Aquatic Sciences (2021) 83:28
https://doi.org/10.1007/s00027-021-00783-x

Aquatic Sciences

RESEARCH ARTICLE

Micronutrients as growth limiting factors in cyanobacterial blooms; a survey of freshwaters in South East Australia

Jordan A. Facey[1] · Terence A. Rogers[1] · Simon C. Apte[2] · Simon M. Mitrovic[1]

Received: 22 October 2020 / Accepted: 25 January 2021 / Published online: 19 February 2021

Abstract

The role of trace metal micronutrients in limiting cyanobacterial growth and structuring the phytoplankton community is becoming more evident. However, little is known regarding the extent of micronutrient limitation in freshwaters or which micronutrient conditions favour potentially-toxic cyanobacteria. To assess how freshwater phytoplankton respond to micronutrient and macronutrient additions, we conducted nutrient amendment bioassays at seven sites across South Eastern-Australia. Sites were variable in cyanobacterial cell densities and phytoplankton community compositions. At two sites, Mannus Lake and Burrendong Dam, micronutrient additions (iron, cobalt, copper, manganese, molybdenum and zinc) increased cyanobacterial growth, indicating micronutrient limitation. Both sites had cyanobacterial blooms present at the onset of the experiment, dominated by *Chrysosporum ovalisporum* at Mannus Lake and *Microcystis aeruginosa* at Burrendong Dam. This suggests that micronutrients may be an important regulator of the severity of cyanobacterial blooms and may become limiting when there is high competition for nutrient resources. The addition of the micronutrient mixture resulted in a higher proportion of cyanobacteria compared to the control and a lower diversity community compared to phosphorus additions, indicating that micronutrients can not only influence cyanobacterial biovolume but also their ability to dominate the phytoplankton community. This reinforces that micronutrient requirements of phytoplankton are often species specific. As micronutrient enrichment is often overlooked when assessing nutrient-constraints on cyanobacterial growth, this study provides valuable insight into the conditions that may influence cyanobacterial blooms and the potential contribution of micronutrients to eutrophication.

Keywords Nutrient limitation · Phytoplankton · Trace metals · Community composition

Introduction

Freshwater phytoplankton communities are highly variable in space and time, and respond rapidly to changes in their physical, chemical and biological environment (Varol and Sen 2018). Highly diverse, low biomass phytoplankton communities are indicative of healthy freshwater systems (Shao et al. 2019). Conversely, high biomass, less diverse phytoplankton communities often dominated by bloom-forming cyanobacteria tend to persist in systems anthropogenically modified through increased nutrients or flow restriction (Mitrovic et al. 2003; Bormans et al. 2004; Dignum et al. 2005; O'Neil et al. 2012). Many cyanobacteria can produce biologically active secondary metabolites, known as cyanotoxins, which can have severe ecological, economic and human health impacts (Bowling 1994; Bormans et al. 1997; Falconer 2001; Pearson et al. 2010; Rastogi et al. 2015).

Optimal growth of phytoplankton depends on the availability of several key nutrients. Among these, phosphorus (P) and nitrogen (N) are required in the largest quantities and are commonly a growth-limiting factor in freshwater systems (Paerl and Otten 2013). The role of these macronutrients in stimulating cyanobacterial blooms is well documented (Hunt and Matveev 2005; Paerl and Otten 2013; Mueller and Mitrovic 2014; Schindler et al. 2016). Micronutrient trace metals also play key roles in a multitude of biological processes and are cofactors in numerous cyanobacterial proteins (Baptista and Vasconcelos 2006; Facey et al. 2019). There is

✉ Jordan A. Facey
Jordan.Facey@uts.edu.au

[1] Freshwater and Estuarine Research Group, School of Life Sciences, University of Technology Sydney, PO Box 123, Broadway, NSW 2000, Australia

[2] CSIRO Land and Water, Lucas Heights, NSW 2234, Australia

emerging evidence they can influence cyanobacterial growth alone or in combination with macronutrients (Lukac and Aegerter 1993; Downs et al. 2008; Molot et al. 2010; Harland et al. 2013; Polyak et al. 2013; Sorichetti et al. 2014).

The availability of macronutrients and micronutrients play a key role in structuring the phytoplankton community (Vyverman et al. 2007). Nutrient requirements within the phytoplankton community are highly variable, leading to interspecific competition for nutrient resources (Sourisseau et al. 2017). As different phytoplankton groups have distinct nutrient requirements and means of nutrient acquisition, the addition of a nutrient can cause differential responses in different segments of the phytoplankton community. This process, termed 'community colimitation', can cause an alteration to the overall structure of the community (Arrigo 2005). For example, Molot et al. 2014 proposed that iron regulates the ability of cyanobacteria to compete with eukaryotic algae and cyanobacterial dominance can be supressed in P-loaded systems by reducing Fe^{2+} availability. Further, the growth of heterocystous cyanobacteria will likely be more dependent on molybdenum availability than non-heterocystous cyanobacteria due to its role in the assimilation of inorganic nitrogen (Glass et al. 2012).

While the importance of trace metal micronutrients for phytoplankton growth is becoming more evident, little is known about the extent of micronutrient limitation in freshwaters or how increased concentrations of micronutrients may alter phytoplankton community structure. Identifying how phytoplankton communities, particularly those that include toxin-producing cyanobacteria, respond to different macronutrient and micronutrient regimes is crucial to making informed, effective catchment management decisions. Our research had two aims, firstly, to understand the extent of micronutrient limitation and/or colimitation of cyanobacterial growth in some South Eastern Australian freshwater systems. Secondly, to understand how various phytoplankton communities change in response to micronutrient amendments and to observe which conditions favour cyanobacteria. We hypothesise that (1) micronutrients will be a limiting factor of cyanobacterial growth in some freshwater systems and (2) changes in phytoplankton community structure will occur with increased micronutrient concentrations. We chose to use a mixture of iron, cobalt, copper, manganese, molybdenum and zinc as these are required by some or all phytoplankton at a biochemical level (Facey et al. 2019).

Materials and methods

Study sites

Seven sites were selected across New South Wales and Victoria, Australia. Study sites were chosen because they were known to have varying levels of cyanobacteria present in warmer months. They comprised of lakes, rivers and creeks and are summarised in Table 1. Sampling occurred between the months of November to February when water temperatures and light intensities were not limiting.

Microcosm enrichment assays

In situ nutrient enrichment microcosms were conducted to determine which nutrients were limiting phytoplankton growth and to test for any nutrient-driven changes in community composition after a 7-day incubation period, similar to Mueller and Mitrovic (2014). Approximately 60 L of surface water was filtered through a 63 µm plankton net into a large plastic container. Water was filtered to exclude zooplankton grazers. 1.0 L clear PET bottles were filled from the container, leaving some air space at the top. Nutrient additions were conducted according to the six treatments outlined in Table 2. All treatments were conducted in triplicate.

