Responses of the phytoplankton functional structure to the spatial and temporal heterogeneity in a large subtropical shallow lake

Respostas de diferentes traços funcionais do fitoplâncton à heterogeneidade espacial e temporal em um extenso lago raso subtropical

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Abstract: Aim: Studies on biological communities that take into account only the species composition and abundances (or biomass) and their relative contributions, most of the time, do not reflect their ecological functions, especially considering the wide spatial and temporal variation of large shallow lakes. This paper aimed at evaluating the influence of environmental spatial and temporal heterogeneity on the functional structure of phytoplankton in a subtropical large shallow lake. **Methods:** Seasonal samplings were carried out in 2010 and 2011, in 19 sampling sites distributed along the entire length (90 km) and width (3-10 km) of Lake Mangueira, a large (820 km^2) and shallow lake (z_{mean} = 2.6 m), comprising the littoral and pelagic zones of the north, central and southern regions. Abiotic variables and phytoplankton functional traits (volume, maximum linear dimension, life forms) and functional groups were analyzed as measures of functional structure. **Results:** The results showed that there was no spatial organization of phytoplankton functional traits during the study. Colonial non-flagellated organisms, organisms with cellular volume between 10^3 and 10^4 µm^3 and greater than 10^4 µm^3, and with maximum linear dimension between 21 and 50 µm prevailed in all zones and regions. Phytoplankton functional groups and traits responded to resource variation, especially increasing their variety and contribution during spring and summer periods. **Conclusions:** The functional structure of the phytoplankton community in Lake Mangueira, here accessed by functional traits and RFGs, was more conditioned by its environmental temporal variability rather than by the spatial variation, indicating that the resources and life conditions seasonal variation strongly influence the phytoplankton in this ecosystem.

Keywords: functional traits; size; functional groups; littoral zone; pelagic zone.

Resumo: Objetivo: Análises de comunidades biológicas que levam em conta apenas a composição e a abundância das espécies e suas contribuições relativas, na maioria das vezes, não reflete suas funções ecológicas, especialmente considerando-se a ampla variação espacial e temporal de extensos lagos

rasos. Este trabalho teve como objetivo avaliar a influência da heterogeneidade espaço-temporal na estrutura funcional do fitoplâncton em um extenso lago raso subtropical. **Métodos:** Foram realizadas amostragens sazonais, em 2010 e 2011, em 19 pontos amostrais distribuídos ao longo de toda a extensão (90 km) e largura (3-10 km) da Lagoa Mangueira, um extenso (820 km²) lago raso ($Z_{média}$ = 2,6 m), compreendendo as zonas litorânea e pelágica das regiões norte, centro e sul. Foram analisadas variáveis abióticas, traços funcionais (volume, máxima dimensão linear, formas de vida) e grupos funcionais como medidas da estrutura funcional. **Resultados:** Os resultados demonstraram que não houve organização espacial dos atributos funcionais do fitoplâncton no período estudado. Formas de vida coloniais não flageladas, organismos com volume celular entre 10^3 e 10^4 μm³ e maiores que 10^4 μm³, e com máxima dimensão linear variando entre 21 e 50 μm prevaleceram em todas as zonas e regiões estudadas. Os grupos funcionais fitoplanctônicos responderam à variação nos recursos, especialmente aumentando sua variedade e contribuição nos meses de primavera e verão. **Conclusões:** A estrutura funcional da comunidade fitoplanctônica da Lagoa Mangueira, aqui acessada pelos traços e grupos funcionais, foi primariamente condicionada pela variação temporal, não apresentando evidente organização espacial, indicando que a variação sazonal das condições de vida e recursos influencia significativamente o fitoplâncton neste ecossistema.

Palavras-chave: traços funcionais; tamanho; grupo funcional; zona litoral; zona pelágica.

1. Introduction

Functional-based approaches are widely used in ecology (Litchman & Klausmeier, 2008) and, once applied in community ecology, have led to considerable progress in understanding the effects of environmental filters on species organization (Jung et al., 2010). They are based on functional traits which may be defined as any morphological, physiological or phenological feature, which impacts fitness indirectly via its effects on growth, reproduction and survival (Violle et al., 2007). Then, describing communities through functional traits may be important for revealing the relations between environmental changes, community composition and ecosystem processes (Lavorel et al., 2008).

Initiatives in classifying phytoplankton regarding its functional features are not new (e.g. Margalef, 1978; Reynolds, 1980, 1997; Reynolds et al., 2002; Salmaso & Padisák, 2007; Kruk et al., 2010, Chen et al., 2015). Due to its simplicity and well-defined traits which are related to ecological niches, phytoplankton may be considered an ideal system for testing functional approaches (Litchman & Klausmeier, 2008). Among the most used approaches, Reynolds' functional grouping system (Reynolds et al., 2002), recently proposed to be named as Reynolds Functional Groups - RFG (Kruk et al., 2017), is one of the most accepted and used (Padisák et al., 2009), and presents advantages over traditional phylogenetic classifications, since it groups organisms based on their survival strategies and their adaptations to environmental conditions (Salmaso & Padisák, 2007).

According to Padisák et al. (2009), among the premises supporting the theory of functional groups are: a species that is functionally well-adapted is likely to tolerate the constraining conditions of factor deficiency more successfully than individuals of a less well-adapted species, and a habitat with certain limiting factors, such as light, phosphorus, carbon or nitrogen, is more likely to be populated by species presenting the appropriate adaptations for overcoming these limiting factors. In addition, sensitivity to grazing is another determining premise of functional groups, as described in the original proposal by Reynolds et al. (2002). Accordingly, a close relationship between functional traits of phytoplankton organisms and environmental variation is expected, as already shown by several studies and functional approaches in temperate (e.g. Huszar et al., 2003; Becker et al., 2010), tropical (e.g. Crossetti & Bicudo, 2008; Gemelgo et al., 2009) and subtropical pelagic communities (e.g. Kruk et al., 2002; Bonilla et al., 2005; Becker et al., 2009).

