



Periphyton nutrient content, biomass and algal community on artificial substrate: response to experimental nutrient enrichment and the effect of its interruption in a tropical reservoir

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Abstract

Periphyton plays an important functional role in the retention of nutrients in aquatic ecosystems, especially phosphorus. We evaluated the effects of enrichment with N and P and the effect after 20 days of no additional N and P on periphyton on artificial substratum in open-bottom mesocosms. The aim was to jointly evaluate periphyton, phytoplankton and zooplankton in the presence of macrophytes. Experimental conditions simulated natural conditions and nutrient addition was based on the maximum concentration recorded in mesotrophic reservoir. Our hypothesis is that the periphyton is sensitive to the effects of N and P enrichment and its interruption, despite the positive response of phytoplankton and zooplankton. Two treatments were designed using open-bottom mesocosms ($n = 3$): control (no nutrient addition); NP+ (combined phosphorus and nitrogen addition). Sampling for the measurement of biotic and abiotic variables was performed, with 10 days of continuous enrichment, on the 3rd, 6th and 11th, and 20 days after enrichment had ended (31st day). Periphyton chlorophyll *a*, dry mass and algal density increased significantly with the addition of N and P and decreased 20 days after the interruption of the enrichment. The highest periphyton P content was found in the NP+ treatment. The enrichment had a positive effect on Chrysophyceae (*Chromulina* spp.) and rotifer (*Polyarthra* spp.) density and the interruption of enrichment favored Bacillariophyceae (*Gomphonema* sp.) and rotifers (*Gastropus stylifer*). Phytoplankton responded positively to enrichment. Along with the high macrophyte coverage over the experimental period, we evidenced the positive effect enrichment had on phytoplankton biomass and zooplankton abundance. Therefore, periphyton on artificial substrate was sensitive to effects of N and P enrichment and its interruption, responding promptly to changes in nutrient availability in a scenario of high competition and grazing.

Keywords Macrophyte coverage · Phytoplankton chlorophyll *a* · Periphyton N and P content · Zooplankton

Introduction

Periphyton plays a significant role in the nutrient cycling, energy flow and food web of aquatic ecosystems (Vadeboncoeur and Steinman 2002). Changes in the biomass and

taxonomic structure of the periphytic algae community can provide information on changes in environmental conditions, and can serve as a tool for ecological assessment (McCormick and Stevenson 1998; Stevenson and Smol 2003). Studies have evidenced that periphyton-based metrics can be reliable indicators of the onset of the eutrophication process (Gaiser et al. 2004, 2006). The success of periphytic algae in colonizing substrates and persisting in the community depends primarily on resource availability and the efficiency of their adaptive strategies to compete for resources (Stevenson 1996). Despite the ecological importance of periphyton, changes in community structure due to environmental changes are still not well understood, particularly in shallow tropical reservoirs and lakes.

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The development of the periphytic algae community depends on a complex series of interactions between abiotic and biotic factors; this includes nutrient and light availability and the substrate type, which act as determining factors (Stevenson 1997). There is consensus in the literature that variations in nutrient availability can promote the redirection of a successional trajectory and change the biomass accrual rate in the periphyton (Sekar et al. 2002). Previous studies have reported the strong effect of enrichment on the colonization and succession of the periphytic algae community in tropical reservoirs (e.g., Ferragut and Bicudo 2012). Experimental studies have evaluated the effect of enrichment on autotrophs and grazers (Jones et al. 2002; Olsen et al. 2015; Zhang et al. 2016), but the interactive effects on periphyton response are poorly explored. Biotic factors may also be determinants for the periphyton community structure, especially competition and grazing (Stevenson 1997). Complex interactions can determine how an ecosystem responds to artificial enrichment (nutrient stress), mainly because enrichment can change the equilibrium between primary producers (Havens et al. 2001). Thus, the response of periphyton to experimental enrichment may be more representative of natural conditions in the presence of its main competitors for resources.

Periphyton responds promptly to physical and chemical disturbances, but the competitive interactions and autogenic changes can greatly influence community development (Sand-Jensen and Borum 1991; Hillebrand and Kahlert 2001). Chemical disturbances, such as enrichment, can also influence the resilience (the ability to return to the pre-disturbance state) of the community to environmental changes (Peterson and Stevenson 1992). Regarding the natural nutrient conditions, the current reservoir is mesotrophic, and phosphorus is considered the primary limiting nutrient of periphytic algal growth (Ferragut et al. 2010). Furthermore, the higher macrophyte aquatic cover plays a significant role in ecosystem functioning, especially due to macrophyte influence on development of the algal communities (Fonseca and Bicudo 2011; Souza et al. 2015). We evaluated the effects of enrichment by N and P, as well as the effect of stopping this chemical disturbance, on periphyton and its relationships with phytoplankton and zooplankton in the presence of macrophytes. Periphyton was evaluated within an experimental scenario a little closer to natural conditions. The response of the periphyton to nutrient enrichment can be affected by some characteristics of the other communities, such as the rapid assimilation of nutrients by phytoplankton (Hwang et al. 1998); the control of P release by photosynthetically active epipelton (Genkai-Kato et al. 2012); shading by macrophytes, which reduces photosynthetic activity (McCormick and Stevenson 1998) and grazing on periphytic algae (Jones et al. 2000). The periphyton response to enrichment was analyzed through changes in nutrient content, biomass and algae community structure. Our hypothesis is that

