Hydrobiologia (2023) 850:1393–1411
https://doi.org/10.1007/s10750-022-04954-1

TRENDS IN AQUATIC ECOLOGY IV

Functional and taxonomic diversities are better early indicators of eutrophication than composition of freshwater phytoplankton

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Received: 3 January 2022 / Revised: 27 June 2022 / Accepted: 29 June 2022 / Published online: 10 August 2022
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Abstract Eutrophication is a key threat to aquatic biodiversity around the world, but especially in the Cerrado biome that has undergone intensive land use conversion and fertilizer use. In this study, we investigated how water conditions and different taxonomic and functional indicators of phytoplankton communities responded to eutrophication over time and also identified which of these variables can act as early indicators of eutrophication. We used a mesocosm experiment to simulate shallow lakes and added nutrients to produce eutrophic and oligo-mesotrophic environments. We found that conductivity and turbidity increased with the nutrient enrichment process. Eutrophication did not change species richness; however, it increased the total phytoplankton density and chlorophyll-a concentration and decreased the diversity indices (Shannon–Wiener diversity and Pielou's equitability) over time, for both taxonomic and functional facets. Our results indicate that eutrophication may not have marked effects on the composition of the initially dominant organisms in the short term but does increase the density of certain organisms and exclude rare species and functional groups in eutrophic environments along the time. Furthermore,

Guest Editors: Koen Martens, Sidinei M. Thomaz, Diego Fontaneto & Luigi Naselli-Flores / Emerging Trends in Aquatic Ecology IV.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10750-022-04954-1.

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the diversity indices are more sensitive indicators of eutrophication compared to abiotic indicators and composition of species and functional groups. Therefore, they may be considered reliable early warnings of ecosystem changes.

Keywords Algae · Freshwater mesocosms · Functional groups · Global change · Nutrient enrichment

Introduction

The relationship between productivity and diversity has been the subject of intense research over the last decades (e.g., Strong, 2010; Vallina et al., 2014; Brun et al., 2019). This is so because studies carried out to date have shown that this relationship can be unimodal, positive, and negative (Mittelbach et al., 2001). In addition, the type of the relationship between productivity and diversity may be further changed by the impacts caused by global changes, including climate change, eutrophication, and land use intensification (e.g., Zhou et al., 2006; Lewandowska et al., 2012; Zhang et al., 2021a).

The intensity of human-induced global changes is expected to increase by the end of this century (IPCC, 2021). Thus, global changes that directly or indirectly impact freshwater environments are troublesome, as these systems harbor high biodiversity, provide several ecosystem services, and are already among the most threatened on the planet (Dudgeon et al., 2006; Strayer & Dudgeon, 2010; Dudgeon, 2019). Furthermore, human-induced changes in watersheds are transferred to aquatic ecosystems as these systems are tightly coupled (Allan, 2004).

Cultural eutrophication consists in increasing nutrient concentrations in aquatic environments by the action of human activities, promoting primary producers growth (Smith & Schindler, 2009; Jeppesen et al., 2010). Urbanization and the conversion of natural land cover to agriculture, for example, are major causes of nutrient enrichment in freshwater ecosystems (Carpenter et al., 1998; Carpenter, 2008; Jeppesen et al., 2010). This process, in turn, is considered one of the main threats to aquatic environments (Smith et al., 1999; Jeppesen et al., 2010), affecting water supply, population health, and well-being (Smith & Schindler, 2009; Chislok et al., 2013). Even more worrying, the negative consequences of eutrophication may be exacerbated by climate change as this process may further reduce the resilience of aquatic systems to eutrophication (e.g., Moss et al., 2011; Meerhoff et al., 2022).

Lakes and ponds are excellent model systems for studying eutrophication (Istvánovics, 2009) due their clearer boundaries, spatial ubiquity, and strong response to current and past changes in watersheds (Adrian et al., 2009). Several approaches have been used to investigate the effects of eutrophication on lakes. Despite the ground-breaking experiments led by D. W. Schindler (Leavitt et al., 2021), there are a predominance of observational studies (e.g., Jeppesen et al., 2000; Ptacnik et al., 2008; Munawar & Fitzpatrick, 2018; Bouraï et al., 2020). Also, experimental studies are mainly conducted in laboratory conditions and based on small experimental units (e.g., microcosms; Soares et al., 2013; Di Carvalho & Wickhan, 2019). Finally, the realistic whole lake experiments (Cottingham & Carpenter, 1998; Schindler, 1998; Wilkinson et al., 2018) usually have

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a limited number of replications. In this sense, experiments in mesocosms can simulate small lakes or ponds, considering the inherent complexity of ecosystems and allowing for the necessary replication for statistical inference.

The transition from an oligotrophic to an eutrophic environment can lead to a regime shift, usually driven by changes in an external forcing variable, as nutrient input (e.g., Donangelo et al., 2010; Buttita et al., 2017). This process can produce abrupt, often irreversible changes, to a turbid water state (Dakos et al., 2019), with high social costs (e.g., Pretty et al., 2003). Studies using the mesocosm approach to detect the effects of eutrophication have been developed in recent years, mainly in temperate and subtropical regions (e.g., Ding et al., 2019; Filiz et al., 2020; Zhang et al., 2021b; but see Crosseti & Bicudo, 2005; Muhid et al., 2013 for tropical regions). Some of these studies simulated episodic nutrient inputs (e.g., Moss et al., 2003; Baho et al., 2017; Zhang et al., 2021b). However, to evaluate how ecosystem properties change over time, in response to continuous nutrient additions (e.g., smoothly, with a shift or catastrophically; see Fig. 1 in Scheffer et al., 2001),

