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Influence of macronutrient and iron enrichment on phytoplankton productivity and community dynamics: an in situ microcosm study in a drinking water supply reservoir

Huy A. Luong [a], Ann-Marie Rohlfs,[b] Jordan A. Facey,[a,c] Anne Colville,[a] Alec W. Davie,[b] Joe B. Pera,[b] and Simon M. Mitrovic[a]

[a]Freshwater and Estuarine Research Group, School of Life Sciences, University of Technology Sydney, Broadway, Australia; [b]Strategic Research and Innovation Group, WaterNSW, Parramatta, Australia; [c]Department of Community and Ecosystem Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

ABSTRACT

This study examines the influence of macronutrients (nitrogen and phosphorus) and trace metals (iron and manganese) on phytoplankton productivity and community composition in Prospect Reservoir, a key component of the drinking water supply for Greater Sydney, Australia. In situ microcosm bioassays were set up at 2 sites (shallow and pelagic) during the Austral growing season (early autumn), and the responses of phytoplankton to various nutrient enrichment scenarios were assessed following an 8-day incubation period. Initial conditions and nutrient additions were compared by analyzing productivity indicators including chlorophyll *a* (Chl-*a*), total phytoplankton, and potentially toxic cyanobacteria biovolume. Phytoplankton community changes were identified using functional group classification and hierarchical cluster analysis. Productivity was colimited by nitrogen and phosphorus. Addition of these nutrients was associated with significant growth and dominance of group **F**, representing green algae such as *Scenedesmus* and *Oocystis*. Significant growth was also observed in meso-eutrophic groups that included the nuisance cyanobacteria *Microcystis* (**M**). An additional enhancement in Chl-*a* and phytoplankton biovolume in the pelagic site was observed when iron was added. Group **D**, represented by the nuisance taxon *Synedra*, seemed to dominate in low-P conditions. The study highlights the importance of informed eutrophication management strategies that address nutrient dynamics including macronutrient colimitation, macronutrient ratios, and iron to mitigate the risks associated with increased phytoplankton productivity and nuisance phytoplankton growth in drinking water reservoirs. This knowledge is particularly significant given the projected increase in macronutrient and trace metal micronutrient inputs and climate change-driven events such as bush fires and flooding.

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Introduction

The management of eutrophication in fresh waterbodies is imperative because it directly impacts water quality and ecosystem stability, both essential for maintaining a healthy society (Khan and Mohammed 2013). Eutrophication often results in the excessive growth of phytoplankton, including problematic taxa that can complicate drinking water treatment by producing toxins (Merel et al. 2013) and clogging filtration systems (Rose et al. 2019). A key factor in controlling phytoplankton productivity and community dynamics in freshwater systems is nutrient availability (Reynolds 2006). Phosphorus (P) and nitrogen (N) are considered macronutrients, with P traditionally considered the primary limiting nutrient driving phytoplankton growth in freshwater systems (Schindler 1977). As a result, most management efforts have focused on closely monitoring and controlling this macronutrient to prevent problematic phytoplankton growth (Wang and Wang 2009, Schindler et al. 2016). However, others advocate for dual macronutrient control, with growing evidence indicating that colimitation by N and P is common (Scott and McCarthy 2010, Mueller and Mitrovic 2015, Paerl et al. 2016, Gardner et al. 2017, Facey et al. 2019, Shatwell and Köhler 2019, Lewis et al. 2020). This understanding is valuable for water managers because it highlights the unique response of freshwater systems to nutrient pollution and the need for

CONTACT Huy A. Luong Huy.Luong@uts.edu.au Freshwater and Estuarine Research Group, School of Life Sciences, University of Technology Sydney, PO Box 123, Broadway, NSW, 2007, Australia

system-specific knowledge of nutrient limitation for effective, targeted water quality management.

Trace metals, including iron (Fe) and manganese (Mn), are essential micronutrients that facilitate crucial biological functions in phytoplankton such as electron transport, chlorophyll synthesis, and oxygen evolution (Facey et al. 2019). Although the role of these trace metals in limiting phytoplankton productivity is well documented in oceanic systems (Sunda 2012, Tripathy and Jena 2019, Browning et al. 2021, Nishioka et al. 2021, Hawco et al. 2022), research in freshwater systems is scarce. The studies available indicate trace metals alone or in tandem with macronutrients can promote phytoplankton growth and/or alter phytoplankton community structure in some aquatic systems (Sterner et al. 2004, Huang et al. 2020, Facey et al. 2021, Xiao et al. 2022, Dengg et al. 2023). Notably, Facey et al. (2021) used in situ nutrient enrichment microcosm bioassays to identify nutrients limiting phytoplankton growth in various freshwater systems across Southeast Australia and uncovered varied combinations of N, P, and trace metal limitation, but the study did not identify the specific trace metal/s responsible for growth limitation. In another study on Lake Superior, a major North American lake, Sterner et al. (2004) observed that P limited phytoplankton growth and that co-addition with Fe further enhanced productivity. These findings underscore the need to examine specific trace metals in nutrient limitation studies to enhance our understanding of their role in freshwater phytoplankton dynamics.

Prospect Reservoir is a critical part of the drinking water supply network for Greater Sydney, in southeast Australia. The reservoir receives water transfers from multiple sources, resulting in complex water quality dynamics that require targeted studies to understand linkages between phytoplankton dynamics and nutrient availability. A recent examination of over a decade of monitoring data from Prospect Reservoir (Luong et al. 2024) revealed that increases in flow activity, particularly following severe bushfires and heavy flooding, were associated with elevated levels of nutrients such as N, Fe, and Mn and a corresponding increase in productivity. Interestingly, the study found no correlation between total P (TP) and the phytoplankton productivity indicator chlorophyll *a* (Chl-*a*), despite its occurrence in most aquatic systems (Carlson 1977, Quinlan et al. 2021). Studies suggests that Chl-*a* and TP relationships depend on trophic status and nutrient ratios, and clear relationships between Chl-*a* and TP would be observable in oligo-mesotrophic lakes (Seip et al. 2000, Liang et al. 2020). The absence of a distinct trend in Chl-*a* and TP in the long-term study by Luong et al. (2024) may suggest that the study lacks the sensitivity to detect such relationships (Dokulil and Teubner 2005), particularly given that TP levels in Prospect Reservoir are consistently low. Luong et al. (2024) also identified Fe and Mn as critical factors impacting phytoplankton dynamics, with strong correlations observed between total Mn with cyanobacteria and Chl-*a*, and a general trend of increasing phytoplankton productivity aligned with rising Fe levels. Given this insight, understanding the role of micronutrient trace metals like Fe and Mn in controlling phytoplankton growth and community dynamics is important for water quality management and can enhance understanding to better monitor and predict phytoplankton dynamics in contexts where no clear correlation exists with traditional limiting nutrients such as P. Such insights are also vital for designing effective management interventions that reduce the impacts of eutrophication and ensure safe drinking water, especially considering the projected increase in macronutrient and trace metal inputs due to climate change (Paul et al. 2022).

In the present study, microcosm bioassays were set up in situ to understand linkages between phytoplankton growth and macronutrients (N and P) and trace metals (Fe and Mn) that may explain the relationships seen in the long-term study (Luong et al. 2024). We hypothesized that phytoplankton productivity would be promoted following co-macronutrient (N and P) addition, and that trace metal addition would further increase productivity. Furthermore, we anticipated that increased cyanobacterial growth would be linked to Mn addition, as suggested by Luong et al. (2024).

Materials and method

Study site

Prospect Reservoir, a 50.2 GL water supply reservoir, is part of a supply network that delivers water to 5.3 million residents in Greater Sydney, Australia. When full, it has an average and maximum depth of 9 and 20 m respectively. To ensure complete mixing of the water column, a bubble-plume destratifier operates almost constantly, except during short periods of routine maintenance. Microcosm incubations were performed at 2 sites in Prospect Reservoir, chosen to represent the difference in reservoir environmental characteristics (Fig. 1). Site 1 (shallow site; 33.819N, 150.879E) is a shallow (2–4 m), macrophyte-dense site. Site 2 (pelagic site; 33.821N, 150.909E) is a deeper (10–12 m), more wind exposed site. The study was conducted in March 2023 (Austral early autumn).

