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KARINE BORGES MACHADO

COMPOSIÇÃO TAXONÔMICA, FUNCIONAL E MOLECULAR DA MICROBIOTA PLANCTÔNICA: INTEGRANDO DIFERENTES NÍVEIS TRÓFICOS NA ECOLOGIA AQUÁTICA

Orientador: Dr. João Carlos Nabout

Goiânia
2019

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KARINE BORGES MACHADO

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Aos vinte e quatro dias do mês de janeiro de 2019 (24/01/2019), às treze horas e trinta minutos (13h30min), no Auditório do ICB V, UFG, reuniram-se os componentes da banca examinadora: **Prof. Dr. João Carlos Nabout, ICB/UFG - UEG/Anápolis; Profa. Dra. Mariana Pires de Campos Telles, ICB/UFG; Prof. Dr. Luis Mauricio Bini, ICB/UFG; Profa. Dra. Fernanda Melo Carneiro, UEG/Goiânia; Profa. Dra. Luzia Cleide Rodrigues, UEM/PR**; para, em sessão pública presidida pelo (a) primeiro(a) examinador(a) citado(a), procederem à avaliação da defesa de tese intitulada: **"Composição taxonômica, funcional e molecular da microbiota planctônica: integrando diferentes níveis tróficos na ecologia aquática"**, em nível de doutorado, área de concentração em Ecologia e Evolução, de autoria de **Karine Borges Machado**, discente do Programa de Pós-Graduação em Ecologia e Evolução da Universidade Federal de Goiás. A sessão foi aberta pelo(a) presidente(a), que fez a apresentação formal dos membros da banca. A palavra, a seguir, foi concedida a(o) autor(a) da tese que, em cerca de 37 minutos, procedeu à apresentação de seu trabalho. Terminada a apresentação, cada membro da banca arguiu a(o) examinada(o), tendo-se adotado o sistema de diálogo sequencial. Terminada a fase de arguição, procedeu-se à avaliação da tese. Tendo-se em vista o que consta na Resolução nº 1127 de dezembro de 2012 do Conselho de Ensino, Pesquisa, Extensão e Cultura (CEPEC), que regulamenta o Programa de Pós-Graduação em Ecologia e Evolução, a tese foi Aprovado, considerando-se integralmente cumprido este requisito para fins de obtenção do título de Doutor(a) em Ecologia e Evolução pela Universidade Federal de Goiás. A conclusão do curso dar-se-á quando da entrega da versão definitiva da tese na secretaria do programa, com as devidas correções sugeridas pela banca examinadora, no prazo de trinta dias a contar da data da defesa. Cumpridas as formalidades de pauta, às 17 h e 05 min., encerrou-se a sessão de defesa e, para constar, eu, Suely Ana Ribeiro, secretária executiva da Universidade Federal de Goiás - UFG, lavrei a presente ata que, após lida e aprovada, será assinada pelos membros da banca examinadora em três vias de igual teor.

Prof. Dr. João Carlos Nabout
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Luzia Cleide Rodrigues
Profa. Dra. Luzia Cleide Rodrigues
UEM/PR

Dedico a minha família, meu porto seguro, razão pela qual nunca pensei em desistir.

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SUMÁRIO

RESUMO

A microbiota aquática tem um papel relevante no funcionamento dos ecossistemas. Porém, os fatores que estão associados à estruturação das comunidades microbiológicas assim como suas respostas às mudanças climáticas ainda não estão bem esclarecidas. Além disso, a maior parte das avaliações não consideram os efeitos sobre os diferentes níveis tróficos simultaneamente. Este estudo teve como objetivo associar o conhecimento taxonômico, funcional e molecular sobre micro-organismos aquáticos, buscando investigar as causas dos padrões geográficos da sua diversidade assim como suas respostas ao aquecimento global. Este trabalho foi conduzido utilizando dados de 27 lagoas da planície de inundação do rio Araguaia e ainda pela construção de um experimento em microcosmo. Nós verificamos que fatores ambientais e espaciais não exercem influência sobre a microbiota planctônica 18S rDNA nas lagoas avaliadas, embora exista uma alta diversidade beta e a composição seja espacialmente estruturada ao longo da planície. Por outro lado, constatamos que o aquecimento simulado experimentalmente promove uma alteração na composição taxonômica e funcional dos organismos planctônicos, devido a uma substituição de espécies e ao predomínio de organismos com menor tamanho. Além disso, a microbiota de diferentes níveis tróficos apresenta um padrão de resposta similar ao aquecimento. Esses resultados contribuem para predizer como diferentes níveis biológicos e grupos tróficos da microbiota aquática respondem a variações nas condições ambientais. Essa abordagem é relevante, uma vez que modificações na composição das comunidades podem afetar inúmeros processos e serviços ecossistêmicos.

Palavras - chave: aquecimento global, fitoplâncton, metacomunidades, protistas

ABSTRACT

The aquatic microbiota play an important role in the ecosystems functioning. However, the factors that are associated with the structuring of microbiological communities as well as their responses to climate change are still not well understood. In addition, most evaluations do not consider the effects on different trophic levels simultaneously. This study aimed to associate taxonomic, functional and molecular knowledge on aquatic microorganisms, seeking to investigate the causes of the diversity geographic patterns as well as their responses to global warming. This work was carried out using data from 27 lakes in the Araguaia river floodplain and also by the construction of an experiment in microcosm. We found that environmental and spatial factors do not influence planktonic microbiota 18S rDNA in the lakes evaluated, although there is a high beta diversity and the composition is spatially structured along the plain. On the other hand, we found that experimentally simulated warming promotes a change in the taxonomic and functional composition of planktonic organisms, due to a species substitution and a predominance of smaller organisms. Furthermore, the microbiota of different trophic levels presents a similar response pattern to warming. These results contribute to predict how different biological levels and trophic groups of the aquatic microbiota respond to variations in environmental conditions. We highlight that this approach is relevant, because changes in the composition of communities can affect many ecosystem processes and services.

Key-words: global warming, phytoplankton, metacommunities, protists

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INTRODUÇÃO GERAL

A microbiota aquática representa um dos grupos mais diversos no planeta terra e tem um papel fundamental no funcionamento dos ecossistemas, através da produtividade primária, ciclagem de nutrientes e decomposição (Chen et al. 2008, Furhrman 2009). No entanto, os fatores que estão associados à estruturação das comunidades microbiológicas (Lindström & Langenhender 2012) assim como as suas respostas às mudanças climáticas não estão bem esclarecidas (p. ex. Moss et al. 2003, Yvon-Durocher et al. 2015). Além disso, a maior parte dos estudos têm analisado as comunidades apenas em um nível biológico e não consideram os efeitos sobre a composição taxonômica, funcional e molecular em conjunto.

Diversos fatores determinam os níveis de composição das comunidades. Estes são resultados de processos locais, regionais e históricos (Martiny et al. 2006, Lindström & Langenhender 2012) que atuam sobre múltiplas escalas espaciais e temporais. Por muito tempo, acreditou-se que a distribuição dos micro-organismos era limitada apenas pelas características do ambiente devido ao seu tamanho reduzido, elevada abundância e alta capacidade de dispersão (Finlay 2002, Fenchel & Finlay 2004). Porém, de acordo com a escala em que o estudo está sendo realizado ou nível biológico avaliado é possível encontrar padrões de distribuição distintos (Martiny et al. 2006).

Nos últimos anos, alguns estudos verificaram o efeito do ambiente e de eventos históricos para as espécies e seus atributos funcionais (p. ex. Beisner et al. 2006, Algarte et al. 2014, Machado et al. 2016). Porém, com o avanço das técnicas de biologia molecular (Logares et al. 2012), torna-se possível investigar os padrões ecológicos dos micro-organismos em nível de ácidos nucleicos. Na abordagem definida como metagenética, o DNA ambiental é amplificado e sequenciado utilizando *primers* universais para fragmentos de genes ribossomais eucarióticos ou procarióticos (Pawlowski 2014). Desse modo, todo o material genético existente na comunidade é considerado coletivamente sem a necessidade de técnicas de cultura (p. ex. Logares et al. 2013, Wallace et al. 2015). A relação dos dados metagenéticos com as condições do ambiente podem revelar inúmeras funções desempenhadas pelas comunidades microbianas nos ecossistemas (Simon & Daniel 2011). Assim, essa estratégia pode trazer um resultado mais refinado e expressar padrões distintos dos que seriam obtidos na composição taxonômica e funcional.

Por outro lado, as consequências do aquecimento global são percebidas em todos os níveis de organização biológica (Woodward et al. 2010) com efeitos diretos previstos para as diferentes formas de vida aquática (Huertas et al. 2011), dentre o qual se destaca o fitoplâncton por seu papel na produtividade primária (Häder et al. 2014). O fitoplâncton é extremamente sensível a mudanças climáticas (Hallegraeff 2010) e pode responder a alterações no ambiente por meio da plasticidade fenotípica em suas características (muitas vezes funcionais), substituição de espécies (que também pode levar a substituição de atributos ou grupos funcionais) ou adaptação genética (Litchman et al. 2012).

Alguns estudos têm demonstrado que o aquecimento deve beneficiar espécies pertencentes a grupos que atualmente já são adaptados a temperaturas elevadas, tais como as cianobactérias (ver Moss et al. 2003, Yvon-durocher et al. 2011, Chen 2015) e dinoflagelados (ver Jeppesen et al. 2009, Sommer et al. 2015), enquanto grupos adaptados a temperaturas mais amenas, como as diatomáceas, devem ser desfavorecidos (Finkel et al. 2010, Sommer & Lewandowska 2011, Chen 2015). Funcionalmente, as espécies fitoplanctônicas podem ser classificadas em grupos funcionais de acordo com suas características morfológicas (p. ex. Kruk et al. 2010) ou considerando suas preferências de habitat e tolerância às condições do ambiente (p. ex. Reynolds et al. 2002, Padisák et al. 2009). Assim, os efeitos da temperatura também podem ser visualizados sobre os atributos funcionais das espécies, auxiliando na compreensão de suas respostas diante das alterações ambientais (Litchman et al. 2012, Chen 2015). Micro-organismos menores geralmente possuem um ciclo de vida curto, são generalistas, oportunistas e metabolicamente mais ativos quando comparados aos de tamanho maior, enquanto micro-organismos grandes são restritivos quanto as suas necessidades de habitat (Moss et al. 2003). Assim, o aquecimento pode alterar a composição dos grupos funcionais levando ao predomínio daqueles representados por organismos pequenos (p. ex. Petchey et al. 1999, Mckee et al. 2003, Yvon-Durocher et al. 2011) devido a modificações na frequência dos atributos funcionais relacionados ao tamanho e formato da célula (p. ex redução do biovolume; Montagnes & Franklin 2001, Yvon-Durocher et al. 2011; Sommer et al. 2015).

As consequências do aquecimento também podem ser identificadas através das ferramentas metagenéticas. Essa estratégia pode trazer um nível de precisão mais elevado (Handselman et al. 2009), uma vez que toda microbiota existente na água será avaliada em conjunto (p. ex. fungos, amebóides, algas, ciliados, cercozoários, etc), incluindo os micro-organismos raros que nem sempre são identificados pela taxonomia tradicional. De fato, comparações entre classificações obtidas por dados moleculares e a identificação morfológica indicam grupos taxonômicos discrepantes entre as duas abordagens (p. ex. Medinger et al. 2010, Brandford et al. 2013) produzindo informações complementares que podem ser utilizadas para fins de biomonitoramento. Desse modo, a combinação de diferentes estratégias (taxonômica, funcional e molecular) faz-se necessária para acessar o efeito das mudanças climáticas sobre a microbiota aquática.

Na abordagem metagenética, as sequências são classificadas dentro de Unidades Taxonômicas Operacionais (OTUs) de acordo com a sua similaridade (p. ex. Preheim et al. 2013). Desse modo, é possível mensurar os efeitos do aquecimento sobre a riqueza e frequência das OTUs (p. ex. Plebani et al. 2015, Smale et al. 2017), distinguir a influência da temperatura sobre as OTUs abundantes e raras ou sobre o grupo taxonômico ao qual cada OTU está associada (e.g. Stratil et al. 2013, Semenova et al. 2015). Além disso, ainda é possível classificar os organismos em diferentes níveis tróficos (p. ex. Simon et al. 2015, Fujimoto et al. 2016, Khomich et al. 2017) e avaliar o efeito do aquecimento sobre as espécies autotróficas, heterotróficas e mixotróficas separadamente.

Caracterizar a microbiota aquática em diferentes níveis biológicos pode ajudar a esclarecer os processos de estruturação das comunidades assim como as suas respostas ao aquecimento global. Nesse contexto, esse trabalho teve como objetivo associar o conhecimento taxonômico, funcional e molecular sobre micro-organismos aquáticos, buscando investigar as causas dos padrões geográficos da sua diversidade assim como suas respostas ao aquecimento global. Essa tese foi dividida em quatro capítulos.

No primeiro deles, investigamos a composição e diversidade (alfa, beta e gama) de protistas planctônicos em 27 lagoas de inundação do Brasil central, usando o sequenciamento do gene 18S rDNA. Além disso, foi avaliado o padrão espacial das comunidades, assim como efeito de variáveis ambientais e espaciais para composição da microbiota num contexto taxonômico e funcional. No segundo capítulo, através de uma abordagem experimental, identificamos o efeito do aumento da temperatura e do tempo de experimento sobre a composição taxonômica e funcional de comunidades fitoplanctônicas em diferentes cenários futuros de aquecimento global. Para o terceiro capítulo, utilizamos o mesmo desenho experimental do capítulo 2. Nós buscamos avaliar se o aquecimento produz efeitos sobre a composição da microbiota procariota e eucariota determinada através do sequenciamento para os genes 16S rDNA e 18S rDNA. Esses efeitos foram avaliados para cada nível trófico separadamente, ou seja, organismos autotróficos, heterotróficos e mixotróficos. Além disso, verificamos se os diferentes níveis de organização biológica (procariotos e eucariotos) e níveis tróficos (autotróficos, heterotróficos e mixotróficos) têm padrões de resposta semelhantes ao incremento da temperatura. O capítulo quatro foi constituído por um artigo de divulgação científica, para demonstrar ao público fora do meio acadêmico parte dos resultados obtidos na tese.

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CAPÍTULO I

Diversity patterns of planktonic protist communities in tropical floodplain lakes based on 18S rDNA gene sequences**

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Abstract

The aquatic microbiota plays key roles in ecosystem processes, however the mechanisms that influence their biogeographic patterns are not yet fully understood. Using high-throughput 18S rDNA gene sequencing, we investigated the composition of planktonic protist in 27 floodplain lakes of the Araguaia River, central Brazil and explored the influence of environmental and spatial factors for communities considering taxonomic and trophic groups. We found 807 OTUs. Chlorophyta and Charophyta were the groups with greater abundance. Beta diversity was high and the similarity of communities decreased as the geographic distance increased. We found a shared explanation between environmental and spatial predictors for total and autotrophic microbiota. However, environmental variables influence only mixotrophic microbiota. These results suggest an OTU turnover along the floodplain, and a spatially structured composition. This spatial pattern can be derived from the association with extrinsic factors, such as spatially structured environmental variables, that generate spatial dependence. However, the relationship of the microbiota to environmental conditions is still unclear. Thus, the metabarcoding strategy should be combined with traditional taxonomic studies, seeking to produce more complete information about metacommunity and biomonitoring of aquatic microbiota.

Keywords: Araguaia River, Illumina Miseq, Metabarcoding, Metacommunities, Microeukaryotes

Introduction

Aquatic microbiota represent a considerable fraction of the total planktonic biomass (Cotner and Biddanda, 2002) and are key elements in aquatic food webs (Chen *et al.*, 2008). Aquatic microbes are responsible for nutrient cycling and degradation of natural and anthropogenic residues, acting in numerous biogeochemical processes (Rodríguez-Valera, 2004). Thus, understanding which factors shape microbial community structure is one of the greatest challenges in ecology (Logares *et al.*, 2013).

Local, regional and historical factors act at different spatial and temporal scales to determine species richness (e.g., alpha and gamma diversity) and turnover (e.g., beta diversity) (Martiny *et al.*, 2006; Lindström and Langenhender, 2012). Two components are typically used to explain the variation of organisms in space: environmental selection and dispersal capacity. According to the niche theory, environmental variables (e.g., habitat conditions, resource availability, abiotic factors) and ecological interactions determine species composition in a community (Hutchison, 1957). On the other hand, spatial variables, such as geographical distance, can influence the structuring of communities by limiting dispersal (e.g., Heino *et al.*, 2014; Ren *et al.*, 2015; Gong *et al.*, 2015). Finally, historical processes may also play a role in community structuring (e.g., Chase, 2003; Chase, 2010), such as dispersion limitation and stochastic events.

Dispersal processes and environmental factors are combined in the metacommunity theory, which used four paradigms to explain community structuring: patch dynamics, species sorting, mass effects and neutral dynamics (Leibold *et al.*, 2004). The patch dynamic paradigm assumes that patches are identical and that the patterns of diversity are determined by the dispersal capacity of the species. In the species sorting paradigm, there is environmental filtering, and species occur only in environmentally appropriate sites. In this case, dispersion allows the species to migrate according to alterations in the environment, promoting changes in composition. In the mass effect paradigm, high dispersion rates promote a homogenization of the communities since species are able to occur even in sites that are not environmentally favourable. In the neutral perspective paradigm, species are ecologically equivalent, and the patterns of diversity are determined by the mechanisms of loss or gain of species.

Recently, mass effects and patch dynamics have been considered as subcategories of the species sorting paradigm, with distinction by the dispersal rate that exists between the communities: low for most species in the patch dynamic paradigm, intermediate in the species sorting paradigm and high in the mass effect paradigm (Winegardner *et al.*, 2012). Thus, patterns of composition in metacommunities are determined by environmental conditions and species dispersion (Winegardner *et al.*, 2012). For aquatic ecosystems such as streams and lakes, species sorting predominates, although these patterns can be altered according to the type of system and spatial scale (Heino *et al.*, 2015).

Despite advances in studies on metacommunities, the processes that explain microbial biogeography are not fully understood (Chen *et al.*, 2008; Hanson *et al.*, 2012; Lindström and Langenhender, 2012). For a long time, it was considered that microbial distribution was determined only by environmental characteristics given their small size, high abundances and high dispersal rates (Finlay, 2002; Fenchel and Finlay, 2004). This is in line with Baas-Becking's (1934) principle, which states "everything is everywhere, but the environment selects". However, it has also been proposed that dispersal limitation may occur in microbes (Martiny *et al.*, 2006). Nevertheless, today, we know that environmental characteristics of aquatic environments, such as pH (Heino *et al.*, 2014; Gong *et al.*, 2015), conductivity (Simon *et al.*, 2015a), phosphorus concentration (Wang *et al.*, 2015; Triadó-Margarit and Casamayor, 2012), luminosity (Charvet *et al.*, 2014), primary productivity (Bradford *et al.*, 2013; Simon *et al.*, 2015a; Wang *et al.*, 2015), temperature and depth (Gong *et al.*, 2015; Wang *et al.*, 2015) can affect microbial community composition. On the other hand, spatial variables have also been tested as predictors for community structuring and play an important role together with environmental conditions (e.g., Soininen *et al.*, 2011; Gong *et al.*, 2015).

For microorganisms, advances in DNA sequencing can help us to understand numerous ecological processes at the community level (Handelsman, 2009). Thus, by means of metabarcoding, environmental DNA can be amplified and high-throughput sequenced using universal molecular markers for both eukaryotic and prokaryotic genes (Pawlowski, 2014). Thus, DNA sequences corresponding to multiple organisms present in the communities are classified into operational taxonomic units (OTUs) according to their similarity (e.g., Heino *et al.*, 2014; Fonseca *et al.*, 2014, Logares *et al.*, 2014). The latter strategy complements the information obtained via traditional morphological identification (Bradford *et al.*, 2013; Santoferrara *et al.*, 2015), allowing the study of rare (e.g., Logares *et al.*, 2014; Grattepanche *et al.*, 2016) or little-known taxa (Bik *et al.*, 2012) and enabling the evaluation of the effects of different functional groups (i.e., different trophic roles in ecosystems) simultaneously (e.g., Simon *et al.*, 2015b; Genitsares *et al.*, 2016; Khomich *et al.*, 2017).

Comparisons between results obtained using high-throughput sequencing and classic microscopy-based morphological identification have shown similar spatial distribution patterns (e.g., see Santoferrara *et al.*, 2015 for ciliates; Hirai *et al.*, 2015 for copepods). The characterization of communities using high-throughput sequencing may facilitate the identification of very small taxa whose morphological characteristics are difficult to differentiate visually (Simon *et al.*, 2015b). Considering that DNA-based classification produces a large amount of information in a short period of time, its use can assist in the taxonomic characterization with higher resolution, reduce the time and costs employed in the process and enable a larger sampling effort (Keck *et al.*, 2017).

Although the number of studies using high-throughput sequencing has grown in recent years, the majority have focused on microorganisms in marine environments (e.g., Countway *et al.*, 2010; Bik *et al.*, 2012; Charvet *et al.*, 2014; Fonseca *et al.*, 2014; Logares *et al.*, 2014; Gong *et al.*, 2015; Genitsaris *et al.*, 2016; Grattepanche *et al.*, 2016; Zhang *et al.*, 2017), while investigations in freshwater environments are scarce (Simon *et al.*, 2015b) and have been mostly conducted in temperate zones (e.g., Amaral-Zettler, 2013; Leperé *et al.*, 2013; Heino *et al.*, 2014; Filker *et al.*, 2015; Kammerlander *et al.*, 2015). An exception was found in a tropical Australian river where it was observed that the composition of the eukaryote community was influenced by environmental variables (Brandford *et al.*, 2013).

In this study, we investigated the composition and diversity (alpha, beta and gamma) of planktonic protists in 27 floodplain lakes in Araguaia River, central Brazil, using the 18S rDNA gene sequencing of targeted markers. To the best of our knowledge, this is one of the first studies to analyse the planktonic microeukaryotes in the Araguaia River, an important river in the Cerrado biome, through a metabarcoding approach and one of the few conducted in the tropical region. Thus, our aims are as follows: i) to describe the diversity of planktonic protists for this region, ii) to determine the spatial diversity patterns of these communities along the floodplain, and iii) to evaluate the relative influence of environmental and spatial variables on the microbiota composition considering taxonomic and trophic groups. We hypothesized that environmental variables may be the major predictors of community composition since floodplain lakes are connected to each other along the plain by the main channel of the river.

Methods

Study area

The Araguaia River is located in the Tocantins-Araguaia basin and represents one of the most important rivers in the central region of Brazil (Valente *et al.*, 2013). Along its course, it is divided into three regions: high, medium and low Araguaia (Latrubesse and Steuvax, 2006). The medium course is characterized by the confluence of important tributaries such as the Crixás, Mortes and Cristalino rivers (Latrubesse and Steuvax, 2006) and by the presence of the floodplain with numerous lakes (Morais *et al.*, 2005), which contribute to maintaining the diversity and functioning of ecosystems in this region.

In this study, we sampled 27 lakes in the central region of Brazil along 500 km in the Araguaia River floodplain and its tributaries, including the Crixás, Vermelho, Mortes and Cristalino River (Figure 1). Sampling was performed in January 2012, which represents the flood period on the Araguaia River (Aquino *et al.*, 2008), a period that allows better access to the studied area. These lakes are considered oligotrophic, with low nutrient concentrations (Marcionilio *et al.*, 2016). Soil cover is predominantly composed of native Cerrado vegetation (a type of savanna), with some areas used for agriculture and livestock (Machado *et al.*, 2016).

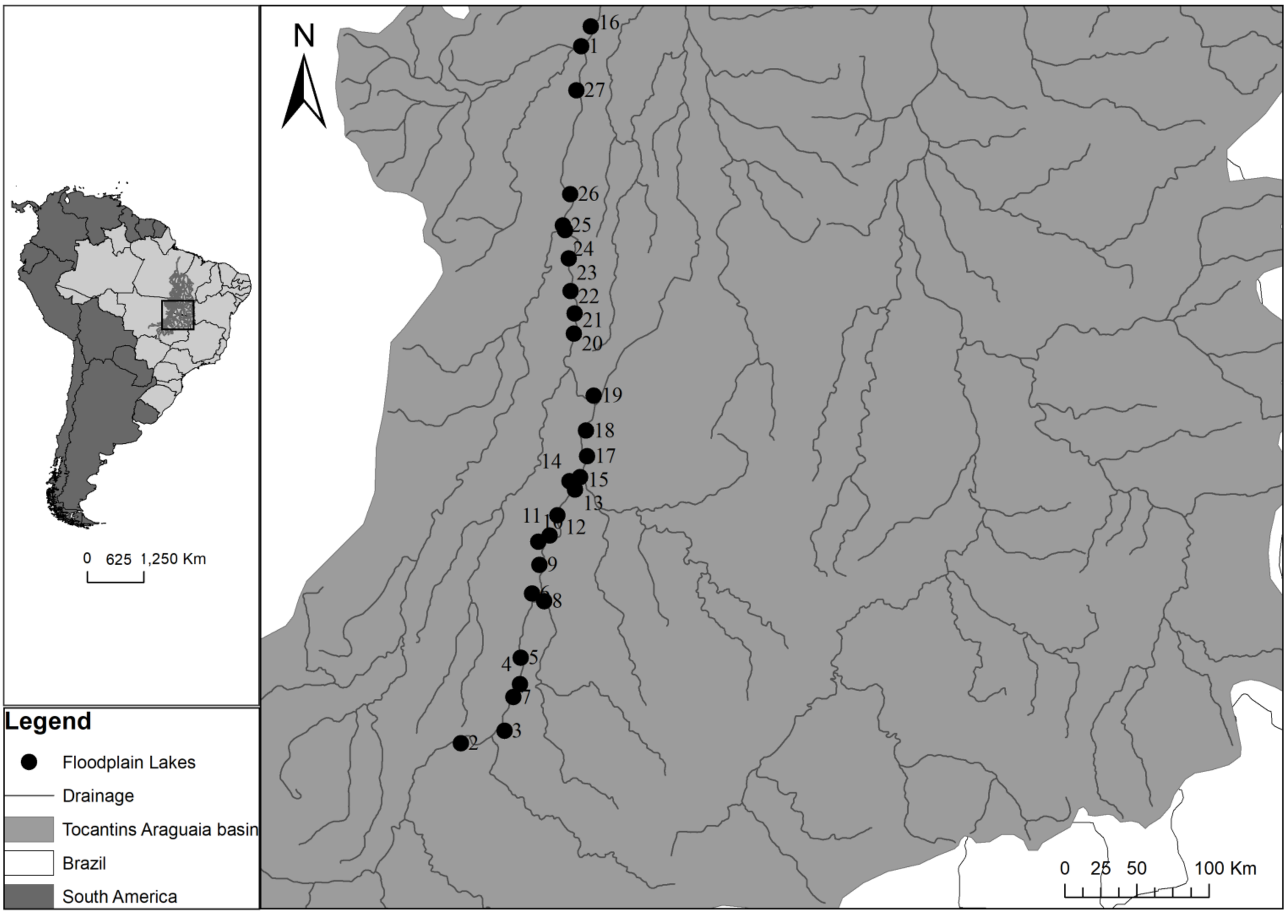

Figure 1 – Map and location of Araguaia River basin, showing the sampled lakes. The numbers indicated lake´s codes.

DNA extraction, amplification, sequencing and bioinformatics

Aquatic microbial samples were collected from the water subsurface (0.5 m). Approximately 500 L of water was filtered through a plankton net (68 µm mesh size), which resulted in 250 mL of concentrated plankton that was stored in polyethylene bottles in a refrigerator (approximately 2 °C). The second filtration of the concentrated sample was performed within 12 hours of sampling using Millipore cellulose filters (3 µm pore size) and a vacuum pump. Thus, the size fraction investigated was 3-68 µm. The filters were stored in liquid nitrogen at -80 °C.

Total genomic DNA was extracted and purified following the PowerWater DNA Isolation kit (MoBio, USA) protocol for each collection point. The extracted DNA was visualized on a 1% agarose gel. A hypervariable fragment, approximately 380 base pairs (bp) of the V4 region of the 18S rDNA gene was amplified using the universal primers TAReuk454FWD1 and TAReuKREV3

(Stoeck *et al.*, 2010), modified with addition of the sequences complementary to the Illumina indices (i7 and i5, Nextera XT). The V4 region of the 18S small subunit (SSU) rDNA gene was targeted with the universal primers. We used 5 ng/µL of DNA and 1 µM of each primer. Polymerase chain reaction (PCR) was performed using the Taq PCR Master Mix Kit (Qiagen) in triplicate for each point to minimize the PCR bias. PCR cycles were as follows: 98ºC for 1 minute; followed by 98ºC for 30 seconds; 53ºC for 30 seconds; and 72ºC for 30 seconds; and a final extension at 72ºC for 10 minutes.

Then, the Illumina indices (i7 and i5) were inserted into the fragments using a limited-cycle PCR programme. We used the Nextera XT index kit v2 set B. Finally, the amplicon libraries were purified, and the short library fragments were removed using Agencourt AMPure XP Beads (Beckman Coulter). The libraries were quantified by real-time PCR with the KAPA Library Quantification Kit, and the amplicon size was estimated using an Agilent High-Sensitivity DNA Kit on a Bioanalyzer. The libraries were normalized to 4 nM and pooled for sequencing using the MiSeq Reagent Kit v3 (300 cycles) on the Illumina MiSeq platform.

The sequence quality was evaluated using FastQC software (available from https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (Andrews, 2010). Sequences with reads < 100 bp or bases with a Phred score < 20 were excluded using the Trimmomatic software (Bolger *et al.*, 2014). The OTU prediction was carried out following the UPARSE pipeline, available at http://drive5.com/usearch/manual/ uparse_pipeline.html and in Edgar, (2013), which consists of i) merging sequences; ii) grouping the sequences of all samples; iii) identification of unique sequences (dereplication); iv) identification of OTUs with representative sequences (clustering by 97% similarity) and chimaera filtering; and v) construction of an OTU table by point. The taxonomic prediction was performed by a BLAST search (Altschul *et al.*, 1990) of the representative OTU sequences against the Silva 119.1 database (available inhttps://www.arb-silva.de/) using a percent identity of 97%. The non-protistan sequences (e.g., Metazoa) were removed from the total microbiota data set.

The OTUs for which it was possible to assign a taxonomic classification were classified into trophic groups according to their nutrition type (See Simon *et al.*, 2015a; Genitsares *et al.*, 2016; Khomich *et al.*, 2017). We considered autotrophic organisms those with chlorophyll-a that act as primary producers (e.g., Chlorophyta, Charophyta, Glaucophyta, Rhodophyta). Organisms classified as mixotrophic (see Flynn *et al.*, 2013 for a detailed description of this nutrition mode) include those that acquire carbon through photosynthesis or heterotrophy (e.g., some putative members of the groups Cryptophyta, Chrysophyta, Ochrophyta, Dinophyta, etc.). Heterotrophic organisms were represented by predators, parasites and decomposers (e.g., fungi, Amoebozoa, Ciliophora, Choanoflagellata, etc.). See Table 1 for the complete OTU classification of trophic groups. Thus, four matrices for OTU abundance were used, hereinafter denominated as follows: i) total microbiota (represented by all OTUs obtained in sequencing, excluding metazoans, non-planktonic organisms and those for which it was not possible to obtain a taxonomic classification); ii) autotrophic microbiota (includes only primary producers); iii) mixotrophic microbiota (includes only putative mixotrophic organisms); and iv) heterotrophic microbiota (includes only heterotrophic organisms).

Environmental variables

In each lake, we measured conductivity, dissolved oxygen, pH, depth, water temperature, transparency, turbidity, oxygen saturation and total dissolved solids. The total nitrogen, total phosphorus and chlorophyll-a estimates were performed in the laboratory according to the methods described in Zagatto *et al.*, (1981) and Golterman *et al.*, (1978). We also determined the areas and widths of lakes and percentage of native Cerrado vegetation, pasture and agriculture. Details of the limnological, morphometric and land use variable estimations as well as the description of their values are presented in Machado *et al.*, (2016) and Marcionilio *et al.*, (2016).

We used a principal component analysis (PCA) to evaluate the environmental heterogeneity among lakes. The PCA was constructed using the correlation matrix and the data standardized by the z score method (limnological variables) or transformed to arcsines of their square roots × 180/π (land use data). The variance inflation factor (VIF) was used to estimate the collinearity between the environmental variables, and those with VIF values greater than 10 were considered collinear (Alin, 2010, see more details in supplementary data). The variables conductivity, pH, water temperature, total dissolved solids, transparency, depth, total phosphorus, total nitrogen, chlorophyll-a and lake width did not present collinearity and thus constituted the set of environmental variables used in the redundancy analysis.

Spatial Variables

In this study, we used two types of spatial filters, "*Principal Coordinates of Neighbour Matrices"* (PCNM, Borcard and Legendre, 2002) and *"Asymmetric Eigenvector Maps*" (AEM, Blanchet *et al.,* 2008a). The PCNM considers the linear distances (Borcard and Legendre, 2002) and may indicate a non-directional dispersion process. The AEM is calculated through the directional connectivity between the sampling points (Blanchet *et al.,* 2008a) and can represent the dispersion along the river course (see more details in supplementary data). Thus, the PCNM and AEM filters represent different hypotheses about connectivity between sites (Heino *et al.,* 2015). The spatial component was represented by those PCNM and AEM filters that showed the highest correlation with OTU abundance. The filters were selected using the forward.sel function (Blanchet *et al.,* 2008b) of the "packfor" package (Dray *et al.,* 2011). The PCNM filters were constructed using the package "vegan" (Oksanen *et al.,* 2016) and the AEM filters using the package "AEM" (Blanchet *et al.,* 2008a), both in R software (R Core Team, 2016).

Data Analysis

All statistical analyses were performed using the "vegan" package (Oksanen *et al.,* 2016) of R software (R Core Team, 2016). We constructed a species accumulation curve to verify how well the OTUs were sampled. If the curve reached the asymptote before the inclusion of all the sampled sites, this indicated that the sampling effort may have been adequate. We used subsampling rarefaction to correct the bias that can be generated by comparing samples with different sizes since a larger number of sequences in a sample leads to a greater number of OTUs (e.g., Bradford *et al.,* 2013; Gong *et al.*, 2015). The rarefaction was conducted through a random subsampling in which the sample size was represented by the lowest number of sequences, i.e., 2,673 reads, recovered from our floodplain lakes (Hurlbert, 1971). This sample size was adequate to represent the community patterns using the Illumina platform (Caporaso *et al.*, 2011) and similar to many studies for protists (e.g., Bradford *et al.*, 2013; Gong *et al.*, 2015). The curve was constructed using the "exact" method of the "specaccum" function, and rarefaction analysis was performed using the “rarefy”, “rrarefy” and “rarecurve” functions. The rarefied richness and abundance data were used for all the statistical analyses described below.

The alpha diversity was represented by the number of OTUs (richness) and the Shannon and Simpson indices (e.g., Gong *et al.*, 2015; Khomich *et al.*, 2017). The alpha diversity indices were calculated using the diversity function. The gamma diversity was expressed as the total number of OTUs obtained considering the whole set of samples. The total beta diversity was estimated using the Sørensen index and partitioned into turnover and nestedness (Baselga, 2010). The Sørensen index varies from 0 to 1, with a value closer to 1 representing greater total beta diversity (Baselga, 2010). High values for turnover indicate that changes in community composition occur mainly due to OTU replacement along the river, while a high value for nestedness indicates that lakes with low species richness are populated by a subset of OTUs that occur in lakes with higher species richness. Beta diversity was estimated using the “betapart” package (Baselga and Orme, 2012). We used non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities to evaluate the community composition along the floodplain. The abundance data were Hellinger transformed. Alpha and beta diversity were estimated for the total microbiota.

The Mantel correlogram was used to evaluate whether the planktonic microbiota is spatially structured, that is, if the lakes have similar or different OTU compositions according to the distance between them (see Legendre and Fortin, 1989; Fortin and Dale, 2005). This analysis was also conducted considering the total microbiota. The OTUs were correlated to a geographic matrix with five distance classes through a Mantel test, and the results were plotted in a correlogram. The number of classes was defined according to Sturge's rule (See Legendre and Legendre, 1998). The OTU abundance was Hellinger transformed. In this analysis, the OTU abundance per point was previously converted into a Bray-Curtis distance matrix and the geographic coordinates into a Euclidean distance matrix.

To determine the influence of environmental variables and geographic distance on the community composition, we used a partial redundancy analysis (pRDA, see Legendre and Legendre, 1998). Thus, we determined what proportion of the variation in community composition was explained by the environment (component a), spatial variables (component c), environment and spatial distance together (component b), and unexplained as the residual fraction (component d). The pRDAs were performed separately for the total, autotrophic, mixotrophic and heterotrophic microbiota. pRDA analysis was also performed between total microbiota, trophic groups (autotrophic, mixotrophic and heterotrophic microbiota) and the major taxonomic groups against each environmental variable separately. The purpose of this analysis was to verify whether specific environmental variables were more strongly related than others to the community structure. In this analysis, the environmental variables were standardized using the Z score, and the OTU abundance data were Hellinger transformed.

Results

Composition and diversity of protist communities

The sequencing of all samples generated 3,287,448 reads. After quality filtering, these reads resulted in 357,933 merged sequences that were predicted in 807 OTUs. The mean number of OTUs was 163 and ranged from 52 to 589 OTUs between the samples. For reads, the mean number was 12,439, ranging from 2,673 to 54,176 between the floodplain lakes sampled. The rarefaction led to a reduction in the number of OTUs (630), with a mean richness of 95, ranging from 28 to 223 OTUs per lake. The OTU abundance was standardized to 2,673 reads per sample. Of the 630 OTUs, it was possible to perform taxonomic annotation only for 342 OTUs (54%) since not all sequences had representatives in the reference database. Of the 342 taxonomically-classified OTUs, 90 OTUs referred to non-protistan sequences (e.g., metazoan) and eight to non-planktonic organisms. Thus, they were excluded, resulting in 244 OTUs used for all analyses (Supplementary data, Table SI). Of these 244 OTUs, 81 (33%) referred to autotrophic organisms, 115 (47%) to heterotrophic organisms and 48 (20%) to mixotrophic organisms.

Considering the total microbiota, the Charophyta and Chlorophyta groups presented the highest abundance, with 48% and 42% of reads, respectively (Table 1). Among the autotrophic organisms, the most relevant were also Charophyta (48%) and Chlorophyta (42%); among the mixotrophic, Cryptophyta (3%) and Chrysophyta (0.86%), and among the heterotrophic, Ciliophora (3%) featuring the highest number of reads (Table 1).

Table 1 – Operational Taxonomic Units richness (OTUs number) and OTUs abundance (% of reads number) according to the taxonomic and trophic groups.

Taxonomic group	**OTUs Richness**	**Number of reads (%)**	**Trophic role**	**References**
Amoebozoa	1	0.006	Heterotrophic	Lesen *et al.*, 2010
Apusozoa	1	0.01	Heterotrophic	Boenigk and Ardnt, 2002
Bacillariophyta	11	0.41	Autotrophic	Khomich *et al.*, 2017
Bicosoecida	5	0.06	Heterotrophic	Khomich *et al.*, 2017
Centrohelida	3	0.06	Heterotrophic	Khomich *et al.*, 2017
Cercozoa	11	0.14	Heterotrophic	Khomich *et al.*, 2017
Charophyta	23	48	Autotrophic	Khomich *et al.*, 2017
Chlorophyta	44	42	Autotrophic	Simon *et al.*, 2015a; Khomich *et al.*, 2017
Choanoflagellata	2	0.01	Heterotrophic	Simon *et al.*, 2015a; Khomich *et al.*, 2017
Chrysophyta	28	0.86	Mixotrophic	Jones, 2000;
Ciliophora	55	3	Heterotrophic	Beaver and Crisman, 1989; Simon *et al.*, 2015a
Colpodellida	1	0.01	Heterotrophic	Myl'nikova and Myl'nikov, 2009
Colponemidia	1	0.006	Heterotrophic	Tikhonenkov *et al.*, 2014
Cryptophyta	14	3	Mixotrophic	Simon *et al.*, 2015a
Dictyochopytes	1	0.01	Autotrophic	Khomich *et al.*, 2017
Dinoflagellata	6	0.75	Mixotrophic	Stoecker, 1999
Eustigmatophyceae	1	0.01	Autotrophic	Fietz *et al.*, 2005
Fungi	19	0.57	Heterotrophic	Simon *et al.*, 2015a
Hyphochytriomycota	1	0.03	Heterotrophic	Beakes and Thines, 2016
Ichthyosporea	2	0.01	Heterotrophic	Glockling *et al.*, 2013
Perkinsidae	4	0.47	Heterotrophic	Mangot *et al.*, 2011
Peronosporomycetes	9	0.55	Heterotrophic	Dick, 2001
Raphidophytes	1	0.03	Autotrophic	Khomich *et al.*, 2017

For the total microbiota, 23 taxonomic groups were identified, and Ciliophora (55), Chlorophyta (44) and Chrysophyta (28) showed the largest number of OTUs considering all floodplain lakes evaluated (Table 1). Among the autotrophic organisms, the most representative were Chlorophyta (44) and Charophyta (23); among the mixotrophic, Chrysophyta (28) and Cryptophyta (14); while for the heterotrophic organisms, Ciliophora (55) and fungi (19) showed the greatest OTU richness (Table 1).

The OTU alpha diversity was different between lakes. The OTU richness (Figure 2A) as well as the Shannon and Simpson indices (Figure 3) varied greatly between them. We also observed a predominance of autotrophic groups in relation to the OTU richness (Figure 2A) and read number in most lakes (Figure 2B). However, no mixotrophic organisms were found in lakes 11 and 13. The gamma diversity was 807 OTUs. After the rarefaction of samples, this value decreased to 630. The rarefaction curves indicated that the diversity of the planktonic microbiota was not fully recovered in most of the lakes (Figure 4A). However, the species accumulation curve plateaued when all samples were considered, indicating that the gamma diversity was recovered (Figure 4B). This indicates that the OTU diversity in this region was well sampled. A high beta diversity value (βSorensen = 0.92) was observed, mostly associated with OTU replacement along the floodplain (β *turnover* = 0.85; β *nestedness* = 0.07). The NMDS analysis indicates that communities display biogeographical patterns in their composition since different taxonomic groups were associated with different lakes (Figure 5).

