Harmful Algae 54 (2016) 87–97

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Review

The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms

Christopher J. Gobler [a,*], JoAnn M. Burkholder [b,1], Timothy W. Davis [c,1], Matthew J. Harke [a,1], Tom Johengen [d,1], Craig A. Stow [c,1], Dedmer B. Van de Waal [e,1]

[a] School of Marine and Atmospheric Sciences, Stony Brook University, 239 Montauk Hwy, New York 11968, USA
[b] Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606, USA
[c] NOAA Great Lakes Environmental Research Laboratory, Ann Arbor, MI 48108, USA
[d] Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, Ann Arbor, MI 48109, USA
[e] Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Post Office Box 50, Wageningen 6700 AB, The Netherlands

ARTICLE INFO

Article history:
Received 29 September 2015
Accepted 30 January 2016

Keywords:
Nitrogen
Phosphorus
Cyanobacteria
Microcystis
Diazotrophy
Nutrients

ABSTRACT

Historically, phosphorus (P) has been considered the primary limiting nutrient for phytoplankton assemblages in freshwater ecosystems. This review, supported by new findings from Lake Erie, highlights recent molecular, laboratory, and field evidence that the growth and toxicity of some non-diazotrophic blooms of cyanobacteria can be controlled by nitrogen (N). Cyanobacteria such as *Microcystis* possess physiological adaptations that allow them to dominate low-P surface waters, and in temperate lakes, cyanobacterial densities can be controlled by N availability. Beyond total cyanobacterial biomass, N loading has been shown to selectively promote the abundance of *Microcystis* and *Planktothrix* strains capable of synthesizing microcystins over strains that do not possess this ability. Among strains of cyanobacteria capable of synthesizing the N-rich microcystins, cellular toxin quotas have been found to depend upon exogenous N supplies. Herein, multi-year observations from western Lake Erie are presented demonstrating that microcystin concentrations peak in parallel with inorganic N, but not orthophosphate, concentrations and are significantly lower ($p < 0.01$) during years of reduced inorganic nitrogen loading and concentrations. Collectively, this information underscores the importance of N as well as P in controlling toxic cyanobacteria blooms. Furthermore, it supports the premise that management actions to reduce P in the absence of concurrent restrictions on N loading may not effectively control the growth and/or toxicity of non-diazotrophic toxic cyanobacteria such as the cosmopolitan, toxin-producing genus, *Microcystis*.

Contents

* Corresponding author.
E-mail address: christopher.gobler@stonybrook.edu (C.J. Gobler).
[1] These authors contributed equally to this manuscript and are listed alphabetically.

http://dx.doi.org/10.1016/j.hal.2016.01.010
1568-9883/

1. Introduction

Toxic cyanobacteria are of worldwide concern because persistent blooms threaten drinking water supplies, recreation, tourism, and fisheries (Chorus and Bartram, 1999; World Health Organization, 2011, and references therein). Such blooms are commonly promoted by excessive nutrient loading (Wetzel, 1983, 2001; Paerl et al., 2011; O'Neill et al., 2012). Thus, there is significant interest in implementing improved management actions to control the nutrients responsible for promoting blooms. The paradigm that primary production in freshwater is controlled by phosphorus (P) (USNAS, 1969; Schindler, 1974) was established decades ago within oligotrophic lakes in Canada (e.g. Dillon and Rigler, 1974; Jones and Bachmann, 1976; Schindler, 1977). It is based largely on the premise that when inorganic nitrogen (N_i) levels are low, diazotrophic or N_2-fixing cyanobacteria balance ecosystem N deficiencies (Schindler et al., 2008; Schindler, 2012; Scott and McCarthy, 2010). As the total P concentration in many freshwater bodies has increased and total N:P ratios have decreased, a shift has been reported in phytoplankton assemblages toward cyanobacteria dominance (Smith, 1983; Trimbee and Prepas, 1987; Watson et al., 1997).

Over the past several decades, many lakes have been driven increasingly out of stoichiometric balance due to disproportionate anthropogenic inputs of N and P, or management efforts targeting reduction of one nutrient (usually P in freshwaters) but not the other (Conley et al., 2009; Glibert et al., 2011; Burkholder and Glibert, 2013; and references therein). Concurrently, thought has evolved from consideration of only one limiting nutrient to recognition of the importance of ecological stoichiometry in directly and/or indirectly controlling phytoplankton assemblage structure and productivity (Conley et al., 2009; Glibert et al., 2011; Burkholder and Glibert, 2013, and references therein). Consequently, the literature is rich with examples of the importance of P (Schindler, 1977; Wetzel, 2001; Sterner, 2008; and references therein), and N (e.g. Gobler et al., 2007; Davis et al., 2010; Beversdorf et al., 2013, 2015) in controlling cyanobacteria blooms as well as with examples of N and P co-limitation (Elser et al., 1990, 2007; Lewis and Wurtsbaugh, 2008; Xu et al., 2010; Chaffin et al., 2013; Bridgeman and Chaffin, 2013; Davis et al., 2015).

N limitation in freshwater systems has been most commonly reported during warmer months when planktonic cyanobacteria blooms are most common (Gobler et al., 2007; Xu et al., 2010; Chaffin et al., 2013; Bridgeman and Chaffin, 2013; Davis et al., 2015). Although N_2 fixation by cyanobacteria has been thought to minimize the role of N in controlling blooms, various physiological and ecological lines of evidence have indicated that the energetic demands of diazotrophy can restrict the extent to which N_2 fixation can offset N demands and limitation, particularly when concurrent rates of denitrification are considered (Scott and McCarthy, 2010). Moreover, some of the most common toxigenic genera of cyanobacteria, such as *Microcystis* and *Planktothrix* (Chorus and Bartram, 1999; World Health Organization, 2011), are not diazotrophs but, rather, depend on exogenous N supplies for growth and toxin synthesis (Berman and Chava, 1999; Vézie et al., 2002; Davis et al., 2010; Monchamp et al., 2014, and references therein). A strong relationship between the growth of non-diazotrophic cyanobacteria and exogenous dissolved N supplies has commonly been reported. For example, in laboratory studies increased N_i has promoted the growth and toxicity of *Microcystis* (Watanabe and Oishi, 1985; Codd and Poon, 1988; Orr and Jones, 1998) and enhanced input of N_i to systems with elevated P has led to succession from diazotrophs to non-diazotrophs (Bunting et al., 2007; Davis et al., 2010; Chaffin et al., 2013; Harke et al., 2016).

This manuscript reviews recent information regarding the role of N and P in supporting the growth and toxicity of cyanobacteria blooms, emphasizing non-diazotrophs. Conditions that render ecosystems prone to cyanobacterial blooms are considered, as well as recent molecular, laboratory, and field studies that support the premise that the toxicity, and sometimes the biomass, of cyanobacterial blooms is influenced by N_i availability. Finally, open questions and research priorities are identified toward the goal of strengthening insights regarding nutrient controls on cyanobacterial blooms and toxin production.

2. Seasonal cycles in N loading, P loading, and cyanobacterial blooms

Due to the seasonality of N and P inputs into temperate freshwater ecosystems, late summer cyanobacteria blooms occur when N_i delivery from river sources is often at an annual minimum (Turner et al., 2003) and, thus, most likely to control the growth of primary producers. Similar trends have been observed in smaller lakes more influenced by groundwater flow than riverine input (Gobler et al., 2007). In Lake Erie, which has sustained major cyanobacterial blooms during the past two decades (Brittain et al., 2000; Conroy et al., 2005; Stumpf et al., 2012; Wynne and Stumpf, 2015), one of the dominant nutrient sources, the Maumee River, has an annual TN:TP minimum during the summer months when cyanobacteria blooms are most likely and summer N limitation has been demonstrated (Stow et al., 2015; Chaffin et al., 2013, 2014).

Species and sources of N present in lakes can differ in their seasonal dynamics, which may also influence cyanobacterial blooms. Nitrate concentrations tend to be highest in winter-spring and decline to low levels as summer progresses in many north temperate lakes (Reynolds, 1984; Wetzel, 2001; Chaffin et al., 2011; Bridgeman and Chaffin, 2013). These low N conditions can be alleviated by periodic summer storms that deliver "new" N and/or by diazotrophic cyanobacteria that release ("leak") amino acids and ammonia during N_2 fixation (Wetzel, 2001 and references therein) although nitrogen fixation has been shown to not offset N ecosystem level demands (Scott and McCarthy, 2010).

In contrast to NO_3^- dynamics, ammonia (NH_3) and ionized ammonia (NH_4^+) are released from sediments and some benthic fauna during warmer months through decomposition processes (Wetzel, 2001, and references therein; Zhang et al., 2008). Substantial water-column NH_4^+ supplies from benthic sources in summer have also been reported in areas with moderate to high densities of bivalve molluscs (Burkholder and Shumway, 2011). For example, the western basin of Lake Erie has sustained major invasions of dreissenid mussels capable of delivering large amounts of NH_4^+ to the water column (Higgins et al., 2006, and references therein; Zhang et al., 2008). In lakes showing symptoms of N limitation during late summer, cyanobacteria such as *Microcystis* have been shown to become dominant by rapidly assimilating recycled ammonium (e.g. Takamura et al., 1987; Ferber et al., 2004, Chaffin et al., 2011). Further, *Microcystis* has been shown to have a high affinity for NH_4^+ and, thus, is highly competitive for recycled, reduced N (McCarthy et al., 2009; Glibert et al., 2015, and references therein). Reduced N forms are rapidly recycled; increased loads of reduced N, such as ammonia in partially treated or untreated sewage, and high NH_4^+/NO_3^- ratios, tend to promote cyanobacteria such as *Microcystis* over diatoms in phytoplankton assemblages (McCarthy et al., 2009; Glibert et al., 2015).