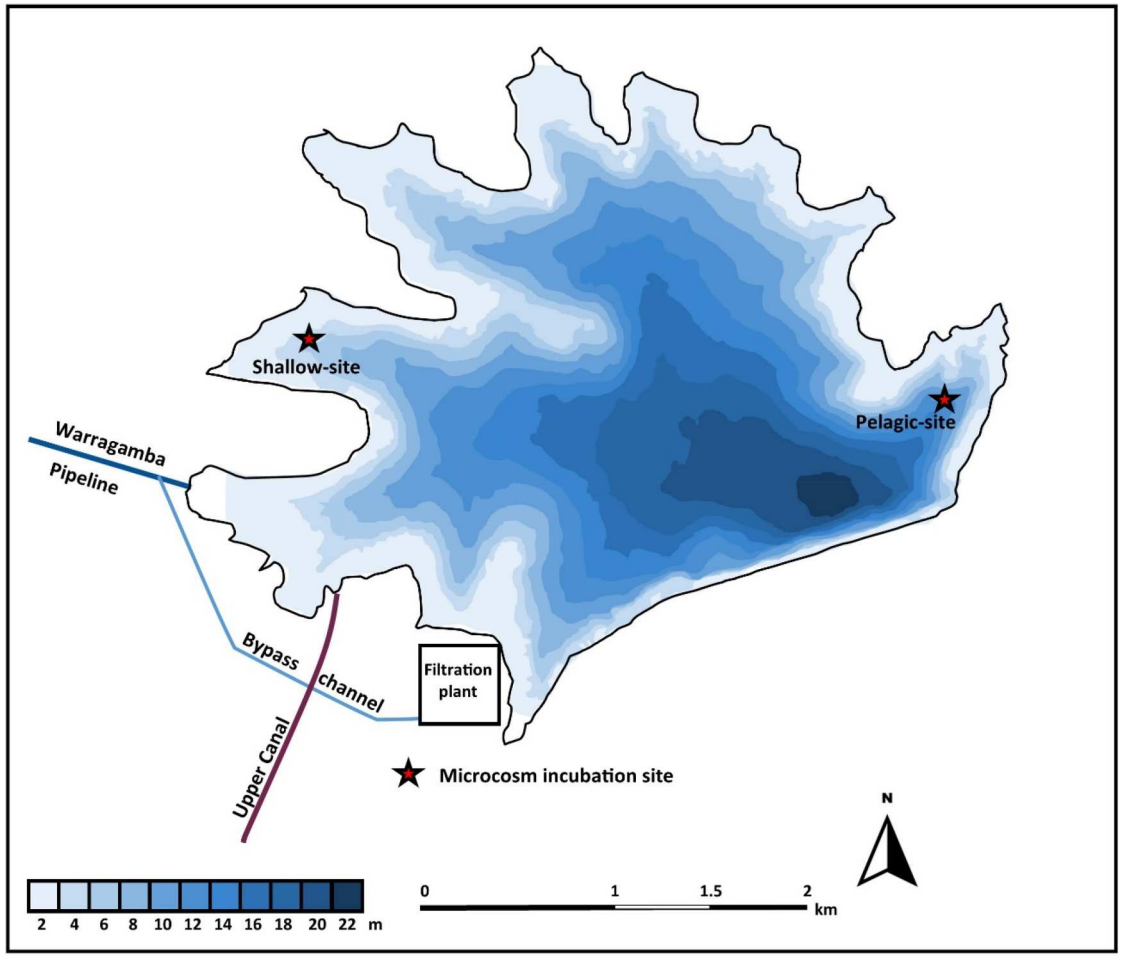

Figure 1. Bathymetry of Prospect Reservoir with microcosm sites locations.

Microcosm enrichment assays

Limiting nutrients were identified using nutrient enrichment in situ microcosms, comparable to Facey et al. (2021). Prior to experimental setup, the PET microcosms were rinsed inside with 10% HCl, followed by 3 repeated rinses with Milli-Q water. The inoculating nutrient solutions (Table 1) were prepared using pre-washed glassware and stored in 50 mL Falcon tubes pre-soaked overnight in an acid bath (10% HCl) and rinsed repeatedly with Milli-Q water reduce nutrient contamination. Approximately 50 L of site-specific surface water was filtered through a 63 µm plankton net into a large plastic tub to exclude phytoplankton grazing by zooplankton. Clear PET microcosms (3 L) were filled, leaving some air space. Nutrient additions were added in accordance with the 8 treatments (Table 1), all in triplicate.

After nutrient addition, the bottles were tightly capped to maintain a closed system then mixed by rotation and tied together in random order. The microcosms were secured to a mooring for the duration of the experiment and held near the water surface using a series of polystyrene floats (~90% surface irradiance). To prevent macronutrient limitation, N and P concentrations were selected to ensure effective growth

Table 1. Initial measurements of physical and chemical variables and productivity indicators (mean [standard error]). Physical chemical variables include: water temperature (WT), electrical conductivity (EC), pH, dissolved oxygen (DO), % saturation (% sat) and mg/L, and nutrients: soluble reactive phosphorus (SRP), oxidized nitrogen (NOx), soluble iron (Fe), and soluble manganese (Mn). Productivity indicators include chlorophyll *a* (Chl-*a*), total phytoplankton, and potentially toxic cyanobacteria (PTC) biovolume.

Initial conditions	Shallow	Pelagic
Physical and chemical variables		
WT (°C)	24.08 (0.012)	24.09 (0.01)
EC (µS/cm)	124 (0.26)	124 (0.36)
pH	7.87 (0.0067)	7.72 (0.0088)
DO (%sat)	111 (0.12)	105 (0.67)
DO (mg/L)	9.5 (0)	9.06 (0.045)
SRP (mg/L)	<0.001 (0)	0.0043 (0.0028)
NOx (mg N/L)	0.015 (0)	0.018 (00057)
Soluble Fe (mg/L)	0.077 (0.0033)	0.077 (0.0033)
Soluble Mn (mg/L)	<0.001 (0)	<0.001 (0)
Productivity indicators		
Chl-*a* (µg/L)	14.8 (0.26)	9.36 (1.14)
Phytoplankton biovolume (mm^3/L)	1.73 (0.12)	1.64 (0.3)
PTC biovolume (mm^3/L)	0.0063 (0.003)	0.015 (0.0062)

stimulation and avoid toxic effects (Müller and Mitrovic 2015, Facey et al. 2021). Trace metal addition (Fe and Mn) followed concentrations of the algal growth medium, MLA (Bolch and Blackburn 1996), and were low enough to avert toxic effects. Samples for macronutrients, trace metals, physical and chemical measurements, Chl-*a*, and phytoplankton enumeration were taken in triplicate from the filtered water at the initiation of the experiment to determine initial concentrations. Macronutrient and trace metal samples were also collected from surrogate microcosms with added nutrients to determine the total concentration of the nutrient addition plus the initial concentration. After an 8-day incubation period, which prior research suggest is adequate for significant changes in phytoplankton productivity and community dynamics (Müller and Mitrovic 2015, Facey et al. 2021), samples were collected from each microcosm. Samples for macronutrient, trace metal, physical, chemical, and Chl-*a* measurements and phytoplankton enumeration were collected after mixing the bottles by rotation.

Nutrient and phytoplankton sampling and analysis

Nutrient samples were collected and preserved according to standard methods after filtering with 0.45 µm pore size glass fibre filters (APHA 2017). Nutrient analysis followed standard methods (APHA 2017). Phytoplankton samples were immediately preserved with Lugol's solution to 0.5% v/v and counted using an upright light microscope and a Lund cell (Hötzel and Croome 1999). Taxa were identified to at least the genus level. Algal cells were approximated as geometric shapes to calculate their surface area (representing the maximal cross-sectional area of the cell), and their dimensions were measured to derive a mean biovolume conversion factor for each taxonomic group. These factors were then multiplied by the cell counts to determine the biovolumes for each taxonomic group. Taxa were then classified into phytoplankton functional groups (FG; Reynolds et al. 2002, Padisák et al. 2009). Only the dominant groups, defined as those present in at least 3 samples and making up >2% of phytoplankton biovolume, were statistically analyzed.

Chlorophyll a analysis

A 500 mL sample water was filtered on site via vacuum filtration onto GF/C glass fibre filters (Whatman) and frozen for preservation. For samples with noticeably higher phytoplankton productivity through visual inspection, 250 mL of water was filtered and Chl-*a* analyzed according to Müller and Mitrovic (2015). Chl-*a* extraction was performed by immersing the glass fibre filters in 10 mL 90% ethanol, followed by heating the sample in a 75 °C water bath for 10 min. Unwanted filter material was removed by centrifugation at 2000 RCF for 10 min. The supernatant was analyzed immediately using Varian Cary 50 Bio UV Spectrophotometer at wavelengths 665 and 750 nm.

Statistical analysis

Phytoplankton productivity differences between initial conditions and nutrient addition treatments were analyzed by comparing productivity indicators including Chl-*a*, phytoplankton biovolume, and potentially toxic cyanobacteria (PTC) biovolume using permutational analysis of variance (PERMANOVA) at a significance level of $\alpha = 0.05$. To cluster community data, we used hierarchical cluster analysis based on Euclidean-distance similarities of phytoplankton FG biovolume, applying the average linkage method. Distance transformation was performed using the *vegan* package in RStudio 4.3.1 and cluster analysis using the *cluster* package. Statistically significant clusters were identified through PERMANOVA. A PCA ordination plot was generated using CANOCO 5.15 to visualize the separation of clusters and identify the FG accounting for these differences. Community data were log-transformed (x+1) prior to analysis. All PERMANOVA analyses were performed using the PRIMER + PERMANOVA software version 6 (Anderson 2001).