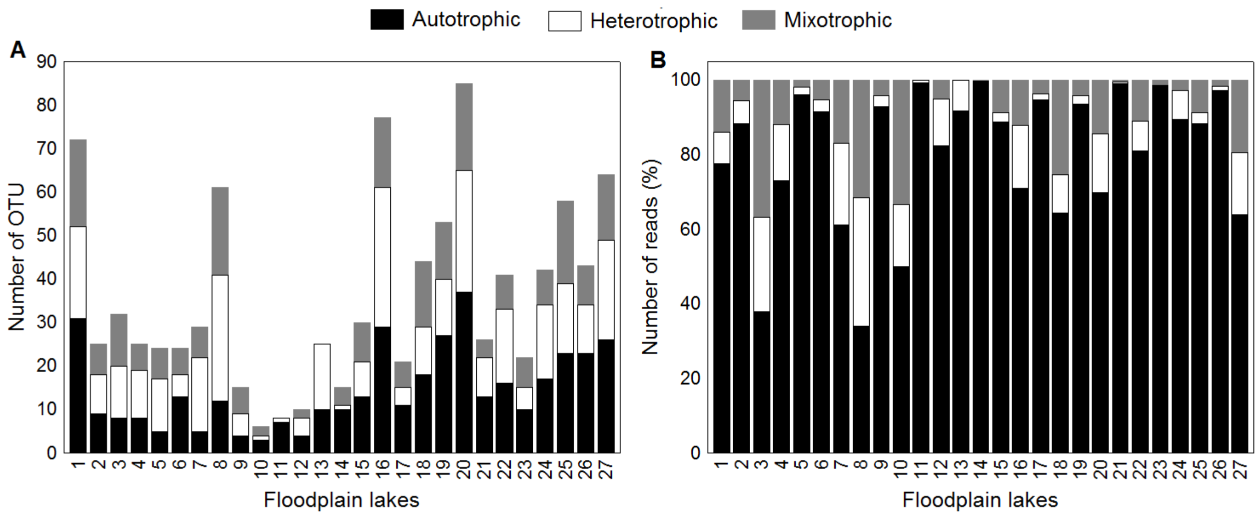

Figure 2 – Number of OTUs (A) and number of reads OUT (B) per lake along the Araguaia river floodplain, divided into trophic groups. The sum of all groups indicates the OTUs richness in each floodplain lake (total microbiota).

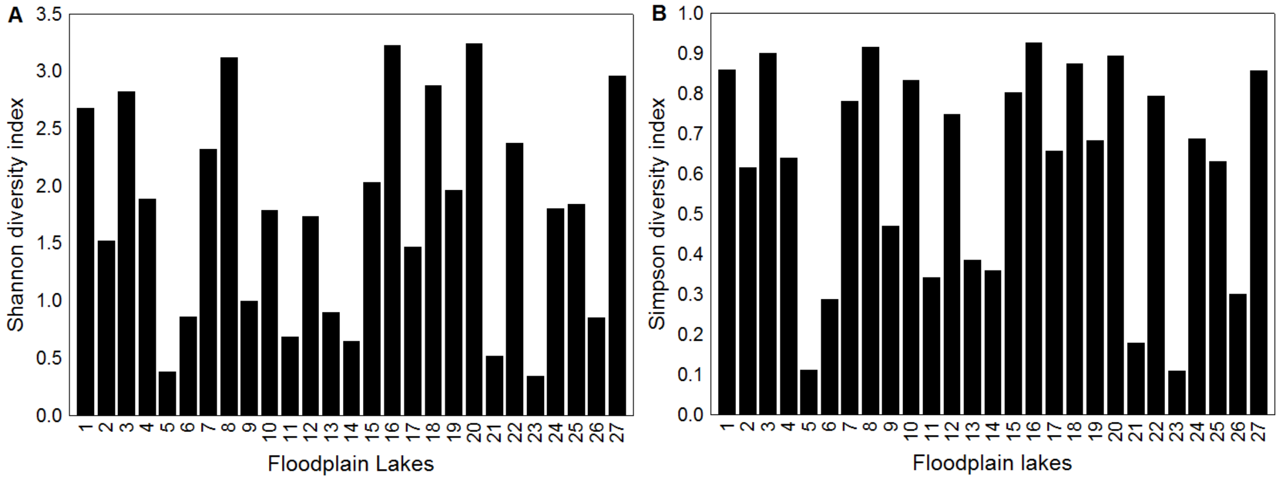

Figure 3 – Shannon (A) and Simpson diversity indices (B) for total microbiota along the Araguaia river floodplain.

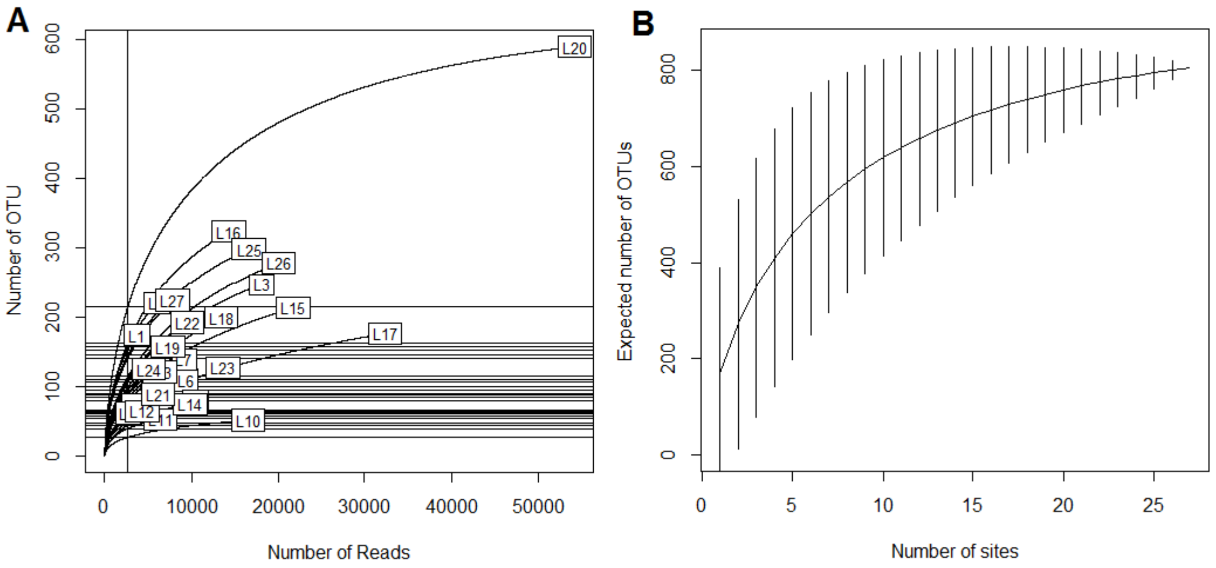

Figure 4 – Rarefaction curves for 27 samples (A) and species accumulation curve for total microbiota sampled in floodplain Araguaia river (B). The vertical line in the rarefaction curve indicates the level of subsampling (2,673 reads per sample). The vertical bars in species accumulation curve indicate the mean and standard error for each number of sites sampled. Only L20 and L10 show a plateau, suggesting that planktonic protist was not completely covered in the most of lakes. However, species accumulation curve stabilized before inclusion of all samples. OTUs = Operational Taxonomic Units, L = lake.

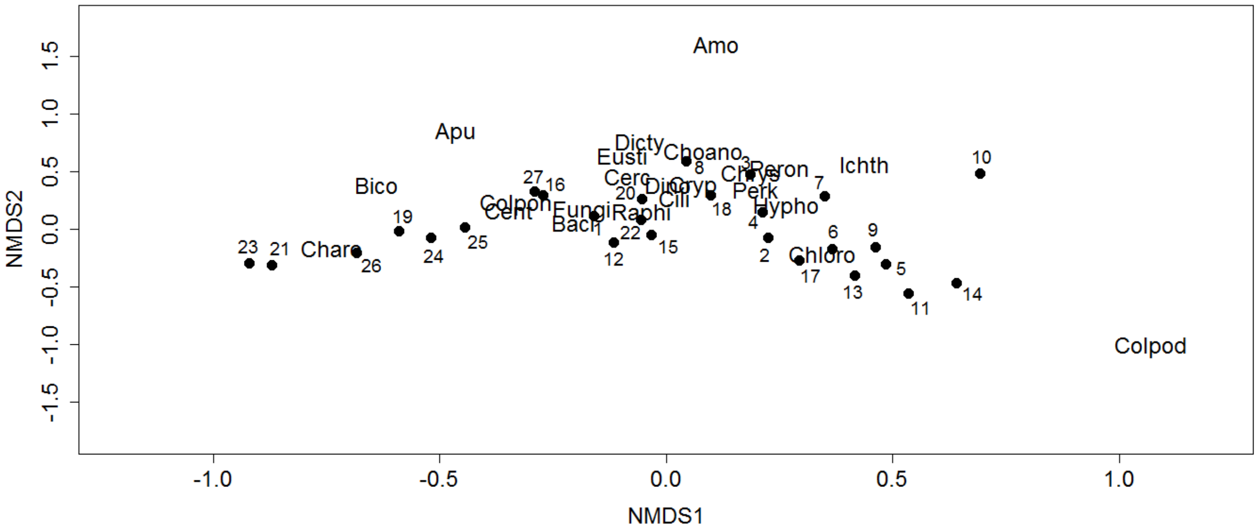

Figure 5 - Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities for protist abundance. The codes used to describe the major taxonomic groups are, Amo = Amoebozoa, Apu = Apusozoa, Baci = Bacillariophyta, Bico = Bicosoecida, Cent = Centrohelida, Cerc =

Cercozoa, Charo = Charophyta, Chloro = Chlorophyta, Choano = Choanoflagellata, Chrys = Chrysophyta, Cili = Ciliophora, Colpod = Colpodellida, Colpon = Colponemidia, Cryp = Cryptophyta, Dicty = Dictyochopytes, Dino = Dinoflagellata, Eusti = Eustignematophyceae, Fungi = Fungi, Hypho = Hyphochytriomycota, Ichth = Ichthyosporea, Perk = Perkinsidae, Peron = Peronosporomycetes, Raphi = Raphidophytes.

Environmental variables

The first and second axes of the PCA represented 42% of the variability in the environmental data. Floodplain lakes displayed heterogeneous environmental characteristics (Figure 6), and some lakes were more associated with certain environmental variables than others. In fact, lakes 6, 7, 10, 11, 12, 13, 14, and 15 were associated mainly with native vegetation, turbidity, total nitrogen, total phosphorus, dissolved oxygen, pH and oxygen saturation. Lakes 3, 4, 8, 9, 18, 19, 22, 23 were associated with conductivity and total dissolved solids. The variables transparency, temperature, chlorophyll-a, grassland and agriculture were important for lakes 1, 21, 24, 25 and 26. Lakes 16, 20 and 27 were associated with lake width.

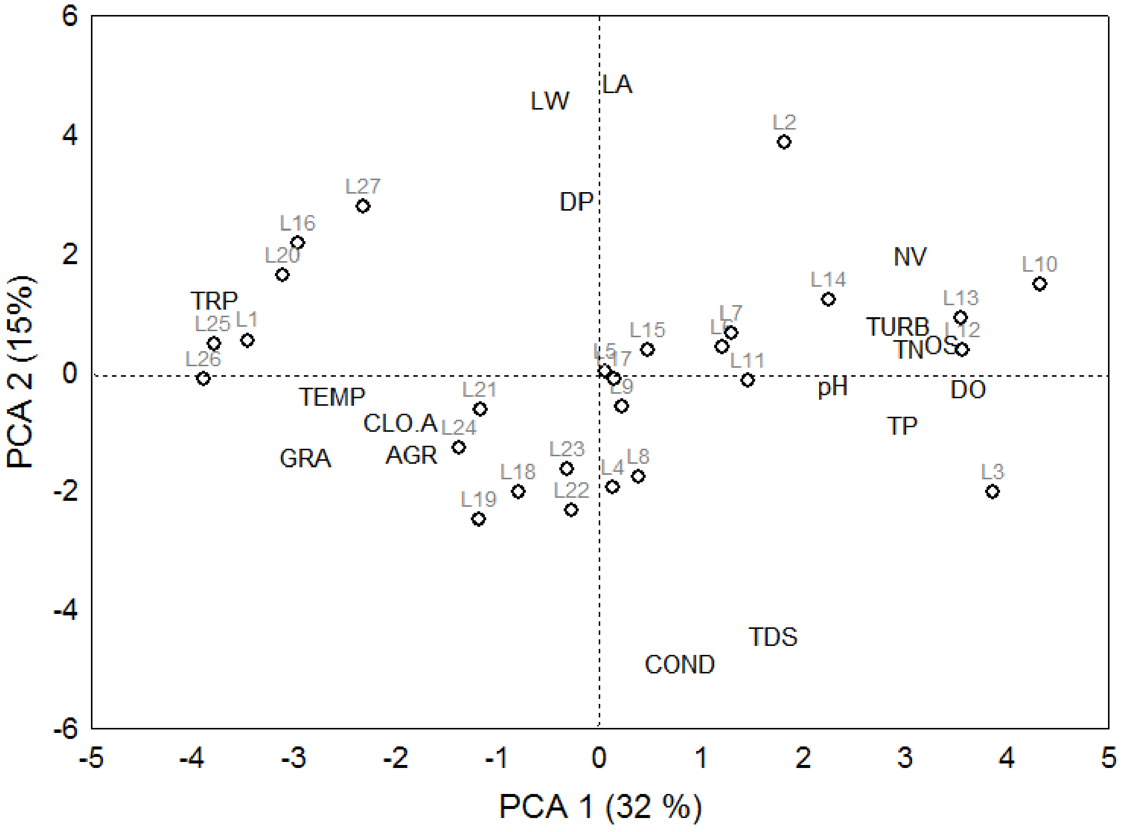

Figure 6 – First and second axis of the Principal Components Analysis applied to environmental variables in the Araguaia river floodplain lakes. The codes of the environmental variables are: COND = conductivity, DO = dissolved oxygen, pH = potential of hydrogen, TEMP = temperature, TURB = turbidity, TDS = total dissolved solids, TRP = transparency, OS = oxygen saturation, DP = depth, TP = total phosphorus, TN = total nitrogen, CHL-A = chlorophyll-a, LA = lake area, LW = lake width, NV = native vegetation, GRA = grassland, AGR = agriculture.

Spatial pattern and variance partitioning

We found a spatial pattern for aquatic microbiota along the Araguaia River (Figure 7). The OTU composition was similar between floodplain lakes located < 70 km from each other. Beyond this distance, the similarity in composition decreased drastically. The environmental and spatial predictors (both directional and non-directional) were not able to explain the variation in the total microbiota composition, as well as the autotrophic and heterotrophic variation (Table 2). However, we found that the environmental variables predicted the composition of the mixotrophic microbiota (Table 2). We also found considerable importance for the shared explanation component between environmental and spatial variables for the total and autotrophic microbiota (Table 2), although its significance cannot be statistically tested. The mixotrophic microbiota was weakly associated with pH (Table 3). The influence of the measured environmental variables on the structure of most taxonomic groups was weak to non-existent (Supplementary data, Table SII).

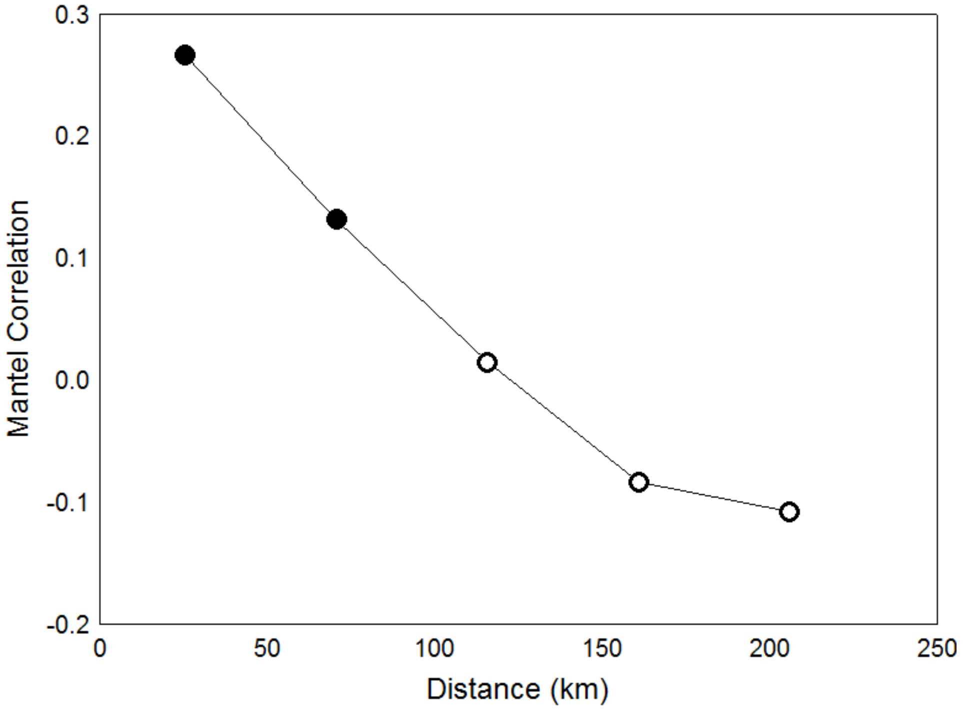

Figure 7 - Mantel correlogram for total microbiota in the Araguaia River floodplain. Bold symbols indicate significant results (*P*≤0.05).

Table 2 - Partial redundancy analysis performed between OTUs abundance (total, autotrophic, heterotrophic and mixotrophic microbiota), environmental variables and spatial predictors. We considered a significance level of 5% ($P \leq 0.05$). R^2 adj= R^2 adjusted. In each analysis, the following spatial filters were selected by the "for selection" function. Total microbiota = PCNM 1 and 2, AEM 1 to AEM 3; Autotrophic = PCNM 1 and 4, AEM 1 to AEM 4; Heterotrophic = PCNM 1, AEM 1; Mixotrophic = PCNM 1, AEM 1 to AEM 3. Components (b) and (d) are not testable significance.

Groups	Environment		Environment and filters	Filters		Residual
	R^2 adj	*P*	R^2 adj	R^2 adj	*P*	R^2 adj
Total microbiota	0	0.74	0.22	0.07	0.13	0.71
Autotrophic	0	0.79	0.32	0.05	0.31	0.63
Heterotrophic	0.01	0.30	0.003	0.01	0.27	0.98
Mixotrophic	**0.12**	**0.03**	0.02	0.04	0.17	0.82

Table 3 - Coefficient of determination (R^2adj = R^2 adjusted) and *P* values obtained for the component "a" (environment) of partial redundancy analysis performed between OTUs abundance (total, autotrophic, heterotrophic, mixotrophic) and each of the environmental variables. In this

analysis, we considered one environmental variable at a time controlled by other environmental variables and spatial filters. Significant values are highlighted in bold (*P*≤0.05). TDS: Total dissolved solids.

	Total microbiota		Autotrophic		Heterotrophic		Mixotrophic	
	R^2 adj	P	R^2 adj	P	R^2 adj	P	R^2 adj	P
Conductivity	0	0.90	0	0.93	0	0.78	0.02	0.16
pH	0	0.44	0	0.39	0.0004	0.37	**0.05**	**0.02**
Temperature	0	0.88	0	0.75	0	0.87	0.005	0.32
TDS	0	0.98	0	0.98	0	0.59	0.003	0.34
Transparency	0	0.63	0	0.41	0	0.87	0	0.69
Depth	0	0.75	0	0.71	0	0.40	0.02	0.10
Total phosphorus	0.02	0.16	0.04	0.07	0	0.71	0	0.76
Total nitrogen	0.003	0.37	0.003	0.35	0.02	0.08	0.01	0.22
Chlorophyll-a	0	0.65	0	0.69	0.02	0.10	0.01	0.23
Width	0.03	0.10	0.02	0.13	0.002	0.40	0.02	0.07

Discussion

High-throughput sequencing can help to unravel ecological factors that influence microbial biogeography (Leray and Knowlton, 2016). In this study, we evaluated the composition and diversity of planktonic protists in floodplain lakes of the Araguaia River, exploring the influence of environmental and spatial factors on shaping these communities at different trophic levels. The floodplain lakes are environmentally heterogeneous and dominated by autotrophic organisms. We detected a spatial pattern in the microorganism distribution and a high beta diversity value. However, contrary to our initial expectation, the environmental variables and spatial filters did not influence the variation in OTU composition for total, autotrophic and heterotrophic microbiota. We found effects of environmental variables only for mixotrophic microbiota; pH seems to be the most important variable to determine their composition, although with a low percentage of explanation.

A high diversity of organisms can be found by the use of molecular techniques (see Medinger *et al.*, 2010; Bradford *et al.*, 2013 for comparisons between OTU-based taxonomic classification and traditional morphological studies). However, it is not always possible to carry out the taxonomic annotation since many sequences are not yet available in the databases (Richards *et al.*, 2005; Huson *et al.*, 2009). In this study, it was not possible to perform the taxonomic annotation for 54% of the data. So far, most of the studies have been conducted in marine ecosystems, and although tropical ecosystems have great biodiversity, they are still rarely studied (Simon *et al.*, 2015b). As a consequence, most database sequences are from marine organisms. Therefore, due to similarity analyses with rigid criteria, there is a high number of freshwater eukaryotes that do not have corresponding OTUs in the SILVA 18S database. This justifies the importance of conducting further studies on freshwater ecosystems.

Considering the organisms for which it was possible to attribute a taxonomic group, representatives of Chlorophyta, Charophyta and Ciliophora were dominant in read numbers and OTU richness. This result is in agreement with studies carried out in rivers (Bradford *et al.,* 2013) and lakes (Medinger *et al.,* 2010; Simon *et al.,* 2015b; Schiaffino *et al.,* 2016) as well as with a taxonomic evaluation previously conducted in many of these floodplain lakes (e.g., Chlorophyceae, Nabout *et al.,* 2006; Machado *et al.,* 2015). During the flood pulse, organic matter enrichment is expected, increasing the particles in suspension, turbidity and consequent reduction in transparency, which could make the conditions of the environment unfavourable for primary productivity (Junk *et al.,* 1989) and lead to the replacement of photosynthetic organisms by mixotrophs during this period (Gallardo *et al.,* 2012). However, the number of primary producers in our lakes remained high. This probably occurred due to local environmental characteristics, such as the entrance of important tributaries of the Araguaia River, which bring more transparent waters (e.g., Mortes River). In addition, nutrients from flood pulses may intensify the primary productivity of phytoplankton in lakes with an intermediate connection level (Schiemer *et al.,* 2006); a high productivity of macrophytes and aquatic plants is also expected in floodplain lakes (Schiemer *et al.,* 2006, Davies *et al.,* 2008).

The total OTU richness was higher than those found in a few studies conducted in marine (Countway *et al.,* 2010) and freshwater environments, although many other studies have shown greater richness than that in the floodplains of the Araguaia River (Brandford *et al.*, 2013, Fonseca *et al.*, 2014, Filker *et al.*, 2015, Genitsaris *et al.*, 2015, Kammerlander *et al.,* 2015, Schiaffino *et al.,* 2016). The beta diversity along the floodplain was determined mainly by the substitution or replacement of OTUs. A high substitution of OTUs suggests that these environments contain microbiota that are lake specific (e.g., Schiaffino *et al.*, 2016). This was supported by the ordination pattern of lakes along the plain, in which some OTUs were more closely associated with certain floodplain lakes. In fact, we found a decrease in composition similarity, and geographically close lakes had more similar OTU compositions than distant lakes. A decrease in similarity is usually attributed to differences in environmental conditions (which generally increase as the geographic distance increases), landscape characteristics or biological limitations that regulate the dispersal rate of organisms (Soininen *et al.*, 2007). The effects of geographic distance (spatial factors) on communities have constantly been associated with processes that act on a regional scale, such as dispersal capacity (Lindström and Langenheder, 2012). However, in the Araguaia River floodplain, none of these variables could explain the OTU variation in the total microbiota.

In this study, we selected environmental variables that have already been considered important in shaping the planktonic communities in previous studies (Li *et al.*, 2012; Triadó-Margarit and Casamayor, 2012; Bradford *et al.*, 2013; Heino *et al.*, 2014; Simon *et al.*, 2015a; Wang *et al.*, 2015). However, we did not find significant effects of these predictors for the total microbiota, contrary to our initial expectations. In plankton, microbial eukaryotes are represented by taxonomically distinct groups (e.g., amoebas, fungi, ciliates, and primary producers, among others, Pawlowski, 2014), which differ in their morphological, genetic and functional characteristics (Countway *et al.*, 2010). Thus, environmental variables that are important to one group may not always be related to others, for example, depth is associated to Dinophyta, but not to Chlorophyta, fungi or Cercozoa; temperature is associated with Cryptophyta and Choanoflagellida, but not with Chlorophyta, Apicomplexa and Ciliophora; and chlorophyll-a is associated with Apicomplexa but not with Bacillariophyta or Perkinsea (see Gong *et al.*, 2015 and their supplementary material). Here, we found that most taxonomic groups were not associated with measured environmental variables.

Considering the trophic groups, the environmental component was important only for mixotrophic microbiota. In fact, environmental variables such as those used in our study have already been identified as important to determine the composition of mixotrophic organisms (e.g., Genitsaris *et al*., 2016; Saad *et al.*, 2016). However, the absence of an explanation of environmental and spatial components has already been observed for microbiota trophic groups (Khomich *et al.*, 2017). When evaluated alone, only pH was weakly associated with the mixotrophic composition. Thus, due to the absence of a purely environmental explanation, the relationship of the microbiota to environmental conditions in our lakes remains unclear.

Indeed, some studies have demonstrated the absence of a clear relationship between environmental variables and planktonic microorganisms (Simon *et al.,* 2015b, Genitsaris *et al.*, 2016; Grantepanche *et al.,* 2016), while others show a very low explanation percentage (Heino *et al.,* 2014; Khomich *et al.,* 2017). An absence of environmental and spatial predictors has also been found for phytoplankton in this same region using a traditional taxonomic approach (Nabout *et al.*, 2009). Thus, we believe that other variables that are not strictly limnological or morphometric, such as biotic interactions, can also be considered in future studies involving microbiota (e.g., Charvet *et al.,* 2014; Sullam *et al.,* 2017) or human degradation gradients (e.g., Tolkkinen *et al.,* 2015; Volant *et al.,* 2016). On the other hand, the presence of a shared explanation component and a spatial pattern of decay in the similarity allows us to assume the presence of spatially structured environmental variables acting on the determination of those communities (Borcard *et al.,* 1992), although their significance could not be tested (Legendre and Legendre, 1998).

The spatial patterns of communities can be derived from intrinsic factors of the organism (e.g., migration rate, dispersion capacity, competition, predation), which promote an autocorrelation in the data, or extrinsic factors (interaction with other spatially structured factors, such as environmental characteristics) that generate spatial dependence (Sokal and Oden, 1978; Legendre and Legendre, 1998). We consider that the spatial patterns observed in communities through the Mantel correlogram can be derived from spatial dependence. Thus, closer sites may feature similar environmental conditions and, consequently, similar OTU compositions (Soininen *et al.,* 2007). This fact is corroborated by the existence of a shared component between the environment and space, indicating the existence of spatially structured environmental variables. However, despite adopting spatial variables that represent directional (Borcard and Legendre, 2002) and non-directional (Blanchet *et al.,* 2008a) dispersion processes, we did not find evidence of a purely spatial explanation in the pRDA. This indicates the absence of spatial autocorrelation caused by intrinsic microbiota processes or by spatially structured environmental variables that were not included in the model (Legendre, 1993).

The reduction in the similarity of the community composition according to the increase in geographic distance is not enough to evaluate the effects of dispersion in the context of metacommunities since this reduction can be derived from environmental similarity or geographic distance (Moritz *et al.,* 2013). Thus, it was not possible to attribute a specific paradigm (i.e., patch dynamics, species sorting, mass effect or neutral dynamic) in the metacommunity context for the protists evaluated. This indicates that a combination of different factors may be responsible for the decay in similarity (Soininen *et al.,* 2007; Moritz *et al.,* 2013), which is often specific to each area of study (Heino *et al*., 2015). For the Araguaia River, our results demonstrate a combination of these two factors, with a reduction in environmental similarity according to geographic distance.

Previous studies have shown that environmental variables are important to explain the composition based on the morphological identification of autotrophic organisms at the taxonomic and functional levels (Machado *et al.,* 2016). Our results demonstrate that this does not occur for the composition obtained by molecular techniques. Another possible explanation for this discrepancy is the absence of a strong environmental gradient. Most lakes are oligotrophic (Marcionilio *et al.,* 2016) and are surrounded by native Cerrado vegetation (Machado *et al.,* 2016). Thus, although the environment explains the taxonomic and functional organism composition, there is no strong environmental pattern restricting certain groups to certain environments. These findings demonstrate the importance of incorporated data, using both traditional morphological identification and high-throughput sequencing since they produce complementary information.

Conclusions

The metabarcoding approach is an efficient tool for the study of planktonic organisms (Hirai *et al.,* 2015) and may produce patterns distinct from the traditional taxonomic approach. In the Araguaia River, using this approach, we characterized the diversity of planktonic protists whose composition is spatially structured along the plain. Thus, our study adds information on the planktonic diversity of microeukaryotes, which is still rarely explored in tropical regions. Although we did not find direct effects of the environment and spatial distance on the OTU composition, we observed a considerable value for the shared explanation component, indicating that spatially structured environmental variables may be acting to determine the composition of these communities. Considering that shallow lakes are important reservoirs of eukaryotic diversity (Simon *et al.*, 2015b), this metabarcoding strategy should be combined with traditional taxonomic studies, seeking to predict the factors influencing communities in a more complete and efficient way.

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Data Archiving

The sequences used in this study were deposited in the GenBank's Sequence Reads Archive (SRA) database (https://submit.ncbi.nlm.nih.gov/) under the access number SUB3701164: MH022894 - MH023194 (Bioproject Accession PRJNA422037 and BioSample Accession SAMN08554969 - SAMN08554997).

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Supplementary Data

Methods - Additional information

Selection of environmental variables

In variance inflation factor (VIF), each variable is correlated with the others by a multiple linear regression and the coefficient of determination (R^2) obtained in each regression is submitted to the following equation: $VIF=1/(1-R^2)$. Thus, variables with VIF values greater than 10 were considered collinear (Alin, 2010).

Spatial variables

In the Principal Coordinates of Neighbor Matrices (PCNM), the geographic coordinates are converted into a euclidean distance matrix used for the construction of eigenvectors, according to the methods described in Borcard and Legendre, (2002). Eigenvectors with positive eigenvalues are considered as the spatial descriptors, since they represent the spatial relationship between the evaluated sites (see Legendre and Legendre, 1998). By considering linear distances, the PCNM may indicate a non-directional dispersion process. The Asymmetric Eigenvector Maps (AEM) is calculated through the directional connectivity between the sampling points (Blanchet *et al.*, 2008), in which the upstream lakes are connected to the downstream lakes (see Legendre and Legendre, 1998). Thus, these filters can represent the dispersion along the river course. These two approaches are complementary (Huszar *et al.*, 2015) and represent different hypotheses about connectivity between sites (Heino *et al.*, 2015). Thus, they may help to better understand how planktonic protists communities are structured throughout space.

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Table SI – List of Operational Taxonomic Units (OTUs) and their taxonomic classification found in Araguaia River floodplain. Were considered the lowest taxonomic level for which it was possible to assign a taxonomic classification.

OTU	Taxonomic group	Minor taxonomic group	Trophic group	References	Similarity (%)
2	Charophyta	*Hyalotheca dissiliens*	Autotrophic	Khomich *et al*., 2017	99.2
3	Chlorophyta	*Gonium viridistellatum*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	97.6
6	Chlorophyta	*Gonium pectorale*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.9
16	Cryptophyta	*Cryptomonas marssonii*	Mixotrophic	Simon *et al*., 2015	100
17	Chlorophyta	*Volvox globator*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.9
30	Cryptophyta	*Cryptomonas* sp.	Mixotrophic	Simon *et al*., 2015	99.4
36	Charophyta	*Staurodesmus validus*	Autotrophic	Khomich *et al*., 2017	100
41	Chlorophyta	*Volvox tertius*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	97
45	Charophyta	*Gonatozygon monotaenium*	Autotrophic	Khomich *et al*., 2017	99.2
47	Charophyta	*Cosmarium ovale*	Autotrophic	Khomich *et al*., 2017	99.7
51	Charophyta	*Hyalotheca mucosa*	Autotrophic	Khomich *et al*., 2017	99.4
52	Ciliophora	*Arcuospathidium cultriforme*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.4
57	Cryptophyta	*Komma caudata*	Mixotrophic	Simon *et al*., 2015	100
59	Ciliophora	*Obertrumia georgiana*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.9
64	Charophyta	*Cyperus alternifolius*	Autotrophic	Khomich *et al*., 2017	99.7
68	Ciliophora	*Vorticella campanula*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.1
71	Perkinsidae	Perkinsidae	Heterotrophic	Mangot *et al*., 2011	97
75	Chrysophyta	*Ochromonas* sp.	Mixotrophic	Jones, 2000	100
81	Ciliophora	*Campanella umbellaria*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.2
84	Ciliophora	*Dileptus* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
86	Dinoflagellata	Thoracosphaeraceae	Mixotrophic	Stoecker, 1999	98.4
87	Perkinsidae	Perkinsidae	Heterotrophic	Mangot *et al*., 2011	97.8
92	Ciliophora	Choreotrichia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
94	Ciliophora	Choreotrichia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.6
95	Charophyta	*Najas minor*	Autotrophic	Khomich *et al*., 2017	99.4
102	Bacillariophyta	*Discostella* sp.	Autotrophic	Khomich *et al*., 2017	99.7
104	Ciliophora	*Strombidium* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.3

105	Bacillariophyta	*Aulacoseira crenulata*	Autotrophic	Khomich *et al*., 2017	99.4
106	Charophyta	*Groenbladia neglecta*	Autotrophic	Khomich *et al*., 2017	98.9
107	Peronosporomycetes	*Aphanomyces astaci*	Heterotrophic	Dick, 2001	98.9
108	Ciliophora	*Tintinnidium balechi*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
109	Ciliophora	*Halteria* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.2
111	Dinoflagellata	*Biecheleria* sp.	Mixotrophic	Stoecker, 1999	99.4
122	Chlorophyta	*Choricystis parasitica*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
127	Chlorophyta	Oedogoniales	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.2
129	Chrysophyta	*Synura petersenii*	Mixotrophic	Jones, 2000	100
133	Cryptophyta	Cryptophyceae	Mixotrophic	Simon *et al*., 2015	99.7
134	Fungi	Fungi	Heterotrophic	Simon *et al*., 2015	98.9
137	Dinoflagellata	*Symbiodinium* sp.	Mixotrophic	Stoecker, 1999	99.7
138	Chlorophyta	*Monomastix minuta*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.6
140	Ciliophora	*Didinium* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.6
141	Ciliophora	Choreotrichia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
142	Eustigmatophyceae	*Pseudocharaciopsis minuta*	Autotrophic	Fietz *et al*., 2005	100
143	Peronosporomycetes	Peronosporomycetes	Heterotrophic	Dick, 2001	98.4
152	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	99.2
155	Charophyta	*Closterium wallichii*	Autotrophic	Khomich *et al*., 2017	99.2
157	Chrysophyta	*Ochromonas* sp.	Mixotrophic	Jones, 2000	98.4
158	Bacillariophyta	*Gomphonema affine*	Autotrophic	Khomich *et al*., 2017	100
159	Dinoflagellata	*Peridiniopsis jiulongensis*	Mixotrophic	Stoecker, 1999	100
160	Chrysophyta	*Ochromonas* sp.	Mixotrophic	Jones, 2000	100
163	Ciliophora	*Chilodonella piscicola*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.8
172	Chlorophyta	Ulvophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.4
176	Fungi	*Rhizoclosmatium* sp.	Heterotrophic	Simon *et al*., 2015	100
177	Chlorophyta	*Monomastix* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.6
179	Chlorophyta	Chlorophyta	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
181	Peronosporomycetes	Peronosporomycetes	Heterotrophic	Dick, 2001	100
182	Charophyta	*Salvinia natans*	Autotrophic	Khomich *et al*., 2017	98.6
183	Bacillariophyta	*Synedra ulna*	Autotrophic	Khomich *et al*., 2017	98.9

184	Chrysophyta	Chromulinales	Mixotrophic	Jones, 2000	98.9
186	Chlorophyta	*Chlorella sorokiniana*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
191	Fungi	Chytridiales	Heterotrophic	Simon *et al*., 2015	98.4
192	Centrohelida	Acanthocystidae	Heterotrophic	Khomich *et al*., 2017	100
194	Cryptophyta	*Cryptomonas* sp.	Mixotrophic	Simon *et al*., 2015	99.4
197	Dinoflagellata	*Biecheleria* sp.	Mixotrophic	Stoecker ,1999	98.6
198	Chrysophyta	Chrysophyta	Mixotrophic	Jones, 2000	99.7
208	Cryptophyta	Cryptophyceae	Mixotrophic	Simon *et al*., 2015	99.7
210	Cercozoa	Cercozoa	Heterotrophic	Khomich *et al*., 2017	97.2
211	Ciliophora	*Prorodon teres*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.8
217	Chlorophyta	*Aphanochaete repens*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.4
220	Ciliophora	*Strombidium* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.4
224	Ciliophora	*Stichotricha aculeata*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.6
227	Peronosporomycetes	*Pythium graminicola*	Heterotrophic	Dick, 2001	97.4
228	Fungi	*Endochytrium* sp.	Heterotrophic	Simon *et al*., 2015	100
230	Ciliophora	*Spirostrombidium* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.3
231	Centrohelida	*Raphidiophrys heterophryoidea*	Heterotrophic	Khomich *et al*., 2017	99.4
235	Bicosoecida	Bicosoecida	Heterotrophic	Khomich *et al*., 2017	100
236	Fungi	*Cladochytrium replicatum*	Heterotrophic	Simon *et al*., 2015	99.4
238	Bacillariophyta	*Eunotia formica*	Autotrophic	Khomich *et al*., 2017	100
243	Charophyta	*Pleurotaenium ehrenbergii*	Autotrophic	Khomich *et al*., 2017	97.6
245	Cryptophyta	Cryptophyceae	Mixotrophic	Simon *et al*., 2015	100
251	Perkinsidae	Perkinsidae	Heterotrophic	Mangot *et al*., 2011	98.9
257	Chlorophyta	Chlorophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.7
261	Ciliophora	*Strobilidium caudatum*	Heterotrophic	Beaver and Crisman ,1989; Simon *et al*., 2015	98.6
262	Chlorophyta	*Tetracystis tetraspora*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.1
264	Chlorophyta	Mamiellophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.4
269	Peronosporomycetes	Pythiaceae	Heterotrophic	Dick, 2001	97.2
270	Chlorophyta	Chlamydomonadaceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
273	Charophyta	*Desmidium grevillei*	Autotrophic	Khomich *et al*., 2017	99.2
275	Charophyta	*Spirogyra* sp.	Autotrophic	Khomich *et al*., 2017	98.7

285	Chlorophyta	*Oedogonium angustistomum*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.1
290	Ciliophora	*Hemiophrys procera*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
291	Ciliophora	Peritrichia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.7
295	Cryptophyta	*Cryptomonas paramecium*	Mixotrophic	Simon *et al*., 2015	98.9
296	Perkinsidae	Perkinsidae	Heterotrophic	Mangot *et al*., 2011	100
308	Ciliophora	Cyrtophoria	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.7
311	Chlorophyta	Chlorophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.4
312	Ciliophora	*Lagynophrya acuminata*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.7
315	Ciliophora	*Haptoria* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.7
316	Chlorophyta	Chlorophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.7
319	Chlorophyta	Chlorophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	97.6
320	Chlorophyta	*Tetracystis* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.4
323	Ciliophora	*Cyrtolophosis mucicola*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.3
329	Fungi	Chytridiales	Heterotrophic	Simon *et al*., 2015	97.1
334	Cercozoa	*Heteromita* sp.	Heterotrophic	Khomich *et al*., 2017	98.7
335	Fungi	Chytridiales	Heterotrophic	Simon *et al*., 2015	98.7
342	Bacillariophyta	*Nitzschia palea*	Autotrophic	Khomich *et al*., 2017	100
343	Ciliophora	Haptoria	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.4
346	Charophyta	*Micrasterias laticeps*	Autotrophic	Khomich *et al*., 2017	100
347	Ciliophora	*Opercularia microdiscum*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.1
348	Chrysophyta	Chromulinales	Mixotrophic	Jones, 2000	97.6
349	Chlorophyta	*Chaetophora* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.2
355	Chlorophyta	*Botryococcus braunii*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
358	Peronosporomycetes	*Phytophthora parsiana*	Heterotrophic	Dick, 2001	100
367	Cryptophyta	*Cryptomonas obovoidea*	Mixotrophic	Simon *et al*., 2015	100
370	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	99.2
373	Cercozoa	Cercozoa	Heterotrophic	Khomich *et al*., 2017	100
374	Ciliophora	*Prorodon* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.2
378	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	98.7
379	Chrysophyta	*Dinobryon sertularia*	Mixotrophic	Jones, 2000	98.9
383	Dictyochopytes	*Pseudopedinella* sp.	Autotrophic	Khomich *et al*., 2017	98

394	Chrysophyta	*Synura uvella*	Mixotrophic	Jones, 2000	100
402	Fungi	*Trichosporon chiarellii*	Heterotrophic	Simon *et al*., 2015	98.1
403	Charophyta	*Zygnema pseudogedeanum*	Autotrophic	Khomich *et al*., 2017	99.2
413	Cryptophyta	*Cryptomonas ovata*	Mixotrophic	Simon *et al*., 2015	98.3
416	Chlorophyta	*Wislouchiella planctonica*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.4
425	Charophyta	*Gonatozygon kinahani*	Autotrophic	Khomich *et al*., 2017	99.7
438	Bicosoecida	*Siluania monomastiga*	Heterotrophic	Khomich *et al*., 2017	98.4
441	Ciliophora	*Tetrahymena* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.7
445	Ciliophora	*Paramecium multimicronucleatum*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
447	Fungi	Fungi	Heterotrophic	Simon *et al*., 2015	100
455	Fungi	Fungi	Heterotrophic	Simon *et al*., 2015	100
457	Ciliophora	Haptoria	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.7
458	Chrysophyta	*Ochromonas sphaerocystis*	Mixotrophic	Jones, 2000	99.4
459	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	99.7
460	Ciliophora	Prostomatea	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97
462	Ciliophora	*Lembadion bullinum*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.1
470	Hyphochytriomycota	*Rhizidiomyces* sp.	Heterotrophic	Beakes and Thines, 2016	99.7
471	Chlorophyta	*Chlamydomonas* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.4
474	Chlorophyta	*Oedogonium* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.4
480	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	97.4
484	Cryptophyta	*Cryptomonas pyrenoidifera*	Mixotrophic	Simon *et al*., 2015	97.7
485	Charophyta	*Spirogyra juergensii*	Autotrophic	Khomich *et al*., 2017	98.9
494	Ciliophora	Haptoria	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.7
497	Ciliophora	*Trichodina hypsilepis*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.8
498	Charophyta	*Spondylosium pulchellum*	Autotrophic	Khomich *et al*., 2017	98.9
502	Colponemidia	*Colponema edaphicum*	Heterotrophic	Tikhonenkov *et al*., 2014	98.3
506	Centrohelida	*Pterocystis tropica*	Heterotrophic	Khomich *et al*., 2017	98.1
508	Chrysophyta	*Lagynion scherffelii*	Mixotrophic	Jones, 2000	99.2
512	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	97.6
513	Cryptophyta	*Chroomonas* sp.	Mixotrophic	Simon *et al*., 2015	97.8
518	Chlorophyta	*Vitreochlamys fluviatilis*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	97.6