Both bottom up and top down controls for phytoplankton are modulated by climatological, hydrological (e.g. residence time) and hydrographical (e.g. mixing pattern) conditions (Talling, 1986; De Senerpont Domis et al., 2013). In large shallow lakes, hydrodynamics may largely influence physical, chemical, and biological variability (Scheffer, 1998). Changes in the water column in lentic systems, related to water circulation patterns or even to water level, are considered one of the main environmental forces that affect the dynamics of phytoplankton (Crossetti et al., 2007). In addition, turbulence and resource availability are recognized as the most important variables in determining its local variability (Margalef, 1978; Reynolds, 2006). The unequal distribution of resources

(light and nutrients), in spatially heterogeneous environments have been related to phytoplankton functional diversity (Caputo et al., 2008; Rychtecký & Znachor, 2011; Crossetti et al., 2014).

In this sense, the present study aimed at evaluating the influence of spatial and temporal heterogeneity on the functional structure of the phytoplankton community in a large subtropical shallow lake. Large variability of functional diversity, accessed through different phytoplankton functional traits (volume, maximum linear dimension, life forms) and functional groups is expected to be related to the environmental heterogeneity in the studied ecosystem. Biological regulation by grazing, which may be important in controlling species composition, was not considered in this study.

2. Material and Methods

2.1. Study area

The study was carried out in Lake Mangueira, located in the Taim Hydrological System (SHT), in the southern part of Rio Grande do Sul state, southern Brazil (32º20' and 33º00' S and 52º20' and 52º45' W). The region has a subtropical climate (Cfa in the Köppen classification; Alvares et al., 2014). Lake Mangueira is a large shallow coastal lake which has a maximum depth of 7 m, mean depth of 2.6 m, and is 90 km long and 3-10 km wide, covering a total area of 820 km² (Figure 1). The main axis of the lake is northeast-southwest, aligned with the prevailing winds (Fragoso Junior et al., 2008), being classified as a continuous warm polymictic system (Lewis Junior, 1983), with daily mixtures due to the intense wind action and rare periods of stratification. The lake is connected with wetlands to the north and extensive macrophyte banks (e.g. *Myriophyllum* spp., *Potamogeton* spp., *Cabomba caroliniana* Gray, *Egeria densa* Planch., *Ceratophyllum demersum* L., *Utricularia* sp., *Zizaniopsis bonariensis* Speg. and *Schoenoplectus californicus* (Mey.) Soják) on its margins, especially in the south where the macrophytes cover ~27% of the littoral area. The lake is considered oligotrophic, but during
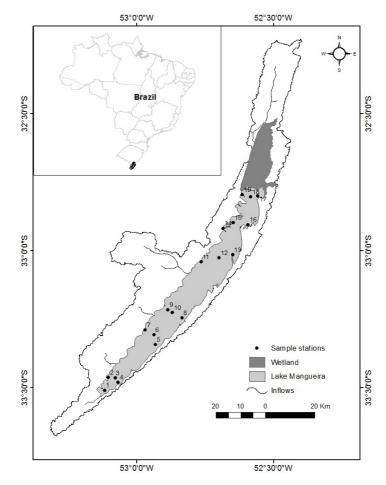

Figure 1. Map and location of Lake Mangueira, South Brazil and the respective sampling stations (black dots).

the spring and summer, the lake mesotrophic conditions occur when water is withdrawn to irrigate rice fields (~2 L ha^{-1} s^{-1} for 100d), and there are higher nutrient loads from the watershed (Fragoso Junior et al., 2008).

2.2. Sampling (abiotic and biological variables)

Subsurface water samples were collected in 2010 and 2011, on a seasonal scale (4 samplings per year), at 19 sites along the entire length of the lake, comprising the pelagic (sampling sites 1, 3, 6, 10, 12, 15, and 18), left littoral (western sampling sites 2, 7, 9, 11, 14, and 19), and right littoral (eastern sampling sites 4, 5, 8, 13, 16, and 17) zones (Figure 1). Littoral samples were taken avoiding macrophyte stands.

Wind direction and velocity and precipitation data were provided by the closest meteorological station (at Santa Vitória do Palmar city, INMET, 2012), which measures climate variables at different times of the day. Data were interpolated according to the time spent at each sampling site. The following parameters were analysed: total phosphorus - TP, soluble reactive phosphorus - SRP, total nitrogen - TN, ammonium - N-NH$_4$$^+$ and N-NO$_3$$^-$ (Mackeret et al., 1989), soluble reactive silica – SRSi and total suspended solids - TSS (APHA, 2012). The water transparency (Secchi disk), water temperature, pH, electrical conductivity and dissolved oxygen (YSI 6920 probe) were also measured. Humic substances and turbidity were determined by spectrophotometry (APHA, 2012). The carbon forms were evaluated using the TOC V equipment (Shimadzu 5000). The humic acids were evaluated (350 nm) using a spectrofluorimeter (BBE-Moldaenke).

