



Effects of temperature increase and nutrient enrichment on phytoplankton functional groups in a Brazilian semi-arid reservoir

Efeito do aumento da temperatura e enriquecimento de nutrientes nos grupos funcionais fitoplanctônicos de um reservatório brasileiro do semiárido

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Abstract: Aim: Our study aimed to evaluate changes in the phytoplankton functional groups brought about by increases in temperature and nutrients predicted by the Intergovernmental Panel on Climate Change (IPCC) scenario for semi-arid regions. **Methods:** Two experiments were performed, one in the rainy season and another in the dry season. The nutrient enrichment was based on the annual mean values (August 2012-August 2013) of soluble reactive phosphorus and nitrate verified in the reservoir. The microcosms were exposed to two different temperatures, the five-year average of air temperature in the reservoir (control) and 4°C above the control temperature (warming). The experiment was conducted over 12 days; every three days water samples of approximately 60 mL in volume were taken from the reservoir for chemical and phytoplankton analysis. All species were classified by Reynolds Functional Groups (RFG). **Results:** The functional groups H_1 , X_1 , L_0 and S_1 were the most representative in both seasons (rainy and dry). Our results showed that bloom-forming cyanobacteria, in particular the species of functional groups H_1 and M , commonly reported in reservoirs in semi-arid regions of Brazil, were not significantly benefited by the warming and nutrient enrichment. The recruitment of other blue-green species, as well as diatoms and green algae, could be observed. **Conclusions:** The effects of warming and/or nutritional enrichment can change the structure of the phytoplankton community. However, as not expected as the pessimist scenario, in our study the bloom-forming phytoplankton functional groups did not show changes in relative biomass. Instead, the recruitment of diatoms and green algae currently found in enriched environments was verified, specifically in the rainy period, when nutrient dilution typically occurs.

Keywords: cyanobacteria; experiment; warming; reservoir.

Resumo: Objetivo: Nosso estudo teve como objetivo avaliar, através de escala experimental, mudanças dos grupos funcionais fitoplanctônicos devido ao aumento de temperatura e nutrientes fornecidos pelo cenário do IPCC para a região semiárida. **Métodos:** Foram realizados dois experimentos, um no período chuvoso e outro no período seco. A água coletada no reservatório foi enriquecida com nutrientes (nitrato e fósforo solúvel reativo) em diferentes concentrações. Os microcosmos foram submetidos a duas temperaturas diferentes, média de cinco anos da temperatura do ar no



reservatório (controle) e 4°C acima da temperatura de controle (aquecimento). O experimento demorou 12 dias e a cada 3 dias cerca de 60 mL de água eram retiradas para as análises químicas e do fitoplâncton. Todas as espécies foram classificadas pelos grupos funcionais de Reynolds. **Resultados:** os grupos funcionais H_1 , X_1 , L_0 e S_1 foram os mais representativos em ambas as estações (chuvosa e seca). Nossos resultados mostraram que as cianobactérias formadoras de florações, especialmente as espécies de grupos funcionais H_1 e M , que são comumente relatadas em reservatórios da região semiárida brasileira, não foram significativamente beneficiadas pelo enriquecimento de nutrientes e aquecimento. O recrutamento de outras espécies de cianobactérias, bem como diatomáceas e algas verdes foram observadas. **Conclusões:** Os efeitos do aquecimento e/ou do enriquecimento nutricional podem alterar a estrutura da comunidade fitoplanctônica. Entretanto, como não esperado no cenário pessimista, em nosso estudo, os grupos funcionais formadores de floração não mostraram mudanças na sua biomassa relativa. Em vez disso, o recrutamento de diatomáceas e algas verdes, atualmente encontradas em ambientes enriquecidos, foi verificado, especialmente no período chuvoso, quando geralmente há diluição dos nutrientes.

Palavras-chave: cianobactéria; experimento; aquecimento; reservatório.

1. Introduction

Global temperatures are rising, causing changes in seasonality and precipitation (IPCC, 2013). Current predictions indicate that temperatures will increase significantly compromising water availability, water quality, agricultural production and economies, and ecosystems. In freshwater bodies, nutrient inputs and increasing temperatures may increase eutrophication and trigger the occurrence of cyanobacteria (Moss et al., 2011). Climate change is therefore a strong catalyst for the expansion of blue-green algae blooms (Paerl & Huisman, 2009).

