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Morpho-functional groups as an efficient tool for monitoring and management of the Billings reservoir (São Paulo, Brazil)

Grupos morfofuncionais como ferramenta facilitadora ao monitoramento e gestão do reservatório Billings (São Paulo, Brasil)

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ABSTRACT

This research applied the Morphology-Based Functional Groups (MBFGs) combined with classical approaches, such as community descriptor species and phytoplankton classes in the Billings reservoir. This local is the largest freshwater reservoir in the metropolitan region of São Paulo that has around 22 million inhabitants. Two sampling stations differing in predominant uses were studied: Rio Grande, classified as mesotrophic, and Central Body, as super and hypereutrophic. The phytoplankton and limnological variables were collected over six months (n=12). The trophic state index was calculated using phosphorus concentrations. Three canonical correspondence analyses were calculated aiming to examine the effect of environmental variables on the phytoplankton structure from each classification method (descriptor species, classes, and MBFGs), identifying the influence of environmental variables (independent variables) on the abundance patterns of the phytoplankton community (dependent variables). The highest concentrations of chlorophyll *a* and phytoplankton density were recorded at the site with the highest concentrations of total phosphorous (average of 99.86 µg/L in Central Body). This site was also associated with the highest electrical conductivity, total dissolved solids, turbidity, and concentrations of total nitrogen and chlorophyll *a*. The main factors associated with phytoplankton distribution were the trophic state and the operational system. It was concluded that the phytoplankton approach through MBFG efficiently responds to fluctuations in the ecological attributes of phytoplankton, and because they are based on morphological features, MBFG reduces the complexity of identifying and classifying organisms at a specific level.

Keywords: bioindicator; environmental diagnosis; eutrophication; microalgae; morphology-based functional groups; trophic state index.

RESUMO

Esta pesquisa aplicou os Grupos Funcionais Baseados na Morfologia (GFBMs) combinados com abordagens clássicas, como espécies descritoras e classes fitoplanctônicas no reservatório Billings. Este local é o maior reservatório de água doce da região metropolitana de São Paulo que tem cerca de 22 milhões de habitantes. Duas estações amostrais que apresentam diferenças nos usos predominantes foram utilizadas: Rio Grande, classificado como mesotrófico e Corpo Central, como super e hipereutrófico. A coleta de fitoplâncton e variáveis limnológicas foi feita ao longo de seis meses (n=12). O índice de estado trófico foi calculado usando concentrações de fósforo. Três análises de correspondência canônica foram calculadas com o objetivo de examinar o efeito das variáveis ambientais na estrutura do fitoplâncton de cada método de classificação (espécie descritora, classe e GFBM), identificando a influência das variáveis ambientais (variáveis independentes) nos padrões de abundância da comunidade fitoplanctônica (variáveis dependentes). As maiores concentrações de clorofila *a* e densidade fitoplanctônica foram registradas no local com as maiores concentrações de fósforo total (média de 99,86 µg/L no Corpo Central). Este local também foi associado à maior condutividade elétrica, sólidos dissolvidos totais, turbidez e concentrações de nitrogênio total e clorofila *a*. Os principais fatores associados à distribuição fitoplanctônica foram o estado trófico e o sistema operacional. Concluiu-se que a abordagem fitoplanctônica por meio do GFBM é eficiente para responder às flutuações nos atributos ecológicos do fitoplâncton e, por serem baseadas em características morfológicas, o GFBM reduz a complexidade de identificar e classificar organismos em um nível específico.

Palavras-chave: bioindicador; diagnóstico ambiental; eutrofização; microalgas; grupos funcionais baseados na morfologia; índice de estado trófico.

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Introduction

Ecological studies in aquatic ecosystems are highly relevant as the relationship between biotic and abiotic factors directly affects the trophic network, thus impacting the different trophic levels. However, anthropogenic actions lead to environmental degradation with pollution and contamination reaching aquatic environments, disrupting the dynamic balance of the interrelationships, as well as causing negative impacts and countless environmental, economic, and social losses (Häder et al., 2020; Akhtar et al., 2021; Prakash and Verma, 2022).

When assessing anthropogenic impacts on water quality, variations in space and time and the biological, physical, and chemical processes of natural systems must be considered (Lobato et al., 2015). These analyses rely on the long-known changes in the composition of numerous species in aquatic ecosystems, including phytoplankton. Planktonic organisms are excellent bioindicators of environmental conditions and the health of the aquatic environment, being sensitive to changes in water quality, responding promptly, for example, to changes in dissolved oxygen levels, nutrient concentrations, and inputs of toxic contaminants, among others (Casé et al., 2008; Chandel, et al., 2024; Essa et al., 2024).

The construction of reservoirs influences aquatic biodiversity and the provision of ecosystem services. The richness and abundance of species directly reflect the determining factors in communities' structure, generating important information about ecosystem function; therefore, understanding the environmental variability on phytoplankton richness and abundance in tropical reservoirs is essential (Moura et al., 2021).

Phytoplankton responds quickly to changes in the environmental features of the aquatic ecosystem, especially nutrient concentrations, and extreme conditions (Padisák and Naselli-Flores, 2021; Salmaso and Tolotti, 2021; Di Pane et al., 2022; Costa et al., 2024). The responses of phytoplankton assemblages to different biotic and abiotic factors were reviewed by Welbara et al. (2024), who highlighted the influences of light, temperature, water column stability (mixing processes), and nutrient deficiencies such as nitrogen and phosphorus.

Several studies have used ecological classification to group phytoplankton species to predict the consequences of environmental variations on the composition and dynamics of this community (Brasil and Huszar, 2011; Kruk et al., 2011; Machado et al., 2019; Znachor et al., 2020). The adaptive strategies of the phytoplankton community and especially cyanobacteria make these microorganisms excellent indicators of environmental changes (Reynolds, 2006).

In addition to taxonomic classification, studies of phytoplankton should consider their form and function. This community fits into the definition of "functional group" for considering species with morphological, physiological, and ecological similarities, seeking to group microorganisms according to their specific adaptations and needs, e.g., high affinity for phosphorus or carbon, need for a siliceous skeleton, efficiency in capturing light, among others (Reynolds et al., 2002).

Kruk et al. (2010) proposed an approach based exclusively on the morphology of the organisms, considering their relationships with the physiology of the species to be potentially well-defined. The authors described that classification based on simple morphological features, such as individual volume, surface area, and maximum length can capture most of the variability in the functional properties of phytoplankton organisms, thus concluding that morphology would be a good indicator of the functional traits of species. Based on the premise that the competitive abilities (e.g., assimilation of nutrients and light, growth and buoyancy mechanisms) of different groups of species reflect on morphological attributes (size, presence of flagella, or mucilage), the authors proposed the use of Morphology-Based Functional Groups (MBFGs) or Morpho-Functional Groups. Besides, several authors have demonstrated the efficiency of this tool in different environments (Kruk et al., 2011; Caroni et al., 2012; Pineda et al., 2020; Zanon et al., 2021; Silva and Jati, 2024).

