. Therefore, cellular N/P variation mainly resulted from the greater plasticity of cellular C/P. This is consistent with the observations that phytoplankton species can adjust to environmental nutrient availability since resource requirements and uptake abilities are species-specific (Klausmeier et al., 2004), with C/P showing a greater flexibility because P is a less abundant component than N within cell.

Elemental homeostasis reflects the degree an organism varies its cellular nutrient composition in response to nutrient supplies, and its ability to acclimate to a changing nutrient availability in the environment (Sterner & Elser, 2002). Non-homeostasis could be regarded as an adaptive trait of those species which can sustain growth under low nutrient concentrations. In the present study, phytoplankton assemblage, with 1/H sowing a value of about 0.60, exhibited a low degree of homeostasis (Persson et al., 2010). Phytoplankton in this tropical reservoir was weakly homeostatic with respect to N and P, since the increase in the supplied N or P was accompanied by an increased N or P cellular content. Our study indicated a varying homeostatic extent depending on the concentration of supplied N and P. At low P levels, phytoplankton exhibited an increased homeostasis. For various N levels, phytoplankton exhibited different levels of flexibility. As

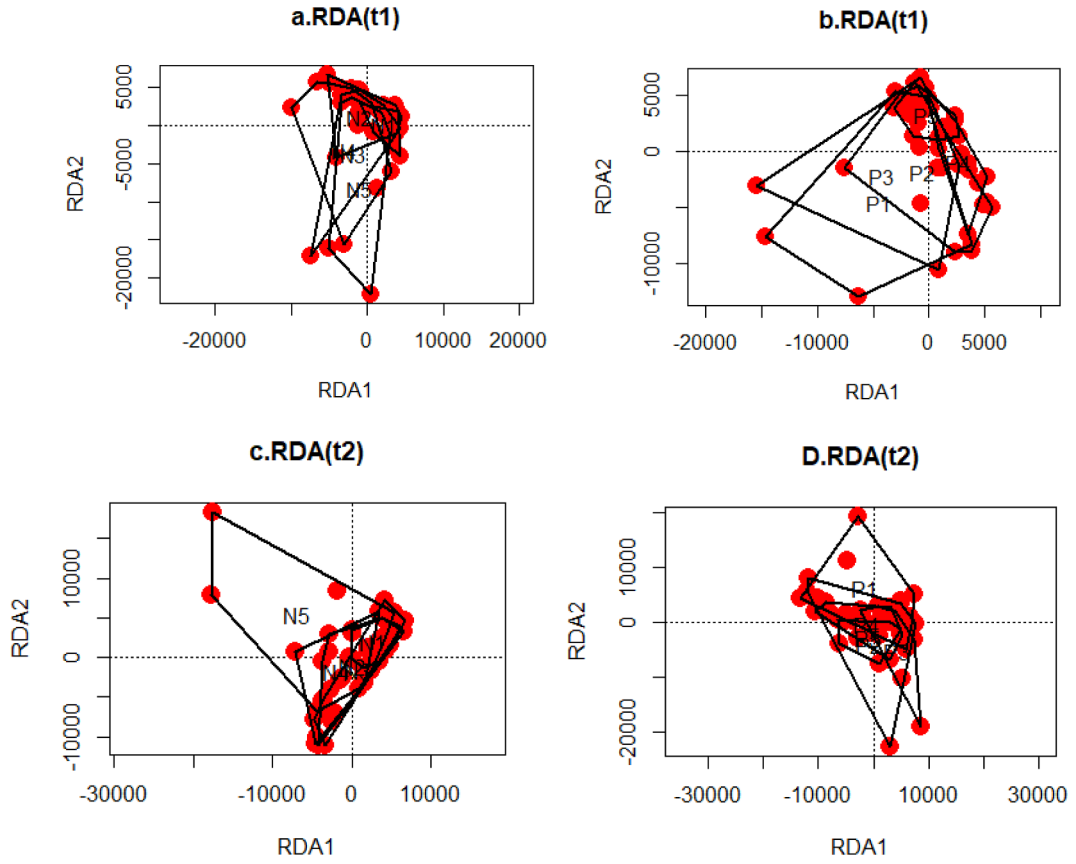

Fig. 4. RDA plot of phytoplankton assemblage among N (a, c) and P (b, d) treatments (t1: exponential phase; t2: stationary phase).