Lake sediments and porewaters are generally enriched in inorganic P (P_i) relative to the water column, although the extent to which sediments retain or export these nutrients varies seasonally. The PO_4^{-3} ion binds preferentially with ferric oxides in sediments under oxygenated conditions, but during summer months as temperatures warm and microbial degradation of sedimentary organic matter accelerates, sediment and near-sediment oxygen

levels are progressively depleted and often become anoxic (Wetzel, 2001, and references therein; Hupfer and Lewandowski, 2008). Under such conditions, PO_4^{-3} dissociates from ferric oxides and is released to the overlying water (Carlton and Wetzel, 1988) making anoxic sediments a substantial source of P_i during warm months, particularly in systems where benthic fluxes of P_i reach surface waters. Phosphate release from organic matter directly depends on rates of microbial decomposition which are typically temperature-dependent and, thus, also maximal during summer (Wetzel, 2001; Reitzel et al., 2007; Hupfer and Lewandowski, 2008). Although NH_4^+ is also released from organic matter decomposition in sediments during warm periods, the N:P ratio of sedimentary fluxes can be enriched in P relative to N, particularly in eutrophic lakes where cyanobacteria blooms are common and anoxic sediments can promote P release and denitrification (Fukushima et al., 1991; Downing and McCauley, 1992; Søndergaard et al., 2003). Hence, maximal benthic fluxes during summer can be a stronger source of P relative to N, contributing toward N limitation, particularly in shallow, well-mixed systems.

3. Bloom-forming, toxic non-diazotrophic cyanobacteria in low P_i waters

While P can limit cyanobacterial growth in freshwater systems, some common non-diazotrophs such as *Microcystis* thrive in low P_i surface waters, conditions common in many temperate lakes during late summer (Heron, 1961; Bertram, 1993; Wilhelm et al., 2003; Harke et al., 2016). Such low water-column P_i conditions, of course, "mask" the fact that TP in biomass typically is high in eutrophic lakes (Wetzel, 2001, and references therein). Within an ecosystem setting, aforementioned benthic P sources may be especially important to cyanobacteria such as *Microcystis* which can manipulate its internal cell pressure via gas vesicles to migrate down through the water column at night, then back near the surface at dawn (Reynolds and Walsby, 1975), a process that facilitates both light and nutrient access as well as herbivore avoidance (Wetzel, 2001, and references therein). In shallow and/or stratified lakes, this vertical migration allows cyanobacteria such as *Microcystis* to access benthic nutrient sources more than phytoplankton without such migratory capabilities (Barbiero and Welch, 1992; Brunberg and Bostrom, 1992; Visser et al., 1997; Verspagen et al., 2004; Xie, 2006; Cottingham et al., 2015, and references therein) and partly accounts for the ability of *Microcystis* to form a bloom in the absence or near-absence of surface water P_i (Heron, 1961; Bertram, 1993; Wilhelm et al., 2003; Harke et al., 2016). In shallow, well-mixed lakes, by contrast, buoyancy regulation by cyanobacteria may be less of a competitive advantage.

Beyond accessing sedimentary P, at a cellular level, culture studies of *Microcystis* have demonstrated that this cyanobacterium has the ability to maintain rapid growth rates under low or no P_i conditions. For example, dense batch culture of *Microcystis* have been shown to grow equally well across a wide range of P_i concentrations, with comparable growth rates at starting media concentrations of 1.75 and 175 μM P_i (~56 and 560 μg L^{-1}; Saxton et al., 2012). Others have reported that cultured *Microcystis* clones continue exponential growth for at least 8 days in P-deplete media (Sbiyyaa et al., 2009). In experiments with *Microcystis aeruginosa* clone LE-3 from Lake Erie, cultures transferred to media lacking P_i ($-P_i$) have displayed growth that exceeded those of cultures in P_i-replete media for more than two weeks before experiencing slowed growth (Fig. 1).

The ability of cyanobacteria such as *Microcystis* to grow well under low P_i conditions is specifically facilitated by a series of key physiological traits that include a high-affinity PO_4^{-3} uptake system that is activated at low P_i concentrations (Harke et al., 2012), high cellular storage capacity for P (Whitton et al., 1991; Harke et al., 2012), and the production of extracellular polyphosphatase enzymes to access organic P or DOP (Healey, 1982; Harke et al., 2012). Although the reported substrate affinity (K_s) for P uptake by *Microcystis* of ~0.6 μM (19 μg PO_4^{-3}-P L^{-1}; Baldia et al., 2007) is comparable to that of other algae (e.g., Reynolds, 1984; Smayda, 1997), *Microcystis* responds to P limitation by a rapid increase in P_i uptake and increasing maximal uptake rates (V_{max}; Jacobson and Halmann, 1982; Kromkamp et al., 1989). Additionally, phosphoesters dominate DOP pools in aquatic environments (Kolowith et al., 2001) and the degradation of these compounds requires phosphatases ranging from acidic to alkaline (Wetzel, 2001, Dyhrman and Ruttenberg, 2006 and references therein). High rates of alkaline phosphatase activity have been reported for cultured *Microcystis aeruginosa* both on a population and a per-cell basis (Giraudet et al., 1997, 1998; Štrojsová et al., 2005; Harke et al., 2012).

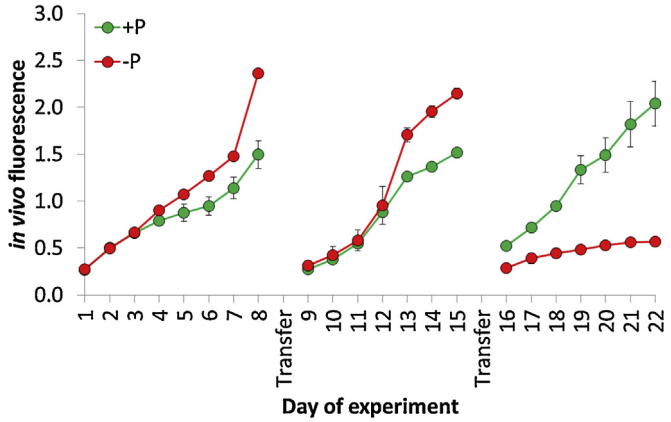

Fig. 1. Phosphorus limitation experiment wherein cultures of *Microcystis aeruginosa* LE-3 harvested from P-replete media were grown under 0 μM inorganic P (−P) and 1200 μM inorganic P (+P). Cultures were repeatedly diluted back to starting densities during the "Transfer" for this time series in order to fully limit the population. In vivo fluorescence levels of this culture were highly correlated with cell densities (Harke et al., 2012).

Gene expression studies on cultured *Microcystis* have revealed the molecular pathways that facilitate rapid growth under low P_i. In $-P_i$ media, *Microcystis* has been shown to strongly upregulate (by 50- to 400-fold) two high-affinity, phosphate-binding proteins (*pstS* and *sphX*) and an alkaline phosphatase (*phoX*), allowing it to maintain rapid growth under extremely low or no P_i conditions (Harke et al., 2012). These genes were highly conserved among ten cultures of *Microcystis aeruginosa* isolated from different geographic regions, and the expression of *phoX* was significantly correlated with alkaline phosphatase activity (Harke et al., 2012). A broader, whole transcriptome study demonstrated that when deprived of P_i, *Microcystis* differentially expressed nearly one-fourth of its genome (Harke and Gobler, 2013). In addition to the *phoX*, *sphX*, and *pstS* genes, transcript levels of many other genes within the Pst-P transport system (*pstSCAB*) and *phoU* increased (Harke and Gobler, 2013). The degree of up-regulation of these P_i scavenging genes varied, suggesting different affinities among transporters as observed for other cyanobacteria (Pitt et al., 2010), which may extend the dynamic range over which *Microcystis* can incorporate P_i. In addition, sulfate-binding and permease proteins were up-regulated under low external P_i (Harke and Gobler, 2013), suggesting that *Microcystis* may switch to sulfolipids in place of P-based membrane lipids to reduce cellular P quotas as another adaptation to the low-P_i conditions (Van Mooy et al., 2006).

While these culture studies identified physiological mechanisms by which *Microcystis* might form blooms under low P conditions, recent field studies of *Microcystis* blooms in Lake Erie

provide ecosystem-based evidence. Harke et al. (2016) performed surveys and experiments from one of the largest tributary P sources in Lake Erie, the Maumee River, out to the distal regions of the western basin, where P_i levels declined from micromolar (μM) to nanomolar (nM) levels. Under high orthophosphate conditions around the Maumee River mouth, *Anabaena* and *Planktothrix* were the dominant cyanobacterial genera (Harke et al., 2016). In contrast, in the more offshore regions of the lake with low P_i concentrations, *Microcystis* became the dominate cyanobacterium, as it upregulated genes associated with P scavenging (*pstSCAB*, *phoX*) as well as P storage (*ppk1*; Harke et al., 2016). Furthermore, experimental enrichment of Lake Erie water with P_i increased the abundance of the total cyanobacterial population but resulted in a decrease in the abundance of *Microcystis* (Harke et al., 2016). Collectively, these findings suggested that *Anabaena* is adapted to the high P regions of western Lake Erie, whereas *Microcystis* dominates and persists under low-P conditions (Harke et al., 2016). Similar niche differentiation of diazotrophic and non-diazotrophic cyanobacteria in high- and low-P environments have also been observed in the Baltic Sea and Lake Taihu, China (Andersson et al., 2015; Paerl and Otten, 2015).

