

Effects of changes in N and P stoichiometry on epipelon and phytoplankton chlorophyll-*a* under eutrophic condition

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ABSTRACT – (Effects of changes in N and P stoichiometry on epipelon and phytoplankton chlorophyll-*a* under eutrophic condition). Nitrogen and phosphorus are essential elements for the growth and performance of organisms, and the environmental N:P ratio can determine the biomass and structure of algal communities. We investigated the effects in the N:P molar ratio changes on epipelon and phytoplankton chlorophyll-*a* under eutrophic conditions. An enrichment experiment was performed in open-bottom mesocosms to simulate P limitation, N limitation, and good N and P availability. Under eutrophic conditions, the combined availability of N and P enhanced phytoplankton bloom, while an increase in P limitation resulted in the loss of phytoplankton biomass. Under conditions of high P limitation, there was significantly loss of epipelon biomass. No change in the N:P ratio led to a significant increase in algal biomass in the epipelon. In conclusion, changes in the N:P molar ratio can affect the growth of phytoplankton and epipelonic algae under eutrophic conditions. Our results suggest that photosynthetic biomass increase in the epipelon depends on an optimal relationship between light and P.

Keywords: enrichment, eutrophic reservoir, N and P limitation, mesocosm experiment

RESUMO – (Efeitos das mudanças na estequiometria de N e P sobre a clorofila-*a* do epipélon e do fitoplâncton em condição eutrófica). Nitrogênio e o fósforo são elementos essenciais para o crescimento e desempenho dos organismos e a razão N:P ambiental pode determinar a biomassa e estrutura das comunidades algais. Nós investigamos os efeitos de mudanças na razão molar N:P sobre a clorofila-*a* do epipélon e fitoplâncton em condições eutróficas. Em mesocosmos de fundo aberto, realizou-se um experimento de enriquecimento para simular a limitação de P, limitação de N e boa disponibilidade de N e P. Em condições eutróficas, a disponibilidade combinada de N e P intensificou a floração do fitoplâncton, enquanto um aumento na limitação de P resultou em perda na biomassa do fitoplâncton. Sob condições de alta limitação de P, houve perda significativa da biomassa do epipélon. Nenhuma mudança na relação N:P levou a um aumento significativo na biomassa algal no epipélon. Em conclusão, mudanças na razão molar N:P podem afetar o crescimento do fitoplâncton e algas epipelônicas em condições eutróficas. Nossos resultados sugerem que um aumento na biomassa fotossintética no epipélon depende de uma relação ótima entre luz e disponibilidade de P.

Palavras-chave: enriquecimento, limitação por N e P, mesocosmo, reservatório eutrófico

Introduction

Globally, eutrophication is the environmental impact that most affect aquatic ecosystems, in which phytoplankton bloom is an immediate response (Smith & Schindler 2009, Ho *et al.* 2019). Intense phytoplankton bloom is reported globally, and restored lakes are still very rare (Ho *et al.* 2019). The phytoplankton bloom control is a crucial goal to reverse eutrophication. However, achieving and maintaining the oligotrophication of a recovered ecosystem is always challenging, especially due to internal P loading

(Jeppesen *et al.* 2007, Søndergaard *et al.* 2013). N and P are the primary limiting nutrients of the algal communities and the main triggers of eutrophication. The changes in the N:P ratio can affect the trophic chain, structure, and function of biological communities, determining the ecological status of ecosystems. The changes in the N:P ratio, regardless of nutrient concentration, can promote changes in the community structures (Sterner & Elser 2017), including in the algal communities (Stelzer & Lamberti 2001). N:P ratio can act to control algal growth potential and interfere with ecological interactions, such as the relationship between

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epipelon and phytoplankton (Hillebrand *et al.* 2013, Zhang *et al.* 2015). However, the effects of light and nutrient availability on algal communities can be interactive (Fanta *et al.* 2010, Hill *et al.* 2011, Sanches *et al.* 2011). Studies demonstrate that the interactive effects between light and nutrients may be more determinant of periphytic biomass changes than the isolated effect of these factors (Hill & Fama 2008, Hill *et al.* 2009, Hill *et al.* 2011, Sanches *et al.* 2011), including changes in the epipelon (Tavares *et al.* 2018).

The changes in water N:P ratios can affect epipelon growth in shallow lakes and reservoirs (Lambert *et al.* 2008), as widely observed in phytoplankton. The epipelon can substantially contribute to primary productivity and total biomass in lakes and reservoirs (Vadeboncoeur *et al.* 2003, Cano *et al.* 2016). Additionally, epipelon can oxidize the surface layer of the sediment and immobilize P, which contributes to the decreased P concentrations in the water column (Wetzel 2001, Dodds 2003). In restored eutrophic lakes, epipelon growth can minimize the P self-fertilization problem. Thus, the ability of epipelon to immobilize P in sediment can contribute to eutrophication control and maintenance of oligotrophication in restored lakes (Liboriussen & Jeppesen 2006, Genkai-Kato *et al.* 2012). Despite the positive aspects, an increase in the frequency of algal blooms in the benthic environment in lakes has been reported, especially in cyanobacteria (Quiblier *et al.* 2013). A greater understanding of epipelon responses to changes in algal growth limiting potential may improve predictions of ecosystem processes, such as those involving restoration.