Learning about the composition/structure of the phytoplankton community and its relationship with the environment is key to understanding the dynamics of these aquatic systems and providing data/information for management and recovery programs for degraded environments. Therefore, this research applied the MBFGs, combined with classical approaches, such as community descriptor species and phytoplankton classes. The aim of the present study was to describe three approaches commonly used in ecological studies of this community (descriptor species, classes, and MBFGs) to determine the most appropriate procedure for analyzing how the structure of the phytoplankton community responds to environmental variations in the Billings reservoir.

Thus, it is possible to verify the feasibility of applying phytoplankton MBFG in different trophic states and environmental variations as a tool to facilitate the monitoring and management of multiple-use reservoirs.

Material and Methods

Study site

The São Paulo Metropolitan Region (RMSP, acronym in Portuguese) has an estimated 22 million inhabitants (IBGE, 2022). The Upper Tietê River Basin (BAT, in Portuguese) is the largest in the region, covering around 70% of the RMSP territory (FABH-AT, 2019).

Billings is the largest freshwater reservoir in the RMSP, with an area of 127 km^2, a volume of $1{,}228.7 \times 10^6$ m^3, and a maximum depth of 18 meters (Marcondes et al., 2021). The water of the Billings reservoir covers six municipalities: São Bernardo do Campo, São Paulo, Diadema, Ribeirão Pires, Rio Grande da Serra, and Santo André (Figure 1), and is part of the water quality monitoring system for the state of São Paulo, comprising the 6th Water Resources Management Unit (UGRHI 6, in Portuguese), according to the Environmental Company of São Paulo State (CETESB in Portuguese, 2017).

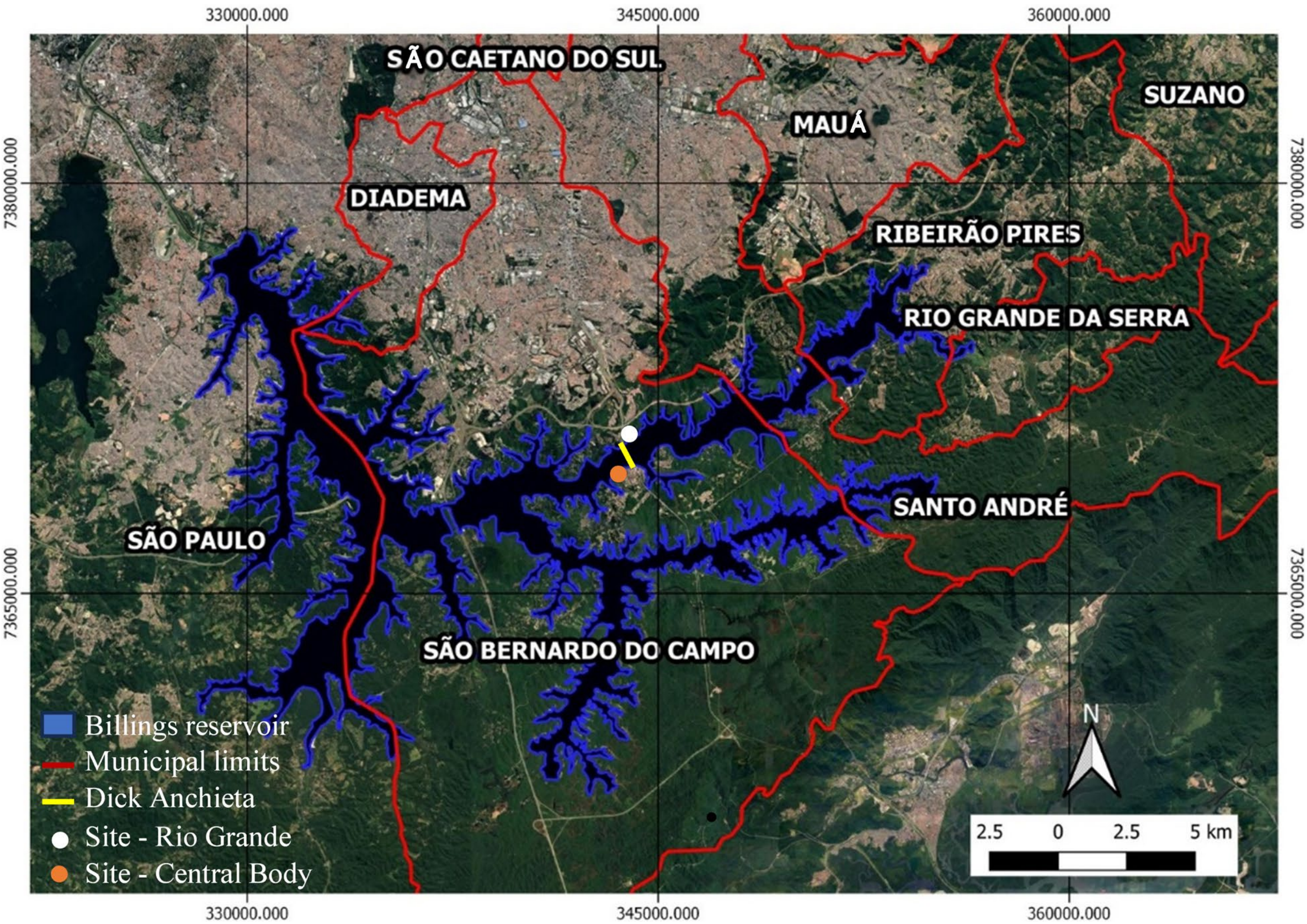

Figure 1 – Satellite image of the two sampling stations located in different regions of the Billings reservoir in São Bernardo do Campo: Rio Grande and Central Body. The dots indicate the approximate sampling location.
Source: adapted from Google Earth Pro. Image collected on January 12, 2024.

The Billings reservoir was originally built to increase electricity generation capacity for the city of São Paulo through the Henry Borden plant (in Cubatão city) (EMAE, s.d.). Currently, it has multiple uses, such as regulating the level of the Pinheiros River by transposing water for flood control, professional and artisanal fishing, water sports, tourism, primary contact recreation (as occurs at Riacho Grande beach, in Central Body), as well as public water supply (in Rio Grande arm) (Penteado et al., 2017; Souza and Duarte, 2024). The increasing use and occupation of the surrounding watershed has caused the continuous process of degradation of the reservoir.

Two sampling stations in the Billings reservoir differ in terms of their predominant uses (Figure 1): 1. Rio Grande (RG), that is close to the water catchment by the São Paulo State Basic Sanitation Company (SABESP, in Portuguese) in the RG arm – classified as mesotrophic by CETESB (2021); and 2. Central Body (CB), located in the central body of the Billings reservoir, classified as super and hypereutrophic by CETESB (2021), which is isolated from the RG arm by the Via Anchieta dike (SABESP, 2023).

The rainfall data series (mm) were provided by the Brazilian National Institute of Meteorology (INMET, 2023), and the water storage capacity of the sites studied (reservoir volume – RV%) were obtained from the database of Producer Systems provided online by the SABESP Portal dos Mananciais (SABESP, 2023).

Water analysis and trophic state index

Water samples were collected bimonthly from March to November 2020. In RG, the samples were collected near the SABESP water collection system, and in CB the samples were collected in the Riacho Grande beach (Figure 1), totaling 12 samples.