For the quantitative analysis of the phytoplankton community, samples were fixed with 1% acetic lugol. Phytoplankton quantification followed Utermöhl (1958) and the sedimentation time and accuracy (counting error < 5%) followed Lund et al. (1958). The biomass (mm^3 L^{-1}) was expressed by means of biovolume measurements, calculated for each species based on geometric solids that were closest to the cell form, isolated or combined (Hillebrand et al., 1999), based on the average measurements of 20 to 30 individuals, whenever possible. Phytoplankton functional structure was accessed through different functional traits. Thus, phytoplankton species were classified based on their: (i) life forms (UF = unicellular flagellated, CF = colonial flagellated, UNF = unicellular non-flagellated, CNF = colonial non-flagellated,

and FI = filamentous), (ii) maximum linear dimension (MLD, I < 10 μm, II = 11 to 20 μm, III = 21 to 50 μm, IV > 50 μm), (iii) volume (I < 10 μm^3, II = 10^2 to 10^3 μm^3, III (10^3 to 10^4 μm^3, IV > 10^4 μm^3). Species were also sorted into Reynolds Functional Groups (RFG) (Reynolds et al., 2002; Padisák et al., 2009).

2.3. Data analyses

Descriptive statistical analyses of the environmental and biological variables were performed to explore the amplitude of their variation. Detrended correspondence analysis (DCA) was performed to indicate the unimodal or linear ordering method to be used in the integration of the biological and abiotic variables (Ter Braak & Šmilauer, 1998). After the result of DCA, two Redundancy Analyses (RDA) were performed: one considering the phytoplankton functional groups and other considering the functional traits, both expressed in absolute biomass. The abiotic variables used in the RDAs (see Table 1) were previously selected after an exploratory analysis (Pricipal Component Analysis, PCA) given in Freitas-Teixeira et al. (2016), based on the same data set, being the higly correlated variables (r>0.8) excluded. Climatic variables were included in the abiotic matrix considering their importance to the ecosystem dynamics, as showed by previous studies (Cardoso et al., 2012; Fragoso Junior et al., 2008). The RDA biplot were built indicating the variables with higher correlations with the canonic axes. For that, data were transformed (log x + 1), except by pH. For these analyzes, the software PC-ORD, version 6 (McCune & Mefford, 2011) was used.

3. Results

3.1. Spatial and temporal heterogeneity of the abiotic environment

The northern region of Lake Mangueira showed the highest concentrations of SRP (56 μg L^{-1}), N-NO$_3$$^-$ (411 μg L^{-1}) and TN (905 mg L^{-1}), as well as the highest values of turbidity (80 NTU) and TSS (28 mg L^{-1}); and in the south region occurred the highest values of transparency (2.7 m) and dissolved oxygen (11.8 mg L^{-1}) and the lower values of conductivity (0,2 mS cm^{-1}) (Table 1). Higher values of temperature (24.6°C), pH (9.2), alkalinity (101 mg.L^{-1} CaCO$_3$) and SRSi (4.2 mg L^{-1}) were observed in spring and summer periods (Table 1), meanwhile in autumn, higher values of precipitation (13.7 mm) and wind velocity (9 m s^{-1}) were registered (Table 1). Humic acids – 350nm (0.05)

Table 1. Minimum and maximum (mean ± standard deviation) values of the climatological, abiotic and biological variables sampled in the South (n = 56), Center (n = 48) and North (n = 48) regions of Lake Mangueira, considering the Left margin (n = 48), Pelagic zone (n= 56) and Right margin (n = 48), during the Spring (n = 38), Summer (n = 38), Autumn (n = 38) and Winter (n = 38).