Results

Initial conditions

Field physical and chemical variables including water temperature (WT), electrical conductivity (EC), and pH at shallow and pelagic sites varied within a similar range (Table 2). Soluble phosphorus (SRP) in the shallow site was below the limit of detection (<LOD) of 0.001 mg/L, contrasting with the pelagic site with measurable concentrations of 0.0043 (standard error 0.0028) mg/L. The initial trace metals concentrations, including filterable Mn, were consistently <LOD (<0.001 mg/L) and were comparable across sites. Initial Chl-*a* was higher in the shallow site (14.8 [0.26] µg/L) compared to the pelagic site (9.36 [1.14] µg/L), corresponding with relatively higher DO levels in the shallow site. By contrast, PTC biovolume at the shallow site was less than the pelagic site, although the concentrations at both sites were relatively low. The initial phytoplankton biovolume were similar between both sites.

Table 2. Results of the 2-way PERMANOVA on 3 productivity indicators: chlorophyll *a* (Chl-*a*), phytoplankton biovolume, and potentially toxic cyanobacteria (PTC) biovolume. The 2 factors were site (shallow and pelagic) and treatment (control and 7 nutrient enrichments). df = degrees of freedom; *F* = *F* ratio; *p* = level of significance. Significant *p* values (<0.05) are in bold.

Productivity indicator	Source of variation	df	Pseudo-*F*	*p*
Chl-*a*				
	Site (A)	1	0.022	0.873
	Treatment (B)	8	221.09	**0.001**
	Interaction of A × B	8	0.639	0.741
	Error	36		
	Total	53		
Phytoplankton biovolume				
	Site (A)	1	11.737	**0.002**
	Treatment (B)	8	51.705	**0.001**
	Interaction of A × B	8	6.5095	**0.001**
	Error	36		
	Total	53		
PTC biovolume				
	Site (A)	1	2.987×10^{-4}	0.586
	Treatment (B)	8	2.24×10^{-2}	**0.001**
	Interaction of A × B	8	5.01×10^{-4}	0.775
	Error	36		
	Total	53		

Nutrient addition and productivity indicator changes

Nutrient measurements of surrogate microcosms indicated that amended concentrations of NOx and SRP, were above the estimated concentration but were similar across sites. Mean (standard error) NOx and SRP concentrations were 0.7 (0.01) µg N/L and 0.4 (0) µg/L, respectively (Fig. 2). For trace metals, soluble Fe and Mn, were close to estimated concentrations at 0.1 (0) µg/L and 0.281 (0) µg/L, respectively. Following the 8-day microcosm incubation, NOx in the N treatment decreased to an average of 0.64 (0) µg N/L. In treatments N + P with and without trace metals (N + P + X), reductions were more pronounced (Fig. 2a). A similar pattern was observed for SRP (Fig. 2b), although final concentrations in the N + P + X treatments did not reduce to initial levels as closely as NOx. Mean final concentrations of soluble Fe all reduced to below initial concentrations (Fig. 2c). Similarly, for soluble Mn, treatments with Mn addition also reduced to initial levels, with most replicates <LOD (Fig. 2d).

Two-way PERMANOVA comparing site (shallow vs. pelagic) and treatment (nutrient addition) revealed no significant effect of site on productivity indicators including Chl-*a* and PTC biovolume (Table 2) following the 8-day incubation. Two-way PERMANOVA revealed a significant effect of site for phytoplankton biovolume (Table 2), with the pelagic site having significantly higher levels, accounted for by N + P + X treatments (Fig. 3b). Two-way PERMANOVA showed a significant effect of treatment for all biological indicators and a significant interaction between site and treatment for phytoplankton biovolume (Table 2).

Chl-*a* concentration in the control and single-nutrient additions (P, N, Fe) excluding Mn, were significantly lower than the initial concentrations (Fig. 3a). The addition of N + P + X resulted in a significant increase in Chl-*a* production compared to initial concentrations, the control, and the single-nutrient addition treatments. Additionally, the N + P + Fe treatment was statistically higher than N + P.

Phytoplankton biovolume significantly increased following the 8-day incubation for all treatments and sites (Fig. 3b). At the shallow site, the biovolume in the control and Mn treatments were significantly higher than N, P and Fe treatments. The N + P + X treatment had the highest phytoplankton biovolume for the shallow site (10.36 [0.53] mm^3/L), whereas the N + P + Fe treatment measured the highest levels (19.70 [1.25] mm^3/L) at the pelagic site.

PTC biovolume trends were similar to the other 2 biological variables, whereby the N + P + X treatment resulted in higher concentrations than most other treatments (control and single-nutrient addition; Fig. 3c). The exception was the N + P + Fe treatment, which was statistically similar to Fe alone and significantly lower than N + P + Mn.

Phytoplankton and functional groups

We identified 52 phytoplankton taxa from initial conditions and the 8-day microcosm incubations (Table 3), classified into 20 FG, from which 17 were identified as dominant: **B**, **D**, **F**, **J**, **K**, **Lo**, **M**, **N**, **P**, **T**, **Tc**, **W1**, **W2**, **X1**, **X2**, **X3**, **Y** (Table 3).

PCA and cluster analysis

The first 2 axes of the PCA explained 86.86% of the variability in the FG community (axis 1 = 71.96%; axis 2 = 14.9%; Fig. 4a). Some of the most important groups for axis 1 ordination include **F** (1.15), **J** (1.09), and **Y** (0.89) on the positive side, and **D** (−0.88) and **X1** (−0.67) on the negative. For axis 2, the most important group was **D** (1.73) and **K** (1.07) on the positive side. The community data displayed in the PCA were further separated into 4 significantly distinct clusters (PERMANOVA: $p < 0.05$) identified through hierarchical cluster analysis.

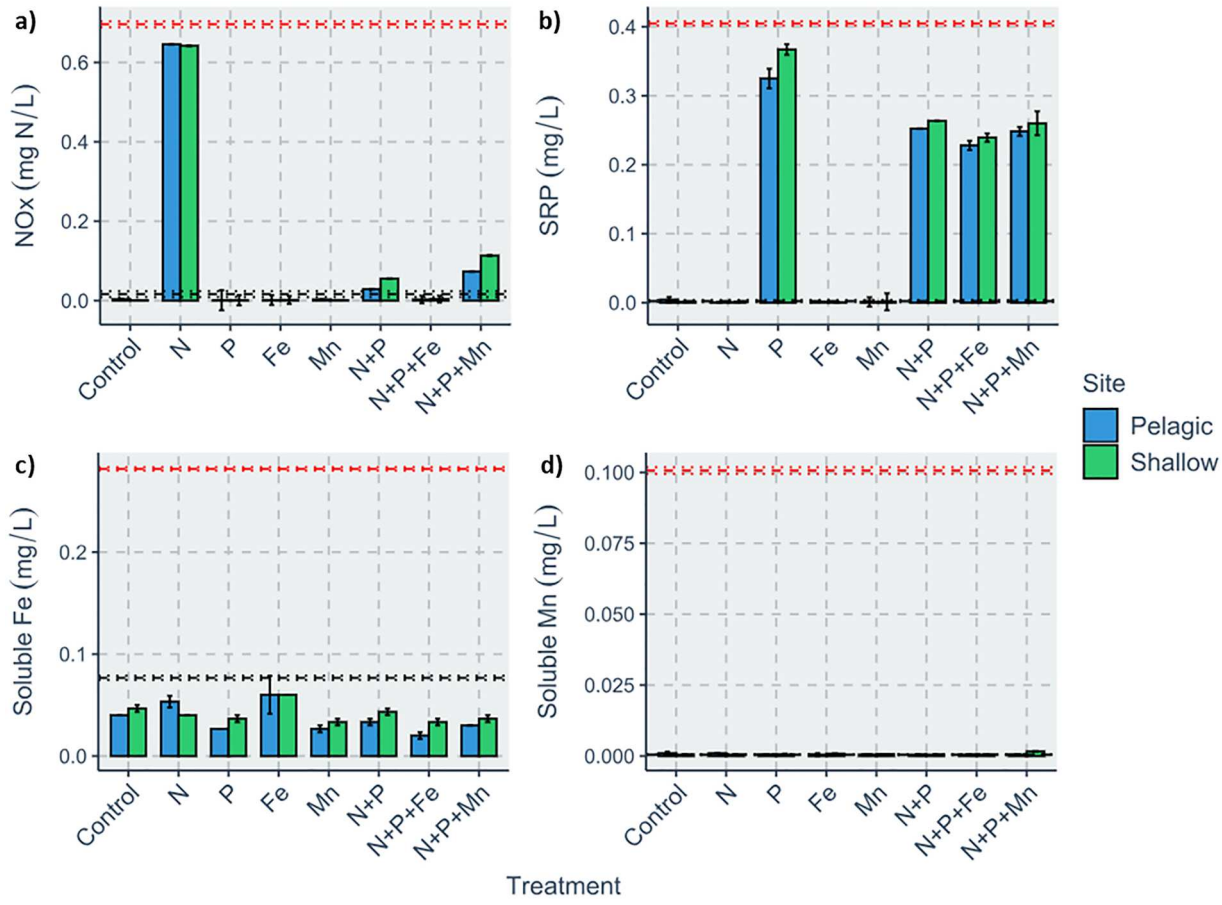

Figure 2. Soluble nutrient concentrations at the termination of the 8-day microcosm incubation (with standard error): (a) oxidized nitrogen (NOx), (b) reactive phosphorus (SRP), (c) iron (Fe), and (d) manganese (Mn). Red dashed line shows the mean soluble nutrient-addition concentration; red dotted lines indicate standard error; black dashed line represents initial soluble nutrient concentrations; and black dotted lines indicate standard error.