The samples were taken from the littoral zone using a metal measuring bucket. In the field, water temperature (°C), dissolved oxygen (mg/L), oxidation-reduction potential (mV), electrical conductivity (μS/cm), turbidity (NTU), total dissolved solids (mg/L), and potential of hydrogen (pH) were determined using a multi-parameter probe (Horiba model U-50). Samples of 1.0 L of water were collected to determine nutrient concentrations and phytoplankton biomass in the laboratory.

Using a Millipore AP-20 filter with a pore size of 0.45 μm, 0.5 L of each sample was immediately filtered in a vacuum pump at the Limnology Reference Laboratory Unit (ULRL) of the São Paulo Fishing Institute to determine the estimate of phytoplankton biomass based on chlorophyll *a* concentration (Marker et al., 1980; Sartory et al., 1984). Analyses to determine the concentrations of total phosphorus (μg/L) and total nitrogen (mg/L) were performed following the methodology described by Valderrama (1981).

The Trophic State Index (TSI) of Carlson (1977) adapted by Lamparelli (2004) for lentic environments was calculated from the results of total phosphorus (TP) and chlorophyll *a* (Chl*a*) (Equations 1 to 3):

$$TSI\ (TP)=10\times[6-((1.77-0.42x\ \ln TP)/\ln 2)] \quad (1)$$

$$TSI\ (Chla)=10\times[6-((0.92-0.34x\ \ln Chla)/\ln 2)] \quad (2)$$

$$Mean\ TSI=TSI(TP)+TSI\ (Chla)/2 \quad (3)$$

Where:
TP=total phosphorus concentration (μg/L);
Chl*a*=chlorophyll *a* concentration (μg/L); and
ln=natural logarithm.

Mean TSI≤47=Ultraoligotrophic; 47<Mean TSI≤52=Oligotrophic; 52<Mean TSI≤59=Mesotrophic; 59<Mean TSI≤63=Eutrophic; 63<Mean TSI≤67=Supereutrophic; Mean TSI>67= Hypereutrophic (CETESB, 2021).

Phytoplankton community structure and morpho-functional grouping

The samples for the qualitative analysis of the phytoplankton community were collected using a 20 μm plankton net (n=12) and preserved in a 4% formaldehyde solution. The taxa were identified using a standard light microscope (Olympus BX51). The identification of taxa at the generic level was used by Bicudo and Menezes (2017). Additionally, specific bibliographies covered other taxonomic categories: Komárek et al. (2014) for cyanobacteria, Krienitz and Bock (2012) for Chlorophyceae, and van den Hoek et al. (1995) for the other classes. The class identification system chosen is based on Wehr and Sheath (2003) and Bicudo and Menezes (2017).

The samples for the phytoplankton quantitative analysis (n=12) were collected with a metal measuring bucket and fixed in 1% Lugol's solution. Phytoplankton populations (org/mL) were examined in random fields, using the sedimentation technique (Utermöhl, 1958) on a Leica DMIL inverted microscope (640x), in 2 and 10 mL sedimentation chambers. The sedimentation time was 3 h/cm (Lund et al., 1958). The counting limit was established using the species rarefaction curve until reaching 100 individuals (cells, colonies, cenobia, and filaments) of the most common species (Bicudo, 1990). Values were expressed as density (org/mL). Taxon richness was measured as the total number of taxa found per sample. Community descriptor species consisted of those whose total relative density accounted for 2% (or more) of the total abundance of the sample, and together, they accounted for more than 80% of the total density of the sample.

Phytoplankton species were grouped into classical taxonomic approach (descriptor species and class) and eight MBFGs, following the methodology proposed by Kruk et al. (2010) and Reynolds et al. (2014). These groups were divided based on nine morphological and physiological traits—volume (V), surface area (S), maximum linear dimension (MLD), surface-to-volume ratio (s.v-1), presence of mucilage, flagella, aerotopes, heterocysts, and demand for silica—all of which were identified for each organism using an optical microscope. According to a study by Reynolds et al. (2014), the eighth morpho-functional group corresponds to nitrogen-fixing cyanobacteria. The relative contribution of each MBFG was calculated for the total richness and density of each sample. The groups were characterized as follows:

Group I: Composed of small organisms with a high individual growth rate and high numerical abundance, it has the lowest values of MLD and V and the highest values of s/v. It comprises picoplankton organisms (MLD<2 μm), small unicellular organisms (flagellated or not), some representatives of small colonies, and some filamentous organisms (V=0.3–120 μm^3).

Group II: Composed of small, flagellated organisms with exoskeletal structures formed by silica and medium to low s/v (Chrysophyceae) (V=4.7–2.783 μm^3).

Group III: Composed of large filamentous organisms with aerotopes (cyanobacteria), low growth rate, and high s/v. Some individuals can fix atmospheric nitrogen (heterocysts) and form resistance structures (acini) (V=8.1–8,708 μm^3 and MLD=2.5–259 μm).

Group IV: Composed of medium to large unicellular, colonial, and filamentous organisms lacking specialized morphological features (e.g., aerotope, flagella, heterocyst, mucilage, and silica exoskeleton) (V=12.7–48,255 μm^3).

Group V: Composed of mostly unicellular and flagellated species, with intermediate size and maximum linear dimension (MLD) values, low growth rate, biomass, and abundance (V=2.4–164,779 μm^3).

Group VI: Composed of non-flagellated organisms with a silica exoskeleton (Bacillariophyceae), well diversified in terms of cell size, with high growth rates and biomass (V=7.8–57,106 μm^3).

Group VII: Composed of all species that form large mucilaginous colonies. Some individuals may have aerotopes and lipid structures that aid buoyancy. They have high V and S values, low growth rates, and s/v (V=10.9–2.4x106 μm^3).

Group VIII: Composed of heterocyst cyanobacteria, i.e., those able to fix atmospheric nitrogen (heterocyst) (V=8.1–8,708 μm^3 and MLD=2.5–259 µm).

Data analysis

The results of the environmental variables were analyzed using descriptive statistics, based on the arithmetic mean as a measure of central tendency and the standard deviation (SD) as a measure of the degree of data absolute dispersion. The standards defined for Class 2 according to the CONAMA Resolution 357/2005 (Brasil, 2005) for lentic environments were used to evaluate the two sampling stations since the Billings reservoir fits into this category based on the classes defined by State Decree No. 10.755/1977 (São Paulo, 1977).

To understand the effect of spatiotemporal patterns on environmental changes in the reservoirs studied, it was applied the principal component analysis (PCA), the correlation matrix between environmental variables, sites, and sampling period. Variables with significant correlation were considered—those that presented r>0.5 with axes 1 and 2 of the ordering. Data were transformed by the amplitude of variation "ranging" [$(X-X_{min})/(X_{max}-X_{min})$].