periphyton responds differently during and after the short-term addition of N and P, despite the positive response of phytoplankton and zooplankton. Specifically, we intend to answer two questions: (1) Does N and P enrichment have a positive effect on biomass and change the periphyton structure? (2) Can enrichment by N and P and its stoppage change the taxonomic structure and biomass accrual in periphyton? The present study aims to contribute to a better understanding of periphyton responses to variations in the nutrient concentration in tropical shallow lakes and reservoirs.

Materials and methods

Study area

Periphyton was studied in Ninfeias Reservoir (23°38'18.95"S and 46°37'16.3"W), located in the Parque Estadual das Fontes do Ipiranga (PEFI), São Paulo, Brazil. This reservoir is small, shallow and mesotrophic. The reservoir has surface area of 5433 m², mean depth of 1.32 m, maximum depth of 3.6 m, and mean theoretical residence time of 7.2 days (Bicudo et al. 2002). The reservoir has an extensive littoral zone with abundance of aquatic macrophytes, such as *Nymphaea* spp. (rooted and leaves floating), *Utricularia foliosa* L. (free floating) and *Panicum repens* L. (rooted grass).

Experimental design

The effect of the variation in nutrient availability on the periphyton was evaluated for 10 days of continuous enrichment and 20 days after enrichment had ended (16 September–17 October 2014). The enrichment experiment was performed in situ in open-bottom mesocosms, which were made of transparent acrylic cubes (1 m × 0.6 m × 0.6 m, 316.8 l volume). The experiment consisted of two replicated treatments ($n = 3$): C, control (no N and P addition); NP+, combined P and N addition. Three mesocosms of each treatment (triplicate) were placed in the shallowest part of the reservoir (depth 0.8 m). The mesocosms were firmly pressed and buried 10 cm deep in the sediment. Each mesocosm was secured to a metal rod that prevented displacement. After the installation of the mesocosms, there was an interval for acclimatization (09/12–10/15/2014).

We determined daily DIN (dissolved inorganic nitrogen) and P-PO₄ concentration in the mesocosms. Based on the daily determination of the DIN and P-PO₄ concentrations in the mesocosms, we added enough nitrogen (NaNO₃ Merck) and phosphorus (KH₂PO₄ Merck) to maintain the N:P molar ratio at 16 in the NP+ treatment. The enrichment aimed to increase the phosphorus availability to 38 µg P-PO₄ l⁻¹ and keep the N:P molar ratio equal or close to 16 (a lower ratio recorded in the reservoir). The value of the selected P-PO₄

concentration refers to the highest TP concentration found in the pelagic region, because ambient concentrations were always below the detection limit of the method ($< 4 \mu\text{g l}^{-1}$).

Periphyton on artificial substrate was sampled on the 3rd, 6th, 11th and 31st days of colonization. On these days, water sampling for the physical, chemical and biological variables was performed. Six transparent acrylic slides (199.92 cm^2) were inserted and fixed in a vertical position at a depth of 25 cm inside each mesocosm for periphyton colonization. One substrate colonized by periphyton was randomly removed from the mesocosms on the sampling day. The periphyton was removed from the substrate by scraping with a soft bristle brush and distilled water jets in the laboratory. Periphyton subsamples were separated for the determination of community attributes.

Abiotic and biotic variables analyzed

Surface water samples were collected from the mesocosms using polyethylene bottles for determining abiotic variables and characterizing phytoplankton communities. The following abiotic variables were determined on the sampling day: temperature, electric conductivity, pH (Horiba U-51), nitrite (N-NO_2) and nitrate (N-NO_3) (Mackereth et al. 1978), ammonium (N-NH_4) (Solorzano 1969), orthophosphate (P-PO_4) and total dissolved phosphorus (TDP) (Strickland and Parsons 1960), total nitrogen (TN), total phosphorus (TP).

In the mesocosms, 5 l of water was collected for the determination of zooplankton density using a tube sampler (PVC tube with a 10-cm diameter); the water was then filtered through a 50- μm mesh net. In order to avoid the contraction of organisms, the process of narcotization with CO_2 saturation was accomplished through the addition of carbonated water.

The macrophyte present in the mesocosms was *Nymphaea* spp., which is quite abundant in the reservoir. The *Nymphaea* leaves were cut to standardize the number of leaves in the mesocosms during the experimental period (on average 14 leaves). Macrophyte coverage was determined by multiplying the number of leaves by the mean leaf area (cm^2) in each mesocosm. The leaf area was calculated using the Image J program 1.47 (Rasband 2008).