Following the nutrient additions, bottles were mixed by rotation and tied together in random order. They were suspended at the same depth within the euphotic zone using floats (approximately 90 % surface irradiance). Concentrations of nitrate and phosphate were selected to alleviate any macronutrient limitation while remaining within levels typically found in natural Australian systems. They resembled those used by Mueller and Mitrovic (2014) (500 µg/L N, 200 µg/L P), as they effectively stimulated

Table 1 Summary of study sites, sampling dates and locations

Site	Date	Coordinates	Description
Hunter River at Morpeth	Nov-2017	− 32.724, 151.651	Upper estuary
Windeyers Creek	Nov-2017	− 32.779, 151.738	Small free flowing stream
Mannus Lake	Feb-2018	− 35.812, 147.976	Shallow artificial reservoir
Lake Lyell	Jan-2020	− 33.516, 150.077	Large dam
Burrendong Dam	Jan-2020	− 32.685, 149.146	Large dam
Murray River at Mildura	Jan-2020	− 34.176, 142.165	Weir pool
Murray River at Euston	Jan-2020	− 34.582, 142.745	Weir pool

Table 2 Summary of treatments and nominal concentrations of the target nutrient additions

Treatment	Salt	Concentration (µg/L)
Control	–	–
Nitrogen (N)	KNO_3	500
Phosphorus (P)	KH_2PO_4	300
Nitrogen + phosphorus (NP)	KNO_3	500
	KH_2PO_4	300
	$COl_2{\cdot}5H_2O$	2
Micronutrients (M)	$CuSO_4{\cdot}5H_2O$	2
	$FeCl_3{\cdot}6H_2O$ (+ $Na_2EDTA.2H_2O$)	200
	$MnCl_2{\cdot}4H_2O$	100
	$ZnSO_4{\cdot}7H_20$	3
Nitrogen + phosphorus + micronutrients (NPM)	KNO_3	500
	KH_2PO_4	300
	$CoCl_2{\cdot}6H_20$	2
	$CuSO_4{\cdot}5H_2O$	2
	$FeCl_3{\cdot}6H_2O$ (+ $Na_2EDTA.2H_2O$)	200
	$MnCl_2{\cdot}4H_2O$	100
	$Na_2MoO_4.2H_2O$	3
	$ZnSO_4{\cdot}7H_20$	3

growth and had no toxic effects. Micronutrient additions resembled the concentrations of the cyanobacterial growth medium, MLA (Bolch and Blackburn 1996) and were low enough to avoid any toxic effects. Samples for micronutrients, nitrate/phosphate, physiochemistry, chlorophyll *a* and phytoplankton enumeration were taken in triplicate from the filtered water at the onset of the experiment. Nitrate/phosphate and micronutrient samples were also taken from surrogate bottles with added nutrients and micronutrients to determine the total concentration of the addition plus the ambient concentration. Samples for chlorophyll *a* and phytoplankton enumeration were taken after 7 days from each sample bottle.

Nutrient sampling and analysis

In the field, 50 mL of water sample was filtered through a prerinsed 0.45 µm cellulose acetate syringe filter (Sartorius) and frozen immediately. Bioavailable nitrate and phosphate concentrations were determined photometrically using Flow Injection Analysis on a QuikChem 8500 Lachat nutrient analyser. For analysis, frozen samples were slowly thawed to room temperature. Soluble reactive phosphorus (srP) was measured by the reduction of ascorbic acid using the molybdate blue method (Murphy and Riley 1962). Nitrate and nitrite (NOx) was determined following reduction by a cadmium column using the sulphanilamide method (APHA 1998).

Trace metal micronutrient analysis

In the field, 25 mL of water sample was filtered through a 0.45 µm cellulose acetate syringe filter (Sartorius) prerinsed with 50 mL of 10 % nitric acid followed by 100 mL milli-Q water. Samples were collected in 50 mL falcon tubes and refrigerated. Falcon tubes had been soaked overnight in an acid bath (10 % nitric acid v/v) and rinsed repeatedly with Milli-Q water. Within 24 h of collection, samples were acidified with ultra-pure nitric acid to 0.2 % v/v. The concentrations of micronutrients in the filtered solution were analysed by inductively coupled atomic emission spectrometry (ICP-AES) (Varian 730 ES). The spectrometer was operated according to the standard operating procedures outlined by the manufacturer. The instruments were calibrated using matrix-matched standards. At least 10 % of samples were conducted in duplicate to ensure the precision of the analyses. To check for potential matrix interferences at least 10 % of samples had spike recoveries performed.

Phytoplankton identification and enumeration

200 mL grab samples were taken at the beginning of the experiment (day 0) from the large container and from individual microcosms on day 7 after homogenization by mixing and preserved with Lugol's Iodine solution (~ 0.25 % v/v). Samples were identified and enumerated at 200 times magnification using a light microscope (Olympus BX41) and Sedgwick-Rafter counting chamber. If required, samples

were concentrated 5× prior to counting by settling in 50 mL measuring cylinders for 24 h. The upper 40 mL was removed after checking all phytoplankton had settled and were no longer present in the upper layer. Phytoplankton taxa were identified to a genus level using identification literature by Prescott (1978), except for potentially toxic cyanobacteria which were identified to species. Counting precision was performed to ± 10 % with at least 100 units of the dominant taxa counted following Hötzel and Croome (1999). Biovolumes were calculated using the most appropriate conversion factors from Newcombe (2012) and Olenina et al. (2006).

Chlorophyll a analysis

200 mL of sample water was filtered on site via vacuum filtration onto GFC glass fibre filters (Whatman) and frozen for preservation. Chlorophyll *a* was analysed according to (Mueller and Mitrovic 2014). The glass fibre filters were extracted in 10 mL 90 % ethanol heated in a 75 °C water bath for 10 min. Unwanted filtered material was removed by centrifuging at 3000 rpm for 10 min. The supernatant was analysed immediately using a Varian Cary 50 Bio UV Spectrophotometer at wavelengths 665 and 750 nm.

Statistical analysis

Phytoplankton biovolume, cyanobacterial biovolume and chlorophyll *a* were analysed using permutational analysis of variance (PERMANOVA) with PRIMER + PERMANOVA software ver. 6 (Anderson 2001) with a significance level of $\alpha = 0.05$. Community analyses (nMDS, PERMANOVA, SIMPER and Inverse Simpson Diversity Index) were performed using the *vegan* package (Oksanen et al. 2019). A square root transformation was performed on the community data prior to analysis to reduce the influence of extreme values and plots were created with the *ggplot2* package (Wickham 2016) using the software R Version 1.2.1335 (Team 2018). Inverse Simpson Diversity was measured in terms of biovolume (Behl et al. 2011) and used algal data identified to the genus level as this is a useful resolution for assessing changes in community structure (Nielsen et al. 1998).