Cyanobacteria blooms, in particular potentially toxic blooms, have been reported in many parts of the world (Sukenik et al., 2012). This expansion has been attributed to global temperature increases and the enrichment of nutrients such as phosphorus and nitrogen in bodies of water (Schindler, 1975; Anderson et al., 2002; Paerl & Huisman, 2008; Moss et al., 2011). In general, cyanobacteria grow quickly in temperatures above 25°C, being faster than eukaryotic algae (Coles & Jones, 2000; Butterwick et al., 2005; Reynolds, 2006).

Climate models indicate that arid and semi-arid areas are more susceptible to climate change (IPCC, 2013). Semi-arid watersheds in Brazil tend to be more sensitive to reduced rainfall and water flow compared to humid tropical systems (Roland et al., 2012), which may lead to changes in water levels and hydraulic retention times. These factors may contribute to nutrient concentration and intensified eutrophication (Jeppesen et al., 2015). Some studies performed during drought events in semi-arid regions have shown changes in phytoplankton dynamics, including a predominance of cyanobacteria (Dantas et al., 2011; Bittencourt-Oliveira et al., 2013; Medeiros et al., 2015).

In order to understand the effects of global warming on phytoplankton, many researchers have carried out experiments in which temperature and/or nutrient levels are manipulated. Most experiments that evaluated the response of phytoplankton to increased temperatures were conducted in monocultures using temperatures higher than those predicted by climate models (Coles & Jones, 2000; Butterwick et al., 2005; Lüring et al., 2013). Few studies have assessed the effects of increased temperature on natural communities (Moss et al., 2003; Lassen et al., 2010). In these communities, it is expected that the warming will influence the composition and abundance of phytoplankton by favoring the tolerant species (Winder & Sommer, 2012).

The phytoplankton functional groups approach (Reynolds et al., 2002) applied to aquatic systems has provided important information for understanding of the dynamics of species selection. Several studies in phytoplankton ecology have adopted this functional approach in order to understand changes in aquatic ecosystems based on the physiological, morphological, and ecological traits of the species (Becker et al., 2010; Kruk et al., 2017; Santana et al., 2017). This approach (RFG or Reynolds Functional Groups, according to Kruk et al., 2017), is widely used in ecological studies of freshwater phytoplankton and is applicable to different types of environments around the globe. Nowadays the classification includes a total of 40 RFGs (Padisák et al., 2009).

Several studies in semi-arid regions note that drought increases the risk of salinization, thereby aggravating the symptoms of eutrophication and increasing the intensity of cyanobacteria blooms (Costa et al. 2016; Brasil et al., 2016). Then, our study aimed to evaluate the influence of temperature increase and nutrient enrichment on

phytoplankton functional groups, in a microcosm experiment, of a tropical reservoir located in the Brazilian semi-arid region, based on IPCC (2007) scenarios. In light of the extreme events predicted for semi-arid tropical regions, including severe drought and eutrophication in water bodies, we hypothesized that the experimental warming and/or nutrient enrichment would favor bloom-forming cyanobacteria functional groups.

2. Material and Methods

2.1. Study area

Subsurface water samples were collected in the rainy (May) and dry (September) seasons of 2013, in the pelagic zone of the Boqueirão de Parelhas reservoir (6°42'38"S; 36°36'39"W), located in the Piranhas-Assú watershed in the semi-arid region of northeastern Brazil. The regional climate is tropical semi-arid (*BS'h'*, Alvares et al., 2013), characterized by irregular rainfall, high evapotranspiration rates, and negative water balance during most of the year. The reservoir has a maximum capacity of $85 \times 10^6 \text{ m}^3$, although its volume does not exceed $10 \times 10^6 \text{ m}^3$ of water and its maximum depth is 13 m. In 2012, the reservoir was considered mesotrophic without thermal or chemical stratification (Galvão et al., 2014). But, due to a prolonged drought, evidenced in 2013, the reservoir changed from mesotrophic to eutrophic state (Gomes, 2016). From 1960 to 2006, the mean annual precipitation was 726 mm, with a well-defined rainy season between January and July, and an almost complete lack of precipitation (dry season) during the remaining months (Brasil et al., 2016). The reservoir is used for multiple purposes including drinking water, irrigation, recreation, and fishing.