525	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	97.1
529	Cercozoa	*Arachnula impatiens*	Heterotrophic	Khomich *et al*., 2017	97.1
534	Chrysophyta	Chrysophyta	Mixotrophic	Jones, 2000	99.7
536	Chlorophyta	*Chaetopeltis orbicularis*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
538	Bacillariophyta	*Urosolenia eriensis*	Autotrophic	Khomich *et al*., 2017	97.6
541	Bacillariophyta	*Chaetoceros* sp.	Autotrophic	Khomich *et al*., 2017	100
542	Raphidophytes	*Merotricha bacillata*	Autotrophic	Khomich *et al*., 2017	98.7
543	Chlorophyta	*Chlamydomonas* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	97.8
548	Chlorophyta	*Hafniomonas turbinea*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.6
550	Ciliophora	Lacrymariidae	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.6
552	Fungi	*Physoderma dulichii*	Heterotrophic	Simon *et al*., 2015	99.2
553	Ciliophora	Peritrichia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.9
557	Colpodellida	*Colpodella edax*	Heterotrophic	Myl'nikova and Myl'nikov, 2009	99.4
559	Chrysophyta	*Ochromonas* sp.	Mixotrophic	Jones, 2000	100
560	Choanoflagellata	Codosigidae	Heterotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.4
561	Chlorophyta	Chlamydomonadales	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.4
562	Chlorophyta	*Tetracystis* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.2
563	Chrysophyta	*Poterioochromonas malhamensis*	Mixotrophic	Jones, 2000	100
564	Apusozoa	Apusomonadidae	Heterotrophic	Boenigk and Ardnt, 2002	98.3
571	Chrysophyta	*Mallomonas matvienkoae*	Mixotrophic	Jones, 2000	99.2
577	Chlorophyta	Dunaliellaceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
578	Ciliophora	*Paramecium* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
579	Ciliophora	Colpodida	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
581	Chrysophyta	*Synura echinulata*	Mixotrophic	Jones, 2000	98.4
588	Fungi	Fungi	Heterotrophic	Simon *et al*., 2015	98.9
590	Cercozoa	*Bodomorpha minima*	Heterotrophic	Khomich *et al*., 2017	99.7
591	Bicosoecida	Bicosoecida	Heterotrophic	Khomich *et al*., 2017	100
598	Ciliophora	*Gonostomum strenuum*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.3
601	Chlorophyta	Chlamydomonadaceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	97.6
602	Fungi	Chytridiomycota	Heterotrophic	Simon *et al*., 2015	100
609	Fungi	Fungi	Heterotrophic	Simon *et al*., 2015	99.4

614	Chrysophyta	Chrysophyceae	Mixotrophic	Jones, 2000	99.4
618	Ciliophora	*Chilodonella acuta*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
619	Ciliophora	*Platyophrya bromelicola*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.1
623	Ciliophora	*Prorodon* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.2
630	Ciliophora	*Rimostrombidium* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	100
636	Charophyta	*Coleochaete orbicularis*	Autotrophic	Khomich *et al*., 2017	99.4
646	Chrysophyta	*Synura spinosa*	Mixotrophic	Jones, 2000	98.6
650	Dinoflagellata	*Peridinium inconspicuum*	Mixotrophic	Stoecker ,1999	99.7
665	Choanoflagellata	*Diaphanoeca* sp.	Heterotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
669	Fungi	Fungi	Heterotrophic	Simon *et al*., 2015	99.7
673	Chlorophyta	*Pseudomuriella* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.6
677	Ciliophora	*Trachelius ovum*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.3
684	Peronosporomycetes	Pythiaceae	Heterotrophic	Dick, 2001	98.7
695	Charophyta	*Closterium venus*	Autotrophic	Khomich *et al*., 2017	98.1
696	Bacillariophyta	*Sellaphora minima*	Autotrophic	Khomich *et al*., 2017	97.3
698	Ciliophora	Peritrichia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.9
708	Chlorophyta	Mamiellophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
711	Ciliophora	Oxytrichidae	Heterotrophic	Beaver and Crisman 1989; Simon et al., 2015	100
713	Cercozoa	Cercozoa	Heterotrophic	Khomich *et al*., 2017	99.2
725	Chrysophyta	*Paraphysomonas* sp.	Mixotrophic	Jones, 2000	100
733	Fungi	*Candida incommunis*	Heterotrophic	Simon *et al*., 2015	100
739	Ciliophora	Peniculia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.2
748	Charophyta	*Mougeotia* sp.	Autotrophic	Khomich *et al*., 2017	99.4
753	Chlorophyta	*Chlamydomonas* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	97.8
758	Chrysophyta	Chromulinales	Mixotrophic	Jones, 2000	98.9
760	Ciliophora	*Linostomella* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.1
765	Chlorophyta	*Chlamydomonas raudensis*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.6
782	Bacillariophyta	*Pinnularia subcapitata* var. *elongata*	Autotrophic	Khomich *et al*., 2017	98.9
787	Fungi	*Karlingiomyces* sp.	Heterotrophic	Simon *et al*., 2015	99.2
799	Chlorophyta	*Bracteacoccus* sp.	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.4
809	Cercozoa	Cercozoa	Heterotrophic	Khomich *et al*., 2017	100

810	Bacillariophyta	*Aulacoseira* sp.	Autotrophic	Khomich *et al*., 2017	97.9
813	Cryptophyta	*Cryptomonas* sp.	Mixotrophic	Simon *et al*., 2015	99.4
814	Cercozoa	*Cercomonas* sp.	Heterotrophic	Khomich *et al*., 2017	100
820	Peronosporomycetes	*Aphanomyces* sp.	Heterotrophic	Dick, 2001	97.4
823	Chlorophyta	Chlorophyceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
824	Ciliophora	*Stichotrichia* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.9
827	Ciliophora	*Glaucoma scintillans*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.6
828	Cercozoa	*Leptophrys vorax*	Heterotrophic	Khomich *et al*., 2017	99.2
829	Cryptophyta	*Rhodomonas* sp.	Mixotrophic	Simon *et al*., 2015	98.9
836	Peronosporomycetes	*Aquastella attenuata*	Heterotrophic	Dick, 2001	99.7
838	Ciliophora	Peritrichia	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98.1
841	Fungi	Cladochytriaceae	Heterotrophic	Simon *et al*., 2015	98.7
863	Chlorophyta	Chlamydomonadaceae	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.4
865	Chlorophyta	*Scherffelia dubia*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	99.2
875	Chlorophyta	*Atractomorpha echinata*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	100
878	Ichthyosporea	Ichthyosporea	Heterotrophic	Glockling *et al*., 2013	97.6
880	Chrysophyta	*Mallomonas* sp.	Mixotrophic	Jones, 2000	100
881	Cercozoa	*Rhogostoma* sp.	Heterotrophic	Khomich *et al*., 2017	98.9
885	Charophyta	*Micrasterias fimbriata*	Autotrophic	Khomich *et al*., 2017	98.1
894	Bicosoecida	Bicosoecida	Heterotrophic	Khomich *et al*., 2017	97.7
898	Ciliophora	*Colpoda magna*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.2
907	Ichthyosporea	Ichthyosporea	Heterotrophic	Glockling *et al*., 2013	98.1
910	Cercozoa	Cercozoa	Heterotrophic	Khomich *et al*., 2017	100
932	Ciliophora	*Arcuospathidium* sp.	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	98
940	Amoebozoa	*Vermamoeba vermiformis*	Heterotrophic	Lesen *et al*., 2010	99.4
944	Bicosoecida	Bicosoecida	Heterotrophic	Khomich *et al*., 2017	97
950	Chlorophyta	*Tetraselmis subcordiformis*	Autotrophic	Simon *et al*., 2015; Khomich *et al*., 2017	98.1
973	Fungi	Chytridiales	Heterotrophic	Simon *et al*., 2015	99.7
985	Ciliophora	*Balantidion pellucidum*	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	97.1
1006	Ciliophora	Prostomatea	Heterotrophic	Beaver and Crisman, 1989; Simon *et al*., 2015	99.7
1011	Charophyta	*Aulacomnium androgynum*	Autotrophic	Khomich *et al*., 2017	98.4

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Table SII - Coefficient of determination (R^2adj = R^2 adjusted) and *P* values obtained for the component "a" (environment) of partial redundancy analysis performed between OTUs abundance for major taxonomic groups and each of the environmental variables. In this analysis, we considered one environmental variable at a time controlled by other environmental variables and spatial filters. Significant values are highlighted in bold ($P \leq 0.05$). The codes of the environmental variables are: COND = conductivity, pH = potential of hydrogen, TEMP = temperature, TDS = total dissolved solids, TRP = transparency, DP = depth, TP = total phosphorus, TN = total nitrogen, CHL-A = chlorophyll-a, LW = lake width. The codes used to describe the major taxonomic groups are, Amo = Amoebozoa, Apu = Apusozoa, Baci = Bacillariophyta, Bico = Bicosoecida, Cent = Centrohelida, cerc = Cercozoa, Charo = Charophyta, Chloro = Chlorophyta, Choano = Choanoflagellata, Chrys = Chrysophyta, Cili = Ciliophora, Colpod = Colpodellida, Colpon = Colponemidia, Cryp = Cryptophyta, Dicty = Dictyochopytes, Dino = Dinoflagellata, Eusti = Eustignematophyceae, Fungi = Fungi, Hypho = Hyphochytriomycota, Ichth = Ichthyosporea, Perk = Perkinsidae, Peron = Peronosporomycetes, Raphi = Raphidophytes.

	COND		pH		TEMP		TDS		TRP		DP		TP		TN		CHL-A		LW	
	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*	R^2adj	*P*
Amo	0	0.63	0	0.62	0	0.69	0	0.86	0	0.64	0	0.7	0	0.4	0	0.63	0.07	0.11	0	0.94
Apu	0	0.77	0	0.54	0	0.56	0	0.52	0	0.91	0.11	0.11	0	0.41	0	0.34	0.13	0.09	0	0.33
Baci	0	0.98	0	0.51	0	0.54	0.0004	0.42	0.01	0.27	0.00008	0.42	0.01	0.28	0.01	0.26	0	0.61	0	0.47
Bico	0	0.99	0	0.94	0	0.53	0	0.95	0.03	0.16	0	0.7	0	0.72	0	0.48	0	0.44	0.01	0.28
Cent	0.03	0.19	0	0.34	0.03	0.19	0	0.38	0.02	0.26	0.01	0.24	0	0.56	0	0.47	0	0.5	0.01	0.29
Cerc	0	0.99	0	0.94	0	0.68	0	0.94	0	0.62	0	0.77	0	0.96	0	0.42	0	0.73	0	0.45
Charo	0	0.77	0.01	0.27	0.04	0.1	0	0.74	0	0.72	0.008	0.31	0.03	0.12	0.04	0.1	0	0.44	0.03	0.11
Chloro	0	0.74	0	0.35	0.03	0.15	0	0.76	0	0.56	0	0.89	0	0.48	0.01	0.25	0	0.73	0	0.83
Choano	0	0.61	0	0.93	0	0.92	0	0.99	0.006	0.32	0	0.97	0	0.82	0	0.58	0	0.45	0.03	0.23
Chrys	0	0.97	0.02	0.14	0.009	0.28	0	0.64	0.009	0.26	0	0.6	0	0.67	0.03	0.06	**0.04**	**0.04**	0	0.54
Cili	0.0004	0.43	0.01	0.27	0	0.88	0.008	0.29	0	0.75	0.02	0.13	0.0006	0.45	0.03	0.06	**0.06**	**0.007**	0.004	0.34
Colpod	0	0.45	0	0.6	0	0.37	0	0.69	0	0.47	0	0.63	0	0.27	0	0.63	0	0.83	0.03	0.23
Colpon	0	0.68	0	0.92	0	0.37	0	0.43	0	0.63	0	0.35	0	0.88	0	0.47	0	0.92	0	0.34
Cryp	0	0.19	**0.07**	**0.02**	0.03	0.1	0	0.47	0	0.72	**0.07**	**0.03**	0	0.44	0.01	0.2	0	0.49	0.02	0.17
Dicty	0	0.54	0	0.71	0	0.59	0	0.79	0	0.44	0	0.59	0	0.43	0	0.64	0	0.55	0.11	0.09
Dino	0	0.7	0.02	0.23	0	0.46	0.02	0.19	0	0.56	0.003	0.4	0	1	0	0.55	0.01	0.29	**0.06**	**0.05**
Eusti	0	0.99	0	0.62	0	0.7	0	0.97	0	0.54	0	0.7	0	0.49	0	0.97	0	0.8	0	0.52
Fungi	0	0.99	0.001	0.46	0	0.83	0	0.53	0	0.5	0	0.79	0	0.76	0.001	0.43	0.01	0.32	0	0.86
Hypho	0	0.79	0.01	0.26	0	0.98	0	0.66	0	0.45	0	0.33	0	0.41	0.01	0.03	0.09	0.11	0.03	0.22
Ichth	0	0.37	0	0.87	0.005	0.37	0	0.7	0.01	0.34	0.1	0.1	0	0.83	0	0.08	0	0.9	0.12	0.06
Perk	0	0.37	0.006	0.34	0	0.9	0	0.64	0.01	0.31	0.003	0.34	0	0.94	0.07	0.08	0	0.9	0	0.68
Peron	0	0.9	0.008	0.3	0.03	0.2	0	0.95	0.05	0.14	0	0.83	0	0.67	0	0.5	0	0.84	0	0.38
Raphi	0	0.51	0	0.76	0	0.22	0	0.59	0	0.92	0	0.82	0.03	0.21	**0.16**	**0.05**	0	0.74	0	0.73

CAPÍTULO II

Predicting the dynamics of taxonomic and functional phytoplankton compositions in different global warming scenarios**

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Abstract

It is important to predict how phytoplankton will respond to global warming, as changes in their composition can affect ecosystem functions. We evaluated the effect of water warming on the taxonomic and functional composition of phytoplankton and on chemical characteristics that affect their occurrence, such as dissolved oxygen, pH and conductivity. Microcosms were constructed outdoors and monitored over time. The temperature was manipulated to simulate different scenarios predicted for the future. Warming caused a reduction in dissolved oxygen, while the pH and conductivity remained unchanged. We found a joint effect of temperature and time on chlorophyll-a as well as on the species and functional groups. The substitution of species and groups occurred in a similar way between treatments. However, a greater number of Cyanophyceae individuals were found at higher temperatures, while Bacillariophyceae and Euglenophyceae species were found more commonly in the lower warming treatments. These results indicate that warming altered the taxonomic and functional composition of phytoplankton, causing species substitution as well as a change in their functional characteristics, which led to the predominance of small organisms. Thus, contribute to predicting how an increase in temperature might alter the patterns of dominance, homogenization and community dynamics in future warming scenarios.

Keywords: climate change**,** microcosm, microorganisms, temperature

Introduction

Understanding the impact of climate change on freshwater ecosystems is of extreme importance mainly because this ecosystem generates needed resources for water supply and is still among the most threatened ecosystems on the planet (Dudgeon et al., 2006). In this context, predicting the effects of climate change on freshwater species has increasingly attracted the interest of the scientific community (Heino et al., 2009; Woodward et al., 2010; Jeppesen et al., 2014). In fact, many climatic variables may impact species; nonetheless, the changes in temperature are highlighted since this variable is responsible for mediating various metabolic and biochemical processes in organisms (Jeppesen et al., 2010). Thus, an increase in temperature can alter the composition, abundance and richness of species and alter the viability of populations (Heino et al., 2009; Jeppesen et al., 2014). In addition, changes in the growth, respiration rates and phenological patterns of organisms are expected, and reproductive events are anticipated (Jeppesen et al., 2010).

Depending on the emissions scenario used, estimates from the Intergovernmental Panel on Climate Change (IPCC) indicate an increase of 0.3 °C to 4.8 °C by the end of the century (IPCC, 2014). This increase in temperature can modify numerous chemical and physical parameters of aquatic environments, such as increased evaporation rates, denitrification, evaporation (Senerponte Domis et al., 2013) and sediment release (Jeppesen et al., 2010). These alterations influence the dynamics of planktonic organisms (Senerponte Domis et al., 2013). The consequences of climate change are reflected at different biological organization levels; for example, climate change will change the respiration rates, growth and body size of individuals; abundance and reproduction in populations; and the diversity of traits, species and community size, as well as the rates of decomposition and nutrient cycling at the ecosystem level (Woodward et al., 2010).

In a climate change context, phytoplankton represent an excellent model for understanding the effects of global warming (Burgmen & Hillebrand, 2011; Dong et al., 2015). This group has an important role in the food webs of aquatic ecosystems, acting as one of the main primary producers (Field et al., 1998). Furthermore, because phytoplankton have a short life cycle (Kruk et al., 2012), several generations can be monitored in a short period of time. The ecological niche of phytoplankton is composed of different axes, such as the availability of resources, interactions with predators and parasites, and environmental variables (Reynolds, 2006). In the environmental context, temperature constitutes an important variable that determines the phytoplankton niche (Litchman & Klausmeier, 2008; Litchman et al., 2012) and, together with other physical and chemical variables (e.g., nutrient availability, dissolved oxygen, turbidity, and conductivity; Reynolds, 2006), is a factor that determines their presence in the environment (Huertas et al., 2011). At the community level, warming effects on chlorophyll-a concentrations (e.g., Feuchtmayr et al., 2009; Hennemann & Petrucio, 2010), species composition (e.g., Yvon-Durocher et al., 2015), and characteristics that reflect cell size (e.g., Yvon-Durocher et al., 2011; Burgmer & Hillebrand, 2011; Adams et al., 2013; Yvon-Durocher et al., 2015) have already been observed.

Experimental or observational evidence has shown that warming should favour groups that are already adapted to survive at high temperatures, such as cyanobacteria (e.g., Burgman & Hillebrand, 2011; Huertas et al., 2011; Chen, 2015) and dinoflagellates (Finkel et al., 2009; Jeppesen et al., 2009), and disfavour those groups that currently prefer cooler waters, such as diatoms (Finkel et al., 2009; Jeppesen et al., 2009; Sommer & Lewandowska, 2011; Chen, 2015). Changes in composition can occur either by species substitution within the same taxonomic group or by changes in the predominance of these groups (Thomas et al., 2016). Thus, an increase in temperature may promote changes in dominance patterns (Yvon-Durocher et al., 2011), often reducing the diversity in warmer environments (Rasconi et al., 2017). In addition, alterations in phytoplankton biomass due to warming can be reflected at higher trophic levels, reducing bottom-up control and increasing the top-down effect (Kratina et al., 2012). Changes in composition to a predominance of smaller species may, for example, alter the dynamics of food webs by changing the predation pattern of zooplankton (Lewandowska & Sommer, 2010).

Most studies have evaluated the effects of temperature on species composition (e.g., Moss et al., 2003; Senerpont Domis et al., 2007; Feuchtmayr et al. 2009; Lewandowska & Sommer, 2010; Yvon-Durocher et al., 2015; Yvon-Durocher et al., 2017). However, since climate change acts at different levels of biological organization, it is important to investigate how future warming can affect community structure on the basis of functional characteristics (Woodward et al., 2010; Lurgi et al., 2012). For phytoplankton, two functional classification approaches are well known and widely used in ecological studies (Kruk et al., 2011). The Reynolds functional groups (RFG, Reynolds et al., 2002; Padisák et al., 2009) classify species into 41 groups according to their ecological characteristics, habitat preferences, tolerance and sensitivity to environmental conditions. Morphologically based functional groups (MBFG, Kruk et al., 2010) organize the species into seven functional groups based on the morphological characteristics of organisms, such as cell size, biological form, the presence or absence of flagella, mucilage, silica structures and aerotopes.

In fact, some of the morphological or physiological features used to classify species into functional groups may bring advantages in a global warming scenario (e.g., small cell size), while others will make individuals more susceptible to its impacts. Organisms with more general habitat requirements tend to deal better with environmental variations than those with a restricted niche (Lurgi et al., 2012). On the other hand, high temperatures tend to intensify cellular metabolism, and smaller cells should be favoured in global warming scenarios (Burgmen & Hillebrand, 2011; Sommer & Lewandowska, 2011; Yvon-Durocher et al., 2011; Kratina et al., 2012; Sommer et al., 2015; but see Yvon-Durocher et al., 2015 for the opposite effect) since these are metabolically more active and grow, reproduce, and utilize resources more rapidly than large cells (Brasil & Huszar, 2011).

Furthermore, phytoplankton may be affected by an indirect effect of temperature depending on their functional characteristics. With warming, some zooplankton groups will be favoured (see

Ekvall & Hanson, 2012; Nicolle et al., 2012 for copepods), increasing predation pressure on phytoplankton (O'Connor et al., 2009). On the other hand, warming increases vertical stratification (Senerpont Domis et al., 2013) and reduces water viscosity, making it difficult to migrate vertically (Paerl & Huisman, 2009). Thus, characteristics that make predation difficult, such as mucilage (Reynolds, 2007), or structures that allow floating regulation and facilitate movement in the water column (e.g., aerotopes and flagella), may confer advantages in different warming scenarios.

Given the wide spectrum of climate change scenarios (Moss et al., 2010), predicting global warming effects on aquatic ecosystems is still a challenge with a high level of uncertainty (Feuchtmayr et al., 2009). Thus, experimentation using different temperature levels serves as an important tool to help clarify this issue (Mckee et al., 2000; Senerpont Domis et al., 2007). In addition, the experimental procedures allow the warming effects to be isolated from the effects of other variables that could interact with temperature, which occurs in natural environments (e.g., nutrients, conductivity, and salinity; Hoang et al., 2018).

In this study, we developed a microcosm experiment to identify the effects of increased temperature and the time of the experiment on phytoplankton taxonomy (species abundance) and functional composition (abundance of functional groups). We used three scenarios of future warming, ranging from an optimistic forecast (2 °C increase) to an extreme warming scenario (8 °C increase), and sampled the microcosms after different time periods. We expect that there will be differences in species abundance and functional groups among the different global warming scenarios. We hypothesized the higher abundance of species already adapted to high temperature conditions, such as cyanobacteria, and a greater abundance of functional groups represented by species with small cell sizes and generalists in terms of their habitat needs. In addition, over time, we expected a homogenization of communities in warmer environments since species poorly adapted to high temperatures can be lost, while those with rapid growth can be favoured.

Methods

Microcosm

Each microcosm was a glass rectangular aquarium that was 29 cm high, 20 cm wide, and 45 cm long and had a capacity of 25 L of water (see Figure 1 in Online Resource 1). An electric heater coupled to a thermostat (aquarium heater, Roxin HT 1300 - 25 Watts model) was positioned on the sidewall of each aquarium and was used to warm the experimental units (e.g., Flury et al., 2010; Burgmer & Hillebrand, 2011). A water circulation pump (model JAD SP 500 - 150 L / h) was placed in the bottom of the aquaria to avoid species sedimentation (e.g., Flury et al., 2010; Sommer et al., 2015). During the entire experiment, the heaters and circulation pumps remained connected to electricity. To avoid the entry of dirt or other residues that could interfere with the results, the aquaria were covered with transparent nylon screens. The microcosms were placed outdoors for exposure to natural light and environmental conditions. During the experiment, the mean air temperature was 23.6 °C, with a minimum value of 21 °C and a maximum value of 27 °C. Considering that light could enter the water column through the sidewalls of the aquaria, which does not occur in a natural aquatic environments, a black nylon screen was placed around the experimental site to reduce the light intensity.

Experimental design

The experiment was conducted in April 2016 at the Goiás State University (UEG) campus, Anápolis, Goiás, Brazil (16°22’52.86” S e 48°56’45.43” W). For Goiás State, located in the Brazilian Cerrado, this period represents a transition from the rainy to the dry season (Simegho, 2017). Twenty microcosms were randomly distributed among four treatments (Figure 1). The control treatment corresponded to the mean annual temperature for the Cerrado region where the experiment was performed. This estimate was obtained from the Meteorology and Hydrology System of Goiás State (Simegho, 2017).

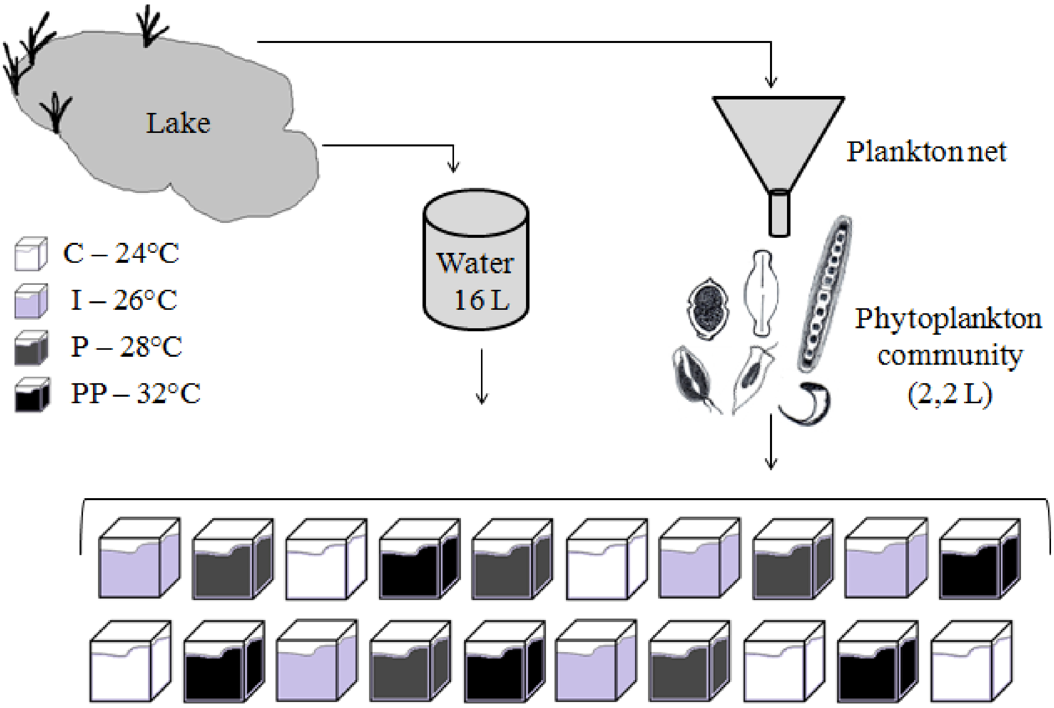

Fig. 1 Experimental design to evaluate the effects of temperature warming and time on phytoplankton communities. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

The temperature increase was established according to the forecasts proposed by the Atmosphere Ocean Global Circulation Model (AOGCM) – Community Climate System Model (CCSM), available in the Ecoclimate database (Lima-Ribeiro et al., 2015). This model has four future warming scenarios based on the radiative forcing levels predicted for the year 2100 (Representative Concentration Pathways - RCP). The RCP 2.6 scenario is considered optimistic, RCP 4.5 and RCP 6.0 are considered intermediate, while the RCP 8.5 scenario is considered pessimist (Moss et al., 2008).

Due to the uncertainties of future temperature trends, three warming scenarios were used. Thus, the experiment was composed of five replicates of the following treatments: i. Control (C - 24 °C): represents the current annual mean temperature for the region where the experiment was constructed; ii. Intermediate (I - 26 °C): represents the mean of the temperature increases predicted in the optimistic and intermediate scenarios, which are equivalent to approximately 2 °C; iii.

Pessimist (P - 28 °C): represents a pessimistic global warming scenario, with an increase of 4 °C in relation to the current mean temperature; and iv. Pessimist + Pessimist (PP - 32 °C): indicates an extreme situation of warming based on a double pessimistic scenario, with an increase of 8 °C in relation to the current mean temperature. These values correspond to the temperature established for the beginning of the experiment. The temperature was manipulated in all treatments using the electric heaters, including the control. Throughout the experiment, following the oscillations in the weather (i.e., sunny or rainy days), the treatment temperatures were an average of 3 °C higher or lower than the previously established values (i.e., 24 °C, 26 °C, 28 °C and 32 °C). Temperature oscillations around previously established values have already been observed in previous experimental studies (e.g. McKee et al., 2003; Flury et al., 2010). However, in all the measurements, the warming treatments maintained higher temperatures than the control (see Figure 2 in Online Resource 1).

There is large variation in the duration time for experiments involving phytoplankton, including days (e.g., Gonzáles et al., 2013), weeks (e.g., Burgmer & Hillebrand, 2011) or even years (e.g., Moss et al., 2003). However, it is important to consider the lifetime of the organisms (Yodzis, 1988). Phytoplankton have a short life cycle, and it is possible to follow numerous generations in a few days. In fact, in a pilot experiment carried out in January 2016 (see more details in additional information topic in Online Resource 1), we verified that changes in the composition of phytoplankton communities cease to occur on the 15th day of the experiment (see Figure 6 in Online Resource 1). For this reason, the experiment was three weeks in duration (20 days).

Each microcosm was filled with 16 L of water collected from an oligo-mesotrophic lake located on the UEG Campus. The mean water temperature recorded for the lake at the time of collection was 24.5 °C. Although pre-filtering of samples to remove zooplankton cells is a common procedure in microcosm studies involving phytoplankton (e.g., Carter et al., 2005; Larson & Belovsky, 2013), this strategy may cause failures since it also removes large phytoplankton cells (greater than 100 µm) from the samples, changing the species composition (Nogueira et al., 2014). Thus, in this experiment, collection of water to fill the aquaria was performed without prior filtration.

The phytoplankton species were collected from the same lake using a plankton net with a mesh of 20 µm. This strategy was used to maximize the collection of less abundant species and to ensure that most of the taxonomic groups were represented in the experimental units. The concentrated sample was distributed in equal volumes (2.2 L) between the experimental units. Thus, at the beginning of the experiment, each microcosm had 18.2 L of water (16 L of water collected without filtration and 2.2 L of water resulting from the net filtration).

To avoid nutrient depletion, we added 5.40 mg L^{-1} of sodium nitrate ($NaNO_3$) and 0.34 mg L^{-1} of potassium phosphate (KH_2PO_4), which represent respectively, 0.2 mg L^{-1} of nitrate (NO_3^-) and 0.002 mg L^{-1} of phosphate (PO_4^{-3}). Since the phytoplankton community was concentrated through collection using the plankton net, the amount of nutrients added was established based on the natural concentrations of nitrate and orthophosphate in the lake and weighted by the abundance of individuals. That is, the abundance of individuals in a concentrated sample and in a non-concentrated sample was calculated. We found that there were twice as many individuals in the concentrated sample as in the non-concentrated sample. Thus, considering the Redfield proportion of 1:16 (Reynolds, 2006), we added twice as many nutrients as the amount originally present in the lake. We adopted this strategy, as the phytoplankton community was collected through the plankton net to fill the microcosms. This technique allowed a large representation of all taxonomic groups but concentrated our community into a larger number of cells than originally occurred in the lake. Thus, the addition of nutrients was performed every four days to avoid depletion. On the 10th day of the experiment, 500 mL of deionized water was also added to each aquarium to compensate for losses by evaporation (e.g., McKee et al., 2000; Ekvall & Hansson, 2012).

After filling (04 April 2016), the microcosms were heated for 48 hours until they all reached the previously determined temperatures based on the treatment type. Changes in temperature can affect the physiology of organisms even in a short time (see Staerh & BiKerland, 2006 for changes in 1-2 hours). Thus, the experiment count started on April 6, 2016 – day zero. During the experiment, a heater related to treatment C malfunctioned. Thus, this replicate was excluded from all subsequent analyses.

Limnological variables

Using the Manta 2 Eureka multiparameter probe, the chlorophyll-a concentrations, dissolved oxygen, conductivity and pH were measured daily (totalling twenty sampling days). At the final time of the experiment, concentrations of nitrate, nitrite, total nitrogen, orthophosphate and total phosphorus were measured in 500 mL samples from all microcosms to show that there were no differences in nutrient concentrations between temperature treatments. The analysis of the samples for nutrients was carried out following the methods described in Golterman et al. (1978).

Phytoplankton

Phytoplankton samples were collected from the water surface layers using dark bottles (100 mL) and fixed with modified acetic acid (Vollenweider, 1974). Sampling was performed every four days, totalling six collection times (T1 = day 0, T2 = day 4, T3 = day 8, T4 = day 12, T5 = day 16, T6 = day 20). In addition to the movement of water through the circulation pump, before each collection, the water was manually homogenized to avoid species loss by sedimentation. The counting of individuals was conducted using the sedimentation technique (Uthermöl, 1958) with an inverted microscope at a magnification of 400x. Individuals (cells, filaments and colonies) were counted in at least 100 random fields until no more species were added (Bellinger & Sigee, 2010). Identification was performed to the lowest possible taxonomic level, and the abundance was expressed as individuals per mL (ind mL^{-1}). The phytoplankton species were then distributed into functional groups according to the classifications proposed by Reynolds et al. (2002) and adapted by Padisák et al. (2009) and Kruk et al. (2010). Thus, the abundance of each functional group refers to the sum of the abundances of all species classified within the group for a given treatment.

Data analysis

To ensure that there were no differences in nutrient concentrations between treatments, we performed analysis of variance for nitrate, nitrite, total nitrogen and total phosphorus. To evaluate the effects of temperature and sampling time on chlorophyll-a, dissolved oxygen, conductivity and pH concentrations, a factorial variance analysis for repeated measurements (ANOVA, see Zar, 2010) was used. In each ANOVA, the limnological variables measured daily represented the dependent variables, while the temperature (4 levels) and sampling time (20 levels) were the factors. Values were log-transformed (log x + 1) to satisfy the normality assumptions and variance homogeneity. The significance level was established according to the Bonferroni criterion for multiple tests (α/tests number, see Legendre & Legendre, 1998); thus, P values < 0.0125 were considered significant. When significant differences were found, Tukey's test was performed to verify for which treatments and time periods the differences occurred.

We used the Berger Park dominance index (Berger & Park, 1974) to assess the existence of taxonomic or functional groups dominant in the different treatments. The Berger Park index (BP) is calculated by the equation $BP = ni/N$, where ni represents the abundance of a taxonomic or functional group in the sample and N is the total abundance of the sample. The dominance was estimated for each sampling time and treatment separately. The dominance values range from 0 to 1, and the closer a value is to 1, the greater the taxon dominance. We also calculated Shannon diversity (H´) as a measure of community evenness using the abundance of species and functional groups among different treatments and sampling times.

A factorial analysis of variance for repeated measures was also used to verify whether there were differences in phytoplankton species richness related to the treatment and sampling time. Here, time was represented by the phytoplankton sampling periods, which were performed every 4 days. In this analysis, species richness represented the dependent variable, while the treatment type (4 levels) and sampling time (6 levels) were the categorical factors. We also used a factorial ANOVA for repeated measures to assess differences in Shannon diversity between different treatments and sampling time for both species composition and the functional groups MBFG and

RFG. According to the Bonferroni criterion, ANOVA values of $P < 0.05$ were considered significant for richness, and ANOVA values of $P < 0.016$ were considered significant for Shannon diversity. ANOVAs were calculated using the Vegan package (Oksanen et al., 2017) in program R (Core Team R, 2016).

To evaluate the temperature and sampling time effects on the abundance of individuals by species and by functional groups RFG and MBFG, analysis of permutational variance (PERMANOVA, see Zar, 2010) was used. In this analysis, the taxonomic and functional data acted as the response variables, while the treatments (4 levels) and sampling time (6 levels) were the factors. Using matrices with the abundance of individuals by species, the MBFG and RFG groups were transformed into Bray Curtis dissimilarity matrices for PERMANOVA. PERMANOVA was performed for each biological level (i.e., species, MBFG and RFG). In cases where significant PERMANOVA results were observed (P values < 0.016 according to the Bonferroni criterion), the data were submitted to principal coordinate analysis (PCoA, see Legendre & Legendre, 1998) to identify for which treatments or time periods these differences occurred. We also used Bray Curtis dissimilarity for PCoA. In both analyses, the data were logarithmized (log x +1). PERMANOVA and PCoA were performed using the Vegan package (Oksanen et al., 2017) in program R (Core Team R, 2016). All figures were constructed using the Statistica software (Version Statistica Single User, License: 134-125-134).

Results

Limnological variables

We did not find significant differences for nutrient concentrations, which ensured that these conditions remained controlled between treatments (Figure 2). Only the sampling time caused differences in pH, while the interaction between the temperature and sampling time was important to explain the differences in conductivity, dissolved oxygen and chlorophyll-a concentrations (Table 1). On average, the chlorophyll-a concentrations were different within each treatment and increased over time when comparing the initial and final days of the experiment (see Table 1 in Online

Resource 2). Treatment C, for example, showed a mean increase of 57.5 µg L^{-1} chlorophyll-a compared to the beginning of the experiment, while treatment I showed a mean increase of 67.6 µg L^{-1}; treatment P, 57.4 µg L^{-1}; and treatment PP, 67.9 µg L^{-1}. We also observed significant differences between the treatments C & I, C & P, C & PP, I & P, I & PP and P & PP from the 10th sampling day (see Table 1 in Online Resource 2 and Figure 3). Treatment C presented lower concentrations of chlorophyll-a in relation to treatments I, P and PP; treatment I presented lower concentrations of chlorophyll-a in relation to treatments P (except between days 16 and 20) and PP; and treatment P showed less chlorophyll-a in relation to treatment PP in all sampling time (Figure 3).

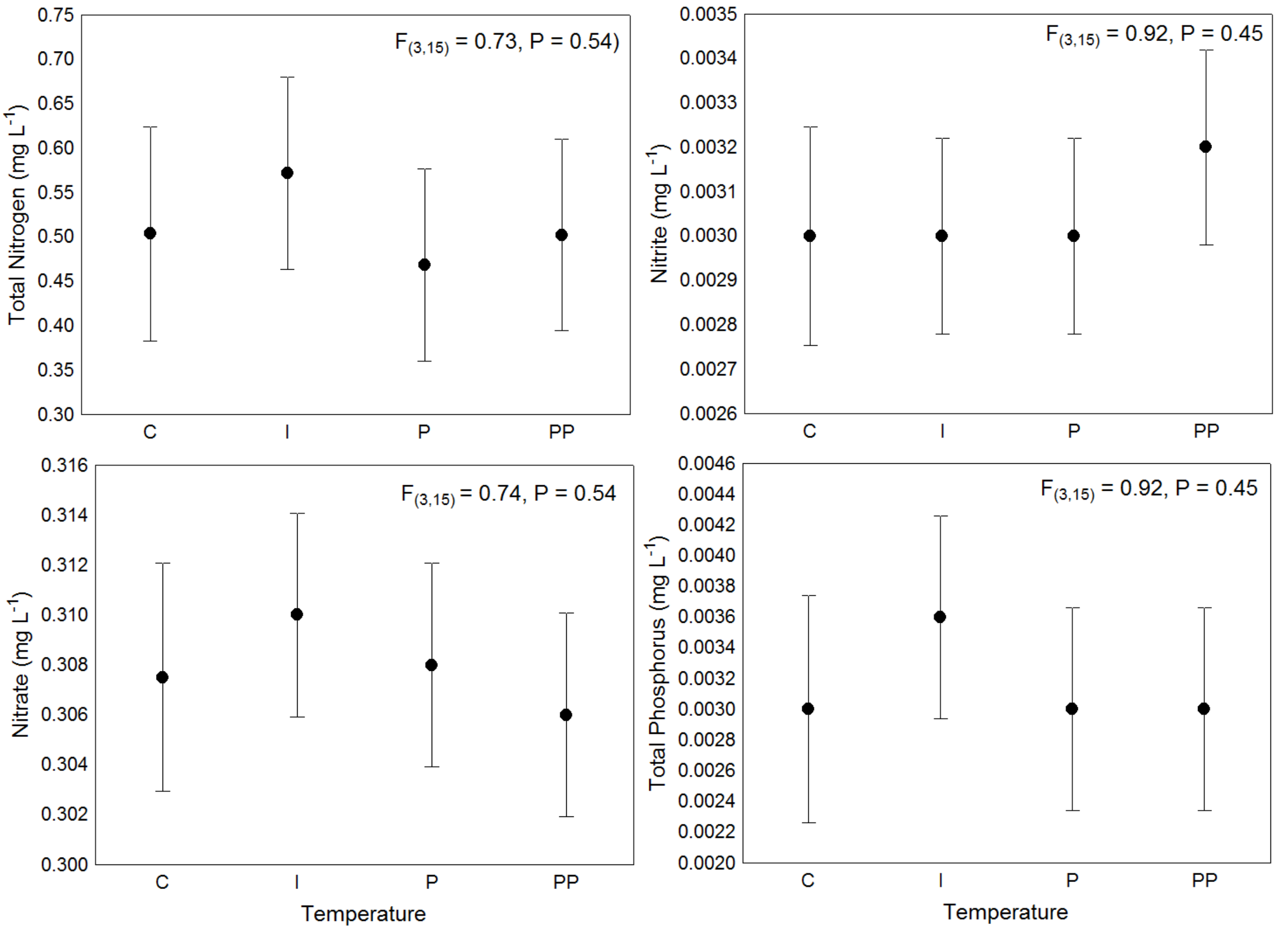

Fig. 2 Analysis of variance for nutrient concentrations in the four treatments of temperature obtained at the final time of the experiment. The non-significant result ($P>0.05$) indicated that nutrient concentrations were similar among treatments. The orthophosphate concentrations were equal between replicates of all treatments (< 0.003 mg L^{-1}). Thus, it was not possible to perform

analysis of variance. The points indicate the mean and the vertical bars the 95% confidence interval. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature. For comparisons between the initial and final nutrient concentration, see the additional information in Online Resource 1

Table 1 F and *P* values for the factorial analysis of variance for repeated measures performed between the limnological variables, treatment and sampling time. Values of $P < 0.0125$ were considered significant (see methods for Bonferroni criterion for multiple tests). DF = degrees of freedom, pH = potential of hydrogen, OD = dissolved oxygen

	Temperature			Time			Temperature x Time		
	F	*P*	DF	F	*P*	DF	F	*P*	DF
Chlorophyll - a	0.92	0.06	3	425.36	**<0.001**	20	2.02	**<0.001**	60
Conductivity	0.80	0.51	3	460.53	**<0.001**	20	3.89	**<0.001**	60
OD	30.2	**<0.001**	3	51.6	**<0.001**	20	2.7	**<0.001**	60
pH	0.38	0.77	3	59.27	**<0.001**	20	1.34	0.06	60

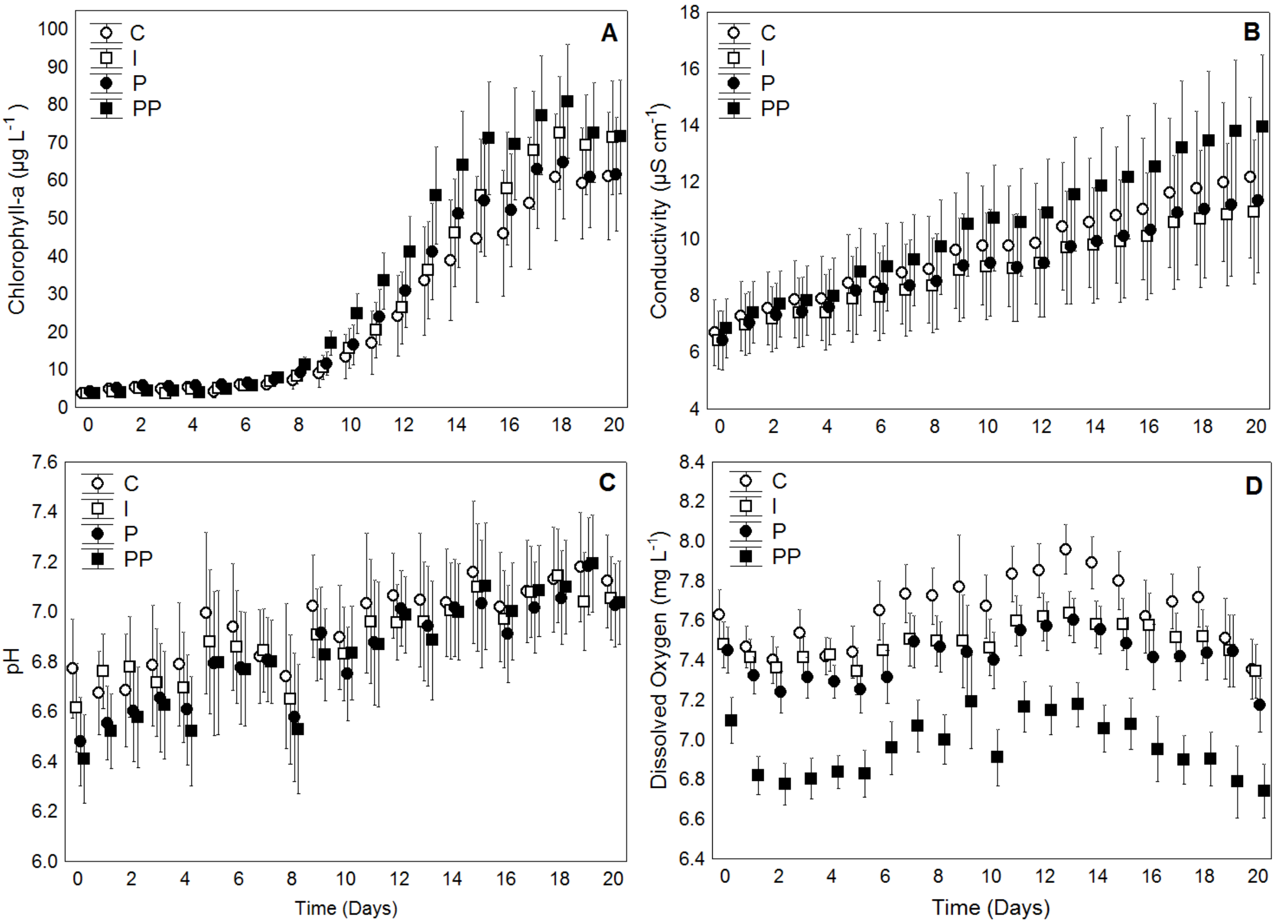

Fig. 3 Mean values observed for chlorophyll-a (A), conductivity (B), pH (C) and dissolved oxygen (D) among treatments over the 20-day experiment. Vertical bars indicate a 95% confidence interval

For conductivity, differences occurred mainly among treatments, with a small increase in their values over time (Figure 3). For treatment C, we observed a mean increase of 5.47 µs cm^{-1} between the first and last day of the experiment. The increase was 4.52 µs cm^{-1} in treatment I and 4.94 µs cm^{-1} in treatment P, while for PP, there was an increase 7.1 µs cm^{-1}. The differences between the temperatures were less evident and occurred in only some time periods for treatments C & PP and I & PP (Figure 3, Table 2 in Online Resource 2). However, treatment PP presented higher conductivity than C and I in all time periods for which significant differences were found. The dissolved oxygen concentrations were lower in the warming treatments compared to the control. Since the initial sampling days, differences between treatments C & PP, I & PP and P & PP were observed, and these differences persisted until the final time of the experiment (Figure 3, Table 3 in Online Resource 3). In general, the PP treatment had a mean dissolved oxygen value 2 mg L^{-1} less than that of C, I and P. The pH values did not differ between the treatments but showed small changes over time (Figure 3, Table 4 in Online Resource 2). Despite oscillations, the pH remained close to neutral for all the sampling time.