Results

The effect of nutrient additions on phytoplankton communities was highly variable based on locations. Limitation by either macronutrients or micronutrients are indicated by increases in the biovolume of some or all groups within the phytoplankton community (Table 4). At two locations that had cyanobacterial dominance and high cell concentrations (Burrendong Dam and Mannus Lake), the micronutrient mixture stimulated cyanobacterial growth, suggesting that one or multiple micronutrients were limiting cyanobacterial growth. This was not observed at the other bloom sites on the Murray River at Mildura and Euston (Fig. S2), both of which had very low nitrogen and phosphorus concentrations at the beginning of the experiments (Table 3). Nitrogen, phosphorus or a combination of the two (co-limitation) regularly limited phytoplankton growth, as observed at Morpeth, Windeyers Creek, Lake Lyall, Burrendong Dam, Mildura and Euston (Table 4).

Cyanobacterial biovolume was strongly influenced by the addition of micronutrients at Burrendong Dam and Mannus Lake (Fig. 1). At Mannus Lake there was no significant difference between cyanobacterial biovolume in the control (C), nitrogen (N treatment), phosphorus (P treatment) or nitrogen + phosphorus (NP treatment) treatments (PERMANOVA: p value > 0.05). However, in the micronutrient treatments (M and NPM) cyanobacterial biovolume was significantly greater than the control and all other treatments (PERMANOVA: NPM vs. control p value = 0.028, M vs. control p value = 0.011). Similarly, at Burrendong Dam the addition of micronutrients alone increased cyanobacterial biovolume relative to the control (p value = 0.001). Nitrogen alone also had a stimulatory effect on cyanobacteria relative to the control (p value = 0.001). There was no significant difference between chlorophyll *a* results across the different

Table 3 Ambient concentrations of macronutrients and micronutrients

Parameter	Windeyers Ck	Mannus Lake	Burrendong Dam	Mildura	Euston	Morpeth	Lake Lyell
NOx	401.950 ± 27.330	90.905 ± 0.138	61.200 ± 2.620	8.468 ± 0.160	2.915 ± 0.568	139.600 ± 5.141	62.067 ± 6.257
FRP	25.900 ± 3.148	7.884 ± 0.105	1.770 ± 0.067	1.878 ± 0.090	1.786 ± 0.217	13.867 ± 1.118	8.867 ± 0.268
Cobalt	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Copper	< 2	3.879 ± 0.101	< 2	< 2	< 2	< 2	3.977 ± 0.054
Iron	295.340 ± 2.752	496.667 ± 7.20	14.600 ± 3.285	16.889 ± 0.787	32.486 ± 10.436	11.076 ± 0.952	29.281 ± 2.225
Manganese	83.643 ± 0.090	206.667 ± 2.722	74.450 ± 0.579	0.783 ± 0.091	2.893 ± 1.062	19.705 ± 1.056	48.656 ± 0.715
Molybdenum	1.196 ± 0.034	< 1	< 1	< 1	< 1	1.733 ± 0.237	11.632 ± 0.064
Zinc	16.417 ± 1.982	5.453 ± 0.442	14.289 ± 6.813	1.414 ± 0.250	17.791 ± 1.935	34.643 ± 0.479	4.634 ± 0.042

All values are in µg/L, n = 3

Table 4 Summary of results from seven nutrient amendment bioassays across South-Eastern Australia

Site	Date	Bloom present at onset?	Dominant group	Limiting nutrient/s	Notable changes in community
Morpeth	Nov-2017	No	Green algae, Diatoms	N + P	Minimal changes
Windeyers Creek	Nov-2017	No	Diatoms	P	P: dinophyceae+, diatoms−
Mannus Lake	Feb-2018	Yes	Cyanobacteria (*Chrysosporum ovalisporum*)	M	M: cyanobacteria+, P: green algae+
Lake Lyall	Jan-2020	No	Euglenoids	N + P	N + P: green algae+
Burrendong Dam	Jan-2020	Yes	Cyanobacteria (*Microcystis aeruginosa*)	N, M	M: cyanobacteria+, P: green algae+
Mildura	Jan-2020	Yes	Cyanobacteria (*Aphanocapsa* sp., *Dolichospermum crassum*)	N, P	P: cyanobacteria+, N: cyanobacteria−
Euston	Jan-2020	Yes	Cyanobacteria (*Aphanocapsa* sp., *Dolichospermum crassum*)	P	P: cyanobacteria+

Limiting nutrients are any nutrient treatments that had a greater chlorophyll or total biovolume than the control