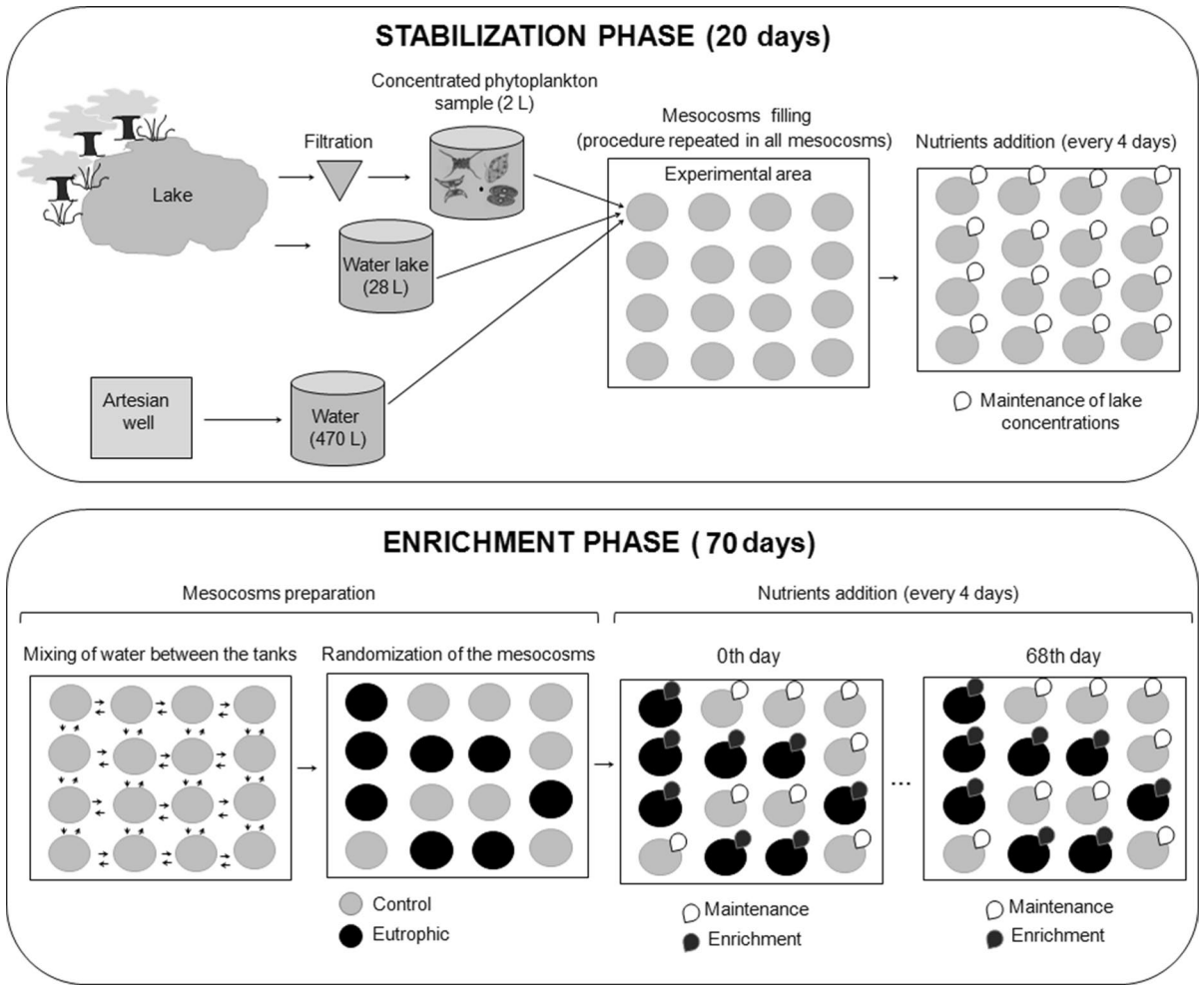


Fig. 1 Experimental design used to assess the effects of gradual eutrophication on phytoplankton communities. The procedure was initiated with the stabilization phase (when nutrients were added to reach the concentrations of the lake) followed by the enrichment phase (when nutrients were added to the eutrophic treatment in increasing concentrations with the time). Before the enrichment phase, the water was mixed among all tanks

one needs to precisely simulate this gradual process which, in general, is more common in nature. This is important for water quality monitoring because early-warning indicators are likely to fail in detecting a catastrophic shift as the state of the system changes little before a certain threshold of nutrient concentration (Scheffer et al., 2001).

Several environmental changes can be observed during the eutrophication process, including reduced water transparency and oxygen depletion (Callisto et al., 2014). In relation to biological components, phytoplankton is one of the first groups of organisms to respond to eutrophication, which in turn triggers changes in ecosystem processes, such as productivity and biogeochemical cycles (Salmaso & Tolotti, 2021). Microalgae are usually limited by phosphorus and nitrogen (Reynolds, 2006) and can proliferate excessively under high-nutrient concentrations and suitable temperature (Smith, 2016). Moreover, some bloom-forming Cyanobacteria species can produce toxins, causing serious damage to human health and aquatic organisms (e.g., Falconer & Humpage, 2005; Paerl & Otten, 2013; Huisman et al., 2018).

Biodiversity measures (e.g., species richness, Shannon–Wiener diversity (H'), and Pielou's equitability (J') indexes) and the abundance of some taxonomic groups of microalgae have been suggested as indicators of eutrophication (e.g., Cottingham & Carpenter, 1998; Soares et al., 2013; Baho et al., 2017; Filiz et al., 2020). Previous studies have found a temporal increase in algal biomass (Donald et al., 2013; Dong et al., 2018), cell biovolume (Ferragut & Bicudo, 2012; Baho et al., 2017), abundance (Jeppesen et al., 2000; Ferragut & Bicudo, 2012), as well as the predominance of Cyanobacteria (e.g., Ptacnik et al., 2008; Lira et al., 2011) in eutrophic environments. Also, some studies found a reduction in species richness and diversity (Shannon–Wiener) as a consequence of nutrient enrichment over time (Cottingham & Carpenter, 1998; Soares et al., 2013; Baho et al., 2017; but see Jeppesen et al., 2000 and Ferragut & Bicudo, 2012 for opposite results).

Despite the importance of taxonomic-based indicators, it has become increasingly evident that functional characteristics of organisms can better indicate how they respond to environmental impacts, including eutrophication (e.g., Weithoff, 2003; Litchman & Klausmeier, 2008; Kruk et al., 2012). For example, Chlorophyceae include unicellular, filamentous, and colonial taxa and each of these groups of algae may respond differently to environmental changes (Jin et al., 2020). Also, other functional characteristics of phytoplankton (e.g., cell size and shape, as well as presence of heterocytes, mixotrophy, and mucilage) may affect the uptake and storage of nutrients, which may favor species that have these characteristics in conditions where nutrients are limiting (Reynolds, 2007; Litchman & Klausmeier, 2008; Litchman et al., 2010).

The Morphologically Based Functional Groups (MBFG) were proposed to delimit phytoplankton functional groups based on morphological characteristics, including cell size, cell shape and presence of flagella, silica exoskeleton, mucilage, aerotopes, and heterocytes (Kruk et al., 2010). The MBFG-based measures tend to show stronger relationships with different environmental gradients than taxonomic-based measures (Kruk et al., 2011; Machado et al., 2015; Rangel et al., 2016), although similar strengths of relationships have also been observed (Santana et al., 2018; Machado et al., 2019).

In this study, we tested the effects of continued increases in nutrient concentrations on phytoplankton community using experimental mesocosms. We expect that (i) temporal increases in phytoplankton density and biomass (as measured by chlorophyll-a concentration) and Cyanobacteria dominance will be larger in enriched than in control mesocosms. Consequently, we predict that (ii) temporal decreases in biodiversity indices (species richness, H' and J' for both taxonomic and functional facets) will be larger in enriched than in control mesocosms. Considering these changes, we expect that community structures (as summarized by ordination axes) in control and enriched mesocosm will differ over time. Finally, we (iv) hypothesize that functional indicators better respond to eutrophication than taxonomic indicators.

Methods

Study design

The study was carried out in an experimental facility at the Universidade Estadual de Goiás (UEG; City of Anápolis, Goiás State, Brazil; Mesocosm, 2021), between August and November 2019. This period represents the transition between the dry (ending around September) and the rainy season (beginning between October and November) in the Brazilian Cerrado. During the months of the experiment, the mean atmospheric temperature was 25 °C and the mean rainfall was 70 mm (INMET, 2021). The Cerrado biome represents one of the world's biodiversity hotspots (Klink & Machado, 2005). However, it has gone through an intense process of land use conversion, and the application of fertilizer in agricultural catchments can intensify the eutrophication (Hunke et al., 2015). In addition, human-induced fires in this biome generate a large amount of ash, which can affect water quality (Brito et al., 2021). Thus, it is important to understand the effects of eutrophication in this region.