Variable	South	Center	North	Left margin	Pelagic zone	Right margin	Spring	Summer	Autumn	Winter
Temperature (°C)	10-25 (18 ± 5)	11-25 (18 ± 4.9)	12-25 (19 ± 4.8)	10-25 (18 ± 4.9)	10-25 (18 ± 4.9)	10-25 (18 ± 4.8)	21-26 (23 ± 1.4)	20-28 (23 ± 0.9)	13-18 (17 ± 1.5)	10-13 (12 ± 0.8)
Wind velocity (m s⁻¹)	1-9 (2.8 ± 2.5)	0-8 (3.1 ± 2.5)	1-8 (4.2 ± 2.6)	0-9 (3.4 ± 2.6)	0-9 (3.3 ± 2.5)	0-9 (3.4 ± 2.7)	2-8 (4.7 ± 2.2)	0-2 (1.3 ± 0.7)	0-9 (4.5 ± 3.3)	1-8 (2.8 ± 1.8)
Precipitation (mm)	0-2.1 (0.3 ± 0.7)	0-13.7 (1.7 ± 4.6)	0-13.7 (1.7 ± 4.6)	0-13.7 (1.2 ± 3.8)	0-13.7 (1.1 ± 3.6)	0-13.7 (1.2 ± 3.8)	0-0 (0 ± 0.0)	0-0 (0 ± 0.0)	0-13.7 (4.7 ± 0.0)	0-0.1 (0.03 ± 0.04)
Transparency (m)	0.6-2.7 (1.3 ± 0.5)	0.3-2.4 (0.9 ± 0.4)	0.4-1.7 (0.9 ± 0.4)	0.3-2.4 (1 ± 0.4)	0.3-2.7 (1 ± 0.5)	0.3-2.4 (1.1 ± 0.5)	0.5-1.6 (0.9 ± 0.3)	0.7-2.1 (1.1 ± 0.4)	0.6-2.35 (1.1 ± 0.4)	0.3-2.7 (1.1 ± 0.7)
Depth (m)	1.4-5.7 (2.9 ± 1.4)	0.8-7.0 (3.2 ± 1.9)	1.1-3.7 (2.3 ± 0.7)	0.8-2.7 (1.6 ± 0.4)	1.5-7 (3.6 ± 1.6)	1-5.2 (2.9 ± 1.1)	1-7 (3.3 ± 1.6)	0.9-6.3 (2.6 ± 1.3)	0.8-6.3 (2.6 ± 1.5)	1.2-6.8 (3.1 ± 1.6)
pH	7.4-8.9 (7.9 ± 0.4)	7.4-9.1 (7.9 ± 0.5)	7.4-9.2 (7.9 ± 0.5)	7.4-9 (7.9 ± 0.4)	7.4-9 (7.9 ± 0.4)	7.4-9 (7.9 ± 0.5)	7.4-8.6 (7.9 ± 0.3)	7.4-9.2 (8.4 ± 0.5)	7.4-8.3 (7.9 ± 0.2)	7.4-8.0 (7.6 ± 0.2)
Conductivity (mS cm⁻¹)	0.2-0.4 (0.3 ± 0.05)	0.3-0.5 (0.3 ± 0.05)	0.3-0.5 (0.3 ± 0.06)	0.2-0.5 (0.3 ± 0.06)	0.3-0.5 (0.3 ± 0.05)	0.3-0.5 (0.3 ± 0.05)	0.3-0.5 (0.4 ± 0.06)	0.2-0.4 (0.3 ± 0.02)	0.3-0.4 (0.3 ± 0.05)	0.3-0.3 (0.3 ± 0.01)
Dissolved oxygen (mg L⁻¹)	7.8-11.8 (9.3 ± 1.0)	8.1-11.7 (9.5 ± 0.9)	8.0-11.9 (9.6 ± 0.8)	7.8-11.8 (9.4 ± 0.9)	7.8-11.9 (9.5 ± 1.0)	7.9-11.8 (9.5 ± 0.9)	7.8-9.0 (8.5 ± 0.4)	8.6-9.9 (9.0 ± 0.4)	8.8-10 (9.6 ± 0.3)	10-11 (10.7 ± 0.7)
Alkalinity (mg L⁻¹ CaCO₃)	48-84 (69 ± 8.2)	59-84 (70 ± 6.4)	59-101 (71 ± 8.8)	47-101 (68 ± 9)	58-84 (70 ± 7)	57-85 (70 ± 7)	68-101 (74 ± 6.1)	59-84 (72 ± 10.4)	57-70 (64 ± 3.5)	47-74 (68 ± 5.9)
Turbidity (NTU)	3-59 (17.7 ± 14.3)	6-52 (22 ± 14.3)	1-80 (25 ± 17.7)	1-80 (22 ± 16)	3-59 (20 ± 15)	4-55 (21 ± 15)	18-80 (37 ± 15)	5-55 (24 ± 12)	1-17 (8 ± 3.6)	3-41 (15 ± 9.6)
Total suspended solids (mg L⁻¹)	4-28 (13 ± 5.9)	5-25 (13.2 ± 5.2)	1.5-27.5 (12.9 ± 6.1)	1.5-28 (13.3 ± 5.7)	3-25 (13.1 ± 5.4)	2-28 (12.7 ± 6.2)	2-27 (13.0 ± 5.2)	7.5-23 (12.9 ± 3.4)	1.5-25 (11.5 ± 6.6)	5-28 (14.8 ± 6.7)
Total phosphorus (μg L⁻¹)	11.6-85.2 (30.2 ± 19.8)	15-80.5 (35.9 ± 16.8)	14-75 (42 ± 14.7)	13-82 (34 ± 17)	11.6-82 (35.7 ± 18.8)	15.6-37 (37 ± 17)	14.8-85 (49 ± 23)	17.0-53 (33 ± 9)	11.6-48 (25 ± 8.7)	12.8-80 (34 ± 17.3)
Soluble reactive phosphorus (μg L⁻¹)	6.9-56.3 (19.3 ± 13.7)	1.5-52.1 (19.8 ± 11.4)	9.1-55.1 (27.8 ± 12.2)	1.5-56.3 (20.8 ± 13.3)	7.1-44 (20.4 ± 12.4)	7.4-54 (22.6 ± 13.6)	9.5-56 (32.1 ± 17.7)	6.9-38 (16.9 ± 8.4)	1.5-45 (17.9 ± 8.4)	8-37 (17.9 ± 8.5)
Total nitrogen (μg L⁻¹)	110-561 (345 ± 131)	105-562 (349 ± 131)	86-905 (380 ± 182)	110-597 (360 ± 144)	105-721 (352 ± 151)	86-905 (361 ± 153)	86-617 (340 ± 162)	161-721 (370 ± 126)	110-543 (325 ± 134)	205-905 (394 ± 166)
Ammonium (μg L⁻¹)	10-2000 (107 ± 268)	2.6-235 (58 ± 53)	8-277 (63 ± 53)	8-264 (62 ± 62)	2.6-2000 (94 ± 266)	10-301 (74 ± 66)	11-348 (109 ± 90)	9-159 (43 ± 31)	10-2000 (83 ± 319)	2-230 (75 ± 55)
Nitrate (μg L⁻¹)	10-293 (96 ± 66)	10-325 (107 ± 72)	9-410 (105 ± 78)	10-410 (107 ± 79)	10-293 (96 ± 68)	9-299 (104 ± 68)	35-410 (125 ± 70)	10-325 (127 ± 95)	9-73 (40 ± 21)	14-192 (117 ± 33)
Soluble reactive silica (mg L⁻¹)	1.5-4.1 (2.8 ± 0.6)	1.6-4.2 (2.9 ± 0.6)	1.3-4.2 (2.7 ± 0.7)	1.3-4.2 (2.7 ± 0.7)	1.5-4.1 (2.8 ± 0.6)	1.6-4.2 (2.8 ± 0.6)	2.4-4.2 (3.3 ± 0.7)	2.7-3.5 (3.0 ± 0.2)	1.7-3.3 (2.7 ± 0.3)	1.3-2.8 (2.1 ± 0.4)

Table 1. Continued...