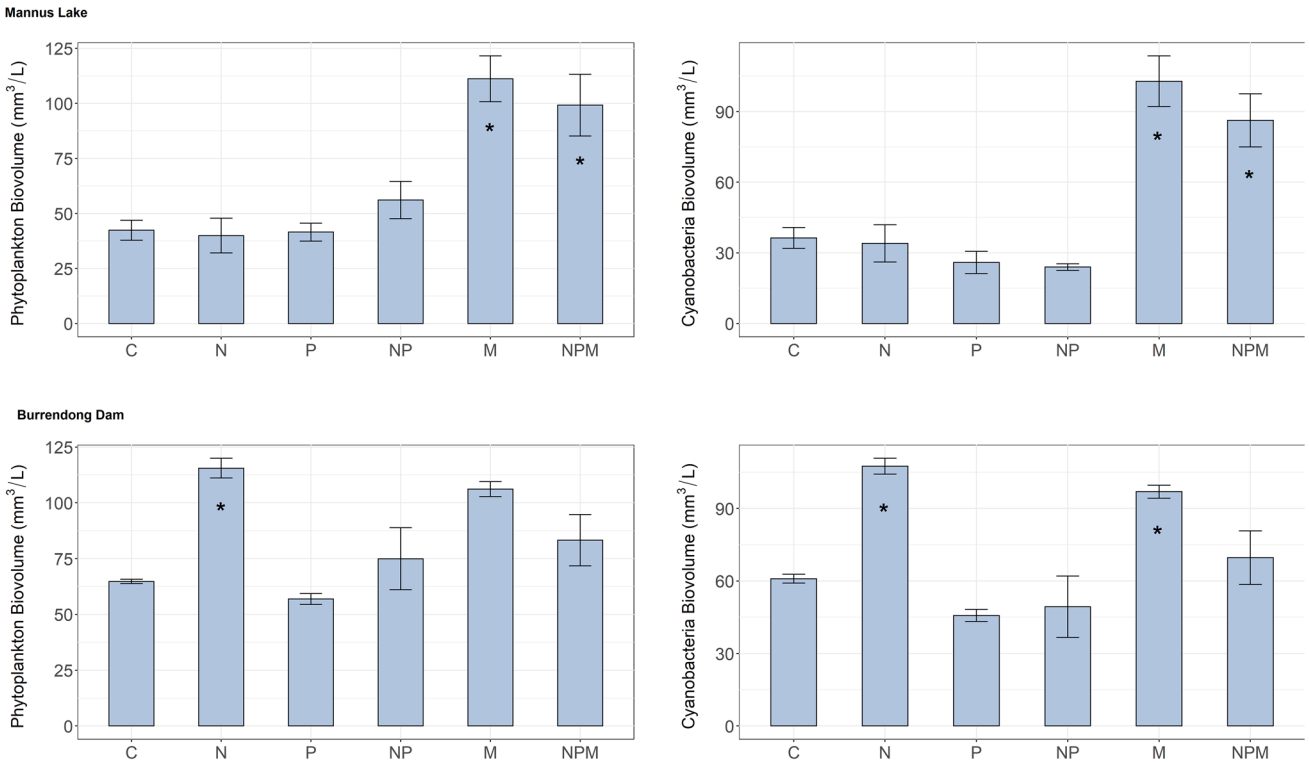

Fig. 1 Total phytoplankton and cyanobacterial biovolume in Mannus Lake and Burrendong Dam microcosms. Asterisk represents significant difference compared to the control (PERMANOVA, p value < 0.05). The nutrient concentrations added for each treatment are listed in Table 2. Error bars are standard error of the mean, n = 3

treatments at either Burrendong Dam (PERMANOVA: p value = 0.188) or Mannus Lake (p value = 0.448) (Fig. S1).

At Mannus Lake the phytoplankton community composition was also significantly affected by macronutrient additions (Fig. 2) (PERMANOVA: p value = < 0.001). In the P treatment, growth of green algae was stimulated. However, phosphorus additions did not cause a significant change in cyanobacterial biovolume relative to the control (PERMANOVA: p value = 0.325). Conversely, the addition of the micronutrient mixture (M), even in the presence of phosphorus (NPM), increased the growth of cyanobacteria, which was already dominant. There was a clear distinction between phytoplankton communities at Mannus Lake in treatments with the micronutrient mixture and those without it (Fig. 2). SIMPER analysis demonstrated that the largest contributor to the differences between all treatments was *Chryosporum ovalisporum*. The increase in *C. ovalisporum* in the M treatment contributed up to ~ 95 % of dissimilarity compared to

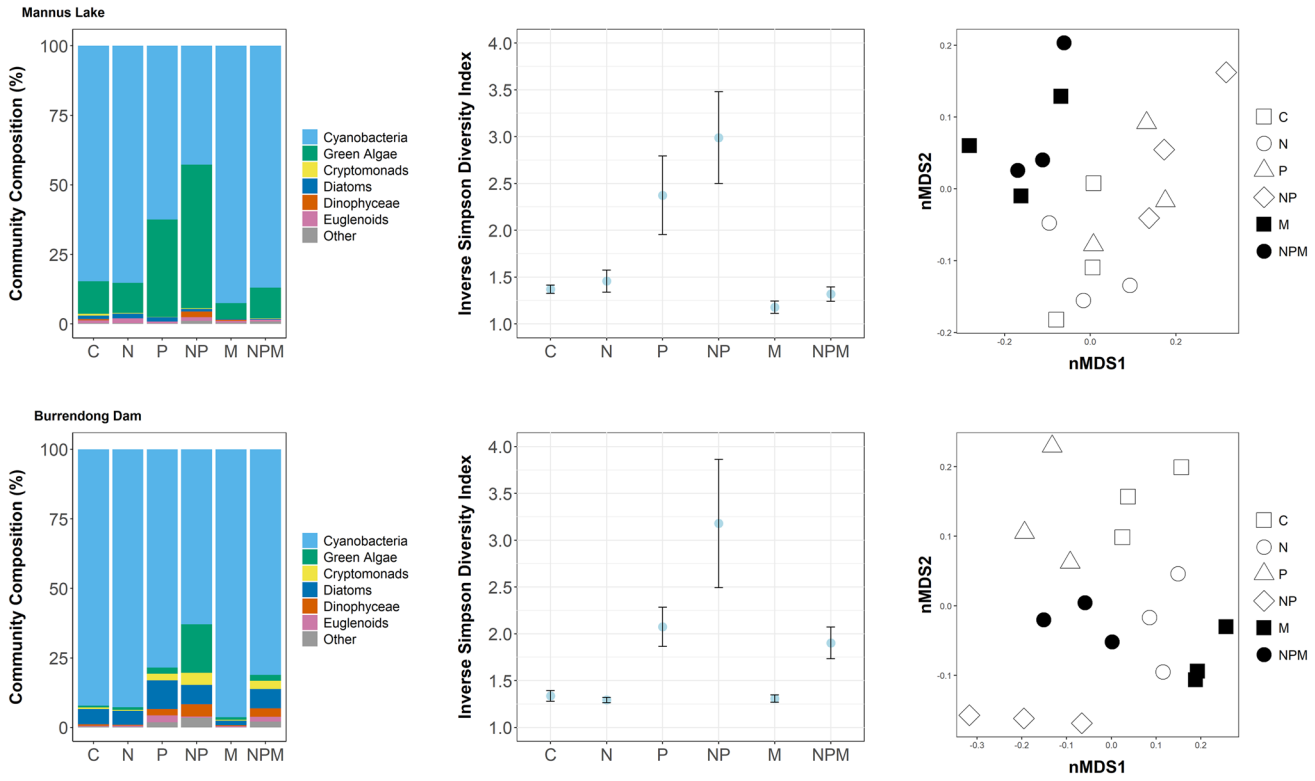

Fig. 2 Proportion of community made up of several key phytoplankton groups at Mannus Lake and Burrendong Dam (left). Shannon Diversity Index (middle) and nMDS plots (right) illustrating differences in phytoplankton community structure between treatments. A square root transformation was performed on the community data for nMDS. Stress < 0.2. Error bars are standard error of the mean, n = 3

the control, while the reduction in *C. ovalisporum* in the NP treatment contributed 71 % of the dissimilarity compared to the control. *Mougeotia* and *Dictyosphaerium* were the key genera of green algae that responded to phosphorus addition in the P and NP treatments. Similarly, at Burrendong Dam the M treatment had a higher proportion of cyanobacteria compared to the control while the phosphorus addition favored a reduction in the proportion of cyanobacteria and a higher diversity community (Fig. 2). SIMPER analysis demonstrated that *Microcystis* and *Radiocystis* were the largest contributors to differences between all treatments, while *Scenedesmus*, *Cryptomonas* and *Chlamydomonas* were the largest non-cyanobacterial responders to the NP addition compared to the control.

Discussion

In situ nutrient bioassays were conducted at seven locations throughout South Eastern Australia to assess the extent of trace metal micronutrient limitation of cyanobacterial growth and to identify how increased micronutrient availability influences phytoplankton community structure. Of the seven freshwater systems examined, two exhibited signs of micronutrient limitation of cyanobacterial growth: Mannus Lake and Burrendong Dam. At Mannus Lake, a dense cyanobacterial bloom had established which was dominated by the heterocystous cyanobacteria *Chrysosporum ovalisporum*, a producer of the toxin cylindrospermopsin (Shaw et al. 1999; Quesada et al. 2006; Yilmaz et al. 2008; Fadel et al. 2014). Cyanobacterial biovolume significantly increased in treatments containing the micronutrient mixture (NPM and M treatments) (Fig. 1) primarily driven by increased growth of the bloom forming *C. ovalisporum*. The addition of nitrogen and phosphorus alongside micronutrients (NPM treatment) did not increase the effect size as there were no significant differences to the phytoplankton response to micronutrients alone. Interestingly, the Murray River at Euston and Mildura experiments were also undergoing a bloom of a filamentous, nitrogen-fixing cyanobacteria, *Dolichospermum crassum*, but the response from the micronutrient addition was not observed at either location on the Murray River. Instead, phosphorus was the limiting factor for cyanobacterial growth at Mildura, and to a lesser extent at Euston (Fig. S2). A similar study conducted by Sterner et al. (2004) found phosphorus limitation at Lake Superior and did not observe any limitation of algal growth by micronutrient

trace metals (manganese, iron or zinc). However, Sterner proposed that the system was on the cusp of micronutrient limitation but suggests this may have been clouded by the simultaneous limitation of phosphorus (North et al. 2007).

Chrysosporum ovalisporum is often dominant in low nitrogen concentrations where heterocystous cyanobacteria have an advantage over other phytoplankton (Fadel et al. 2014). Nitrogen fixation requires high levels of iron (Sterner et al. 2004; Molot et al. 2014), molybdenum (ter Steeg et al. 1986; Paerl et al. 2006) and cobalt (Rodriguez and Ho 2015), as the N_2 fixing enzyme nitrogenase contains metal cofactors. This causes heterocystous cyanobacteria to require some trace metals in higher amounts than other phytoplankton (Schoffman et al. 2016) and may make them more prone to micronutrient limitation (Kustka et al. 2002; Molot et al. 2010; Romero et al. 2013) observed significant increases in nitrogen fixation upon addition of both iron and molybdenum and suggested co-limitation involving trace metals is common in lakes. A similar phenomenon may have caused the increase in *C. ovalisporum* growth in Mannus Lake upon the addition of the trace metal micronutrient mixture. *C. ovalisporum* had already established a dense bloom so it is possible that nutrient constraints were beginning to come into effect. Given that iron was relatively available at the onset of the Mannus Lake experiment (Table 4), molybdenum and cobalt are more likely to be the limiting micronutrients. Both were below detection limit.

At Burrendong Dam, which was dominated by the microcystin-producing genera *Microcystis aeuginosa* and *Radiocystis* sp. (Vieira et al. 2003; Rastogi et al. 2015), the micronutrient treatment (M) had a slightly higher proportion of cyanobacteria than the control, and the NPM treatment had a higher proportion of cyanobacteria than the NP treatment. This indicates that cyanobacteria may be more successful competitors in the phytoplankton community with higher micronutrient concentrations. The addition of micronutrients alone (M) and nitrogen (N) stimulated cyanobacterial growth relative to the control. Although the NPM treatment was higher, it was not statistically different (p value > 0.05) to the control (Fig. 1). *M. aeruginosa* and *Radiocystis* remained dominant under all treatments. The large stimulatory effect of nitrogen on cyanobacteria at Burrendong Dam was not observed at Mannus Lake where the heterocystous *Chrysosporum ovalisporum* dominated. It has been suggested that reduced nitrogen input will cause an increase in the proportion of N_2 fixing cyanobacteria (Schindler et al. 2008). The relatively low availability of NOx at the onset of the Mannus Lake experiment was likely a contributing factor to the dominance of *C. ovalisporum* and given its ability to fix atmospheric nitrogen, nitrate is unlikely to limit *C. ovalisporum* growth. Conversely, *Microcystis* and *Radiocystis* depend on dissolved nitrogen for growth, which had become limiting by the onset of the Burrendong Dam experiment.

As *Microcystis* and *Radiocystis*, the dominant cyanobacterial genera at Burrendong Dam, are non-nitrogen fixing, the limitation of growth by micronutrients in this system was unlikely to be related to nitrogen fixation. Iron is also required for the reduction of nitrate to ammonia prior to assimilation (via nitrate and nitrite reductase) (Schoffman et al. 2016). Sub-optimal iron availability appears to be able to limit nitrate uptake in natural waters (DiTullio et al. 1993). At low iron concentrations, and without the presence of highly bioavailable ammonia, the phytoplankton community can be co-limited by iron and nitrogen (Saito et al. 2008; Schoffman et al. 2016). For example, North et al. (2007) suggested that iron enrichment reduced nitrogen limitation by allowing NO_3 assimilation in nutrient enrichment bioassays. However, this is not supported by our results as the addition of nitrate alone in the N treatment stimulated cyanobacterial growth at Burrendong Dam, suggesting there was sufficient Fe in the ambient water to allow for nitrate reduction and assimilation. The simultaneous limitation of the community by nitrate and micronutrient trace metals at Burrendong Dam, combined with the lack of response in the NP and NPM treatments, is difficult to elucidate.

These results demonstrate that micronutrient trace metals can stimulate cyanobacterial growth *in-situ* and may act as an important regulator of the severity of cyanobacterial blooms. This study joins a growing list that have observed an important role of micronutrients in structuring phytoplankton communities and increasing cyanobacterial growth in physically and chemically diverse freshwater systems. For example, Downs et al. (2008) noted a stimulation of the cyanobacterium *Anabaena flos-aquae* upon addition of cobalt, copper, manganese and a trace metal mixture, while a number of studies have observed iron limitation of cyanobacteria growth (Wever et al. 2008; Molot et al. 2010; Harland et al. 2013; Fujii et al. 2016).

Phosphorus-driven changes in community structure

In both experiments the addition of phosphorus promoted higher diversity in the phytoplankton community composition. Green algae, diatoms and dinoflagellates made up a larger proportion of the community in P and NP treatments (Fig. 2). This trend is surprising as the addition of P decreases the N:P ratio, which is generally expected to favour cyanobacterial growth (Tew et al. 2014; Li et al. 2018). However, the opposite effect was observed at both Burrendong Dam and Mannus Lake. The change in community composition may indicate that the systems were also phosphorus limited at the time and green algae and diatoms were able to respond faster to the sudden phosphorus pulse due to their faster growth rate compared to cyanobacteria (Lürling et al. 2013; Deng et al. 2014). Alternatively, each species is likely to have different nutrient requirements and

therefore some species can be nutrient limited whereas others are not (Baptista and Vasconcelos 2006; Mueller and Mitrovic 2014). This may explain why phosphorus limitation was not evident when assessing total phytoplankton biomass. This trend is particularly evident in Burrendong Dam where phosphorus concentrations were very low. Interestingly, when the phosphorus addition (P or NP) was coupled with the micronutrient mixture (NPM) the communities were composed of a notably higher proportion of cyanobacteria, particularly at Mannus Lake. This suggests that micronutrients impart a competitive advantage to cyanobacteria over other components of the phytoplankton community even under high phosphorus conditions. This may be because of specific micronutrient requirements of cyanobacteria or a result of a more efficient metal uptake system (Baptista and Vasconcelos 2006; Sunda 2012), for example via the production of metallophores (Kraemer et al. 2015).

Implications for management and research

Micronutrient trace metals appear to be an important regulator of the severity of cyanobacterial blooms in some freshwater systems. Improving our understanding of how specific micronutrients influence phytoplankton community structure and cyanobacterial growth could be an important aspect of catchment management plans and may be critical to securing freshwater resources into the future. In both micronutrient limited sites, high-density cyanobacterial blooms had established by the onset of the experiment. Limiting micronutrient inputs may help to reduce the severity of such blooms. All the micronutrients used in this study are common additions to many fertilizers (Molina et al. 2009). Over application of fertilizers and subsequent runoff may be a significant source of trace metals in freshwater systems as well as N and P. This risk could be minimized through more targeted application of fertilizers or by increasing vegetation in the riparian zone to act as a buffer for micronutrient inflows, which are already effective measures for reducing macronutrient inflows (Aguiar et al. 2015).

Many trace metals (such as Co, Cu, Fe, Mn and Zn) can be released from sediments under anoxic conditions caused by thermal stratification (Shipley et al. 2011). These micronutrients can become available to cyanobacteria who may vertically migrate to nutrient-rich hypolimnial waters (Bormans et al. 1999; Wagner and Adrian 2009; Molot et al. 2014), particularly in shallow reservoirs such as Mannus Lake. Further, when the water column mixes after periods of thermal stratification upwelling occurs, increasing the availability of micronutrients in surface waters. Breaking down or supressing the formation of thermal stratification via maintaining high flow velocities in rivers or by installing mixers (such as fans or bubble plumes) are commonly used to manage blooms in systems where cyanobacterial buoyancy mechanisms are a primary driver of their dominance (Mitrovic et al. 2011; Visser et al. 2016; Bormans et al. 2016). These mixers may also be effective in reducing sediment-derived micronutrients in systems prone to cyanobacterial blooms by preventing anoxic conditions at the water-sediment interface.

Conclusions

This study has provided insight into the extent of micronutrient limitation of cyanobacterial growth in Australian freshwater systems and how the phytoplankton community changes in response to micronutrient additions. We hypothesised that micronutrients will be a limiting factor of cyanobacterial growth in some freshwater systems. Two sites out of seven exhibited signs of micronutrient limitation. Both of these sites had high cyanobacterial biovolume at the onset of the bioassays, suggesting that micronutrients may become limiting during high competition for nutrient assimilation during bloom events. This suggests that micronutrient trace metals can regulate the severity of cyanobacterial blooms in some freshwater systems. Micronutrients also influenced phytoplankton community structure, supporting our second hypothesis. At both sites showing micronutrient limitation of cyanobacteria, the addition of the micronutrient mixture resulted in higher proportion of cyanobacteria compared to the control, suggesting that micronutrients can not only influence cyanobacterial biovolume but also their ability to compete with other phytoplankton. These results may have important implications for the management of micronutrients and cyanobacterial blooms in freshwater systems.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00027-021-00783-x.

Acknowledgements We wish to thank Josh King for his assistance with ICP-MS analysis and Nikolai Love for his help with phytoplankton identification and enumeration.

Author contributions JAF, SMM and SCA contributed to the study conception and design. Sample collection was performed by JAF and TAR. Analysis was performed by JAF with advice from SMM and SCA. The first draft of the manuscript was written by JAF and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding No funding was received to assist with the preparation of this manuscript.

Availability of data and material The datasets generated during the current study are available from the corresponding author on reasonable request.

Code availability The code used in this study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Aguiar TR, Rasera K, Parron LM et al (2015) Nutrient removal effectiveness by riparian buffer zones in rural temperate watersheds: the impact of no-till crops practices. Agric Water Manag 149:74–80. https://doi.org/10.1016/j.agwat.2014.10.031

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Aust Ecol. https://doi.org/10.1046/j.1442-9993.2001.01070.x

APHA WEF (1998) AWWA, 1995. Standard methods for the examination of water and wastewater. Amer Pub Heal Assoc, Washington DC

Arrigo KR (2005) Marine microorganisms and global nutrient cycles. Nature 437:349–355. https://doi.org/10.1038/nature04159

Baptista MS, Vasconcelos MT (2006) Cyanobacteria metal interactions: requirements, toxicity, and ecological implications. Crit Rev Microbiol 32:127–137. https://doi.org/10.1080/10408410600822934

Behl S, Donval A, Stiborb H (2011) The relative importance of species diversity and functional group diversity on carbon uptake in phytoplankton communities. Limnol Oceanogr. https://doi.org/10.4319/lo.2011.56.2.0683

Bolch CJS, Blackburn SI (1996) Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. J Appl Phycol. https://doi.org/10.1007/BF02186215

Bormans M, Maier H, Burch M, Baker P (1997) Temperature stratification in the lower River Murray, Australia: implication for cyanobacterial bloom development. Mar Freshw Res 48:647–654. https://doi.org/10.1071/mf97058

Bormans M, Sherman BS, Webster IT (1999) Is buoyancy regulation in cyanobacteria an adaptation to exploit separation of light and nutrients? Mar Freshw Res 50:897–906

Bormans M, Ford PW, Fabbro L, Hancock G (2004) Onset and persistence of cyanobacterial blooms in a large impounded tropical river, Australia. Mar Freshw Res 55:1–15

Bormans M, Maršálek B, Jančula D (2016) Controlling internal phosphorus loading in lakes by physical methods to reduce cyanobacterial blooms: a review. Aquat Ecol. https://doi.org/10.1007/s10452-015-9564-x

Bowling L (1994) Occurrence and possible causes of a severe cyanobacterial bloom in Lake Cargelligo, New South Wales. Mar Freshw Res 45:737–745. https://doi.org/10.1071/MF9940737

Deng J, Qin B, Paerl HW et al (2014) Effects of nutrients, temperature and their interactions on spring phytoplankton community succession in Lake Taihu, China. PLoS One 9:1–19. https://doi.org/10.1371/journal.pone.0113960

Dignum M, Matthijs HCP, Pel R et al (2005) Nutrient limitation of freshwater cyanobacteria. In: Huisman J, Matthijs HCP, Visser PM et al (eds) Harmful cyanobacteria. Springer Netherlands, Dordrecht, pp 65–86

DiTullio GR, Hutchins DA, Bruland KW (1993) Interaction of iron and major nutrients controls phytoplankton growth and species composition in the tropical North Pacific Ocean. Limnol Oceanogr. https://doi.org/10.4319/lo.1993.38.3.0495

Downs TM, Schallenberg M, Burns CW (2008) Responses of lake phytoplankton to micronutrient enrichment: a study in two New Zealand lakes and an analysis of published data. Aquat Sci 70:347–360. https://doi.org/10.1007/s00027-008-8065-6

Facey JA, Apte SC, Mitrovic SM (2019) A review of the effect of trace metals on freshwater cyanobacterial growth and toxin production. Toxins (Basel) 11:1–18. https://doi.org/10.3390/toxins11110643

Fadel A, Atoui A, Lemaire BJ et al (2014) Dynamics of the toxin cylindrospermopsin and the cyanobacterium *Chrysosporum (Aphanizomenon) ovalisporum* in a mediterranean eutrophic reservoir. Toxins (Basel) 6:3041–3057. https://doi.org/10.3390/toxins6113041

Falconer IR (2001) Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. Phycologia 40:228–233. https://doi.org/10.2216/i0031-8884-40-3-228.1

Fujii M, Dang TC, Bligh MW, Waite TD (2016) Cellular characteristics and growth behavior of iron-limited *Microcystis aeruginosa* in nutrient-depleted and nutrient-replete chemostat systems. Limnol Oceanogr 61:2151–2164. https://doi.org/10.1002/lno.10360

Glass JB, Axler RP, Chandra S, Goldman CR (2012) Molybdenum limitation of microbial nitrogen assimilation in aquatic ecosystems and pure cultures. Front Microbiol 3:1–11. https://doi.org/10.3389/fmicb.2012.00331

Harland FMJ, Wood SA, Moltchanova E et al (2013) Phormidium autumnale growth and anatoxin-a production under iron and copper stress. Toxins (Basel) 5:2504–2521. https://doi.org/10.3390/toxins5122504

Hötzel G, Croome R (1999) A phytoplankton methods manual for Australian rivers. LWRRDC Occasional Paper 22/99. Land and Water Resources Research and Development Corporation, Canberra

Hunt RJ, Matveev VF (2005) The effects of nutrients and zooplankton community structure on phytoplankton growth in a subtropical Australian reservoir: an enclosure study. Limnologica 35:90–101. https://doi.org/10.1016/j.limno.2005.01.004

Kraemer SM, Duckworth OW, Harrington JM, Schenkeveld WDC (2015) Metallophores and trace metal biogeochemistry. Aquat Geochem 21:159–195. https://doi.org/10.1007/s10498-014-9246-7

Kustka A, Carpenter EJ, Sañudo-Wilhelmy SA (2002) Iron and marine nitrogen fixation: progress and future directions. Res Microbiol 153:255–262. https://doi.org/10.1016/S0923-2508(02)01325-6

Li J, Hansson L-A, Persson MK (2018) Nutrient control to prevent the occurrence of cyanobacterial blooms in a Eutrophic Lake in Southern Sweden, used for drinking water supply. Water 10:919

Lukac M, Aegerter R (1993) Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. Toxicon 31:293–305. https://doi.org/10.1016/0041-0101(93)90147-B

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

Mitrovic SM, Oliver RL, Rees C et al (2003) Critical flow velocities for the growth and dominance of *Anabaena circinalis* in some turbid freshwater rivers. Freshw Biol 48:164–174. https://doi.org/10.1046/j.1365-2427.2003.00957.x

Mitrovic SM, Hardwick L, Dorani F (2011) Use of flow management to mitigate cyanobacterial blooms in the Lower Darling River, Australia. J Plankton Res 33:229–241. https://doi.org/10.1093/plankt/fbq094

Molina M, Aburto F, Calderón R et al (2009) Trace element composition of selected fertilizers used in Chile: phosphorus fertilizers as a source of long-term soil contamination. Soil Sediment Contam 18:497–511. https://doi.org/10.1080/15320380902962320

Molot LA, Li G, Findlay DL, Watson SB (2010) Iron-mediated suppression of bloom-forming cyanobacteria by oxine in a eutrophic lake. Freshw Biol 55:1102–1117. https://doi.org/10.1111/j.1365-2427.2009.02384.x

Molot LA, Watson SB, Creed IF et al (2014) A novel model for cyanobacteria bloom formation: the critical role of anoxia and ferrous iron. Freshw Biol 59:1323–1340. https://doi.org/10.1111/fwb.12334

Mueller S, Mitrovic SM (2014) Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: progressing from the phosphorus limitation paradigm. Hydrobiologia 744:255–269. https://doi.org/10.1007/s10750-014-2082-3

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. https://doi.org/10.1016/S0003-2670(00)88444-5

Newcombe G (2012) International guidance manual for the management of toxic cyanobacteria. International Water Association, London

Nielsen DL, Shiel RJ, Smith FJ (1998) Ecology versus taxonomy: is there a middle ground? Hydrobiologia 387–388:451–457. https://doi.org/10.1007/978-94-011-4782-8_58

North RL, Guildford SJ, Smith REH et al (2007) Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. Limnol Oceanogr 52:315–328

O'Neil JM, Davis TW, Burford MA, Gobler CJ (2012) The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14:313–334. https://doi.org/10.1016/j.hal.2011.10.027

Oksanen J, Blanchet FG, Friendly M et al (2019) vegan: Community ecology package. R package version 2.5–6. https://CRAN.R-project.org/package=vegan

Olenina I, Hajdu S, Edler L et al (2006) Biovolumes and size-classes of phytoplankton in the Baltic Sea. In: HELCOM Balt. Sea Environ. Proc., vol 106