Cluster 1 (Fig. 4a) represented the initial conditions, and the P treatment and was relatively low in biovolume (2.61 [0.312] mm^3/L). On average, cluster 1 was mostly dominated by group B (37.1%), followed by similar dominance of **F** (17%), **Lo** (16.4%), and **D** (12.8%; Fig. 4b). Cluster 2 (orange ellipse) was identified as samples from the control and single-nutrient additions (N, Fe, Mn), excluding P addition. Group **D** (65.8%) dominated the community of cluster 2, with an average total biovolume more than double the concentration of cluster 1 (6.13 [0.414] mm^3/L). Cluster 3 and 4 (grey and green ellipses, respectively), shared similar dominant FG, with **F** dominating the community (55.7% and 63.0% for cluster 3 and 4, respectively), followed by a similar dominance in **B**, **J,** and **Y**. A major difference in clusters 3 and 4 is that the average total biovolume of cluster 4 (20.9 [0.11] mm^3/L) is almost double that of cluster 3 (12.3 [0.58] mm^3/L). The average total biovolume of clusters 3 and 4 were also >4 and 8 times higher than cluster 1, respectively. Cluster 4 included 2 samples from the N + P + Fe treatment at the pelagic site while cluster 3 included all other samples from the N + P + X treatment.

Discussion

In situ microcosm incubations were performed at 2 sites (shallow and pelagic) within Prospect Reservoir to assess macronutrient and trace metal limitation on phytoplankton growth and composition. Both sites, which exhibited similar initial physical and chemical conditions and phytoplankton communities, had mostly consistent responses to the various nutrient-enrichment bioassays. The most consistent changes observed at both sites following the 8-day incubation included significantly higher Chl-*a*, phytoplankton biovolume, and PTC biovolume in the N + P treatments with and without trace metal addition (N + P + X) relative to the control, indicating macronutrient colimitation. Productivity indicators for single-macronutrient treatments typically did not statistically differ from the

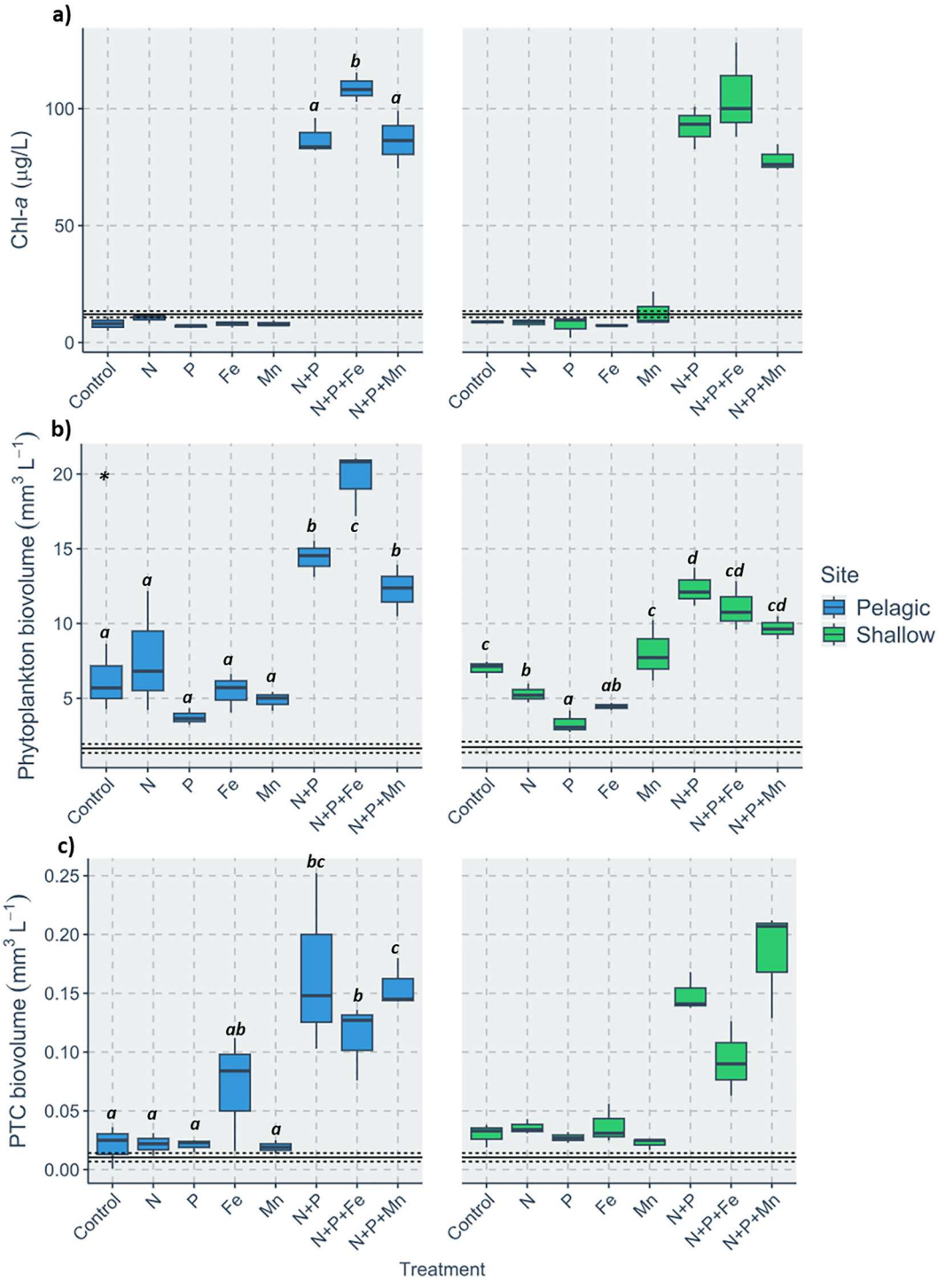

Figure 3. Productivity indicator values at the termination of the 8-day incubation: (a) chlorophyll *a* (Chl-*a*), (b) phytoplankton biovolume, and (c) potentially toxic cyanobacteria (PTC) biovolume. * indicates a significant difference in indicator between sites ($p < 0.05$); *i* indicates initial concentration was significantly higher than the treatment. Box-plots sharing no letter or the same letter denote statistically similar treatments; treatments with different letters are significantly different; and treatments with double letters (e.g., *ab*) indicate statistical similarity to treatments associated with both letters. For Chl-*a* and PTC biovolume, Site × Treatment pairwise comparisons were not conducted because PERMANOVA revealed no significant interaction. Consequently, treatment pairwise comparisons are displayed on the pelagic site box-plot.

control, demonstrating the presence of simultaneous colimitation rather than independent limitation of resources (Harpole et al. 2011). Previous studies have shown similar outcomes of simultaneous macronutrient colimitation (Müller and Mitrovic 2015, Paerl et al. 2016, Facey et al. 2021), highlighting the prevalence of

Table 3. Phytoplankton functional groups (FG) found in initial conditions at Prospect Reservoir and following an 8-day microcosm incubation. The species represented by each FG are listed as well as the corresponding higher-level taxonomic groups. FG included in the principal component analysis are indicated by an asterisk. Dominant taxa within the FG are highlighted in bold.