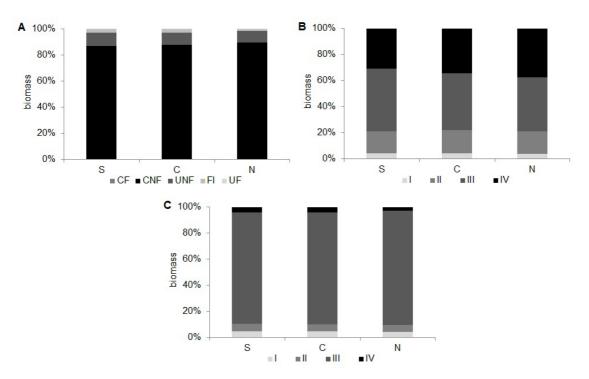| Variable	South	Center	North	Left margin	Pelagic zone	Right margin	Spring	Summer	Autumn	Winter
Dissolved inorganic carbon (mg L^{-1})	9.4-26 (14.8 ± 4.1)	8.0-117 (16.1 ± 15.4)	9.6-24 (15.6 ± 3.8)	9.4-23 (14.5 ± 3.8)	9.4-24 (14.6 ± 3.8)	8.0-117 (16.5 ± 15.4)	8.9-23 (23.3 ± 16.7)	8.8-16 (12.9 ± 1.7)	9.4-15 (13.1 ± 1.3)	8.0-117 (17.1 ± 16.9)
Humic acids-350 nm	0.004-0.05 (0.01 ± 0.01)	0.004-0.03 (0.01 ± 0.01)	0.007-0.04 (0.02 ± 0.01)	0.006-0.05 (0.02 ± 0.01)	0.004-0.04 (0.01 ± 0.01)	0.005-0.05 (0.02 ± 0.01)	0.01-0.04 (0.02 ± 0.01)	0.0-0.03 (0.01 ± 0.01)	0.01-0.05 (0.01 ± 0.01)	0.01-0.05 (0.02 ± 0.01)
Total biomass (mm^3 L^{-1})	0.2-12.5 (5.7 ± 2.8)	1.4-23.6 (7.1 ± 3.9)	2.3-19 (7.8 ± 4.4)	0.2-23.6 (7.4 ± 4.6)	0.2-17.5 (6.7 ± 3.8)	1.8-17.2 (6.4 ± 25.8)	3.4-23.6 (8.7 ± 4.3)	0.2 - 18.6 (8.0 ± 4.7)	2.3-9.9 (4.5 ± 1.6)	1.8-12.8 (6.0 ± 2.3)

and DIC (16.9 mg L^{-1}) values were higher in winter period. Higher mean concentrations of N-NH$_4^+$ (95 µg L^{-1}) were observed in the pelagic zone. Considering the temporal variability, the availability of SRP and TP were higher during spring time (mean values 32.1 and 50 µg L^{-1}, respectively; Table 1). In general, the abiotic data evidenced a clearer temporal variability, demonstrating less spatial variation, even though a north-to-south spatial gradient of nutrients has been observed.

3.2. Spatial and temporal heterogeneity of phytoplankton traits and Reynolds Functional Groups (RFG)

At total, 117 phytoplankton species belonging to seven major algal groups (Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cyanobacteria, Dinophyceae, Euglenophyceae, and Zygnemaphyceae) were identified. Among the phytoplankton life forms, the colonial non-flagellated organisms were the most representative in all regions, accounting for 87, 88 and 90% of the total biomass registered (see Table 1 for biomass values), respectively, in the southern, central and northern regions of Lake Mangueira, followed by the unicellular non-flagellated organisms contribution

(10, 10, and 9%, respectively) (Figure 2A). The filamentous organisms accounted for 2.5% of the total biomass in southern and central regions of the lake, meanwhile the other life forms showed lower biomass values (Figure 2A).
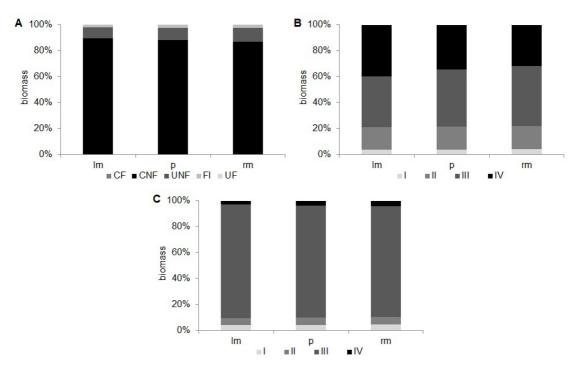
Considering the volume, organisms sorted into the categories III (from 10^3 to 10^4 µm^3) and IV (> 10^4 µm^3) were the most representative, contributing with 48, 31 and 43%, and 34, 41 and 37% in the south, center and north regions, respectively (Figure 2B). As to the maximum linear dimension, organisms sorted into the class III (between 21 and 50 µm) showed higher biomass contribution in these same regions (85% in the south, 86% in the center and 88% in the north) (Figure 2C).