2.2. Experiment design

Water samples were collected from the reservoir, transferred to 20L bottles, and immediately taken to the laboratory to begin the experiments. The experiments were conducted by using transparent glass 1L beakers, which were filled with water from the sample, with four replicates, for temperature manipulation and nutrient addition. Each day the beakers were manually shaken and submitted to a 12:12 hours light/dark photoperiod. The experiment went on for 12 days; every three days, 60 mL of water was collected from each beaker for chemical analysis and phytoplankton quantification.

For the temperature manipulation, the scenario A2 of IPCC (2007) was considered, taking into

account an increase of 4 °C. Thus, based on the mean values of five years of temperature recorded for the reservoir region, the control temperature was considered 26°C e 27°C for dry and rainy season, respectively, and that of the treatment simulating the heating was 30°C and 31 °C. For this, two independent incubators were used.

To simulate nutrient loading, solutions of NaNO_3 and KH_2PO_4 were added to the microcosms on the first day of experiment in an attempt to simulate a punctual release of nutrients. The nutrient enrichment was based on the annual mean values (August 2012 - August 2013) of soluble reactive phosphorus and nitrate verified in the reservoir ($4.47 \mu\text{g L}^{-1}$ and $237.30 \mu\text{g L}^{-1}$, respectively). The enrichment was performed as follows: (i) treatment C, control treatment, without nutrient enrichment; (ii) treatment P2, enriched with twice the mean concentration of soluble reactive phosphorus; (iii) treatment N2, enriched with twice the mean concentrations of nitrate; (iv) treatment P2+N2, the combination of P2 and N2; (v) treatment P4, enriched with four times the mean concentration of soluble reactive phosphorus; (vi) treatment N4, enriched with four times the mean concentration of nitrate; and (vii) treatment P4+N4, the combination of P4 and N4.

2.3. Phytoplankton community

Phytoplanktonic organisms were identified, whenever possible, at the species level. For quantitative analysis, phytoplankton abundance was estimated according to Utermöhl (1958), in inverted microscope, at 400x magnification. Individuals (cells, cenobia, colonies and filaments) were listed in random fields (Uhelinger, 1964). The biovolume ($\text{mm}^3 \text{ L}^{-1}$) was calculated on the basis of approximate geometric models (Hillebrand et al., 1999), assuming the unit of fresh weight expressed in mass (mg L^{-1}). Species richness was estimated by the number of taxa and the diversity index as established by Shannon & Weaver (1963).

The descriptor species were defined as those that contributed with more than 5% of the total biomass. All species were classified into functional groups, based on Reynolds et al. (2002) and Padišák et al. (2009).

2.4. Data analysis

Box plot analysis was performed to access the biological data variation. Two-way blocking analysis of variance (ANOVA) was performed to evaluate the effect of warming (block) and nutrient enrichment

on phytoplankton functional groups. The data were transformed ($\log_{10}(x+1)$) to meet the assumptions of ANOVA. In all statistically significant situations ($p < 0.05$) the Tukey Honest Significant Difference (HSD) test was applied. The statistical analyses were performed using STATISTICA® software v.11.

3. Results

A total of 28 phytoplankton species of three major taxonomic categories (Cyanobacteria, Chlorophyceae and Bacillariophyceae) were distributed in 12 phytoplankton functional groups (Table 1). The phytoplankton functional groups **H₁**, **X₁**, **L₀** and **S₁** were the most representative in both seasons.