The experiment included 16 mesocosms (500 l polyethylene water tanks) exposed to external conditions and buried in the ground to avoid overheating (Fig. S1 in Online Resource 1). To avoid changes in nutrient concentrations due to animal activities and to prevent the proliferation of disease-bearing insects, the mesocosms were covered with transparent nylon screens (e.g., Muhid et al., 2013; Baho et al., 2017). The experimental area was fenced to prevent access of large animals.

Mesocosms were seeded with phytoplankton from a shallow oligo-mesotrophic lake, located nearby the experimental area at the ecological reserve of the UEG Campus (16° 23′ 11″ S and 48° 56′ 35″ W). This is a small and shallow reservoir, with a surface area of approximately 1500 m^2, formed by the damming of the Barreiro stream, surrounded by native Cerrado vegetation (Cerradão) and abandoned pasture (Curado & Angeline, 2006). This reservoir has a diverse flora with representatives of several phytoplankton taxonomic groups, such as Bacillariophyceae, Chlorophyceae, Cyanobacteria, Cryptophyceae, Chrysophyceae, Euglenophyceae, and Dinophyceae (Machado et al., 2019). Each mesocosm initially received 30 l of lake water, consisting of 2 l of a concentrated sample obtained through filtration, using a plankton net with a mesh size of 20 µm, and the remaining 28 l collected directly from the lake, without prior filtration. We used 2 l of filtered water to maximize the chances of representation of the main taxonomic groups and of species with low abundance in the mesocosms, whereas the 28 l of water were used to maximize the representation of species smaller than 20 µm. The total volume of the mesocosms was completed with approximately 470 l of water from an artesian well. We found low-nutrient concentrations in the artesian well, which characterizes it as oligotrophic (orthophosphate < 0.01 mg l^{-1}, nitrate = 0.10 mg l^{-1}, organic nitrogen = 0.28 mg l^{-1}, total nitrogen = 0.38 mg l^{-1}).

The experiment was divided into two phases: stabilization and enrichment (Fig. 1). During the stabilization phase, we added N-NO_3^- and P-PO_4^{-3} obtained from solutions of $NaNO_3$ and KH_2PO_4 in all mesocosms. We added nutrients to reach concentrations similar to that of the reservoir where the samples were obtained (0.80 mg l^{-1} N-NO_3^- and 0.01 mg l^{-1} P-PO_4^{-3}). These concentrations were adjusted to follow the Redfield proportion of 16 N:1P (Reynolds, 2006), resulting in the addition of 0.16 mg l^{-1} N-NO_3^- and 0.01 mg l^{-1} P-PO_4^{-3}. This procedure was repeated every four days, until all mesocosms reached chlorophyll-a concentrations similar to those of the reservoir (mean = 4.4 µg l^{-1}). This strategy was adopted so that all tanks started the enrichment phase in a trophic state similar to each other and also to that of the lake, that is, oligo-mesotrophic. We used the nutrient concentrations of the reservoir as a baseline in the control mesocosms to avoid using an unrealistic comparison (as it would be the case if the control mesocosms had too low-nutrient concentrations, in other words, similar to those of the water well). The stabilization phase lasted for 20 days (August 22, 2019—September 10, 2019) and promoted the growth and stabilization of phytoplankton communities, with species densities at the end of this phase similar to those observed in the lake (see Table S1 in Online Resource 1).

On the first day of the enrichment phase (September 11, 2019), the water of the different mesocosms were mixed to homogenize the communities. The control and nutrient treatments (hereinafter named as eutrophic) were randomly assigned to the mesocosms,

resulting in eight replicates for each group. In the control, we added nutrients every four days to avoid nutrient depletion and maintain the concentration similar to that observed at the oligo-mesotrophic lake, where the water samples were collected. Thus, the control always received 0.16 mg l^{-1} of $N\text{-}NO_3^-$ and 0.01 mg l^{-1} of $P\text{-}PO_4^{-3}$. After the chlorophyll-a concentration of the control mesocosms reached the limit established for a mesotrophic environment (11.03 µg l^{-1}; Carlson, 1977 adapted by Lamparelli, 2004), nutrients were added only if subsequent reductions in chlorophyll-a concentrations were observed.

In the eutrophic treatment, we simulated a gradual nutrient enrichment until the mesocosms reached the eutrophic state, based on the classification of Carlson (1977) adapted by Lamparelli (2004). For that, we added nutrients every four days with an initial concentration 10% higher than that used in control mesocosms and, subsequently, with an increase of 10% over the previous concentration. Thus, in the first addition, the eutrophic treatment received 0.176 mg l^{-1} of $N\text{-}NO_3^-$ and 0.011 mg l^{-1} of $P\text{-}PO_4^{-3}$. In the last enrichment event, on November 18, 2019 (70th day of the enrichment phase; 18th nutrient addition event), these concentrations were 0.48 mg l^{-1} $N\text{-}NO_3^-$ and 0.028 mg l^{-1} $P\text{-}PO_4^{-3}$ (for the concentration of each enrichment event, see Table S2 in Online Resource 1). All the concentrations followed the Redfield proportion 16 N:1P (Reynolds, 2006). The total concentrations of nutrients added to the eutrophic mesocosms were 5.62 mg l^{-1} of $N\text{-}NO_3^-$ and 0.35 mg l^{-1} of $P\text{-}PO_4^{-3}$. On the last day of the experiment, the concentrations in the enriched mesocosms were, on average, 0.09 mg l^{-1} for nitrate and 0.12 mg l^{-1} for total phosphorus (see Fig. S2 in Online Resource 1).

The chlorophyll-a concentration in the eutrophic mesocosms stabilized at around 20 µg l^{-1} at the end of the experiment (after 70 days). Throughout the experiment (stabilization and enrichment phases), the replacement of the water lost by evaporation was done naturally by the incidence of rain. When this was not enough, we completed the volume of the mesocosms using water from the well.

Abiotic variables

Temperature (°C), conductivity (µS cm^{-1}), dissolved oxygen (mg l^{-1}), pH, and turbidity (NTU) were monitored in control and eutrophic mesocosms using a multiparameter probe (model Manta 2—Eureka, Austin, Texas, USA). During the stabilization phase, data were taken every four days, totaling five sampling events. During the enrichment phase, measurements were made every two days (a total of 36 sampling events), always in the same period of the day (early morning). Water samples (500 mL) were taken weekly from all mesocosms to assess nutrient concentrations for control and eutrophic treatment (see Fig. S2 in Online Resource 1). The samples were immediately frozen to -20 °C for further analyses in the laboratory. Orthophosphate and total phosphorus concentrations were determined using the ascorbic acid and molybdate method (Golterman et al., 1978; method 4500-P E in APHA 2005), while nitrate and ammoniacal nitrogen were measured through the cadmium reduction method (Method 4500-NO3-E and 4500-NH3 F, respectively; APHA 2005). Unfortunately, it was not possible to perform nutrient analyses at the 9th week of the experiment due to technical problems with sampling storage.

Phytoplankton sampling and functional grouping

We used a multiparameter probe (model Manta 2—Eureka, Austin, Texas, USA) to measure chlorophyll-a concentrations every four days and every two days during the stabilization and enrichment phases, respectively. With the same sampling schedule, subsurface samples of phytoplankton were collected from the mesocosms using dark flasks (100 mL) and immediately fixed with 5 mL of Lugol's solution (Vollenweider, 1974). Before each sampling event, we mixed the water of each mesocosm. Individuals were identified and counted under an inverted microscope with 400 × magnification, using the sedimentation technique (Utermöhl, 1958). Phytoplankton counting, considering cells, filaments, and colonies, was performed in random fields until no more new species were found (Bellinger & Sigee, 2010). Taxa were identified to the lowest possible taxonomic level and the density was expressed as individuals per mL (ind.mL^{-1}). The richness was considered as the number of species per sample.