The functional traits did not show any trend considering the littoral and pelagic zones of Lake Mangueira (Figure 3). Colonial non-flagellated organisms dominated on the left margin, pelagic zone and right margin (90, 88 and 87% of the total biomass, respectively) (Figure 3A). Following the same pattern observed for the size classes in the southern, central and northern regions of the lake, the volume categories III and IV and organisms with maximum linear dimension (class III) showed

Figure 2. Relative phytoplankton biomass (%) of phytoplankton shared by life forms (A), volume (B) and maximum linear dimension, MLD (C) categories in the southern (S, n = 56), central (C, n = 48) and northern (N, n = 48) regions of Lake Mangueira. UF = unicellular flagellated, CF = colonial flagellated, UNF = unicellular non-flagellated, CNF = colonial non-flagellated, and FI = filamentous. Volume and MLD categories are given in methods section.

Figure 3. Biomass (%) of phytoplankton life forms (A), volume (B) and maximum linear dimension (C) in the littoral (left margin – lm, n = 48; right margin – rm, n = 48) and pelagic (pel, n = 56) zones of Lake Mangueira. UF = unicellular flagellated, CF = colonial flagellated, UNF = unicellular non-flagellated, CNF = colonial non-flagellated, and FI = filamentous. Volume and MLD categories are given in methods section.

higher contribution in both littoral and pelagic zones (Figure 3B, 3C).

Eighteen RFGs were identified, with only six of them accounting for at least 90% of the total phytoplankton biomass (Table 2). The functional groups **K** and **L$_O$** (Table 2) presented the higher biomass values in the south (40 and 47%, respectively), center (43 and 43%) and north sampling sites (49 and 40%). In the southern of Lake Mangueira, higher relative biomass of the functional groups **Y** (*Cryptomonas erosa* Ehrenberg*)*, **E** (*Dinobryon sertularia* Ehrenberg, *Mallomonas* sp.), **H1** (*Dolichospermum solitarium* (Klebahn) Wacklin, L.Hoffmann & Komárek), **D** (Table 2), **X1** (*Monoraphidium* spp.) and **W1** (*Euglena* sp., *Phacus* sp.) was verified. In the central sampling sites, **S$_N$** (*Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju), **X2** (*Chlamydomonas planctogloea* Skuja, *Phacotus* sp.), **N$_A$** (*Cosmarium* spp.), **C** (*Cyclotella meneghiniana* Kützing), **J** (Table 2), **S1** (Table 2) and **MP** (*Pseudanabaena catenata* Lauterborn, *P. galeata* Böcher, *Mougeotia* sp.) presented higher biomass. The functional groups **M** (*Microcystis aeruginosa* (Kützing) Kützing, **F** (Table 2) **P** (*Fragilaria crotonensis* Kitton,

Closterium aciculare T.West) and **W1** (*Euglena* sp., *Phacus* sp.) presented higher contribution in the north. (Figure 4A).

Regarding the littoral and pelagic zones, the functional groups **K** and **LO** were the most representative on the left margin (50 and 39% of total biomass, respectively), pelagic region (44 and 43%) and in the right margin (41 and 46%) (Figure 4B). Groups **M** and **Y** were not registered in the left margin of Lake Mangueira.

In general, no spatial variation was observed regarding life forms, volume or MLD traits. Regarding RFGs, most of the groups' biomass was higher in the pelagic zone, and the varied between the lake regions.
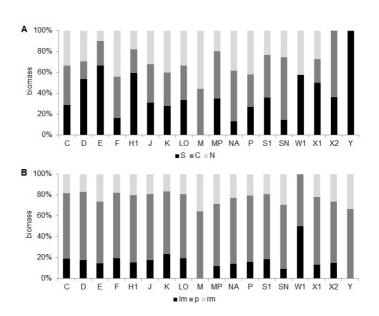
3.3. Phytoplankton and environment relationships

The integrated analysis of the functional groups and traits *versus* the abiotic variables recorded during the study period was carried out through RDA. A total of 77.4% of the data variability was explained by the two first axes (64.2 and 13.2%, respectively). A strong correlation between the matrices and the first two axes was found (Axis 1, r = 0.75; Axis 2, r = 0.50). The Monte Carlo indicated that the ordination was statistically

Table 2. Main phytoplankton Reynolds Functional Groups (RFG) in Lake Mangueira, South Brazil, and their respective main representative species, life forms (LF), maximum linear dimension (MLD), volume (VOL) and habitat description, tolerances and sensitivities for each functional group*. UNF = unicellular non-flagellated, CNF = colonial non-flagellated, and FI = filamentous.

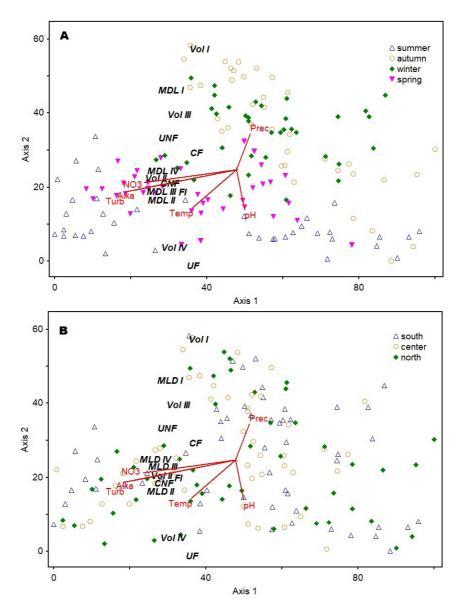| RGF	Main Species	LF	MLD	VOL	Habitat*	Tolerances*	Sensitivities*
D	*Fragilaria acus* (Kützing) Lange-Bertalot	UNF	IV	III	Shallow, enriched turbid waters	Flushing	Nutrient depletion
F	*Oocystis lacustris* Chodat	CNF	III	III	Clear epilimnia	Low nutrients, high turbidity	CO_2 deficiency
J	*Tetraedron minimum* (A.Braun) Hansgirg	UNF	II	II	shallow, enriched lakes, ponds and rivers		Settling into low light
	Scenedesmus obtusus Meyen	CNF	III	IV			
K	*Aphanocapsa conferta* (West & G.S.West) Komárková-Legnerová & Cronberg	CNF	III	IV	Short, nutrient-rich columns		Deep mixing
	Anathece smithii Komárková-Legnerová & Cronberg) Komárek, Kastovsky & Jezberová	CNF	III	II			
	Aphanothece sp.	CNF	III	II			
	Aphanothece stagnina (Sprengel) A.Braun	CNF	II	II			
	Synechocystis aquatilis Sauvageau	UNF	I	I			
L$_o$	*Chroococcus dispersus* (Keissler) Lemmermann	CNF	III	II	Summer epilimnia in mesotrophic lakes	Segregated nutrients	Prolonged or deep mixing
	Chroococcus giganteus West	CNF	II	II			
	Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O.Komárek & Zapomelová	CNF	III	III			
	Chroococcus planctonicus Bethge	CNF	III	III			
	Coelomeron sp	CNF	III	III			
	Gomphosphaeria aponina Kützing	CNF	III	III			
S1	*Planktolyngbya contorta* (Lemmermann) Anagnostidis & Komárek	FI	IV	II	Turbid mixed layers	High light deficiency	Flushing