Three canonical correspondence analyses (CCA) were performed to examine the effect of environmental variables on the phytoplankton structure from each classification method (descriptor species, classes, and MBFGs). The main objective of this analysis was to identify the influence of environmental variables (independent variables) on the abundance patterns of the phytoplankton community (dependent variables). Environmental variables and phytoplankton data were transformed by the amplitude of variation "ranging" (Legendre and Legendre, 2012) to reduce the influence of species and avoid biased data, thus allowing the use of linear methods (Peres-Neto et al., 2006). The significance of the axes was tested using the Monte Carlo simulation test (McCune and Mefford, 2011). The variation partitioning of the canonical analysis was used to test and determine the probability of environmental variables explaining the patterns of the phytoplankton community structure. Therefore, as recommended by Peres-Neto et al. (2006), inertia values and the adjusted coefficient of determination of R-squared values (ratio of permutations of inertias) were used as a measure of comparison between the classic taxonomic approach and morpho-functional groups.

The multivariate analyses applied to the abiotic and biotic data were performed using the PC-ORD 6.0 for Windows program (McCune and Mefford, 2011).

Results and Discussion

Water analysis and trophic state index

Rainfall and water temperature remained as expected for the region, with the highest volumes of rainfall recorded in the summer and the lowest recorded in the months with the lowest temperature values (Figure 2).

Table 1 shows the results of the environmental variables for the studied reservoirs and the comparison in terms of compliance with current legislation. In general, CB showed higher values for the environmental variables analyzed than RG. Both environments presented similar pH values above 7. The water storage capacity in CB showed higher variation (SD=14.05) and lower average volume (46.57%) than in RG (80.16%; SD=7.92).

RG and CB belong to Class 2 according to the definition by State Decree No. 10.755/1977 (São Paulo, 1977). Comparison standards were those defined by CONAMA Resolution 357/2005 (Brasil, 2005) for lentic environments. The values of the limnological variables remained within the limits established by the current resolution, except for the total phosphorus variable, whose recommended concentration is <30 µg/L.

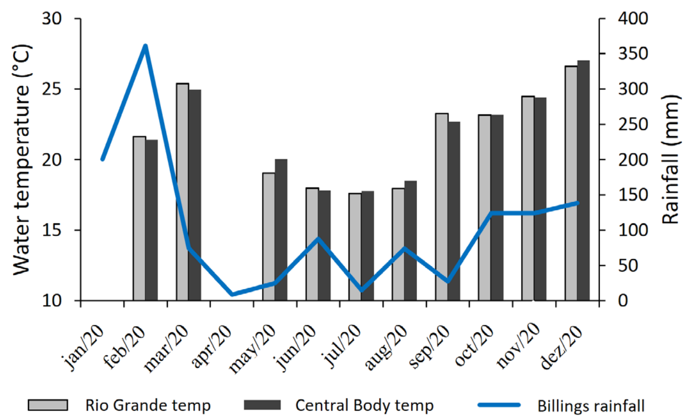

Figure 2 – Annual variation in water temperature and mean monthly rainfall for the study period in the Billings reservoir.

Table 1 – Mean values and standard deviation of the limnological variables of the samples collected in the Billings reservoir: Rio Grande and Central Body (n=6).

	Rio Grande	Central Body	CONAMA 357/2005 Class 2
Temperature (°C)	21.32±3.42	21.35±3.07	---
pH	7.64±0.87	7.81±1.01	6–9
Conductivity (µS/cm)	85.28±12.04	151.72±19.13	---
Turbidity (NTU)	2.70±1.07	13.36±9.22	<100
Dissolved oxygen (mg/L)	8.17±0.37	8.65±0.65	>5
TDS (mg/L)	0.06±0.01	0.10±0.01	<500
Total nitrogen (µg/L)	76.29±3.52	115.88±3.52	<2,180
Total phosphorus (µg/L)	53.47±2.01	99.86±2.01	<30
Chlorophyll *a* (µg/L)	6.98±0.56	13.17±0.56	<30
Mean TSI	57.00±0.39	61.00±0.15	---
Volume (%)	80.16±7.92	46.57±14.05	---

The data have no standards defined in the Resolution.
pH: potential of hydrogen; TDS: total dissolved solids; TSI: trophic state index; Volume: water storage capacity of the reservoir.

In RG, total phosphorus concentrations were higher than recommended, with average concentrations of 53.47 µg/L, and in CB, concentrations were 3x higher than indicated by the resolution, with averages of 99.86 µg/L. Considering the classification of the reservoirs in terms of trophic status, RG was categorized as mesotrophic throughout the study period, while CB was considered as eutrophic (Table 1).

The joint assessment of the environmental data, using PCA, summarized 80.52% of the total variability of the data in the first two ordination axes, showing statistical significance for the first axis ($p<0.010$) according to the randomization test (Figure 3; Table 2). The first axis was the most important for explaining the spatial distribution of reservoirs as a function of environmental features. The negative side of axis 1 (PC1) arranged the samples from CB associated with the highest concentrations of total nitrogen (TN), total phosphorus (TP), total dissolved solids (TDS), dissolved oxygen (DO), turbidity, and chlorophyll *a* (Chl*a*). Both the lowest values and the highest values of these variables were randomized. The lowest values of these variables and the largest volume (water storage capacity) were grouped with the samples linked to the RG.

Table 2 – Pearson's correlation coefficient between the environmental variables of the Rio Grande and Central Body sampling stations, at Billings reservoir, over a seasonal cycle in 2020.

	Abbreviations	PC 1	PC 2
Temperature (°C)	T	-0.155	-0.906
pH	pH	-0.310	-0.758
Oxi-reduction potential (mV)	ORP	0.308	-0.616
Electric conductivity (µS/cm)	Cond	-0.964	0.153
Turbidity (NTU)	Tur	-0.742	-0.488
Dissolved oxygen (mg L^{-1})	DO	-0.435	-0.445
Total dissolved solids (mg L^{-1})	TDS	-0.963	0.141
Total nitrogen (mg L^{-1})	TN	-0.972	0.076
Total phosphorus (µg L^{-1})	TP	-0.976	0.105
Chlorophyll *a* (µg L^{-1})	Chl*a*	-0.987	0.004
Reservoir volume (%)	RV%	0.898	-0.310
	Explainability	58.99%	21.53%