4. Evidence for N control of toxic cyanobacteria blooms

Recently, N has been recognized as a key factor influencing cyanobacterial blooms. Kosten et al. (2012) assessed 143 lakes along a latitudinal transect ranging from subarctic Europe to southern South America, and found that temperature and TN concentrations were the strongest explanatory variables for cyanobacterial biomass. Similarly, Beaulieu et al. (2013) assessed cyanobacteria blooms in 1147 lakes and reservoirs of differing trophic status across the U.S. and found that the best multiple linear regression model to predict these events was based on TN and water temperature. This finding is also consistent with the strong positive association between N_i concentrations and microcystin levels that has been reported across many U.S. lakes (Yuan et al., 2014). In 102 north German lakes, Dolman et al. (2012) found that the positive relationship between total cyanobacterial biovolume and P concentration disappeared at high TP concentrations, but continued to increase with increasing TN concentration. This may suggest that some cyanobacteria have higher N:P requirements and, thus, are potentially N limited within highly P-enriched lakes. Conversely, research in large experimental lake studies has shown that reduction of N_i inputs can result in a decline in cyanobacterial abundance (Scott and McCarthy, 2011).

As other recent examples showing the importance of N in controlling cyanobacteria assemblages, Davis et al. (2010) compared N versus P influence on dense natural *Microcystis* blooms in a tidal (brackish) tributary and a eutrophic lake, and found that in both systems during nutrient amendment experiments, all *Microcystis* populations tested were stimulated by N more frequently than by P. Monchamp et al. (2014) assessed three shallow, mesotrophic to hypereutrophic lakes in southwestern Quebec, Canada, and found TN, NH_4^+, and DON significantly influenced the cyanobacterial assemblage structure, and that the relative biomass of *Microcystis* spp. was significantly, positively related to DON concentrations. Davis et al. (2015) found that in blooms dominated by *Planktothrix agardhii/suspensa*, cyanobacterial growth and microcystin (MC) concentrations increased as inorganic N concentrations increased, and that loading of N_i combined with P_i most often lead to the highest MC concentrations.

Water-column N_i concentrations have also been shown to promote diazotroph-to-non-diazotroph succession in cyanobacteria assemblages. For example, based on two years of observations in highly eutrophic Lake Mendota, WI, USA, Beversdorf et al. (2013) reported that cyanobacteria assemblage changes were strongly correlated with dissolved N_i concentrations and that N_2-fixation by the diazotroph *Aphanizomenon* provided N supplies for toxic *Microcystis*. *Microcystis* populations increased in cell density several days after the first significant N_2-fixation rates were measured, and then *Microcystis* became dominant following a short period of low-N_i. In the year when N_2-fixation rates were much greater, the MC concentrations were also higher. Importantly, this system was sufficiently eutrophic to support blooms of diazotrophic or non-diazotrophic cyanobacteria depending on prevailing conditions. Within a nutrient-enriched setting, temporary low-N can cause an initial decrease in non-diazotrophs which can subsequently form toxic blooms when provided N from diazotrophs.

5. Exogenous N influences on cellular toxin composition and quotas

Beyond N influence on the *occurrence* of cyanobacteria blooms, there is evidence that the *toxicity* of blooms formed by non-diazotrophic cyanobacteria such as *Microcystis* is also highly influenced by N availability, beginning at the cellular level with toxin composition and cell quota. As noted by Glibert et al. (2015), various researchers have reported positive, direct relationships between N availability and toxin production in *Microcystis* and other toxigenic cyanobacteria (e.g., Lee et al., 2000; Vézie et al., 2002; Downing et al., 2005; Van de Waal et al., 2009; Harke and Gobler, 2013). The common cyanotoxins MCs, nodularins (NODs), cylindrospermopsins (CYNs), and saxitoxins (STXs) all either contain amino acids or require amino acid precursor(s) (Fig. 2) (Sivonen and Jones, 1999; Kellmann et al., 2008). Synthesis of amino acids, in turn, depends on N availability (Flores and Herrero, 2005; Tapia et al., 1996; Van de Waal et al., 2010). Thus, N can play a central role in determining the quantity of toxins produced by cyanobacteria.

High levels of N_i are needed to synthesize the N-rich MCs, and high levels of exogenous N_i have been shown to promote higher cellular quotas of MCs in the non-diazotrophs *Microcystis* and *Planktothrix* (Lee et al., 2000; Vézie et al., 2002; Downing et al.,

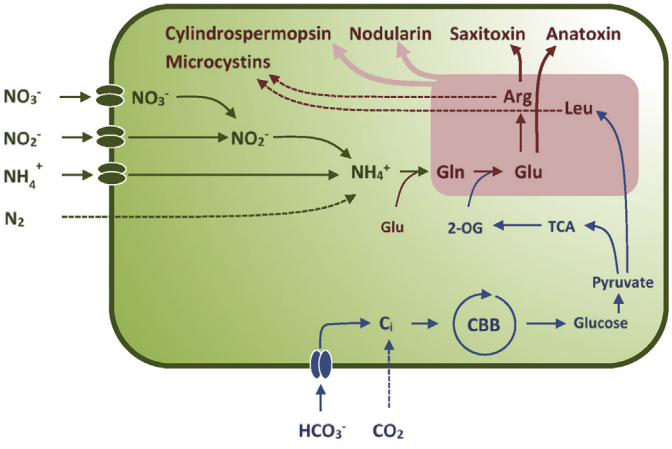

Fig. 2. Cell model describing the coupling of C and N assimilation with synthesis of some cyanobacterial toxins, and showing the importance of N in toxin production. N_i is assimilated to amino acids by the incorporation of cellular NH_4^+ into C skeletons through the glutamine synthetase-glutamate synthase pathway. The toxins are synthesized from the cellular amino acid pool (light red arrows), or from distinct amino acids (dark red arrow). C_i, cellular inorganic carbon; CBB, Calvin–Benson–Bassham cycle; TCA, tricarboxylic acid; 2-OG, 2-oxoglutarate; Gln, glutamine; Glu, glutamate; Arg, arginine; Leu, leucine. Note, N_2 fixation occurs in a heterocyte, and that toxins are produced by different cyanobacteria species. Here, all cyanobacteria are shown in the same cell model for simplicity. Modified after Van de Waal et al. (2010).

2005; Harke and Gobler, 2013; Horst et al., 2014; Van de Waal et al., 2010, 2014). One of the first studies to suggest a positive relationship between MC production in *Microcystis* and available external N_i supplies was by Long et al. (2001), who found a positive correlation between N-dependent growth rates and the cellular MC quota, as well as MC production rates. Later research showed that cellular MC quota depends on cellular N availability and decreases when N_i is limiting (Downing et al., 2005; Van de Waal et al., 2009, 2014; Harke and Gobler, 2013; Horst et al., 2014). At the molecular level, the microcystin synthetase gene cassette (*mcy* genes) appears to be responsive to N supply. For instance, N-deprived cultures of *Microcystis* downregulated genes involved in peptide synthesis (*mcy*ABCDE) and a decrease in cellular quota of MC under N-deplete conditions (Harke and Gobler, 2013).

In the field, addition of NH_4^+ compared with NO_3^- has led to an increase in MC concentrations and bloom maintenance for a longer duration (Donald et al., 2011). Glibert et al. (2011) noted a common phenomenon among freshwater and brackish systems that had been subjected to P reductions but not N reductions in management efforts: In systems receiving substantial NH_4^+ inputs, once the "sediment pump" of stored P began to increase P supplies to the overlying water, an interplay of P sequestration and NH_4^+ tolerance influenced shifts to new dominant taxa such as *Microcystis*. Under P limitation, N-rich toxins would be expected to be favored as a mechanism whereby N could accumulate in excess (Granéli and Flynn, 2006; Van de Waal et al., 2014, and references therein).

Recent research conducted by Beversdorf et al. (2015) is germane in this regard, and indicates that N supply and speciation can control MC synthesis: In Lake Mendota (WI, USA), the toxic phase of the annual cyanobacterial blooms occurred during a transition of high NO_3^- but declining NH_4^+ concentrations, coinciding with upregulation of the MC synthetase gene operon, and leading to high MC levels in the ecosystem. In addition, concentrations of MCs peaked at the same time as the TN/TP ratios, suggesting the importance of an elevated N supply in supporting MC production. These findings are consistent with prior laboratory studies, wherein MC production was tightly coupled to N-dependent growth rates (Long et al., 2001; Harke and Gobler, 2013) and field studies showing that N enrichment enhanced MC levels and the expression of peptide synthesis genes involved in MC production in *Microcystis* (*mcyBEG*; Harke et al., 2016).

Compared to the plethora of laboratory and field studies showing the strong link between MC synthesis and elevated nitrogen levels, two studies have reported an increase in cell quota of MCs, and/or MC synthetase gene expression under N limitation of *Microcystis* (Ginn et al., 2010; Pimentel and Giani, 2014). Such an apparently counterintuitive increase of MC synthesis may be linked to the putative role of MCs in protection against increased oxidative stress (Pimentel and Giani, 2014; Zilliges et al., 2011; Meissner et al., 2013, 2015). Thus, cellular MC quota depends on the relative availability of external bioavailable N, as N is needed for MC synthesis, but this dependency may in turn be affected by the function of MCs which determines when the compounds are required.