*from Reynolds et al. (2002).

Figure 4. Relative biomass (%) of phytoplankton Reynolds Functional Groups in the southern (S, n = 56), central (C, n = 48) and northern (N, n = 48) regions (A) and in the littoral (left margin - lf, n = 48; right margin – rm, n = 48) and pelagic (pel, n = 56) zones (B) of Lake Mangueira.
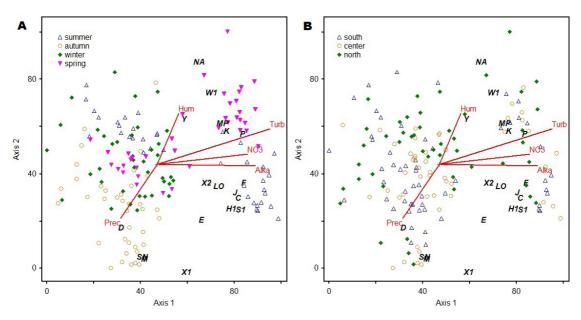
significant (p = 0.001) (Figure 5A and 5B). For the first axis, the most important variables were turbidity (r = -0.731), alkalinity (-0.664) and nitrate (-0.601), while for axis two it was pH (-0.579), temperature (-0,551) and precipitation (-0.523). Higher biomass of most of the traits were ordinated to the summer and spring sample units, under higher values of N-NO$_3^-$, turbidity, alkalinity and temperature. Associated to the higher values of precipitation and to the autumn and winter sample units it was found the Volume I and MLD I classes. In general,

the phytoplankton functional traits varied more temporally than spatially (Figure 5A and 5B)

The RDA performed with RFGs and the abiotic variables resumed 55.2% of the total variability of the data, in the two first axes (40.4 and 14.8%, respectively). A strong correlation between the matrices and the first two axes was found (Axis 1, r = 0.80; Axis 2, r = 0.57) and the Monte Carlo indicated that the ordination was statistically significant (p = 0.001) (Figure 6A and 6B). For the first axis, the most important variables were turbidity (r = 0.740), alkalinity (0.643) and N-NO$_3^-$ (0.590),

Figure 5. Redundancy analysis (axis 1 and 2) of phytoplankton traits in relation to abiotic variables of Lake Mangueira, emphasizing the temporal (A) and spatial (B) variation. Vol= phytoplankton volume, MLD = maximum linear dimension, CF = colonial flagellated, UNF = unicellular non-flagellated, CNF = colonial non-flagellated, FI = filamentous, Turb = turbidity, Alk = alkaliniy, NO3 = nitrate, Temp = water temperature, Prec = precipitation.

Figure 6. Redundancy analysis (axis 1 and 2) of RFGs in relation to abiotic variables of Lake Mangueira, emphasizing the temporal (A) and spatial (B) variation. Turb = turbidity, Alk = alkaliniy, NO3 = nitrate, Hum = humic substances, Prec = precipitation.

while for axis two it was precipitation (-0.586) and humic acids (0.553). The RFGs **P**, **K**, **N$_A$**, **MP**, **Y** and **W1** were grouped to the higher values of humic acids, nitrate and turbidity and to the spring sample units. **F**, **L$_O$**, **X2**, **J**, **C**, **H1**, **S1** and **E** were associated to the higher values of alkalinity and nitrate, and to the summer sample units. The groups **M**, **S$_N$**, **X1** and **D** were associated with the highest values of precipitation in the autumn. In general, the analysis evidenced the strong temporal organization of functional groups when considering the abiotic scenario (Figure 6A and 6B).

4. Discussion

The results of the present study showed that most of the phytoplankton functional structure, based both on traits and RFGs, was temporally organized, varying according to the seasonal variability of Lake Mangueira. Then, no clear spatial trend was verified neither for the pelagic and littoral zones nor the southern, central and northern regions when integrating the biological data with the abiotic scenario (Figure 5 and 6). This result was contrary to what was expected since the presence of macrophytes, usually found in the littoral regions of shallow lakes, can alter the availability of conditions and resources, as already observed in other studies (Fonseca & Bicudo, 2010; Villamagna & Murphy, 2010). Comparatively, the open water region in shallow lakes tends to be less variable from the point

of view of phytoplankton resources (in the absence of macrophytes) (Padisák et al., 2003).