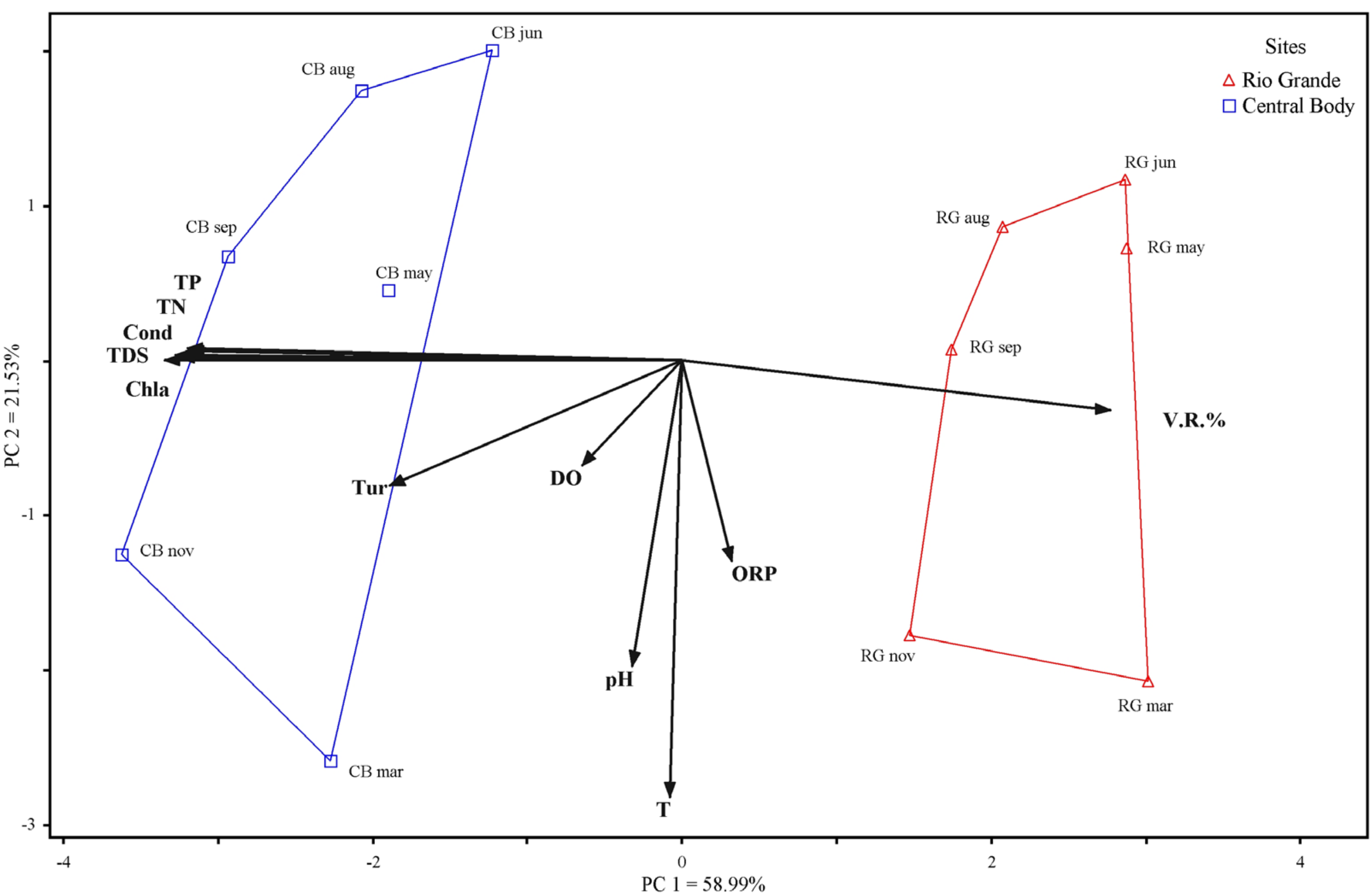

Figure 3 – Biplot ordination for the principal component analysis, between the values of the environmental variables of the Central Body reservoir and Rio Grande arm over a complete seasonal cycle in 2020. See Table 2 for the correlations of the environmental variables with axes 1 and 2 of the ordination, and their respective abbreviations.

The results showed differences between the sampling stations in the Billings reservoir in terms of limnological features and trophic state classification (RG arm – mesotrophic class and CB – eutrophic class). The samples from CB were associated with the highest values of electrical conductivity, TDS, turbidity, and concentrations of TN, TP, and Chl*a* (Figure 3). These values may be related to the surrounding activities, because of intense irregular occupation, lack of urban planning, poor sanitation, inadequate waste disposal, and loss of vegetation cover (Cardoso-Silva et al., 2014; Cunha et al., 2016; Araújo, 2018; Lobo et al., 2021). Added to these factors is the transposition of water from the Pinheiros River, a polluted river, as a way of controlling flooding in São Paulo city (Penteado et al., 2017). On the other hand, the lowest concentrations of nutrients were recorded in the RG arm; these findings may be linked to the separation of the sampling stations by the Via Anchieta dike, which is designed to preserve the quality of the water in the RG arm for public supply (Pompêo et al., 2015) and the release of algaecides due to the use of water for public supply (Wengrat and Bicudo, 2011; Gargiulo, 2021).

The input of nutrients, the morphology, and operating system of reservoirs, seasonal dynamics, and climate change can cause a series of modifications in the phytoplankton community structure (Silva et al., 2022; Costa et al., 2023; Pires et al., 2023). Therefore, this community can be an excellent bioindicator of the environmental variations and water management that occur in reservoirs. As observed herein, the highest concentrations of Chl*a* and phytoplankton density were recorded in CB, the site with the highest concentrations of TP (average of 99.86 µg/L) (Table 1).

Phytoplankton community structure and morpho-functional grouping

Phytoplankton community structure

We identified 138 taxa of microalgae and cyanobacteria in the environments studied, with 33 taxa recorded exclusively in RG and 34 taxa recorded only in CB. These taxa are distributed in 15 taxonomic classes, presented below in descending order: Cyanobacteria (42 taxa – sum of the classes Synechococcales and Oscillatoriales, both with 17 taxa and Nostocales with 8 taxa); Chlorophyceae (38 taxa); Trebouxiophyceae (18 taxa); Zygnematophyceae (11 taxa); Euglenophyceae (10 taxa); diatoms (6 taxa – sum of the classes Bacillariophyceae, Coscinodiscophyceae, and Fragilariophyceae); Chrysophyceae (5 taxa); Dinophyceae (4 taxa); Cryptophyceae (2 taxa), Eustigmatophyceae (1 taxon), and Xanthophyceae (1 taxon) (Figure 4A).

These taxa were distributed in the following eight MBFGs: I (23 taxa); II (5 taxa); III (3 taxa); IV (44 taxa); V (16 taxa); VI (6 taxa); VII (33 taxa), and VIII (8 taxa). Group VIII consisted of genera of heterocystous cyanobacteria that potentially cause toxic blooms, such as *Aphanizomenon*, *Cuspidothrix*, *Dolichospermum*, and *Raphidiopsis* (Figure 4B).

There was no significant variation in the taxonomic and morpho-functional richness of the reservoirs, being slightly higher from March to June for CB and from August to December for RG (Figure 4). The cyanobacteria group was more representative in CB, while in RG, there was an alternation in the contribution to total richness between cyanobacteria and Chlorophyceae.