FG	Species	Dominant Taxa
A	*Achnanthidium* sp.	Diatom
B*	***Aulacoseira* sp.**, *Cyclotella* sp.	Diatom
D*	*Nitzschia sp., Skeletonema* sp., ***Synedra* sp.**	Diatom
F*	*Dictyosphaerium* sp., *Elakatothrix* sp., *Kirchneriella* sp., *Nephrocytium* sp., ***Oocystis* sp.**, ***Scenedesmus* spp.**, *Ankistrodesmus* sp.	Green algae
J*	*Coelastrum* sp., *Crucigenia* sp., *Pediastrum* sp., *Tetraedron* sp., *Tetrastrum* sp.	Green algae
K*	***Aphanocapsa* sp.**, *Aphanothece* sp., *Cyanocatena* sp., ***Cyanogranis* sp.**, *Cyanonephron* sp., *Rhabdogloea* sp.	Cyanobacteria
Lo*	*Chroococcus* sp., *Merismopedia* sp., *Peridinium* spp.	Dinoflagellate
M*	*Microcystis* spp.	Cyanobacteria
MP	*Navicula* sp.	Diatom
N*	*Cosmarium* sp., ***Spondylosium* spp.**, *Staurastrum* sp.	Green algae
P*	*Fragiliaria* sp.	Diatom
S1	*Limnothrix* sp., *Planktolyngbya* sp.	Cyanobacteria
T*	*Mougeotia* sp.	Green algae
Tc*	*Leptolyngbya* sp.	Cyanobacteria
W1*	***Euglena* sp.**, *Gonium* sp.	Euglenoid
W2*	*Trachelomonas* sp.	Euglenoid
X1*	*Monoraphidium arcuatum*	Green algae
X2*	*Carteria* sp., ***Chroomonas* sp.**, *Chrysochromulina* sp., *Pteromonas* sp.	Cryptomonad
X3*	*Chlamydomonas* sp.	Green algae
Y*	***Cryptomonas* sp.**, ***Gymnodinium* sp.**	Cryptomonad/ dinoflagellate

this particular resource limitation in freshwater systems. The addition of N + P + Fe significantly promoted Chl-*a* production and phytoplankton biovolume at the pelagic site, suggesting that Fe can further enhance phytoplankton growth. By contrast, no significantly distinct effects were observed in treatments with Mn addition (Fig. 5).

Macronutrient co-limitation and trace metals

Phytoplankton community structure in the N + P + X treatments was grouped into either cluster 3 or 4, both dominated by group **F**. This group is mainly represented by green algae including *Scenedesmus* and *Oocystis* and prefers meso-eutrophic lakes (Padisák et al. 2009). Clusters 3 and 4 had higher growth rates in several groups including **J**, **K**, **M,** and **Y** (Fig. 4a), all of which respond positively to nutrient enrichment (Reynolds et al. 2002, Padisák et al. 2009). Notably, *Microcystis* spp., the representative alga for group **M** and a primary concern for water managers because of its potential to produce cyanotoxins (Merel et al. 2013), increased in biovolume under addition of N + P + X. The concentration and percent composition remained relatively low following the 8-day incubation; however, over time concentrations may reach concerning levels (WaterNSW 2022). Green algae typically exhibit faster growth rates than cyanobacteria following nutrient additions (Lürling et al. 2013), suggesting they are the immediate responders. The response of cyanobacteria might be delayed, with potential succession occurring later (De Tezanos Pinto and Litchman 2010, Müller and Mitrovic 2015, Wang et al. 2015, Facey et al. 2022a), a dynamic not identifiable in short-term studies. Müller and Mitrovic (2015) found that changes in PTC were not discernible in 4-day microcosm experiments but were significant in 18-day mesocosm incubations. Similarly, Facey et al. (2022a) observed in Mannus Lake, Southeast Australia, that an initial proliferation of green algae and diatoms in response to nutrient pulses was subsequently followed by a dominance of *Microcystis*.

Chl-*a* concentration was significantly higher in the N + P + Fe treatment than in N + P, indicating that Fe may elevate phytoplankton productivity in tandem with co-macronutrient addition, a response previously observed in Lake Superior (Sterner et al. 2004). However, while Chl-*a* showed no significant difference between sites following the 8-day incubation, higher phytoplankton biovolume was found in the pelagic site compared to the shallow site, despite similar initial conditions, a difference accounted for by the N + P + X addition. Moreover, phytoplankton biovolume following N + P + Fe addition in the pelagic site was significantly higher than N + P and N + P + Mn treatments and was approximately double the concentration from the N + P + X addition at the shallow site. The pelagic N + P + Fe treatment was also dominated by the cluster 4 community, which had approximately double the average phytoplankton biovolume as cluster 3, suggesting site characteristics may affect the severity of phytoplankton growth (biovolume) to nutrient addition.

Single-nutrient additions and phytoplankton dynamics

Despite mostly similar responses in productivity indicators (Chl-*a*, total phytoplankton, and PTC biovolume) between the control and single-nutrient addition treatments (N, P, Fe, and Mn), phytoplankton FG community analysis revealed key differences between the initial phytoplankton community and P addition, and the control and other single-nutrient additions (N, Fe, and Mn). Hierarchical cluster analysis identified the initial community and P addition treatment as statistically similar. They were grouped in cluster 1, which was characterized by a dominance of the functional group **B** (*Aulacoseira*) followed

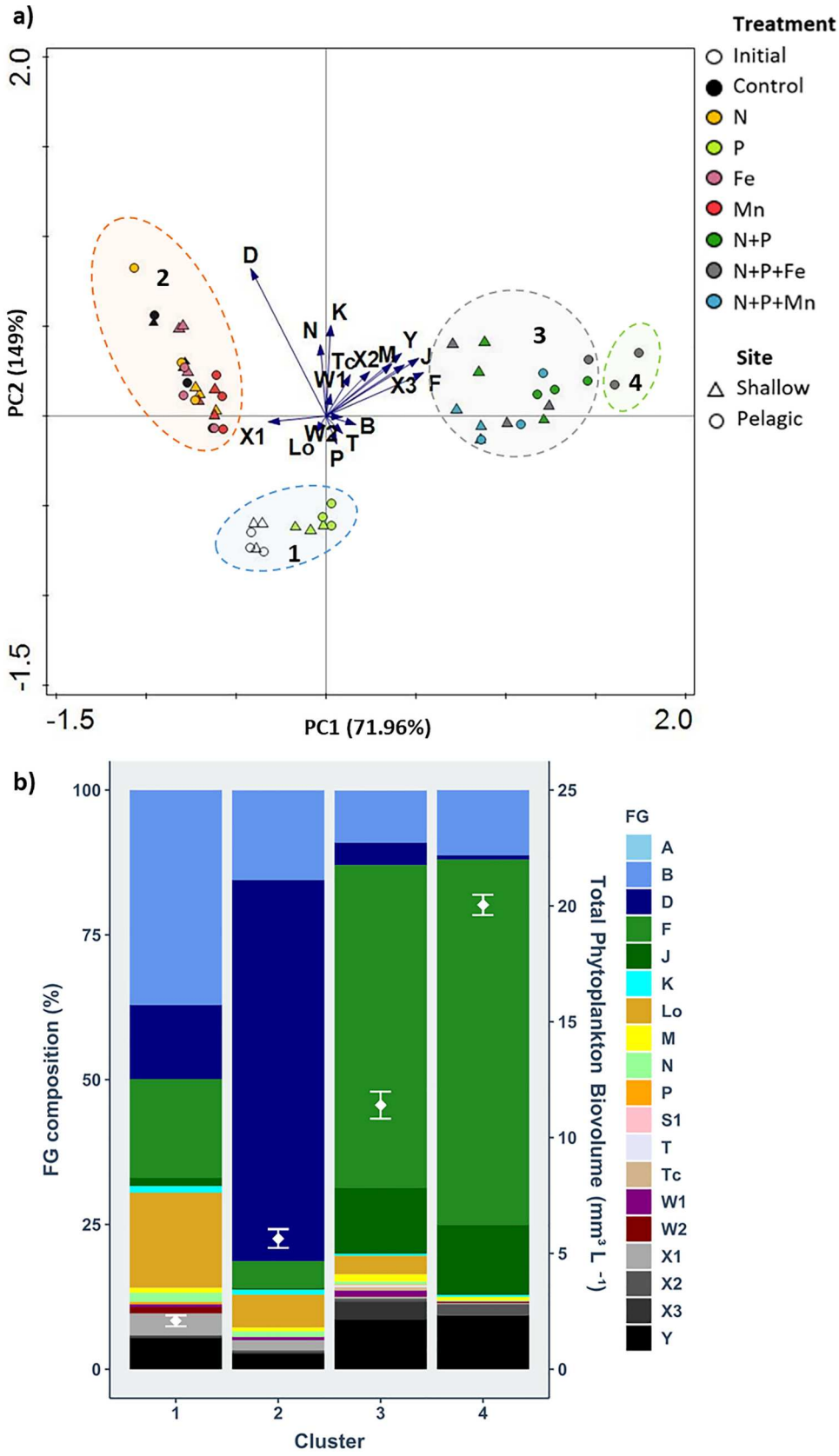

Figure 4. (a) Principal component analysis (PCA) applied to functional groups (FG) in initial lake water and microcosm incubations. Hierarchical cluster analysis identified 4 significant clusters indicated by numbered ellipses. Ellipses were created manually to aid visual interpretation of their distribution within the ordination. (b) FG community composition of the 4 clusters (left axis) and their average (standard error) biovolume (right axis, white diamonds [standard error]).