Biotic factors, such as high phytoplankton biomass and macrophyte coverage, may also play a crucial role in epipelon growth by interfering with light and nutrient availability, especially in shallow eutrophic systems (Zhang *et al.* 2015, Fernández & Alcocer 2017). In addition to the N and P concentration changes, the N and P stoichiometric balance can also affect the phytoplankton growth potential (Vrede *et al.* 2009). In eutrophic conditions, high phytoplankton biomass coverage can substantially reduce the amount of incident light that reaches the epipelon affecting the algal biomass negatively (Vadeboncoeur *et al.* 2001, Spears *et al.* 2010, Amaral *et al.* 2020). Besides shading, phytoplankton can interfere with nutrient availability for the epipelon (Zhang *et al.* 2015, Tavares *et al.* 2019). The immobilization of P by the epipelon can control phytoplankton production (Carlton & Wetzel 1988, Genkai-Kato *et al.* 2012). The effect of changing light and nutrient availability on algal communities can be interactive (Hill & Fanta 2008, Hill *et al.* 2009, Fanta *et al.* 2010 and Hill *et al.* 2011, Sanches *et al.* 2011). The interactive effects between light and nutrients on alga communities may be more crucial than their isolated effects (Eriksson *et al.* 2007, Sanches *et al.* 2011). Studies show that the light:nutrient ratio can explain part of the temporal variability of periphyton biomass in rivers and streams (Hill & Fama 2008, Hill *et al.* 2009, Hill *et al.* 2011), coastal lagoons (Sanches *et al.* 2011) and shallow reservoirs (Tavares *et al.* 2018, Lambrecht 2019). Despite the consensus in the literature that changes in the N and P ratio influence changes in the biomass and

structure of algal communities, the potential for algal limitation in eutrophic conditions still has gaps, particularly in the epipelon. In addition, in eutrophic ecosystems, the restoration processes generally alter the N:P ratio tending to reduce it (Zamparas & Zacharias 2014, Schindler *et al.* 2016). In the present study, we promoted changes in the N:P ratio and investigated their effects on epipelon and its relationship with phytoplankton responses in a eutrophic reservoir. The reduction of nutrient availability, especially P, can negatively affect phytoplankton (Schindler 2016, Rosińska *et al.* 2019) and influence the epipelon (Amaral *et al.* 2020). Thus, we hypothesized that the P-limitation condition negatively affects phytoplankton and can favor algal growth in the epipelon.

Material and methods

Study Area - The mesocosm experiment was performed at Garças Reservoir (23°38'20,03"S; 46°37'19,20"W), located in the Parque Estadual das Fontes do Ipiranga, São Paulo State, Brazil. The reservoir has an area of 88,156 m², a volume of 188,785 m³ and a maximum depth of 4.7 m. The reservoir is classified as super-eutrophic with frequent phytoplankton blooms (Bicudo *et al.* 2020). To minimize rain effects on the enriched treatments, the experiment was carried out during winter (June - July) when the total accumulated rainfall was 2.3 mm, and air temperature ranged between 10-19 °C (<http://www.estacao.iag.usp.br/boletim.php>).

Experimental design - We performed an enrichment experiment to simulate the P-limiting, N-limiting conditions, and good N and P availability. Triplicate treatments were designated as Control, without nutrient addition; P+, isolated P addition (N-limiting); N+, isolated N addition (P-limiting); and NP+, combined N and P addition (no limitation).

Twelve open-bottom mesocosms constructed with PVC cylinders (0.5 m diameter, 151 L volume) were placed in the littoral zone for the enrichment experiment. All mesocosms were buried in the sediment at one meter from each other, maintaining the water column depth at 80 cm. The experimental period was from June 30 to July 20, 2017, with enrichment (T0) performed after five acclimatization days (July 6). Sampling of the water, phytoplankton, and epipelon was performed after 7 and 14 days. However, the N:P molar ratio was monitored in each treatment on days 1, 4, 8, and 11, aiming at maintaining the proposed environmental conditions. The experimental design is summarized in figure 1.

On average, the initial concentrations of dissolved inorganic nitrogen (DIN) and PO₄-P were 735.1 µg N L⁻¹ and 5.0 µg P L⁻¹, respectively. In the enriched treatments, the N:P molar ratios were adjusted to 16:1 in NP+ treatment, <16:1 in N+, and >16:1 in P+. These ratios were reached by adding defined amounts of salts (KH₂PO₄ and NH₄NO₃ Merck) to the mesocosms, according to Redfield (1958). To obtain realistic DIN and PO₄-P concentrations, the enrichment was based on the range of values obtained from monitoring between 1997 and 2017 (Database of

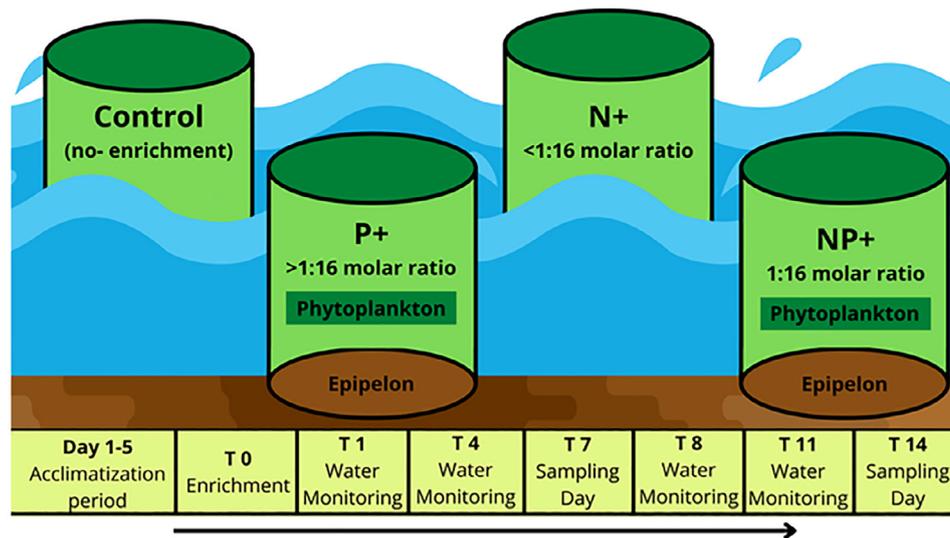


Figure 1. Scheme showing the types of treatments, the communities sampled and a summary of the experimental design. D: days of acclimatization period. T: days of experimental period.

the Aquatic Ecology Laboratory, Instituto de Pesquisas Ambientais - São Paulo).