There are more than 90 known MC congeners (Schmidt et al., 2014) that differ in two variable amino acids (Welker and von Döhren, 2006). There is high variation in the structure and C:N composition of MCs (Van de Waal et al., 2009, and references therein) and MCs may be important in redox control within cyanobacterial cells (Neilan et al., 2013; Glibert et al., 2015). It has also been suggested that toxin production may be associated with pathways of energy balance or cellular stoichiometric rebalancing (e.g., Glibert and Burkholder, 2011; Van de Waal et al., 2014, and references therein; Glibert et al., 2015). Two common MC variants are MC-LR and MC-RR which consist of a leucine (i.e. L) and arginine (i.e. R), or of two arginine molecules (RR) on the two variable positions. These variants differ in toxicity, with respective LD_{50} values (i.p. on mice) of 33–73 and 310–630 $\mu g\ kg^{-1}$ (Sivonen and Jones, 1999; Chen et al., 2006) and N:C ratios (0.20 and 0.27, respectively). N availability can alter the synthesis of specific MCs, with a shift from MC-LR to the more N-rich MC-RR at higher N availability (Van de Waal et al., 2009, 2010). The extent to which such shifts may occur in an ecosystem setting have yet to be evaluated.

Cyanobacteria NODs, CYNs, and STXs are generally produced by N_2-fixing cyanobacteria (Chorus and Bartram, 1999; Sivonen and Jones, 1999). Early work on CYN production by *Cylindrospermopsis raciborskii* revealed that N species differentially influenced CYN production (Saker and Neilan, 2001). Subsequent studies that have investigated the molecular response of CYN synthetase genes to N, however, suggested that CYN synthesis does not depend on N availability or species (e.g. Shalev-Malul et al., 2008). Other laboratory (Davis et al., 2014) and field (Burford et al., 2014) experiments clarified that CYN is constitutively produced; therefore, changes in CYN concentrations likely are due to changes in ratios of CYN-producing and non-CYN-producing genotypes (i.e., intraspecific or strain differences; Orr et al., 2010 – and see below). The synthesis of STXs and NODs in N_2-fixing cyanobacteria has also shown counterintuitive responses to N additions, and appears to be inhibited by NH_4^+ (Kabir and El-Shehawy, 2012; Stucken et al., 2010). For example, Stucken et al. (2014) quantified intra- and extra-cellular toxin content in cultured *C. raciborskii* (CYN producer) and *Raphidiopsis brookii* (STX producer) at early stages of growth under NO_3^-, NH_4^+, urea, and N-free media, and showed that the N source did not influence either CYN or STX production in the strains tested. In media without N added, however, precursor toxins decreased, and *R. brookii* also produced less STX under growth with NH_4^+. These observations for cyanobacteria differ markedly from the strong, predictable dependencies on N availability that have been observed in the STX-producing dinoflagellate *Alexandrium* (Boyer et al., 1987; Anderson et al., 1990; Flynn et al., 1994; John and Flynn, 2000; Hattenrath et al., 2010; Van de Waal et al., 2013).

6. Intraspecific differences in N influence: toxic versus nontoxic strains

Consistent with studies of marine toxigenic algae, within a given genus of cyanobacteria, strains differ in toxin composition and cellular toxin quota, and nontoxic strains not only co-occur but can be major components of blooms (Burkholder and Glibert, 2006; and references therein). In natural cyanobacteria populations, total cellular toxin quotas resemble average values for an entire population and strongly depend on the contribution of toxigenic versus nontoxic genotypes (Janse et al., 2005; Kardinaal et al., 2007; Briand et al., 2008; Davis et al., 2009, 2010; Orr et al., 2010; Burford et al., 2014). Shifts in the genotypic composition of a population will cause changes in both the average cellular toxin quota but also the toxin composition (Bittencourt-Oliveira et al., 2001; Ame and Wunderlin, 2005; Zurawell et al., 2005; Monchamp et al., 2014). While N can strongly influence the relative abundance of toxic versus nontoxic strains of cyanobacteria, intraspecific variation (strain differences) in toxin production are poorly understood but of paramount importance in the dynamics of overall bloom toxicity and N controls.

While environmental drivers such as N have been shown to influence cell quotas of MCs, most studies have shown only up to four-fold changes in such quotas (Sivonen and Jones, 1999; Horst et al., 2014; Harke and Gobler, 2013). During cyanobacterial blooms, however, changes in MCs and other cyanotoxins can often vary many times greater than four-fold (Chorus and Bartram,

1999; Zurawell et al., 2005; and references therein). Therefore, changes in community composition between cells with the genetic ability to produce cyanotoxins (i.e. toxigenic cells), and those lacking that capability (nontoxic cells; Davis et al., 2009) are likely to play a key role in influencing bloom toxicity.

Laboratory studies of *Microcystis* have shown that toxigenic (MC+) strains yield faster growth rates than nontoxic (MC−) strains at high N_i concentrations (Vézie et al., 2002, Zurawell et al., 2005). In contrast, MC− strains of *Microcystis* require lower N_i concentrations to achieve maximal growth rates in comparison to MC+ strains (Vézie et al., 2002) and MC− *Microcystis* strains have been shown to outcompete MC+ strains when N_i concentrations are low (Vézie et al., 2002; Davis et al., 2010). In field research, bloom populations of *Microcystis* in a temperate, tidal (brackish) tributary and a eutrophic lake shifted from dominance of MC+ strains to MC− strains as N_i concentrations decreased through the summer (Davis et al., 2010). Other researchers working in various lakes have observed a similar seasonal succession of toxic to nontoxic *Microcystis* populations (Fastner et al., 2001; Welker et al., 2007; Briand et al., 2009; Otten et al., 2012, Singh et al., 2015; Beversdorf et al., 2015) or have noted the dominance of MC− strains during the peak of a *Microcystis* bloom event (Welker et al., 2003, 2007; Kardinaal et al., 2007). Since inorganic nutrient levels are generally depleted by dense algal blooms (Wetzel, 2001, Sunda et al., 2006, and references therein), the predominance of MC− strains in established (and senescing) blooms has been hypothesized to be a function of their ability to outcompete MC+ strains when nutrient levels are lower (Vézie et al., 2002; Davis et al., 2010). Thus, under low N conditions, MC+ strains would be succeeded by MC− strains, and/or MC synthesis would be down-regulated. Overall, toxic *Microcystis* cells appear to have a higher N requirement than nontoxic cells (Vézie et al., 2002; Davis et al., 2010), likely related at least in part to the additional N requirements associated with the enzymes involved in MC synthesis (Tillett et al., 2000) and perhaps with additional light-harvesting pigments (Hesse et al., 2001). MC is a N-rich compound (average of 10 N atoms per molecule) and MC can represent up to 2% of cellular dry weight of toxic *Microcystis* cells (Nagata et al., 1997). Accordingly, in many eutrophic systems MC concentrations have more commonly been reported to increase in response to increasing N than increasing P (Gobler et al., 2007; Donald et al., 2011, Chaffin et al., 2013; Bridgeman and Chaffin, 2013; Davis et al., 2015).

Interactions between N and other environmental factors further influence cyanotoxin concentrations (Chorus and Bartram, 1999; Zurawell et al., 2005; and references therein). For example, when MC+ strains dominate assemblages in the early bloom phase when N concentrations are high, there is lower overall biomass and, thus, higher average light intensities. MC+ strains have been shown to grow well under higher light intensities, better than their MC−-counterparts (Zilliges et al., 2011), while MC− strains are better competitors at low light intensities characteristic of dense blooms (Kardinaal et al., 2007). Hence, while N plays a primary role in shaping the relative abundance of MC-producing cells in an ecosystem setting, other biotic and abiotic factors likely act and interact to influence these populations as well.

7. The dynamics of N, P, *Microcystis*, and micocystins in western Lake Erie

In support of prior studies showing the importance of exogenous N in controlling the toxicity of cyanobacterial blooms, data from western Lake Erie, a region long known for cyanobacterial blooms and recently experiencing a re-intensification of these events (Stumpf et al., 2012; Obenour et al., 2014), has been synthesized for this review. Inter-annual differences in the duration, intensity, and toxicity of cyanobacterial blooms were considered in relationship to in-lake and tributary nutrient concentrations. Monitoring surveys totaling 14, 19, and 21 events in 2012, 2013, and 2014, respectively, were conducted across an area of ~300 km^2 (mean depth ~5 m, total volume 1.5 km^3) wherein sampling occurred at four fixed stations weekly (July–September) to biweekly or monthly (May, June, October). Discharge for the Maumee River was obtained as USGS daily averages (Waterville gaging station 4193500; http://waterdata.usgs.gov/usa/nwis/uv?site_no=04193500, last accessed in July 2015). River nutrient concentrations were obtained from the Water Quality Laboratory at Heidelberg College, Tifton (OH, USA) and were averaged as daily means when more than one sample was generated on a given day.

Maumee River discharge was significantly lower in 2012 than in the other two years, especially during spring and summer when flows are likely to have the greatest potential influence on the size and intensity of western Lake Erie cyanobacterial blooms (Stumpf et al., 2012). The river discharge for that period in 2012 (0.42 km^3) was only 16 and 17% of that observed in 2013 (2.71 km^3) and 2014 (2.47 km^3; Fig. 3A). Similarly in the lower Maumee River, the mean NO_3^- concentration in April–September 2012 (1.03 mg L^{-1}) was 28% of that in 2013 and 2014 (3.82 and 3.60 mg L^{-1}, respectively; Fig. 3B). NO_3^- concentrations in June and July 2012 were significantly lower than those present in during those months in 2013 and 2014 (ANOVA; $p < 0.01$). Average PO_4^{-3} in the lower Maumee River in April–September 2012 (26 μg L^{-1}) was lower than in 2013 and 2014 (50.2 and 57.4 μg L^{-1}, respectively; Fig. 3C) although extended periods of significant differences were not detected.