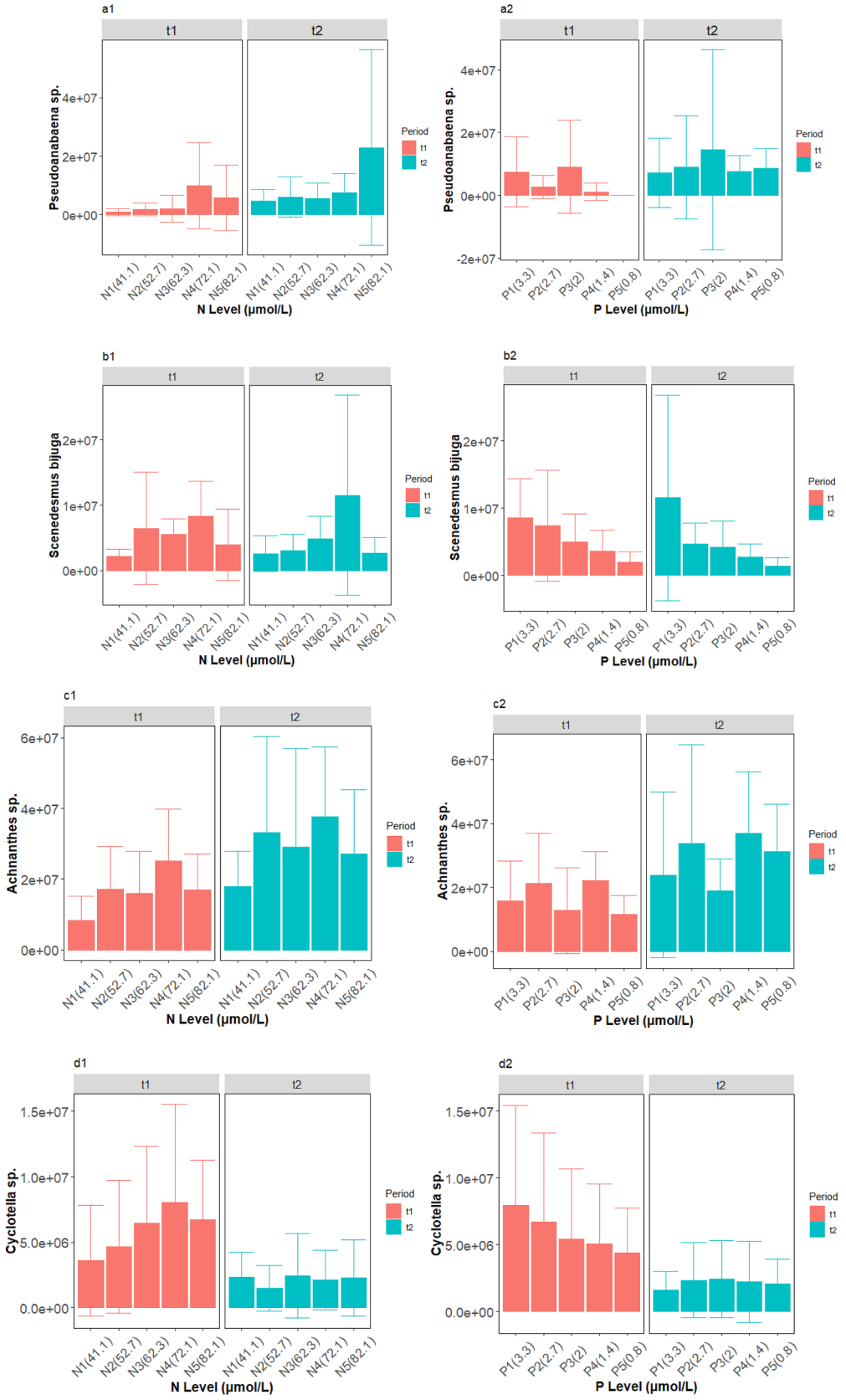

Fig. 5. Biomass variation of *Pseudoanabaena* sp. (a1,2), *Scenedesmus bijuga* (b1,2), *Achnanthes* sp. (c1,2) and *Cyclotella* sp. (d1,2) along supplied N and P levels in two phases (t1: exponential phase, t2: stationary phase).