We determined phytoplankton and periphyton chlorophyll *a* (corrected for phaeophytin) concentration from subsamples filtered with glass-fiber filters (GF/F Whatman, Maidstone, UK), following 24 h extraction with 90% ethanol in the dark (Sartory and Grobbelaar 1984). Periphyton dry mass (DM) was determined by filtration of subsamples into pre-calcined glass-fiber filters (GF/F Whatman), which were stored ($100 \text{ }^\circ\text{C}$) and weighed daily until constant mass was obtained (APHA 2005).

Periphyton subsamples were preserved with a 4% formalin solution for qualitative analysis and with an acetic Lugol solution for quantitative analysis. Algal quantifications were performed under a Zeiss Axiovert microscope (400 \times) according to Utermöhl (1958), and subsample sedimentation time was measured in sedimentation chambers following Lund et al. (1958). The counting limit was determined using the species rarefying curve. We considered descriptor species those with relative density $\geq 10\%$ and dominant species those with a relative density $\geq 50\%$.

Periphyton phosphorus content was determined by combustion of the subsamples at $550 \text{ }^\circ\text{C}$ for 1 h, and then the samples were heated with 1 N HCl at $80 \text{ }^\circ\text{C}$ for 30 min (Andersen 1976; Pompêo and Moschini-Carlos 2003). Subsequently, the P-PO_4 concentration was determined using the ascorbic acid method (Strickland and Parsons 1960). Periphyton nitrogen content was determined using the micro-Kjeldahl method. Nitrogen (TN) and phosphorus (TP) content was expressed as percentage of dry mass (% DM).

The quantitative analysis of the zooplankton was performed on an acrylic plate, checked under a microscope with 50 \times magnification, for Cladocera and Copepoda. The counts of Rotifera and Copepoda nauplii were carried out in a Sedgewick-Rafter chamber under an optical microscope with a magnification of 100 \times . The limit count was determined using the species rarefaction curve.

Data analysis

We used two-way ANOVA to determine statistical differences in the DIN, P-PO_4 and N:P ratio between treatments and time, and two-way repeated-measures ANOVA (RM ANOVA) to determine the effects of enrichment and time on periphyton attributes (N and P content, chlorophyll *a*, dry mass and algal density) and zooplankton density. The Tukey and Student Newman–Keuls (SNK) tests, a posteriori comparison of means, were used to detect the minimum significant difference between treatments ($\alpha = 0.05$), and were performed using the statistical program SigmaPlot 11.0.

Cluster analysis was performed to determine the similarity in species composition of the periphytic algae community between treatments. This analysis was performed with an algal density matrix, using mean association (UPGMA) and the Simpson index application. Non-parametric permutational multivariate analysis of variance (two-way PERMANOVA) was applied to analyze the effect of enrichment on the taxonomic structure of the periphytic algae community. This analysis was performed using Bray–Curtis similarity and 4999 permutations, and performed using Past 3.14 (Hammer et al. 2001).

Results

Abiotic variables

During the enrichment period (4–15 days), P-PO₄ concentration in the NP+ treatment was higher and significantly different from the control on the 3rd and 11th days (SNK: $p < 0.001$). DIN concentration was higher and significantly different in the NP+ treatments from the control on the 3rd and 6th days (SNK: $p < 0.03$). Twenty days after nutrient interruption, DIN concentration in the control was higher and significantly different from NP+ treatment (SNK: $p = 0.006$). The N:P molar ratio in the control ranged from 11.9 to 21.1 and in the NP+ treatment, it ranged from 16.2 to 77.7 during the enrichment period. After the enrichment period the N:P molar ratio in the control was 61.9 and was 11.2 in the NP+ treatment (Table 1).

Periphyton

Periphyton chlorophyll *a* and dry mass were significantly influenced by enrichment, colonization time, and the interaction of these two factors (Fig. 1a, b; ANOVA: $p < 0.05$). Periphyton chlorophyll *a* was significantly different between the control and NP+ treatment on the 3rd, 6th and 11th days (Tukey test: $p < 0.05$). In the NP+ treatment, the maximum value of periphyton chlorophyll *a* was reduced by 3.4 times, 20 days after enrichment had ended. Periphyton dry mass increased exponentially in NP+ treatment and fluctuated in the control, but the significant difference occurred only on the 3rd day of colonization (Tukey test: $p < 0.05$).

Periphyton P content showed a significant difference between NP+ treatment and the control on the 3rd, 6th and 11th days (Fig. 1c; Tukey test: $p < 0.05$). We found

the lowest periphyton P content in the NP+ treatment on the 31st day of colonization. Periphyton N content showed statistically significant interaction between enrichment and time factors (Fig. 1d). Periphyton N and P content had significant difference among treatments on the 3rd and 11th days (Tukey test: $p < 0.05$).

Regarding periphytic algae community structure, we found that algal density was significantly influenced by enrichment, colonization time, and the interaction of these two factors (Fig. 2; ANOVA: $p < 0.05$). Multiple comparisons for enrichment factor showed that periphyton algal density was significantly different between the control and NP+ treatment on the 3rd, 6th and 11th days (Tukey test: $p < 0.05$).

A total of 59 periphytic algae species were identified in the treatments. These species belong to the following algal classes: Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Cyanobacteria, Euglenophyceae and Zygnemaphyceae. Chrysophyceae and Bacillariophyceae were the most representative algal classes in the community structure (Fig. 3). In the control, Chrysophyceae (32%), Cyanobacteria (22%) and Euglenophyceae (19%) had high relative densities on the 6th day of colonization, but later Chrysophyceae dominated (> 50%). Chrysophyceae (60%) was the class most representative in the control and NP+ treatment during the enrichment period. Bacillariophyceae (50%) was dominant in NP+ treatment and Chrysophyceae maintained dominance in the control 20 days after enrichment had ended (31st day).

Periphyton species composition changed over time in the control and NP+ treatment (Fig. 4). In the control, *Chromulina* spp. and *Chroococcus* sp. were the most abundant taxa in the periphyton on the 3rd and 6th days of colonization, while *Chromulina* spp. and *Synechocystis aquatilis* were more abundant on the 11th and 31st days. In the NP+ treatment, *Chromulina* spp. was the most representative species on the 3rd, 6th and 11th days of colonization. Despite the dominance of *Chromulina* spp., the relative density was higher in the NP+ treatment compared to the control (Fig. 4b). The highest *Chromulina* spp. density occurred on the 6th day in the NP+ treatment, representing 60% of the total density. Diatom *Gomphonema* sp. had the highest relative density in the NP+ treatment 20 days after enrichment ended (31st day).

Two-way PERMANOVA showed that the periphytic algae community structure was significantly influenced by enrichment and time factors ($p < 0.01$), but there was no significant interaction between the two factors.

Clustering analysis showed the formation of two groups at a similarity level of 54%: control and treatment (Fig. 5). The highest similarity in species composition was found between the 6th and 11th days (70%) in the control group.

Table 1 Average and standard deviation of DIN, P-PO₄ and NP molar ratio in the control and treatment with N and P combined addition (NP+) on sampling day

Days	Treatments	Variables		
		DIN (μg l ⁻¹)	P-PO ₄ (μg l ⁻¹)	NP ratio
3rd day	Control	9.0 ± 2.1	< 4.0	11.9 ± 2.7
	NP+	102.5 ± 83.7*	7.7 ± 3.2*	33.9 ± 35.7
6th day	Control	14.7 ± 2.4	< 4.0	19.4 ± 3.2
	NP+	129.2 ± 25.8*	< 4.0	77.7 ± 17.5*
11th day	Control	28.4 ± 9.1	< 4.0	21.1 ± 11.2
	NP+	97.0 ± 16.3	13.3 ± 1.4*	16.2 ± 3.6
31st day	Control	136.6 ± 96.8	4.3 ± 2.3	61.9 ± 24.8
	NP+	15.4 ± 13.0*	< 4.0	11.2 ± 2.2*