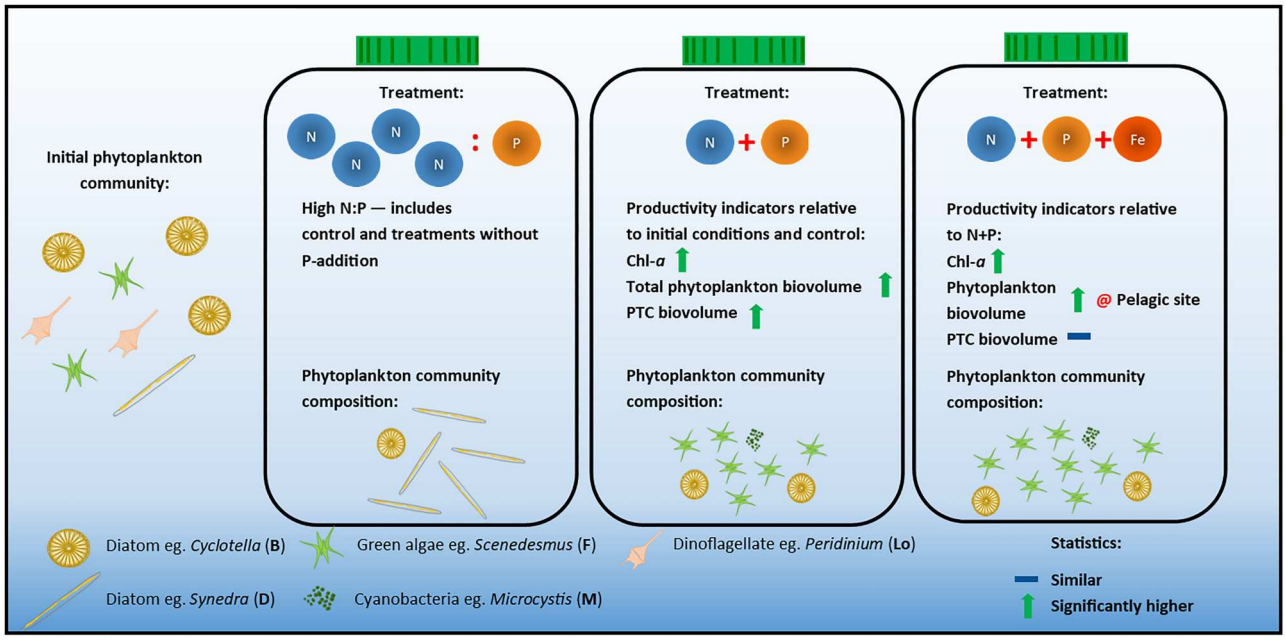

Figure 5. Visual summary of the 8-day in situ microcosm study. Productivity indicators and phytoplankton community responses to 3 nutrient treatments: (1) high nitrogen to phosphorus ratio (N:P), including control and treatments without phosphorus addition; (2) nitrogen and phosphorus (N + P); and (3) nitrogen, phosphorus, and iron (N + P + Fe). Productivity indicators include chlorophyll *a* (Chl-*a*), phytoplankton biovolume, and potentially toxic cyanobacteria (PTC) biovolume. Statistical differences are indicated by arrows: An up arrow denotes significantly higher values ($p < 0.05$), while horizontal lines indicate no significant difference.

by an equal distribution of **D**, **F,** and **Lo**. By contrast, the control and other single-nutrient additions were represented by cluster 2 (Fig. 4b), which was more productive (higher phytoplankton biovolume) and dominated by group **D** (*Synedra*), a diatom typically found in shallow and turbid environments (Padisák et al. 2009). *Synedra* is problematic for water managers because of its large cell size that can clog filters (Joh et al. 2011) and its potential to produce taste and odor compounds (Rose et al. 2019). These issues indicate that Chl-*a* may not be sensitive enough to elucidate significant differences in phytoplankton productivity (biovolume) and community response to nutrient enrichment that could be attributed to species-specific physiological variation in Chl-*a* production (Bowles 1982). Additionally, the results indicate that P-enrichment or macronutrient co-enrichment could suppress the dominance of *Synedra*. Facey et al. (2021) found that P addition in microcosm incubations at Windeyers Creek, which was initially dominated by diatoms, resulted in their suppression and replacement by dinoflagellates. Laboratory studies have also shown that *Synedra* has a low P saturation constant, and thus is better adapted to low P conditions (Tilman et al. 1982, Youn et al. 2020), which further supports our findings.

At the time of this study, N, Mn, and Fe enrichment alone did not significantly influence phytoplankton productivity and community dynamics because these treatments were statistically similar to the control. These results are similar to previous studies that suggest significant trace metal effects only occur in conjunction with macronutrient co-addition (Sterner et al. 2004, Huang et al. 2020) or in systems with particularly low trace metal concentrations (Dengg et al. 2022). However, the results also contrast with the strong positive relationship between Chl-*a* and total Mn observed from analyzing historical monitoring data from Prospect Reservoir (Luong et al. 2024), suggesting that the correlation between Mn and Chl-*a* may not be causative. Facey et al. (2022b) measured the growth of *Microcystis aeruginosa* under trace metal-limited laboratory conditions and found that while both Fe and Mn-limited conditions significantly reduced growth, the inhibition from Mn-limitation was less pronounced, and clear effects were not apparent until after >20 days. This observation may be attributed to a surplus absorption of Mn, as suggested by Sunda (2012), who reported cyanobacteria can acquire 2–4 times more Mn than necessary to support maximum growth. Consequently, the incubation period of the present study may not be long enough to detect any limiting effects of Mn.

Management recommendations

The present study suggests that the control of one or both key macronutrients is essential to reduce

productivity and the growth of problematic cyanobacteria like *Microcystis* in Prospect Reservoir. A study by Kolzau et al. (2014), who conducted monthly nutrient limitation bioassays on 4 German lakes, observed that colimitation occurred when both SRP and dissolved inorganic nitrogen (DIN) fell below thresholds of 0.001 and 0.1 mg/L, respectively, levels consistent with those recorded in our study and initially identified by Maberly et al. (2002) as critical points for the development of N or P limitation. Kolzau et al. (2014) also found that single-macronutrient limitation generally occurred when the particular macronutrient was below its aforementioned threshold. However, instances of single-nutrient limitation were recorded even when both nutrients were below these thresholds, suggesting that ambient nutrient concentrations may not consistently predict colimitation. This irregularity was linked to the "luxury uptake" of macronutrients by certain phytoplankton taxa, highlighting the importance of understanding the physiological traits of the prevailing phytoplankton community when predicting nutrient limitations of a waterbody. Additionally, the observed weak association between Chl-*a* and phytoplankton biomass and composition in this study suggests relying predominantly on Chl-*a* to monitor phytoplankton-related water quality threats could prove unreliable. Therefore, to ensure effective assessment and response to phytoplankton-related disturbances, we recommended that regular algal speciation and quantitative counts continue to be incorporated in monitoring programs of crucial water supplies.

Recognition of the benefits of controlling both N and P to effectively manage eutrophication is growing (Paerl et al. 2016, Gardner et al. 2017, Shatwell and Köhler 2019). Managing both macronutrients simultaneously removes the need to assess which macronutrient to prioritise and accommodates all potential temporal variations in nutrient limitation. Traditionally, strategies have predominantly focused on reducing P loading because P is commonly regarded as the primary macronutrient controlling productivity, and such strategies are well-established management techniques (Schindler et al. 2016, Wagner and Erickson 2017). However, our findings reveal that P reductions alone could inadvertently promote the dominance of *Synedra* in low P environments, highlighting the need for a nuanced approach to managing eutrophication that considers complex ecological dynamics, such as nutrient stoichiometry, to ensure an optimal outcome. Controlling for both N and P simultaneously would reduce the likelihood of high N:P conditions that can promote *Synedra* growth (Youn et al. 2020, Pera et al. 2022), but the associated costs could limit its implementation in water management schemes (Håkanson and Bryhn 2010).

Control measures that simultaneously address N and P inputs may not always be necessary to mitigate undesirable phytoplankton growth. The phytoplankton community response in the control treatment suggests that initial nutrient conditions are already conducive to *Synedra* over-production. Because the microcosm setup involved constant surface water irradiance, the phytoplankton community response observed in the control may be linked to stable light conditions. Therefore, the phytoplankton response scenarios observed in the experiment may not occur in Prospect Reservoir, given its hydromorphological characteristics, including a deep and thoroughly mixed water column. Hamilton et al. (1995) illustrated a scenario in Prospect Reservoir where phytoplankton did not respond to sediment-nutrient release during stratification, which would then be accessible for phytoplankton following destratification. However, this increase in phytoplankton productivity was delayed and only occurred after the water column had restratified. Light limitation could therefore be a significant factor in controlling phytoplankton dynamics in Prospect Reservoir, and the continuous operations of the destratifier may be integral in preventing undesirable productivity (Wagner and Erickson 2017).