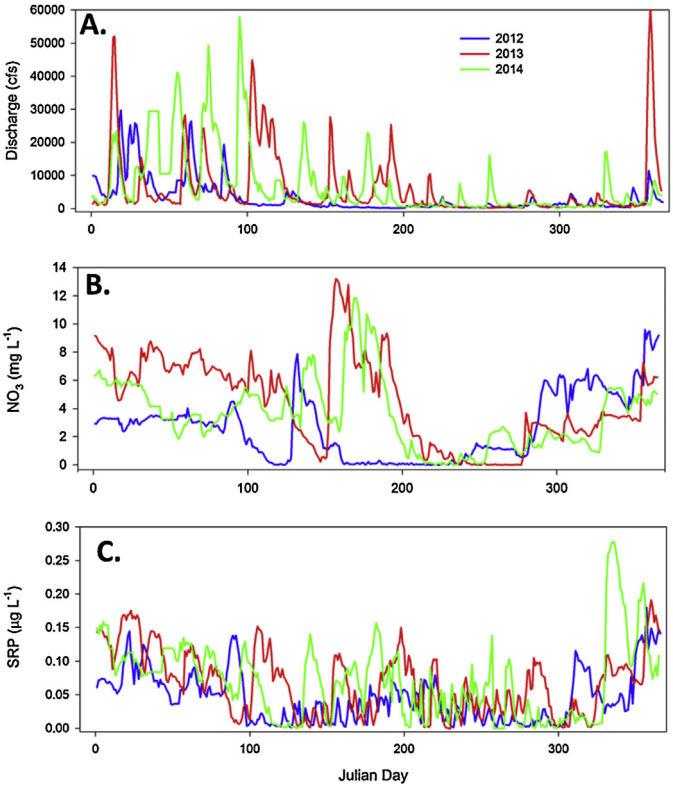

Fig. 3. (A) Maumee River discharge (cubic feet per second, cfs), from daily data taken by the USGS at gaging station (B, C), with concentrations of (B) NO_3^- (mg L^{-1}) and (C) SRP (soluble reactive phosphorus: μg L^{-1}) in the Maumee River (mg L^{-1}). Data from the NOAA Great Lakes Environmental Research Laboratory, Ann Arbor, MI, U.S.A.

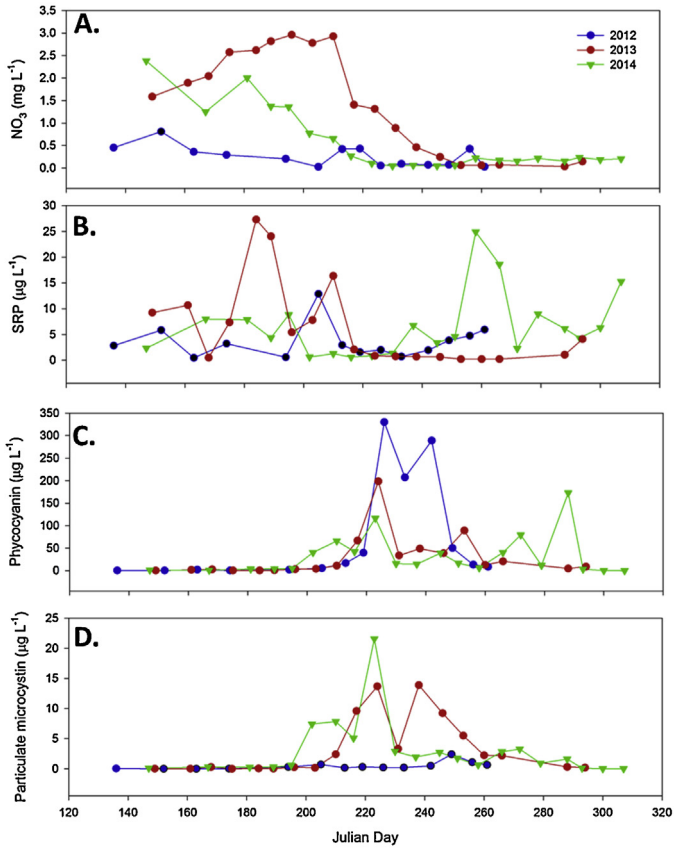

Fig. 4. Concentrations of (A) nitrate (mg L^{-1}), (B) SRP (soluble reactive phosphorus; µg L^{-1}), (C) phycocyanin (µg L^{-1}), and (D) particulate microcystin (µg L^{-1}) in western Lake Erie during 2012–2014.

Reflective of the tributary loads, in-lake NO_3^- concentrations varied substantially among years both in terms of the timing and magnitude of peak levels, as well as, the rates of decline during the bloom season (Fig. 4). Maximum NO_3^- levels in 2012 were 25 and 30% of levels seen in 2013 and 2014, with minimum concentrations present 1–2 months earlier in the season (Fig. 4A). NO_3^- concentrations reached their seasonal minima of <0.1 mg L^{-1} several weeks before the onset of the 2012 cyanobacterial blooms in August and remained below that level during the ~6 weeks of elevated biomass (Fig. 4A). The concentrations of NO_3^- during the May, June, and July were significant lower in 2012 compared to the same period during 2013 and 2014 (ANOVA; $p < 0.01$) whereas PO_4^{-3} concentrations were not significantly different among years during this time period.

Levels of the cyanobacterial pigment, phycocyanin, varied seasonally and annually but showed clear seasonal maxima from mid-July to September (Fig. 4C). The highest cyanobacterial biomass in this area of the lake was measured in 2012 when river flows and nutrient inputs were lower (Figs. 3 and 4C). Maximum levels of phycocyanin (200–300 µg L^{-1}) were about twice as high in 2012 than in the other two years (Fig. 4C), a finding emphasizing the ability of *Microcystis* (the dominant genera present during blooms) to form dense, open water blooms under low P conditions (Wilhelm et al., 2003; Harke et al., 2016). Regarding MC, however, in the summer of 2012, there was almost no detectable increase in whole-cell (particulate) MC (Fig. 4D), suggesting that nontoxic *Microcystis* strains comprised a significant portion of the bloom biomass and that the toxic strains that were present likely had low MC cellular quotas, conclusions supported by prior sections of this manuscript. At the beginning of September 2012, when the bloom appeared to wane and NO_3^- increased slightly and MC finally increased but remained relatively low (maximum, 2.5 µg L^{-1}; Fig. 4D). In contrast, during summers of 2013 and 2014, NO_3^- levels remained higher for a longer period (over 2.0 and 0.6 mg L^{-1} at the end of July) and peak MC concentrations exceeded 13 and 21 µg L^{-1}, respectively. MC concentrations during August 2013 and 2014 were significantly higher than the levels present during August of 2012 (ANOVA; $p < 0.01$). NH_4^+ dynamics were similar to those for nitrate, higher in 2013 and 2014 compared to 2012, and depleted in August but slightly elevated before and after that month (data not shown). Except for one sampling date in each of the latter two years, particulate MC concentrations declined once NO_3^- decreased to <0.5 mg L^{-1}. In contrast, there was no apparent relationship between particulate MC concentrations and SRP (P_i; Fig. 4). Hence, the significantly lower overall MC concentrations in 2012 compared to 2013 and 2014, as well as declines in MC in those years, coincided with lower NO_3^- levels.

These ecosystem observations are consistent with studies outlined above which have shown that MC concentrations during toxic *Microcystis* and *Planktothrix* blooms in western Lake Erie have been controlled by the availability of water-column N_i (Horst et al., 2014; Davis et al., 2015; Harke et al., 2016). Furthermore, these findings suggest the intriguing possibility that declines in N loading in western Lake Erie (Stow et al., 2015) could yield blooms of lower toxicity, even if P_i levels rise or remain stable. Therefore, in order to manage Lake Erie toward smaller blooms of lower toxicity, managing N and P should be considered. The response of Lake Erie to changes in nutrient conditions as management actions are implemented will offer an opportunity to learn more about these processes in the context of a large-scale ecosystem manipulation. It will be important to develop rigorous, testable hypotheses, and execute a well-conceived research and monitoring program to take advantage of this opportunity, and refine management priorities as the lake responds.

8. Conclusions and challenges ahead

Although P has traditionally been considered the primary nutrient influencing harmful cyanobacterial blooms in freshwater systems, this review of recent findings from laboratory studies and lakes throughout the world demonstrates that N can be important in controlling the timing, density, *and* toxicity of some non-diazotrophic cyanobacterial blooms (Fig. 5). Moreover, some

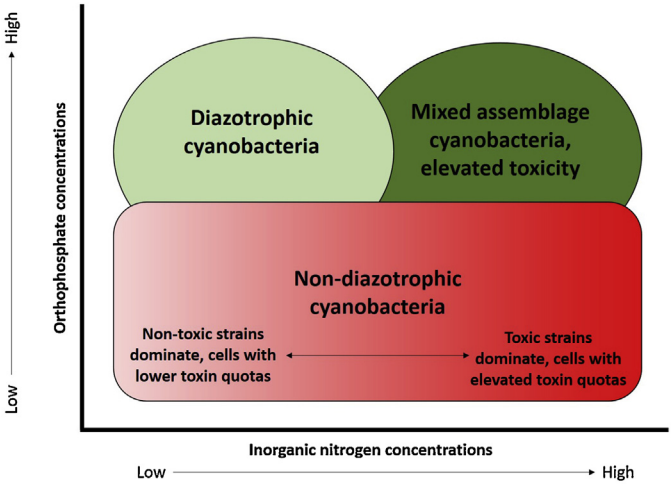

Fig. 5. Conceptual diagram of shifts in cyanobacterial populations that can be facilitated by high and low levels of N and P. While ecosystem-specific exceptions to this general depiction are likely, this paper emphasizes mechanisms that control instances wherein changes in N_i and P_i concentrations facilitate shifts among cyanobacterial populations.

non-diazotrophic cyanobacteria seem well-adapted to low P_i environments (Fig. 5). Finally, it seems clear that high N_i environments favor cyanobacterial capable of synthesizing MC and permit those cells to maximize the amount of toxin synthesized per cell (Fig. 5). Hence, while cyanobacteria dominate high N and high P environments, reducing levels of P, but not N, could result in a shift in cyanobacterial diversity, but not necessarily toxicity (Fig. 5).