*Significant difference between control and NP+ treatment

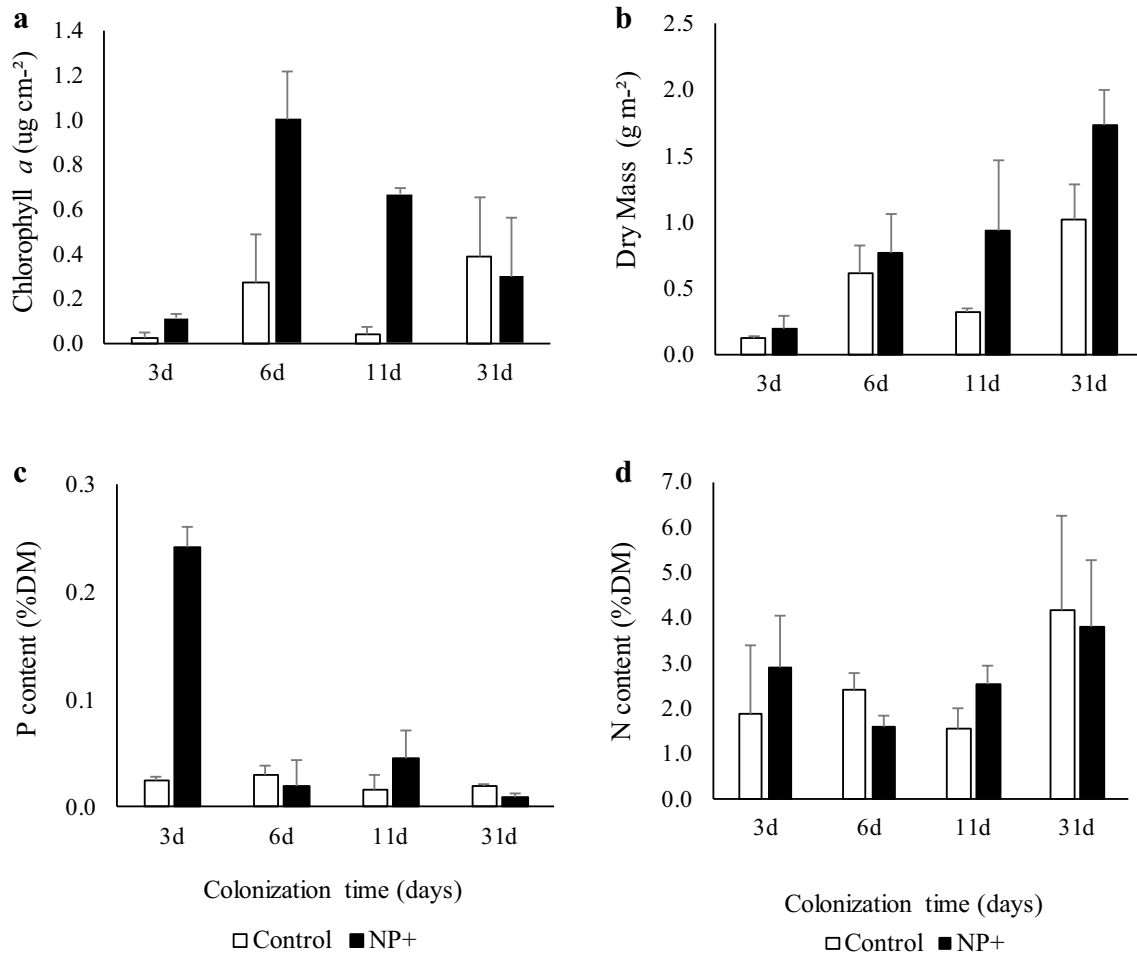


Fig. 1 Periphyton chlorophyll *a* (a), dry mass (b), phosphorus (c) and nitrogen (d) content ($n = 3; \pm$ SD) in control and combined N and P addition treatment (NP+) during the enrichment continuous period (3rd, 6th, 11th days) and 20 days after its interruption (31st day)

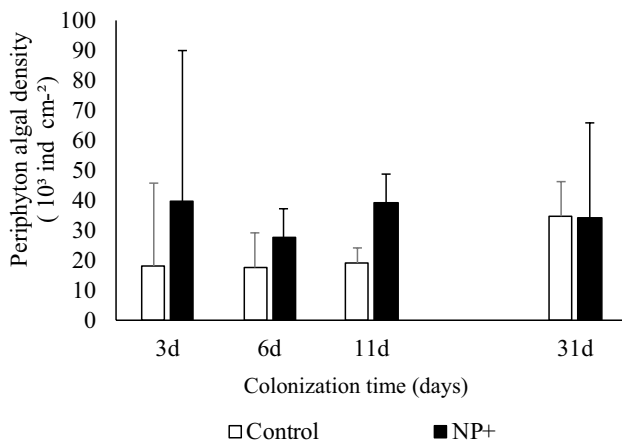


Fig. 2 Periphyton total density in control (C) and combined N and P addition treatment (NP+) during the enrichment continuous period (3rd, 6th, 11th days) and 20 days after its interruption (31st day)

The same result was also observed in the NP+ treatment (75% similarity).

Macrophyte and phytoplankton

The *Nymphaea* spp. coverage stayed constant in both the control and NP+ treatment during the experimental period, ranging from 49.3 to 57.0% in the control and 57.0–66.1% in NP+ treatment during the enrichment period. After the enrichment period, the *Nymphaea* spp. average coverage was 70% in the control and 79% in the NP+ treatment.

Phytoplankton chlorophyll *a* varied from 3.5 to 14.1 $\mu\text{g l}^{-1}$ in the control and from 22.0 to 78.2 $\mu\text{g l}^{-1}$ in the NP+ treatment (Fig. 6a). After the enrichment period, phytoplankton chlorophyll *a* was 12.9 $\mu\text{g l}^{-1}$ in the control and 9.1 $\mu\text{g l}^{-1}$ in the NP+ treatment.

Fig. 3 Relative density of periphytic algae class ($n = 3$) in combined N and P addition treatment (NP+) during the enrichment continuous period (3rd, 6th, 11th days) and 20 days after its interruption (31st day)