Significant decrease of phytoplankton total biomass ($F = 3.7$; $p = 0.05$) was verified under the temperature increase in the dry season experiments (Figure 1). Following this same tendency, the warming effect also significantly influenced the biomass increase of Bacillariophyceae in the dry period ($F = 7.7$; $p = 0.007$) and its decrease in the rainy season ($F = 7.6$; $p = 0.007$), which was also observed for Chlorophyceae biomass ($F = 8.4$;

$p = 0.005$). Species richness increase in the rainy season ($F = 4.0$; $p = 0.04$) and decrease in the diversity values ($F = 9.2$; $p = 0.003$) in the dry season were also assigned to the isolated effect of temperature rising (Figures 2, 3). Significant influence of nutrient enrichment on the phytoplankton total biomass, major group contribution, species richness or diversity of both rainy and dry periods was not verified.

Biomass of phytoplankton functional groups **B**, **S₁** and **X₁** were significantly influenced by the temperature manipulation and nutrient enrichment only during the rainy season experiments (Figure 4). The warming led to the decrease in the biomass of **B** ($F = 9.4$; $p = 0.003$) and **S₁** ($F = 6.2$; $p = 0.015$) and increase of **X₁** ($F = 69.5$; $p = 0.000$). The nutrient enrichment, in turn, led to the significant increase of the biomass of the functional group **B** in the treatment P4+N4 ($F = 3.0$; $p = 0.012$), of **S₁** biomass in the treatment P2 ($F = 4.5$; $p = 0.000$) and **X₁** functional group biomass in all the enriched treatments, specially P4+N4 ($F = 3.6$; $p = 0.003$).

Regarding the biomass of the functional groups in the dry season experiments, the warming effect

Table 1. Functional groups, descriptor species and corresponding taxonomic groups found during rainy and dry seasons in microcosm experiments.

Functional Group	Descriptor(s) specie(s)	Taxonomic Group
H₁	<i>Dolichospermum planctonica</i> (Brunnthal) Wacklin, L. Hoffmann & Komárek 2009	Cyanobacteria
	<i>Aphanizomenon gracile</i> Lemmermann	
K	<i>Aphanocapsa</i> sp. Nägeli	Cyanobacteria
	<i>Aphanothece</i> sp. Nägeli	
Z	<i>Chroococcus</i> sp. Nägeli	Cyanobacteria
L₀	<i>Coelosphaerium</i> cf. <i>aerugineum</i> Lemmermann	Cyanobacteria
	<i>Coelosphaerium</i> sp. Nägeli	
	<i>Radiocystis</i> sp. Skuja	
	<i>Lemmermanniella</i> sp. Geitler	
	<i>Gloeococystis</i> sp. Nägeli	Chlorophyceae
M	<i>Microcystis aeruginosa</i> (Kützing) Kützing	Cyanobacteria
S₁	<i>Pseudanabaena mucicola</i> (Naumann & Huber-Pestalozzi) Schwabe	Cyanobacteria
	<i>Planktolyngbya limnetica</i> (Lemmermann) Komárková-Legnerová & Cronberg	
	<i>Planktothrix isoethrix</i> (Skuja) Komárek & Komárková	
	Pseudanabaenaceae K.Anagnostidis & J.Komárek	
B	<i>Cyclotella</i> sp. (Kützing) Brébisson	Bacillariophyceae
D	<i>Nitzschia</i> sp. Hassal	Bacillariophyceae
J	<i>Crucigenia</i> sp. Morren	Chlorophyceae
	<i>Senedsmus</i> sp. Meyen	
X₁	<i>Synechocystis salina</i> Wislouch	Cyanobacteria
	<i>Monoraphidium irregulare</i> (G.M.Smith) Komárková-Legnerová	Chlorophyceae
F	<i>Kirchneriella lunaris</i> (Kirchner) Möbius	Chlorophyceae
	<i>Kirchneriella obesa</i> (West) West & G.S.West	
	<i>Kirchneriella</i> sp. Schmidle	
	<i>Eutetramorus</i> sp. Walton	
P	<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	Bacillariophyceae