Considering the fact that greater phytoplankton diversity has been related to more stable situations when comparing to continuous and intense stress conditions (Sommer et al., 1993), the greater stability of the pelagic region could favor phytoplankton species by offering more time and resources (higher light availability, for example) to recruit species with different traits, which was not observed in the present study. One of the possible reasons for this homogeneous distribution of the traits pattern of life forms and phytoplankton size classes along Lake Mangueira is the hydrodynamic of this ecosystem. Previous studies have demonstrated that wind and fetch, as well as the reduced depth are responsible for promoting the continuous mixing of the water body, leading to a great environmental homogeneity (Freitas-Teixeira et al., 2016).

In highly turbulent and turbid shallow lakes, and with margins densely colonized by aquatic macrophytes, such as Lake Mangueira, planktonic spatially structured community could be expected, as mentioned above, especially assigned to the shading or competition for nutrients by macrophytes that may inhibit phytoplankton growth (Fonseca & Bicudo, 2010). However, some studies have demonstrated that the horizontal distribution of communities in lakes might be closely related to the ecosystem size, which may influence

several limnological processes (Scheffer & Van Nes, 2007). This relationship has been shown in some studies (e.g. Post et al., 2000; Borics et al., 2011), suggesting that the ecosystem size may also matter for phytoplankton horizontal distribution. For instance, phytoplankton homogeneous distribution in large lakes has already been reported (Padisák & Dokulil, 1994; Freitas-Teixeira et al., 2016), as has the occurrence of species with no active locomotion ability and high sinking rates in large waterbodies associated with the suitable habitat provided by well-mixed water columns (Borics et al., 2016). This tendency was equally verified in the present study.

In Lake Mangueira, former studies have also shown a spatial pattern of resources assigned to the increased availability of dissolved nutrients, the reduced water transparency and higher concentrations of suspended solids and turbidity, reflecting the influence of the adjacent wetland in the northern region of the lake (Cardoso, et al., 2012; Crossetti et al., 2013, 2014). Comparatively, in the present study, the RFGs were apparently more sensible in demonstrating some slight spatial differences in Lake Mangueira (Figure 4 and 6) than the functional traits. However, despite the minor differences showed by both RDAs (humic substances were important to the RFGs ordination, especially the coda **Y** and **W1** and pH was important for the functional traits structuring), both approaches described clearly the temporal variation of Lake Mangueira, indicating higher phytoplankton biomass especially in summer and spring periods, with higher values of alkalinity, turbidity and N-NO$_3^-$, which may be eventually limiting in Lake Mangueira (Freitas-Teixeira et al., 2016). Ecological interest in grouping species based on functional traits is increasing since they can better predict or explain the structure of communities and their responses to environmental conditions (Brasil & Huszar, 2011). Regarding phytoplankton functional features, its wide diversity of shapes and size is clearly related to kinetics for resource utilization and susceptibility to loss processes (Reynolds et al., 2002).

The morphological diversity of phytoplankton constitutes indispensable survival strategy. Phytoplankton species may evolves to minimize its losses by sedimentation, by decreasing the body size (raising the risk of predation), decreasing its specific gravity (e.g. gas vacuoles of cyanobacteria and the accumulation of oil droplets as a storage product), or increasing its resistance to sedimentation through

the form (Padisák et al., 2003). The presence of flagella is also an important morphological feature to avoid sedimentation losses (Reynolds, 1997). On the other hand, the sedimentation rate can increase significantly with maximum linear dimensions (Kruk et al., 2010). Size structure might also be crucial for preventing predation. Most herbivores consume only a certain variation in size within the full spectrum of available food particles (Brasil & Huszar, 2011). Mucilage sheaths reduce the palatability of algae by making them too large for microzooplankton to ingest, difficult to manipulate by mesozooplankton and mechanically obstructive to cladocerans (Brasil & Huszar, 2011).

The functional groups observed in Lake Mangueira represented, in their majority, the environmental conditions observed, represented by organisms adapted to the high inorganic turbidity (functional groups **D** and **S1**) and to reduced nutritional concentrations (**F** and **LO**) (Reynolds et al., 2002; Padisák et al., 2009). Although functional groups **J** and **K** have been primarily associated with nutritionally enriched environments, their occurrences in nutrient deficient aquatic ecosystems have been reported (Becker et al., 2008). Morphology describes well the ecological functions of the phytoplankton community, while the functional groups are better suited to predicting community composition (Kruk et al., 2011) and their efficiency associated with spatial and temporal heterogeneity have been demonstrated previously (Rychtecký & Znachor, 2011; Crossetti et al., 2014).

Recently, another study demonstrated that the temporal variability determined the phytoplankton structure over the spatial organization in Lake Mangueira, just as observed in the present study with the functional structure of this community. Although the northern region has presented higher values of dissolved nutrients, phytoplankton RFGs were probably limited by the lower values of water transparency. The prevalence of non-flagellated colonial organisms, organisms with a cell volume between 10^3 and 10^4 μm^3 and greater than 10^4 μm^3, and with maximum linear dimension varying between 21 and 50 μm in all studied zones and regions were observed. The prevalence of relatively large organisms and the spatial homogeneity observed may be a consequence of the high environmental variability which is closely related to the local climatological variation and the lake hydrodynamics, as already showed in previous studies (Freitas-Teixeira et al., 2016; Crossetti et al.,

2014; Cardoso et al., 2012). Finally, it is possible to conclude that the functional structure of the phytoplankton community in Lake Mangueira, here accessed by functional traits and RFGs, is more conditioned by its environmental temporal variability rather than by the spatial variation.

Acknowledgements

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