We also grouped species into Morphologically Based Functional Groups (MBFG) according to the classification proposed by Kruk et al. (2010). This classification differentiates seven groups based on morphological traits, including cell size and shape, flagella, silica exoskeleton, mucilage, aerotopes, and heterocytes. These traits have been shown to respond to nutrient limitation and eutrophication (Weithoff, 2003; Litchman & Klausmeier, 2008; Litchman et al., 2010).

Data analysis

Data obtained during the stabilization phase were used only to monitor the development of communities and their stabilization. Thus, we used data from the enrichment phase to test our hypotheses.

We used different variables to describe the phytoplankton communities in the mesocosms: chlorophyll-a, total density, taxonomic species richness, Shannon–Wiener diversity (H'), and Pielou's equitability (J') indices. H' and J' were also calculated using the functional data. The richness of functional groups (MBFG) was not informative as these groups were present in all replicates of nutrient treatment and control in most time periods. Given our large number of samples, we opted to not measure biovolume as the time necessary to properly measure it (as detailed, for example, by Hillebrand et al., 1999; Bellinger & Sigee, 2010) would be prohibitively long (but see the Discussion section).

We used a Non-Metric Multidimensional Scaling Analysis (NMDS) to reduce the dimensionality of the phytoplankton data (Legendre & Legendre, 2012), based on log (density + 1) of species and of MBFG sampled along the enrichment phase. The NMDS was set to two axes and performed using the Bray–Curtis dissimilarity (Clark, 1993).

We used analysis of variance with repeated measures (ANOVA-rm; Zar, 2010) to test whether the abiotic variables (i.e., temperature, conductivity, pH, dissolved oxygen, and turbidity), chlorophyll-a concentrations, species richness, total species density, diversity indices (H' species, H' MBFG, J' species, J' MBFG), and community composition variables (NMDS Axes – species, NMDS Axes – MBFG) differed between control and eutrophic treatment and over sampling time, as well as to test the interaction between these factors. For this analysis, we evaluated the assumptions of normality, homogeneity of variances, and sphericity, and when these assumptions were not achieved, the data (except pH) were log transformed. A constant of 1.0 was added, before log-transformation, for variables with zero values. The Greenhouse–Geisser correction was applied when the sphericity assumption was violated.

We calculated the Hedges' g (and the associated 95% confidence interval) to measure the difference between control and eutrophic mesocosms (Borenstein, 2009). This analysis was performed for each sampling event, considering only the abiotic and biological variables that showed significant effects of nutrients or significant interactions in ANOVA-rm, to identify when the differences between the eutrophic treatment and control became more evident over time (see also below). Positive values of g indicated that the values of the variable were higher in the nutrient-enriched mesocosms than in the controls, whereas negative values indicated the opposite (eutrophic mesocosms < control).

For each indicator, we also used change-point analysis to find the time at which changes in mean and variance occurred in the time series of the Hedges' g (Killick et al., 2016). Taken together, the best early-warning indicators of eutrophication would be those with significant differences between control and nutrient-enriched mesocosms (ANOVA-rm), with high effect sizes (Hedges' g) and that also showed the earliest changes (as indicated by the change-point analysis).

All statistical analyses were performed using the R program (R Core Team, 2020). The diversity and NMDS analyses were performed using the vegan package (Oksanen et al., 2020). The ANOVA-rm was conducted using the rstatix package (Kassambara et al., 2020). The Hedges'g was estimated using the metafor package (Viechtbauer, 2010), while the change-point analysis was conducted using the changepoint package (Killick et al., 2016).

Results

We found significant interactions between nutrient and time for temperature, pH, conductivity and turbidity (Table 1). The significant interactions indicate that the differences between eutrophic treatment

Table 1 Effects of nutrient enrichment and sampling time (36 events) on limnological variables assessed using Analysis of Variance with repeated measures

	Nutrient		Time		Nutrient × Time		Change
	$F_{(1,7)}$	*P*	$F_{(35,245)}$	*P*	$F_{(35,245)}$	*P*	
Temperature	1.7	0.024	**153.8**	**< 0.001**	**1.7**	**0.008**	48
pH	0.005	0.946	**50.2**	**< 0.001**	**1.5**	**0.022**	70
Dissolved Oxygen	4.8	0.063	**41.2**	**< 0.001**	1.2	0.162	–
Conductivity	**94.1**	**< 0.001**	**1148.6**	**< 0.001**	**42.8**	**< 0.001**	22
Turbidity	**9.2**	**0.019**	**31.9**	**< 0.001**	**2.9**	**< 0.001**	28

Bold values indicate significant results ($P < 0.05$). The last column (Change) indicates the time (in days) in which the difference between the eutrophic treatment and control began to be observed according to the change-point analysis. This analysis was employed only when a significant effect of nutrients or a significant interaction was detected

and control were not constant over time. Despite the significant interaction, temperature and pH varied mainly through time and similarly in both groups (Fig. 2a and b). Dissolved oxygen concentrations varied through time only, with variable and lower values at the end of the experiment (Fig. 2c). The temporal trajectories of conductivity (Fig. 2d) and turbidity (Fig. 2e) were similar between eutrophic treatment and control at the beginning of the experiment; however, later on, the enriched mesocosms showed higher values of both variables than the control mesocosms.

We identified 25 phytoplankton species, distributed in seven taxonomic classes. Chlorophyceae was the richest group (11 species), followed by Cyanobacteria (3), Dinophyceae (3), Zygnematophyceae (3), Cryptophyceae (2), Euglenophyceae (2), and Bacillariophyceae (1). Cyanobacteria and Chlorophyceae reached the highest densities in both control and eutrophic mesocosms. The algae were classified into five functional groups, with predominance of groups I (small organisms with a high surface-to-volume ratio and IV (organisms of medium size and lack of specialized traits).

Species richness varied significantly over time only but did not show a clear trend (Table 2, Fig. 3a). We found a significant interaction between nutrient and time for chlorophyll-a (Table 2). Thus, the differences between eutrophic treatment and control, with higher concentrations in the nutrient-enriched mesocosms than in the control mesocosms, progressively increased over time (Fig. 3b). Total density was significantly higher in nutrient enriched than in control mesocosms as shown in Table 2. In addition, total density significantly increased over time in both groups (Table 2; Fig. 3c).

The temporal trajectories of all diversity measures (H' and J' for both taxonomic and functional data) differed between nutrient-enriched and control mesocosms, as indicated by the significant interactions (Table 2). These trajectories can be divided into three phases: a period with high values in the nutrient-enriched and control mesocosms, followed by a period of decline (which was more intense for the eutrophic treatment) and a final period of stabilization. During this last phase, the diversity measures tended to be higher in the control than in the eutrophic treatment (Fig. 3d–g). In general, these results can be explained by the higher increase in density and dominance in the eutrophic treatment over time, when compared to the control mesocosms. Thus, despite the similarity between eutrophic treatment and control for species richness, there was a higher decrease in evenness (J' and, consequently, in H') at the nutrient-enriched mesocosms.