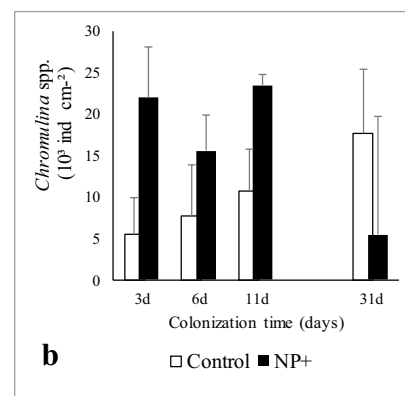
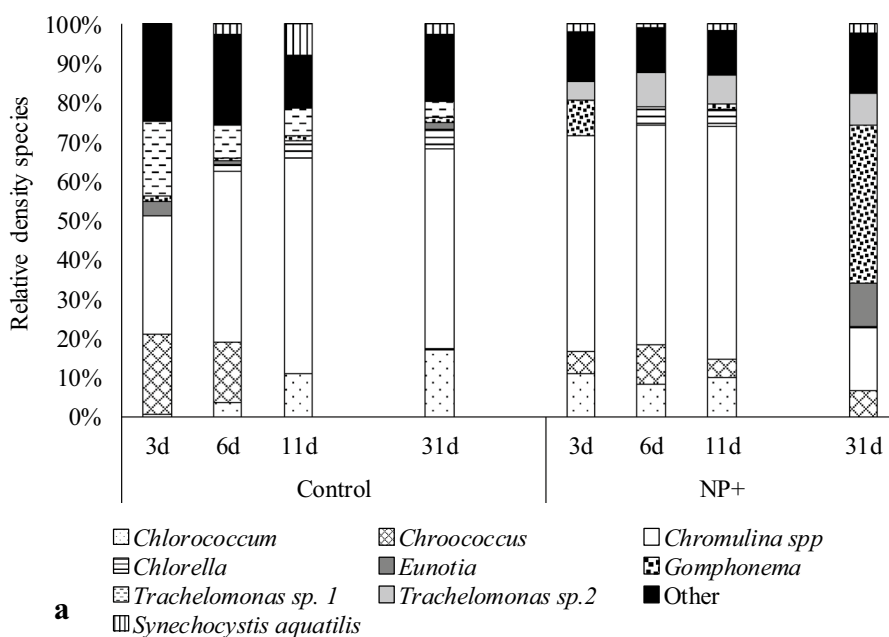
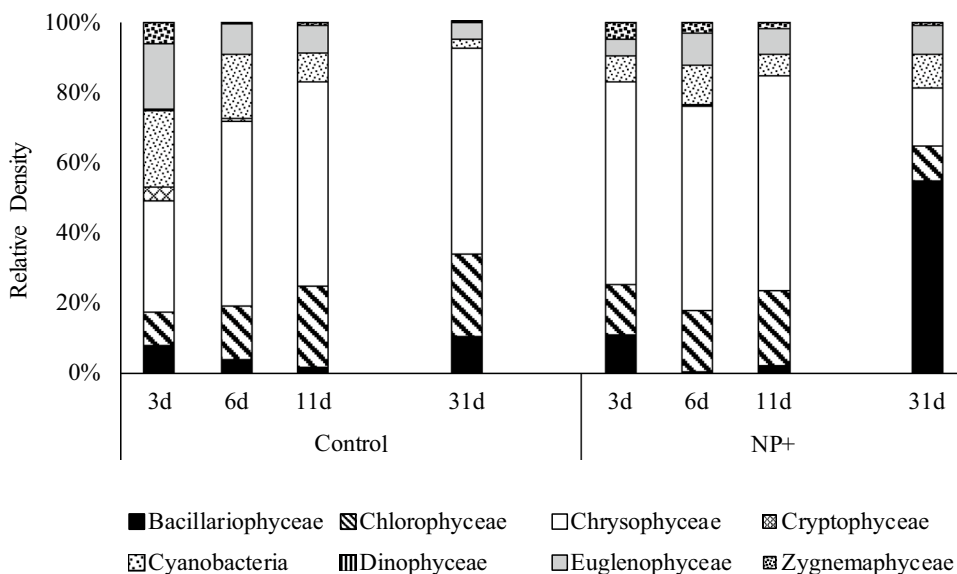


Fig. 4 Relative density of periphytic descriptor species (a) and *Chromulina* spp. density (b) in control and combined N and P addition treatment (NP+) during the enrichment continuous period (3rd, 6th, 11th days) and 20 days after its interruption (31st day)

Zooplankton

Zooplankton total density was significantly different between the control and the NP+ treatment during the enrichment period (Fig. 6b; RM ANOVA: $F = 13.00$; $p = 0.023$). Total density was significantly different only on the 11th day (Tukey test: $p < 0.05$) and was 2.3 times higher than amounts found in the control on the 11th day. After 20 days

without enrichment, zooplankton total density was not significantly different between treatments, but a significant difference could be observed between the 11th and 31st days in the NP+ treatment.

Considering taxa with a contribution greater than 10% to the community abundance, we verified that *Polyarthra* spp. (Rotifera) was the most favored species (Table 2). Twenty days after enrichment ended (31st day), we