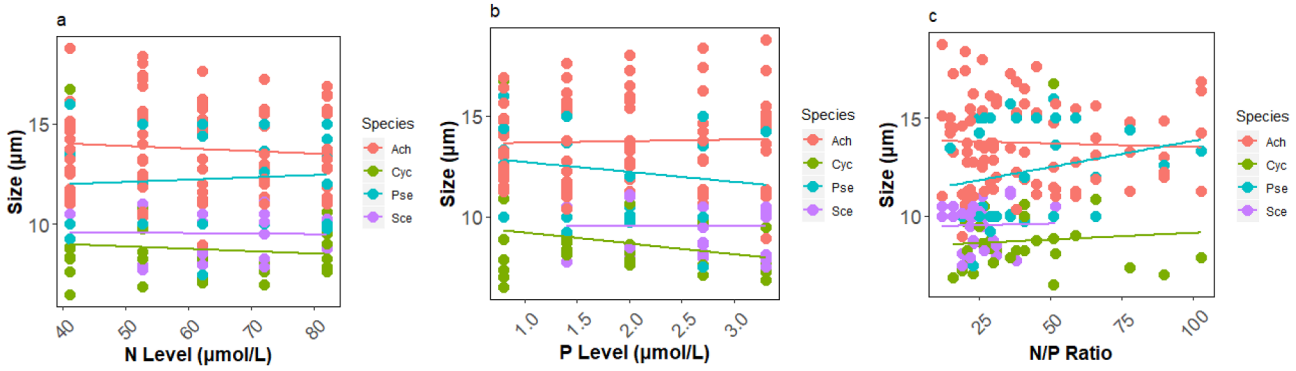

Fig. 6. Variation of cell size in the most abundant species along different supplied N (a), P (b) concentration and N/P (c) (Ach: *Achnanthes* sp.; Cyc: *Cyclotella* sp.; Pse: *Pseudoanabeana* sp.; Sce: *Scenedesmus bijuga*).

mentioned earlier, phytoplankton in this subtropical reservoir was regarded as P-limited, but our findings suggest that its increased homeostasis at low P concentrations provide an efficient strategy to survive in such environment. Since Phytoplankton is not a uniform entity and each member of the assemblage has species-specific requirements for resources (Reynolds, 2006), the cellular elemental ratio represented the overall average value of the single species stoichiometry in the considered assemblage as a result of trade-off among the different functional traits which characterize the different species in a phytoplankton assemblage (Glibert, 2016).

4.3. Phytoplankton assemblages

The different phytoplankton groups showed different response patterns to nutrient treatments and so did their stoichiometry. The N/P ratio, rather than the absolute concentration of N and P, has been considered in several studies as a factor affecting the phytoplankton assemblage structure. However, the importance of relative N and P concentration is strongly based on their absolute concentration. If the absolute concentration drops below the limiting threshold for a given algal species, an unbalanced N/P ratio in the environment might be an important factor structuring phytoplankton community (Reynolds, 1999). Under such conditions, only species with greater tolerance to nutrient limitation would become dominant in the assemblage. More in general, the species-specific physiological limits to storage lead to species-specific optimal N and P requirements, and the ecological interactions among species (i.e. competition) determine the structure of the assemblage. Therefore, different combinations of N and P can result in various species-coexistence in the assemblage. This is consistent with the resource ratio competition theory which predicts that the outcomes