The first two NMDS axes derived from the taxonomic data and the first NMDS axis derived from the functional data varied significantly over time and between eutrophic and control mesocosms as indicated by the significant interactions (Table 2; Fig. 4). The second NMDS axis derived from the functional data varied significantly only between control nutrient treatment (Table 2; Fig. 4).

In general, the Chlorophyceae *Chlamydomonas* sp., *Monoraphidium contortum* (Thuret) Komárková-Legnerová, and the Cyanobacteria *Cyanobium plancticum* (G.Drews, H.Prauser & D.Uhlmann)

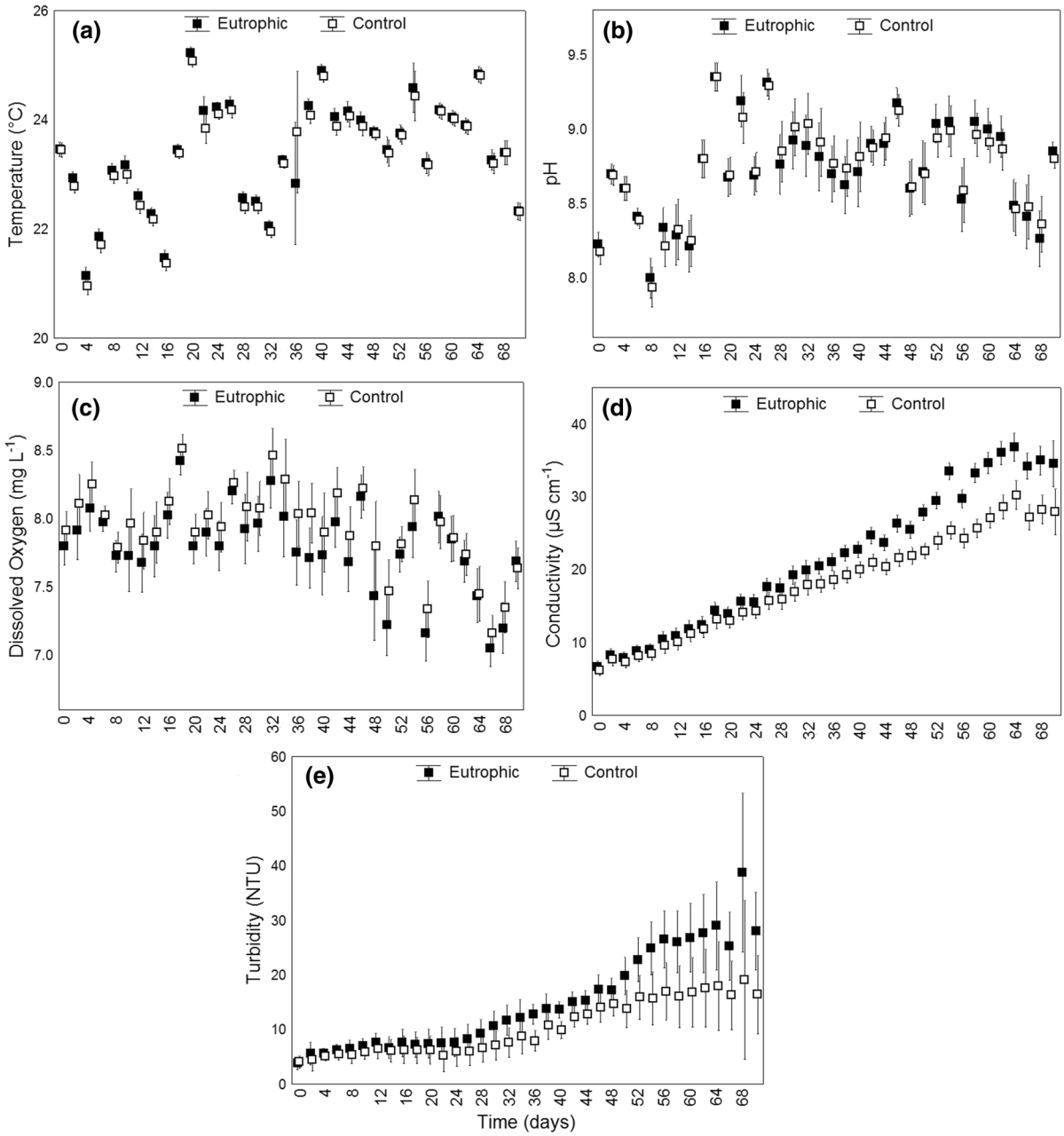


Fig. 2 Temporal variation in mean temperature (**a**), pH (**b**), dissolved oxygen (**c**), conductivity (**d**), and turbidity (**e**) for eutrophic treatment and control, considering the sampling events. Open and filled squares indicate data for control and eutrophic, respectively. Vertical bars represent 95% confidence intervals

Komárek, J.Kopecký & Cepák had high densities in both eutrophic treatment and control. However, the patterns of dominance of these species changed over time. At the beginning of the experiment, *Chlamydomonas* sp. showed high density in both eutrophic treatment and control, followed by *C. plancticum*. From the 34th day on, density of *Chlamydomonas* sp. decreased and of *C. plancticum* increased, followed by *M. contortum*. Then, *C. plancticum* and *M. contortum* remained as the two dominant species throughout

Table 2 Effects of nutrient enrichment and sampling time (36 events) on chlorophyll-a, total density, diversity metrics, and community composition (axes 1 and 2 of the Non-Metric Multidimensional Scaling Analysis—NMDS) assessed using Analysis of Variance with repeated measures

	Nutrient		Time		Nutrient x Time		Change
	$F_{(1,7)}$	*P*	$F_{(35, 245)}$	*P*	$F_{(35,245)}$	*P*	
Species richness	0.5	0.475	**2.2**	**< 0.001**	1.2	0.185	–
Log Chlorophyll-a	**36.6**	**< 0.001**	**91.0**	**< 0.001**	**6.6**	**< 0.001**	26
Log Total Density	**14.2**	**0.007**	**37.8**	**< 0.001**	1.2	0.191	52
H' Species	1.4	0.272	**22.4**	**< 0.001**	**1.9**	**0.002**	16
H' MBFG	1.3	0.291	**15.1**	**< 0.001**	**1.7**	**0.010**	16
J' Species	1.1	0320	**18.1**	< 0.001	**1.7**	**0.007**	70
J' MBFG	1.2	0.298	**14.3**	< 0.001	**1.7**	**0.008**	16
NMDS 1 Species	**119.1**	**< 0.001**	**8.4**	**< 0.001**	**8.3**	**< 0.001**	26
NMDS 2 Species	**41.1**	**< 0.001**	1.4	0.052	**6.4**	**< 0.001**	70
NMDS 1 MBFG	**130.1**	**< 0.001**	**9.8**	**< 0.001**	**6.1**	**< 0.001**	70
NMDS 2 MBFG	**8.5**	**0.02**	0.9	0.544	1.2	1.159	70

Bold values indicate significant results ($P < 0.05$)

H' Shannon diversity, *J'* Pielou's equitability, *MBFG* Morphologically Based Functional Groups. The last column (Change) indicates the time (in days) in which the difference between the eutrophic treatment and control began to be observed according to the change-point analysis. This analysis was employed only when a significant effect of nutrients or a significant interaction was detected

the end of the experiment in both eutrophic treatment and control (Fig. S3 in Online Resource 1).

Despite the lack of clear differences in species composition between eutrophic treatment and control, five rare species were found exclusively in the control. *Ankistrodesmus falcatus* (Corda) Ralfs, *Euglena* sp., and *Staurastrum furcatum* Brébisson only occurred in one sampling event (day 4, 62, and 68, respectively). Furthermore, *Cryptomonas marssonii* Ehrenberg and *Frustulia* sp. were found in two and three sampling events, respectively, distributed over the time of the experiment. These species were also the most associated with the first and second axes of the NMDS (see Table S1 in Online Resource 2).

For the MBFG, we found a temporal pattern similar to that observed for species composition (Fig. 4). At the beginning of the experiment, MBFG V (unicellular organisms with medium to large size and the presence of flagella) was predominant in both eutrophic treatment and control, followed by group I (Fig. S3 in Online Resource 1). From day 34th on, the density of MBFG V decreased, whereas the density of MBFG I increased, becoming dominant in both eutrophic treatment and control, followed by MBFG IV (Fig. S3 in Online Resource 1). Among the least frequent functional groups, MBFV VI was found exclusively in the control mesocosms, while MBFG III was found most often in the eutrophic mesocosms. These groups were also the most important for the composition of the first two axes of the NMDS (See Table S2 in Online Resource 2).

Among the water quality indicators, we found that the largest effect sizes (Hedges' *g*) of gradual nutrient enrichment were observed for conductivity and turbidity (Fig. S4 in Online Resource 1). Large effect sizes were also found for Chlorophyll-a and total density (Fig. S5 in Online Resource 1). Our exploratory change-point analysis also showed that conductivity, turbidity, and chlorophyll-a were the variables showing the earliest changes in effect sizes at day 22, 28, and 26, respectively (Tables 1 and 2). In short, conductivity, turbidity, and chlorophyll-a were simultaneously the variables that most differentiated the eutrophic treatment and control and did so early. However, according to the change-point analysis, H' (for both taxonomic and MFBG data) and J' (functional MFBG data), despite their smaller effect sizes (Fig. S6 in Online Resource 1), were the earliest indicators of differentiation between nutrient-enriched and control mesocosms (at the 16th day; Table 2).

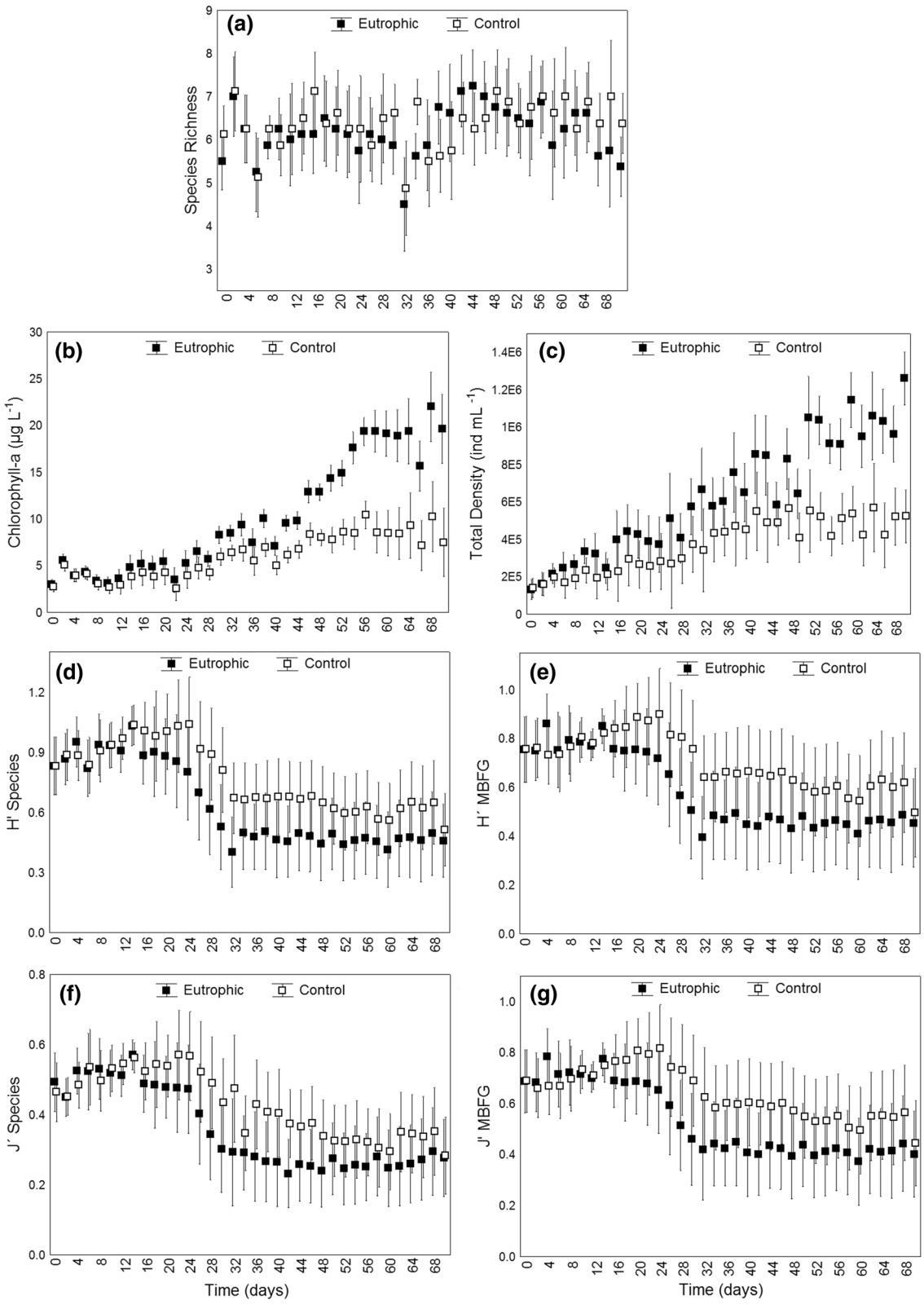
Discussion

Our experiment revealed the expected responses of abiotic and biological (phytoplankton) indicators to progressive eutrophication. In line with our expectations, we found an increase in total density and chlorophyll-a concentration, as well as a reduction in the diversity indices (Shannon–Wiener diversity and Pielou's equitability) of species and MBFG in the enriched mesocosms. The dominant species occurred in control and nutrient-enriched mesocosms, while rare species and MBFG tended to be exclusive to eutrophic treatment or control, accounting for most of the differences between them. This indicates that eutrophication process may not have marked effects on the composition of the initially dominant organisms, but may strongly decrease the density of rare species and groups. Furthermore, although with lower effect sizes, diversity indicators (H' for species and MBFG, J' for MBFG) were the earliest indicators of eutrophication, whereas abiotic and chlorophyll-a changes were more evident, but occurred later during eutrophication.

Eutrophication causes several changes in aquatic ecosystems (Smith et al., 1999; Chislock et al., 2013). Increase in nutrient concentrations enhances cell growth and algal biomass (Cottingham & Carpenter, 1998; Smith, 2016; Bouraï et al., 2020). In this sense, chlorophyll-a has been used as a proxy for algal biomass or as an indicator of eutrophication (Ptacnik et al., 2008; Liu et al., 2019; Millette et al., 2019). Indeed, we found that the highest effect sizes (i.e., the differences between nutrient-enriched and control mesocosms) and the clearest trends in their magnitudes were found for chlorophyll-a concentration and phytoplankton density. It is also worth emphasizing the theoretically consistent temporal order in which the variables showed the clearest changes in the effect sizes (as indicated by the change-point analysis). Thus, as expected during the eutrophication process, the change-point analysis indicated firstly a shift in the effect size based on the ionic concentration (22nd day). After, the earliest shifts were found for the effect sizes based on chlorophyll-a (26th day) and turbidity (28th day). Interestingly, a clear shift in the effect size for phytoplankton density was found only much later (52nd day).

We did not find evidence of an association between eutrophication and phytoplankton species richness, which varied over time but not consistently between eutrophic treatment and control. Some studies have shown that the relationship between phytoplankton species richness and nutrient enrichment is unimodal, being lower in oligotrophic and eutrophic conditions and reaching peaks in mesotrophic conditions (Jeppessen et al., 2000). The absence of a large gradient in trophic status may account for the insensitivity of species richness to eutrophication, as found in many studies (Smith, 2007). However, this explanation is unlikely as we found a wide range of trophic states over the experiment. Other studies indicate that the shift from eutrophic to oligotrophic conditions tends to increase species richness, as it causes a reduction in the biomass of dominant groups, allowing the coexistence of a higher number of species (Barçante et al., 2020). Still others found that this relationship can be specific to each group of organisms or environment (Dodson et al., 2000). Furthermore, the relationship between productivity and species richness can vary in different ways (positive or negative) depending on the range of the eutrophication gradient (Wang et al., 2017). In addition, other factors such as ecosystem size (e.g., Smith, 2005; Baho et al., 2017), physical structure of the aquatic environment (Liu et al., 2019), or biological interactions between trophic levels (e.g., Korhonen et al., 2011) may also interact with nutrients or be more important to explain variations in species richness.

In line with previous studies (Smith, 2016; Wang et al., 2017; Filiz et al., 2020), we found an increase in the total phytoplankton density due to nutrient enrichment. The increase in total phytoplankton density may be mainly driven by the increase in abundance of nuisance and bloom-forming species (Smayda, 1997; Heisler et al., 2008). Our results are in line with previous studies showing a dominance of few species in nutrient-enriched mesocosms, which promoted a decrease in species and MBFG diversity. Thus, eutrophication can lead to further decrease in the abundance of rare species or functional groups and to the dominance of those that thrive over a wider range of nutrient concentrations (Ansari et al., 2010). Thus, the increased rarity of these organisms in enriched mesocosms coupled with an increased dominance of a few ones caused a decline in H' and J', as also found in previous studies (Cottingham & Carpenter, 1998; Soares et al., 2013; Baho et al., 2017).


(a)
Eutrophic
Control
Species Richness
(b)
Chlorophyll-a (µg L-1)
(c)
Total Density (ind mL-1)
(d)
H' Species
(e)
H´ MBFG
(f)
J´ Species
(g)
J' MBFG
Time (days)
Time (days)

◂**Fig. 3** Temporal variation in mean species richness (**a**), chlorophyll-a (**b**), total density (**c**), H' species (**d**), H' MBFG (**e**), J' species (**f**), and J' MBFG (**g**) for eutrophic treatment and control. The open and filled squares indicate data for the control and eutrophic, respectively. The vertical bars represent 95% confidence intervals. H' = Shannon diversity; J' = Pielou's equitability. MBFG = Morphologically Based Functional Groups

In general, different classes of algae are found in lakes, each one showing tolerance to a wide range of nutrient concentrations and propensity to form blooms (Carvalho et al., 2013; Munawar & Fitzpatrick, 2018). Many eutrophic environments are dominated by Cyanobacteria and Chlorophyceae at high nitrogen and phosphorus concentrations, respectively (Jensen et al., 1994; Ferragut & Bicudo, 2012); however, each species may have a different response to nutrient enrichment (Piehler et al., 2004; Kissman et al., 2013; Donald et al., 2013). The dominant species observed in this study are cosmopolitans and occur in both high- and low-nutrient concentrations, although *C. plancticum* (Drews et al., 1961) and *Chlamydomonas* (Soares et al., 2013) are mainly found in eutrophic environments and *M. contortum* in water bodies ranging from oligotrophic to mesotrophic (John & Tsarenko, 2002). This wide niche breadth may explain why these species became dominant in both eutrophic and control mesocosms.

Less abundant species occurred mainly in the control. Thus, our main finding was that the initially dominant species (and with wide niche breadth) were favored, while those species that were naturally rare tended to disappear in eutrophic conditions or, at least, become even rarer (i.e., up to the point of not being detectable in our samples). Disturbances, which act negatively and selectively on rare species, can lead to biotic homogenization (Smart et al., 2006; Petsch, 2016), losses of ecosystem functioning (Lyons et al., 2005), and services (Dee et al., 2019), as their functional roles may be disproportionate to their abundance (Leitão et al., 2016).

Cell size is a key characteristic that affects reproduction, protection against herbivores, and the acquisition of resources by phytoplankton (Litchman & Klausmeier, 2008). The large area–volume ratio of small species, as those classified in MBFG I, favors the rapid uptake of nutrients compared to larger species (Litchman et al., 2010). In addition, although with larger size when compared to MBFG I, other functional characteristics of MBFG V and IV may also have favored the growth of these groups over time in the eutrophic treatment and control and allowed their co-dominance together with MBFG I. These include the presence of flagella in MBFG V and structures to prevent sinking in MBFG IV, such as processes and spines (Kruk et al., 2010). Traits related to motility, sinking, and cell length can increase the uptake of nutrients, favoring the growth of species that possess them (Litchman et al., 2010). These characteristics may have favored the growth of species belonging to these functional groups in both eutrophic and control mesocosms.

As stated above, the eutrophic treatment and control were similar in terms of MBFG dominance. Thus, even at lower-nutrient concentrations, we also found species with characteristics associated with motility, sinking, cell length, and small size belonging to MBFG I, IV, and V. This implies that the rapid change from an oligo-mesotrophic to a eutrophic environment may not have produced marked effects on the composition of dominant functional groups or species, but on the number of individuals of these groups and species (e.g., Rhew et al., 1999).

Forestalling undesirable water quality changes, as those triggered by eutrophication, is an area of growing interest in ecology, and several studies have investigated ways to detect these changes and produce early warnings (e.g., Batt et al., 2013; Buttita et al., 2017; Aubriot et al., 2020). In this context, we found that, with the progress of the eutrophication process, ionic and chlorophyll-a concentrations became higher in the nutrient-enriched mesocosms as compared to the control mesocosms. Subsequently, biogenic turbidity also became higher in the nutrient-enriched mesocosms. These were the variables with both the highest effect sizes and with the earliest responses to eutrophication. Nutrient enrichment also led to an increase in the total density of individuals, but the shift in effect size, as detected by the change-point analysis, occurred later. Therefore, whereas the importance of chlorophyll-a as an indicator of eutrophication is well known (Wilkson et al., 2018), our study adds evidence that chlorophyll-a has the potential to be used as an early-warning indicator of eutrophication with high effect size.