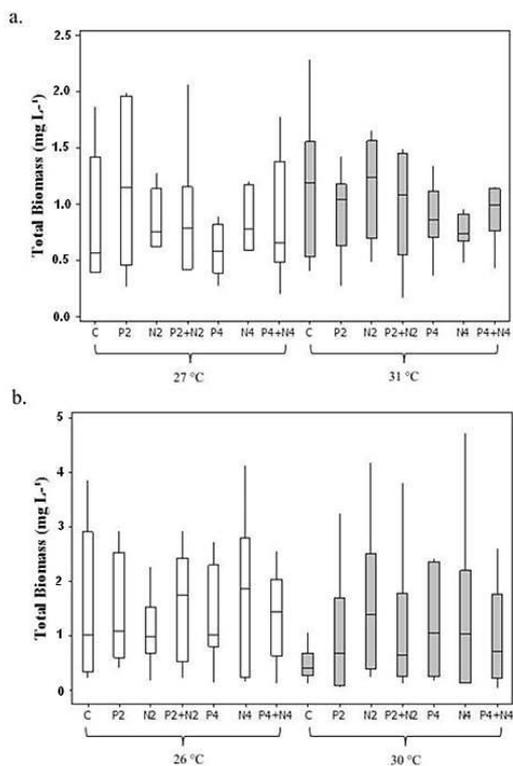


Figure 1. Variation (medians and quartiles, $n = 8$) of the total biomass (mg L^{-1}) in different nutritional enrichment and temperature treatments, for the rainy season (a) and dry season (b). White bars represent the temperature control treatments and the ashes the warming temperature treatments.

significantly influenced the decrease of L_O ($F = 7.1$; $p = 0.009$). The functional group **F** was the only one influenced by the nutritional enrichment in the dry season ($F = 3.5$; $p = 0.003$), by its biomass increase observed specially in the treatment P4+N4 (Figure 4). Although there was a trend of increasing biomass in the **H₁** group by the nutritional enrichment in the dry season (Figure 4), it was not considered significant by ANOVA.

4. Discussion

Our study evidenced that temperature and/or nutrient manipulation influences the structure and composition of the phytoplankton community in Boqueirão de Parelhas reservoir.

Climate models predict increases in temperature in the coming years, and these changes are associated with nutrient enrichment in water bodies. The effects of nutrient increase include the proliferation of potentially toxic cyanobacteria (Paerl & Paul, 2012). This proliferation was expected in the present study, however, our results showed

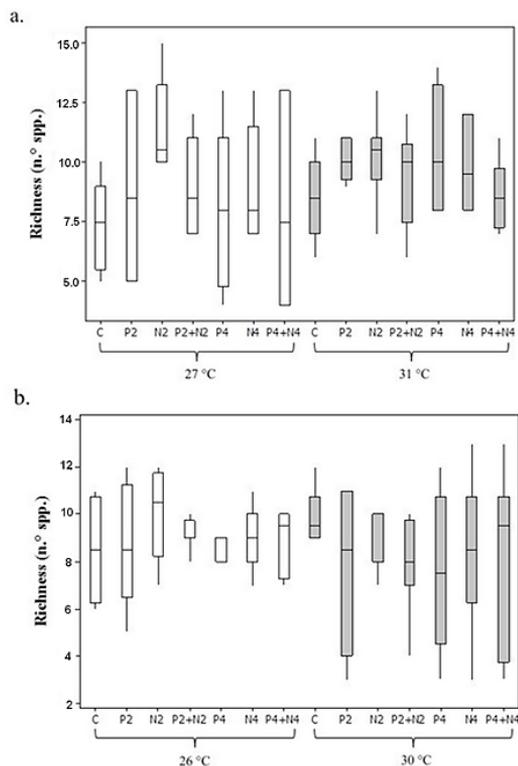


Figure 2. Variation (medians and quartiles, $n = 8$) of the species richness ($n^\circ \text{ spp.}$) in different nutritional enrichment and temperature treatments, for the rainy season (a) and dry season (b). White bars represent the temperature control treatments and the ashes the warming temperature treatments.

that bloom-forming cyanobacteria, specifically species of functional groups **H₁** and **M** which are commonly reported in reservoirs of the Brazilian semi-arid region (Costa et al., 2009; Dantas et al., 2011; Bittencourt-Oliveira et al., 2013), were not significantly more abundant after exposure to higher temperatures and nutrient enrichment. Instead, the recruitment of other cyanobacteria species as well as diatoms and green algae could be observed.

Although there is clear consensus in the literature that cyanobacteria will proliferate in increasing temperatures and that eukaryotic algae growth rates decline in temperatures that are optimal for blue-green algae (Paerl & Otten 2013; Kosten et al., 2012; Reynolds, 2006), some studies have shown that higher temperatures do not offer a clear advantage for cyanobacteria over its competitors, even when temperatures are ideal. Studies have also shown that there are no significant differences between the replication rates of Cyanobacteria and Chlorophyceae when subjected to a temperature of 29 °C (Lurling et al., 2013). Moss et al. (2003)

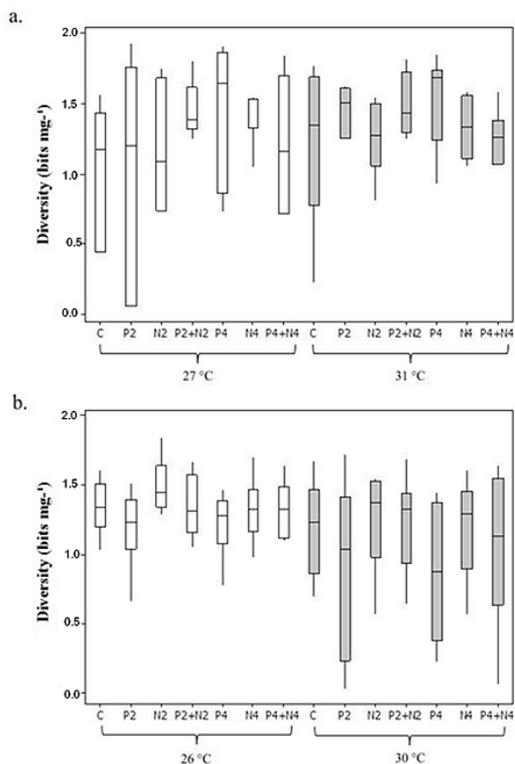


Figure 3. Variation (medians and quartiles, n = 8) of the diversity (bits mg⁻¹) of the phytoplankton community in different nutritional enrichment and temperature treatments, for the rainy season (a) and dry season (b). White bars represent the temperature control treatments and the ashes the warming temperature treatments.

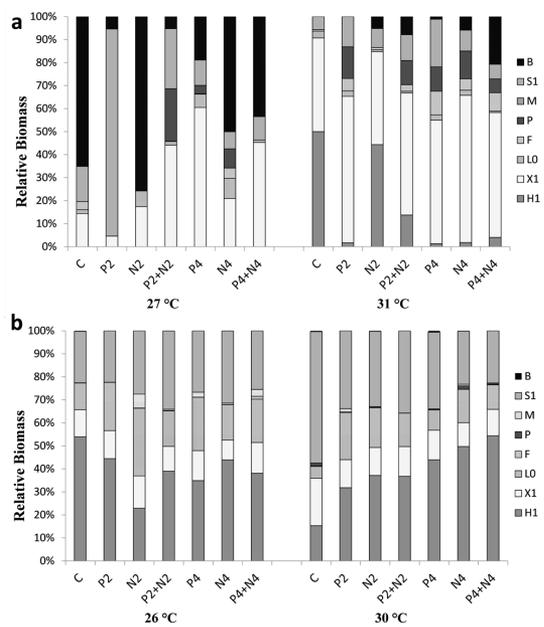


Figure 4. Relative biomass (%) of the Reynolds Functional Groups (RFG) in different nutritional enrichment and temperature treatments, for the rainy season (a) and dry season (b).

demonstrated that two species of green algae (*Tetraedron minimum* and *Micractinium pusillum*, which belong to the functional groups **J** and **F**, respectively) proliferated significantly in higher temperatures. Cyanobacteria species were not affected by temperature manipulation. The same results were observed in this study. For example, the functional group **F** was the only one recruited in both rainy and dry seasons; it was influenced by temperature increase (rainy and dry seasons) and nutrient enrichment, particularly in treatment P4+N4 (dry season). This codon is usually associated with meso-eutrophic conditions (Padisák et al., 2009).