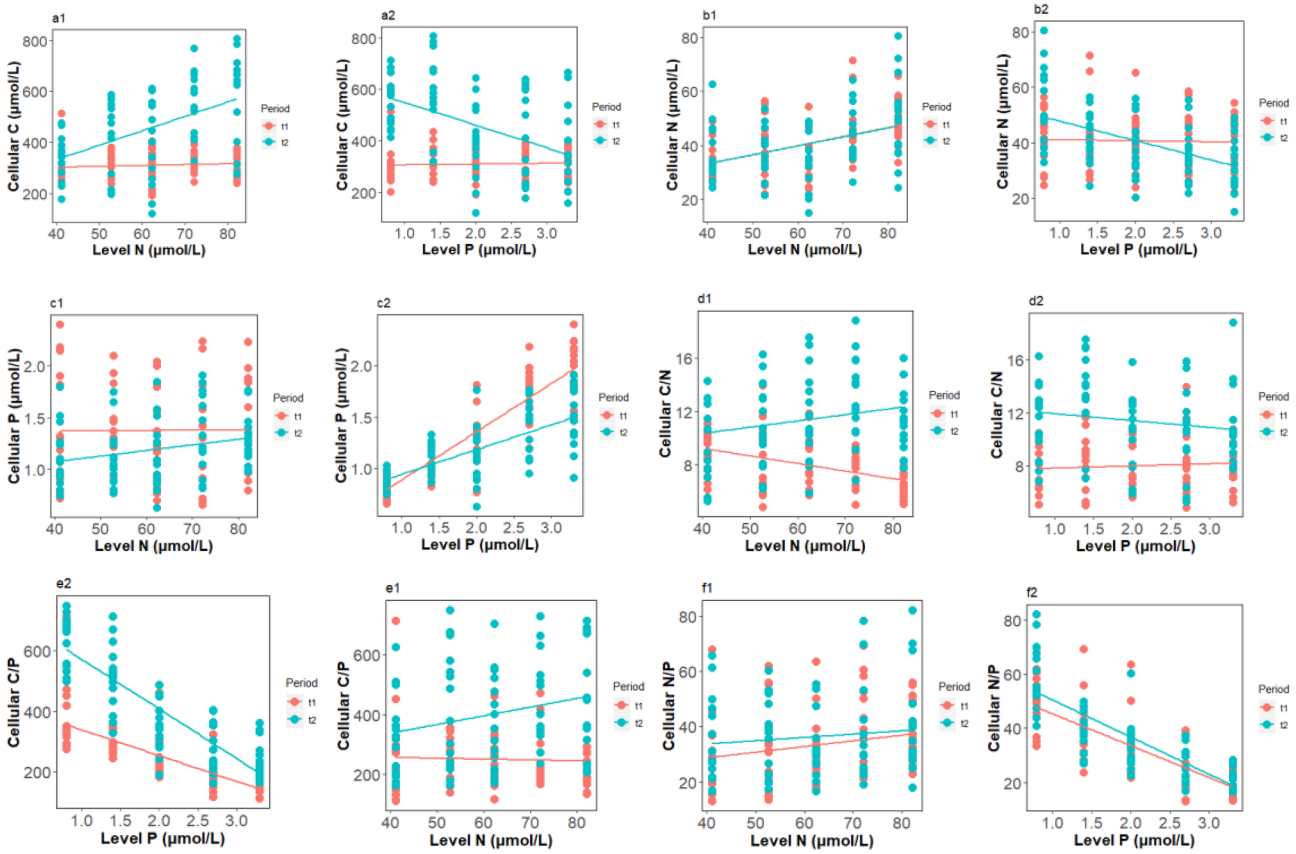

Fig. 7. Relationship between cellular C (a1,2), N (b1,2) and P (c1,2) with supplied N and P, cellular C/N (d1,2), C/P (e1,2) and N/P (f1,2) ratios with supplied N/P ratios in two phases (t1: exponential phase; t2: stationary phase).

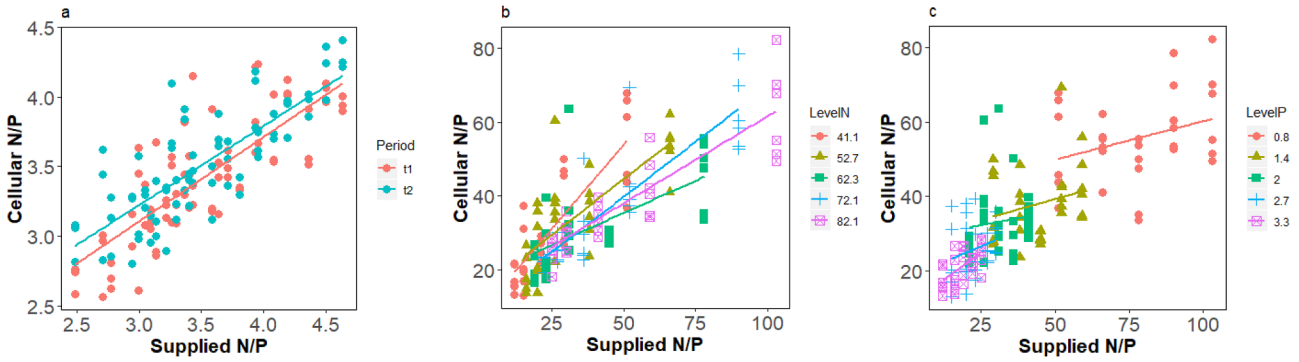

Fig. 8. Homeostasis test as the relationship between cellular N/P and supplied N/P (a. in t1: exponential and t2: stationary phase; b. by supplied N levels; c. by supplied P levels).

of competition will be determined by differential abilities to compete for important resources (Tilman, 1982). Superior competitors dominate at their optimal N and P ratios with other species at the suboptimal N and P ratios (Reynolds, 2006). The significant results obtained in the present study when comparing N/P and the structure of the phytoplankton assemblages could be achieved by multiplying both the absolute concentrations of N and P by a constant x. The ratio cannot therefore be used as a reliable predictor of the phytoplankton structure.