The subsurface water samples were collected for the analysis of abiotic variables and phytoplankton. A PVC tube with a 5 cm diameter was used to collect samples from the surface sediment up to 1 cm deep. The sampling site was drawn and scored to avoid resampling. In the laboratory, the sediment samples were diluted to a known volume, and aliquots were separated for epipelon analysis.

Variables analyzed - Temperature, electrical conductivity, and pH were measured with a multiparameter probe (Horiba U-53, Kyoto, Japan). Dissolved oxygen (DO) concentration (azide-modification method), alkalinity (titration method), free CO_2 (calculate from alkalinity and pH), nitrate (cadmium-reduction method), ammonium (phenol hypochlorite method), orthophosphate (ascorbic acid method), orthosilicate (molybdate method), and total nitrogen (TN), total phosphorus (TP) (alkaline persulfate method) were determined according to APHA (2012). The light was measured in the subsurface of the water column and above the sediment layer using a quantum sensor (LI-250A, LI-COR, Lincoln, NB, USA). The light attenuation coefficient (k) was calculated according to Kirk (1994). The ratio between light availability ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and total phosphorus concentration ($\mu\text{g L}^{-1}$) in the water subsurface was calculated according to Fanta *et al.* (2010).

The phytoplankton and epipelon biomass were estimated by the chlorophyll-*a* concentration. The samples of water and epipelon were filtered through a glass fiber filter (Whatman GF/F), and the extraction of chlorophyll-*a* (corrected for pheophytin) was performed with ethanol 90% (Sartory & Grobblelar 1984). The loss or gain of photosynthetic biomass in the phytoplankton and epipelon was calculated by subtracting the chlorophyll-*a* concentration in the enriched treatments from the control mean concentration. The determination of chlorophyll-*a* was carried out within a maximum period of one month. Data analysis - Experimental abiotic conditions were evaluated using principal components analysis (PCA) with

a covariance matrix and log-transformed data (PC-ORD 6.0, McCune and Mefford 2011). The two-way repeated-measures analysis of variance (two-way RM-ANOVA, $\alpha = 0.05$) was applied to abiotic and biological variables to detect significant differences between treatments and days. Tukey's test was used for multiple mean comparisons. The data were logarithmized to comply with the analysis assumptions, normality, and homogeneity of variance. These analyses were performed using SigmaPlot 12.0 (Systat Software, Inc.).

Results

As shown in Table 1, all variables except pH exhibited significant changes associated with treatment and time (table 1). Environmental conditions DIN, TN, TP, N:P molar ratio, and light attenuation coefficient were different between treatment (RM-ANOVA: $p < 0.002$) and days (RM-ANOVA: $p < 0.05$). TP concentration differed significantly between all treatments (Tukey: $p < 0.018$). The environmental conditions in the Control and N+ treatments were P-limiting (>16) and P+ treatment was N-limiting (<16) on both sampling days (figure 2a). In the NP+ treatment, although the N:P ratio was reduced by 64.2% compared to the control on day 7, the condition remained P-limiting. However, on day 14, the ratio was close to 16. The light at the bottom was low in all treatments (figure 2 b). However, the highest light availability was found in the N+ treatment, where the mean was different from the control and other treatments on day 7 (Tukey: $p < 0.043$). Additionally, Light:P ratio was different between treatments (figure 2 c; RM-ANOVA: $p < 0.001$). However, the ratios found were extreme (>24.9 or <4.7).

PCA summarized 83.05% of the total variability of the abiotic data on the first two axes (figure 3). On the positive side of axis 1, the P+ and NP+ treatments were ordered and associated with high dissolved and total P availability. In contrast, the Control and N+ treatments were ordered on the negative side of axis 1 associated with high $\text{NH}_4\text{-N}$ and

Table 1. Mean and standard error of the limnological variables in treatments on experimental days 7 and 14 and RM-ANOVA results. Treat: treatment. Inter: Interaction. ns: no significant.