Phytoplankton

We found a total of 81 phytoplankton species, with representatives of the classes Zygnemaphyceae (22), Chlorophyceae (20), Bacillariophyceae (20), Cyanophyceae (7), Euglenophyceae (5), Cryptophyceae (4), Dinophyceae (2) and Chrysophyceae (1). Species richness was distinct among the six sampling time (Figure 4), but no significant temperature effects were observed (Temperature: $F_{(3)} = 1.78$, $P = 0.19$; Time: $F_{(5)} = 6.42$, $P<0.001$; Time x Temperature: $F_{(15)} = 1,27$, $P = 0.23$). Shannon diversity for species and functional groups decreased over time in all treatments (Figure 5). We found no significant temperature effects for Shannon diversity estimated for the species, although sampling time was an important factor (Table 2). For the MBFG functional groups, the temperature and sampling time interacted to explain the differences in Shannon diversity (Table 2); however, for the RFG groups, only sampling time was important (Table 2).

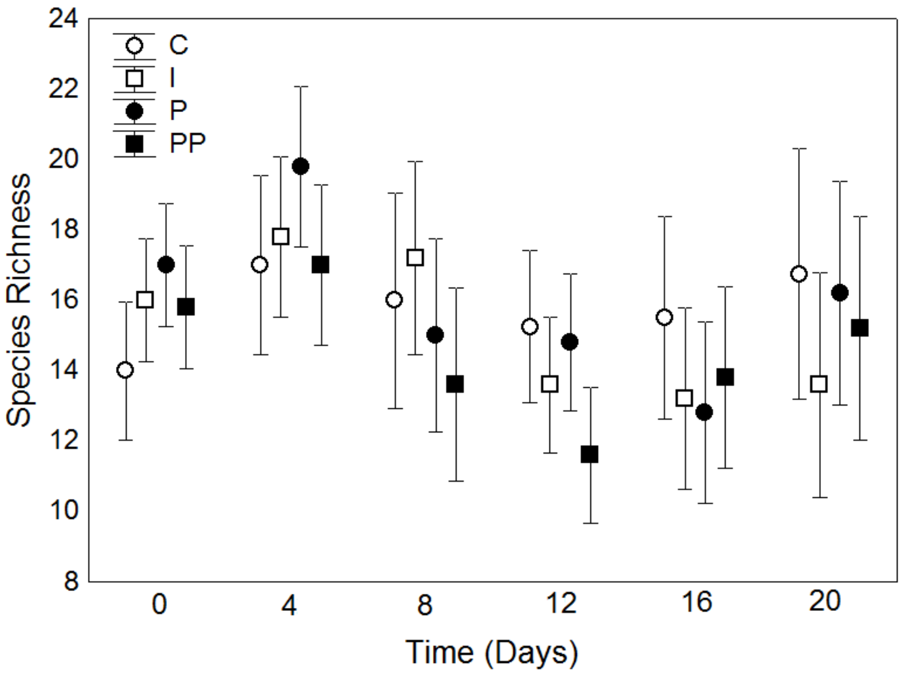

Fig. 4 Phytoplankton species richness (mean) over time between different treatments. Vertical bars indicate a 95% confidence interval. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

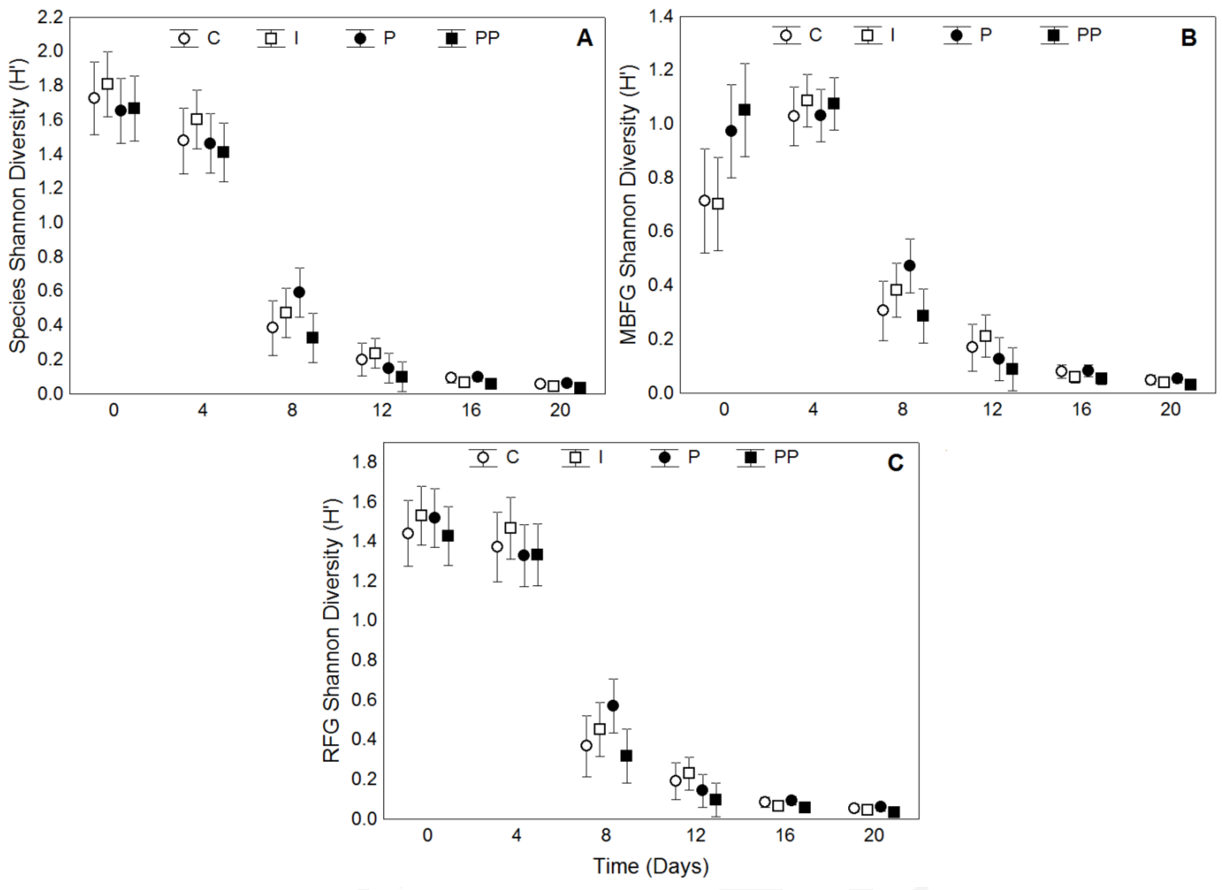

Fig. 5 Shannon diversity index for species (A), morphologically based functional groups (B) and Reynolds functional groups (C) between the treatments and sampling time. Vertical bars indicate a 95% confidence interval. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

Table 2 F and P values for the factorial analysis of variance for repeated measures performed between the Shannon diversity (H'), treatment and sampling time. Values of $P < 0.016$ were considered significant (see methods for Bonferroni criterion for multiple tests). RFG = Reynolds functional groups, MBFG = morphologically based functional groups, DF = Degrees of freedom

	Temperature			Time			Temperature x Time		
	F	P	DF	F	P	DF	F	P	DF
H’ Species	3.26	0.05	3	610.83	0.001	5	0.99	0.47	15
H’ RFG	2.85	0.07	3	634.23	0.001	5	1.02	0.44	15
H’ MBFG	1.78	0.19	3	352.84	0.001	5	3.69	0.001	15

The abundance of species varied between treatments and sampling time (Table 5 in Online Resource 2). We found 19 RFG groups and representatives of the seven MBFG functional groups

(Table 3). The temperature, sampling time and interaction between these two factors were statistically significant in explaining the variation in the abundance of species and functional groups (Table 4). The communities showed rare species found in only some sampling periods as well as generalist taxa present in all treatments. Among rare taxa, species of Bacillariophyceae and Chlorophyceae, such as *Encyonema* sp., *Gomphonema* sp., *Navicula* sp., *Nupela* sp., *Ankistrodesmus tortus* Komárek & Comas González, *Coelastrum astroideum* De Notaris, *Eudorina illinoisensis* (Kofoid) Pascher, and *Westella botryoides* (West) De Wildeman, occurred in the lower warming treatments, C and I (See Table 5 in Online Resource 2). Although species of Cyanobacteria, such as *Synechococcus* sp., was found in all treatments, the more individuals occurred in the P and PP temperatures for most of the sampling time (see Table 5 in Online Resource 2).

Table 3 List of morphologically based functional groups (MBFG) and Reynolds functional groups (RFG) found in the experiment

Functional Groups	Some representative species found in our experiment
MBFG	
I	*Romeria gracilis*, *Synechococcus* sp.
II	*Dinobryon sertularia* Ehrenberg
III	*Limnothrix mirabilis* (Böcher) Anagnostidis
IV	*Scenedesmus* sp., *Closterium* sp.
V	*Cryptomonas* sp., *Peridinium* sp., *Euglena* sp.
VI	*Aulacoseira* sp., *Eunotia* sp. , *Navicula* sp.
VII	*Dictyosphaerium pulchellum* H. C. Wood, *Chroococcus minutus* (Kützing) Nägeli
RFG	
B	*Aulacoseira* sp., *Sellaphora* sp.
D	*Encyonema* sp., *Nitzschia* sp.
E	*Dinobryon sertularia* Ehrenberg
F	*Botryococcus sudetica* Lemmerman, *Westella botryoides* (West) De Wildeman
G	*Eudorina illinoensis* Ehrenberg
J	*Coelastrum astroideum* De Notaris, *Scenedesmus armatus* (Chodat) Chodat
K	*Synechococcus* sp.
Lo	*Chroococcus minutus* Kützing (Nägeli), *Peridinium volzii* Lemmermann
MP	*Encyonopsis* sp., *Gomphonema* sp.
N_A	*Cosmarium depressum* Bailey, *Staurodesmus dejectus* (Brébisson) Teiling
P	*Closterium* sp.
R	*Romeria gracilis* (Koczwara) Koczwara
S1	*Limnothrix mirabilis* (Böcher) Anagnostidis, *Pseudanabaena limnetica* (Lemmermann) Kómarek
Tb	*Eunotia* sp., *Frustulia* sp.

Td	*Pleurotaenium minutum* (Ralfs) Hilse, *Gonatozygon monotaenium* De Bary
W1	*Phacus pleuronectes* (O.F.Müller) Nitzsch ex Dujardin, *Euglena* sp.
W2	*Trachelomonas hispida* (Perty) F.Stein
X1	*Monoraphidium* sp.
Y	*Cryptomonas* sp.

Table 4 F and *P* values for the analysis of permutational variance performed between species density, Reynolds functional groups (RFG), morphologically based functional groups (MBFG), temperature treatment and sampling time. Values of $P < 0.016$ were considered significant (see methods for Bonferroni criterion for multiple tests). DF = Degrees of freedom

	Temperature				Time				Temperature x Time			
	F	R^2	*P*	DF	F	R^2	*P*	DF	F	R^2	*P*	DF
Species	3.75	0.04	**0.001**	3	29.9	0.53	**0.001**	5	1.9	0.10	**0.001**	15
RFG	3.62	0.03	**0.001**	3	42.1	0.60	**0.001**	5	2.4	0.10	**0.001**	15
MBFG	4.0	0.02	**0.002**	3	55.8	0.65	**0.001**	5	3.1	0.11	**0.001**	15

At the beginning of the experiment (approximately 48 hours after the microcosms were installed and the temperature stabilized, day zero), it was possible to observe a clear separation between treatments C, I, P and PP (Figure 7A) and the initial sample used to fill the microcosms (Figure 3 in Online Resource 1). The same species presents in the lake (initial sample) occurred in all treatments and the differences between these were in relation to the number of individuals (Table 5 in Online Resource 2). All treatments showed a mean higher abundance for the Chlorophyceae (C = 551 ind mL^{-1}, I = 671 ind mL^{-1}, P = 1,181 ind mL^{-1}, PP = 1,089 ind mL^{-1}) and Dinophyceae classes (C = 82 ind mL^{-1}, I = 138 ind mL^{-1}, P = 709 ind mL^{-1}, PP = 1,222 ind mL^{-1}), but treatments P and PP showed a greater number of individuals (Figure 8). During the first sampling time, species of the class Chlorophyceae, namely, *Tetraedron minimum* (A. Braun) Hansgirg and *Treubaria triappendiculata* C. Bernard, were dominant in treatments C (BP = 0.47 and 0.14, respectively) and I (BP = 0.37 and 0.27, respectively), while species of Cyanophyceae, namely, *Synechococcus* sp. (BP = 0.35), and of Dinophyceae, namely, *Peridinium volzii* Lemmermann (BP = 0.20), were dominant in the P treatment. For the PP treatment, *Tetraedron minimum* (PB = 0.20) and *Peridinium volzii* (BP = 0.37, see also Figure 6 and Table 1 in Online Resource 1) were dominant.

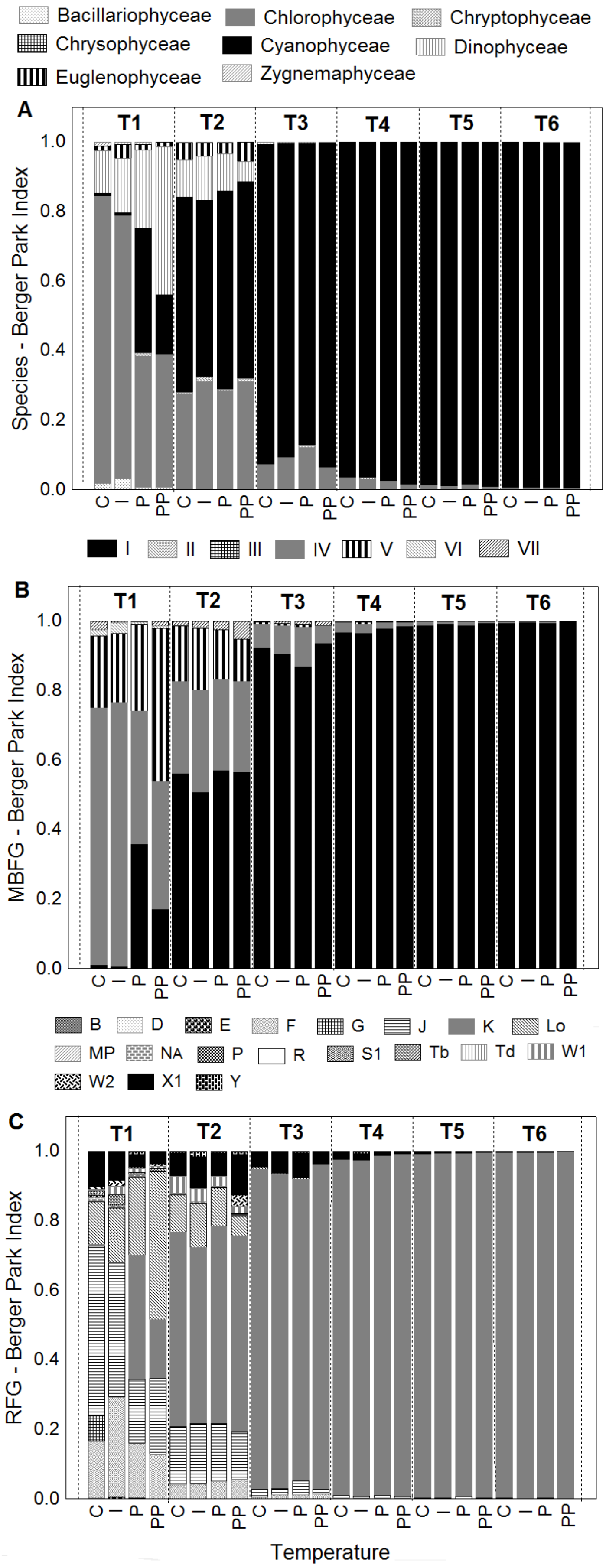
Bacillariophyceae
Chlorophyceae
Chryptophyceae
Chrysophyceae
Cyanophyceae
Dinophyceae
Euglenophyceae
Zygnemaphyceae
A
T1
T2
T3
T4
T5
T6
Species - Berger Park Index
1.0
0.8
0.6
0.4
0.2
0.0
C I P PP C I P PP C I P PP C I P PP C I P PP C I P PP
I
II
III
IV
V
VI
VII
B
MBFG - Berger Park Index
B
D
E
F
G
J
K
Lo
MP
NA
P
R
S1
Tb
Td
W1
W2
X1
Y
C
RFG - Berger Park Index
Temperature

Fig. 6 Berger Parker dominance index (BG) for taxonomic class (A), morphologically based functional groups (B) and Reynolds functional groups (C) between the treatments and sampling time. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature. T1 = 0 day; T2 = 4th day; T3 = 8th day; T4 = 12th day; T5 = 16th day; T6 = 20th day

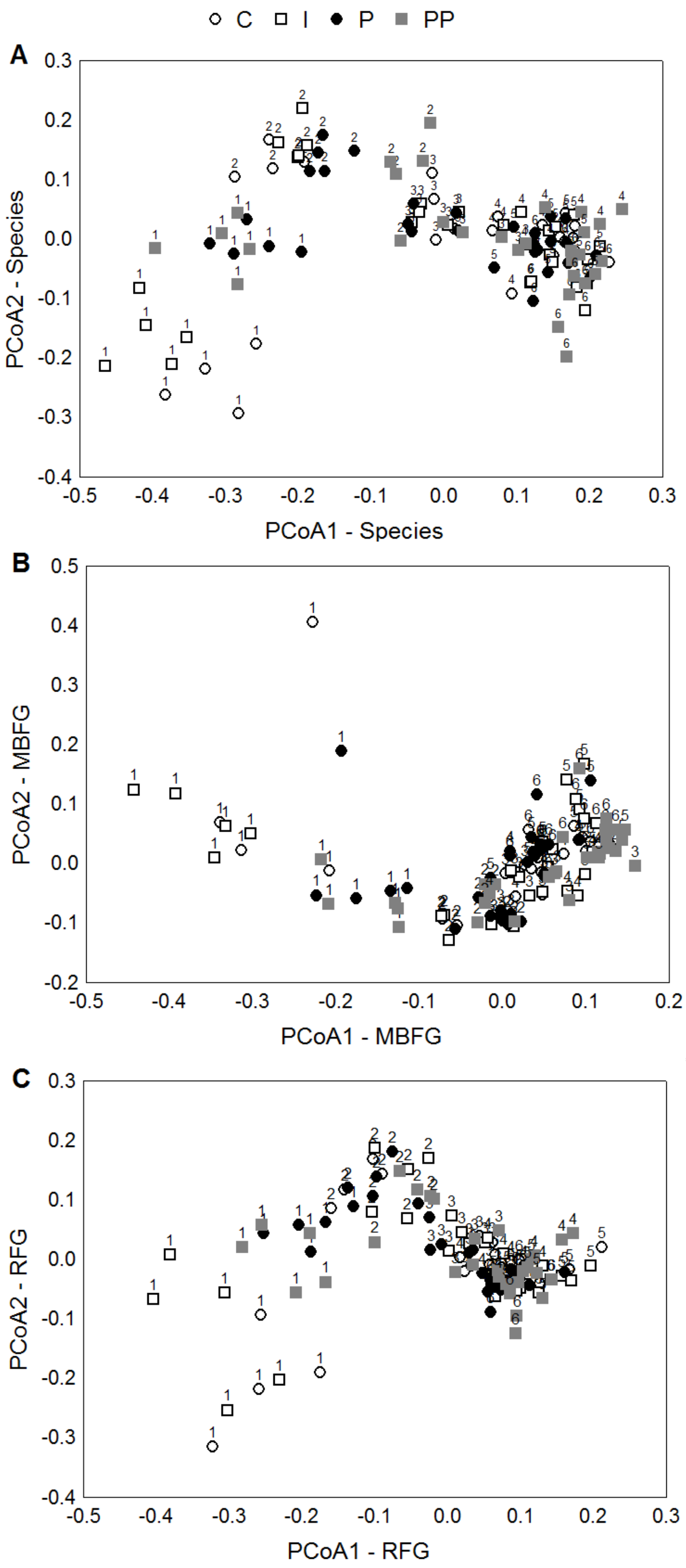
○ C □ I ● P ■ PP
A
PCoA2 - Species
PCoA1 - Species
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
B
PCoA2 - MBFG
PCoA1 - MBFG
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
C
PCoA2 - RFG
PCoA1 - RFG
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

Fig. 7 Principal Coordinate Analysis (PCoA) for phytoplankton communities between treatments and sampling time. "A" represents the variation in species abundance; "B" the variation in morphologically based functional groups (MBFG) and "C" the variation in Reynolds functional groups (RFG). 1 = 0th day (approximately 48 hours after the microcosms were installed and the temperature stabilized); 2 = 4th day; 3 = 8th day; 4 = 12th day; 5 = 16th day; 6 = 20th day. Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

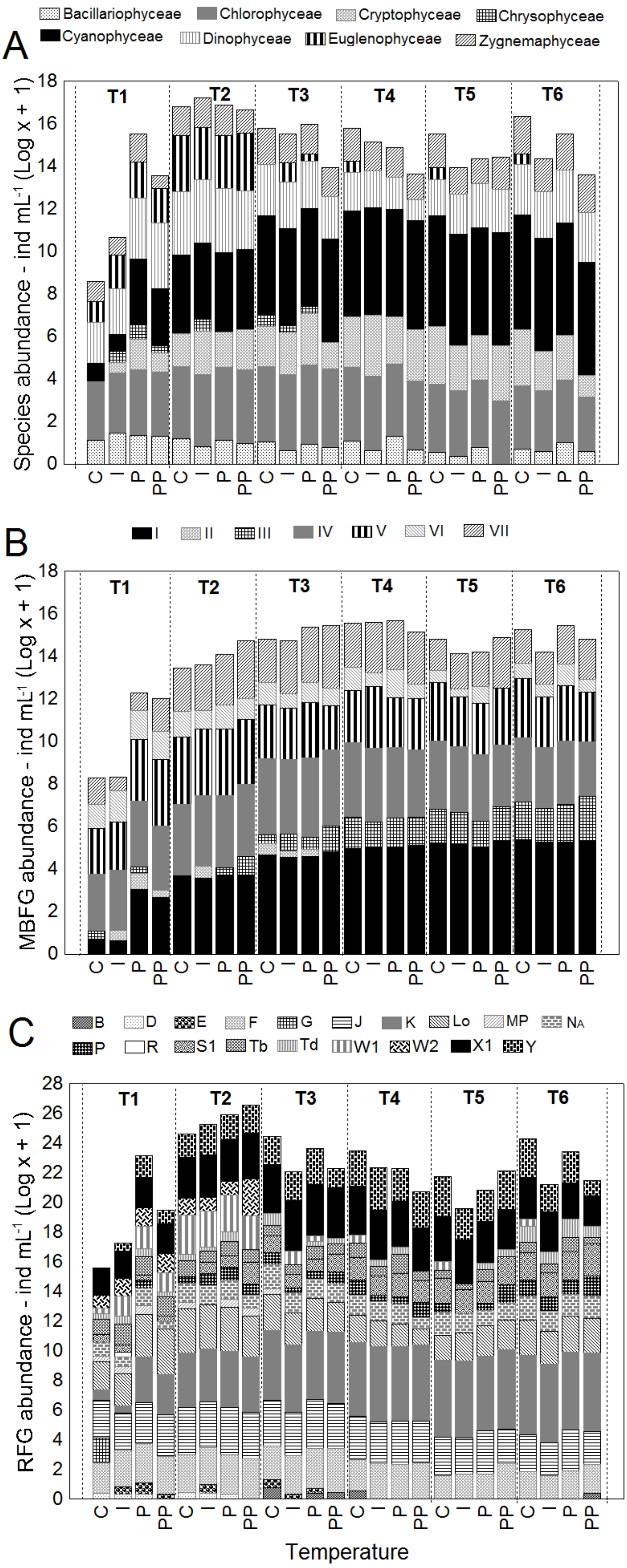
A
Bacillariophyceae
Chlorophyceae
Cryptophyceae
Chrysophyceae
Cyanophyceae
Dinophyceae
Euglenophyceae
Zygnemaphyceae
Species abundance - ind mL-1 (Log x + 1)
0
2
4
6
8
10
12
14
16
18
T1
T2
T3
T4
T5
T6
C
I
P
PP
B
I
II
III
IV
V
VI
VII
MBFG abundance - ind mL-1 (Log x + 1)
C
B
D
E
F
G
J
K
Lo
MP
NA
P
R
S1
Tb
Td
W1
W2
X1
Y
RFG abundance - ind mL-1 (Log x + 1)
20
22
24
26
28
Temperature

Fig. 8 Mean species abundance by taxonomic class (A), morphologically based functional groups (B) and Reynolds functional groups (C) according to temperature and sampling time. T indicates the experiment period in which the sampling was performed, as follows: T1 = 0 day (approximately 48 hours after the microcosms were installed and the temperature stabilized); T2 = 4th day; T3 = 8th day; T4 = 12th day; T5 = 16th day; T6 = 20th day). C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature. To visualize the abundance values as a percentage, please see Figure 5 in Online Resource 1

From the second period (fourth day), this distinction occurred between temperatures C and I and more pessimist scenarios (P and PP, Figure 7A). In this period, Cyanophyceae showed an increase in the number of individuals in all treatments (Time 1 – C = 7 ind mL^{-1}, I = 5 ind mL^{-1}, P = 1,124 ind mL^{-1}, PP = 488 ind mL^{-1}; Time 2 – C = 4,895 ind mL^{-1}, I = 3, 774 ind mL^{-1}, P = 5,448 ind mL^{-1}, PP = 5,395 ind mL^{-1}, see also Figure 8A) and became a dominant group in the communities (BP *Synechococcus* sp. > 0.50), together with Chlorophyceae (BP *Tetraedron minimum* > 0.13) and Dinophyceae (BP *Peridinium volzii* > 0.05). On the other hand, compared to the previous sampling time (Figure 8A), the number of individuals in the Bacillariophyceae class was lower in the warming treatments (Time 1 – C = 12 ind mL^{-1}, I = 28 ind mL^{-1}, P = 22 ind mL^{-1}, PP = 20 ind mL^{-1}; Time 2 – C = 15 ind mL^{-1}, I = 6 ind mL^{-1}, P = 12 ind mL^{-1}, PP = 9 ind mL^{-1}).

From the third sampling time (8th day), a distinction was observed only between the PP treatment and the others. Species of Cyanophyceae and Chlorophyceae were predominant in all treatments (see figure 6 and Table 1 in Online Resource 1), and over time, Cyanophyceae increased and Chlorophyceae decreased in abundance (Figure 8A). From the fourth sampling time, despite the dominance of *Synechococcus* sp. (Cyanophyceae) in all treatments (BP index > 0.96, see Table 1 in Online Resource 1), the main difference between them was the number of individuals. On average, P and PP temperatures presented the highest number of individuals of Cyanophyceae species since the beginning of the experiment (Figure 8A). From the fourth collection period, representatives of the Euglenophyceae class were found at only treatment C (Figure 8A). Similarly, Bacillariophyceae species were found less frequently in the extreme PP treatment (Figure 8A).

Similar patterns of variation between the treatments and sampling periods were found for taxonomic as well as functional classification. In the MBFG functional classification, there was a separation between the C, I, P and PP treatments in the first sampling time (Figure 7B). We observed the predominance of groups IV (BP index > 0.36) and V (BP index > 0.19) in all treatments (see Figure 6) but a higher number of individuals in those with higher temperatures (P and PP, Figure 8B). From the second sampling time, group I was dominant (BP index > 0.50, see Figure 6). In the fourth sampling period, group II disappeared in all treatments. On average, group VI was present at a lower abundance in the PP treatment, while group VII had the highest abundance at this temperature (Figure 8B). For the functional RFG classification, a clear separation among treatments C, I, P and PP was found in the first sampling period (Figure 7C). In the first sampling time, we found a predominance of representatives of groups J (BP index > 0.18), Lo (BP index > 0.12) and F (BP index > 0.12). In the second sampling time, groups K and J were predominant (BP index > 0.50 and 0.13, respectively). However, group K had greater dominance and a greater number of individuals until the final time of the experiment (See figure 6 and Figure 8C). The W1 and NA groups were found in higher abundance in treatments C, I and P. In general, the predominant groups for the classification of MBFG and RFG presented a greater number of individuals at temperatures P and PP (Figure 8C).

Discussion

In this study, using an experimental approach that considered different future warming scenarios, we evaluated the effects of temperature and sampling time on taxonomic and functional phytoplankton community composition. In addition, we detected possible effects on some physical and chemical characteristics of the environments where these communities are found. Warming scenarios led to a reduction in dissolved oxygen concentrations, while pH and conductivity remained unchanged. The treatments and time influenced the taxonomic and functional composition of the phytoplankton communities, but the sampling time had a greater effect on richness and evenness. We observed a substitution of species and functional groups that were dominant over time, promoting a homogenization of the communities and the higher abundance of cyanobacteria in all treatments. However, the largest number of individuals in this group was found at higher temperatures (P and PP). Furthermore, rare species and functional groups were detected mainly in the treatments with lower temperatures, and these species and groups are probably responsible for the differentiation in composition between treatments.

Changes in oxygen and chlorophyll-a concentrations due to a temperature increase have already been observed in previous studies, and these changes have produced contrasting results (See McKee et al., 2003 and Feuchtmayr et al., 2009 for oxygen decrease; Hennemann & Petrucio, 2010 for oxygen increase; Jeppesen et al., 2009 and Moura et al., 2017 increase in chlorophyll-a; Feuchtmayr et al., 2009 and Pulina et al., 2016 for a decrease in chlorophyll-a; Mckee et al., 2003 for effects absent on chlorophyll-a). In our study, we found that warming caused a decrease in the quantity of dissolved oxygen and an increase in chlorophyll-a concentrations. The oxygen decrease may be associated with lower solubility at high temperatures or an increase in organisms' metabolic rates because of warming (Esteves, 2011; Diaz & Breitburg, 2009). In fact, studies have indicated that warming intensifies the respiration of planktonic organisms (Yvon-Durocher et al., 2015) and decomposition rates (Geraldes et al., 2012), both of which are processes that involve oxygen consumption. On the other hand, chlorophyll-a concentrations have already been used as an indicator of primary productivity and algal biomass (Carneiro et al., 2014; Santos et al., 2014). Thus, its increase may indicate an increase in the number of individuals in the water column, as was observed in our experiment, with increasing abundance over time. Although previous studies have found a reduction in pH (McKee et al., 2003) and an increase in conductivity in warming scenarios (McKee et al., 2003; Feuchtmayr et al., 2009), in our experiment, the conductivity was higher in the PP treatment in only some temporal periods, while the pH was influenced by only the sampling period, always maintaining values close to neutral.

Our results indicate that different biological classifications responded similarly to changes in temperature. This finding is uunderstandable since many functional groups are defined according to taxonomic groups (e.g., VI: Bacillariophyceae, II: Chrysophyceae; see Kruk et al., 2010; W1: Euglenophyceae; N_A: Zygnemaphyceae; see Padisák et al., 2009). However, the characteristics used for inclusion of individuals in each functional group are not always exclusive to a certain taxonomic class (e.g., mucilage, flagella, mixotrophic habit), and these characteristics may influence the preference of certain species to particular environmental conditions, including temperature (Litchman & Klausmeier, 2008). For example, flagellate species of group Y have already been associated with the available light and mixing regime in water (Becker et al., 2010); species with silica structures, such as groups A and D, have been associated with low transparency and high turbulence (Nabout & Nogueira, 2007); and small species of the K group are associated with high temperatures (Becker et al., 2010).

We found an effect of both the temperature and the sampling time on the taxonomic and functional composition of the phytoplankton communities. In addition, the abundance of communities has become less evenness over time, probably due to the great abundance of cyanobacteria in all treatments. In fact, we observed changes in the dominance pattern over time, with the replacement of Chlorophytes and Dinoflagellates by cyanobacteria, promoting a homogenization of the communities, mainly due to presence of the generalist species *Synechococcus* sp. in all treatments. Thus, although these groups have different temperature sensitivities (Thomas et al., 2016), this does not seem to be an important factor in determining their succession in our experiment. A similar situation was detected in previous studies (e.g., Senerponte Domis et al., 2007, Sommer et al., 2015), in which the taxonomic groups were replaced over time in a similar way at different temperatures. Thus, with adequate nutrient and light conditions, the temperature seems to be less important to determine succession patterns.

Contrary to our expectations, we did not exclusively find any predominance of species or taxonomic classes already adapted to high temperatures in the warming treatments in any of the sampling time. Cyanobacteria higher abundance occurred at all temperatures, including the C treatment. Cyanophyceae are known for their wide distribution and limited need for specialized habitat requirements (Broady & Merican, 2012). This group has a wide range of growth temperatures (Broady & Merican, 2012; Lurling et al., 2013), and under favourable nutrient conditions, temperature seems to be less important for their development (see Moss et al., 2003). In fact, in our experiment, nutrients were added every four days, ensuring that they were not a limiting factor. This addition provided a higher concentration of total nitrogen and nitrate compared to the initial concentrations (see the topic additional information in Online Resource 1). Thus, this group had the opportunity to expand, regardless of temperature. However, the effect of nutrient addition occurred in all treatments. Furthermore, even at the final time of the experiment, after all additions, the concentrations of nutrients were very low, and the trophic status of the aquaria remained oligotrophic, similar to the lake in which the samples were collected. Although Cyanophyceae occurred at all temperatures, the main difference between treatments was associated with a higher abundance of individuals in this group in warmer environments (P and PP). This finding corroborates the results obtained in previous studies, in which species of this group can be favoured in global warming scenarios (e.g., Staehr & Birkeland, 2006; Burgman & Hillebrand, 2011; Yvon-Durocher et al., 2011; Kosten et al., 2012; Rasconi et al., 2017).

Cyanobacteria expansion occurred mainly due to the presence of the species *Synechococcus* sp. (See Figure 4 in Online Resource 1). This unicellular alga (which can be organized in the form of microcolonies) has a small size (picoplankton 0.2 - 2 μm) and high plasticity to adapt to different environmental changes, such as luminosity, temperature, nutrient availability and noxious agents (Callieri, 2017). Their rapid growth caused a change in the number of individuals in the communities, where the predominance of Chlorophyceae and Dinophyceae observed in the first sampling time was changed to a high number of cyanobacteria individuals. In fact, the temperature increase showed a positive effect on the growth of this species (e.g., Fu et al., 2007). This result indicates that the concern with warming should be about not only the growth rates of the different groups, but also the changes in dominance patterns (Kosten et al., 2012; Lurgi et al., 2013) and the blooms that certain species may cause (e.g., Liu et al., 2011; Granéli et al., 2011). This concern is particularly high in the case of toxin-producing species, which can cause serious damage to the health of humans and other animals (Parhel & Otten, 2013 and their references).

Taxonomic groups with thermal characteristics and distinct evolutionary histories may respond differently to global warming (Thomas et al., 2016). Although we did not observe a dominance of species or functional groups being restricted to the warming treatments, rare species belonging to Bacillariophyceae and Euglenophyceae as well as the NA, W1, and VI groups were more commonly observed at the control temperature. It is known that organisms can respond in three different ways to warming: i. dispersion to sites with conditions more suitable to their survival, ii. adaptation to the new temperature conditions using their high phenotypic plasticity, or iii. adaptation through genetic changes via an evolutionary process (Hofmann & Todghan, 2010). As our microcosms were constructed to prevent species dispersion and the experiment time was too short to track genetic adaptation, we can attribute the changes in the composition over time to an alternate composition process. In this process, species with low tolerance to high temperatures probably disappeared, while the growth of those that were well adapted was favoured. Considering that the microcosm constitutes a closed system preventing the entry of new colonizers, the tendency over time is that the communities will become more similar. However, in the more extreme temperatures, we expected that temperature exerted a great effect on homogenization. This probably did not occur due to the presence of species that can adapt and survive in both milder and elevated temperatures, such as *Synechococcus* sp., which higher abundance occurred at different temperatures and time intervals.

Acclimatization processes can occur even in short time periods, altering different metabolic processes of phytoplankton, such as growth rate, respiration and photosynthesis (Staehr & Birkeland, 2006). In situations where the organisms cannot adapt rapidly to new temperature conditions, a stress condition is created in which vital activities continue less efficiently and can lead to death (Fogg, 2001). In fact, the change from a natural lake with a mean temperature of 24.5 °C and low nutrient concentration (e.g. nitrate = 0.1 mg L^{-1} and phosphate = 0.001 mg L^{-1}) to an experimental environment that simulates these conditions, can influence the dynamics of communities. Thus, it is justifiable that shortly after the experiment was started, changes in community composition, both in taxonomic and functional classification, were already observed. Diatoms (see Thomas et al., 2016) and MBFG VI (Segura et al., 2018) have optimal growth rates at lower temperatures compared to other taxonomic or functional groups. Thus, the presence of diatoms (also represented by VI group) preferentially in treatment C and I is in accordance with previous studies that predict a diatom reduction in environments with high temperatures (e.g., Lewandowska & Sommer 2011, Javaheri et al., 2015, but see Anderson, 2000 for a greater effect of other environmental factors). In addition, especially under conditions with limited silicon in the water (Javaheri et al., 2015), warming may exert effects on the morphological patterns of the silica structure. Thus, temperature seems to be an important factor in controlling the abundance of these species as well as the functional groups in which they are classified. Likewise, functional groups W1 and NA were found to be less abundant at PP temperature. The W1 group has as typical representative species of Euglenophyceae that inhabit sites with high organic matter content, whereas NA is composed of many species of Zygnemaphyceae (Padisák et al., 2009). Although Zygnemaphyceae is found in a wide temperature range (Stamenkovic' & Hanelt, 2016), very high temperatures, as represented in PP, may not be the most suitable for their development. Similarly, the high rate of organic matter degradation at elevated temperatures (Geraldes et al., 2012) may be unfavourable for Euglenophyceae.