There exist important gaps in the understanding of how N influences cyanobacterial blooms. Information is often lacking regarding N speciation within freshwater bodies, especially separate data for NO_3^- and NH_4^+ (Glibert et al., 2011, and references therein), as management efforts often focus on TN (e.g. U.S. Environmental Protection Agency, 2000). This practice can prohibit gaining insights about the influence of specific N species on cyanobacteria and other phytoplankton groups, particularly given that the TN pool can include mostly N contained within cyanobacteria cells and other plankton. In many aquatic ecosystems, a poor understanding of external and internal N loading and biological N_2-fixation rates prevents accurate assessment of the relative importance of N versus P in controlling cyanobacteria toxicity and blooms. In systems where external N loading rates are constrained, assessments of how bloom toxicity is related to N loading, as presented above for Lake Erie, are needed.

Laboratory and ecosystem studies provide many examples of N controlling the biomass and toxicity of cyanobacteria. The extent to which N and/or P controls the density and toxicity of cyanobacterial blooms is likely to vary as a function of watershed N and P loads, lake geomorphology, and resident cyanobacterial assemblages. The relative importance of N_i species versus key components of DON in controlling bloom toxicity remains poorly understood, especially considering the DON concentrations in freshwaters are rarely quantified, despite representing the largest dissolved N pool in aquatic systems (Berman and Bronk, 2003). Research is needed to strengthen insights regarding the competitive outcomes between diazotrophs, non-diazotrophs, and eukaryotic algae under varying N and P regimes and influences of physical and biological interactions such as temperature, mixing, parasitism, and grazing. While Fig. 5 is presented as a framework for understanding the extent to which varying N and P concentrations may influence such competition, there remain significant knowledge gaps on this topic. The precise role of nutrients in driving MC congener production and total bloom toxicity also remains to be determined (e.g., Van de Waal et al., 2009, 2010; Monchamp et al., 2014). For example, do differing N sources and concentrations result in differing MC congeners or overall MC concentrations in an ecosystem setting, or does N affect congener production only indirectly through cyanobacteria assemblage structure?

It is unclear what causes the differing dependencies of toxin synthesis on N availability in N_2-fixing cyanobacteria versus non-N_2 fixing cyanobacteria or other STX-producing algae. Thus far, studies on N regulation of toxin production in cyanobacteria have been limited and, thus, further studies will be required to elucidate the intriguing interplay between N_2-fixing capability and the synthesis of cyanotoxins. A substantial portion of cellular MC and NOD can bind to proteins, particularly when cells are under oxidative stress, suggesting a role of these toxins in protection from free radicals and the potential for oxidative stress to influence their production in conjunction with N (Zilliges et al., 2011; Meissner et al., 2013). Approaches are needed to assess the extent to which shifts in toxin speciation, cellular toxin quotas, and total cellular toxicity result from changes N concentrations and speciation, changes in toxin binding, and/or additional exogenous forcing factors (Meissner et al., 2013).

Finally, the information presented in this review has implications for the management of freshwater bodies. During the past decade, compelling information has accumulated in support of a need to control both N and P to mitigate algal blooms in freshwater and estuarine ecosystems (Conley et al., 2009; Glibert et al., 2011; U.S. Environmental Protection Agency, 2015). Although the U.S. Environmental Protection Agency (2000) required U.S. states to develop numeric nutrient criteria to reduce TN and TP loads to surface waters, most states have not yet developed numeric criteria for N species (Barvenik et al., 2009; U.S. Environmental Protection Agency, 2014; and see http://cfpub.epa.gov/wqsits/nnc-development/). For example, present management practices in states bordering the western basin of Lake Erie call only for reductions in P, not N (Ohio Environmental Protection Agency 2013, Great Lakes Water Quality Agreement Annex 4 Report, 2015). How reductions in N, as well as P, will shape cyanobacterial blooms at an ecosystem level is not yet fully clear, and will depend on a range of abiotic and biotic factors. This review has demonstrated that key, non-diazotrophic cyanobacteria such as *Microcystis* can sustain high biomass even when P_i is near or below detection limits, and that populations can become more toxic within high-N_i environments (Fig. 5). Hence, reductions of both N and P loading will be required to lessen the intensity and toxicity of blooms caused by *Microcystis* and other non-diazotrophs.

Acknowledgments

This work was supported by a Chicago Community Fund grant NOAA-ECOHAB program being funded by the National Oceanic and Atmospheric Center for Sponsored Coastal Ocean Research under award no. NA10NOS4780140 to C.J.G. This is GLERL contribution number 1809 and ECOHAB contribution number 844.[SS]

References

Ame, M.V., Wunderlin, D.A., 2005. Effects of iron, ammonium and temperature on microcystin content by a natural concentrated *Microcystis aeruginosa* population. Water Air Soil Pollut. 168, 235–248.

Anderson, D.M., Kulis, D.M., Sullivan, J.J., Hall, S., Lee, C., 1990. Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. Mar. Biol. 104, 511–524.

Andersson, A., Höglander, H., Karlsson, C., Huseby, S., 2015. Key role of phosphorus and nitrogen in regulating cyanobacterial community composition in the northern Baltic Sea. Estuar. Coast. Shelf Sci. 164, 161–171.

Baldia, S.F., Evangelista, A.D., Aralar, E.V., Santiago, A.E., 2007. Nitrogen and phosphorus utilization in the cyanobacterium *Microcystis aeruginosa* isolated from Laguna de Bay, Philippines. J. Appl. Phycol. 19, 607–613.

Barbiero, R.P., Welch, E.B., 1992. Contribution of benthic blue-green algal recruitment to lake populations and phosphorus translocation. Freshw. Biol. 27, 249–260.

Barvenik, S., Chirigotis, A., Engelberg, D., Fuller, L., Hamann, J., Reed, M., 2009. EPA needs to accelerate adoption of numeric nutrient water quality standards. Evaluation Report 09-P-0223. Office of Inspector General, U.S. Environmental Protection Agency, Washington, DC.

Beaulieu, M., Pick, F., Gregory-Eaves, I., 2013. Nutrients and water temperature as significant predictors of cyanobacterial biomass in a 1147 lakes dataset. Limnol. Oceanogr. 58, 1736–1746.

Beversdorf, L.J., Miller, T.R., McMahon, K.D., 2013. The role of nitrogen fixation in cyanobacterial bloom toxicity in a temperate, eutrophic lake. PLOS ONE 8, e56103, http://dx.doi.org/10.1371/journal.pone.0056103.

Beversdorf, L.J., Miller, T.R., McMahon, K.D., 2015. Long-term monitoring reveals carbon-nitrogen metabolism is key to microcystin production in eutrophic lakes. Front. Ecol. 6, 1–12.

Berman, T., Chava, S., 1999. Algal growth on organic compounds as nitrogen sources. J. Plankton Res. 21, 1423–1437.

Berman, T., Bronk, D.A., 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat. Microb. Ecol. 31, 279–305.

Bertram, P.E., 1993. Total phosphorus and dissolved oxygen trends in the central basin of Lake Erie, 1970–1991. J. Gt. Lakes Res. 19, 224–236.

Bittencourt-Oliveira, M.C., Cabral de Oliveira, M., Bolch, C.J.S., 2001. Genetic variability of Brazilian strains of the *Microcystis aeruginosa* complex (Cyanobacteria/Cyanophyceae) using the phycocyanin intergenic spacer and flanking regions (cpcBA). J. Phycol. 37, 810–818.

Boyer, G.L., Sullivan, J.J., Andersen, R.J., Harrison, P.J., Taylor, F.J.R., 1987. Effects of nutrient limitation on toxin production and composition in the marine dinoflagellate *Protogonyaulax tamarensis*. Mar. Biol. 96, 123–128.

Briand, E., Gugger, M., Francois, J.-C., Bernard, C., Humbert, J.-F., Quiblier, C., 2008. Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming *Planktothrix agardhii* (cyanobacterium) population. Appl. Environ. Microbiol. 74, 3839–3848.

Briand, E., Escoffier, N., Straub, C., Sabart, M., Quiblier, C., Humbert, J.-F., 2009. Spatiotemporal changes in the genetic diversity of a bloom-forming *Microcystis aeruginosa* (cyanobacteria) population. ISME J. 3, 419–429.

Bridgeman, T.B., Chaffin, J.D., 2013. Diversity of *Microcystis* across trophic gradients. Final Report to the Ohio Lake Erie Commission. Lake Erie Center and Department of Environmental Sciences, University of Toledo, Oregon, OH.

Brittain, S.M., Wang, J., Babcock-Jackson, L., Carmichael, W.W., Rinehart, K.L., Culver, D.A., 2000. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. J. Gt. Lakes Res. 26, 241–249.

Brunberg, A.K., Bostrom, B., 1992. Coupling between benthic biomass of *Microcystis* and phosphorus release from the sediments of a highly eutrophic lake. Hydrobiologia 235, 375–385.

Bunting, L., Leavitt, P.R., Gibson, C.E., McGee, E.J., Hall, V.A., 2007. Degradation of water quality in Lough Neagh, northern Ireland, by diffuse nitrogen flux from a phosphorus-rich catchment. Limnol. Oceanogr. 52, 354–369.

Burford, M.A., Davis, T.W., Orr, P.T., Sinha, R., Willis, A., Neilan, B.A., 2014. Nutrient-related changes in the toxicity of field blooms of the cyanobacterium, *Cylindrospermopsis raciborskii*. FEMS Microbiol. Ecol. 89, 135–148.