Variables	two-way RM-ANOVA												
	7 day						14 day						
	C	N+	P+	NP+	C	N+	P+	NP+	Treat	F	p	Inter	
Free CO ₂ (mg L ⁻¹)	14.1 ±8.5	9.2 ±1.6	1.4 ±1.4	0.1 ±0.01	33.9 ±19.9	17.8 ±5.2	8.3 ±0.8	4.6 ±1.6	45.08	<0.001	66.67	9.35	0.005
DO (mg L ⁻¹)	6.7 ±0.8	10.0 ±1.6	10.9 ±0.7	14.2 ±0.8	5.9 ±0.02	6.7 ±1.3	6.0 ±2.2	6.9 ±0.4	7.44	0.011	36.49		ns
Light at surface (μmol m ⁻² s ⁻¹)	254.0 ±75.1	334.5 ±203.8	183.2 ±113.1	435.3 ±163.7	222.5 ±30.7	452.4 ±231.9	464.3 ±137.0	678.3 ±210.4	5.78	0.021	19.41	6.60	0.015
Light at bottom (μmol m ⁻² s ⁻¹)	23.8 ±12.8	44.8 ±16.1	23.4 ±19.1	17.4 ±9.5	28.3 ±9.4	26.9 ±11.6	17.9 ±10.9	21.5 ±8.5	6.62	0.015	9.16	5.40	0.025
Light attenuation coefficient	3.15 ±0.45	2.52 ±0.42	2.88 ±0.58	4.32 ±0.11	2.61 ±0.24	3.61 ±1.00	4.26 ±0.26	4.63 ±0.43	6.33	<0.001	6.50	2.25	ns
Light:P ratio	55.2 ±5.4	74.0 ±10.8	7.7 ±4.8	24.9 ±6.3	55.6 ±7.7	79.8 ±4.9	4.6 ±2.3	4.7 ±1.3	162.04	<0.001		5.70	0.022
NH ₄ -N (μg L ⁻¹)	247.5 ±160.6	294.8 ±198.1	11.0 ±0.28	10.8 ±0.00	553.3 ±246.2	631.8 ±258.1	189.9 ±250.9	341.5 ±26.4	26.76	0.002	48.14	8.97	0.008
NO ₃ -N (μg L ⁻¹)	143.6 ±23.5	859.6 ±97.7	7.0 ±6.0	317.7 ±21.1	69.8 ±8.7	1313.3 ±414.9	13.3 ±5.8	751.0 ±106.3	487.53	<0.001	52.16	53.26	<0.001
pH	6.9 ±0.23	7.0 ±0.05	8.0 ±0.47	8.9 ±0.02	6.2 ±0.40	6.8 ±0.11	7.1 ±0.03	7.4 ±0.16		ns			ns
Suspended Particulate Matter (g L ⁻¹)	18.60 5.05	17.13 2.05	19.72 3.29	31.91 5.14	24.04 5.34	42.31 11.17	34.35 9.06	28.55 2.98	5.44	0.025	33.03	7.12	0.012
PO ₄ -P (μg L ⁻¹)	<4.0	<4.0	29.1 ±29.0	14.7 ±0.8	<4.0	<4.0	104.9 ±37.2	133.2 ±20.9	84.89	<0.001	16.83	9.04	0.006
N:P molar ratio	180.1 ±75.4	550.0 ±46.7	3.0 ±1.5	64.4 ±5.0	288.1 ±107.4	883.1 ±70.9	1.6 ±0.9	19.7 ±2.8	233.32	<0.001	10.35	34.57	<0.001
TP (μg L ⁻¹)	100.2 ±39.8	85.8 ±14.1	282.2 ±42.2	388.7 ±15.7	92.4 ±23.5	227.4 ±19.9	383.5 ±112.6	432.9 ±72.5	37.31	<0.001	45.83	22.03	<0.001
TN (μg L ⁻¹)	598.3 ±166.7	1957.5 ±145.7	277.3 ±137.3	1194.3 ±91.1	817.8 ±468.2	1901.0 ±157.5	776.9 ±248.2	1697.2 ±596.7	31.25	<0.001	5.13	1.98	ns
Temperature (°C)	16.7 ±0.23	16.6 ±0.02	16.6 ±0.02	16.7 ±0.03	14.5 ±0.38	14.9 ±0.07	15.1 ±0.07	15.4 ±0.12	5.51	0.024	827.1	13.59	0.002

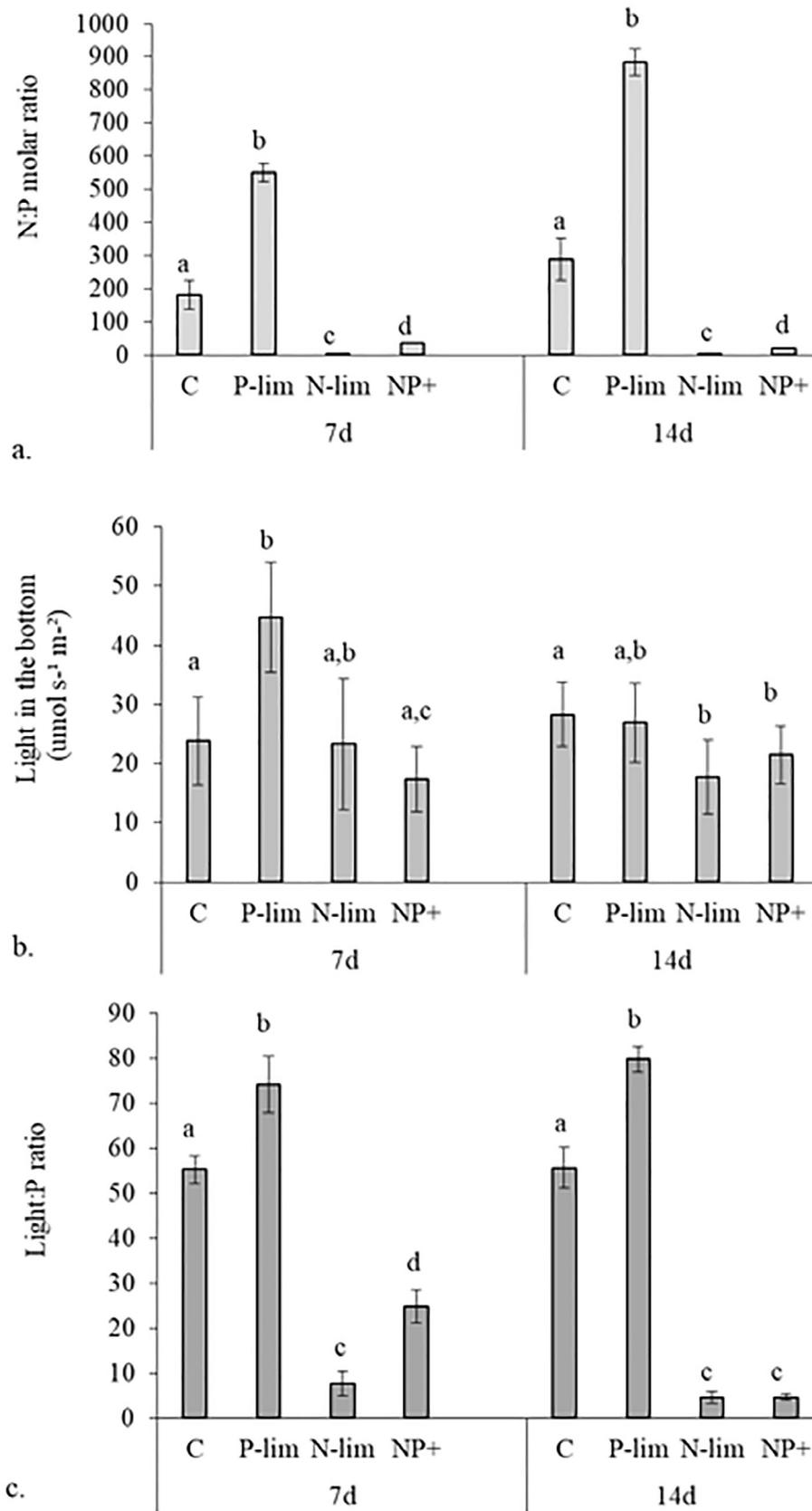


Figure 2. N:P molar ratio of the water column (a), incident light above the surface of the sediment (b) and subsurface water column Light:P ratio (c) ($n = 3$, SE) in the control and enriched treatments on day experimental 7 and 14. Within each experimental day, treatments marked with the same letter are not significantly different by Tukey test ($\alpha < 0.05$).

$\text{NO}_3\text{-N}$ concentrations and N:P molar ratios. PCA axis 1 represented the variations in nutrient availability indicating algal growth potential.

Phytoplankton - The phytoplankton chlorophyll-*a* was highest in the NP+ treatment on days 7 and 14 and was six times higher than in the Control (figure 4 a). Phytoplankton chlorophyll-*a* differed between treatments (RM-ANOVA: $F = 36.56$; $p = 0.003$) and days (RM-ANOVA: $F = 17.11$; $p < 0.001$), and the interaction between factors was significant (RM-ANOVA: $F = 5.11$; $p = 0.021$). Phytoplankton chlorophyll-*a* in the NP+ treatment was different from the control and other treatments on days 7 and 14 (Tukey: $p < 0.001$). In the N+ treatment, phytoplankton chlorophyll-*a* decreased and was different from the other treatments on day 7 (Tukey: $p < 0.035$). The highest gains in phytoplankton biomass were found in the NP+ treatments, while losses occurred in the N+ treatment on day 7 (figure 4b). We observed that phytoplankton was co-limited by N and P.

Epipelton - On day 7, the epipelton chlorophyll-*a* in the N+ treatment was six times lower than in the Control (figure 5 a), evidencing a substantial loss of biomass (figure 5 b). Additionally, differences in the epipelton chlorophyll-*a* were found between treatments (RM-ANOVA: $F = 73.42$; $p < 0.001$) and days (RM-ANOVA: $F = 4.90$; $p = 0.013$), a significant interaction between factors (RM-ANOVA: $F = 5.38$; $p = 0.009$). However, epipelton chlorophyll-*a* in treatment N+ was different from other treatments on day 7 (Tukey: $p < 0.04$) when there was a biomass loss. Concerning time, we observed an increase in epipelton biomass in all treatments on day 14 when compared to day 7 (Tukey: < 0.0012).

Discussion

Our findings showed that altering the N and P stoichiometry of water through isolated and combined N and P enrichment affected the photosynthetic biomass of phytoplankton and epipelton, allowing identification of the limiting nutrient. The response of phytoplankton and epipelton to the change in the N:P ratio showed that the stoichiometric balance can determine the increase in algal biomass even in a hypereutrophic environment. In eutrophic lakes, reducing the N:P ratio can result in biomass loss and a significant shift in the phytoplankton assemblage (Crossetti & Bicudo 2005, Vrede *et al.* 2009). Changes in phytoplankton biomass can influence algal growth in the epipelton mainly due to changes in the degree of sediment shading and competition for nutrients (Cano *et al.* 2016, Tavares *et al.* 2018, Amaral *et al.* 2020). Based on a global lake dataset, one study suggested that low N:P stoichiometry and high probability of co-limitation tend to occur in eutrophic systems (Zhou *et al.* 2022). In the present study, we discuss the responses of phytoplankton and chlorophyll-*a* epipelton to the change in the N:P ratio of water under eutrophic conditions, highlighting the influence of the stoichiometric balance on algal communities.

Regarding treatment NP+, our results showed that an improvement in the N:P ratio (closer to 16) intensified phytoplankton bloom, evidencing a community co-limited by N and P. The 10-fold average reduction in N:P ratio significantly increased phytoplankton chlorophyll-*a* (7 and 14d). As a consequence of biotic responses, there was an increase in light attenuation which only reached 5%

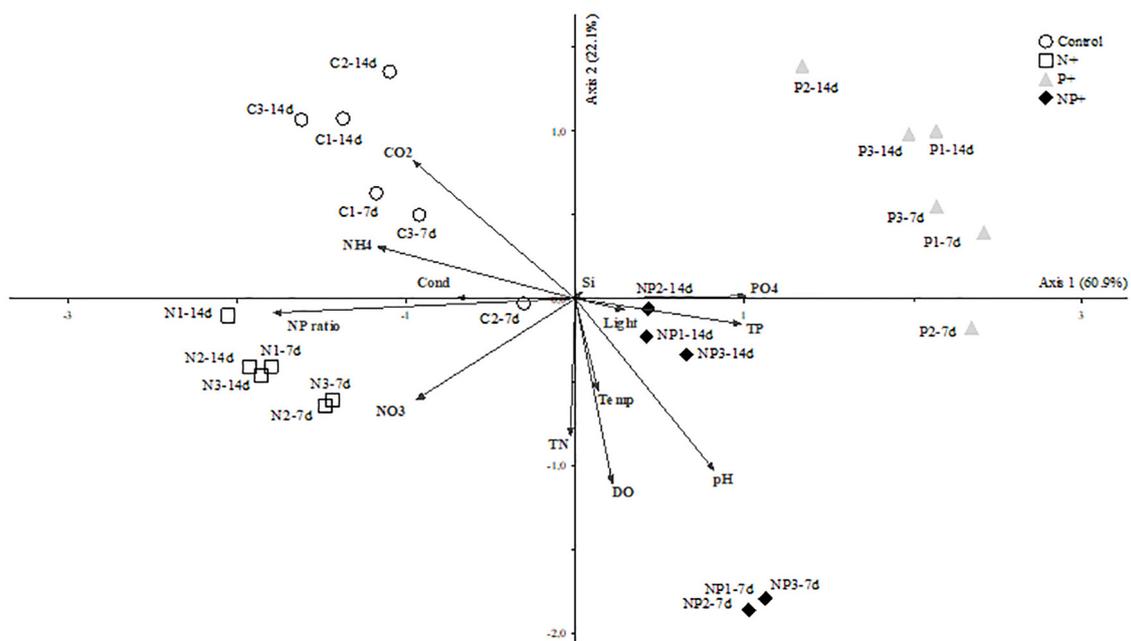


Figure 3. PCA of abiotic variables in the treatments. Abbreviations: the first two characters indicate the treatment (C, N+, P+ or NP+), and the last two characters indicate the sampling day (7 or 14). Vectors - Cond: electrical conductivity. CO₂: free CO₂. Light: Light Attenuation. pH: pH. DO: dissolved oxygen. NH₄: ammonium. NO₃: Nitrate. TN: Total Nitrogen. TP: Total Phosphorus. Temp: Temperature.

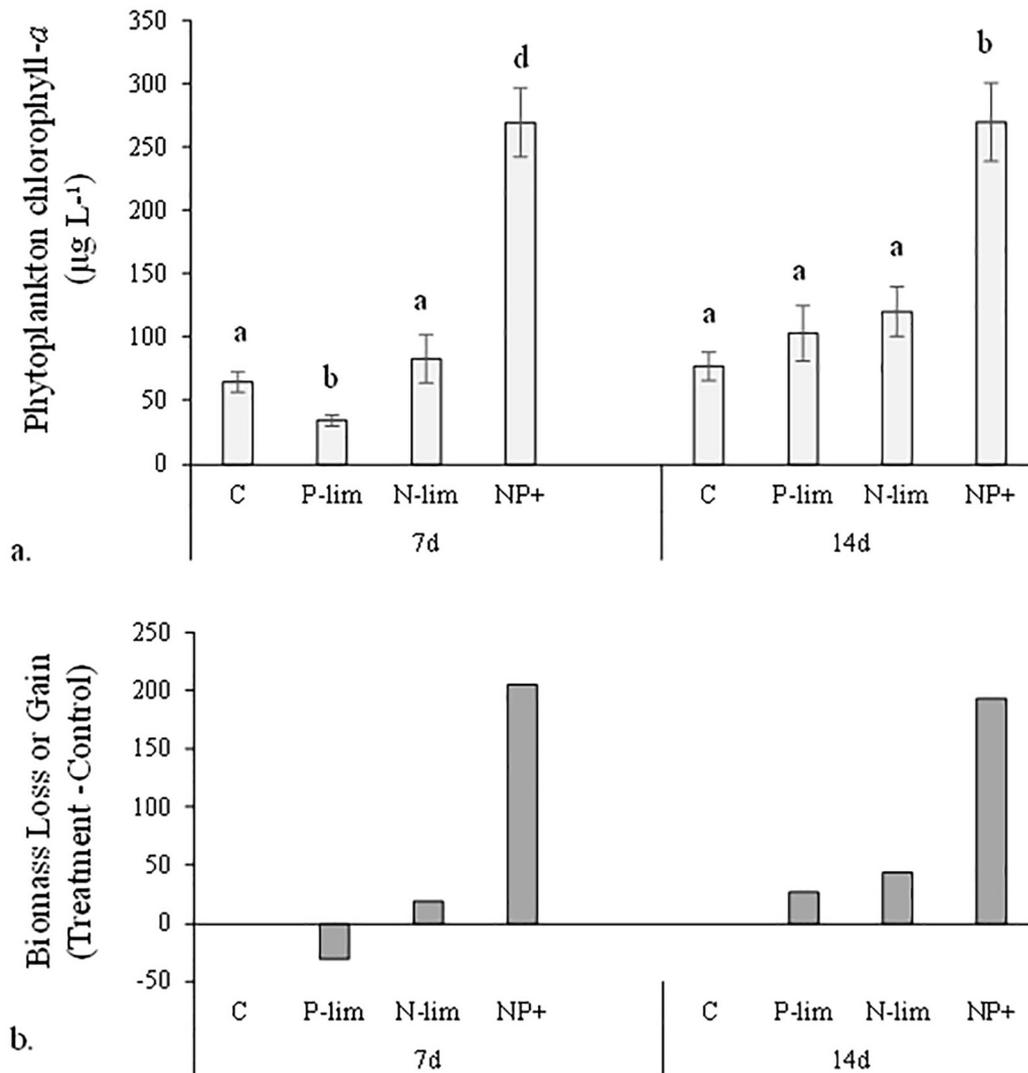


Figure 4. Phytoplankton chlorophyll-*a* ($n = 3$; SE) concentration (a) and the difference in the chlorophyll-*a* concentrations of control and treatments (b). Within each experimental day, treatments marked with the same letter are not significantly different by Tukey test ($\alpha < 0.05$).

on the sediment surface in NP+ treatment. Therefore, the increase in phytoplankton blooms led to a worsening in light availability at the sediment surface, where conditions remained unfavorable for algal growth. In this scenario, there was no epipelton biomass response, that is, the community did not respond to the increase in nutrient availability. The inverse relationship between phytoplankton and epipelton is described in shallow eutrophic lakes (Liboriussen & Jeppesen 2006, Pouličková *et al.* 2014). The phytoplankton overgrowth promotes the reduction of the euphotic zone, negatively impacting the phototrophic epipelton growth. Although light is not usually a limiting factor for algal growth in the epipelton of shallow lakes (Vadeboncoeur *et al.* 2014), it can become limiting in cases where phytoplankton blooms restrict the euphotic zone (Bicudo *et al.* 2007).

In the N+ treatment, seven days of extreme P limitation (N:P = 550) led to a significant decrease in phytoplankton chlorophyll-*a* when compared with control. Additionally, there was an increase in light availability (46.8%) at the bottom under extreme P-limiting conditions. Despite the

apparent improvement in environmental conditions with a decrease in the phytoplankton biomass in N+ treatment, there was a significant loss of epipelton biomass. Some studies have reported little or even no response of the epipelton to enrichment (Cano *et al.* 2016, Tavares *et al.* 2019) since nutrients from the sediment are considered more critical for epipellic algae than those from the water column (Vinebrooke & Leavitt 1999). In addition, the nutrient competition with phytoplankton can be a determining factor for benthic algal biomass response to enrichment (Zhang *et al.* 2015). Another aspect is the Light:P ratio, which also can explain the algal community growth, as demonstrated in streams (Hill & Fanta 2008, Fanta *et al.* 2010). In the present experiment, the Light:P ratio increased in N+ treatment when compared with the control and other treatments, indicating an imbalance between light and P availability. Thus, our results suggest that the competition for nutrients and Light: Nutrients ratio may have contributed to the loss of epipelton biomass in P-limiting conditions. The effect of light-nutrient co-limitation on the epipelton needs to be