This study raises concerns about the impact of increased Fe inputs on phytoplankton productivity, particularly when macronutrients are also available, a scenario that may occur following increased water transfers (Fornarelli and Antenucci 2011, Luong et al. 2024). To mitigate this risk, macrophytes could present a sustainable management option with a low risk to biological communities when compared to conventional chemical-based approaches (Wagner and Erickson 2017, Drewek et al. 2022). Macrophytes can influence the phytoplankton community through various physical, chemical, and biological processes (Scheffer et al. 1993, Song et al. 2019), including the uptake of macronutrients and micronutrient trace metals (Nabi 2021, Lv et al. 2023) and the production of allelochemicals that suppress phytoplankton growth (Hu and Hong 2008). The immediate presence of macrophytes in Prospect Reservoir in the shallow site may explain the observed differences in phytoplankton productivity. Although the role of macrophytes in controlling phytoplankton in deep lakes is not well understood, their potential impact should not be overlooked (Hilt et al. 2010, Hilt 2015). Further research should be considered to understand the extent that macrophytes can mitigate eutrophication in deep reservoirs like Prospect Reservoir.

Conclusion

This study advances our understanding of macronutrient (N and P) and trace metal (Fe and Mn) influence on phytoplankton productivity and community dynamics in a water drinking supply reservoir. We hypothesised that colimitation by macronutrients would primarily limit phytoplankton growth and that trace metals would further promote productivity and cyanobacterial growth. Our findings confirmed macronutrient colimitation at both study sites (shallow and pelagic), and productivity (Chl-*a*) was also enhanced with Fe addition, but Mn did not significantly affect cyanobacterial productivity. Low P conditions promoted the growth of *Synedra*, a problematic taxon. These insights highlight the need for management strategies that simultaneously address N and P levels and consider the role of Fe to effectively control eutrophication.

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ORCID

Huy A. Luong http://orcid.org/0009-0004-3988-0022

References

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Aust Ecol. 26 (1):32–46.

[APHA] American Public Health Association. 2017. Standard methods for the examination of water and wastewater, 23rd ed. Washington (DC): American Public Health Association – American Water Works Association.

Bolch CJS, Blackburn SI. 1996. Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. J Appl Phycol. 8:5–13. doi:10.1007/BF02186215

Bowles BA. 1982. Nutrient criteria for inland waters. Environmental Studies Series 394. Victoria, Melbourne (Australia): Ministry for Conservation.

Browning TJ, Achterberg EP, Engel A, Mawji E. 2021. Manganese co-limitation of phytoplankton growth and major nutrient drawdown in the Southern Ocean. Nat Commun. 12(1):884. doi:10.1038/s41467-021-21122-6

Carlson RE. 1977. A trophic state index for lakes 1. Limnol Oceanogr. 22(2):361–369. doi:10.4319/lo.1977.22.2.0361

De Tezanos Pinto O, Litchman E. 2010. Interactive effects of N:P ratios and light on nitrogen-fixer abundance. Oikos. 119(3):567–575. doi:10.1111/j.1600-0706.2009.17924.x

Dengg M, Stirling CH, Reid MR, Verburg P, Armstrong E, Kelly LT, Wood SA. 2022. Growth at the limits: comparing trace metal limitation of a freshwater cyanobacterium (*Dolichospermum lemmermannii*) and a freshwater diatom (*Fragilaria crotonensis*). Sci Rep. 12 (1):467. doi:10.1038/s41598-021-04533-9

Dengg M, Stirling CH, Safi K, Lehto NJ, Wood SA, Seyitmuhammedov K, Reid MR, Verburg P. 2023. Bioavailable iron concentrations regulate phytoplankton growth and bloom formation in low-nutrient lakes. Sci Tot Environ. 902:166399. doi:10.1016/j.scitotenv.2023.166399

Dokulil MT, Teubner K. 2005. Do phytoplankton communities correctly track trophic changes? An assessment using directly measured and palaeolimnological data. Freshw Biol. 50:1594–1604. doi:10.1111/j.1365-2427.2005.01431.x

Drewek A, Rybak M, Drzewiecka K, Niedzielski P, Polak J, Klimaszyk P. 2022. The impact of iron coagulant on the behavior and biogeochemistry of freshwater mussels *Anodonta cygnea* and *Unio tumidus* during lake restoration. J Environ Manage. 318:115535. doi:10.1016/j.jenvman.2022.115535

Facey JA, Apte SC, Mitrovic SM. 2019. A review of the effect of trace metals on freshwater cyanobacterial growth and toxin production. Toxins. 11(11):643. doi:10.3390/toxins11110643

Facey JA, Michie LE, King JJ, Hitchcock JN, Apte SC, Mitrovic SM. 2022a. Severe cyanobacterial blooms in an Australian lake; causes and factors controlling succession patterns. Harmful Algae. 117:102284. doi:10.1016/j.hal.2022.102284

Facey JA, Rogers TA, Apte SC, Mitrovic SM. 2021. Micronutrients as growth limiting factors in cyanobacterial blooms; a survey of freshwaters in South East Australia. Aquat Sci. 83(28):1–11. doi:10.1007/s00027-021-00783-x

Facey JA, Violi JP, King JJ, Sarowar C, Apte SC, Mitrovic SM. 2022b. The influence of micronutrient trace metals on *Microcystis aeruginosa* growth and toxin production. Toxins. 14(11):812. doi:10.3390/toxins14110812.

Fornarelli R, Antenucci JP. 2011. The impact of water transfers on water quality and the disturbance regime in a reservoir. Water Res. 45(18):5873–5885. doi:10.1016/j.watres.2011.08.048

Gardner WS, Newell SE, McCarthy MJ, Hoffman DK, Lu K, Lavrentyev PJ, Hellweger FL, Wilhelm SW, Liu Z, Bruesewitz DA, Paerl HW. 2017. Community biological ammonium demand: a conceptual model for cyanobacteria blooms in eutrophic lakes. Environ Sci Technol. 51(14):7785–7793. doi:10.1021/acs.est.6b06296

Håkanson L, Bryhn AC. 2010. Controlling eutrophication in the Baltic Sea and the Kattegat. In: Ansari AA, Gill SS, Lanza GR, Rast W, editors. Eutrophication: causes, consequences and control. Dordrecht (Netherlands): Springer; p. 17–67.

Hamilton DP, Schladow G, Fisher IH. 1995. Controlling the indirect effects of flow diversions of water quality in an Australian reservoir. Environ Int. 21(5):583–590. doi:10.1016/0160-4120(95)00061-O

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken ME, Elser JJ, Gruner DS, Hillebrand H, Shurin

JB, Smith JE. 2011. Nutrient co-limitation of primary producer communities. Ecol Lett. 14(9):852–862. doi:10.1111/j.1461-0248.2011.01651.x

Hawco NJ, Tagliabue A, Twining BS. 2022. Manganese limitation of phytoplankton physiology and productivity in the Southern Ocean. Glob Biogeochem Cycles. 36(11): e2022GB007382. doi:10.1029/2022GB007382

Hilt S. 2015. Regime shifts between macrophytes and phytoplankton – concepts beyond shallow lakes, unravelling stabilising mechanisms and practical consequences. Limnetica. 34(2):725–733. doi:10.23818/limn.34.35

Hilt S, Henschke I, Rücker J, Nixdorf B. 2010. Can submerged macrophytes influence turbidity and trophic state in deep lakes? Suggestions from a case study. J Environ Qual. 39(2):725–733. doi:10.2134/jeq2009.0122

Hötzel G, Croome R. 1999. A phytoplankton methods manual for Australian freshwaters. Canberra (Australia): Land and Water Resources Research and Development Corporation.

Hu H, Hong Y. 2008. Algal-bloom control by allelopathy of aquatic macrophytes – a review. Front Environ Sci Eng. 2:421–438. doi:10.1007/s11783-008-0070-4

Huang Y, Li Y, Ji D, Nwankwegu AS, Lai Q, Yang Z, Wang K, Wei J, Norgbey E. 2020. Study on nutrient limitation of phytoplankton growth in Xiangxi Bay of the Three Gorges Reservoir. China Sci Tot Environ. 723:138062. doi:10.1016/j.scitotenv.2020.138062

Joh G, Choi YS, Shin JK, Lee J. 2011. Problematic algae in the sedimentation and filtration process of water treatment plants. J Water Supply: Res Technol-AQUA. 60 (4):219–230. doi:10.2166/aqua.2011.035

Khan MN, Mohammed F. 2013. Eutrophication: challenges and solutions. In: Ansari AA, Gill SS, editors. Eutrophication: causes, consequences and control. New York: Springer; p. 1–15.