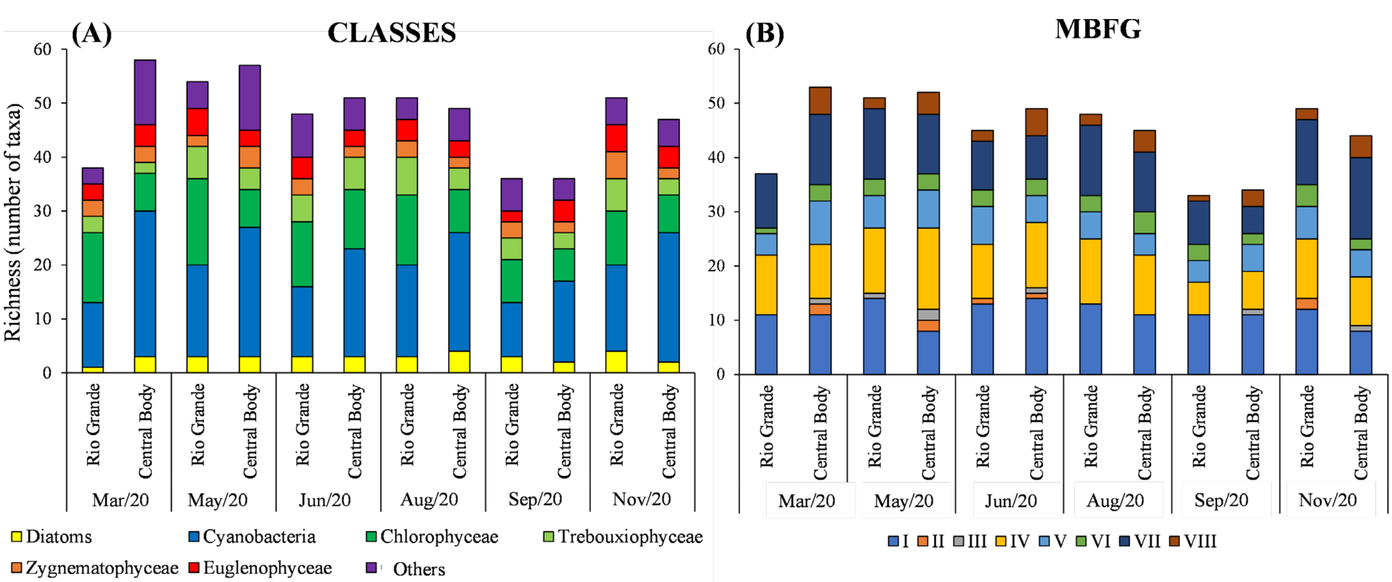

Figure 4 – Temporal variation in the richness of phytoplankton taxonomic classes (A) and morpho-functional groups (B) at the Rio Grande and Central Body sampling stations during the six sampling periods in 2020. (Others=Cryptophyceae, Chrysophyceae, Xanthophyceae, and Dinophyceae).

Cyanobacteria were associated with different morpho-functional groups (I, III, VII, and VIII) recorded in the two sampling stations. The taxa *Dolichospermum planctonicum*, *Microcystis wesenbergii*, *Phormidium* sp., *Planktothrix agardhii*, and *Raphidiopsis brookii* were recorded exclusively in CB. A further species common to this environment was *Raphidiopsis raciborskii* (previously known as *Cylindrospermopsis raciborskii*) (MBFG VIII). These species are mentioned in the literature as potential toxin producers.

Twenty of the 138 taxa identified had an average density ≥2% of the total density and were classified as descriptor species of the phytoplankton community in the Billings reservoir (Figure 5A). Cyanobacteria was the most representative group of the phytoplankton community for the studied environments, contributing with 59% of the total density recorded, followed by the green algae Chlorophyceae and Trebouxiophyceae, both with 11% of the total density, and diatoms, which accounted for 9% (Figure 5B). MBFG I (Chlorophyceae and cyanobacteria with small linear dimensions) represented the highest densities of the phytoplankton community throughout the period for the studied environments, contributing with 40% of the total density observed, followed by organisms from MBFGs IV and VII, accounting for 19 and 16%, respectively (Figure 5C).

The classes with the greatest contributions to the total number of taxa were cyanobacteria (38 taxa) and Chlorophyceae (28 taxa). At the RG arm, there was an alternation in the contribution of richness and abundance between cyanobacteria and Chlorophyceae, along with the presence of organisms with smaller linear dimensions representing the Cryptophyceae, diatoms, Euglenophyceae, and Zygnematophyceae classes. The following species described the community: *Monoraphidium minutum*, *M. contortum*, *M. nanum*, and *Fragilaria* sp., as well as the mucilaginous cyanobacterium *Aphanocapsa delicatissima*. According to Zanon et al. (2021), the relevance of the nanoplanktonic green algae richness in environments with high turbidity (low light availability) lies in their high surface-to-volume ratio, making them more efficient in terms of resources utilization. The operational system of the reservoir may contribute to the establishment of these nanoplanktonic and picoplanktonic organisms, as samples were obtained close to the SABESP water collection system in the RG arm. This system produces around 5 m^3/s of water and is responsible for supplying approximately 1.5 million people (SABESP, 2023). In RG, the residence time for 2020 was 277 days and the water storage capacity remained at around 80% of the reservoir volume, while in CB, the residence time was 575 days and the reservoir volume was 47% (CETESB, 2021).

Cyanobacteria was the most abundant group in CB, accounting for around 80% of phytoplankton density, with the following species as descriptors: *Raphidiopsis raciborskii*, *Anagnostidinema amphibium*, *Geitlerinema* sp., and *Synechococcus nidulans*. The highest densities occurred during the rainy season (summer and fall) and were associated with the highest values of DO, turbidity, electrical conductivity, TP, and TN. *Ceratium furcoides* (MBFG V), an exotic species considered invasive in Brazilian water bodies, was recorded by the present study exclusively in CB in the rainy season (higher temperature). Matsumura-Tundisi et al. (2010) recorded a bloom of *C. furcoides* in the Billings reservoir and linked this occurrence to the mixing of the water column, which may have caused a sharp increase in the phosphorus content, favoring the rapid growth of this taxon.