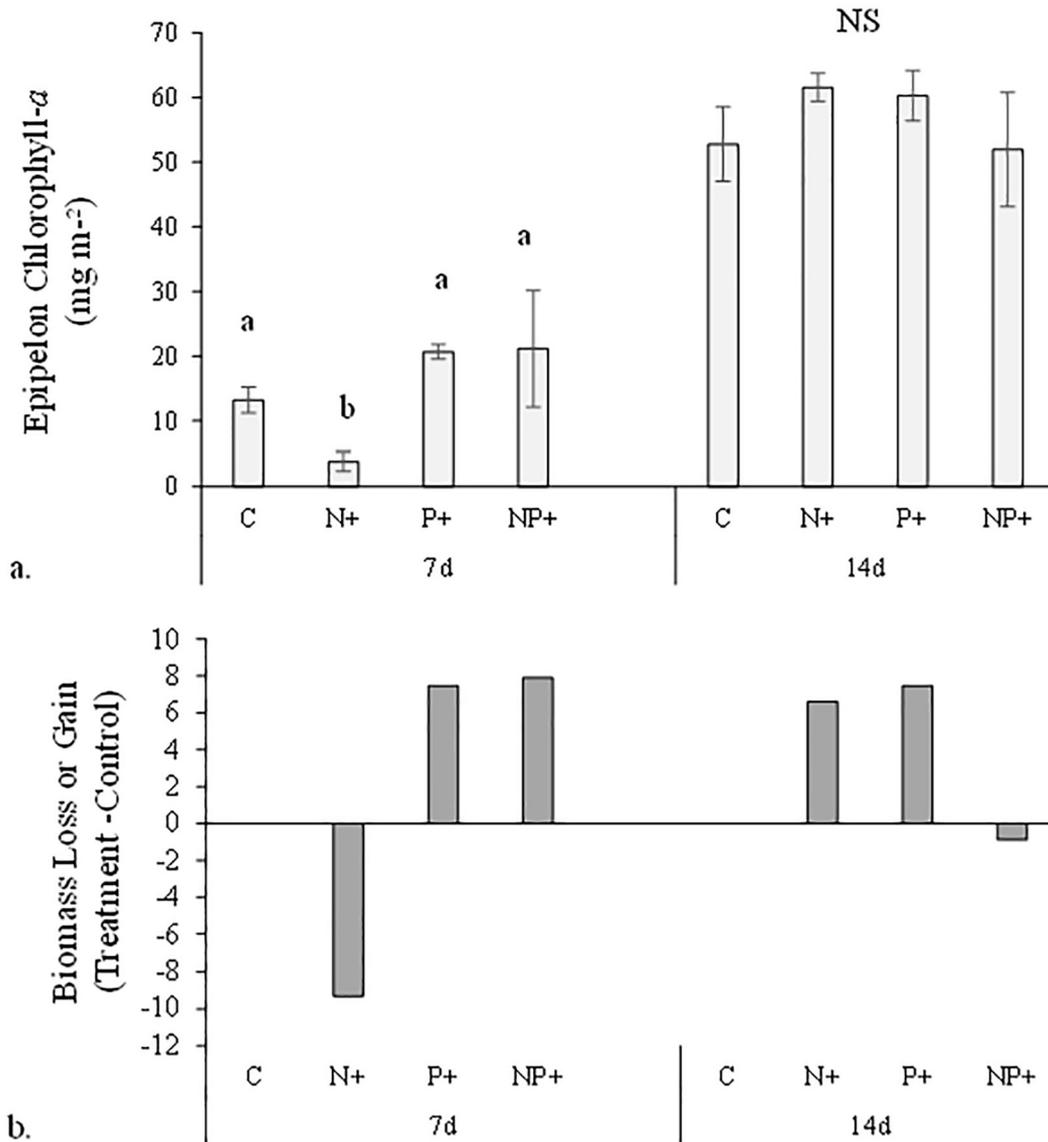


Figure 5. Epipelton chlorophyll-*a* concentrations ($n = 3$; SE) (a) and the difference of the chlorophyll-*a* concentration of the Control and enriched treatments (b). Within each experimental day, treatments marked with the same letter are not significantly different by Tukey test ($\alpha < 0.05$). NS = non-significant.

better understood, particularly in eutrophic environments, where the growth of the phototrophic epipelton is usually restricted.

Regarding the effect of time, there was an increase in epipelton biomass in the treatments compared to the experimental days. Several aspects may have favored the increase in epipelton biomass through time, such as phytoplankton sedimentation increase and reduced physical disturbance (Vadeboncoeur *et al.* 2014, Broman *et al.* 2019). Regards sedimentation, we did not find the loss of phytoplanktonic biomass in the treatments, but, in contrast, there was the loss of epipelton biomass in the N+ treatment. Thus, we did not find clear evidence about the interference of phytoplankton sedimentation influencing epipelton chlorophyll. For example, the loss of phytoplankton biomass in the N+ treatment should have increased epipelton biomass, but this did not occur. Another factor that affects

the increase in epipelton biomass is wave action, which can disturb the lacustrine benthic environment. The effect of the structuring force of waves depends on the depth, size, and physical exposure of a specific location within a lake (Mariotti and Fagherazzi 2012). Additionally, the mixing process can also disturb the benthic environment and act as a structuring factor (MacIntyre & Melack 1995), especially in lakes with low littoral macrophyte coverage (James *et al.* 2003). Thus, physical disturbances cause instability and can impact the benthic algae community (Vadeboncoeur *et al.* 2014, Cano *et al.* 2016). However, the mesocosms structure minimizes or eliminates the effect of horizontal waves, and wind and water column mixing on the epipelton. As highlighted by Vadeboncoeur *et al.* (2014), the role of disturbance resulting from the action of internal waves may be determined for the epipelton, but not for other periphytic communities. Thus, our results

suggested that the reduction in physical disturbances may also have favored the increase in epipelon biomass during the experimental period. Another crucial aspect is competition for nutrients, in which phytoplankton have faster access to added dissolved nutrients than epipelon. Phytoplankton can quickly assimilate nutrients from water, while epipelon can store nutrients (Hwang *et al.* 1998). Thus, the nutrient storage capacity of the epipelon and the reduction of physical disturbances to the community can explain the increase in epipelon biomass over time in all treatments. The P addition and its consequent decrease of P-limitation positively influenced the phytoplankton growth in eutrophic conditions. More specifically, the increased P availability intensified the phytoplankton bloom (isolate and combined addition), while extreme P limitation reduced it (N⁺ treatment). However, there was no positive effect on the epipelon biomass, regardless of the N:P ratio. Thus, our findings partially supported our hypothesis. Our findings reinforce the importance of eliminating the nutrient input, particularly P, for the control of phytoplankton bloom, as widely reported in the literature (*e.g.*, Schindler 2016). There was a significant loss of epipelon biomass under conditions of high P limitation even with a slight improvement in light availability. Thus, our results suggested that epipelon growth could depend on the optimal relationship between light and P availability, as Hill and Fanta (2008) demonstrated for stream periphyton. In conclusion, changes in the N:P ratio affected phytoplankton and epipelon under eutrophic conditions, meanwhile, epipelon growth seems to depend on an optimal relationship between light and P availability. In eutrophic systems, understanding the simultaneous response of communities to the change in N:P can help in restoration processes that alter the stoichiometric balance of nutrients, such as the oligotrophication process.

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Author contributions

Luyza Mayary Amaral: developed the experimental designed and contributes to the epipelon and phytoplankton data; analyzed the results; wrote the manuscript.

Ruan de Oliveira Carneiro: contributed with abiotic data and the critical review.

Carla Ferragut: contributes to the concept and experimental design; analyzed the results; wrote the manuscript.

Conflict of interests

There is no conflict of interest.

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