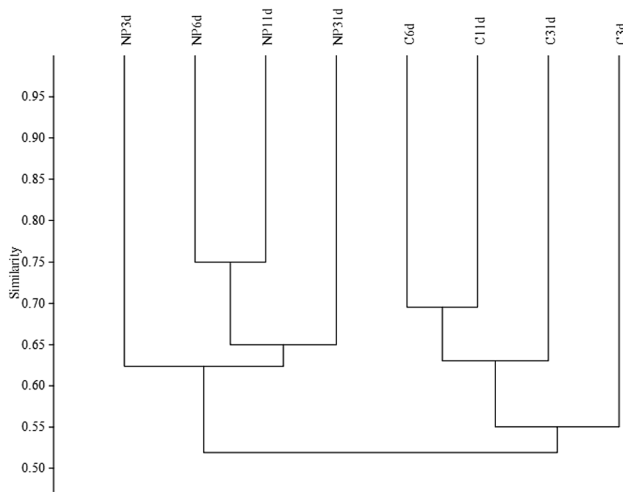


Fig. 5 Similarity of species composition of periphytic algae in control (C) and combined N and P addition treatment (NP+) during the enrichment continuous period (3rd, 6th, 11th days) and 20 days after its interruption (31st day)

observed a decrease in average density of *Polyarthra* spp. and an increase of *Gastropus stylifer* (Rotifera) in the NP+ treatment.

Discussion

Our experimental results showed that combined addition of N and P had a positive and significant effect on biomass, P content and algal density in the periphyton on artificial substrate. In addition, the taxonomic structure of the periphytic

algae community changed with the combined addition of N and P, but changes also occurred 20 days after enrichment had ended. On the 31st day of colonization, there was a reduction in algal growth and biomass accumulation in periphyton. The enrichment period favored the increase of rotifer density in the water, mainly *Polyarthra* spp., which is a raptorial rotifers and commonly plant-associated (Obertegger and Flaim 2015; Romo et al. 2004). Although water temperature is a determining factor for population dynamics of the *Polyarthra* (Virro 1995), enrichment can act significantly on rotifer density (Romo et al. 2004). Based on the feeding habits of the most abundant species of zooplankton, we believe that the grazing pressure was low on the periphyton. We showed that the positive response of phytoplankton biomass and zooplankton density did not minimize the enrichment effect on the periphyton.

Our findings have shown that the positive response of periphyton chlorophyll *a* and algal density to enrichment was not affected by the high phytoplankton abundance and macrophyte coverage throughout the experimental period. Besides nutrient competition, high phytoplankton abundance and macrophyte coverage could inhibit growth of periphyton due to strong shading (Genkai-Kato et al. 2012; Souza et al. 2015). Previous studies reported that high macrophyte coverage (80–100%) and phytoplankton biomass can negatively influence periphyton biomass accrual (Casartelli and Ferragut 2015). The interactive effects between light and nutrient availability could influence the ability of the periphyton to assimilate and use N and P, which could, in turn, reduce the development of the periphyton (Liess et al. 2009). However, the light:nutrient ratio can have a large impact in determining algal response (Fanta et al. 2010), or low light

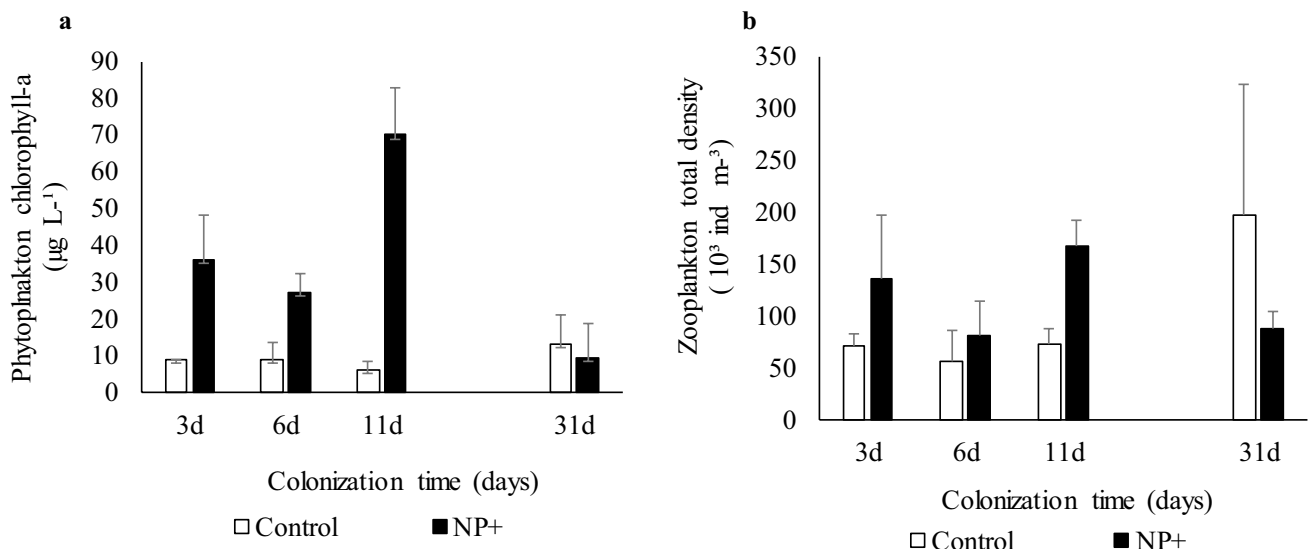


Fig. 6 Phytoplankton chlorophyll *a* (a) and zooplankton total density (b) in control (C) and combined N and P addition treatment (NP+) during the enrichment continuous period (3rd, 6th, 11th days) and 20 days after its interruption (31st day)