Burkholder, J.M., Glibert, P.M., 2006. Intraspecific variability: an important consideration in forming generalizations about toxigenic algal species. Afr. J. Mar. Sci. 28, 177–180.

Burkholder, J.M., Shumway, S.E., 2011. Bivalve shellfish aquaculture and eutrophication. In: Shumway, S.E. (Ed.), Shellfish and the Environment. Wiley, New York, NY, pp. 155–215.

Burkholder, J.M., Glibert, P.M., 2013. Eutrophication and oligotrophication.. In: Levin, S. (Ed.), Encyclopedia of Biodiversity, 2nd edition, vol. 3. Academic Press, Waltham, MA, pp. 347–371.

Carlton, R.G., Wetzel, R.G., 1988. Phosphorus flux from lake sediments: effect of epipelic oxygen production. Limnol. Oceanogr. 33, 562–570.

Chaffin, J.D., Bridgeman, T.B., Heckathorn, S.A., Mishra, S., 2011. Assessment of *Microcystis* growth rate potential and nutrient status across a trophic gradient in western Lake Erie. J. Gt. Lakes Res. 37, 92–100.

Chaffin, J.D., Bridgeman, T.B., Bade, D.L., 2013. Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. Adv. Microbiol. 3, 16–26.

Chaffin, J.D., Bridgeman, T.B., Bade, D.L., Mobilian, C.N., 2014. Summer phytoplankton nutrient limitation in Maumee Bay of Lake Erie during high-flow and low-flow years. J. Gt. Lakes Res. 40, 524–531.

Chen, Y.M., Lee, T.H., Lee, S.J., Huang, H.B., Huang, R., Chou, H.N., 2006. Comparison of protein phosphatase inhibition activities and mouse toxicities of microcystins. Toxicon 47, 742–746.

Chorus, I., Bartram, J. (Eds.), 1999. Toxic Cyanobacteria in Water – A Guide to Their Public Health Consequences, Monitoring and Management. E & FN Spon for the World Health Organization, New York, NY.

Codd, G.A., Poon, G.K., 1988. Cyanobacterial toxins. In: Rogers, L.L., Gallon, J.R. (Eds.), Biochemistry of the Algae and Cyanobacteria. Proceedings of the Phytochemistry Society of Europe, vol. 28. Oxford University Press, Oxford, UK, pp. 283–296.

Conley, Daniel, J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Controlling eutrophication: nitrogen and phosphorus. Science 323, 1014–1015.

Conroy, J.D., Edwards, W.J., Pontius, R.A., Kane, D.D., Zhang, H., Shea, J.F., Richey, J.N., Culver, D.A., 2005. Soluble nitrogen and phosphorus excretion of exotic freshwater mussels (*Dreissena* spp.): potential impacts for nutrient remineralisation in western Lake Erie. Freshw. Biol. 50, 1146–1162.

Cottingham, K.L., Ewing, H.A., Greer, M.L., Carrey, C.C., Weathers, K.C., 2015. Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. Ecosphere 6, 1–19.

Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J., 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae 8, 715–725.

Davis, T.W., Harke, M.J., Marcoval, M.A., Goleski, J., Orano-Dawson, C., Berry, D.L., Gobler, C.J., 2010. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. Aquat. Microb. Ecol. 61, 149–162.

Davis, T.W., Orr, P.T., Boyer, G.L., Burford, M.A., 2014. Investigating the production and release of cylindrospermopsin and deoxy-cylindrospermopsin by *Cylindrospermopsis raciborskii* over a natural growth cycle. Harmful Algae 31, 18–25.

Davis, T.W., Bullerjahn, G.S., Tuttle, T., McKay, R.M., Watson, S.B., 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol. 49, 7197–7207.

Dillon, P.J., Rigler, F.H., 1974. The phosphorus–chlorophyll relationship in lakes. Limnol. Oceanogr. 19, 767–773.

Dolman, A.M., Rücker, J., Pick, F.R., Fastner, J., Rohrlack, T., Mischke, U., Wiedner, C., 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. PLOS ONE 7, e38757, http://dx.doi.org/10.1371/journal.pone.0038757.

Donald, D.B., Bogard, M.J., Finlay, K., Leavitt, P.R., 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. 56, 2161–2175.

Downing, J.A., McCauley, E., 1992. The nitrogen: phosphorus relationship in lakes. Limnol. Oceanogr. 37, 936–945.

Downing, T.G., Sember, C.S., Gehringer, M.M., Leukes, W., 2005. Medium N:P ratios and specific growth rate co-modulate microcystin and protein content in *Microcystis aeruginosa* PCC7806 and *M. aeruginosa* UV027. Microb. Ecol. 49, 468–473.

Dyhrman, S.T., Ruttenberg, K.C., 2006. Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: implications for dissolved organic phosphorus remineralization. Limnol. Oceanogr. 51, 1381–1390.

Elser, J.J., Marzolf, E.R., Goldman, C.R., 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci. 47, 1468–1477.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142.

Fastner, J., Erhard, M., von Dohren, H., 2001. Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl. Environ. Microbiol. 67, 5069–5076.

Ferber, L.R., Levine, S.N., Lini, A., Livingston, G.P., 2004. Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? Freshw. Biol. 49, 690–708.

Flores, E., Herrero, A., 2005. Nitrogen assimilation and nitrogen control in cyanobacteria. Biochem. Soc. Trans. 33, 164–167.

Flynn, K., Franco, J.M., Fernandez, P., Reguera, B., Zapata, M., Wood, G., Flynn, K.J., 1994. Changes in toxin content, biomass and pigments of the dinoflagellate *Alexandrium minutum* during nitrogen refeeding and growth into nitrogen or phosphorus stress. Mar. Ecol. Prog. Ser. 111, 99–109.

Fukushima, T., Amano, K., Muraoka, K., 1991. Factors explaining sediment concentrations of 16 elements in 28 Japanese lakes. Water Sci. Technol. 23, 465–474.

Ginn, H.P., Pearson, L.A., Neilan, B.A., 2010. NtcA from *Microcystis aeruginosa* PCC 7806 is autoregulatory and binds to the microcystin promoter. Appl. Environ. Microbiol. 76, 4362–4368.

Giraudet, H., Berthon, J.L., Buisson, B., 1997. Comparison of daily alkaline phosphatase activity of a cyanobacterium (*Microcystis aeruginosa*) and a diatom (*Synedra capitata*). CR Acad. Sci. III 320, 451–458.

Giraudet, H., Abrial, D., Berthon, J.-L., Buisson, B., 1998. Variations nycthémérales de l'activité phosphatase alkaline d'une Diatomée: *Synedra capitata* et de deux Cyanobactéeries: *Microcystis aeruginosa* et *Aphanizomenon flos aquae*. Ann. Limnol. (Int. J. Limnol.). 34, 367–374.

Glibert, P.M., Burkholder, J.M., 2011. Eutrophication and HABs: strategies for nutrient uptake and growth outside the Redfield comfort zone. Chin. J. Oceanol. Limnol. 29, 724–738.

Glibert, P.M., Fullerton, D., Burkholder, J.M., Cornwell, J.C., Kana, T.M., 2011. Ecological stoichiometry, biogeochemical cycling, invasive species and aquatic food webs: San Francisco Estuary and comparative systems. Rev. Fish. Sci. 19, 358–417.

Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2015. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr., http://dx.doi.org/10.1002/lno.10203.

Gobler, C.J., Davis, T.W., Coyne, K.J., Boyer, G.L., 2007. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. Harmful Algae 6, 119–133.

Granéli, E., Flynn, K.J., 2006. Chemical and physical factors influencing toxin content. In: Granéli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer, Berlin, Germany, pp. 229–241.

Harke, M.J., Berry, D.L., Ammerman, J.W., Gobler, C.H., 2012. Molecular response of the bloom-forming cyanobacterium, *Microcystis aeruginosa*, to phosphorus limitation. Microb. Ecol. 63, 188–189.

Harke, M.J., Gobler, C.J., 2013. Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. PLOS ONE 8 (7), E69834, http://dx.doi.org/10.1371/journal.pone.0069834.

Harke, M.J., Davis, T.W., Watson, S.B., Gobler, C.J., 2016. Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys. Environ. Sci. Technol. 50 (2), 604–615.

Hattenrath, T.K., Anderson, D.A., Gobler, C.J., 2010. The influence of nutrients and climate on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) estuary. Harmful Algae 9, 402–412.

Healey, F.P., 1982. Phosphate. In: Carr, N.G., Whitton, B.A. (Eds.), The Biology of Cyanobacteria. University of California Press, Berkeley, CA, pp. 105–124.

Heron, J., 1961. The seasonal variation of phosphate, silicate, and nitrate in waters of the English lake district. Limnol. Oceanogr. 6, 338–346.

Hesse, K., Dittmann, E., Börner, T., 2001. Consequences of impaired microcystin production for light-dependent growth and pigmentation of *Microcystis aeruginosa* PCC 7806. FEMS Microbiol. Ecol. 37, 39–43.

Higgins, S.N., Hecky, R.E., Guildford, S.J., 2006. Environmental controls of *Cladophora* growth dynamics in eastern Lake Erie: application of the Cladophora Growth Model (CGM). J. Gt. Lakes Res. 32, 629–644.

Horst, G.P., Sarnelle, O., White, J.D., Hamilton, S.K., Kaul, R.B., Bressie, J.D., 2014. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. Water Res. 54, 188–198.

Hupfer, M., Lewandowski, J., 2008. Oxygen controls the phosphorus release from lake sediments – a long-lasting paradigm in limnology. Int. Rev. Hydrobiol. 93, 414–432.