Bacillariophyceae were the dominant group of the phytoplankton assemblages in all the experimental treatments. Firstly, this was due to their dominance in the original assemblage at the beginning of the experiment, and secondly, they generally have low C and N investments in the protective layer surrounding cells due to their major demand of silica. Therefore, diatoms show a greater variability in C/P and N/P (Garcia et al., 2018). On the contrary, green algae has higher C/P and N/P due to the high concentrations of glycoproteins in the cell wall (Gerken et al., 2012). Carrick and Lowe (1988) reported an increase in green algae biovolume under enrichment of N and P. Variation in the stoichiometry of the assemblage may result from interspecific variation in species-specific optimal elemental composition (Sterner & Elser, 2002) and differences in storage capacity. Thus, phytoplankton stoichiometry might provide deceptive indications of nutrient limitation. Chrysophyceae formed 15% of total abundance in the original assemblage, but this group disappeared afterwards in the treatment. With the nutrient enrichment, other groups in the assemblage, such as Bacillariophycea and Chlorophyceae have advantages and outcompete Chrysophyceae. Additionally, the dead cells could provide extra nutrients and fueled the cultures along with supplied N and P.

The non-heterocystous filamentous *Pseudanabaena* sp. was the dominant cyanobacteria species. The abundant supplied N favored this type of species over other diazotrophs. *Cyclotella* sp. and *Scenedesmus* sp. are groups of relatively small diatom and green algae with the advantages to take up nutrients rapidly. The half saturation of growth in several *Cyclotella* and *Scenedesmus* species are known to be around 0.25 and 0.20 μmol P/L respectively, higher than requirements of most species (Reynolds, 2006). In the present experiment, the lowest supplied P was 0.80 μmol P/L, which was above the requirement, and 25 °C setup might fuel the metabolism. These fast-growing species are velocity-adapted, have similar, quite high phosphorus half-saturation constants (compared to other species) and thus are sensitive to phosphorus deficiency (Padisák, 2003). Therefore, they were dominant when nutrients were abundant (especially phosphorus), as indicated by relatively low N/P ratios. *Achnanthes* sp. was the most abundant species with highest frequency of presence in the study. The strong precipitation in spring with increasing water flow provided this benthic species the chance to be present in the upper water column. Its abundance in the original assemblage allowed *Achnanthes* sp. to be the most abundant species in the treatments. *Cyclotella* and *Achnanthes* had relatively higher optimal temperature, and the experimental temperature provided them advantages to outcompete other diatom species, such as *Fragilaria* sp. and *Aulacoseira* sp. In addition, the great surface to volume ratio of *Achnanthes* sp. facilitates nutrient uptake and makes it more competitive when nutrients are low (Baillie, 1987; Carrick and Lowe, 1988).

In our study, cell size did not exhibit significant variations among different treatments. On one side, the experimental duration might be not long enough for cell size change. On the other side, greater P availability might do not directly favor species with larger body size, but rather favor species with higher relative growth rate (Elser et al., 2003). This is consistent with earlier observation that excessive uptake of P was not useful to promote an increased cell size (Skau et al., 2017). Cell size of *Achnanthes* sp. exhibited decreasing trend with supplied N/P, which is consistent with the previous observations that body size is negatively correlated with N/P (Mendez & Karlsson, 2005). However, the decrease in diatom cell size could be more likely due to its high growth during the experiment and to the natural decreasing in daughter cells size.

5. Conclusions

Our results highlighted that variability of phytoplankton cellular elemental ratios mainly came from the plasticity of cellular P. Phytoplankton assemblage, in this reservoir characterized by rather low P concentration, was weakly homeostatic. This mechanism constituted a strategy to survive and adapt to a dynamic nutrient environment. The absolute concentrations of N and P were important for phytoplankton growth and governed the observed assemblage composition shift. Moreover, the observed effects of N/P depended on the absolute concentration of N and P, and should not be applied to estimate nutrient limitation in this subtropical reservoir. Small changes in species composition in our study suggested that these responses cannot be explained simply by analyzing the compositional variations of nutrient available in the water. Due to the multi-species nature of the phytoplankton assemblage, species-specific physiological plasticity provides functional redundancy and masks minor response due to supplied nutrients. Further experiments with multiple environmental factors influencing the growth rate should be considered to investigate the phytoplankton stoichiometry and its relationship with the environmental nutrient conditions.

CRediT authorship contribution statement

Yang Yang: Supervision, Writing - original draft. **Jingyun Pan:** . **Bo-Ping Han:** Conceptualization, Methodology. **Luigi Naselli-Flores:** Validation, Writing - review & editing.

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

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