Our results indicate that species and MBFG composition, as summarized by ordination scores, are not good early indicators of eutrophication. This may be

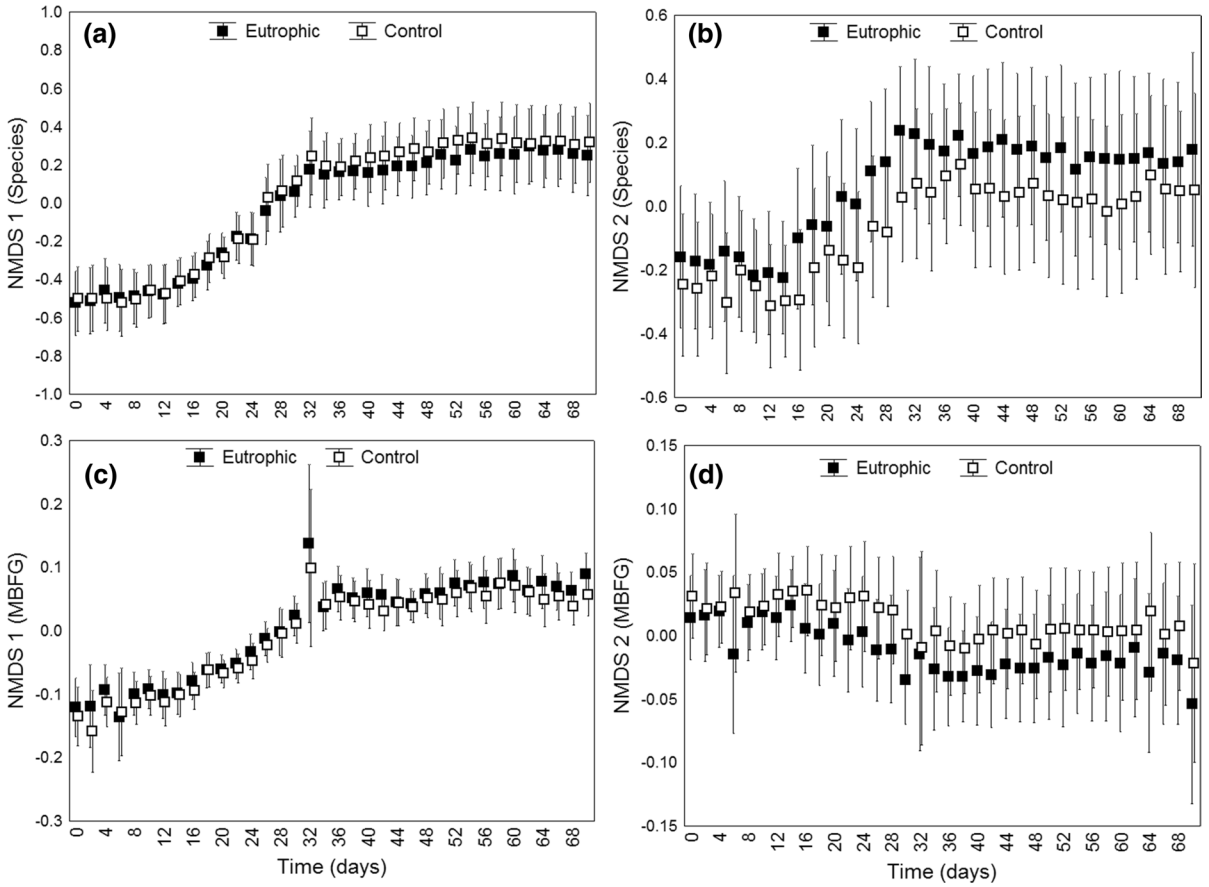


Fig. 4 Temporal variation in the first and second axes of Non-Metric Multidimensional Scaling Analyses (NMDS) for species composition and Morphologically Based Functional Groups (MBFG) for eutrophic treatment and control. Open and filled squares indicate mean values for the control and eutrophic, respectively. Vertical bars represent 95% confidence intervals

related to the diversity of responses to environmental change among species and MBFG and to the high variability of species data, which preclude summarizing a multidimensional data using a few ordination axes (Gauch & Gauch Jr., 1982). However, although with smaller effect sizes as compared to other variables, the diversity equitability indices were the ones that responded earlier to eutrophication. These findings are in line with previous studies showing that diversity and equitability indices are good indicators of eutrophication when compared to species or group composition (e.g., Cottingham & Carpenter, 1998). In addition, most studies have used biomass metrics (i.e., chlorophyll-a) to understand the impacts of eutrophication in lakes (e.g., Chen et al., 2003; Wang & Liu 2005), probably due to the need of specialized skills for identification in species level and greater complexity in interpreting the results from species or group composition analyses (Carvalho et al., 2013). Taken together, our results suggest that a set of abiotic and biological diversity variables would provide the best indicators of eutrophication in terms of effect sizes and earlier detection.

Biovolume is a key variable in different phytoplankton-based studies (e.g., Litchman & Klausmeier, 2008; Kruk et al., 2010). Unfortunately, as explained above, we were unable to obtain this variable. Thus, we cannot rule out that biovolume-based variables would emerge as the best ones in terms of earlier detection of the eutrophication process. However, considering the translation of scientific results (observation and experimental) to applied settings, mainly

biomonitoring, we dispute that biovolume could be widely and routinely measured, at least in Brazil. This is so due to the scarcity of resources for biomonitoring and skilled personnel to proper measure this time-consuming variable.

In general, our study shows that gradual eutrophication events affect abiotic variables as well as taxonomic and functional diversity. We recognize that eutrophication phenomena can also occur in the form of pulses (e.g., Di Carvalho & Wickham, 2019). However, investigating how communities vary with gradual nutrient inputs is important to detect earlier the several water quality problems caused by eutrophication. Furthermore, climate change (Jeppesen et al., 2010; Nazari-Sharabian et al., 2018), lake hydrodynamics (Bhagowati & Ahamad, 2019), and surrounding basin features (Salmaso & Toletti, 2021) can interact with eutrophication further exacerbating its effects. In this sense, the results of our study are conservative, as they do not consider interactions with these factors. However, this study adds relevant information, being able to isolate the effects of eutrophication from other factors and detect early warnings of eutrophication.

Acknowledgements We thank Cleiber Cintra de Morais for their assistance in monitoring the mesocosms and nutrient analyses.

Author contribution KBM, LMB, ASM, PC, FBT, FOR, JCB, AAP, LCGV, RBDC, TS, LJ, MSD, RTM, and JCN contributed to the study conception; KBM, ATA, and MFA performed the experiment and laboratory analysis; KBM and WAGJ performed the species identification; KBM, LMB, and JCN performed the data analysis. All authors contributed to the drafting of the manuscript and revised their final version.

Funding This study was funded by Ministério da Ciência, Tecnologia e Inovações/Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq –process number 465610/2014–5), and Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG), in the context of National Institute of Science and Technology in Ecology, Evolution and Biodiversity Conservation (INCT-EECBio). RBD-C received a postdoctoral fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001. KBM received DTI-B research grant from CNPq. ATA and MFA received scientific initiation scholarship from CNPq and Universidade Estadual de Goiás (UEG), respectively. LMB, ASM, FBT, FOR, AAP, LGCV, LJ, and JCN were supported by CNPq research grants.

Data availability Data are available from the authors upon reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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