Jacobson, L., Halmann, M., 1982. Polyphosphate metabolism in the blue-green alga *Microcystis aeruginosa*. J. Plankton Res. 4, 481–488.

Janse, I., Kardinaal, W.E.A., Kamst-van Agterveld, M., Meima, M., Visser, P.M., Zwart, G., 2005. Contrasting microcystin production and cyanobacterial population dynamics in two *Planktothrix*-dominated freshwater lakes. Environ. Microbiol. 7, 1514–1524.

John, E.H., Flynn, K.J., 2000. Growth dynamics and toxicity of *Alexandrium fundyense* (Dinophyceae): the effect of changing N:P supply ratios on internal toxin and nutrient levels. Eur. J. Phycol. 35, 11–23.

Jones, J., Bachmann, R., 1976. Prediction of phosphorus and chlorophyll levels in lakes. J. Water Pollut. Control Fed. 48, 2176–2184.

Kabir, A.H., El-Shehawy, R., 2012. Expression of nifH, hetR, and ndaF genes in *Nodularia spumigena* under nitrogen sources. Plant Biosyst. 146, 992–1000.

Kardinaal, W.E.A., Janse, I., Kamst-van Agterveld, M., Meima, M., Snoek, J., Mur, L.R., Huisman, J., Zwart, G., Visser, P.M., 2007. *Microcystis* genotype succession in relation to microcystin concentrations in freshwater lakes. Aquat. Microb. Ecol. 48, 1–12.

Kellmann, R., Mihali, T.K., Jeon, Y.J., Pickford, R., Pomati, F., Neilan, B.A., 2008. Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. Appl. Environ. Microbiol. 74, 4044–4053.

Kolowith, L.C., Ingall, E.D., Benner, R., 2001. Composition and cycling of marine organic phosphorus. Limnol. Oceanogr. 46, 309–320.

Kosten, S., Huszar, V.L.M., Bécares, E., Costa, L.S., van Donk, E., Hansson, L.-A., Jeppesen, E., Kruk, C., Lacerot, G., Mazzeo, N., de Meester, L., Moss, B., Lürling, M., Nõges, T., Romo, S., Scheffer, M., 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biol. 18, 118–126.

Kromkamp, J., van den Heuvel, A., Mur, L.R., 1989. Phosphorus uptake and photosynthesis by phosphate-limited cultures of the cyanobacterium *Microcystis aeruginosa*. Br. Phycol. J. 24, 347–355.

Lee, S.J., Jang, M.-H., Kim, H.-S., Yoon, B.-D., Oh, H.-M., 2000. Variation on microcystin content of *Microcystis aeruginosa* relative to medium N:P ratio and growth stage. J. Appl. Microbiol. 89, 323–329.

Lewis, W.M., Wurtsbaugh, W.A., 2008. Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. Int. Rev. Hydrobiol. 93, 446–465.

Long, B.M., Jones, G.J., Orr, P.T., 2001. Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. Appl. Environ. Microbiol. 67, 278–283.

McCarthy, M.J., James, R.T., Chen, Y., East, T.L., Gardner, W.S., 2009. Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical lakes Okeechobee (FL, USA) and Taihu (China). Limnology 10, 215–227.

Meissner, S., Fastner, J., Dittmann, E., 2013. Microcystin production revisited: conjugate formation makes a major contribution. Environ. Microbiol. 15, 1810–1820.

Meissner, S., Steinhauser, D., Dittmann, E., 2015. Metabolomic analysis indicates a pivotal role of the hepatotoxin microcystin in high light adaptation of *Microcystis*. Environ. Microbiol. 17, 1497–1509.

Monchamp, M.-E., Pick, F.R., Beisner, B.E., Maranger, R., 2014. Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. PLOS ONE 9, e85573, http://dx.doi.org/10.1371/journal.pone.0085573.

Nagata, S., Tsutsumi, T., Hasegawa, A., Yoshida, F., Ueno, Y., Watanabe, M.F., 1997. Enzyme immunoassay for direct determination of microcystins in environmental water. J. AOAC Int. 80, 408–417.

Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., Dittmann, E., 2013. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environ. Microbiol. 15, 1239–1253.

Obenour, D.R., Gronewold, A.D., Stow, C.A., Scavia, D., 2014. Using a Bayesian hierarchical model to improve Lake Erie cyanobacteria bloom forecasts. Water Resour. Res. 50, 7847–7860.

O'Neill, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.

Orr, P.T., Jones, G.J., 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. Limnol. Oceanogr. 43, 1604–1614.

Orr, P.T., Rasmussen, J.P., Burford, M.A., Eaglesham, G.K., Lennox, S.M., 2010. Evaluation of quantitative real-time PCR to characterise spatial and temporal variations in cyanobacteria, *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya et Subba Raju and cylindrospermopsin concentrations in three subtropical Australian reservoirs. Harmful Algae 9, 243–254.

Otten, T.G., Xu, H., Qin, B., Zhu, G., Paerl, H.W., 2012. Spatiotemporal patterns and ecophysiology of toxigenic *Microcystis* blooms in Lake Taihu, China: implications for water quality management. Environ. Sci. Technol. 46, 3480–3488.

Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y., Gardner, W.S., 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): the need for a dual nutrient (N & P) management strategy. Water Res. 45, 1973–1983.

Paerl, H.W., Otten, T.G., 2015. Dueling "CyanoHABs": unraveling the environmental drivers controlling dominance and succession among diazotrophic and non-N_2-fixing harmful cyanobacteria. Environ. Microbiol., http://dx.doi.org/10.1111/1462-2920.13035.

Pimentel, J.S.M., Giani, A., 2014. Microcystin production and regulation under nutrient stress conditions in toxic *Microcystis* strains. Appl. Environ. Microbiol. 80, 5836–5843.

Pitt, F.D., Mazard, S., Humphreys, L., Scanlan, D.J., 2010. Functional characterization of *Synechocystis* sp. strain PCC 6803 pst1 and pst2 gene clusters reveals a novel strategy for phosphate uptake in a freshwater cyanobacterium. J. Bacteriol. 192, 3512–3523.

Reitzel, K., Ahlgren, J., Debrabandere, H., Waldebäk, M., Gogoll, A., Tranvik, L., 2007. Degradation rates of organic phosphorus in lake sediments. Biogeochemistry 82, 15–28.

Reynolds, C.S., Walsby, A.E., 1975. Water blooms. Biol. Rev. 50, 437–481.

Reynolds, C.S., 1984. The Ecology of Freshwater Phytoplankton. Cambridge Studies in Ecology. Cambridge University Press, New York, NY.

Saker, M.L., Neilan, B.A., 2001. Varied diazotrophies, morphologies, and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from northern Australia. Appl. Environ. Microbiol. 67, 1839–1845.

Saxton, M.A., Arnold, R.J., Bourbonniere, R.A., McKay, R.M., Wilhelm, S.W., 2012. Plasticity of total and Intracellular phosphorus quotas in *Microcystis aeruginosa* cultures and Lake Erie algal assemblages. Front. Microbiol. 3, 1–9.

Sbiyyaa, B., Loudiki, M., Oudra, B., 2009. Nitrogen and phosphorus intracellular capacity in storage by *Microcystis aeruginosa* Kütz. and *Synechocystis* sp.: toxic cyanobacteria occasionally forming blooms in Marrakesch area (Morocco). Int. J. Limnol. 34, 247–257.

Schindler, D.W., 1974. Eutrophication and recovery in experimental lakes: implications for lake management. Science 184, 897–899.

Schindler, D.W., 1977. Evolution of phosphorus limitation in lakes. Science 195, 260–262.

Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty, K.G., Lyng, M., Kasian, S.E.M., 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc. Nat. Acad. Sci. U. S. A. 105, 11254–11258.

Schindler, D.W., 2012. The dilemma of controlling cultural eutrophication of lakes. Proc. R. Soc. Lond. B 279, 4322–4333.

Schmidt, J.R., Wilhelm, S.W., Boyer, G.L., 2014. The fate of microcystins in the environment and challenges for monitoring. Toxins 6, 3354–3387.

Scott, J.T., McCarthy, M.J., 2010. Nitrogen fixation may not balance the nitrogen pool of lakes over timescales relevant to eutrophication management. Limnol. Oceanogr. 55, 1265–1270.

Scott, J.T., McCarthy, M.J., 2011. Response to comment: nitrogen fixation has not offset declines in the Lake 227 nitrogen pool and shows that nitrogen control deserves consideration in aquatic ecosystems. Limnol. Oceanogr. 56, 1548–1550.

Shalev-Malul, G., Lieman-Hurwitz, J., Viner-Mozzini, Y., Sukenik, A., Gaathon, A., Lebendiker, M., Kaplan, A., 2008. An AbrB-like protein might be involved in the regulation of cylindrospermopsin production by *Aphanizomenon ovalisporum*. Environ. Microbiol. 10, 988–999.

Singh, S., Rai, P.K., Chau, R., Ravi, A.K., Neilan, B.A., Asthana, R.K., 2015. Temporal variations in microcystin-producing cells and microcystin concentrations in two freshwater ponds. Water Res. 69, 131–142.

Sivonen, K., Jones, G., 1999. Cyanobacterial toxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. E. & F.N. Spon, London, UK, pp. 41–111.

Smayda, T.J., 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42, 1137–1153.

Smith, V.H., 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in lake phytoplankton. Science 221, 669–671.

Søndergaard, M., Jensen, J.P., Jeppesen, E., 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia 506, 135–145.

Sterner, R.W., 2008. On the phosphorus limitation paradigm for lakes. Int. Rev. Hydrobiol. 93, 433–445.

Stow, C.A., