Cylindrospermopsis raciborskii dominates under very low and high nitrogen-to-phosphorus ratios

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ABSTRACT

In freshwater ecosystems, a variety of factors mediate phytoplankton community structure, including herbivore community structure, light availability, temperature, mixing, and absolute and relative nutrient concentrations (total nitrogen (TN), total phosphorus (TP)). Ecological stoichiometry examines how the nutrient content of organisms and their environment may mediate population-, community-, and ecosystem-level processes. The manipulation of N:P ratios is a widely regarded tool for managing phytoplankton species composition given that nitrogen-fixing cyanobacteria should dominate algal communities under relatively low N:P (<64:1, by atoms) given their ability to convert dissolved dinitrogen gas into organic nitrogen. However, due to the physiological expense of nitrogen fixation, diazotrophs should be outcompeted by non-nitrogen fixing phytoplankton under higher N:P when other environmental factors are similar. We tested this hypothesis in a field experiment using 2500-L limnocorrals installed in a eutrophic lake (ambient N:P w 40:1 (by atoms); TN w 1360 μg L−1; TP w 75 μg L−1). At the start of the experiment, we randomly assigned limnocorrals among the ambient (40:1) and low (7:1) or high (122:1) N:P treatments (n = 4 replicates/treatment), which were established by adding P or N at the start of the experiment, respectively. The phytoplankton community in the enclosures at the start of the experiment was diverse (i.e., 18 phytoplankton genera) and dominated by chlorophytes (including Coelastrum and Scenedesmus (30% and 13% of total biomass, respectively)) and cyanobacteria (including Anabaena and Cylindrospermopsis (23% and 17% of total biomass, respectively)). In contrast to predictions based on ecological stoichiometry, the phytoplankton community in all N:P treatments increased in abundance and was almost entirely composed of the nitrogen-fixing cyanobacterium, Cylindrospermopsis raciborskii, by the conclusion of the study. Moreover, concentrations of the cyanobacterial neurotoxin, saxitoxin, were enhanced under the two highest N:P conditions. The ability of C. raciborskii to dominate phytoplankton communities under such extreme N:P shows that short-term management of nutrient stoichiometry through fertilization is not likely to be effective for controlling blooms of this noxious cyanobacterium and may help to explain the rapid expansion of this invasive species to temperate latitudes.

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1. Introduction

Ecological stoichiometry, the study of the effects of the elemental composition of organisms and their environment on food web interactions and ecosystem function, has played an important role in basic and applied research by relating the physiology of organisms to ecosystem-level processes, such as biogeochemical cycles, nutrient recycling, and limiting nutrients (Sterner et al., 1992; Sterner and Elser, 2002). In particular, the study of freshwater and marine phytoplankton has played a key role in the development of the field of ecological stoichiometry (Fearsall, 1932; Tilman, 1977; Klausmeier et al., 2008). One classical assumption in ecological stoichiometry studies of marine phytoplankton is the relative constancy of the carbon:nitrogen:phosphorus ratio (i.e., the Redfield ratio; carbon-to-nitrogen-to-phosphorus (C:N:P) = 106:16:1, by atoms), and the Redfield ratio is frequently used in understanding biogeochemical cycles of these elements and nutrient limitation in aquatic systems, in general (Redfield, 1958; Healey and Hendzel, 1980; Howarth, 1988). Observational studies of freshwater lakes have shown that relatively low nitrogen-to-phosphorus ratios (N:P < 64:1, by atoms) tend to favor dominance by nitrogen-fixing cyanobacteria (Schindler, 1977; Smith, 1983; Sterner et al., 1992; Downing et al., 2001; Ferber et al., 2004; but see Nöges et al., 2008; Kosten et al., 2009) through their ability to convert dissolved dinitrogen gas into organic nitrogen. These findings are further supported by field N:P manipulations, which tended to study the effects of reduced N:P (Schindler, 1974; Barica et al., 1980; Barica, 1994; Paterson et al., 2002; Vrede et al., 2009; Finlay et al., 2010; Donald et al., 2011). Although other environmental factors are known to promote cyanobacterial dominance, such as reduced light penetration and turbulence, elevated temperature or pH, or small-bodied zooplankton (Ferber et al., 2004; Paerl and Paul, 2012; Chisholm et al., 2013), controlling nutrient supply and stoichiometry have been central foci of lake management and restoration efforts (Smith and Schindler, 2009) given that eutrophication and concomitant cyanobacterial blooms are the leading cause of water quality impairment of many freshwater and coastal marine ecosystems around the world.

Human activities have accelerated the rate and extent of eutrophication through both point and non-point discharges of limiting nutrients, such as nitrogen and phosphorus (Carpenter et al., 1998; Paerl et al., 2011; Paerl and Paul, 2012), and numerous studies in the ecological literature demonstrate that eutrophication can be controlled by decreasing nutrient inputs (Edmondson, 1970; Schindler, 1974; Jeppesen et al., 2005). However, determining which nutrient is ultimately limiting algal biomass and primary productivity is often a point of contention for basic research and applied management decisions (Schindler et al., 2008; Conley et al., 2009; Paerl, 2009; Scott and McCarthy, 2010), especially considering that some systems are co-limited by both N and P (Elser et al., 2007). Numerous studies have documented the successful management and restoration of freshwater lakes affected by eutrophication by controlling phosphorus inputs, since the classic study by Schindler (1974). The emphasis on controlling eutrophication has been strongly influenced by the phosphorus (P)-limitation paradigm, as phosphorus can be readily removed from pollution sources and possibly because this nutrient was first identified as the mechanism driving eutrophication (Paerl and Scott, 2010). However, there has also been a recent (renewed) surge of interest and advocacy for nitrogen or dual nitrogen and phosphorus control in abatement efforts (Paerl and Scott, 2010; Scott and McCarthy, 2010). Advocates of phosphorus control of eutrophication argue that phosphorus reductions also reduce harmful cyanobacterial blooms caused by nitrogen-fixing species by altering the N:P ratio in favor of other non-nitrogen fixing species (Barica et al., 1980; Tilman et al., 1982; McQueen and Lean, 1987; Stockner and Shortreed, 1988; Jeppesen et al., 2005; Schindler et al., 2008). Alternatively, the P-limitation paradigm also predicts that blooms of N-fixing cyanobacteria are made worse if nitrogen levels are reduced without also decreasing phosphorus concentrations (Schindler, 1977; Smith, 1983). These predictions have received some support from lake survey data and whole-lake experiments (Barica et al., 1980; Findley and Kasián, 1987; Schindler, 1977; Schindler et al., 2008). Several studies have even advocated the addition of nitrogen fertilizers at the whole-lake scale to shift the N:P ratio in favor of more innocuous, non-nitrogen fixing species (Smith, 1983). However, these predictions have rarely been tested using replicated, manipulative field experiments (but see Barica et al., 1980; Vrede et al., 2009; Finlay et al., 2010; Donald et al., 2011). Here we explicitly test the hypothesis that nitrogen-fixing cyanobacteria should dominate under low (7:1, by atoms) and ambient (40:1) N:P but are outcompeted by non-nitrogen fixing species under high N:P (122:1), using a limnocorral experiment in a eutrophic pond.

2. Materials and methods

During a 49-day field experiment, we manipulated total N:total P ratio in 2500-L, clear polyethylene enclosures that were sealed at the bottom, open to the atmosphere, and suspended from a floating platform (EZ-Dock) anchored in Pond S1 at the Auburn University E.W. Shell Fisheries Research Station in Auburn, Alabama. S1 is shallow (maximum depth = 3 m), polymeric, and eutrophic (TN ~1360 µg L⁻¹, TP ~75 µg L⁻¹, SRP ~9 µg L⁻¹; N:P ~40:1, by atoms, Secchi depth ~0.5 m). Sediments were not included because we were interested in epilimnetic algal community structure and expected little algal recruitment from the benthos during the summer. Twelve enclosures were filled on 11 June 2010 (day 0) by pumping water through a 60-µm mesh net to exclude fish and large zooplankton. We then randomly assigned limnocorals to each of the three N:P treatments: (1) ambient (40:1, by atoms), (2) low (7:1), and (3) high (122:1) with four replicates per treatment. The high N:P treatment was almost double the N:P threshold observed by Smith (1983). Ambient enclosures received no initial nutrient additions, low N:P enclosures were fertilized with sodium phosphate to establish an N:P ratio of 7:1, and sodium nitrate was added to high N:P enclosures to create an N:P ratio of 122:1. Given that this field experiment aimed to test the importance of three N:P ratios on phytoplankton community structure, we attempted to maintain treatment N:P using weekly fertilizations at a rate of 10%...
of the initial mean TN and TP concentrations for their corresponding N:P treatment (Fig. 1). Thus, the low and ambient N:P treatments received the same weekly N additions, and the ambient and high N:P treatments received the same weekly P additions. Soluble reactive phosphorus (SRP) was readily available in the low N:P treatment over the course of the experiment but <5 µg L⁻¹ in the ambient and high N:P treatments (Fig. 1D). In contrast, nitrogen fixation occurred in the ambient and low N:P treatments (Figs. 1B and 4B). Thus, despite the high TN and TP in this eutrophic pond, contrasting treatments (Fig. 1D). In contrast, nitrogen fixation occurred in the ambient and low N:P treatments (Figs. 1B and 4B). Thus, despite the high TN and TP in this eutrophic pond, contrasting nutrients were likely limiting in the three N:P ratio treatments over the course of the experiment.

We sampled the pond and enclosures approximately biweekly from 11 June to 29 July 2010. To avoid contaminating additions. Soluble reactive phosphorus (SRP) was readily available in the low N:P treatment received the same weekly P treatments received the same weekly N additions, and the ambient and high N:P treatments received the same weekly P additions. Soluble reactive phosphorus (SRP) was readily available in the low N:P treatment over the course of the experiment but <5 µg L⁻¹ in the ambient and high N:P treatments (Fig. 1D). In contrast, nitrogen fixation occurred in the ambient and low N:P treatments (Figs. 1B and 4B). Thus, despite the high TN and TP in this eutrophic pond, contrasting nutrients were likely limiting in the three N:P ratio treatments over the course of the experiment.

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![Graph](image-url)

Fig. 1 – Dynamics of (A) total N:total P ratio (by atoms), (B) total nitrogen (TN), (C) total phosphorus (TP), and (D) soluble reactive phosphorus (SRP) over the 49-day experiment. Data represent means ± one standard error. Open symbols denote the ambient (40:1, by atoms) N:P treatment, gray symbols indicate the low (7:1) N:P treatment, and closed symbols indicate the high (122:1) N:P treatment.
change in TN and TP across treatments from the beginning to the end of the experiment. We calculated the predicted TN and TP concentrations for each treatment at the conclusion of the study based on the total amount of fertilizer added and initial nutrient concentrations. Relative change (%) in TN or TP was then calculated by subtracting the predicted TN or TP concentration at the conclusion of the experiment (day 49) from the actual TN or TP concentration on day 49, dividing by the predicted concentration, and multiplying by 100. We then calculated a 95% confidence interval for each treatment mean to compare the relative change to 0.

3. Results

At the start of the experiment, the phytoplankton community was diverse (i.e., 18 phytoplankton genera) and comprised primarily of chlorophytes (~50%) and cyanobacteria (~40%), with the N-fixing cyanobacterium, *C. raciborskii*, accounting for ~17% of total phytoplankton biomass (Appendix Fig. C). Average initial phytoplankton biomass was 3 mg L$^{-1}$ and significantly increased in all three N:P treatments over time (6–16× increase; Fig. 2A). Phyttoplankton biomass was significantly higher in the high N:P treatment (122:1) than for the other two treatments over the course of the experiment (Tukey’s test: *P* = 0.540). By day 15, all N:P treatments were becoming dominated by *C. raciborskii* (~40–75%), and, as expected for an N-fixing species, the low N:P treatment (7:1) contained a higher absolute biomass of *C. raciborskii* than the other two N:P treatments (Fig. 2B). Similar patterns were obtained for *C. raciborskii* relative biomass (i.e., *C. raciborskii* relative biomass was negatively related to N:P). However, regardless of N:P treatment, regular nutrient additions resulted in a rapid loss of phytoplankton species diversity and complete dominance by *C. raciborskii* (~99–100%) by the conclusion of the experiment (day 49, Fig. 2C).

Interestingly, saxitoxin concentrations were elevated in the two highest N:P treatments ($F_{2,7} = 50.83, *P* = 0.0001; Fig. 3A) and were significantly highest in the high N:P treatment (Tukey’s test: *P* ≤ 0.002). Furthermore, mean saxitoxin content at the conclusion of the experiment, expressed as a percentage of total phytoplankton dry biomass, was nearly an order of magnitude higher at high N:P than for the other two N:P treatments. In contrast, N:P treatment had no effect on microcystin concentration ($F_{2,8} = 0.90, *P* = 0.44) (Fig. 3B). Anabaena was the only other abundant, toxigenic cyanobacterium present during the experiment, and Anabaena abundance followed declining microcystin production over time. *Cylindrospermopsis* concentrations were below detection limit over the course of the experiment, regardless of treatment.

Although total N:total P ratio was maintained with weekly nutrient additions in the high (122:1) N:P treatment (+1% change from start to end), large increases in N:P were observed in the two lower N:P treatments (7 N:P = +69% change; 40 N:P = +97% change) during the 49-day study (Fig. 1A). Mass balance calculations based on initial nutrient

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**Fig. 2** – Dynamics of (A) phytoplankton dry biomass, (B) *Cylindrospermopsis raciborskii* dry biomass, and (C) relative biomass (%) of *C. raciborskii* over the 49-day experiment. Data represent means ± one standard error. Open symbols denote the ambient (40:1, by atoms) N:P treatment, gray symbols indicate the low (7:1) N:P treatment, and closed symbols indicate the high (122:1) N:P treatment.