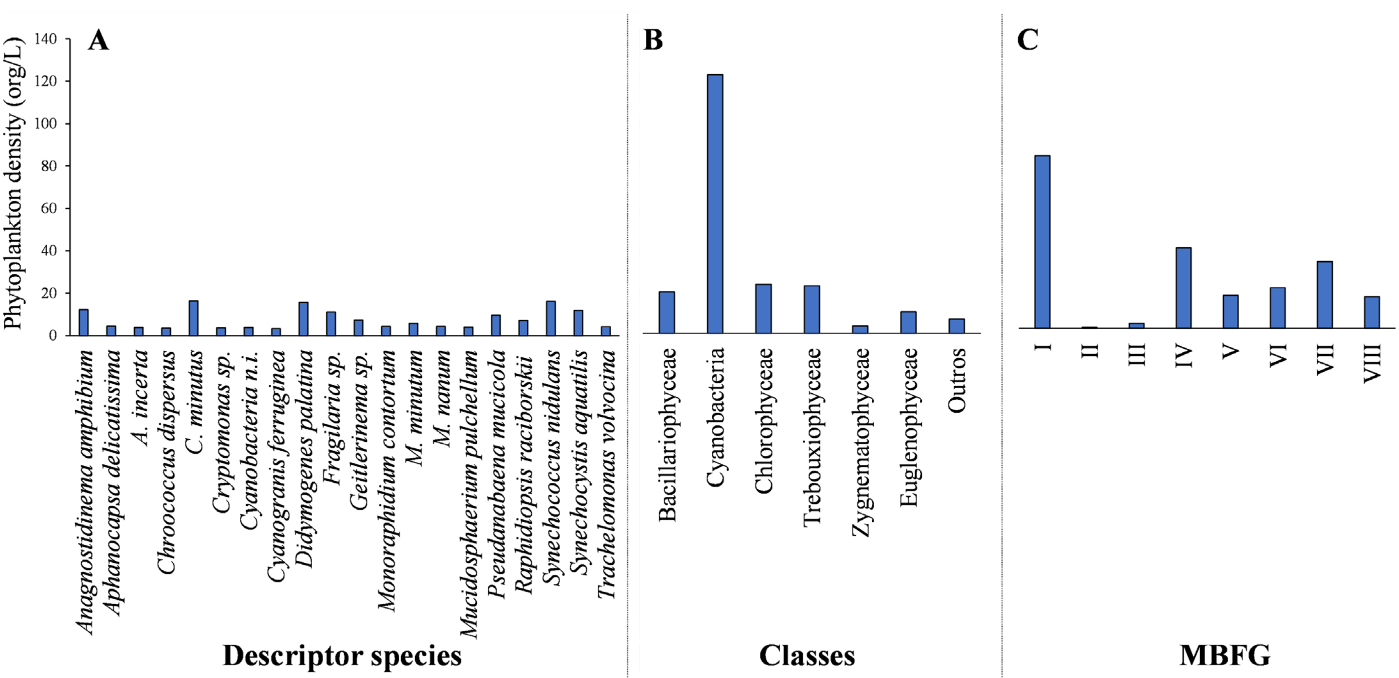

Figure 5 – Average phytoplankton density (org/L) of descriptor species (A), taxonomic classes (B), and morpho-functional groups (C) for the reservoirs studied (n=12). (Others=Cryptophyceae, Chrysophyceae, Xanthophyceae, and Dinophyceae).

Rojas-Castillo et al. (2023) attributed the higher density of *C. furcoides* to the mixing of the water column, linking its occurrence to higher concentrations of iron, manganese, and turbidity, and higher water temperatures.

Indicators of phytoplankton community structure

The density values of the phytoplankton descriptor species, classes, and MBFGs over a seasonal cycle were tested and compared using the multivariate data technique. The results of the ordination analysis (CCA) demonstrated spatial differences for all three approaches, grouping the samples related to CB associated with the highest concentrations of nutrients (electric conductivity, TN, TP, and Chl*a*); on the other hand, RG was related with lower concentrations of the variables mentioned (Figure 6). In all CCA, only the first axis was significant (p=0.010). The highest value of inertia (I) was observed when the descriptor species were analyzed (I=1.03), followed by classes (I=99), and MBFG (I=0.79). Descriptor species, classes, and MBFG showed similar R-square, 0.54, 0.59, and 0.51, respectively.

The results of the CCA for the descriptor species (Figure 6A) showed that CB was related to higher concentrations of electric conductivity, TN, TP, Chl*a* and associated with cyanobacteria *Raphidiopsis raciborskii* (r=-0.71), *Anagnostidinema amphibium* (r=-0.54), *Geitlerinema* sp. (r=-0.58), and *Synechococcus nidulans* (r=-0.48). On the other hand, RG was related with lower concentrations of the variables mentioned and the higher values of DO and associated with *Monoraphidium minutum* (r=0.84), *M. contortum* (r=0.87), *M. nanum* (r=0.63), and the diatom *Fragilaria* sp. (r=0.76).

For phytoplankton classes (Figure 6B), cyanobacteria (r=-0.88), Chrysophyceae (r=-0.82), Xanthophyceae (r=-0.77), and Dinophyceae (r=-0.83) were negatively correlated to the first component containing the samples collected in CB, regardless of the period sampled. The samples collected in RG were ordered on the positive side of the axis, associated with the classes Chlorophyceae (r=0.63) and Diatoms (r=0.53).

The results of the CCA for the MBFG (Figure 6C) showed that groups VIII (r=-0.70), represented by filamentous heterocystous cyanobacteria, as well as IV (r=-0.86) formed by medium-sized cyanobacteria with no specialized features and green algae (classes Chlorophyceae, Trebouxiophyceae and Zygnematophyceae). These groups were associated with the highest concentrations of nutrients and related to the site CB. On the other hand, RG was associated with the largest reservoir volume (r=0.76) and grouped the MBFG VI (r=0.87) (monophyletic group formed by diatoms).

The phytoplankton community structure by each taxonomic and functional approach proved to be sensitive tools for identifying the influence of environmental variables and those related to reservoir management (i.e., volume and residence time). The phytoplankton community reflected these changes well, showing a greater contribution of cyanobacteria in CB and green algae (Chlorophyceae, Trebouxiophyceae, and Zygnematophyceae) in the RG arm.

The management of reservoirs used for public supply and the tools employed for their monitoring have been widely discussed in the literature. The analysis of phytoplankton community structure has proven to be efficient for this approach (Lima, 2017; Costa et al., 2019). The taxonomic approaches of cyanobacteria were adopted by Lima (2017), who determined the concentration of cyanotoxins in a reservoir located in the Agreste and Zona da Mata, in the North region of the state of Pernambuco. The author attributed the high densities of cyanobacteria to harmful human practices, like the dumping of solid waste, domestic and industrial effluents, and siltation. Costa et al. (2019) studied the environmental quality of the Paiaguás Lagoon in Cuiabá (Mato Grosso) by surveying microalgae from the Chlorophyta group. The authors related the periods of flood and drought as conditioning factors for environmental changes and the presence or absence of Chlorophytes and concluded that the group can support the management of water resources due to their role as environmental bioindicators, which can provide information on cause and effect.

The use of the functional grouping approach can provide indications of the reservoir enrichment degree (Cunha and Calijuri, 2011). In our study, the trophic status and operational system of the reservoirs were the main factors associated with the distribution of the phytoplankton community. The joint analysis of data by CCA (Figure 6C) in CB showed that the most prominent MBFG were VIII (organisms with cells specialized in nitrogen fixation) and IV (green algae). The dominance of cyanobacteria can be associated with the nutrient availability (Zanon et al., 2021), likely due to their adaptative strategies in Group VIII, such as the presence of aerotopes and heterocystous (Reynolds et al., 2014). According to these authors, the lengthening of filaments increases organismic size but preserves the high surface-to-volume ratios that aid optimal interception of underwater light in turbid, mixed layers. In fact, in our study, the presence of this group was linked to higher nutrient availability. Additionally, the low reservoir volume and the shorter water renewal time recorded in CB also appeared to influence the functional patterns and abundance of species.

The canonical analysis (Figure 6) applied to MBFGs and the taxonomical methods, such as community descriptor species and phytoplankton classes, showed that the approaches used were key to understanding the degree of nutrient enrichment, reducing the dimensionality of the phytoplankton data, and facilitating interpretation with the environmental variables. The morpho-functional approach provided a better explanation of the phytoplankton-environment relationship when compared with taxonomic (species) approaches in a river-floodplain system (Zanon et al., 2021). The authors concluded that morpho-functional groupings could extract a better response from the interaction between the environment and the phytoplankton community, contributing to a better understanding of the functioning of the ecosystem studied. Costa et al. (2024) stated that MBFG can be used to predict changes in phytoplankton ecology and determine how this can affect the functioning of ecosystems, especially in a world with climate changes such as extreme rainfall events.