Phytoplankton studies in the semi-arid region have shown that cyanobacteria are predominant (Bouvy et al., 2000; Costa et al., 2009; Medeiros et al., 2015; Brasil et al., 2016). However, the phytoplanktonic composition has been shifting between colonial and filamentous morphotypes due to different adaptive strategies driven by the hydrological regime (Medeiros et al., 2015). During the rainy season, the inflow from tributaries and overflow reservoir contributed to the lower phytoplankton biomass, and modified the populations of cyanobacteria, favoring the dominance of filamentous species, such as *Cylindrospermopsis raciborskii* (functional group **S_N**), with these adaptive conditions, turbid, warm and mixed lake (Reynolds et al., 2002). During the dry season, longer periods of water retention favored the growth of colonial cyanobacteria species such as *Microcystis panniformis* and *Sphaerocavum brasiliensis* (group **M**), which contributed to higher phytoplankton biomass values (Medeiros et al., 2015). In addition, during severe drought conditions, low water levels can favor adaptive phytoplankton species with requirement for low light by inorganic particles such as diatoms (functional group **P** and **B**) and cryptomonads (group **Y**). This unusual behavior of the phytoplankton community appears to contradict expectations in climatic change scenarios (Medeiros et al., 2015; Costa et al., 2016).

In this study, structural changes in the phytoplankton community were observed in the two seasons. In the dry period, the biomass of Chlorophyceae and Bacillariophyceae, species diversity, and functional group variability were significantly influenced in the higher temperature treatment only. All the functional groups that presented significant variation in the rainy period were closely related to the nutritional enrichment.

These variations may be explained by the dynamics of the semi-arid reservoirs; during the rainy period these reservoirs have more nutrient dilution due to greater water inflow (Medeiros et al., 2015). This would explain the importance of nutrient enrichment as a critical influence on phytoplankton during the rainy period.

The functional group **S1**, represented primarily by *Planktolyngbya limnetica*, was affected by temperature increase and by nutrient manipulation, particularly in P2. This group, composed of non-N fixing cyanobacteria, is typical of mixed environments, tolerant of shade, and sensitive to the flow of water (Padisák et al., 2009). Note that shade can occur from the proliferation of algae of the **H₁** group, forming “scum”. This functional group may be predominant in the phytoplankton community in periods with no inflow (Devercelli & O’Farrell, 2013) in eutrophic lakes, and it may also be found in conditions with limited nutrients (Reynolds, 2006; Dembowska et al., 2015).

Several studies on the effects of climate change on phytoplanktonic communities have noted that increased total biomass is an expected result; this is thought to be due to the increase in blooms of cyanobacteria in water bodies (Paerl & Huisman, 2008). These blooms lead to a decrease in species richness (Paerl & Paul, 2012; Kosten et al., 2012; Paerl & Otten, 2013). In the present study, temperature manipulation alone influenced the increase of species richness in the rainy season, specifically related to the recruitment of species belonging to functional groups **X₁** and **B**, which have been associated with mixed conditions (Reynolds et al., 2002; Padisák et al., 2009), common in the rainy period in semi-arid regions due to the water input and turbulence (Medeiros et al., 2015).

Studies have shown that if climate change creates conditions that benefit and influence cyanobacteria growth, then the resilience of ecosystems may lead to the development of algal groups other than blue green algae (e.g. Moss et al., 2013). The microcosm has had a role in exploring the consequences of global issues, such as the effect of climate change on species distribution, population dynamic, trophic structure, biodiversity and the impacts of pollution and fisheries on ecosystems (Benton et al., 2007). In addition, the design of experimental studies cannot capture the complexity of ecosystems. More studies are needed to integrate experimental approaches and field observations in order to better

understand the effects that climate change may bring to the dynamics of aquatic ecosystems.

In summary, our study showed that temperature increase and nutritional enrichment influenced the functional structure of the phytoplankton community differently in the rainy and dry seasons of Boqueirão de Parelhas reservoir. Our hypothesis that the experimental warming and/or nutrient enrichment would favor bloom-forming cyanobacterial functional groups was refuted. These functional groups of cyanobacteria did not proliferate as expected. Instead, it was observed the recruitment of diatoms and green algae (typically found in enriched environments), in particular during the rainy period.

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