**Fig. 3** – Dynamics of the cyanotoxins, saxitoxin (A) and microcystin (B), during the 49-day experiment. Data represent means ± one standard error. Open symbols denote the ambient (40:1, by atoms) N:P treatment, gray symbols indicate the low (7:1) N:P treatment, and closed symbols indicate the high (122:1) N:P treatment.
concentrations and weekly N and P additions showed large P losses in all three treatments (range = −19 to −34%, relative to total amounts based on mass balance calculations) (Fig. 4A) and a loss of N in the high N:P treatment (−19%) (Fig. 4B). Interestingly, the two treatments with limiting N showed large N gains (+92 to −114%), presumably through nitrogen fixation (Fig. 4B).

4. Discussion

While controlling phosphorus has played a central role in the management of eutrophication in lakes, limnologists continue to argue whether nitrogen inputs should be controlled as well as (or even instead) of phosphorus (Schindler et al., 2008). Smith (1982, 1983) used resource-based competition theory to support the hypothesis that alterations of N:P ratio in lakes can have strong effects on phytoplankton biomass and species composition. In particular, Smith (1983) hypothesized that nitrogen-fixing cyanobacteria dominate systems with relatively low N:P (<64:1, by atoms), but lose their competitive advantage at higher N:P when nitrogen is readily available. While controlling nutrient supply and stoichiometry have been central to decades of lake management and restoration efforts, the importance of stoichiometry for controlling cyanobacteria has typically only been evaluated using short-term, laboratory bioassays (reviewed by Lewis and Wurtsbaugh, 2008) or large-scale lake surveys (Downing et al., 2001; Smith, 1983) and has been less tested via manipulative field experiments (but see Barica et al., 1980; Stockner and Shortreed, 1988; Paterson et al., 2002; Vrede et al., 2009; Finlay et al., 2010; Donald et al., 2011). In our experiment, the phytoplankton community at the conclusion of the experiment was almost entirely comprised of the nitrogen-fixing cyanobacterium, C. raciborskii, regardless of N:P treatment. Thus, we suggest that management of nutrient stoichiometry through fertilization is not likely to be effective for improving water quality and controlling blooms of this noxious cyanobacterium.

Literature shows that phytoplankton abundance increases with concentrations of total phosphorus (Jones et al., 1998; Brown et al., 2000; Schindler et al., 2008), total nitrogen (Paerl, 1988, 2009; Finlay et al., 2010), or both total nitrogen and total phosphorus (Downing et al., 2001; Dolman et al., 2012). Similarly, absolute and/or relative abundance of cyanobacteria have also been shown to increase with nutrients (Watson et al., 1997; Downing et al., 2001; Elser et al., 2007; Vrede et al., 2009; Finlay et al., 2010; Millie et al., 2006; Donald et al., 2011).

While N:P stoichiometry has played a central role in lake management, whether cyanobacterial abundance is more strongly affected by TP, TN, or N:P is poorly understood (Smith, 1983; Downing et al., 2001; Catherine et al., 2008). In particular, stoichiometric effects on phytoplankton composition are expected under nutrient-stressed conditions (i.e., when nutrient concentrations decrease below phytoplankton cell quotas), thus affecting the growth and response of organisms but are likely less important if the total amount of nutrients is high. In our experiment, weekly nutrient fertilizations promoted algal growth in all N:P treatments, with the highest phytoplankton biomass in the high N:P treatment at the conclusion of the experiment. SRP was low in two of the treatments (ambient and high). Furthermore, we found large increases of N in the ambient and low N:P treatments, presumably through nitrogen fixation. Thus, we observed evidence of phosphorus versus nitrogen limitation in our contrasting N:P treatments despite the elevated total nutrient concentrations in our study system. Our results, combined with the documented ability of C. raciborskii to tolerate wide variations in nutrient availability and fix atmospheric nitrogen (Isvanovics et al., 2000; Moisander et al., 2012), suggest that controlling nitrogen and phosphorus (i.e., dual control) may be necessary to reduce phytoplankton biomass, when this species is prevalent (Isvanovics et al., 2000). Future experiments simultaneously manipulating N:P ratio and nutrient concentrations are necessary to elucidate how the interaction between these factors determine phytoplankton community structure.