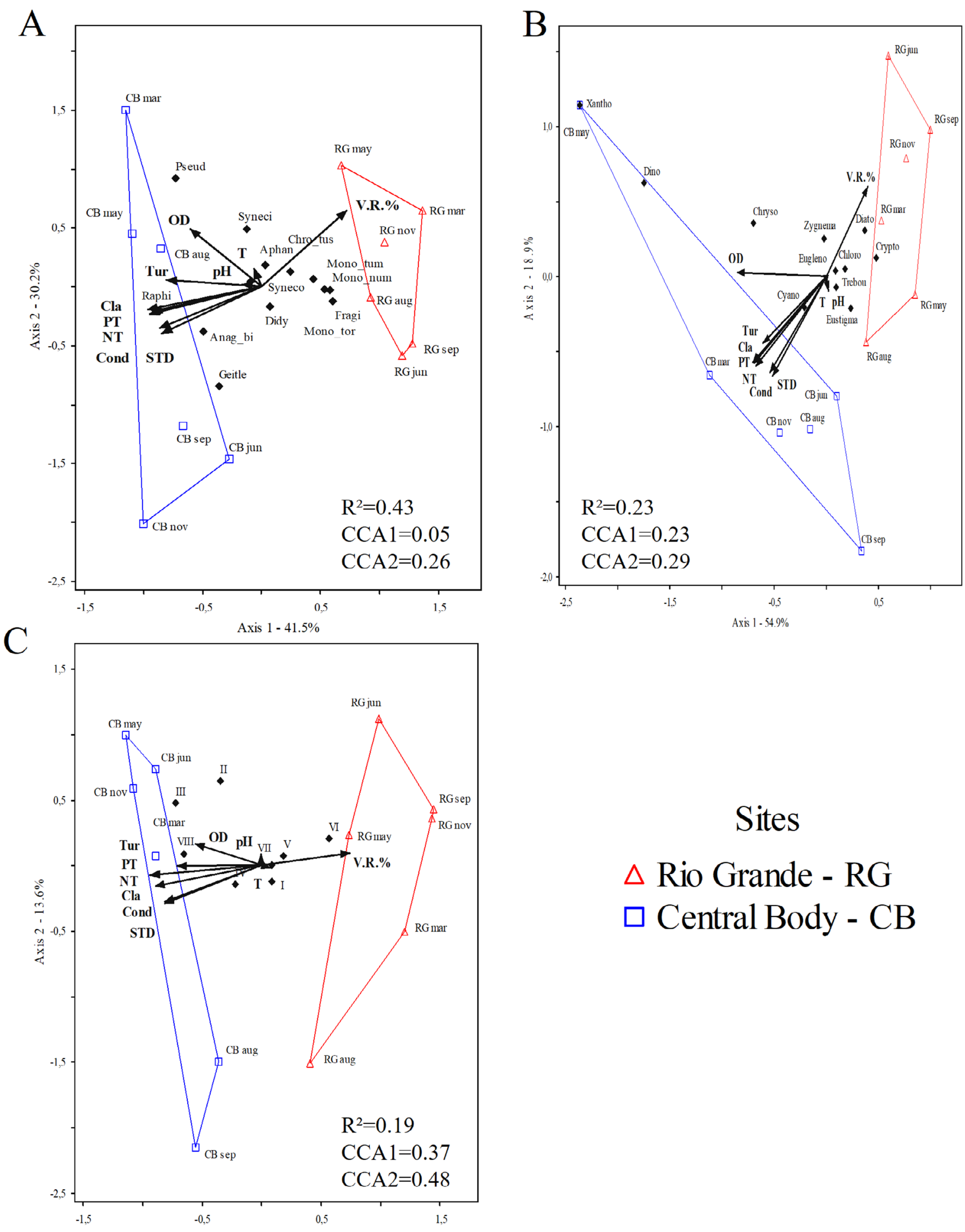

Figure 6 – Biplot ordination for the canonical correspondence analysis of the sampling units Central Body and Rio Grande generated from eleven phytoplankton classes (A), thirteen descriptor species (B), and eight morpho-functional groups (C), throughout 2020.

CB: Central Body; RG: Rio Grande; pH: potential of hydrogen; T: temperature; TP: total phosphorus; TN: total nitrogen; TDS: total dissolved solids; Chla: chlorophyll a; DO: dissolved oxygen; Tur: turbidity; RV%: reservoir volume; Cond: electric conductivity; ORP: oxi-reduction potential; Cyano: cyanobacteria; Chloro: Chlorophyceae; Trebou: Trebouxiophyceae; Zygnema: Zygnematophyceae; Eugleno: Euglenophyceae; Diato: diatoms; Eustigma: Eustigmatophyceae; Crypto: Cryptophyceae; Xantho: Xanthophyceae; Dino: Dinophyceae; Chryso: Chrysophyceae; Raphi_skii: *Raphidiopsis raciborskii*; Anag_bium: *Anagnostidinema amphibium*; Chro_tus: *Chroococcus minutus*; Geitle: *Geitlerinema* sp.; Aphano_ma: *Aphanocapsa delicatissima*; Pseud_cola: *Pseudanabaena mucicola*; Synecho_lans: *Synechococcus nidulans*; Synecho_lis: *Synechocystis aquatilis*; Mono_tortum: *Monoraphidium contortum*; Mono_nutum: *Monoraphidium minutum*; Mono_nanum: *Monoraphidium nanum*; Didy: *Didymogenes palatina*; Fragi: *Fragilaria* sp.

However, the results presented herein showed that using the phytoplankton community through morpho-functional groupings in areas with different trophic status classifications is a means of identifying general patterns and rules that explain, at least partially, phytoplankton associations throughout a given trophic condition. Moreover, the use of morpho-functional approach can simplify the process of identification when compared with the taxonomic approaches that demand familiarity with a high level of taxonomic knowledge (Kruk et al., 2021).

Conclusions

The phytoplankton community structure using taxonomic and morpho-functional methods proved to be sensitive tools for identifying the influence of environmental variables related to the eutrophication process, and those related to reservoir management (i.e., volume and residence time). These findings demonstrate that the phytoplankton approach through MBFG is suitable for responding to fluctuations in the ecological attributes of phytoplankton. In addition, because they are based on morphological features easily observed, morpho-functional groups reduce the complexity of identifying and classifying organisms at a specific level. Therefore, studies that use the morpho-functional approach can reduce the time required for technical analysis, in addition to reducing the dimensionality for interpreting results, helping stakeholders to make more assertive decisions aimed at managing the Billings reservoir.

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Authors' contributions

Peixoto Chamizo, A.C.: formal analysis; investigation; methodology; writing – original draft. **Mercante**, C.T.J.: resources; conceptualization; project administration; writing – original draft. **Moraes**, M.A.B.: conceptualization; data curation; writing – original draft. **Carmo**, C.F.: conceptualization; data curation; writing – original draft. **Oliveira**, M.B.H.: formal analysis; data curation; methodology. **Osti**, J.A.S.: conceptualization; investigation; methodology; supervision; writing – original draft.

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