On the other hand, we observed the predominance of the I and K groups in all treatments, though there were a higher number of individuals in the warmer environments (P and PP). The RFG K group is characterized by unicellular or colonial species of small size that live in shallow and enriched environments (Reynolds et al., 2002; Padisák et al., 2009). The MBFG I group is composed of species with low biovolume (<10 μm^3) and an absence of specialized traits (Kruk et al., 2010). Both groups have typical picoplankton representatives, and their higher abundance in warming microcosms corroborates our initial hypothesis. The patterns reported in the literature further confirm our hypothesis, which is a higher abundance of small species in warming conditions (e.g., Montagnes & Franklin, 2001; Daufresne et al., 2009; Yvon-Durocher et al., 2011).

Alterations in the mean size of communities can occur as a result of an increase in the relative abundance of small species, the substitution of larger species for smaller, or even through the interaction of these two factors (Adams et al., 2013). In our study, we observed an increase in the abundance of small species caused mainly by the predominance of *Synechococcus* sp., classified in groups I and K, while filamentous species with higher biovolume typical of the NA group were disadvantaged. Small species have high growth rates (see Kremer et al., 2017), a higher surface area ratio that gives them greater efficiency in resource acquisition (Litchman & Klausmeier, 2008; Brazil & Huszar, 2011) and a more intense metabolism. These are typical characteristics of R-strategist species (Reznick et al., 2002). Thus, warming caused a higher frequency of species with less specialized habitat requirements.

Knowing the effects of temperature on organisms is an essential step for understanding the observed patterns in communities and ecosystems (Kremer et al., 2017). Thus, the construction of outdoor experiments provides a level of intermediate realism between laboratory experiments and the dynamics of natural environments (Senerpont-Domis et al., 2007; Feuchtmayr et al., 2007). Despite the fact that climate change intensifies stratification (Senerpont Domis et al., 2013) and increases the occurrence of extreme events (e.g., heatwaves, Bertani et al., 2016), our experimental design is limited in answering these questions, since it was not built for this purpose; moreover, in shallow lakes in tropical regions, stratification did not occur in more parts of the years (Esteves, 2011). In addition, we acknowledge that these factors in conjunction with temperature increase may also be important for predicting changes in phytoplankton composition, especially in species with resistance stages. Thus, the results of the present study were conservative because they did not consider these other effects. These factors can be evaluated in future studies, especially for tropical regions, which are still rarely investigated (Roland et al., 2012).

In general, our initial hypotheses were corroborated by the higher abundance of Cyanophyceae (greater number of individuals) in microcosms with higher temperatures (P and PP) and the lower abundance of Bacillariophyceae in extremely warmed treatments. The functional groups with higher abundance at high temperatures were those of small size that had few habitat restrictions. Our results indicate that warming should alter the taxonomic and functional composition of phytoplankton communities, causing species substitution as well as changes in their functional characteristics. This alteration leads to the higher abundance of small organisms. This pattern was observed for both the warming prediction for 2100 (scenario P) and for an extreme warming simulation (PP scenario). Communities composed predominantly of smaller organisms can alter the predation patterns of zooplankton with serious consequences to food webs (Lewandowska & Sommer, 2010). Considering that each species exerts a function in ecosystems, our results contribute to predicting how the temperature increase should alter dominance patterns, homogenization and community dynamics in future warming scenarios.

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ELETRONIC SUPPLEMENTARY MATERIAL I – ESM I

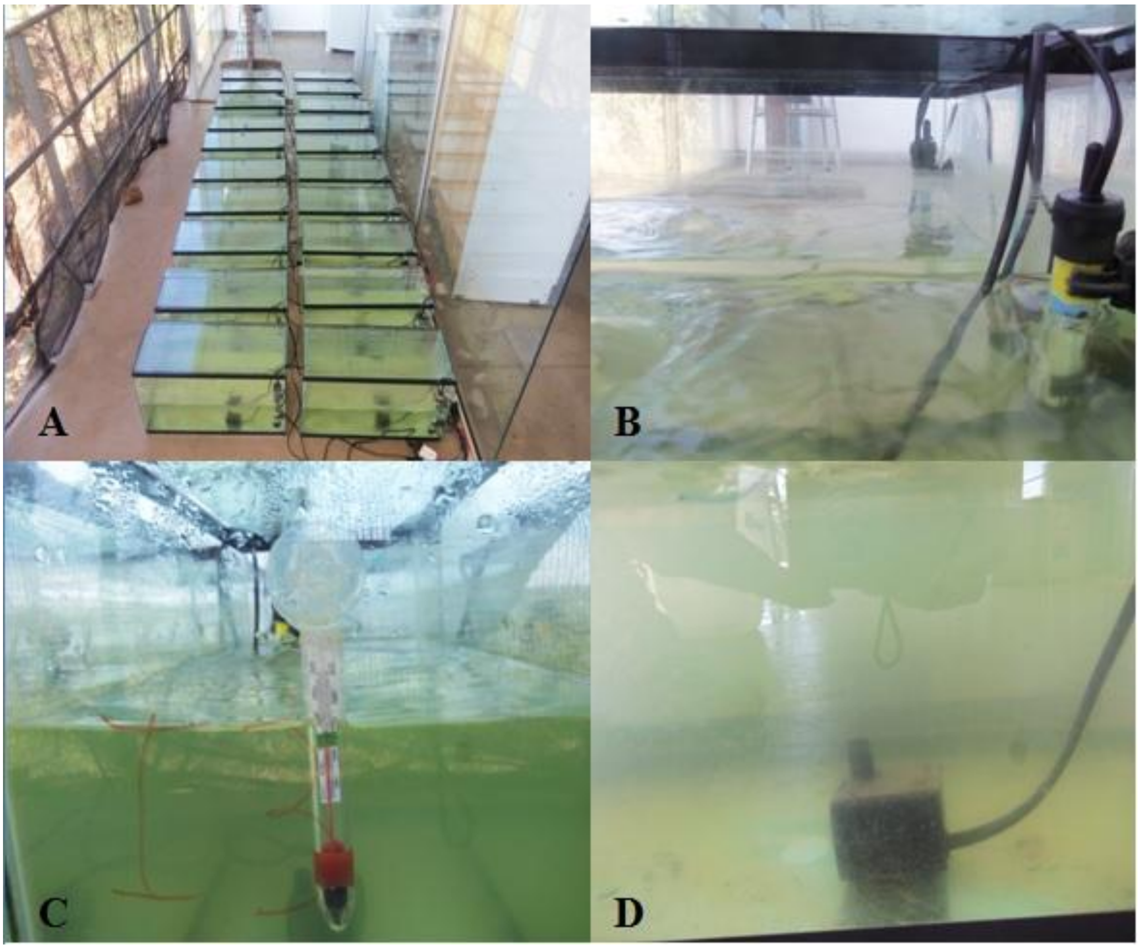

Fig. 1 Microcosm used in experiment. A - overview of the experiment, B - electric heater coupled to a thermostat, C - thermometer, D - water circulation pump. The water temperature was monitored daily with a multiparameter probe

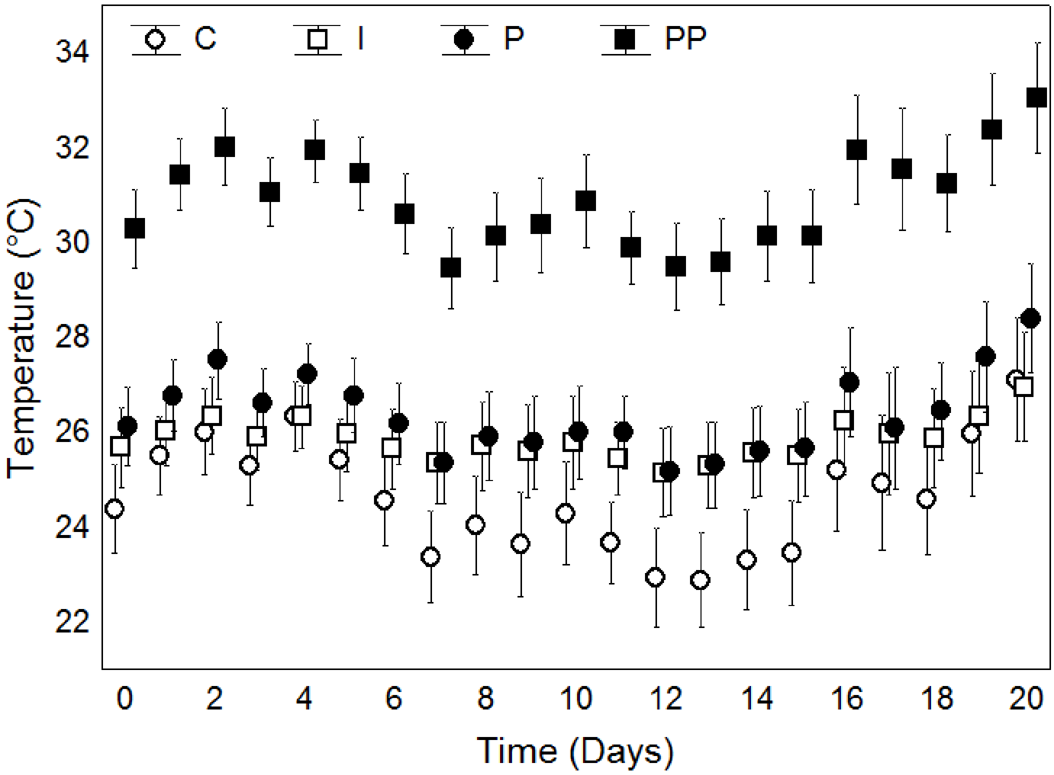

Fig. 2 Mean of temperature values between the replicates of each treatment, measured daily during the experiment. Vertical bars indicate a 95% confidence interval. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

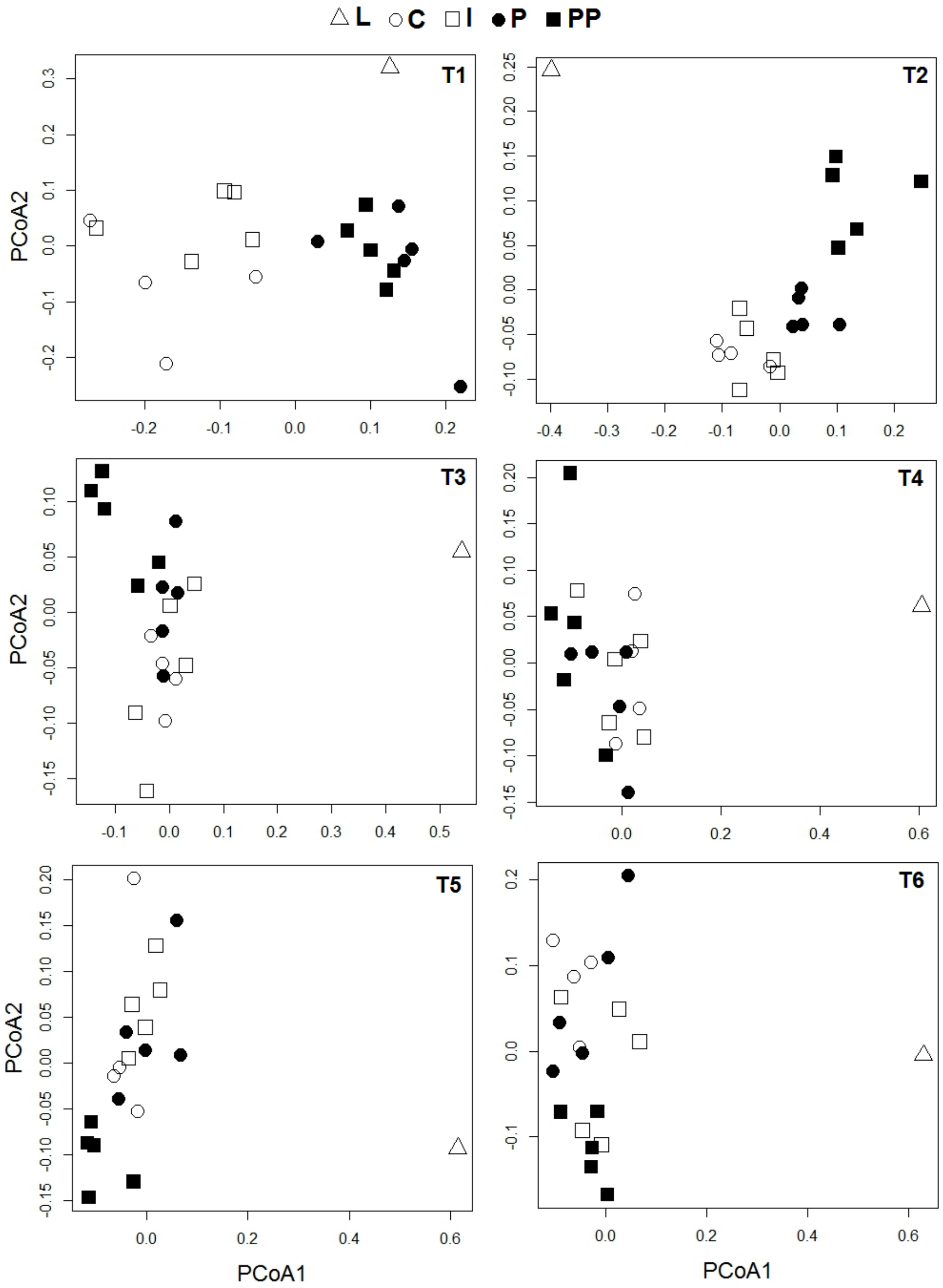

Fig 3 Principal Coordinate Analysis for the phytoplankton species abundance. Here we compared the species composition between the treatments and the untreated sample used to fill the microcosms in different sampling periods. L = Sample obtained in the lake and used to fill all microcosms at the beginning of the experiment, C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of

temperature. T1 = 0 day (approximately 48 hours after the microcosms were installed and the temperature stabilized); T2 = 4th day; T3 = 8th day; T4 = 12th day; T5 = 16th day; T6 = 20th day)

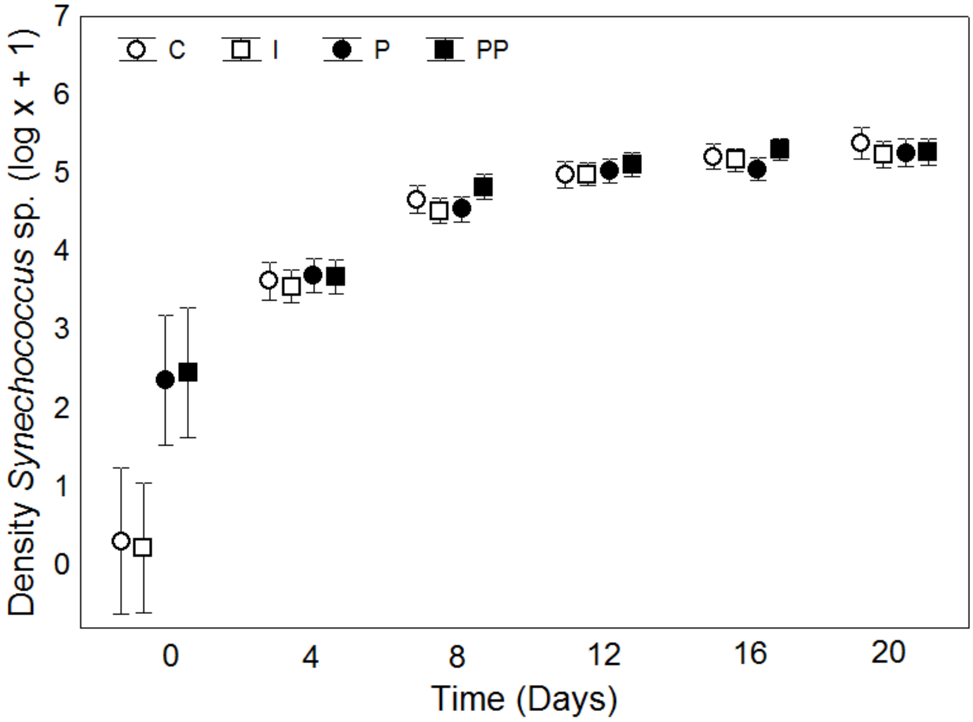

Fig. 4 Mean values for the abundance of the species *Synechococcus* sp. Vertical bars indicate a 95% confidence interval. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

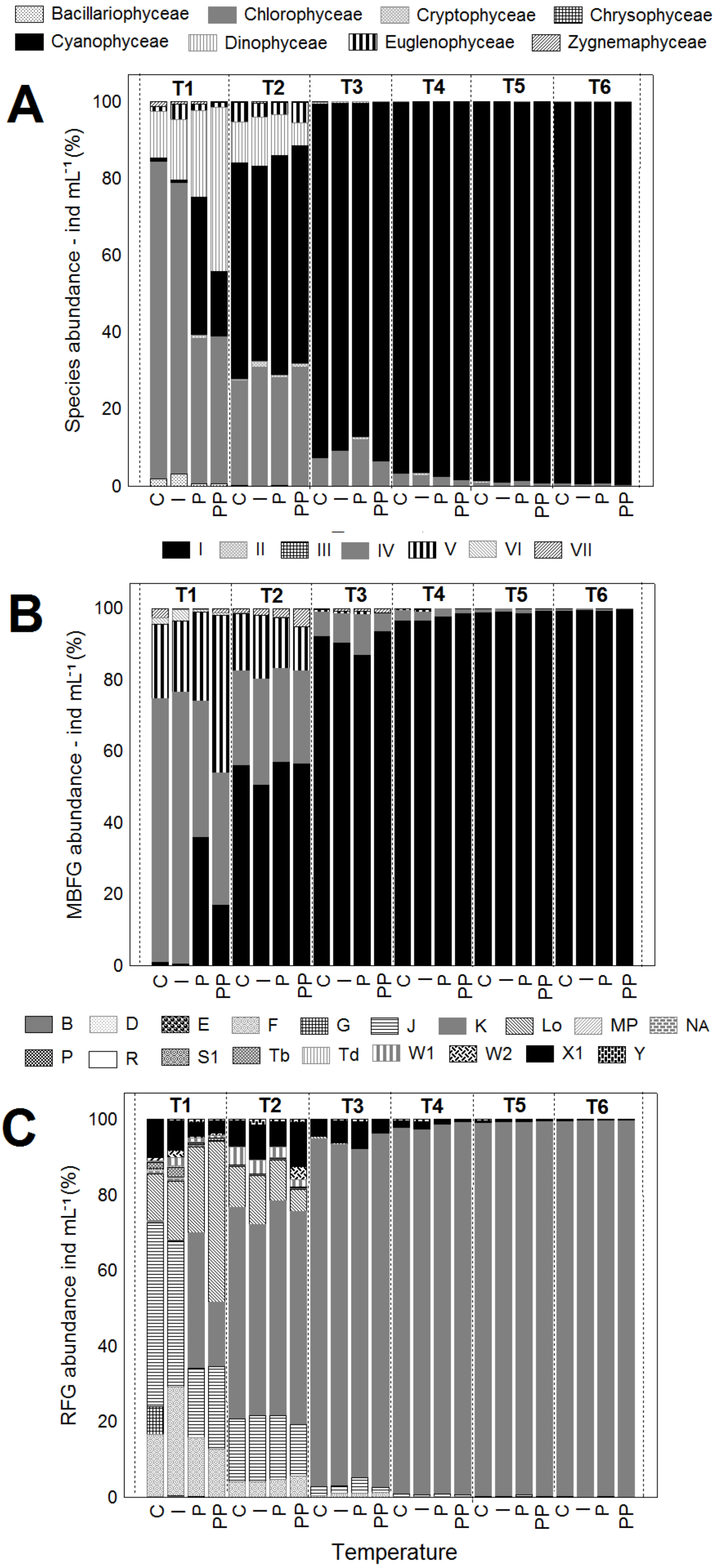
Bacillariophyceae
Chlorophyceae
Cryptophyceae
Chrysophyceae
Cyanophyceae
Dinophyceae
Euglenophyceae
Zygnemaphyceae
A
Species abundance - ind mL⁻¹ (%)
T1
T2
T3
T4
T5
T6
0
20
40
60
80
100
C
I
P
PP
I
II
III
IV
V
VI
VII
B
MBFG abundance - ind mL⁻¹ (%)
B
D
E
F
G
J
K
Lo
MP
NA
P
R
S1
Tb
Td
W1
W2
X1
Y
C
RFG abundance ind mL⁻¹ (%)
Temperature

Fig 5 Mean of species abundance by taxonomic class (A), morphologically based functional groups (B) and Reynolds functional groups (C) according to the temperature and sampling time. T indicates the experiment period in which the sampling was performed, as follows: T1 = 0 day (approximately 48 hours after the microcosms were installed and the temperature stabilized); T2 = 4th day; T3 = 8th day; T4 = 12th day; T5 = 16th day; T6 = 20th day). C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

Table 1 Berger Parker dominance index (BG) for species between the different treatments and sampling time. C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature. T1 = 0 day (approximately 48 hours after the microcosms were installed and the temperature stabilized); T2 = 4th day; T3 = 8th day; T4 = 12th day; T5 = 16th day; T6 = 20th day). * BG > 0 ≤0.01.

	T1	T1	T1	T1	T2	T2	T2	T2	T3	T3	T3	T3	T4	T4	T4	T4	T5	T5	T5	T5	T6	T6	T6	T6
Species	C	I	P	PP	C	I	P	PP	C	I	P	PP	C	I	P	PP	C	I	P	PP	C	I	P	PP
Aulacoseira sp. Ehrenberg	0	0	0	0	0	0	0	0	*	0	0	0	*	0	0	0	0	0	0	0	0	0	0	*
Encyonema sp. Kützing	0	0	0	0	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis sp1 Krammer	0	*	0	0	0	0	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis sp2 Krammer	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis sp3 Krammer	0	0	0	0	0	0	*	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia sp1 Ehrenberg	*	*	*	*	*	*	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia sp2 Ehrenberg	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia sp3 Ehrenberg	*	*	*	*	0	0	*	0	*	*	*	*	*	0	0	0	0	0	0	0	*	*	*	0
Eunotia sp4 Ehrenberg	*	*	*	*	*	*	*	*	*	*	0	*	*	0	*	*	0	*	*	0	0	*	0	*
Eunotia sp5 Ehrenberg	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia sp6 Ehrenberg	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia sp. Rabenhorst	*	*	0	0	0	0	*	0	0	*	*	*	0	*	*	0	*	0	*	0	*	0	*	0
Gomphonema sp. Ehrenberg	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula sp. Bory	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sp. Hassal	*	*	*	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nupela sp. Vyverman & Compère	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia sp. Ehrenberg	0	*	*	*	*	*	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stenopterobia sp. Brébisson ex Van Heurck	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellaphora sp. Mereschkovsky	0	0	0	0	0	0	0	0	*	0	*	*	0	0	0	0	0	0	0	0	0	0	0	0
Surirella sp. Turpin	0	*	0	*	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	0
Ankistrodesmus fasciculatus Lundberg (Komárková)	*	*	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ankistrodesmus tortus Komarék & Comas González	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Botryococcus sudetica Lemmermann	0	0	0	*	*	0	0	0	0	*	0	0	*	0	*	*	*	*	*	0	0	*	0	*
Coelastrum astroideum De Notaris	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Dictyosphaerium pulchellum H. C. Wood	0.01	0	*	*	0	0	0.01	0.05	*	*	*	0.01	*	*	*	*	*	*	*	*	*	*	*	*
Echinosphaeridium nordstedtii Lemmermann	0	0	0	0	0	0	0	*	0	*	0	0	0	*	*	*	0	*	0	*	*	*	*	*
Eremosphaera sp. De Bary	*	*	*	*	0	*	*	*	*	*	*	0	*	0	0	0	*	*	*	*	*	*	*	*
Eudorina illinoisensis Ehrenberg	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Franceia javanica C. Bernard Hortobágyi	0.01	*	*	0.01	0.01	0.01	*	*	0	*	0	0	0	*	0	*	*	0	*	*	0	0	0	0
Golenkinia radiata Chodat	0	0	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	*	*	0	*	0
Monoraphidium arcuatum (Korshikov) Hindák	*	0	0	*	0	0	0	*	0	0	*	*	0	*	0	0	*	0	0	*	0	0	0	0
Monoraphidium contortum (Thuret) Komárková-Legnerová	0.04	0.04	0.02	0.02	0.06	0.08	0.06	0.1	0.03	0.05	0.06	0.03	0.01	0.01	*	*	*	*	*	*	*	*	*	*
Monoraphidium griffithii (Berkeley) Komárková-Legnerová	0.01	*	0	0	0	0	0	0	0	*	0	0	0	*	*	0	0	0	0	0	0	0	0	0
Monoraphidium komarkovae Nygaard	0.04	0.02	0.01	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pachycladella umbrina (G. M. Smith) P. C. Silva	0	0	0	*	*	*	*	0	0	*	0	*	0	0	0	0	*	0	*	*	0	*	0	0
Scenedesmus armatus (Chodat) Chodat	0	*	*	*	*	*	*	*	0	*	*	*	0	*	*	*	*	*	*	*	*	*	*	0
Scenedesmus opoliensis P. G. Richter	0	0	*	0	0	*	0	0	0	0	0	0	0	0	0	*	0	0	0	0	0	0	0	0
Tetraedron minimum (A. Braun) Hansgirg	0.47	0.37	0.18	0.2	0.15	0.15	0.15	0.13	0.02	0.01	0.04	0.01	*	*	*	*	*	*	*	*	*	*	*	*
Treubaria triappendiculata C. Bernard	0.14	0.27	0.15	0.12	0.03	0.04	0.03	*	*	*	*	*	*	*	*	*	*	0	*	0	0	0	*	0
Westella botryoides (West) De Wildeman	0	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptomonas sp. Ehrenberg	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptomonas erosa Ehrenberg	0	0	0	0	0	0	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Cryptomonas obovata Skuja	0	0	*	*	*	*	*	*	*	*	*	0	*	0	0	0	0	0	0	0	0	0	0	0
Cryptomonas pierenoidifera Geitler	0	0	*	*	*	0.01	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Dinobryon sertularia Ehrenberg	0	*	*	*	0	*	0	0	*	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0
Chroococcus minutus	0	0	0	*	*	*	*	*	*	*	0	*	0	*	0	*	0	*	*	0	0	0	0	0

(Kützing) Nägeli																								
Geitlerinema amphibium (C. Agardh ex Gomont) Anagnostidis	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limnothrix mirabilis (Böcher) Anagnostidis	0	0	0	0	0	0	0	0	0	*	0	0	*	*	*	*	*	*	0	*	*	*	*	*
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová & Cronberg	*	0	*	0	0	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pseudanabaena limnetica (Lemmermann) Kómarek	0	*	0	*	0	0	*	*	*	*	*	0	*	*	*	*	*	*	*	*	*	*	*	*
Romeria gracilis Koczwara (Koczwara)	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synechococcus sp. C. Nägeli	*	*	0.35	0.16	0.56	0.5	0.56	0.56	0.92	0.9	0.86	0.93	0.96	0.96	0.97	0.98	0.98	0.99	0.98	0.99	0.99	0.99	0.99	0.99
Peridinium volzii Lemmermann	0.07	0.09	0.2	0.37	0.09	0.11	0.09	0.05	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Peridinium umbonatum F. Stein	0.04	0.06	0.02	0.04	*	0.01	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	*	0	*
Euglena sp1 Ehrenberg	*	*	*	*	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena sp2 Ehrenberg	0	0	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena próxima P. A. Dangeard	0	*	*	*	0	0	0	0	0	*	0	0	*	0	0	0	0	0	0	0	0	0	0	0
Phacus pleuronectes (O.F.Müller) Nitzsch ex Dujardin	0	0.02	*	*	0.04	0.03	0.02	0.01	0	*	*	0	0	0	0	0	*	0	0	0	*	0	0	0
Trachelomonas híspida (Perty) F.Stein	0.01	0.01	*	*	*	*	*	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Closterium acutum Brébisson	0	0	0	0	0	*	*	*	0	0	0	*	*	*	*	*	*	0	*	*	*	*	*	*
Closterium ceratium Perty	0	0	0	0	0	0	0	0	*	*	0	0	0	0	0	0	0	0	0	0	0	0	*	0
Closterium setaceum Ehrenberg ex Ralfs	0	0	*	0	*	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	0	0
Closterium sp. Kuetzingii Brébisson	0	0	0	0	0	*	0	*	*	0	*	*	*	0	0	0	0	0	0	*	*	0	*	*
Cosmarium depressum Bailey	*	0	*	0	*	*	*	0	*	0	0	0	*	*	*	0	0	0	0	0	0	*	0	0
Cosmarium sp1 Corda ex Ralfs	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cosmarium sp2 Corda ex Ralfs	0	0	0	0	0	0	*	*	*	*	0	0	0	*	0	*	*	*	*	0	0	0	0	*
Cosmarium sp3 Corda ex Ralfs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	0	*	*

Euastrum cuneatum Jenner	0	0	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*
Euastrum binale Ehrenberg ex Ralfs	0	0	0	0	0	0	0	0	0	0	0	0	*	0	0	0	*	0	0	0	0	0	0	0
Netrium digitus (Brébisson ex Ralfs) Itzigsohn & Rothe	0	0	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurotaenium minutum (Ralfs) Hilse	*	*	*	*	*	*	*	0	0	0	*	*	0	0	*	0	0	0	0	*	*	*	*	*
Spondylosium sp. Brébisson ex Kützing	0	0	0	0	0	0	0	0	*	0	0	0	0	*	0	0	0	0	0	0	0	0	*	*
Staurodesmus dejectus (Brébisson) Teiling	*	0	*	0	*	*	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Staurastrum furcatum Brébisson	0	0	0	0	*	*	*	0	*	*	*	0	*	0	0	0	*	*	0	0	*	0	0	0
Staurastrum muticum Brébisson ex Ralfs	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	*	0	0	0	0	0	0	0	*
Staurastrum sp. Meyen ex Ralfs	0	0	0	*	0	*	0	0	0	0	0	0	0	0	0	0	*	0	0	0	0	0	0	0
Staurastrum leptacanthum Nordstedt	0	0	0	0	0	0	*	0	0	*	0	*	0	0	*	0	*	0	0	*	*	*	*	*
Staurastrum elegantissimum L. N. Johnson	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	*	0	*	*	*	*
Staurodesmus phimus (W. B. Turner) Thomasson	*	*	*	*	*	*	*	*	*	*	*	*	0	0	*	0	*	0	*	*	*	0	*	*
Teilingia sp. Bourrelly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	0	0	0	0	0
Gonatozygon monotaenium De Bary	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	*	*	0

Table 2 – Points (PCoA 1 and PCoA 2) and eigenvalues (Eig) for Principal Coordinates Analysis for species, morphologically based functional groups (MBFG) and Reynolds functional Groups (RFG). T = Sampling time, = C = Control, I = intermediate scenario of temperature, P = pessimist scenario of temperature, PP = pessimist + pessimist scenario of temperature

	Species			MBFG			RFG		
	PCoA 1	PCoA2	Eig	PCoA 1	PCoA2	Eig	PCoA 1	PCoA2	Eig
T1C1	-0.32721	-0.2182	4.20E+00	-0.31341	0.021657	1.790001	-0.25903	-0.21821	2.09E+00
T1C2	-0.38175	-0.26256	9.95E-01	-0.33874	0.069138	0.68778	-0.32199	-0.31521	7.64E-01
T1C3	-0.25752	-0.17589	8.87E-01	-0.2077	-0.01127	0.310301	-0.2559	-0.09286	3.58E-01
T1C4	-0.28183	-0.29368	5.68E-01	-0.22762	0.405768	0.280905	-0.17492	-0.18952	3.35E-01
T1I1	-0.41763	-0.08177	4.56E-01	-0.33281	0.063362	0.209773	-0.38059	0.008488	3.07E-01
T1I2	-0.46496	-0.21279	4.23E-01	-0.34549	0.010033	0.152425	-0.23154	-0.2026	2.69E-01
T1I3	-0.40927	-0.14454	3.95E-01	-0.39294	0.118193	0.130894	-0.40366	-0.0663	1.95E-01
T1I4	-0.37368	-0.20968	3.73E-01	-0.44235	0.12439	0.077615	-0.30199	-0.25334	1.67E-01
T1I5	-0.35311	-0.1655	3.07E-01	-0.30282	0.050494	0.049271	-0.30555	-0.05624	1.43E-01
T1P1	-0.23983	-0.01174	2.76E-01	-0.19347	0.189668	0.045161	-0.20456	0.057298	1.20E-01
T1P2	-0.28778	-0.02474	2.75E-01	-0.22371	-0.05331	0.037746	-0.18797	0.012059	9.40E-02
T1P3	-0.19526	-0.02126	2.53E-01	-0.11428	-0.04146	0.032089	-0.12976	0.088937	8.43E-02
T1P4	-0.32168	-0.00846	2.20E-01	-0.17508	-0.05806	0.023846	-0.25255	0.043325	6.99E-02
T1P5	-0.26983	0.032675	2.15E-01	-0.13471	-0.04575	0.021952	-0.1672	0.062498	6.18E-02
T1PP1	-0.28307	-0.07705	1.98E-01	-0.21729	0.006691	0.019872	-0.20868	-0.05544	5.82E-02
T1PP2	-0.39688	-0.01497	1.82E-01	-0.20841	-0.06735	0.015463	-0.28232	0.020124	4.74E-02
T1PP3	-0.28283	0.044218	1.71E-01	-0.12445	-0.107	0.012049	-0.18924	0.043495	4.27E-02
T1PP4	-0.26672	-0.01782	1.67E-01	-0.12591	-0.07513	0.010235	-0.16761	-0.03811	3.81E-02
T1PP5	-0.30437	0.00981	1.56E-01	-0.12895	-0.06686	0.009157	-0.25564	0.058432	3.27E-02
T2C1	-0.2345	0.119096	1.42E-01	-0.05422	-0.10353	0.008023	-0.14127	0.11669	3.01E-02
T2C2	-0.19201	0.12938	1.35E-01	0.005421	-0.08898	0.007549	-0.08929	0.143778	2.60E-02
T2C3	-0.28646	0.10463	1.24E-01	-0.07197	-0.09212	0.005979	-0.15925	0.085324	2.55E-02
T2C4	-0.23982	0.167006	1.10E-01	-0.07245	-0.09222	0.005011	-0.10212	0.168381	2.13E-02
T2I1	-0.20042	0.136862	1.03E-01	-0.07	-0.08632	0.004975	-0.10384	0.080177	1.96E-02
T2I2	-0.19869	0.140347	1.03E-01	-0.07354	-0.08815	0.004047	-0.05514	0.067918	1.65E-02

T2I3	-0.19398	0.219322	9.30E-02	0.013973	-0.10536	0.003627	-0.02634	0.170143	1.52E-02
T2I4	-0.22765	0.16294	8.56E-02	-0.06473	-0.12938	0.003391	-0.09987	0.186841	1.35E-02
T2I5	-0.18791	0.157358	7.81E-02	-0.01366	-0.10187	0.002986	-0.05373	0.151718	1.18E-02
T2P1	-0.1845	0.113111	7.25E-02	-0.05703	-0.1107	0.002379	-0.13724	0.119814	9.66E-03
T2P2	-0.17307	0.144685	6.15E-02	0.021987	-0.09787	0.002001	-0.09705	0.139431	8.69E-03
T2P3	-0.16633	0.17413	6.05E-02	0.006503	-0.10272	0.001326	-0.07637	0.181189	7.85E-03
T2P4	-0.16312	0.114659	5.40E-02	-0.01431	-0.08789	0.001131	-0.10274	0.105913	6.88E-03
T2P5	-0.12274	0.148013	5.07E-02	-0.02847	-0.05728	0.00097	-0.0405	0.093222	5.44E-03
T2PP1	-0.06595	0.108894	4.68E-02	0.014343	-0.09781	0.000797	-0.04195	0.117177	4.60E-03
T2PP2	-0.07273	0.128872	4.21E-02	-0.02962	-0.09964	0.000599	-0.01865	0.101941	4.35E-03
T2PP3	-0.0294	0.131715	3.84E-02	-0.02076	-0.03454	0.000354	-0.02307	0.105822	3.57E-03
T2PP4	-0.01893	0.195323	3.52E-02	-0.0206	-0.06669	0.000185	-0.06583	0.147614	2.65E-03
T2PP5	-0.05914	-0.00353	3.20E-02	-0.00831	-0.03516	0.000103	-0.09993	0.028817	2.35E-03
T3C1	-0.01326	0.066762	2.80E-02	0.048459	-0.0528	-5.1E-17	0.06149	0.028706	1.38E-03
T3C2	-0.01094	-0.00112	2.51E-02	0.02643	-0.00325	-2.7E-05	0.057697	-0.00903	1.29E-03
T3C3	-0.01544	0.110321	2.49E-02	0.003621	-0.01633	-6.3E-05	0.043405	0.038336	7.51E-04
T3C4	0.014045	0.017055	1.77E-02	-0.00998	-0.09439	-0.00011	0.02303	-0.01992	3.83E-04
T3I1	-0.03116	0.059843	1.52E-02	0.014186	-0.01518	-0.00029	0.001463	0.014429	1.68E-07
T3I2	0.020605	0.045793	1.36E-02	0.020159	-0.02247	-0.00039	0.029251	0.026508	5.29E-17
T3I3	0.00617	0.023242	1.20E-02	0.099774	-0.01848	-0.00043	0.051075	0.028109	-7.57E-04
T3I4	-0.03392	0.045738	1.08E-02	0.032659	-0.05469	-0.00051	0.019118	0.045609	-8.34E-04
T3I5	-0.04392	0.028806	7.46E-03	0.04815	-0.04823	-0.00052	0.005691	0.072836	-1.08E-03
T3P1	-0.04179	0.058936	6.03E-03	-0.00029	-0.09602	-0.00066	-0.0245	0.07075	-1.51E-03
T3P2	-0.0492	0.025441	4.81E-03	0.010057	-0.08537	-0.00068	-0.00817	0.024226	-1.70E-03
T3P3	-0.04438	0.012683	0.00313	-0.00174	-0.07826	-0.0007	-0.02306	0.015985	-1.84E-03
T3P4	0.019369	0.014029	0.000177	0.050076	-0.02183	-0.00075	0.029329	0.010558	-2.00E-03
T3P5	0.016997	0.044023	-1.9E-16	-0.0011	-0.09058	-0.00091	0.035246	0.015205	-2.21E-03
T3PP1	0.025592	0.010935	-0.00086	0.056108	-0.02275	-0.00099	0.070009	0.048936	-2.34E-03
T3PP2	-0.00058	0.028869	-0.00132	0.080836	-0.06185	-0.00104	0.037916	0.033678	-2.60E-03
T3PP3	0.102183	-0.01906	-0.00363	0.065348	-0.01273	-0.0011	0.035391	-0.0092	-2.76E-03
T3PP4	0.112218	-0.00691	-0.0051	0.15888	-0.0037	-0.00117	0.069286	-0.01876	-3.02E-03

T3PP5	0.078835	0.003828	-0.00617	0.062924	-0.01714	-0.00124	0.010662	-0.0211	-3.11E-03
T4C1	0.073722	0.037816	-0.00633	0.047386	-0.01432	-0.00132	0.073639	-0.01329	-3.51E-03
T4C2	0.135748	0.023186	-0.00724	0.034524	-0.00896	-0.00143	0.086764	-0.0035	-3.68E-03
T4C3	0.066905	0.014079	-0.00901	0.016383	-0.05518	-0.00151	0.017422	0.003057	-3.91E-03
T4C4	0.093076	-0.0912	-0.01108	0.073507	0.015554	-0.0016	0.061622	-0.05335	-4.43E-03
T4I1	0.144964	-0.02693	-0.01227	0.054875	-0.00555	-0.00174	0.060552	-0.03607	-4.64E-03
T4I2	0.11062	-0.00938	-0.01247	0.077875	-0.0457	-0.00179	0.076757	0.008637	-4.77E-03
T4I3	0.106566	0.044592	-0.01386	0.09006	-0.05403	-0.00182	0.054619	0.03546	-4.99E-03
T4I4	0.081211	0.02298	-0.0145	0.010156	-0.01183	-0.00196	0.10597	-0.04235	-5.11E-03
T4I5	0.144785	0.014691	-0.01556	0.107792	0.015964	-0.00208	0.129659	-0.01127	-5.44E-03
T4P1	0.123553	-0.00952	-0.01735	0.037173	0.01898	-0.00218	0.104226	-0.01106	-5.93E-03
T4P2	0.146664	-0.00522	-0.01761	0.050303	-0.01717	-0.00223	0.047878	-0.02324	-6.08E-03
T4P3	0.129435	-0.01904	-0.01871	0.009187	0.020876	-0.00246	0.07826	-0.03941	-6.45E-03
T4P4	0.168259	-0.00562	-0.01903	0.039458	0.012854	-0.00256	0.093606	-0.02166	-6.66E-03
T4P5	0.125642	-0.02231	-0.01995	0.091345	0.039902	-0.00269	0.112845	-0.04339	-6.97E-03
T4PP1	0.175682	-0.03607	-0.0205	0.136163	0.020561	-0.00282	0.097233	-0.03848	-7.53E-03
T4PP2	0.1386	0.052351	-0.02145	-0.0155	-0.05096	-0.00311	0.070263	-0.02911	-7.66E-03
T4PP3	0.18908	0.044601	-0.02169	0.116939	0.016063	-0.00323	0.156322	0.032918	-7.84E-03
T4PP4	0.244142	0.050178	-0.02314	0.127901	0.026046	-0.00346	0.172607	0.043202	-8.30E-03
T4PP5	0.214738	0.025498	-0.02449	0.115042	0.019655	-0.00378	0.119199	0.006629	-8.52E-03
T5C1	0.167701	0.043413	-0.02501	0.08596	0.063058	-0.00401	0.21198	0.020852	-9.15E-03
T5C2	0.159848	0.003094	-0.02561	0.042209	0.010142	-0.00409	0.098423	-0.01744	-9.52E-03
T5C3	0.151055	-0.0277	-0.02688	0.096064	0.043831	-0.00422	0.093283	-0.02235	-9.88E-03
T5C4	0.177061	0.001538	-0.02709	0.092586	0.039845	-0.00455	0.167617	-0.01759	-1.05E-02
T5I1	0.214323	-0.01184	-0.02872	0.092729	0.091015	-0.00474	0.156663	-0.02737	-1.08E-02
T5I2	0.195737	-0.07405	-0.02879	0.09781	0.167338	-0.00488	0.196468	-0.01048	-1.13E-02
T5I3	0.178993	0.040042	-0.02928	0.108003	0.018772	-0.00501	0.082119	-0.02034	-1.19E-02
T5I4	0.152772	0.020585	-0.03042	0.048419	0.035841	-0.00509	0.099841	-0.04755	-1.23E-02
T5I5	0.148713	-0.03881	-0.03275	0.077091	0.140408	-0.00564	0.169835	-0.03583	-1.25E-02
T5P1	0.166496	0.034323	-0.03285	0.047362	0.023516	-0.00592	0.072483	-0.05209	-1.30E-02
T5P2	0.146533	0.037577	-0.03464	0.030212	0.001328	-0.00623	0.063062	-0.02867	-1.44E-02

T5P3	0.095734	0.019849	-0.03533	-0.01469	-0.02407	-0.00654	0.032651	-0.01131	-1.49E-02
T5P4	0.143078	-0.05588	-0.03698	0.105894	0.139526	-0.00707	0.161215	-0.0219	-1.53E-02
T5P5	0.069538	-0.04798	-0.03785	0.034901	0.044538	-0.0075	0.064645	-0.04555	-1.66E-02
T5PP1	0.174297	-0.02033	-0.03909	0.143493	0.039489	-0.00759	0.08033	-0.04142	-1.69E-02
T5PP2	0.192063	0.010734	-0.04104	0.116199	0.009179	-0.00795	0.115224	-0.00057	-1.80E-02
T5PP3	0.185564	-0.02688	-0.04188	0.101785	0.009664	-0.0084	0.122325	-0.02396	-1.89E-02
T5PP4	0.216481	-0.03689	-0.04373	0.125465	0.039985	-0.00988	0.109447	-0.01323	-1.96E-02
T5PP5	0.207458	-0.05873	-0.04524	0.146819	0.056091	-0.01031	0.129827	-0.06473	-2.02E-02
T6C1	0.165243	-0.00417	-0.04664	0.10001	0.020406	-0.01085	0.098659	-0.00182	-2.05E-02
T6C2	0.179306	0.021278	-0.04754	0.048813	0.031139	-0.01211	0.065297	0.007517	-2.20E-02
T6C3	0.180122	-0.02059	-0.04981	0.031185	0.055888	-0.01247	0.08419	-0.05798	-2.35E-02
T6C4	0.22734	-0.03892	-0.05057	0.105491	0.037031	-0.01286	0.126704	-0.04095	-2.48E-02
T6I1	0.19361	-0.12027	-0.05276	0.112658	0.067318	-0.01606	0.125503	-0.0562	-2.66E-02
T6I2	0.118264	-0.07255	-0.05452	0.060753	0.024319	-0.01743	0.065155	-0.06269	-2.70E-02
T6I3	0.11974	-0.07102	-0.05643	0.09768	0.075584	-0.01844	0.097996	-0.02652	-2.85E-02
T6I4	0.197613	-0.03459	-0.05786	0.046245	0.029818	-0.01863	0.094372	-0.053	-3.21E-02
T6I5	0.182948	-0.08153	-0.06017	0.088111	0.108878	-0.02252	0.142078	-0.03405	-3.38E-02
T6P1	0.170568	-0.04036	-0.06385	0.056261	0.032492	-0.02714	0.059385	-0.03585	-3.83E-02
T6P2	0.200806	-0.06376	-0.0698	0.046296	0.032224	-0.02896	0.055219	-0.05378	-4.07E-02
T6P3	0.124981	0.009902	-0.07061	0.008726	0.013466	-0.02944	0.062996	-0.04605	-4.80E-02
T6P4	0.209641	-0.02863	-0.07703	0.129251	0.032084	-0.03385	0.087511	-0.01692	-4.92E-02
T6P5	0.123133	-0.10391	-0.08294	0.041627	0.115151	-0.04859	0.058964	-0.08882	-5.41E-02
T6PP1	0.172779	-0.09342	-0.08491	0.136348	0.055888	-0.05834	0.085443	-0.05763	-5.48E-02
T6PP2	0.179131	-0.06172	-0.0914	0.123921	0.056658	-0.06489	0.103055	-0.01942	-6.73E-02
T6PP3	0.157391	-0.14856	-0.09853	0.092604	0.159989	-0.09939	0.143202	-0.03353	-7.45E-02
T6PP4	0.193303	-0.07469	-0.10196	0.072826	0.044753	-0.13695	0.095203	-0.09481	-9.67E-02
T6PP5	0.168388	-0.19732	-0.1299	0.125327	0.075728	-0.1967	0.092726	-0.12431	-1.30E-01

Additional Information

Pilot Experiment

We conducted a pilot experiment between January 18 and February 5, 2016, to evaluate the operation of electric heaters and circulation pumps and to define some details of the definitive experiment (e.g., time duration). The water samples were obtained from an oligo-mesotrophic lake, located on the UEG Campus, the same lake in which samples were collected for the definitive experiment. The construction and filling of the microcosms occurred in the same way described for the definitive experiment. For the experiment pilot, two treatments were used, control (C = 24 ° C) and warmed (H = 30 ° C), with four replicates each (C1, C2, C3, C4, H1, H2, H3, H4). Phytoplankton collections and nutrient addition were also carried out every four days, in the same concentrations used for the definitive experiment. The pilot experiment lasted 20 days. The collection and screening of phytoplankton samples were performed using the same procedures cited for the definitive experiment. We used factorial analysis of variance for repeated measures to evaluate if the species richness differs between the treatments and the sampling time. To evaluate if there are differences between these same factors for the species abundance, we used a analysis of permutational variance.

We found 83 species belonging to the classes Bacillariophyceae (17), Chlorophyceae (18), Cryptophyceae (3), Cyanophyceae (10), Dinophyceae (2), Euglenophyceae (3) and Zygnemaphyceae (30). The abundance of species varied over time (figure 6). The temperature and sampling day did not affect the species richness (Temperature: $F_{(1)} = 0.19$, $P = 0.67$; Time: $F_{(5)} = 1.77$, $P = 0.14$; Temperature x Time: $F_{(5)} = 0.77$, $P = 0.57$). Significant effects of these two factors were observed for community abundance (Temperature: $F_{(1)} = 3.54$, $R^2 = 0.04$, $P = 0.009$; Time: $F_{(5)} = 6.87$, $R^2 = 0.43$, $P = 0.001$; Temperature x Time: $F_{(5)} = 0.98$, $R^2 = 0.06$, $P = 0.48$), although time was more important. We observed a succession of taxonomic groups throughout the experiment (Figure 6 and 7), with a greater variability of groups in the first collection period (e.g. Bacillariophyceae, Cyanophyceae, Chlorophyceae, Euglenophyceae, Zygnemaphyceae) when compared to the end (e.g. Chlorophyceae, Cyanophyceae and Zygnemaphyceae). In general, Cyanophyceae presented a higher number of individuals in H treatment when compared to C, whereas Euglenophyceae and Dinophyceae were found preferentially in treatment C (Figure 6).

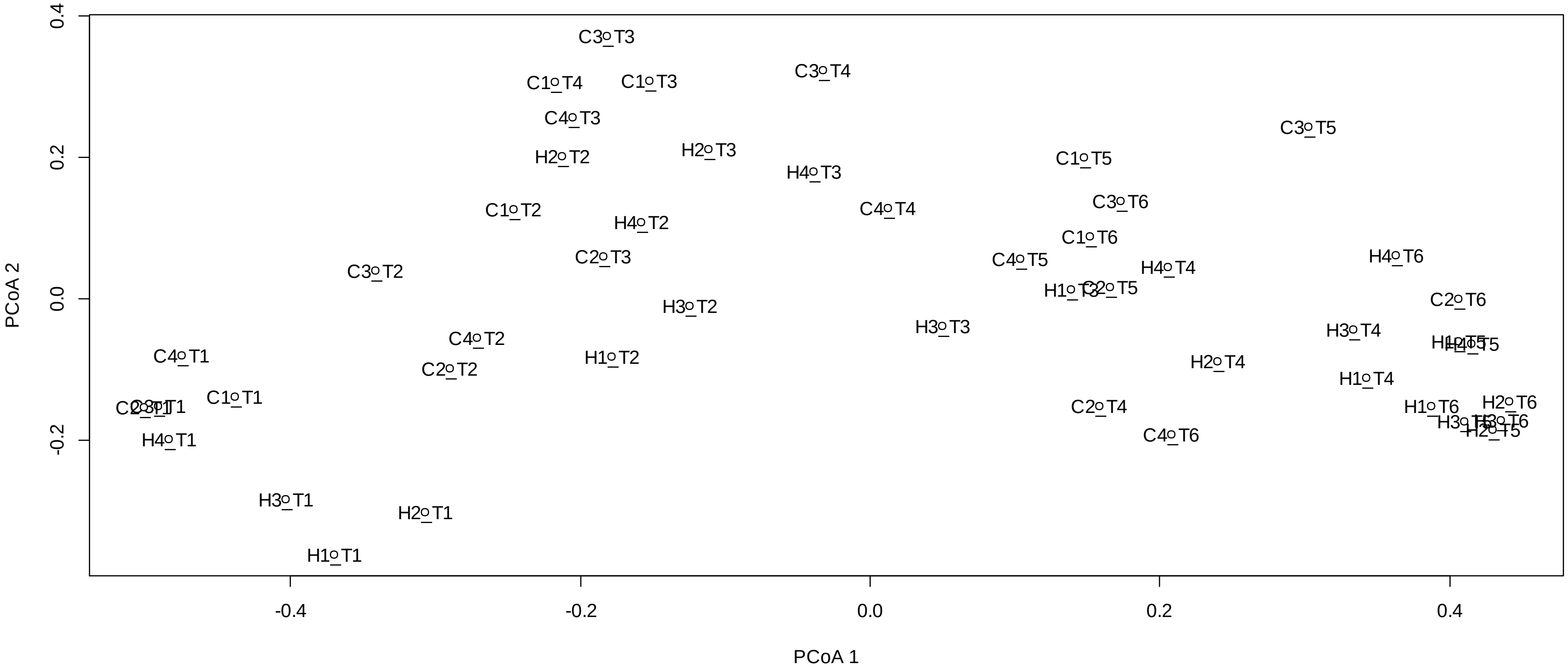

Fig. 6 Principal Coordinate Analysis for the phytoplankton species abundance during the pilot experiment. The pilot was conducted between January 18 and February 5, 2016 to adjust some parameters of the definitive experiment. Two treatments were used, control (C = 24 ° C) and warmed (H = 30

°C), with four replicates each (C1, C2, C3, C4, H1, H2, H3, H4). Samples were collected every four days (T1, T2, T3, T4, T5, T6). It is important to note that changes in species abundance cease to occur from the fourth collection (15th day)

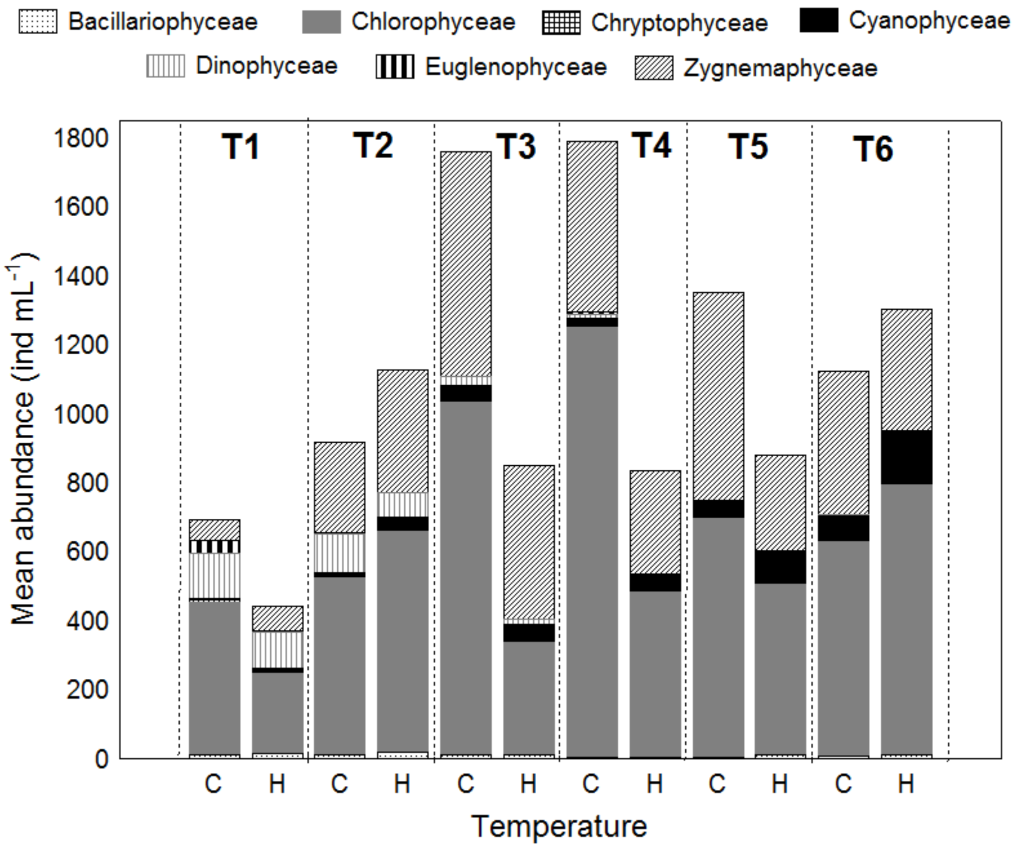

Fig 7 Number of individuals per taxonomic group between the heated (H) and control (C) treatment, at different time periods of the experiment. T1 = 0 day; T2 = 4th day; T3 = 8th day; T4 = 12th day; T5 = 16th day; T6 = 20th day)

Nutrients addition

We performed a paired T test to verify if there were differences in nutrient concentration between the beginning and final of the experiment. Here, the initial concentration was represented by the amount of nutrients in the water used to fill the microcosms. This water was collected in an oligotrophic lake. The final concentration was represented by the amount of nutrients present in the microcosms at the end of the experiment, that is, after all the additions were made. The nitrite and total phosphorus concentrations of the lake were similar to those observed at the end of the experiment in all aquaria (Nitrito: $T_{(18)} = -1$, $P = 0.33$; Total Phosphorus: $T_{(18)} = -1$, $P = 0.3$). For orthophosphate, the concentrations were the same at both the beginning and the end of the experiment in all microcosms (<0.003 mg L^{-1}). We found significant differences between the beginning and end of the experiment for total nitrogen ($T_{(18)}$ = -10.7, $P < 0.001$, initial mean = 0.24 mg/L, final mean = 0.51 mg L^{-1}, difference mean = 0.27 mg L^{-1}) and nitrate ($T_{(18)}$ = 2.19, P = 0.04, initial mean = 0.31 mg L^{-1}, final mean = 0.30 mg L^{-1}, difference mean = 0.1 mg L^{-1}). However, these concentrations were very low and the trophic status of the all microcosms remained as oligotrophic, as that the lake in which the samples were collected. The T test was performed using the Vegan package (Oksanen et al. 2017) in program R (Core Team R 2016).

References

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ELECTRONIC SUPPLEMENTARY MATERIAL II – ESM II

Esses dados estão disponíveis no arquivo Excel, nomeado “ESM II” em https://link.springer.com/article/10.1007%2Fs10750-018-3858-7.

CAPÍTULO III

Respostas da microbiota procariota e eucariota ao aquecimento global: os padrões são concordantes?**

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Resumo

1. A microbiota aquática está envolvida em importantes funções ecossistêmicas. Investigar o efeito do aquecimento global sobre esses organismos é altamente relevante, principalmente diante das inúmeras alterações climáticas previstas até o fim do século. Neste estudo, nós utilizamos uma abordagem experimental para avaliar se os efeitos do aquecimento global predito por diferentes cenários futuros afetam a composição da microbiota aquática procariota e eucariota, além de verificar se os mesmos efeitos ocorrem para cada nível trófico separadamente (autotrófico, heterotrófico e mixotrófico).

2. Nosso experimento demonstrou que a composição da microbiota planctônica tanto para eucariotos como para procariotos é afetada pelo incremento na temperatura e estes apresentam um padrão de resposta similar ao aquecimento. Modificações nas comunidades foram observadas através da substituição de táxons específicos, que ocorreram principalmente no cenário extremo de

aquecimento. Mudanças na composição das comunidades também foram identificadas quando os níveis tróficos foram avaliados separadamente.

3. De modo geral, organismos mixotróficos eucariotos são mais sensíveis ao incremento da temperatura, modificando os padrões de riqueza e composição com um aumento de apenas 2°C. Por outro lado, os protistas e bactérias heterotróficas se mostraram mais resistentes, com alterações nas comunidades visualizadas apenas no cenário extremo de aquecimento. Já os organismos autotróficos não foram afetados pelo aumento na temperatura da água em nenhuma das classificações biológicas avaliadas.

4. Nossos resultados contribuem para predizer como diferentes níveis biológicos e grupos tróficos da microbiota aquática respondem ao aquecimento global. Essa abordagem é relevante pois o aquecimento leva a modificação na composição das comunidades e por sua vez afeta processos ecossistêmicos essenciais ao ambiente aquático.

Palavras-chave: Bacterioplâncton, protistas, 18S rDNA, 16S rDNA, níveis tróficos

Introdução

Compreender como as comunidades biológicas respondem às variações ambientais é um dos objetivos centrais da ecologia (Smale et al., 2017). Inúmeras alterações nas condições dos ambientes, incluindo as derivadas das mudanças climáticas, têm sido observadas nos últimos anos (p. ex. aumento na temperatura, alteração na frequência de precipitação, conversão do uso do solo e enriquecimento dos corpos de água) e estão previstas para se intensificarem até o fim deste século (IPCC, 2014). Embora múltiplos fatores atuem na estruturação das comunidades biológicas, alterações na temperatura merecem destaque, uma vez que essa variável afeta as taxas de crescimento, reprodução, fenologia e dinâmica trófica dos organismos (Jeppesen et al., 2010). Assim, predizer as consequências ecológicas tanto do aumento (p. ex. Bergkemper et al., 2018) como da variação na temperatura (p. ex. Rasconi et al., 2017) sobre os ecossistemas, constitui um tema de alta relevância (Altermatt et al., 2015).

Os ecossistemas aquáticos continentais abrigam uma alta biodiversidade, mas se encontram entre os mais ameaçados do planeta (Dudgeon et al., 2006). Nos últimos anos, inúmeros esforços têm sido concentrados para predizer como as mudanças climáticas, incluindo o aquecimento global, devem afetar esses ambientes (Woodward et al., 2010; Roland et al., 2012; Jeppesen et al., 2014). Efeitos já foram observados sobre as espécies através de mudanças nos padrões de composição (p. ex. Yvon-Durocher et al., 2015) e abundância (Clements et al., 2013), assim como sobre as suas características funcionais (p. ex. redução no tamanho, Sommer et al., 2015; Rasconi et al., 2017). Do mesmo modo, mudanças nas características das comunidades devem alterar a forma como elas se relacionam com os outros níveis tróficos por meio de um efeito top down ou bottom up (Sommer & Lewandowska, 2011; Vadadi-Fülöp et al., 2012; Velthuis et al., 2017). Embora muitos estudos tenham procurado elucidar os efeitos do aquecimento global, a maioria têm como foco apenas um grupo taxonômico e não consideram diferentes níveis tróficos simultaneamente. Essa abordagem limita a compreensão para um único grupo e ignora a interação entre grupos taxonômicos. Nesse sentido, estudos ecológicos que utilizem diferentes níveis tróficos simultaneamente podem auxiliar a compreender se diferentes grupos taxonômicos e tróficos respondem similarmente às alterações ambientais.

Efeitos distintos do aquecimento são esperados sobre os diferentes níveis tróficos que compõem as comunidades aquáticas. De acordo com a teoria metabólica da ecologia (Brow et al., 2004), os processos heterotróficos são mais dependentes da temperatura do que os autotróficos. De fato, estudos têm demonstrado que o aquecimento global deve intensificar as taxas de respiração e decomposição levando ao predomínio de organismos heterotróficos sobre os autotróficos (Hoope et al., 2008; Lara et al., 2013; Von Scheneiber et al., 2014; Bergkemper et al., 2018 mas ver Domaizon et al., 2012 para um efeito contrário). Essa mudança na estrutura das comunidades pode aumentar o controle dos consumidores sobre os produtores em cenários de aquecimento (Yang et al., 2016). Por outro lado, a microbiota mixotrófica possui uma maior plasticidade diante das alterações na temperatura, adotando de acordo com a situação, um comportamento de produtor ou consumidor.

Para esses organismos já foi constatada uma redução na quantidade de clorofila-a (Wilken et al., 2012) e aumento do consumo de bactérias (Wilken et al., 2018), o que levou a sobreposição do metabolismo heterotrófico sobre o autotrófico em maiores temperaturas.

Por outro lado, respostas concordantes entre grupos ou níveis biológicos pode ter uma importante aplicação para o biomonitoramento de ambientes aquáticos. Por exemplo, se dois grupos apresentam elevada concordância, um grupo poderia ser utilizado como indicador ou substituto de outro (p. ex. Jackson & Harvey, 1993; Heino, 2010; Machado et al., 2015). A utilização de grupos substitutos pode reduzir tempo e custos em trabalhos de biomonitoramento (Heino, 2010). A concordância é esperada principalmente para grupos taxonômicos que respondem similarmente aos gradientes ambientais e espaciais, ou ainda apresentam interações biológicas (Grenouillet et al., 2008). Para comparações entre comunidades procariotas e eucariotas, já foram observadas respostas similares ao incremento da temperatura (Smale et al., 2017), assim como a ausência de efeitos nestes dois níveis biológicos (Maugendre et al., 2015). Embora também existam resultados contrastantes, com aumento na abundância bacteriana (Domaizon et al., 2012; Lara et al., 2013; Tuyet et al., 2015; Bergen et al., 2016) e redução da abundância (Domaizon et al., 2012; Vázquez-Domínguez et al., 2012) e biomassa de protistas (Lara et al., 2013), indicando que organismos eucariotos podem ser mais sensíveis aos efeitos do aquecimento global.

Bactérias (Fugimoto et al., 2016) e protistas planctônicos (Khomich et al., 2017) possuem representantes nos diferentes níveis tróficos (autotrófico, heterotrófico e mixotrófico), além de representar grupos evolutivamente distintos (procariotos e eucariotos). Por possuir um tamanho reduzido e um ciclo de vida curto (Altermatt et al., 2015), esses organismos são um excelente modelo para compreender os efeitos do aquecimento global. Através da abordagem metagenética inúmeros grupos taxonômicos com características biológicas distintas, tanto para microbiota procariota (p. ex. Von Scheneiber et al., 2014; Tuyet et al., 2015; Bergen et al., 2016) como para eucariota (p. ex. Domaizon et al., 2012; Moustaka-Gouni et al., 2016) podem ser avaliados simultaneamente. Assim, torna-se importante avaliar efeitos do aquecimento sobre essas comunidades, uma vez que eles desempenham um importante papel nas redes alimentares (Chen et al., 2008) e funcionamento dos ecossistemas, através dos ciclos biogeoquímicos e ciclagem de nutrientes (Rodríguez-Valera, 2004; Chen et al., 2008).

Neste estudo nós utilizamos uma abordagem experimental para simular os efeitos do aquecimento global predito por diferentes cenários futuros sobre a composição da microbiota aquática procariota e eucariota, além de avaliar os efeitos separadamente para cada nível trófico (autotrófico, heterotrófico e mixotrófico). A composição das comunidades foi obtida através do sequenciamento de última geração, utilizando marcadores para os genes 16S rDNA e 18S rDNA. Essa abordagem já foi utilizada anteriormente em experimentos envolvendo ambientes marinhos (p. ex. Domaizon et al., 2012; Bergen et al., 2016; Smale et al., 2017; Huggett et al., 2018). Porém, até onde temos conhecimento, nosso experimento é um dos primeiros a simular os efeitos do incremento da temperatura utilizando uma abordagem metagenética para a microbiota procariota e eucariota de água doce tropical (mas ver Pajares et al., 2013 e Ren et al., 2017 para o bacterioplâncton na zona temperada).

Nós buscamos avaliar se o aquecimento produz efeitos sobre a microbiota aquática e ainda se os diferentes níveis de organização biológica (procariotos e eucariotos) e grupos tróficos (autotróficos, heterotróficos e mixotróficos) têm padrões de resposta semelhantes ao aquecimento. Nós hipotetizamos que aquecimento pode ser mais importante sobre a composição dos organismos eucariotos (aqui representados pelos protistas), uma vez que esses possuem uma alta diversidade de organismos com tolerâncias termais distintas (p. ex. Fungi, Ciliophora, Amoebozoa, Chlorophyta, Chrysophyta, Bacillariophyta, etc). Além disso, conforme predito pela teoria metabólica da ecologia, esperamos uma substituição dos organismos autotróficos pelos heterotróficos diante do aquecimento. Por outro lado, como bactérias e protistas possuem representantes dos três níveis tróficos e estão diretamente associados na rede alimentar, esperamos que ambos os níveis biológicos assim como os diferentes níveis tróficos respondam ao aquecimento de forma similar, uma vez que alterações em um nível pode levar a modificações no outro.

Métodos

Desenho experimental

Cada microcosmo foi representado por um aquário retangular de vidro com capacidade para 25 litros de água. Uma bomba de circulação de água (modelo JAD SP – 500) foi posicionada no fundo dos aquários buscando evitar a sedimentação dos organismos (p. ex. Maugendre et al., 2015; Bergen et al., 2016). A temperatura da água foi mantida utilizando um aquecedor elétrico com termostato (modelo Roxin HT 1300 – 25W). Vinte microcosmos foram distribuídos aleatoriamente em quatro tratamentos de temperatura. O tratamento controle correspondeu à temperatura média anual para a região onde foi executado o experimento. Essa estimativa foi obtida através do Sistema de Meteorologia e Hidrologia do Estado de Goiás (Simehgo, 2017). O incremento da temperatura foi estabelecido de acordo com as previsões propostas pelo Modelo de Circulação Climática Global Atmosfera-Oceano (AOGCM) *Community Climate System Model* (CCSM), disponível na base de dados Ecoclimate (http://ecoclimate.org/; Lima-Ribeiro et al., 2015). Esse modelo possui quatro cenários de aquecimento futuros (*Representative Concentration Pathways* – RCP) elaborados com base nos níveis de forçamento radioativo previstos para o ano de 2100 (RCP 2.6 cenário otimista; RCP 4.5 e RCP 6.0 cenário intermediário; RCP 8.5 cenário pessimista; Moss et al., 2008). O cenário intemediário prevê um aumento médio 2°C em relação a temperatura média atual, enquanto o cenário pessimista supõe um aumento de 4°C.

Os microcosmos foram aquecidos buscando simular esses diferentes cenários de temperatura. Assim o experimento foi construído com cinco réplicas dos seguintes tratamentos: Controle (C – 24°C): temperatura média atual para a região onde foi construído o experimento; Intermediário (I – 26°C): representa uma média para o incremento na temperatura previsto nos cenários otimista e intermediário; Pessimista (P – 28°C) representa um cenário pessimista de aquecimento global e Pessimista + Pessimista (PP – 32°C): indica uma situação extrema no incremento na temperatura, com um cenário pessimista mais um aumento de 100%. O cenário RCP 8.5 é o mais utilizado na literatura para definir um maior estado de aquecimento, no entanto, ele não atua como um limite superior para as possíveis emissões, já que elas dependem diretamente das incertezas nas atividades antrópicas para o próximo século (Hayhoe et al., 2017). Assim, embora o tratamento PP não represente diretamente nenhum cenário de aquecimento real predito para futuro, ele foi utilizado para entender como um aumento na temperatura considerando o dobro do que é experado por um cenário pessimista poderá afetar as comunidades.

O experimento foi conduzido durante o mês de abril de 2016, no campus da Universidade Estadual de Goiás (UEG), Anápolis, Goiás, Brasil (-16°22'52.86" -48°56'45.43"W). Cada microcosmo foi preenchido com 18,2 litros de água coletados em uma lagoa oligo-mesotrófica, situada no Campus da UEG. Destes, 2,2 L foram coletados com rede de plâncton com abertura de malha de 20µm, buscando maximizar a coleta de espécies que ocorrem em menor abundância e garantir que grande parte dos grupos taxonômicos fosse representada nas unidades experimentais. Os outros 16L foram coletados sem filtragem prévia. A obtenção dos organismos diretamente do seu habitat natural permite a utilização de populações geneticamente diversas e que coexistem naturalmente (Altermatt et al., 2015). Após o preenchimento dos microcosmos (dia 04 de abril de 2016) foram aguardadas cerca de 48 horas para a estabilização da temperatura e aclimatação dos organismos. Assim, o experimento teve início no dia 06 de abril de 2016 e foi finalizado em 26 de abril de 2016, totalizando 20 dias. Esse período temporal condiz com tempo de geração dos organismos (Domaizon et al., 2012), e está de acordo com outros estudos experimentais realizados anteriormente envolvendo a microbiota planctônica (p. ex., Lara et al., 2013; Lindh et al., 2013; Bergen et al., 2016; Menden-Deuer et al., 2018).

Para evitar o esgotamento de nutrientes, a cada quatro dias, foram adicionados 5.40 mg L^{-1} de nitrato de sódio ($NaNO_3^-$) e 0.34 mg L^{-1} de fosfato de potássio (KH_2PO_4), os quais representam respectivamente 0.2 mg L^{-1} de nitrato (NO_3^-) e 0.002 de fosfato (PO_4^{-3}). Essas adições tiveram como base a concentração natural de nitrato e ortofosfato da lagoa na qual a água foi coletada, considerando a proporção de Redfield 1:16 (Reynolds, 2006). No 10° dia de experimento também foram adicionados em cada aquário 500 mL de água deionizada, procurando recuperar as perdas por evaporação (p. ex. McKee et al., 2000; Ekvall & Hansson, 2012). Ao longo do experimento, a temperatura nos tratamentos oscilou em média 3°C para mais ou para menos em torno dos valores previamente estabelecidos (24°C, 26°C, 28°C e 32°C) acompanhando as oscilações do tempo (dias de sol ou dias de chuva). No entanto, os tratamentos aquecidos sempre estiveram com temperaturas maiores que o controle. Durante o experimento, um aquecedor referente ao tratamento controle apresentou falhas em seu funcionamento. Assim, essa réplica foi excluída de todas as análises realizadas posteriormente.

Coleta das amostras

No final do experimento foram coletados 500 mL de água em cada microcosmo utilizando frascos de polietileno. As amostras foram armazenados em refrigerador (cerca de 2°C) até o momento da filtragem, que ocorreu em até 24 horas após a coleta. Com auxílio de uma bomba a vácuo, a primeira filtragem foi realizada utilizando filtro de celulose Millipore com espessura de 3µm, o que permitiu capturar a microbiota eucariota presente na amostra. A água resultante desse processo passou por uma nova filtragem, porém com filtro de celulose Millipore com 0,22 µm de espessura. Nesse caso, a intenção era capturar a microbiota procariota. Cada um dos filtros (3 µm e 0,22 µm) foram colocados separadamente em tubos de plástico e armazenados em nitrogênio líquido a -80°C.

Extração do DNA, amplificação e sequenciamento

O DNA total dos organismos eucariotos (filtro de 3 µm) e procariotos (0,22 µm) foi extraído seguindo o protocolo do Kit para extração de DNA *PowerWater® DNA Isolation Kit.* O DNA extraído foi analisado e quantificado em gel de agarose a 1%. Para o DNA eucarioto, um fragmento hipervariável (~400pb) de 18S rDNA foi amplificado através da reação em cadeia da polimerase (PCR) utilizando os *primers* TAReuk45FWD1 e TAReuKREV3 seguindo o protocolo descrito no kit *Taq PCR Master Mix Qiagen*. Para o DNA procarioto, a amplificação foi conduzida utilizando os *primers* BAC341F e BAC805R para 16S rDNA de acordo com o *Kit ReadyMix*^{TM} *TakPCR*

Reation Mix. As amplificações foram realizadas em triplicatas e o DNA resultante analisado em gel de agarose 1,5% e purificado usando o *Agencourt AMPure XP Beads* (Beckman Coulter).

Durante a preparação das bibliotecas Illumina, as triplicatas foram agrupadas em um único tubo por réplica do tratamento e em seguida foram inseridos *indexes* de acordo com o *Kit Nextera XT Index 2*, através de uma reação em cadeia da polimerase (PCR) de ciclo limitado. As amostras foram novamente purificadas utilizando o *AMPure XP Beads*. Os fragmentos para eucarioto apresentaram em média 400pb e os de procarioto 600pb. As bibliotecas indexadas foram quantificadas por *Real Time PCR* com o *Kappa Library Quantification kit* e validadas pelo *Bioanalyzer*. O tamanho de cada amplicon foi estimado para a normalização das bibliotecas. As bibliotecas de cada réplica foram normalizadas para 4 nM e sequenciadas utilizando o *MiSeq Reagent Kit v3* (600 ciclos).

A qualidade das sequências foi avaliada utilizando o software FastQC (Andrews, 2010). Sequências que apresentaram tamanho menor que 100pb ou ainda as bases com valor de Phred < 20 foram excluídas através do software Trimmomatic (Bolger et al., 2014). Nessa etapa também foram removidos os adaptadores. A predição das unidades taxonômicas operacionais (OTUs) foi realizada utilizando o pipeline UPARSE (Edgar et al., 2013), que consiste em (i). montagem das sequências; (ii). separação das sequências por tratamento; (iii). desreplicação com a identificação das sequências únicas e filtragem das quimeras; (iv). agrupamento das sequências com similaridade acima de 97% em uma mesma OTU; (v). obtenção da tabela de OTUs por tratamento. A predição taxonômica foi realizada por meio de um BLASTn (Altschul et al.,1990) das sequências representativas das OTUs contra o banco de dados Silva 128 usando um percentual de identidade de 97%. Sequências de metazoários ou que não pertencem a microbiota aquática (p. ex. plantas) foram removidas para as análises posteriores.

As OTUs para as quais foi possível atribuir um grupo taxonômico foram classificadas em grupos funcionais de acordo com sua fonte de carbono (p. ex. Simon et al., 2015; Fujimoto et al., 2016; Khomich et al., 2017). Organismos que possuem clorofila-a e obtêm carbono exclusivamente através da produtividade primária foram considerados como autotróficos (p. ex. Chlorophyta, Charophyta). Os heterotróficos foram representados por organismos que obtém carbono através da predação, parasitismo ou decomposição (p. ex. Ciliophora, Fungi, Amoebozoa) enquanto organismos que adquirem carbono tanto heterotroficamente como através da fotossíntese, foram considerados como mixotróficos (p. ex. membros putativos de Chryptophyta, Chrysophyta, Ochrophyta).

Assim, sete matrizes de dados foram utilizadas em nossas análises, buscando avaliar o efeito do aquecimento sobre os microorganismos planctônicos. São elas, (i). microbiota eucariota: composição das OTUs obtida pelo sequenciamento do 18S rDNA; (ii). microbiota procariota: composição das OTUs obtida pelo sequenciamento do 16 S rDNA; (iii). microbiota autotrófica procariota: inclui apenas os produtores primários procariotos; (iv). microbiota heterotrófica procariota: inclui apenas os heterotróficos procariotos; (v). microbiota autotrófica eucariota: inclui apenas os produtores primários eucariotos; (vi). microbiota heterotrófica eucariota: inclui apenas os heterotróficos eucariotos; (vii). microbiota mixotrófica eucariota: inclui apenas os mixotróficos eucariotos.

Análise de dados

Todas as análises foram realizadas no programa R (Core Team R, 2016) utilizando o pacote Vegan (Oksanen et al., 2016). Para investigar o efeito do aquecimento sobre a riqueza de OTUs procariotas e eucariotas e ainda para os diferentes níveis tróficos foi utilizada a Análise de Variância (ANOVA). Nessa análise a riqueza de OTUs atuou como a variável resposta e os níveis de temperatura como a variável preditora. Nas situações em que foram encontrados resultados significativos na Anova, os dados foram submetidos a um teste de Tukey buscando verificar entre quais temperaturas essas diferenças ocorrem.

Análises de Variância Permutacional – Permanova (Zar, 2010) foram utilizadas para avaliar o efeito do aquecimento sobre a composição da microbiota procariota, eucariota e dos grupos tróficos. O pressuposto da homogeneidade de variância foi testado através da função *betadisper*

(Anderson, 2006). Todas as matrizes testadas apresentaram uma variação homogênea. Na permanova, a presença e ausência das OTUs representou a variável resposta e os tipos de tratamento o preditor categórico. Nessa análise, a matriz de ocorrência das OTUs foi convertida em uma matriz de distância de Jaccard. Nas situações em que foram constatadas diferenças significativas na Permanova, foi utilizada uma Análise de Escalonamento Multidimensional não Métrico – NMDS (Legendre & Legendre, 1998) para avaliar entre quais tratamentos essas diferenças ocorrem. Para o NMDS a composição de OTUs também foi representada pelos dados de presença e ausência convertidos em uma matriz de distância de Jaccard.

Para avaliar a concordância entre a microbiota eucariota e procariota, assim como entre os diferentes níveis tróficos (microbiota autotrófica, heterotrófica e mixotrófica) foi utilizado o teste de Procrustes (Legendre & Legendre 1998). Nesta análise, a composição de OTUs (presença ou ausência) foi convertida em uma matriz de distância Jaccard e em seguida submetida a uma NMDS. Os dois primeiros eixos da NMDS foram correlacionados para as diferentes classificações biológicas e níveis tróficos através do teste de Procrustes. O resultado do teste de Procrustes (estatística r) varia de 0 (ausência de concordância) a 1 (perfeita concordância).

Resultados

O sequenciamento de todas as amostras resultou em 6,888,566 leituras para a microbiota procariota e 4,352,080 leituras para microbiota eucariota. Após a filtragem de qualidade, foram obtidas 4,506,636 e 2,708,476 leituras para procariotos e eucariotos, respectivamente. O Total de sequências montadas foi de 52,055 para procariotos, as quais foram preditas em 272 OTUs. Já para eucariotos, foram obtidas 339,908 sequências montadas, preditas em 311 OTUs. Para 143 OTUs procariotas e 165 OTUs eucariotas não foi possível atribuir uma classificação taxonômica, já que estes não possuíam representantes na base de referência utilizada para o BLAST. Entre as OTUs classificadas taxonomicamente, seis OTUs procariotas e quatro OTUs eucariotas representavam organismos não planctônicos, enquanto 15 OTUs eucariotas foram atribuídos a metazoários, e, portanto não foram considerados. Assim, 123 OTUs procariotas e 127 OTUs eucariotas constituíram o conjunto de dados utilizados em nossas análises (ver tabela 1S e 2S no material suplementar).

Entre os organismos procariotos, Proteobacteria foi o grupo com maior número de OTUs em todas as temperaturas, seguido de Bacteroidetes nos tratamentos C e I, Bacteriodetes e Actinobacteria no tratamento P e por Cyanobacteria e Actinobacteria no tratamento PP (Tabela 1). Para eucariotos, Cercozoa e Chlorophyta apresentaram o maior número de OTUs no tratamento C, Chlorophyta e Fungi no tratamento I enquanto Chlorophyta, Ciliophora e Fungi no tratamento P e PP (Tabela 1). Entre os organismos procariotos foram encontrados 105 OTUs heterotróficos, 08 autotróficos, enquanto 10 OTUs não apresentaram uma resolução taxonômica suficiente para ser classificados quanto sua fonte de carbono. Para eucariotos, foram encontrados 32 OTU autotróficos, 83 heterotróficos e 12 mixotróficos. O número de OTUs para a microbiota total e por grupo trófico tanto de procariotos (Figura 1) como de eucariotos (Figura 2) não diferiu entre os níveis de temperatura (Tabela 2). A única exceção ocorreu para a riqueza de eucariotos mixotróficos, no qual o tratamento C apresentou em média três OTUs a mais que o tratamento I (Figura 2).

Tabela 1 – Número de OTUs (%) procariotos e eucariotos encontrados nos diferentes cenários de aquecimento futuro, de acordo com o grupo taxonômico. DP = Desvio padrão. C = 24°C, I = 26°C, P = 28°C e PP = 32°C.

	C		I		P		PP	
	Média	(±DP)	Média	(±DP)	Média	(±DP)	Média	(±DP)
Procariotos								
Acidobacteria	1	1	1	1	1	2	1	2
Actinobacteria	8	2	10	3	9	3	8	3
Armatimonadetes	2	2	1	1	1	1	1	1
Bacteroidetes	10	3	11	5	9	1	7	4
Chlorobi	1	1	1	1	0	0	1	1
Cyanobacteria	7	3	6	3	7	2	8	3
Planctomycetes	1	2	2	1	1	1	1	2
Proteobacteria	67	5	66	6	69	3	71	5
Spirochaetes	2	1	1	1	2	1	1	1
Verrucomicrobia	1	1	1	1	1	1	1	1
Eucariotos								
Amoebozoa	4	2	7	5	8	4	9	4
Ancyromonadida	0.5	1	0	0	0	0	0	0
Bicosoecida	3	0.5	3	1	2.5	0.5	1	1
Cercozoa	18	4	16	1	15	1	12	5

Charophyta	6	0.5	7	1	8	1	7	3
Chlorophyta	18	3	20	2	20	3	20.5	4
Choanoflagellida	0	0	0	0	0	0	1.5	1
Ciliophora	16	2	15	7	16	2	16	4
Dinoflagellata	2	1	2	0.5	2	1	4	1
Fungi	16	5	17	6	16	4	16	3
Ichthyosporea	0.5	1	1	1	0	0	0	0
Ochrophyta	15	2	11	3	11	3	12	5
Perkinsidae	1	1	0	0	0	0	0	0
Peronosporomycetes	0	0	0	0	0.5	1	0	0
Protosteliales	0	0	1	1	1	1	1	1

Tabela 2 – Análise de Variância (Anova) realizada entre a riqueza de OTUs e os diferentes níveis de aquecimento. Os números em parênteses indicam o grau de liberdade para cada teste. Comparações par a par pelo teste de Tukey foram realizadas para as situações em que a Anova apresentou resultados significativos (P<0,05). N.A. = Não aplicável.

	SQ	MQ	F	*P*	Teste de Tukey
Microbiota procariota $_{(3,15)}$	753	251.1	1.18	0.35	N.A.
Microbiota eucariota $_{(3,15)}$	565.4	188.5	1.07	0.39	N.A.
Microbiota autotrófica procariota $_{(3,15)}$	1.36	0.45	0.15	0.92	N.A.
Microbiota heterotrófica procariota $_{(3,15)}$	646.8	215.6	1.45	0.26	N.A.
Microbiota autotrófica eucariota $_{(3,15)}$	31.4	10.3	0.58	0.63	N.A.
Microbiota heterotrófica eucariota $_{(3,15)}$	191.9	63.9	0.89	0.46	N.A.
Microbiota mixotrófica eucariota $_{(3,15)}$	30.28	10.1	3.65	0.03	C#I; C=P,C=PP; I=P, I=PP; P=PP

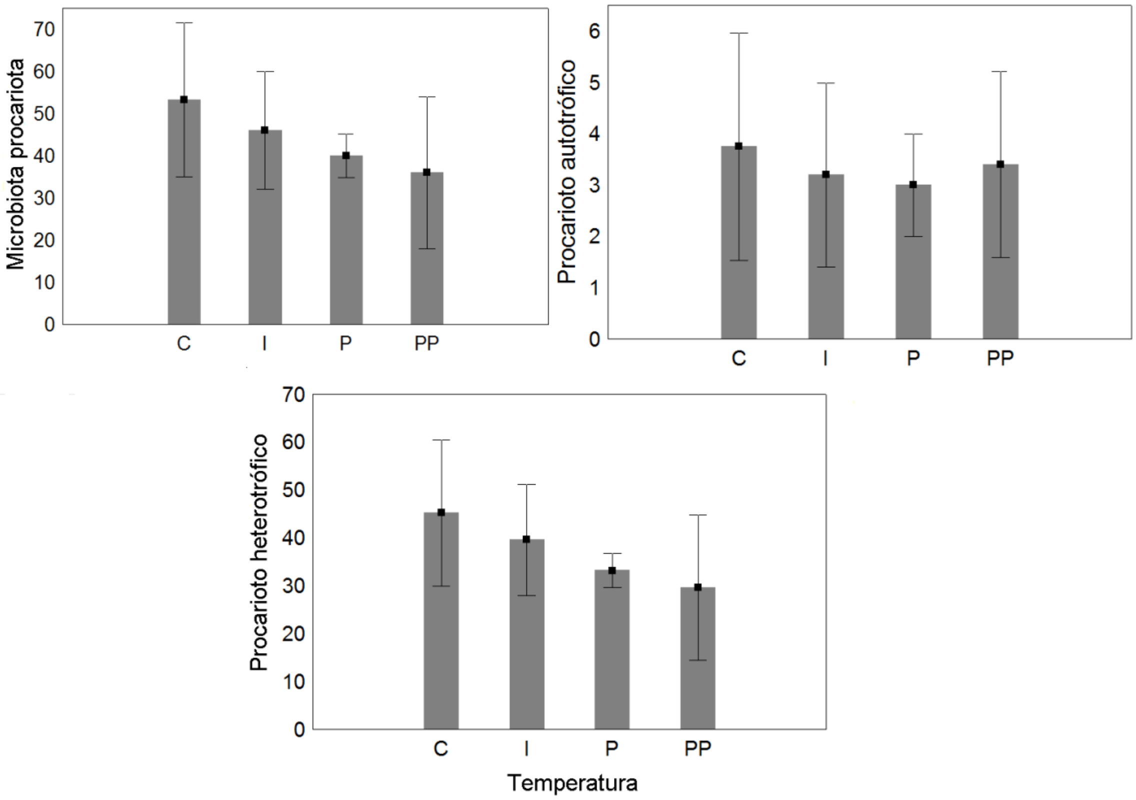

Figura 1 – Riqueza de OTUs total e por grupo trófico para organismos procariotos entre os diferentes níveis de aquecimento. Os símbolos representam o valor médio e as barras verticais o desvio padrão. C = 24°C, I = 26°C, P = 28°C e PP = 32°C.

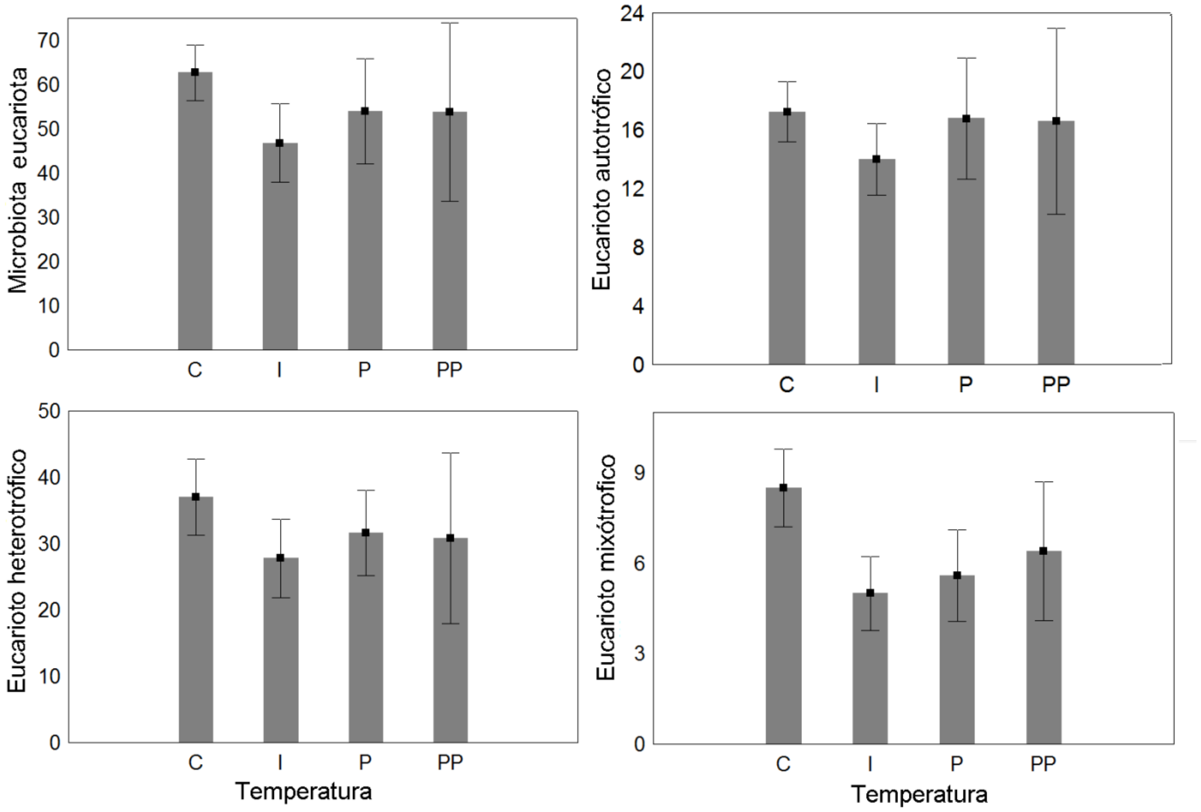

Figura 2 - Riqueza de OTUs total e por grupo trófico para organismos eucariotos entre os diferentes níveis de aquecimento. Os símbolos representam o valor médio e as barras verticais o desvio padrão. C = 24°C, I = 26°C, P = 28°C e PP = 32°C.

O aquecimento influenciou a composição da microbiota procariota e eucariota (Tabela 3). Em ambas as classificações foram encontradas diferenças entre as temperaturas C e PP, I e PP, P e PP, embora para eucariotos essa diferenciação entre os grupos tenha demonstrado um padrão mais claro na ordenação NMDS (Figura 3 e 4). Algumas OTU estiveram diretamente associadas à temperatura PP, possivelmente contribuindo para a distinção na composição das comunidades nessa temperatura em relação às demais. Entre elas destacam-se para procariotos as OTU correspondentes aos grupos Proteobacteria e Cyanobacteria como Rickettsiales (OTU 101), Obscuribacterales (OTU 157), *Rhodopila* sp. (OTU 263), *Bdellovibrio* sp. (OTU 108), *Methylobacterium* sp. (OTU 158), *Acidovorax* sp. (OTU 147), *Pseudoxanthobacter* sp. (OTU 239), *Methylobacillus* sp. (OTU 268), *Elstera* sp. (OTU 54) (Figura 3). Para eucariotos, as OTU associadas à temperatura PP referem-se às espécies *Ochromonas sphaerocystis* (OTU 149), *Pseudomuriella* sp. (OTU 98), *Orphella* *catalaunica* (OTU 191), *Arcella hemisphaerica* (OTU 188), *Scenedesmus muspupukensis* (OTU 122), *Spumella* sp. (OTU 90), Tubilinida (OTU 117) e *Leptopharynx* sp. (OTU 185) (Figura 3).

Tabela 3 – Análise de Variância Permutacional para composição de OTUs procariotos, eucariotos e grupos tróficos. GL = Graus de liberdade.

	GL	R^2	F	*P*
Microbiota procariota	3,15	0,21	1,35	**0.01**
Microbiota eucariota	3,15	0,25	1,67	**0.001**
Microbiota autotrófica procariota	3,15	0,06	0,36	0.96
Microbiota heterotrófica procariota	3,15	0,21	1,40	**0.009**
Microbiota autotrófica eucariota	3,15	0,18	1,17	0.28
Microbiota heterotrófica eucariota	3,15	0,32	2,35	**0.01**
Microbiota mixotrófica eucariota	3,15	0,25	1,73	**0.001**

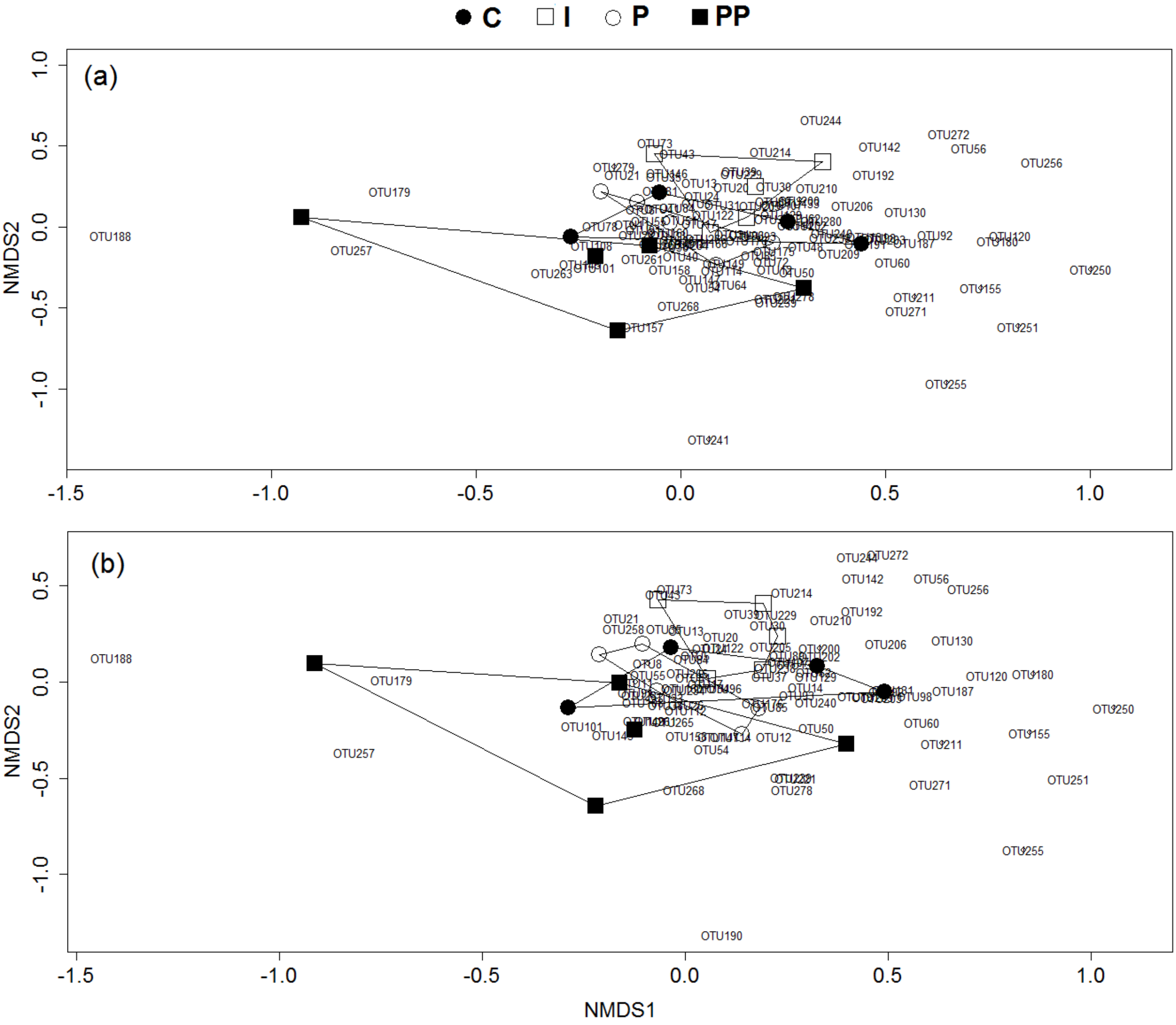

Figura 3 – Análise de escalonamento multidimensional não métrico (NMDS) para composição total de OTUs e por grupos tróficos procariotos. Aqui foram consideradas apenas as classificações biológicas que apresentaram diferenças significativas na Permanova. Em (a): microbiota procariota e em (b): microbiota procariota heterotrófica. Os símbolos indicam a temperatura, sendo C = 24°C, I = 26°C, P = 28°C e PP = 32°C. Ver tabela 1S no material suplementar para a classificação taxonômica das OTUs de acordo com o número indicado.

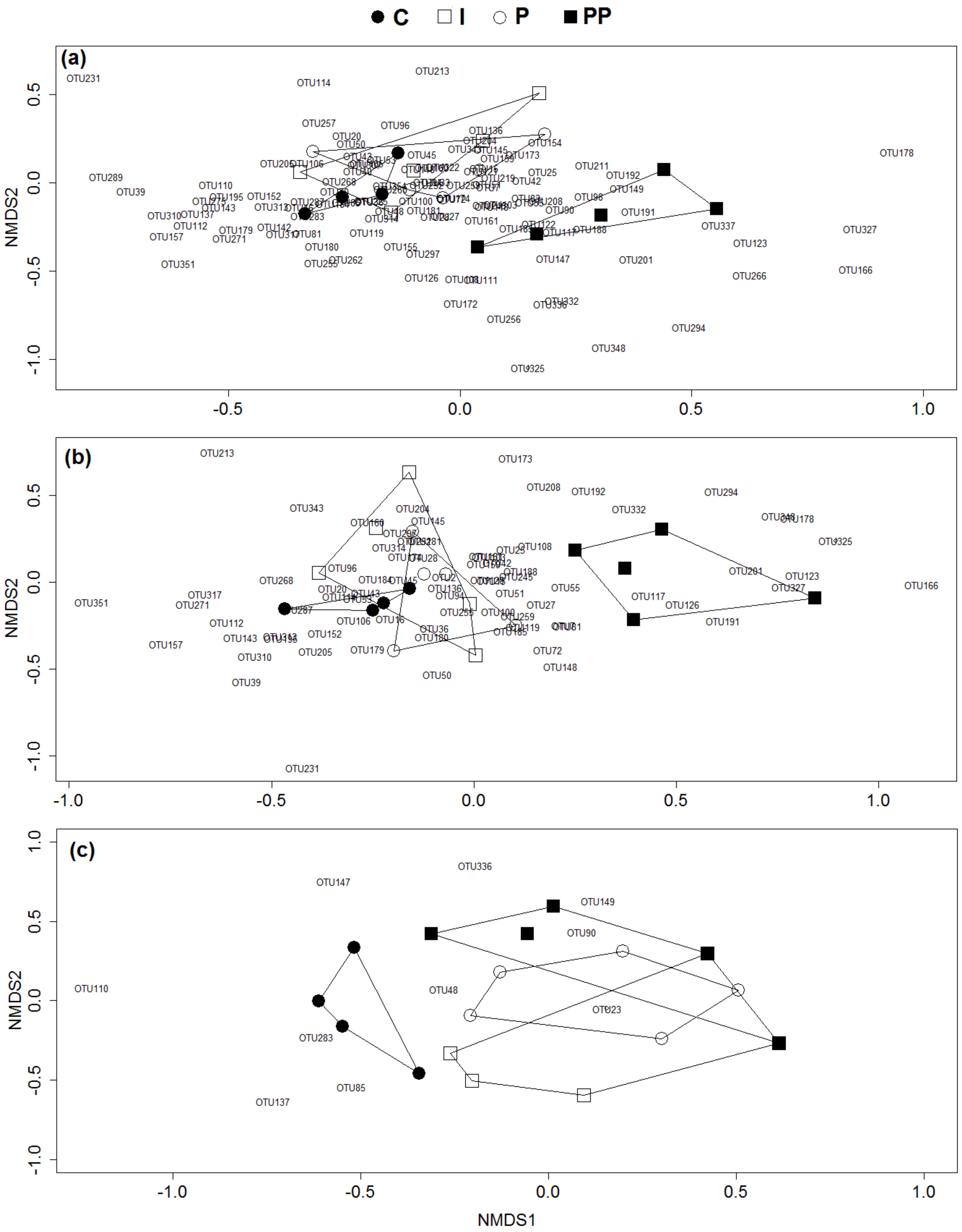

Figura 4 – Análise de análise de escalonamento multidimensional não métrico (NMDS) para composição total de OTUs e por grupos tróficos eucariotos. Aqui foram consideradas apenas as classificações biológicas que apresentaram diferenças significativas na Permanova. Em (a):

microbiota eucariota, (b): microbiota eucariota heterotrófica e (c): microbiota eucariota mixotrófica. Os símbolos indicam a temperatura, sendo C = 24°C, I = 26°C, P = 28°C e PP = 32°C. Ver tabela 2S no material suplementar para a classificação taxonômica das OTUs de acordo com o número indicado.

Algumas OTUs procariotas e eucariotas foram observadas em apenas um tipo de tratamento. Para procariotos, as OTU classificadas taxonomicamente como Armatimonadales (OTU 53), Neisseriaceae (OTU 130), *Zavarzinella* sp. (OTU 150), Cytophagaceae (OTU 180), *Coxiella* sp. (OTU 229), Hyphomicrobiaceae (OTU 250) e *Chromobacterium* sp. (OTU 257) foram exclusivas do tratamento C; Fimbriimonadaceae (OTU 137) para o tratamento I; nenhuma OTU ocorreu exclusivamente no tratamento P, enquanto *Acidocella* sp. (OTU 190), *Dinghuibacter* sp. (OTU 194), Acidimicrobiaceae (OTU 241), Chitinophagaceae (OTU 255), *Rhodopila* sp. (OTU 263) ocorreram apenas no tratamento PP. Entre os eucariotos, as OTU representadas por, *Carchesium polypinum* (OTU 114), *Parvilucifera* sp. (OTU 157), *Ancyromonas* sp. (OTU 310) ocorreram apenas no tratamento C; nenhuma OTU foi exclusiva do tratamento I; *Aphanomyces* sp. (OTU 231) foi exclusiva no tratamento P e *Ophrydium versatile* (OTU 88), *Salpingoeca* sp. (OTU 166), *Mougeotia* sp. (OTU 266), *Cladochytrium replicatum* (OTU 325) e *Leptomyxa reticulata* (OTU 348) no tratamento PP.

Quanto aos grupos tróficos procariotos, o aquecimento gerou efeitos apenas sobre a composição dos organismos heterotróficos (Tabela 3), porém essas diferenças na composição das OTUs foram observadas apenas entre as temperaturas I e PP (Figura 3). As principais OTUs associadas à temperatura I foram *Bdellovibrio* sp. (OTU 20), Rhizobiales (OTU 24), *Legionella* sp. (OTU 122), Sporichthyaceae (OTU 13), *Rhodovarius* sp. (OTU 39), *Azospirillum* sp. (OTU 30) (Figura 3), enquanto na temperatura PP destacam-se Rickettsiales (OTU 101 e OTU 179), *Pedomicrobium* sp. (OTU 145), *Elstera* sp. (OTU 54), *Roseomonas* sp. (OTU 12), *Bryobacter* sp. (OTU 265) e *Methylobacillus* sp. (OTU 268) (Figura 3).

Para eucariotos, o aquecimento afetou os organismos heterotróficos e mixotróficos (Tabela 3), sendo que para a microbiota heterotrófica essas diferenças ocorreram entre as temperaturas C e P, C e PP, I e PP, P e PP enquanto para mixotróficos as diferenças foram evidenciadas entre a temperatura C e as demais (Figura 4). Entre os heterotróficos, as principais OTUs associadas à temperatura C foram Rhinosporideaceae (OTU 287), *Spongomonas* sp. (OTU 53) e táxons representantes de Ciliophora como *Loxophyllum* sp. (OTU 43) e *Carchesium polypinum* (OTU 114). Na temperatura I, *Bodomorpha* sp. (OTU 160), *Paramicrosporidium* sp. (OTU 204), Dactylopodida (OTU 145), *Cercomonas* sp. (OTU 314), *Rhogostoma* sp. (OTU 96), *Hannaella oryzae* (OTU 184). Para a temperatura P destacam-se Cryptomycota (OTU 2), *Cryptodifflugia operculata* (OTU136) e Cercozoa (OTU 94, OTU 255, OTU 36 e OTU 180) enquanto para o tratamento PP Tubilinida (OTU 117), *Tetrahynema orphan* (OTU 126), Cryptomycota (OTU 201) e Cercozoa (OTU 327) (Figura 4). Para os organismos mixotróficos, as principais OTU associadas à temperatura C permitindo a sua diferenciação dos demais tratamentos foram *Lagynion scherffelii* (OTU 283) e Chromulinales (OTU 85), ambas pertencentes à classe Chrysophyceae (Figura 4).

A microbiota procariota e eucariota apresentou concordância na resposta aos diferentes níveis de aquecimento (Tabela 4). Esse mesmo padrão foi observado nas comparações entre eucariotos heterotróficos e os demais níveis tróficos de eucariotos. Porém não observamos concordância entre eucariotos autotróficos e eucariotos mixotróficos (Tabela 4). Para procariotos, nenhum nível trófico apresentou resposta concordante ao outro. Nas comparações para os grupos tróficos entre os níveis biológicos, apenas os organismos heterotróficos tem um padrão de resposta similar ao aquecimento (Tabela 4). Embora tenham sido encontradas respostas similares, o valores de correlação obtidos no teste de procrustes foram baixos ($r < 0,50$).

Tabela 4 – Valores de r e *P* para os testes de Procrustes realizados entre as diferentes classificações biológicas.

Dados comparados	r	*P*
Procarioto e Eucarioto	0,42	**0,04**
Eucarioto heterotrófico e eucarioto autotrófico	0,46	**0,02**
Eucarioto heterotrófico e eucarioto mixotrófico	0,42	**0,05**
Eucarioto autotrófico e eucarioto mixotrófico	0,37	0,15
Procarioto heterotrófico e procarioto autotrófico	0,40	0,07
Eucarioto heterotrófico e procarioto heterotrófico	0,48	**0,02**
Eucarioto autotrófico e procarioto autotrófico	0,29	0,38

Discussão

É relevante predizer como os micro-organismos respondem às variações na temperatura, principalmente diante dos inúmeros distúrbios ambientais observados atualmente e já previstos para o fim do século (Menden-Deuer et al., 2018). Neste estudo, nós avaliamos o efeito do aquecimento sobre diferentes grupos biológicos e níveis tróficos para microbiota planctônica utilizando uma abordagem experimental. Nós não observamos diferenças na riqueza de OTUs entre as diferentes temperaturas para a maioria das classificações adotadas. Porém, nós constatamos que o aumento da temperatura influencia tanto os organismos procariotos quanto os eucariotos, alterando os padrões de composição das comunidades em um cenário pessimista de aquecimento. Além disso, bactérias e protistas tem um padrão de resposta concordante. Os efeitos do aquecimento parecem ser mais severos sobre os mixotróficos eucariotos, uma vez que diferenças na composição já foram constatadas com o aumento de apenas 2°C tanto para riqueza como para composição de OTUs. Para heterotróficos, as diferenças foram observadas apenas entre o cenário extremo de aquecimento PP e os demais. Porém, não observamos efeitos do incremento da temperatura sobre os organismos autotróficos, tanto para eucariotos quanto para procariotos.

A temperatura representa um fator limitante para a maior parte dos organismos aquáticos, determinando importantes processos ecológicos nas comunidades, como a produtividade primária (p. ex. Häder et al., 2014; Yvon-Durocher et al., 2015), decomposição (p. ex. Geraldes et al., 2012), respiração (p. ex. Hoope et al., 2008; Panigrahi et al., 2013), ciclo do carbono (p. ex. Wohlers et al., 2009, Yvon-Durocher et al., 2010). Alguns estudos têm demonstrado que o aquecimento promove uma redução na riqueza de espécies (p. ex. Peblani et al., 2015; Pesce et al., 2018), geralmente diminuindo a diversidade das comunidades (p. ex. Bergkemper et al., 2018). Porém, resultados contrários já foram observados, com a ausência de efeitos (Andrushchyshyn et al., 2009; Domaizon et al., 2012) ou ainda com o aumento na riqueza diante do incremento na temperatura (p. ex. Smale et al., 2017). Em nosso experimento, não observamos diferenças na riqueza de OTUs entre os tratamentos para maioria dos grupos biológicos e níveis tróficos avaliados, com exceção apenas para a riqueza de mixotróficos eucariotos. Isso indica que o aquecimento provavelmente não levou a uma alteração no número de OTUs que poderiam refletir na riqueza, mas sim a sua substituição promovendo a permanência daqueles que representam grupos mais adaptados a temperaturas elevadas. De fato, essa premissa é confirmada quando avaliamos a composição das comunidades, na qual as respostas foram específicas para cada grupo taxonômico, com algumas OTUs ocorrendo em apenas alguns tratamentos.

Em nosso estudo, os principais grupos taxonômicos não foram encontrados exclusivamente em uma única temperatura. As OTUs correspondentes a Proteobacteria ocorreram em todos os tratamentos para procariotos, enquanto eucariotos foram representados principalmente por OTUs atribuídas a Cercozoa, Chlorophyta, Ciliophora e Fungi. Isso indica que as mudanças na composição podem ter ocorrido pelo crescimento de táxons específicos (p. ex. Lindh et al. 2013; Bergen et al., 2016). De fato, algumas OTUs procariotas e eucariotas ocorreram exclusivamente no tratamento PP ou estiveram mais associadas a essa temperatura, o que pode ter contribuído para uma diferenciação deste tratamento em relação aos demais. Muitas dessas OTUs representam táxons que conseguem tolerar altas temperaturas, tais como *Methylobacterim* sp. (Kovaleva et al., 2014) e *Methylobacillus* sp. (Kaparullina et al., 2017) ou que tem o crescimento positivamente associado com o aquecimento como *Bdellovibrio* sp. (Kelley et al., 1997), *Spumella* sp. (Weisse, 1997) e *Arcella hemisphaerica* (Arrieira et al. 2016). Além disso, entre as OTUs encontradas no tratamento PP, estão alguns táxons capazes de causar doenças nos seres humanos, como os procariotos da ordem Ricktissiales (Schrallhammer et al., 2013) e o gênero *Methylobacterium* sp. (Kovaleva et al., 2014). Desse modo, além de gerar consequências ecológicas com a substituição das espécies, o aquecimento global previsto para as próximas décadas também pode trazer ameaças à saúde e bem estar humano através da proliferação de organismos patógenos favorecidos com o aquecimento (Ichiru Kurame, 2010).

Embora alguns estudos já tenham demonstrado o efeito do aquecimento sobre os produtores primários (p. ex. Domaizon et al., 2012; Yvon-Durocher et al., 2015; Pulina et al., 2016; Rasconi et al., 2017), nós não observamos diferenças para a composição dos organismos autotróficos em nenhuma das classificações biológicas. Em todos os tratamentos, os autotróficos eucariotos foram representados principalmente por clorofíceas enquanto os procariotos por cianobactérias. Estudos anteriores apontam que a temperatura ótima para o crescimento desses dois grupos é muito semelhante, especialmente na região tropical (Thomas et al., 2016). Cianobactérias tem crescimento ótimo entre 25°C e 35 °C enquanto Chlorophyceae cresce entre 27.5 e 35°C (Lürling et al., 2013). Desse modo, os dois grupos parecem apresentar uma tolerância termal que lhes permite sobreviver tanto em temperaturas elevadas como nas mais amenas, contribuindo para a ausência de efeitos das diferentes temperaturas utilizadas em nosso experimento.

Por outro lado, os organismos heterotróficos tiveram sua composição influenciada pela temperatura tanto para os procariotos como para os eucariotos. De fato, os processos heterotróficos devem ser mais afetados pelo incremento na temperatura do que os autotróficos (p. ex. Hoope et al., 2008; Panigrahi et al., 2013; Von Scheibner et al., 2014; Huete-Stauffer et al., 2018). Devido a ligação dos organismos heterotróficos a outras comunidades dentro da cadeia alimentar, alterações na sua composição podem trazer sérias consequências para as redes alimentares (Domaizon et al., 2012), levando por exemplo, a um aumento no consumo dentro dos ecossistemas (Yang et al., 2016). Esses organismos são responsáveis pela remineralização da matéria orgânica e reciclagem do CO2 através da respiração (Hoope et al. 2008). Assim, alterações na sua composição podem trazer consequências para funções ecossistêmicas como decomposição e ciclagem de nutrientes.

Em nosso estudo, encontramos representantes mixotróficos apenas para os eucariotos e este foi o nível trófico mais sensível, com diferenças na riqueza e composição evidenciadas já entre os tratamentos C e I. Os organismos mixotróficos combinam o hábito fotoautotrófico e heterotrófico para produção ou obtenção de matéria orgânica (Flynn et al., 2013). A ocorrência da produtividade primária ou secundária depende tanto da temperatura como da disponibilidade de luz no ambiente (p. ex. Princiotta et al., 2013). Assim, na maior parte dos ecossistemas aquáticos com disponibilidade de luz, é provável que exista um contínuo entre os dois hábitos (Flynn et al., 2013). Evidências experimentais indicam que o aquecimento promove o predomínio de um comportamento heterotrófico nos mixotróficos (Wilken et al., 2012; Wilken et al., 2018). Embora nosso experimento não tenha sido desenhado para quantificar essas mudanças de hábitos, podemos sugerir que devido à plasticidade na obtenção de matéria orgânica, pequenas alterações na temperatura podem ser capazes de influenciar a composição da microbiota mixotrófica, levando a uma redução na sua riqueza. No entanto, estudos futuros que analisem essas alterações em nível de comunidades e com interações de outros fatores ambientais são necessários para compreender melhor os efeitos do aquecimento global sobre esse nível trófico.

Em termos de composição, um resultado importante foi a resposta similar obtida entre procariotos e eucariotos diante do aquecimento. Os dois níveis biológicos apresentaram uma substituição de espécies entre o cenário de aquecimento PP e os demais, embora os organismos eucariotos tenham mostrado um padrão de diferenciação mais claro no NMDS. De fato, evidências apontam que eucariotos possuem uma menor tolerância termal quando comparados aos procariotos (Clarck, 2014). Esse fato pode ser decorrente das características biológicas de cada grupo. As bactérias surgiram e evoluíram em um período no qual a terra estava submetida a altas temperaturas (Oschmann et al., 2002) e até os dias atuais são encontrados representantes de bactérias que conseguem sobreviver em temperaturas extremas (Clark, 2014). Por outro lado, protistas são compostos por inúmeros grupos taxonômicos (Pawlowski, 2014) que possuem tolerâncias termais distintas. Isso indica que houve uma resposta similar entre os dois níveis, porém o padrão de substituição provavelmente não ocorreu na mesma intensidade entre os grupos biológicos.

Nossos resultados também demonstram um padrão de resposta concordante entre organismos eucariotos heterotróficos e os demais níveis tróficos avaliados. Também observamos uma resposta similar entre heterotróficos procariotos e eucariotos. De fato, esses diferentes níveis tróficos estão diretamente associados a transferência de energia e nutrientes entre os vários componentes na cadeia alimentar microbiana. Os protistas autotróficos, mixotróficos assim como as cianobactérias são responsáveis pela fixação de CO2 em matéria orgânica, enquanto os protistas heterotróficos e mixotróficos através do consumo de micro-organismos influenciam a liberação de matéria orgânica assim como a decomposição por bactérias heterotróficas e fungos (Jassey et al., 2015; Worden et al., 2015). Isso indica que a alteração em um nível pode gerar consequências para o outro, mesmo que algum deles não tenha sido diretamente afetado pelo aquecimento. A ausência de concordância entre eucariotos autotróficos e mixotróficos ou ainda entre eucariotos e procariotos autotróficos pode ser atribuída a ausência de efeitos do aquecimento sobre os produtores primários em ambas as classificações biológicas.

Embora tenham sido identificadas respostas similares para a maior parte dos níveis avaliados, o valor de correlação entre eles foi baixo. Isso provavelmente ocorreu pois mesmo que as classificações respondam ao aquecimento PP, o padrão de substituição das espécies entre os diferentes níveis tróficos e classificações biológicas ocorreu de forma distinta. Ou seja, eles respondem ao aquecimento, mas o padrão de mudança não ocorre na mesma intensidade. Isso gerou um padrão de ordenação um pouco diferente e ocasionou um baixo valor de concordância no teste de procrustes. Em estudos de biomonitoramento envolvendo organismos aquáticos, correlações acima de 0,70 geralmente são consideradas adequadas para propor a substituição de níveis taxonômicos (p. ex. Gomes et al., 2015; Machado et al., 2015; Vieira et al. 2017) ou grupos biológicos (p. ex. Heino, 2010; Vieira et al. 2017), pois indicam que os níveis avaliados apresentam um padrão de resposta semelhante a variação ambiental. Assim, embora uma resposta similar tenha sido observada, devido ao baixo valor de concordância, recomendamos que os níveis biológicos e tróficos sejam avaliados separadamente para compreender os efeitos do aquecimento sobre a microbiota aquática.

De fato, compreender as respostas de diferentes grupos taxonômicos e funcionais ao incremento da temperatura é um fator crucial para prever a dinâmica das comunidades em um cenário de aquecimento global (Smale et al., 2017). De modo geral, nossos resultados indicam que o aumento de temperatura deve afetar diferentes níveis de organização biológica, alterando a composição da microbiota heterotrófica e mixotrófica. Os organismos procariotos e eucariotos apresentam uma resposta similar ao aquecimento. Porém, devido ao baixo valor de concordância, para fins de biomonitoramento é recomendado que estes níveis sejam avaliados separadamente. As mudanças climáticas aqui simuladas representam uma alteração na temperatura que deve ocorrer ao longo de décadas e a escala de tempo avaliada não é suficiente para determinar a adaptação dos micro-organismos a altas temperaturas (p. ex. Berger et al., 2016). No entanto, os experimentos fornecem uma ligação entre a teoria e os ecossistemas naturais, na qual as condições (p. ex. efeito da temperatura) são simuladas de forma simplificada, mas com alto nível de controle (Altermatt et al., 2015). Assim, nossos resultados contribuem para predizer os efeitos do aquecimento em diferentes níveis de organização biológica e grupos tróficos, principalmente para os ecossistemas aquáticos continentais na região tropical, que possuem uma alta biodiversidade e ainda é pouco estudada.

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MATERIAL SUPLEMENTAR

Tabela 1S – Classificação taxonômica e funcional para as Unidades Taxonômicas Operacionais (OTUs) procarióticas. Os grupos tróficos foram estabelecidos pela consulta a literatura, considerando o menor nível para a classificação taxonômica de cada OTU. OTUs com classificação taxonômica insuficiente não foram funcionalmente agrupadas. Nós consideramos a classificação taxonômica obtida através do Blast. NC = Não classificado.

OTU	**Reino**	**Filo**	**Classe**	**Ordem**	**Família**	***Gênero***	***Espécie***	**Grupo Trófico**	**Referências**
1	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Polynucleobacter*	*Polynucleobacter* sp.	Heterotrophic	Hahn et al., 2011
2	Bacteria	Actinobacteria	NC	Frankiales	Sporichthyaceae	NC	NC	Heterotrophic	Goodfellow et al., 2012
4	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*	*Novosphingobium* sp.	Heterotrophic	Takeuche et al., 2001
5	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Sediminibacterium*	*Sediminibacterium* sp.	Heterotrophic	Rosenberg et al., 2014
6	Bacteria	Cyanobacteria	NC	NC	FamilyI	*Synechococcus*	*Synechococcus* sp.	Autotrophic	Boone et al., 2001
7	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	*Sphingomonas* sp.	Heterotrophic	Rivadeneyra Torres et al., 2013
8	Bacteria	Actinobacteria	NC	Frankiales	Sporichthyaceae	NC	NC	Heterotrophic	Goodfellow et al., 2012
10	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	*Bdellovibrio* sp.	Heterotrophic	Stolp and Starr 1963
11	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*	*Novosphingobium* sp.	Heterotrophic	Takeuche et al., 2001
12	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Roseomonas*	*Roseomonas* sp.	Heterotrophic	Rihs et al., 1993
13	Bacteria	Actinobacteria	NC	Frankiales	Sporichthyaceae	NC	NC	Heterotrophic	Goodfellow et al., 2012
14	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Saprospiraceae	*Haliscomenobacter*	*Haliscomenobacter* sp.	Heterotrophic	Rosenberg et al., 2014
17	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NC	NC	Uncultured	Heterotrophic	Rosenberg et al., 2014
20	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	*Bdellovibrio* sp.	Heterotrophic	Stolp and Starr 1963
21	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NC	NC	NC	Heterotrophic	Erlacher et al., 2015 and their references
24	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NC	NC	NC	Heterotrophic	Erlacher et al., 2015 and their references
26	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	*Methylobacterium* sp	Heterotrophic	Erlacher et al., 2015 and their references
28	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Incertae Sedis	*Reyranella*	*Reyranella* sp.	Heterotrophic	Pagnier et al., 2011
30	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Azospirillum*	*Azospirillum* sp.	Heterotrophic	Triphati et al., 2013
31	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NC	NC	NC	NC
33	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Undibacterium*	*Undibacterium* sp.	Heterotrophic	Vandermaesen et al., 2017
34	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	*Devosia* sp.	Heterotrophic	Erlacher et al., 2015 and their references
35	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Pseudarcicella*	*Pseudarcicella* sp.	Heterotrophic	McBride et al., 2014
37	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NC	NC	NC	Heterotrophic	Hess et al., 2016
38	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sandarakinorhabdus*	*Sandarakinorhabdus* sp.	Heterotrophic	Gich & Overmann, 2006
39	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Rhodovarius*	*Rhodovarius* sp.	Heterotrophic	Kämpfer et al., 2004
40	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NC	NC	NC	NC
41	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NC	NC	NC	NC
43	Bacteria	Actinobacteria	NC	Frankiales	Sporichthyaceae	NC	NC	Heterotrophic	Goodfellow et al., 2012
48	Bacteria	Cyanobacteria	NC	NC	NC	NC	NC	Autotrophic	Boone et al., 2001
50	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Inquilinus*	*Inquilinus* sp.	Heterotrophic	Coenye et al., 2002
51	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	*Candidatus Megaira*	*Candidatus Megaira*	Heterotrophic	Scharallhammer et al.,

							polyxenophila		2013
53	Bacteria	Armatimonadetes	Armatimonadia	Armatimonadales	NC	NC	NC	Heterotrophic	Tamaki et al., 2011
54	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Elstera*	*Elstera* sp.	Heterotrophic	Rahalkar et al., 2012
55	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Incertae Sedis	*Candidatus*	*Candidatus finniella*	Heterotrophic	Hess et al., 2016
56	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Ferruginibacter*	*Ferruginibacter* sp.	Heterotrophic	Rosenberg et al., 2014
60	Bacteria	Armatimonadetes	Chthonomonadetes	Chthonomonadales	Chthonomonadaceae	*Chthonomonas*	*Chthonomonas* sp.	Heterotrophic	Jiang et al., 2016
62	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	NC	NC	Heterotrophic	Fuerst and Sangulenko, 2011
64	Bacteria	Cyanobacteria	NC	NC	FamilyI	NC	NC	Autotrophic	Boone et al., 2001
72	Bacteria	Cyanobacteria	Melainabacteria	Obscuribacterales	NC	NC	NC	Autotrophic	Boone et al., 2001
73	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Sediminibacterium*	*Sediminibacterium* sp.	Heterotrophic	Rosenberg et al., 2014
78	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	NC	NC	NC	NC	NC
81	Bacteria	Cyanobacteria	NC	NC	NC	NC	NC	Autotrophic	Boone et al., 2001
84	Bacteria	Spirochaetes	Spirochaetae	Spirochaetales	Leptospiraceae	*Leptospira*	*Leptospira* sp.	Heterotrophic	Rosenberg et al., 2014
85	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium*	*Sphingobium* sp.	Heterotrophic	Takeuche et al., 2001
89	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	*Chthoniobacter*	*Chthoniobacter* sp.	Heterotrophic	Sangwan et al., 2004
91	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	*Legionella* sp.	*Legionella* sp.	Heterotrophic	Richards et al., 2013
92	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NC	NC	NC	Heterotrophic	Rosenberg et al., 2014
93	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	*Phenylobacterium* sp.	Heterotrophic	Tiago et al., 2004
96	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Undibacterium*	*Undibacterium* sp.	Heterotrophic	Vandermaesen et al., 2017
98	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Heliimonas*	*Heliimonas* sp.	Heterotrophic	Rosenberg et al., 2014
101	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NC	NC	NC	Heterotrophic	Scharallhammer et al., 2013
107	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NC	NC	NC	Heterotrophic	Scharallhammer et al., 2013
108	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	*Bdellovibrio* sp.	Heterotrophic	Stolp and Starr 1963
111	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	NC	NC	Heterotrophic	Doronina et al., 2014
112	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	*Bradyrhizobium* sp.	Heterotrophic	Erlacher et al., 2015 and their references
114	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	*Sphingomonas* sp.	Heterotrophic	Rivadeneyra Torres et al., 2013
120	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Ferruginibacter*	*Ferruginibacter* sp.	Heterotrophic	Rosenberg et al., 2014
122	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	*Legionella*	*Legionella* sp.	Heterotrophic	Richards et al., 2013
129	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas*	*Brevundimonas* sp.	Heterotrophic	Tiago et al., 2004
130	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	NC	NC	Heterotrophic	Klann et al., 2016
131	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	NC	NC	Heterotrophic	Erlacher et al., 2015 and their references
133	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	*Bdellovibrio* sp.	heterotrophic	Stolp and Starr 1963
137	Bacteria	Armatimonadetes	Fimbriimonadia	Fimbriimonadales	Fimbriimonadaceae	NC	NC	Heterotrophic	Im et al., 2012
138	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	*Legionella*	*Legionella* sp.	Heterotrophic	Richards et al., 2013
142	Bacteria	Actinobacteria	NC	Frankiales	Sporichthyaceae	NC	NC	Heterotrophic	Goodfellow et al., 2012
145	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	*Pedomicrobium* sp.	Heterotrophic	Erlacher et al., 2015 and their references
146	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NC	NC	NC	NC
147	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax*	*Acidovorax* sp.	Heterotrophic	Vandermaesen et al., 2017
149	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Ralstonia*	*Ralstonia* sp.	Heterotrophic	Klann et al., 2016
150	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	*Zavarzinella*	*Zavarzinella* sp.	Heterotrophic	Fuerst and Sangulenko, 2011
155	Bacteria	Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	*Armatimonas*	*Armatimonas* sp.	Heterotrophic	Tamaki et al., 2011

157	Bacteria	Cyanobacteria	NC	Obscuribacterales	NC	NC	NC	Autotrophic	Boone et al., 2001
158	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	*Methylobacterium* sp.	Heterotrophic	Erlacher et al., 2015 and their references
160	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NC	NC	Heterotrophic	Kasalicky et al., 2013; Vandermaesen et al., 2017
166	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NC	NC	NC	NC
167	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Sediminibacterium*	*Sediminibacterium* sp.	Heterotrophic	Rosenberg et al., 2014
175	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NC	NC	NC	NC
176	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Sphingomonadaceae	NC	NC	Heterotrophic	Erlacher et al., 2015 and their references
179	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NC	NC	Uncultured	Heterotrophic	Hess et al., 2016
180	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	NC	NC	Heterotrophic	McBride et al., 2014
182	Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Bryobacter*	*Bryobacter* sp.	Heterotrophic	Kielak et al., 2016
187	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Curvibacter*	*Curvibacter* sp.	Heterotrophic	Vandermaesen et al., 2017
188	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NC	NC	NC	Heterotrophic	Rosenberg et al., 2014
189	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	*Chthoniobacter*	*Chthoniobacter* sp.	Heterotrophic	Sangwan et al., 2004
190	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Acidocella*	*Acidocella* sp.	Heterotrophic	Servín Guarcidueñas et al., 2013
191	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Endobacter*	*Endobacter* sp.	Heterotrophic	Ramírez Bahena et al., 2013
192	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NC	NC	Heterotrophic	Kasalicky et al., 2013; Vandermaesen et al., 2017
194	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Dinghuibacter*	*Dinghuibacter* sp.	Heterotrophic	Rosenberg et al., 2014
200	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	*Bdellovibrio* sp.	Heterotrophic	Stolp and Starr 1963
202	Bacteria	Verrucomicrobia	Spartobacteria	Chthoniobacterales	NC	NC	NC	Heterotrophic	Sangwan et al., 2004
203	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NC	NC	NC	Heterotrophic	Hess et al., 2016
205	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	*Sphingomonas* sp.	Heterotrophic	Rivadeneyra Torres et al., 2013
206	Bacteria	Proteobacteria	Alphaproteobacteria	Burkholderiales	Comamonadaceae	*Limnohabitans*	*Limnohabitans* sp.	Heterotrophic	Kasalicky et al., 2013
209	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Holosporaceae	NC	NC	Heterotrophic	Hess et al., 2016
210	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NC	NC	Heterotrophic	Scharallhammer et al., 2013
211	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Comamonas*	*Comamonas* sp.	Heterotrophic	Park et al., 1962
212	Bacteria	Cyanobacteria	NC	NC	Family I	*Snowella*	*Snowella* sp.	Autotrophic	Boone et al., 2001
214	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Holosporaceae	NC	NC	Heterotrophic	Hess et al., 2016
221	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	NC	NC	Heterotrophic	McBride et al., 2014
229	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	*Coxiella*	*Coxiella* sp.	Heterotrophic	Michaud et al., 2009
234	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Incertae Sedis	*Candidatus Odyssella*	*Candidatus Odyssella*	Heterotrophic	Birtles et al., 2000
238	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	NC	NC	Heterotrophic	Lee et al., 2005
239	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NC	*Pseudoxanthobacter*	*Pseudoxanthobacter* sp.	Heterotrophic	Erlacher et al., 2015 and their references
240	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Skermanella*	*Skermanella* sp.	Heterotrophic	Lindsay and Stackebrandt 1999
241	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	NC	NC	NC	NC
244	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NC	NC	NC	Heterotrophic	Hess et al., 2016
250	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	NC	NC	Heterotrophic	Erlacher et al., 2015 and their references

251	Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae subgroup 3	*Paludibaculum*	*Paludibaculum* sp.	Heterotrophic	Kielak et al., 2016
255	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	NC	NC	Heterotrophic	Rosenberg et al., 2014
256	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NC	NC	NC	Heterotrophic	Rosenberg et al., 2014
257	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	*Chromobacterium*	*Chromobacterium* sp.	Heterotrophic	Klann et al., 2016
258	Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae Subgroup 3	*Bryobacter*	*Bryobacter* sp.	Heterotrophic	Kielak et al., 2016
261	Bacteria	Chlorobi	Chlorobia	Chlorobiales	NC	NC	Uncultured	Heterotrophic	Hiras et al.,, 2016
263	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Rhodopila*	*Rhodopila* sp.	Autotrophic	Brenner et al., 2005
265	Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae subgroup 3	*Bryobacter*	*Bryobacter* sp.	Heterotrophic	Kielak et al., 2016
266	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	*Bdellovibrio* sp.	Heterotrophic	Stolp and Starr 1963
268	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	*Methylobacillus*	*Methylobacillus* sp.	Heterotrophic	Doronina et al., 2014
271	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Hyphomicrobium*	*Hyphomicrobium* sp.	Heterotrophic	Erlacher et al., 2015 and their references
272	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NC	NC	NC	Heterotrophic	Hess et al., 2016
278	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	*Devosia* sp.	Heterotrophic	Erlacher et al., 2015 and their references
279	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NC	NC	NC	NC
280	Bacteria	Proteobacteria	Gammaproteobacteria	NC	NC	NC	NC	NC	NC

Tabela 2S – Classificação taxonômica e funcional para as Unidades Taxonômicas Operacionais (OTUs) eucarióticas. Os grupos tróficos foram estabelecidos pela consulta a literatura, considerando o menor nível para a classificação taxonômica de cada OTU. OTUs com classificação taxonômica insuficiente não foram funcionalmente agrupadas. Nós consideramos a classificação taxonômica obtida através do Blast. NC = Não classificado.

OTU	Reino	Filo	Classe	Ordem	Familia	Gênero	Espécie	Grupo Trófico	Referências
2	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
4	NC	Ochrophyta	Chrysophyceae	Ochromonadales	NC	Ochromonas	*Ochromonas sphaerocystis*	Mixotrophic	Jones, 2000
7	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
8	NC	Cercozoa	NC	NC	Vampyrellidae	Arachnula	*Arachnula impatiens*	Heterotrophic	Khomich et al., 2017
9	NC	Chlorophyta	Trebouxiophyceae	NC	NC	Choricystis	*Choricystis* sp.	Autotrophic	Simon et al., 2015; Khomich et al., 2017
11	NC	Chlorophyta	Trebouxiophyceae	Chlorellales	NC	Makinoella	*Makinoella tosaensis*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
12	NC	Charophyta	Zygnematophyceae	NC	NC	Staurastrum	*Staurastrum* sp.	Autotrophic	Khomich et al., 2017
13	NC	Chlorophyta	Trebouxiophyceae	NC	NC	Micractinium	*Micractinium reisseri*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
15	NC	Ciliophora	Spirotrichea	NC	NC	NC	*Stichotrichia* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
16	NC	NC	NC	Bicosoecida	NC	NC	NC	Heterotrophic	Khomich et al., 2017
17	NC	Ochrophyta	Chrysophyceae	Chromulinales	NC	Poterioochromonas	*Poterioochromonas*	Mixotrophic	Jones, 2000;
20	NC	Ciliophora	Litostomatea	NC	NC	Hemiophrys	*Hemiophrys procera*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
23	NC	Dinoflagellata	Dinophyceae	NC	NC	Glenodinium	*Glenodinium* sp.	Mixotrophic	Stoecker, 1999
25	NC	Ciliophora	Oligohymenophorea	NC	NC	Vorticella	*Vorticella* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
27	NC	Ciliophora	Prostomatea	NC	NC	Cryptocaryon	*Cryptocaryon* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015

28	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
33	NC	Ochrophyta	Eustigmatophyceae	Eustigmatales	NC	NC	NC	Autotrophic	Fietz et al., 2005
35	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
36	NC	Cercozoa	Thecofilosea	Incertae Sedis	NC	Pseudodifflugia	*Pseudodifflugia gracilis*	Heterotrophic	Khomich et al., 2017
39	NC	Ciliophora	Spirotrichea	NC	NC	Oxytricha	*Stichotrichia* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
40	NC	Chlorophyta	Mamiellophyceae	Monomastigales	NC	Monomastix	*Monomastix minuta*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
42	Fungi	Cryptomycota	Incertae Sedis	Incertae Sedis	Incertae Sedis	*Paramicrosporidium*	*Paramicrosporidium* sp.	Heterotrophic	Simon et al., 2015
43	NC	Ciliophora	Litostomatea	NC	NC	*Loxophyllum*	*Loxophyllum* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
45	NC	NC	NC	Bicosoecida	NC	NC	Uncultured	Heterotrophic	Khomich et al., 2017
48	NC	Ochrophyta	Chrysophyceae	Ochromonadales	NC	*Paraphysomonas*	*Paraphysomonas vestita*	Mixotrophic	Jones, 2000
50	Fungi	Cryptomycota	Incertae Sedis	Incertae Sedis	Incertae Sedis	*Paramicrosporidium*	*Paramicrosporidium* sp.	Heterotrophic	Simon et al., 2015
51	NC	Ciliophora	Colpodea	Cyrtolophosidida	NC	*Cyrtolophosis*	*Cyrtolophosis* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
53	NC	Cercozoa	Imbricatea	Spongomonadida	NC	*Spongomonas*	*Spongomonas* sp.	Heterotrophic	Khomich et al., 2017
55	NC	Ciliophora	Colpodea	Cyrtolophosidida	NC	*Microdiaphanosoma*	*Microdiaphanosoma arcuatum*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
70	NC	Charophyta	Zygnematophyceae	Desmidiales	NC	*Staurodesmus*	*Staurodesmus omearii*	Autotrophic	Khomich et al., 2017
72	NC	Ciliophora	Spirotrichea	NC	NC	*Anteholosticha*	*Anteholosticha gracilis*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
81	NC	Ciliophora	Oligohymenophorea	NC	NC	*Vorticella*	*Vorticella campanula*	Heterotropic	Beaver & Crisman, 1989; Simon et al., 2015
83	NC	Chlorophyta	Chlorophyceae	Oedogoniales	NC	NC	NC	Autotrophic	Simon et al., 2015; Khomich et al., 2017

85	NC	Ochrophyta	Chrysophyceae	Chromulinales	NC	NC	NC	Mixotrophic	Jones, 2000
88	NC	Ciliophora	Oligohymenophorea	NC	NC	*Ophrydium*	*Ophrydium versatile*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
90	NC	Ochrophyta	Chrysophyceae	Ochromonadales	NC	*Ochromonas*	*Ochromonas* sp.	Mixotrophic	Jones, 2000
94	NC	Cercozoa	NC	NC	NC	NC	Uncultured	Heterotrophic	Khomich et al., 2017
96	NC	Cercozoa	Thecofilosea	Cryomonadida	Rhizaspididae	*Rhogostoma*	*Rhogostoma* sp.	Heterotrophic	Khomich et al., 2017
98	NC	Chlorophyta	Chlorophyceae	NC	NC	*Pseudomuriella*	*Pseudomuriella* sp.	Autotrophic	Simon et al., 2015; Khomich et al., 2017
100	NC	Cercozoa	NC	NC	NC	NC	NC	Heterotrophic	Khomich et al., 2017
103	Fungi	Cryptomycota	Incertae Sedis	Incertae Sedis	Incertae Sedis	*Paramicrosporidium*	*Paramicrosporidium* sp.	Heterotrophic	Simon et al., 2015
105	NC	Chlorophyta	Chlamydomonadaceae	NC	NC	NC	NC	Autotrophic	Simon et al., 2015; Khomich et al., 2017
106	NC	Cercozoa	Thecofilosea	Cryomonadida	Rhizaspididae	*Rhogostoma*	*Rhogostoma* sp.	Heterotrophic	Khomich et al., 2017
108	Fungi	Glomeromycota	Glomeromycetes	Glomerales	NC	*Glomus*	*Glomus* sp.	Heterotrophic	Simon et al., 2015
110	NC	Ochrophyta	Chrysophyceae	Chromulinales	NC	*Spumella*	*Spumella* sp.	Mixotrophic	Jones et al., 2000
111	NC	Charophyta	Zygnematophyceae	Zygnematales	NC	*Netrium*	*Netrium digitus* var. *latum*	Autotrophic	Khomich et al., 2017
112	NC	Ciliophora	Oligohymenophorea	NC	NC	*Vorticella*	*Vorticella* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
114	NC	Ciliophora	Oligohymenophorea	NC	NC	*Carchesium*	*Carchesium polypinum*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
117	NC	Amoebozoa	Tubulinea	Euamoebida	NC	NC	NC	Heterotrophic	Lesen et al., 2000
118	NC	Chlorophyta	Pedinophyceae	Pedinomonadales	NC	*Pedinomonas*	*Pedinomonas* sp.	Autotrophic	Simon et al., 2015; Khomich et al., 2017
119	NC	Ciliophora	Colpodea	Cyrtolophosidida	NC	*Cyrtolophosis*	*Cyrtolophosis mucicola*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
120	NC	Cercozoa	NC	NC	Vampyrellidae	NC	NC	Heterotrophic	Khomich et al., 2017
121	NC	Chlorophyta	Trebouxiophyceae	Chlorellales	NC	*Compactochlorella*	*Compactochlorella*	Autotrophic	Simon et al.,

							kochii		2015; Khomich et al., 2017
122	NC	Chlorophyta	Chlorophyceae	Sphaeropleales	NC	*Scenedesmus*	*Scenedesmus pupukensis*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
123	NC	Ciliophora	Colpodea	Cyrtolophosidida	NC	*Pseudocyrtolophosis*	*Pseudocyrtolophosis alpestris*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
126	NC	Ciliophora	Oligohymenophorea	Hymenostomatia	NC	*Tetrahymena*	*Tetrahymena orphan*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
136	NC	Amoebozoa	Tubulinea	Arcellinida	NC	*Cryptodifflugia*	*Cryptodifflugia operculata*	Heterotrophic	Lesen et al., 2010
137	NC	Ochrophyta	Chrysophyceae	Ochromonadales	NC	*Epipyxis*	*Epipyxis pulchra*	Mixotrophic	Jones 2000
142	NC	Chlorophyta	Chlorophyceae	NC	Chlamydomonadaceae	NC	NC	Autotrophic	Simon et al., 2015; Khomich et al., 2017
143	NC	Ciliophora	Colpodea	Bursariomorphida	NC	*Bryometopus*	*Bryometopus* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
145	NC	Amoebozoa	Discosea	Dactylopodida	NC	NC	NC	Heterotrophic	Lesen et al., 2010
147	NC	Dinoflagellata	Dinophyceae	NC	NC	*Glenodinium*	*Glenodinium* sp.	Mixotrophic	Stoecker, 1999
148	NC	Amoebozoa	NC	Protosteliales	NC	NC	NC	Heterotrophic	Lesen et al., 2010
149	NC	Ochrophyta	Chrysophyceae	Ochromonadales	NC	*Ochromonas*	*Ochromonas sphaerocystis*	Mixotrophic	Jones 2000
152	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
154	NC	Chlorophyta	Chlorophyceae	NC	Chlamydomonadaceae	NC	NC	Autotrophic	Simon et al., 2015; Khomich et al., 2017
155	NC	Chlorophyta	Chlorophyceae	NC	Scenedesmaceae	NC	NC	Autotrophic	Simon et al., 2015; Khomich et al., 2017
157	NC	NC	NC	NC	Perkinsidae	*Parvilucifera*	*Parvilucifera* sp.	Heterotrophic	Mangot et al., 2011
159	NC	Amoebozoa	Tubulinea	Arcellinida	NC	*Vermamoeba*	*Vermamoeba vermiformis*	Heterotrophic	Lesen et al., 2010
160	NC	Cercozoa	NC	Glissomonadida	NC	*Bodomorpha*	*Bodomorpha* sp.	Heterotrophic	Khomich et al., 2017
161	NC	Amoebozoa	Tubulinea	Arcellinida	Echinamoebidae	*Echinamoeba*	*Echinamoeba thermarum*	Heterotrophic	Lesen et al., 2010
166	NC	Holozoa	Choanoflagellida	Craspedida	Salpingoecidae	*Salpingoeca*	*Salpingoeca* sp.	Heterotrophic	Simon et al.,

									2015; Khomich et al., 2017
172	NC	Chlorophyta	Chlorophyceae	NC	NC	*Neochlorosarcina*	*Neochlorosarcina negevensis*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
173	NC	Amoebozoa	NC	Protosteliida	NC	*Protostelium*	*Protostelium mycophagum* var. *crassipes*	Heterotrophic	Lesen et al., 2000
174	NC	Ciliophora	Colpodea	Platyophryida	NC	*Platyophrya*	*Platyophrya vorax*	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
178	NC	Amoebozoa	Tubulinea	Euamoebida	NC	*Saccamoeba*	*Saccamoeba* sp.	Heterotrophic	Lesen et al., 2010
179	Fungi	Chytridiomycota	Chytridiomycetes	NC	Chytridiaceae	NC	NC	Heterotrophic	Simon et al., 2015
180	NC	Cercozoa	Metromonadea	NC	NC	NC	NC	Heterotrophic	Khomich et al., 2017
181	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	*Clavaria*	*Clavaria zollingeri*	Heterotrophic	Simon et al., 2015
184	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Incertae Sedis	*Hannaella*	*Hannaella oryzae*	Heterotrophic	Simon et al., 2015
185	NC	Ciliophora	Nassophorea	NC	NC	*Leptopharynx*	*Leptopharynx* sp.	Heterotrophic	Beaver & Crisman 1989; Simon et al., 2015
188	NC	Amoebozoa	Tubulinea	Arcellinida	NC	*Arcella*	*Arcella hemisphaerica*	Heterotrophic	Lesen et al., 2010
191	Fungi	Kickxellomycotina	Incertae Sedis	Harpellales	Legeriomycetaceae	*Orphella*	*Orphella catalaunica*	Heterotrophic	Simon et al., 2015
192	NC	Amoebozoa	Discosea	Dactylopodida	NC	NC	NC	Heterotrophic	Lesen et al., 2010
195	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	*Clavaria*	*Clavaria zollingeri*	Heterotrophic	Simon et al., 2015
201	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
204	Fungi	Cryptomycota	Incertae Sedis	Incertae Sedis	Incertae Sedis	*Paramicrosporidium*	*Paramicrosporidium* sp.	Heterotrophic	Simon et al., 2015
205	NC	Cercozoa	NC	NC	NC	NC	NC	Heterotrophic	Khomich et al., 2017
208	NC	Cercozoa	NC	NC	NC	*Ammopiptanthus*	*Ammopiptanthus mongolicus*	Heterotrophic	Khomich et al., 2017
211	NC	Chlorophyta	Chlorophyceae	Sphaeropleales	NC	*Atractomorpha*	*Atractomorpha echinata*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
213	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
219	NC	Ochrophyta	Bacillariophyceae	NC	NC	*Eunotia*	*Eunotia* sp.	Autotrophic	Khomich et al., 2017

225	NC	Ochrophyta	Eustigmatophyceae	Eustigmatales	NC	NC	NC	Autotrophic	Fietz et al., 2005
231	NC	NC	Peronosporomycetes	Saprolegniales	NC	*Aphanomyces*	*Aphanomyces* sp.	Heterotrophic	Dick 2001
234	NC	Ciliophora	Colpodea	Bursariomorphida	NC	*Bryometopus*	*Bryometopus* sp.	Heterotrophic	Beaver & Crisman, 1989; Simon et al., 2015
245	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	*Clavaria*	*Clavaria zollingeri*	Heterotrophic	Simon et al., 2015
252	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	*Clavaria*	*Clavaria zollingeri*	Heterotrophic	Simon et al., 2015
255	NC	Cercozoa	NC	NC	NC	NC	NC	Heterotrophic	Khomich et al., 2017
256	NC	Chlorophyta	Chlorophyceae	Chlamydomonadales	NC	*Oogamochlamys*	*Oogamochlamys zimbabwiensis*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
257	NC	Chlorophyta	Chlorodendrophyceae	Chlorodendrales	NC	*Tetraselmis*	*Tetraselmis subcordiformis*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
259	NC	Cercozoa	NC	NC	Vampyrellidae	NC	NC	Heterotrophic	Khomich et al., 2017
260	NC	Chlorophyta	Chlorophyceae	NC	NC	*Lobomonas*	*Lobomonas monstruosa*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
262	NC	Ochrophyta	Eustigmatophyceae	Eustigmatales	NC	NC	NC	Autotrophic	Fietz et al., 2005
266	NC	Charophyta	Zygnematophyceae	NC	NC	*Mougeotia*	*Mougeotia* sp.	Autotrophic	Khomich et al., 2017
268	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
271	NC	Cercozoa	Imbricatea	NC	Euglyphidae	*Euglypha*	*Euglypha filifera*	Heterotrophic	Khomich et al., 2017
275	NC	Chlorophyta	Chlorophyceae	NC	Chlamydomonadaceae	NC	NC	Autotrophic	Simon et al., 2015; Khomich et al., 2017
283	NC	Ochrophyta	Chrysophyceae	Hibberdiales	NC	*Lagynion*	*Lagynion scherffelii*	Mixotrophic	Jones 2000
287	NC	Holozoa	Ichthyosporea	Dermocystida	NC	NC	NC	Heterotrophic	Glocking et al., 2013
289	NC	Chlorophyta	Trebouxiophyceae	NC	NC	*Micractinium*	*Micractinium reisseri*	Autotrophic	Simon et al., 2015; Khomich et al., 2017
294	NC	Cercozoa	Thecofilosea	NC	NC	*Pseudodifflugia*	*Pseudodifflugia gracilis*	Heterotrophic	Khomich et al., 2017
297	Fungi	Basidiomycota	Agaricomycetes	NC	NC	*Xylodon*	*Xylodon* sp.	Heterotrophic	Simon et al., 2015
307	NC	Chlorophyta	Chlorophyceae	NC	NC	NC	NC	Autotrophic	Simon et al.,

									2015; Khomich et al., 2017
310	NC	Incertae Sedis	NC	Ancyromonadida	NC	*Ancyromonas*	*Ancyromonas* sp.	Heterotrophic	Boenigk & Ardnt, 2002
313	Fungi	Cryptomycota	NC	NC	NC	NC	NC	Heterotrophic	Simon et al., 2015
314	NC	Cercozoa	NC	NC	Incertae Sedis	*Cercomonas*	*Cercomonas* sp.	Heterotrophic	Khomich et al., 2017
317	Fungi	Incertae Sedis	Kickxellomycotina	Harpellales	Legeriomycetaceae	*Smittium*	*Smittium orthocladii*	Heterotrophic	Simon et al., 2015
322	NC	Charophyta	Zygnematophyceae	Desmidiales	NC	*Cosmocladium*	*Cosmocladium saxonicum*	Autotrophic	Khomich et al., 2017
325	Fungi	Chytridiomycota	Chytridiomycetes	Cladochytriales	Cladochytriaceae	*Cladochytrium*	*Cladochytrium replicatum*	Heterotrophic	Simon et al., 2015
327	NC	Cercozoa	NC	NC	NC	NC	NC	Heterotrophic	Khomich et al., 2017
332	Fungi	Cryptomycota	Incertae Sedis	Incertae Sedis	Incertae Sedis	*Paramicrosporidium*	*Paramicrosporidium* sp.	Heterotrophic	Simon et al., 2015
336	NC	Dinoflagellata	Dinophyceae	NC	Peridiniales	*Glenodinium*	*Glenodinium inaequale*	Mixotrophic	Stoecker, 1999
337	NC	Charophyta	Zygnematophyceae	Desmidiales	NC	*Closterium*	*Closterium tumidum*	Autotrophic	Khomich et al., 2017
343	NC	Amoebozoa	Tubulinea	Euamoebida	NC	*Saccamoeba*	*Saccamoeba limax*	Heterotrophic	Lesen et al., 2010
348	NC	Amoebozoa	Tubulinea	Leptomyxida	NC	*Leptomyxa*	*Leptomyxa reticulata*	Heterotrophic	Lesen et al., 2010
351	NC	Ciliophora	Nassophorea	Microthoracida	NC	NC	NC	Heterotrophic	Beaver & Crisman ,1989; Simon et al., 2015
354	NC	Charophyta	Zygnematophyceae	Desmidiales	NC	*Desmidium*	*Desmidium grevillei*	Autotrophic	Khomich et al., 2017

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CAPÍTULO 4

Artigo de divulgação científica

Aquecimento global: os micro-organismos aquáticos serão afetados?*

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O aquecimento global vem sendo noticiado cada vez com mais frequência. O aumento da temperatura na superfície da terra deve afetar a saúde e bem-estar dos seres humanos assim como de outros animais. Porém, como ficam os organismos microscópicos nessa situação? Eles também são afetados pelas mudanças climáticas? Um estudo em laboratório ajudou a responder essa questão.

A sensação de estar vivendo dias e noites cada vez mais quentes é compartilhada por várias pessoas em diferentes partes do planeta. Inúmeros estudos científicos apontam que o aumento da temperatura não é apenas uma impressão, mas sim uma realidade que pode trazer sérias consequências para as diferentes formas de vida. De acordo com último relatório do Painel Intergovernamental para Mudanças Climáticas, publicado em 2018, a temperatura na superfície da terra vêm aumentando em uma velocidade sem precedentes e até o final do século, se medidas mitigadoras não forem tomadas, ela pode chegar a um aumento de até 4,8 °C em relação a temperatura média atual. Além dos estudos sobre as causas e cenários de alterações climáticas, os biólogos e cientistas ambientais tem se dedicado a avaliar os efeitos do aquecimento global sobre os organismos terrestres e aquáticos. Ao contrário do que muitas pessoas imaginam, o aumento da temperatura não afeta apenas os seres humanos ou outros animais de grande porte, mas as plantas assim como os micro-organismos também podem apresentar mudanças ecológicas vivendo em ambientes cada vez mais quentes.

A microbiota aquática é representada por organismos microscópicos que vivem livremente na coluna d'água, entre os quais estão incluídas as microalgas, amebas, ciliados, cercozóarios, algumas espécies de fungos, assim como as bactérias e cianobactérias (Figura 1). As microalgas e cianobactérias desempenham para os ambientes aquáticos um papel semelhante ao que as plantas realizam para os ecossistemas terrestres, sendo responsáveis pela produtividade primária. Nesse processo, na presença de luz e nutrientes o gás carbônico é convertido em oxigênio, que se torna disponível para a respiração, e em glicose, que é utilizada como fonte de energia para o metabolismo destes organismos. Os demais grupos representam os consumidores, que se alimentam tanto das microalgas como de outros micro-organismos presentes na coluna de água, desempenhando um papel similar ao dos animais nos ambientes terrestres. Através do consumo, esses micro-organismos promovem o fluxo de nutrientes entre os diferentes níveis que compõem os ecossistemas aquáticos, promovendo uma ligação entre os grupos taxonômicos. Além disso, a presença e quantidade de células desses organismos no ambiente aquático atuam como um indicador da qualidade da água para seus diferentes usos (p. ex. recreação, pesca, consumo e etc). Deste modo, o efeito do aquecimento mesmo que diretamente em somente um grupo de micro-organismos, pode afetar os demais, assim como alterar a relação de produção, consumo e decomposição nos ambientes aquáticos, além de afetar os organismos terrestres que utilizam do recurso aquático, como por exemplo, o homem.

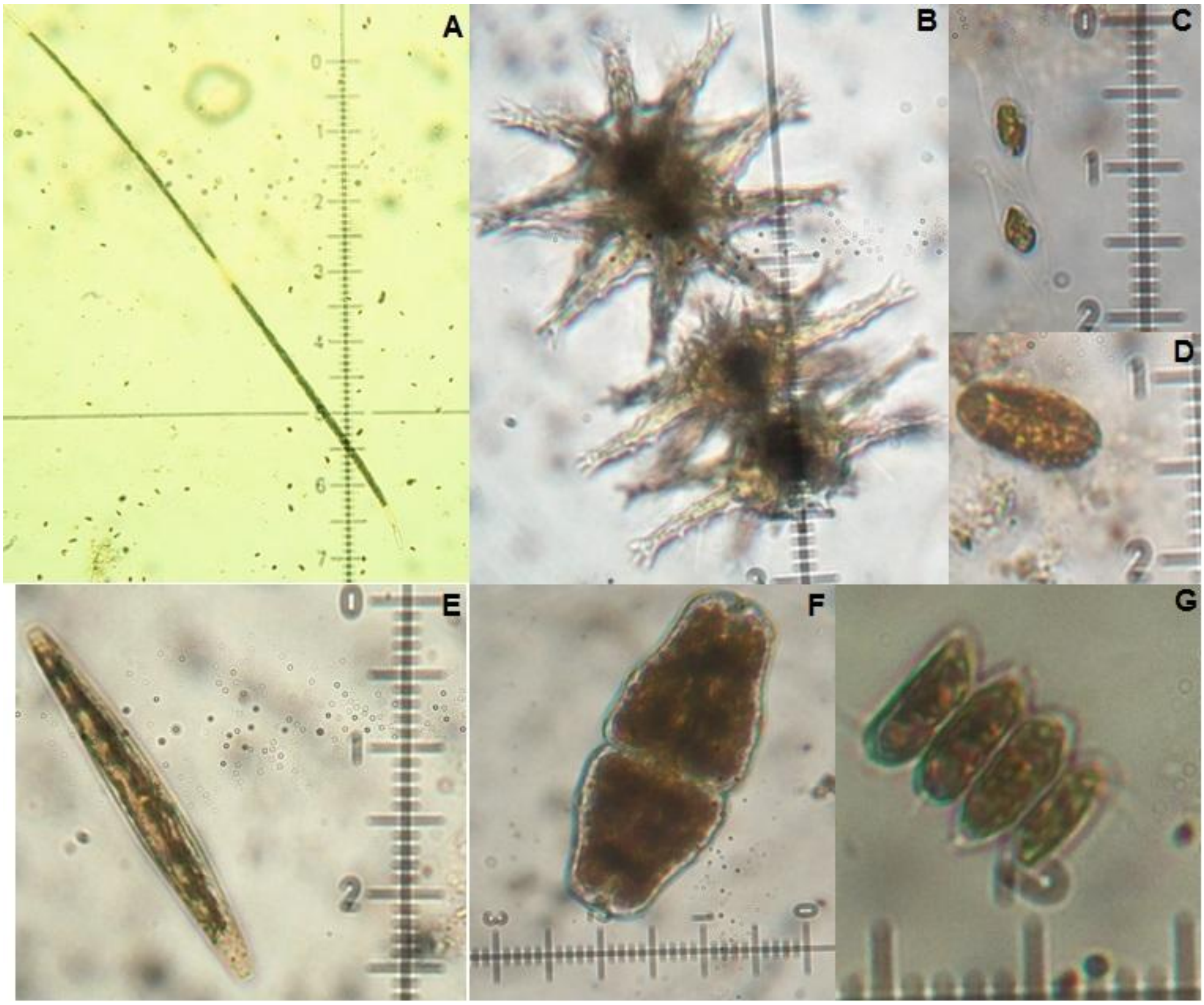

Figura 1 – Alguns representantes da microbiota aquática. Em (A) *Closterium ceratium*, (B) *Staurastrum leptacanthum*, (C) *Dinobryon sertularia*, (D) *Cryptomonas obovata*, (E) *Closterium* sp., (F) *Euastrum cuneatum* e (G) *Desmodesmus armatus*. Esses micro-organismos têm medidas variando de 339 μm^3 (Figura C) até 32,030 μm^3 (Figura B). A coloração dos micro-organismos é devido a um fixador utilizado para preservar as amostras após a coleta.

Existem diferentes formas para avaliar o efeito do aquecimento global sobre os micro-organismos aquáticos. Estudos em campo podem ser realizados com a coleta e análise de amostras em locais com baixa e elevada temperatura. Embora essa estratégia seja muito útil, ela pode sofrer interferências de outros fatores como a disponibilidade de nutrientes, luz e impacto antrópico no entorno do corpo de água. Assim, é difícil discernir quais alterações são observadas pelo aumento da temperatura e quais podem ser atribuídas a esses fatores externos. Outra estratégia muito utilizada é a construção de experimentos para simular o aquecimento global. Embora nem todos os componentes de um ambiente aquático real possam ser representados, essa abordagem traz um maior controle das variáveis testadas e permite detectar quais são os efeitos diretos da temperatura sobre os micro-organismos.

Recentemente, em uma parceria entre pesquisadores das Universidades Federal e Estadual de Goiás e Universidade de Brasília, foi realizado um experimento para simular os efeitos do aquecimento sobre os micro-organismos aquáticos produtores e consumidores. Com essa iniciativa, nós avaliamos as mudanças na ocorrência de espécies através da identificação em microscópio assim como pelo sequenciamento do DNA encontrado nas amostras. O experimento foi construído buscando simular diferentes níveis de aquecimento para a região tropical, com um cenário otimista, no qual houve um pequeno aumento em relação a temperatura atual (2°C), um cenário pessimista com incremento de 4°C, assim como um aumento extremo, simulando o dobro do aquecimento previsto pelo cenário pessimista (8°C) (Figura 2). Os ambientes aquáticos foram simulados utilizando aquários com capacidade para 25 litros de água. A temperatura foi mantida através de aquecedores elétricos e as amostras de água utilizadas para preencher os aquários foram coletadas em uma lagoa natural (Figura 3).

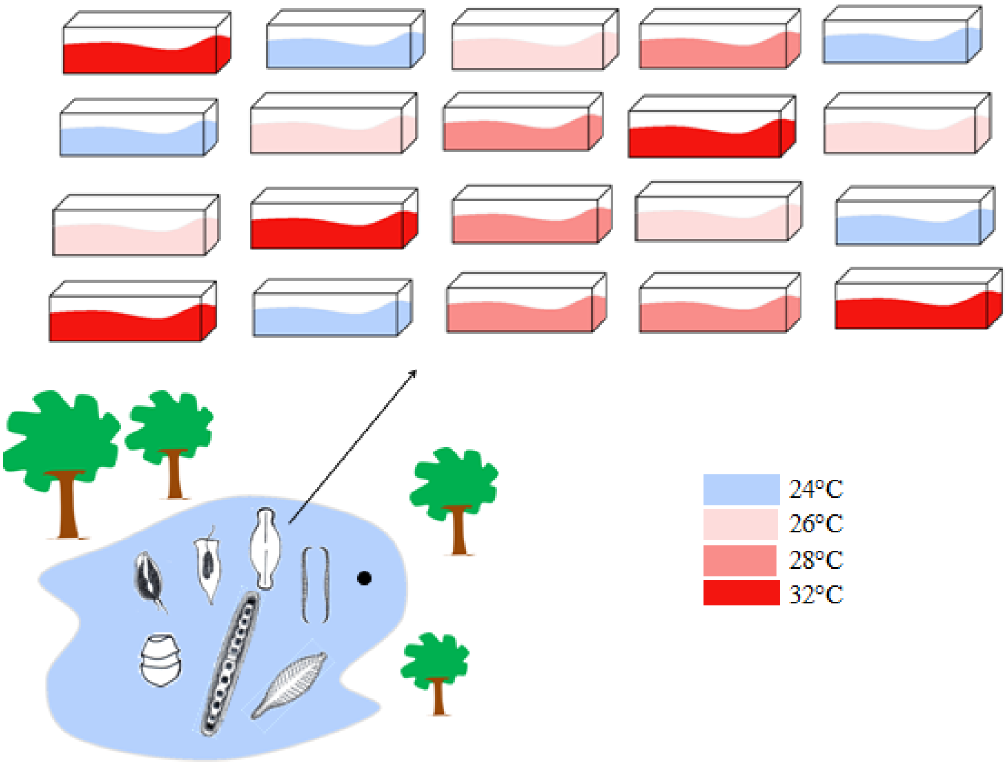

Figura 2 – Experimento utilizado para testar o efeito do aumento na temperatura sobre os micro-organismos aquáticos. A água e as espécies foram coletadas em uma lagoa natural e colocadas em aquários. Cada aquário foi aquecido utilizando aquecedores elétricos, buscando simular a temperatura atual (24°C) e os diferentes cenários de aquecimento previstos para o futuro (26°C, 28°C e 32°C).

Figura 3 – Lagoa na qual foram realizadas as coletas de água e micro-organismos para preencher os aquários utilizados no experimento.

Nós verificamos que a composição de espécies de microalgas é diferente entre os aquários com temperatura atual e os aquecidos (veja os resultados publicados no periódico Hydrobiologia). De modo geral, na temperatura atual houve uma maior variedade de espécies, com tamanhos e formas distintas. Por outro lado, na temperatura elevada, os aquários foram ocupados predominantemente por uma espécie pequena e de formato circular (Figura 4). Muitas espécies desapareceram com o aquecimento. Essa situação é muito preocupante, já que a perda de espécies e o predomínio de algumas em detrimento a outras podem afetar a produtividade primária dos ambientes aquáticos. Além disso, os organismos que se alimentam de microalgas específicas também podem ser afetados, já que o seu recurso alimentar pode ser perdido. Quando os consumidores primários são afetados, os peixes e outros organismos que deles se alimentam também sofrem as consequências e em algum momento esses efeitos chegarão até o homem. Outro fator preocupante observado com o experimento foi o maior número de cianobactérias nas altas temperaturas. Esses organismos são capazes de produzir toxinas, que além de afetar a qualidade da água podem ser prejudiciais ao ser humano e a outros animais. Mas calma! Em nosso experimento não observamos crescimento de espécies tóxicas. Além disso, existem aproximadamente 7.000 espécies desse grupo e nem todas são capazes de produzir toxinas. As cianobactérias são organismos bem pequenos e muito resistentes (elas estão na terra há aproximadamente 2,5 bilhões de anos), e outros estudos de ambientes temperados também encontraram predomínio dessas espécies em ambientes mais aquecidos.

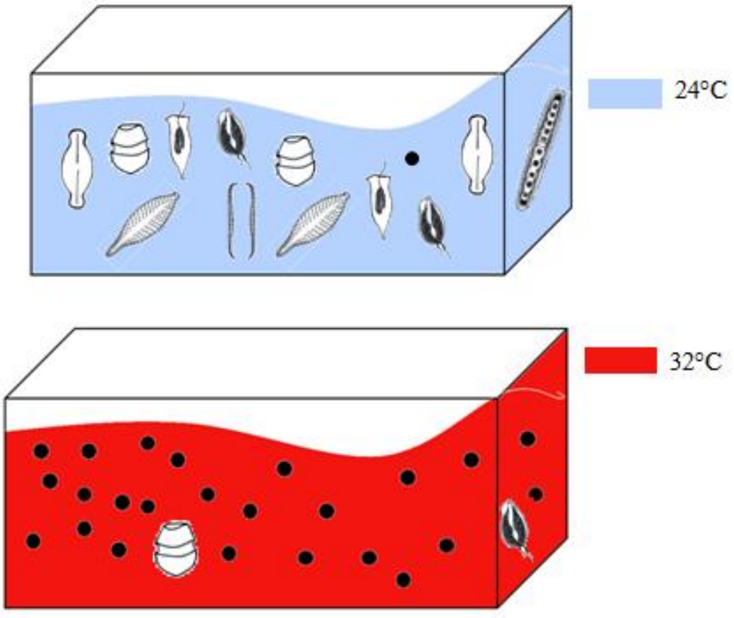

Figura 4 – Efeitos do aquecimento sobre os micro-organismos aquáticos. Cerca de 99% do aquário aquecido foi ocupado por uma espécie pequena e de formato circular. Além disso, muitas espécies foram perdidas com o aumento da temperatura.

Nosso experimento representou um pequeno, mas importante passo para conhecer os efeitos do aquecimento sobre os micro-organismos aquáticos da região tropical. Para tornar os experimentos mais realistas outras alterações esperadas com as mudanças climáticas podem ser consideradas. No entanto, isso exige um maior investimento financeiro, assim como o desenvolvimento de áreas experimentais mais complexas. Uma proposta nesse sentido já foi aprovada e está sendo desenvolvida pelo grupo de trabalho em ecologia aquática dentro do Instituto Nacional de Ciência e Tecnologia em Ecologia, Evolução e Conservação da Biodiversidade (INCT-

EECBio). Aqui, pesquisadores das Universidades Federal e Estadual de Goiás que trabalharam no experimento citado anteriormente , em parceria com outros pesquisadores de oito universidades do país, devem simular os efeitos das alterações climáticas sobre diferentes micro-organismos aquáticos. A área experimental contém um grande número de caixas d’ água que serão utilizadas nas simulações e foi construída na Universidade Estadual de Goiás na cidade de Anápolis – Goiás (Figura 5). Com essa área experimental será possível dar maior realismo nas pesquisas com micro-organismos aquáticos tropicais. Consequentemente, o conjunto de ações com diferentes tipos de experimentos (os já realizados e os futuros) permitirão compreender o impacto das alterações climáticas nos ambientes aquáticos tropicais e nos mecanismos para gestão desses ambientes, pois como encontrado na pesquisa desenvolvida, até mesmo os micro-organismos aquáticos podem ser afetados com as alterações climáticas futuras.

Figura 5 – Área experimental que está sendo construída no campus da Universidade Estadual de Goiás para simular os efeitos das alterações climáticas sobre os micro-organismos aquáticos.

Sugestões de leitura

Machado, K. B., Vieira, L. C. G., Nabout, J. C. 2018. Predicting the dynamics of taxonomic and functional phytoplankton compositions in different global warming scenarios. Hydrobiologia, https://doi.org/10.1007/s10750-018-3858-7.

CONCLUSÕES GERAIS

Esta tese buscou avaliar a resposta da microbiota aquática a gradientes ambientais naturais e simulados experimentalmente. Foram utilizadas diferentes abordagens para acessar a composição das comunidades, representando aspectos taxonômicos (composição de espécies), moleculares (composição de OTUs) e funcionais (composição de grupos funcionais e tróficos). De modo geral, os resultados obtidos indicam que na planície de inundação avaliada, a composição molecular da microbiota aquática não responde ao gradiente de variação ambiental, embora sua composição seja espacialmente estruturada. Assim, a similaridade na composição decresce com o aumento da distância geográfica e as características ambientais do habitat não influenciam. Por outro lado, os micro-organismos responderam ao incremento da temperatura nas três abordagens utilizadas. Foi observada uma substituição de espécies entre os tratamentos de temperatura, promovendo mudanças na composição da microbiota procariota e eucariota, bem como para fitoplâncton avaliado separadamente. Isso indica que os diferentes níveis que compõem a microbiota aquática tem um padrão de resposta similar ao aumento da temperatura. O aquecimento levou ao predomínio de organismos pequenos e generalistas em suas necessidades de habitat na avaliação taxonômica e funcional do fitoplâncton via microscopia tradicional. Por outro lado, através de uma abordagem molecular, a microbiota autotrófica não apresentou respostas ao aquecimento. Assim, a utilização de diferentes abordagens para investigar as comunidades pode produzir resultados complementares. Essa tese contribui para predizer como diferentes grupos da microbiota aquática respondem a variações nas condições do ambiente. Diante das inúmeras alterações climáticas e ambientais preditas para o futuro, estudos como este são relevantes, já que modificações na composição da microbiota aquática podem afetar inúmeros processos e serviços ecossistêmicos.