Given the ability of many cyanobacterial species to fix atmospheric nitrogen and that many species are thought to be poor competitors for inorganic phosphorus (Tilman et al., 1997; Stockner and Shortreed, 1988), the results of our experiment did not agree with theoretical predictions as N:P had no effect on phytoplankton composition. Previous manipulative experiments have found that increased N:P ratio may actually favor cyanobacterial species lacking the ability to fix nitrogen (Barica et al., 1980; Finlay et al., 2010) (e.g., Merismopedia, Phormidium, Microcystis) but capable of producing toxins (Finlay et al., 2010; Donald et al., 2011). However, in our experiment, C. raciborskii accounted for ~100% of total phytoplankton biomass under very low (7:1) and very high (122:1) N:P ratios. Over the course of the experiment, the relative and absolute biomass of Microcystis aeruginosa declined along with levels of microcystin. While Microcystis can tolerate high temperatures and low light characteristic of

![Fig. 4](image-url) - Relative change in (A) total phosphorus (TP) and (B) total nitrogen (TN) from the beginning to the end of the experiment. Using a mass balance approach, we calculated the predicted TN and TP concentrations for each treatment at the conclusion of the study based on the total amount of fertilizer added and initial nutrient concentrations. Relative change in TN or TP was calculated by subtracting the predicted TN or TP concentration at the conclusion of the experiment (day 49) from the actual TN or TP concentration on day 49 and then dividing by the predicted concentration. Data represent means ± 95% confidence interval (CI). If the 95% CI does not include 0, there was a significant change in nutrient concentration.
eutrophic waters in the summer, its colonial growth habit could have comprised its ability to compete with the faster growing *Cylindrospermopsis* (Marinho et al., 2013). Furthermore, as our experiment was conducted in limnocorals that were sealed at the bottom, there was no potential seed bank for the recruitment of *Microcystis* (or other phytoplankton species). However, *Microcystis* typically recruits from the sediments in the spring (Reynolds et al., 1981), and thermal stratification during our summer experiment (Fig. 5) would have prevented significant recruitment from the hypolimnion. In contrast to results for microcystin, levels of the neurotoxin saxitoxin known to be produced by some strains of *C. raciborskii* (Lagos et al., 1999) increased over time. While prior studies suggest that N:P can be important for structuring phytoplankton communities, our results indicate that *C. raciborskii*’s presence and abundance may supersede N:P influence. Indeed, blooms of *C. raciborskii* are becoming increasingly prevalent in tropical, subtropical, and temperate systems around the world (Padisák, 1997). The rapidly expanding geographical range of this invasive phytoplankton species, potential ecosystem-level effects, and human health concerns (Chorus and Bartram, 1999) have stimulated much recent ecological interest in *C. raciborskii* (Appendix Fig. D). In general, ecologists have attributed the success of *C. raciborskii* to resistance to grazing by zooplankton (Panosso et al., 2003), high P uptake and storage capacity (Isvanovics et al., 2000), high ammonium (and nitrate) uptake affinity (Pré sing et al., 1996), tolerance of low light levels (Briand et al., 2004; de Tezanos and Litchman, 2010), ability to fix nitrogen (Moisander et al., 2012), and tolerance of a wide range of temperatures (including temperatures in excess of 30 °C, Butterwick et al., 2005; Bonilla et al., 2012). We did not measure light attenuation in the enclosures to avoid contamination. However, light availability was minimal given the dense algal biomass in the enclosures (Fig. 2A and B; Appendix Fig. B1 and B2). Moreover, calanoid copepods dominated the zooplankton community in our experiment, regardless of N:P treatment (data not shown). Given the inability of calanoid copepods to reduce the biomass of several species of cyanobacteria, including *C. raciborskii*, in laboratory feeding assays (Panosso et al., 2003), it is likely that the grazing resistance and tolerance of high water temperature (Fig. 5) or low light by *C. raciborskii* mediated the effects of N:P in our study. Future experiments are encouraged to test these hypotheses.

5. Conclusions

Our study adds to a growing body of studies demonstrating that *C. raciborskii* has a wide tolerance range for key environmental factors (including N:P ratio), which likely explains its rapid expansion to temperate latitudes. As *C. raciborskii* exhibits optimum growth under high nutrients and temperatures, it is likely that the range of this species will continue to increase with climate change and nutrient enrichment. Based on the results of our field experiment and synthesis of the available information, we conclude that:

- Short-term management of N:P ratio (and stoichiometric control) is not likely to be effective for controlling blooms of *C. raciborskii*.
- Dual N and P reductions may ultimately be needed to reduce the spread of this species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2013.11.022

References