Table 2 Zooplankton taxa with relative density greater than 10% of total density (indm^{-3}) in the control and combined N and P addition treatment (NP+) on sampling day ($n = 3$)

Days	Treatment	Taxa	Group	Average density	%
3rd day	Control	<i>Polyarthra</i> spp.	Rotifers	18,933	26.1
	NP+	<i>Polyarthra</i> spp.	Rotifers	41,867	31.0
6th day	Control	Nauplius	Copepods Calanoid	7533	13.6
		<i>Polyarthra</i> spp.	Rotifers	7700	10.7
	NP+	<i>Polyarthra</i> spp.	Rotifers	25,000	30.8
		<i>Chydorus pubescens</i>	Cladocerans	13,867	10.3
11th day	Control	<i>Polyarthra</i> spp.	Rotifers	9200	8.5
		Nauplius	Copepods Cyclopoid	5933	8.2
	NP+	<i>Polyarthra</i> spp.	Rotifers	62,733	37.5
31st day	Control	<i>Asplanchna brightwellii</i>	Rotifers	49,800	25.4
		<i>Asplanchna sieboldi</i>	Rotifers	48,067	24.5
	NP	<i>Gastropus stylifer</i>	Rotifers	19,867	22.9

availability may not inhibit algae response to high nutrient concentration due to other factors (Rober et al. 2015). As the epiphyton response was different (Santos 2017, unpublished data), we believe that the position of the substrate in the water column may have promoted a light:nutrient ratio favorable to periphyton development, not masking the enrichment effect. The type and position of the substrate can be a determining factor in periphytic biomass accrual and algae community (Cattaneo and Amireault 1992; Vadeboncoeur et al. 2006).

Periphyton chlorophyll *a* and algal density decreased after enrichment had ended, especially when compared to the 11th day of colonization. Differences in periphyton attributes between the control and NP+ treatment were minimized 20 days after enrichment had ended, thus showing the significant effect that enrichment has on periphyton structure. We observed that the periphyton responded promptly to the changes in nutrient availability in the water. Besides, the periphyton P content was significantly higher in the NP+ treatment than the control (on days 3 and 14) during the enrichment period and significantly reduced after the enrichment stopped (on day 35). Thus, periphyton response to nutrient addition was also verified by the P content, which was coupled with water P concentration, as observed in other studies (e.g., Gaiser et al. 2004). The ability of periphyton to retain nutrients is an efficient competitive strategy, especially over time (Havens et al. 1999). On the other hand, periphyton dry mass showed exponential growth even after the enrichment period, indicating high participation of non-algal components in the community, including detrital components. The dissolved organic matter (DOM) can have a great influence on periphyton elemental composition (Frost et al. 2007). Therefore, our results showed that nutrient availability in the water was a determining factor for the development of the periphyton on artificial substrate.

We found that the nutrient enrichment and the effect of its interruption caused changes in the periphyton taxonomic

structure throughout the experimental period. However, the combined N and P addition was not sufficient to alter the representativeness of algal classes in the periphyton, since Chrysophyceae was dominant during the enrichment period (first 11 days), especially *Chromulina* spp. After stopping enrichment, we found a negative effect on the *Chromulina* spp. density. On the 31st day of colonization, the reduction in nutrient availability had a negative effect on periphyton biomass and algal density, favoring the substitution of Chrysophyceae (*Chromulina* spp.) with Bacillariophyceae (*Gomphonema* sp.). Chrysophyceae present competitive advantages, such as mixotrophy and the presence of flagella, which may explain their absolute dominance in the periphyton and their success during the enriched treatment (Sandgren 1988). *Chromulina* have characteristics that guarantee success in adverse conditions, including rapid reproduction, higher surface/volume ratio and presence of a flagellum, which facilitates the obtaining of resources (Happy-Wood 1988). Although the enrichment effect on the algal classes was only quantitative, the reduction of nutrient availability due to stopping enrichment changed the class-level structure, favoring diatoms, especially *Gomphonema* sp. Therefore, the interruption of additional nutrients changed the taxonomic structure of the periphytic algae community in the NP+ treatment.

We have demonstrated that biomass and periphytic algae community structure responded promptly to N and P enrichment, as reported in numerous experiments (e.g., Havens et al. 1999; Hillebrand and Kahlert 2001). However, the interruption of the chemical disturbance reduced the accumulation of biomass and redirected taxonomic structure in the periphyton, including changes in class and species level. The effect of the enrichment on the periphyton on artificial substrate was not masked by an increase in the phytoplankton biomass and the zooplankton abundance in the presence of the high macrophyte coverage. Our findings have shown that periphyton on an artificial substrate is sensitive

to N and P enrichment and the effects of its interruption in a mesotrophic reservoir, responding promptly to changes in nutrient availability in a highly competitive environment for resources, and with grazing pressure.

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