Kolzau S, Wiedner C, Rücker J, Köhler A, Dolman AM. 2014. Seasonal patterns of nitrogen and phosphorus limitation in four German lakes and the predictability of limitation status from ambient nutrient concentrations. PLoS One. 9(4):e96065. doi:10.1371/journal.pone.0096065

Lewis ASL, Kim BS, Edwards HL, Wander HL, Garfield CM, Murphy HE, Poulin ND, Princiotta SD, Rose KC, Taylor AE, et al. 2020. Prevalence of phytoplankton limitation by both nitrogen and phosphorus related to nutrient stoichiometry, land use, and primary producers biomass across the northeastern United States. Inland Waters. 10(1):42–50. doi:10.1080/20442041.2019.1664233

Liang Z, Soranno PA, Wagner T. 2020. The role of phosphorus and nitrogen on chlorophyll *a*: evidence from hundreds of lakes. Water Res. 185:116236. doi:10.1016/j.watres.2020.116236

Luong HA, Rohlfs AM, Facey JA, Colville A, Mitrovic SM. 2024. Long-term study of phytoplankton dynamics in a supply reservoir reveals signs of trophic state shift linked to changes in hydrodynamics associated with flow management and extreme events. Water Res. 256:121547. doi:10.1016/j.watres.2024.121547

Lürling M, Eshetu F, Faassen EJ, Kosten S, Huszar VL. 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshw Biol. 58(3):552–559. doi:10.1111/j.1365-2427.2012.02866.x

Lv C, Tian Y, Huang L, Shan H, Chou Q, Zhang W, Su H, Li K, Zhang X, Ni L, et al. 2023. Buffering capacity of submerged macrophytes against nutrient pulses increase with its coverage in shallow lakes. Chemosphere. 332:138899. doi:10.1016/j.chemosphere.2023.138899

Maberly SC, King L, Dent MM, Jones RI, Gibson CE. 2002. Nutrient limitation of phytoplankton and periphyton growth in upland lakes. Freshw Biol. 47(11):2136–2152. doi:10.1046/j.1365-2427.2002.00962.x

Merel S, Walker D, Chicana R, Snyder S, Baurès E, Thomas O. 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ Int. 59:303–327. doi:10.1016/j.envint.2013.06.013

Müller S, Mitrovic SM. 2015. Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: progressing from the phosphorus limitation paradigm. Hydrobiologia. 744:255–269. doi:10.1007/s10750-014-2082-3

Nabi M. 2021. Heavy metals accumulation in aquatic macrophytes from an urban lake in Kashmir Himalaya, India. Environ Nanotechnol Monit Manag. 16:100509.

Nishioka J, Obata H, Hirawake T, Kondo Y, Yamashita Y, Misumi K, Yasuda I. 2021. A review: iron and nutrient supply in the subarctic Pacific and its impact on phytoplankton production. J Oceanogr. 77(4):561–587. doi:10.1007/s10872-021-00606-5

Padisák J, Crossetti LO, Naselli-Flores L. 2009. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. Hydrobiologia. 621:1–19. doi:10.1007/s10750-008-9645-0

Paerl HW, Scott JT, McCarthy MJ, Newell SE, Gardner WS, Havens KE, Hoffman DK, Wilhelm SW, Wurtsbaugh WA. 2016. It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. Environ Sci Technol. 50 (20): 10805–10813. doi:10.1021/acs.est.6b02575

Paul MJ, LeDuc SD, Lassiter MG, Moorhead LC, Noyes PD, Leibowitz SG. 2022. Wildfires induce changes in receiving waters: a review with consideration for water quality management. Water Resour Res. 58(9):1–28. doi:10.1029/2021WR030699

Pera JB, Davie AW, Rohlfs AM, Mitrovic SM. 2022. Simulating the potential effects of a carp virus fish kill on water quality and phytoplankton in lentic environments. Mar Freshw Res. 73(2):178–192. doi:10.1071/MF20368

Quinlan R, Fizallo A, Mhdiyan O, Shuvo A, Blagrave K, Ewins C, Moslenko L, Gray DK, O'Reilly C, Sharma S. 2021. Relationships of total phosphorus and chlorophyll in lakes worldwide. Limnol Oceanogr. 66(2):392–404. doi:10.1002/lno.11611

Reynolds CS. 2006. The ecology of phytoplankton (ecology, biodiversity and conservation). Cambridge (UK): Cambridge University Press.

Reynolds CS, Huszar C, Kruk C, Naselli-Flores L, Melo S. 2002. Towards a functional classification of the freshwater phytoplankton. J Plankton Res. 24(5):417–428. doi:10.1093/plankt/24.5.417

Rose AD, Kinder JE, Fabbro L, Kinnear S. 2019. A phytoplankton risk matrix: combining health, treatment, and aesthetic considerations in drinking water supplies. Environ Syst Decis. 39:163–182. doi:10.1007/s10669-018-9711-8

Scheffer M, Hosper SH, Meijer ML, Moss B, Jeppesen E. 1993. Alternative equilibria in shallow lakes. Trends Ecol Evol. 8(8):275–279. doi:10.1016/0169-5347(93)90254-M

Schindler DW. 1977. Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. Science. 195(4275):260–262. doi:10.1126/science.195.4275.260

Schindler DW, Carpenter SR, Chapra SC, Hecky RE, Orihel DM. 2016. Reducing phosphorus to curb lake eutrophication is a success. Environ Sci Tech. 50(17):8923–8929. doi:10.1021/acs.est.6b02204

Scott JT, McCarthy MJ. 2010. Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnol Oceanogr. 55(3):1265–1270. doi:10.4319/lo.2010.55.3.1265

Seip KL, Jeppesen E, Jensen JP, Faafeng B. 2000. Is trophic status or regional location the strongest determinant for Chl-*a*/TP relationships in lakes? Aquat Sci. 62:195–204. doi:10.1007/PL00001331

Shatwell T, Köhler J. 2019. Decreased nitrogen loading controls summer cyanobacterial blooms without promoting nitrogen-fixation taxa: long-term response of a shallow lake. Limnol Oceanogr. 64(S1):S166–S178. doi:10.1002/lno.11002

Song Y, Liew JH, Sim DZH, Mowe MAD, Mitrovic SM, Tan HTW, Yeo DCJ. 2019. Effects of macrophytes on lake-water quality across latitudes: a meta-analysis. Oikos. 128:468–481. doi:10.1111/oik.05809

Sterner RW, Smutka TM, McKay RML, Xiaoming Q, Brown ET, Sherrell RM. 2004. Phosphorus and trace metal limitation of algae and bacteria in Lake Superior. Limnol Oceanogr. 49(2):495–507. doi:10.4319/lo.2004.49.2.0495

Sunda WG. 2012. Feedback interactions between trace metal nutrients and phytoplankton in the ocean. Front Microbiol. 3:204. doi:10.3389/fmicb.2012.00204

Tilman D, Kilham SS, Kilham P. 1982. Phytoplankton community ecology" the role of limiting nutrients. Annu Rev Ecol Syst. 13:349–372. doi:10.1146/annurev.es.13.110182.002025

Tripathy SC, Jena B. 2019. Iron-stimulated phytoplankton blooms in the Southern Ocean: a brief review. Remote Sens Earth Syst Sci. 2:64–77. doi:10.1007/s41976-019-00012-y

Wagner T, Erickson LE. 2017. Sustainable management of eutrophic lakes and reservoirs. J Environ Prot. 8(4):436–463. doi:10.4236/jep.2017.84032

Wang H, Wang H. 2009. Mitigation of lake eutrophication: Loosen nitrogen control and focus on phosphorus abatement. Prog Nat Sci. 19(10):1445–1451. doi:10.1016/j.pnsc.2009.03.009

Wang L, Liu X, Qin B, Gu Z, Wu Z, Xu H, Zhu H, Cheng G, Liu H. 2015. Green algae dominance quickly switches to cyanobacteria dominance after nutrient enrichment in greenhouse with high temperatures. J Ecol Environ. 38(3):293–305. doi:10.5141/ecoenv.2015.030

WaterNSW. 2022. Annual water quality monitoring report 2021–2022. Paramatta (Australia): WaterNSW.

Xiao LJ, Xie J, Tan L, Lei KM, Peng L, Wang Z, Naselli-Flores L. 2022. Iron enrichment from hypoxic hypolimnion supports the blooming of *Raphidiopsis raciborskii* in a tropical reservoir. Water Res. 219:117562. doi:10.1016/j.watres.2022

Youn SJ, Yu SJ, Byeon MS. 2020. Occurrence characteristics of *Stephanodiscus* and *Synedra* in relation to water temperature and concentrations of nutrients during spring diatom bloom in Lake Paldang, Korea. Appl Ecol Environ Res. 18(4):5135–5147. doi:10.15666/aeer/1804_51355147