Paerl HW, Otten TG (2013) Harmful cyanobacterial blooms: causes, consequences, and controls. Microb Ecol 65:995–1010. https://doi.org/10.1007/s00248-012-0159-y

Paerl HW, Fulton RS, Graneli E, Turner J (2006) Ecology of harmful marine algae. Springer, Berlin

Pearson L, Mihali T, Moffitt M et al (2010) On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Mar Drugs 8:1650–1680. https://doi.org/10.3390/md8051650

Polyak Y, Zaytseva T, Medvedeva N (2013) Response of toxic cyanobacterium *Microcystis aeruginosa* to environmental pollution. Water Air Soil Pollut. https://doi.org/10.1007/s11270-013-1494-4

Prescott GW (1978) How to know the freshwater algae. W. C. Brown, Pennsylvania State University, State College

Quesada A, Moreno E, Carrasco D et al (2006) Toxicity of *Aphanizomenon ovalisporum* (Cyanobacteria) in a Spanish water reservoir. Eur J Phycol. https://doi.org/10.1080/09670260500480926

Rastogi RP, Madamwar D, Incharoensakdi A (2015) Bloom dynamics of cyanobacteria and their toxins: environmental health impacts and mitigation strategies. Front Microbiol 6:1–22. https://doi.org/10.3389/fmicb.2015.01254

Rodriguez IB, Ho TY (2015) Influence of Co and B12 on the growth and nitrogen fixation of Trichodesmium. Front Microbiol 6:1–9. https://doi.org/10.3389/fmicb.2015.00623

Romero IC, Klein NJ, Sañudo-Wilhelmy SA, Capone DG (2013) Potential trace metal co-limitation controls on N2 fixation and NO-3 uptake in lakes with varying trophic status. Front Microbiol 4:1–12. https://doi.org/10.3389/fmicb.2013.00054

Saito M, Goepfert TJ, Ritt JT (2008) Some thoughts on the concept of colimitation: three definitions and the importance of bioavailability. Limnol Oceanogr 53:276–290. https://doi.org/10.4319/lo.2008.53.1.0276

Schindler DW, Hecky RE, Findlay DL et al (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc Natl Acad Sci 105:11254–11258. https://doi.org/10.1073/pnas.0805108105

Schindler DW, Carpenter SR, Chapra SC et al (2016) Reducing phosphorus to curb lake eutrophication is a success. Environ Sci Technol 50:8923–8929. https://doi.org/10.1021/acs.est.6b02204

Schoffman H, Lis H, Shaked Y, Keren N (2016) Iron–nutrient interactions within phytoplankton. Front Plant Sci 7:1223. https://doi.org/10.3389/fpls.2016.01223

Shao NF, Yang ST, Sun Y et al (2019) Assessing aquatic ecosystem health through the analysis of plankton biodiversity. Mar Freshw Res 70:647–655

Shaw GR, Sukenik A, Livne A et al (1999) Blooms of the cylindrospermopsin containing cyanobacterium, *Aphanizomenon ovalisporum* (Fofti), in newly constructed lakes, Queensland, Australia. Environ Toxicol 14:167–177. https://doi.org/10.1002/(SICI)1522-7278(199902)14:1%3c167::AID-TOX22%3e3.0.CO;2-O

Shipley HJ, Gao Y, Kan AT, Tomson MB (2011) Mobilization of Trace metals and inorganic compounds during resuspension of anoxic sediments from Trepangier Bayou, Louisiana. J Environ Qual 40:484–491. https://doi.org/10.2134/jeq2009.0124

Sorichetti RJ, Creed IF, Trick CG (2014) Evidence for iron-regulated cyanobacterial predominance in oligotrophic lakes. Freshw Biol 59:679–691. https://doi.org/10.1111/fwb.12295

Sourisseau M, Le Guennec V, Le Gland G et al (2017) Resource competition affects plankton community structure; evidence from trait-based modeling. Front Mar Sci 4:52

Sterner RW, Smutka TM, Mckay RML et al (2004) Phosphorus and trace metal limitation of algae and bacteria in Lake Superior. Limnol Oceanogr 49:495–507

Sunda WG (2012) Feedback interactions between trace metal nutrients and phytoplankton in the ocean. Front Microbiol 3:1–22. https://doi.org/10.3389/fmicb.2012.00204

Team R (2018) RStudio: integrated development environment for R (Version 1.2. 1335). RStudio Inc, Boston, MA

ter Steeg PF, Hanson PJ, Paerl HW (1986) Growth-limiting quantities and accumulation of molybdenum in *Anabaena oscillarioides* (Cyanobacteria). Hydrobiologia 140:143–147. https://doi.org/10.1007/BF00007567

Tew KS, Meng P-J, Glover DC et al (2014) Characterising and predicting algal blooms in a subtropical coastal lagoon. Mar Freshw Res 65:191–197

Varol M, Sen B (2018) Abiotic factors controlling the seasonal and spatial patterns of phytoplankton community in the Tigris River, Turkey. River Res Appl 34:13–23. https://doi.org/10.1002/rra.3223

Vieira JMDS, Azevedo MTDP, De Oliveira Azevedo SMF et al (2003) Microcystin production by *Radiocystis fernandoi* (Chroococcales, Cyanobacteria) isolated from a drinking water reservoir in the city of Belém, PA, Brazilian Amazonia region. Toxicon. https://doi.org/10.1016/j.toxicon.2003.08.004

Visser PM, Ibelings BW, Bormans M, Huisman J (2016) Artificial mixing to control cyanobacterial blooms: a review. Aquat Ecol. https://doi.org/10.1007/s10452-015-9537-0

Vyverman W, Muylaert K, Sabbe K, Verleyen E (2007) Ecology of non-marine algae: lakes and large rivers. In: Algae of Australia: Introduction. Australian Biological Resources Study; CSIRO Publishing, Canberra, ACT; Melbourne, VIC, Australia, pp 459–475

Wagner C, Adrian R (2009) Cyanobacteria dominance: quantifying the effects of climate change. Limnol Oceanogr. https://doi.org/10.4319/lo.2009.54.6_part_2.2460

Wever A, De, Muylaert K, Langlet D et al (2008) Differential response of phytoplankton to additions of nitrogen, phosphorus and iron in Lake Tanganyika. Freshw Biol 53:264–277. https://doi.org/10.1111/j.1365-2427.2007.01890.x

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York

Yilmaz M, Phlips EJ, Szabo NJ, Badylak S (2008) A comparative study of Florida strains of *Cylindrospermopsis* and *Aphanizomenon* for cylindrospermopsin production. Toxicon. https://doi.org/10.1016/j.toxicon.2007.08.013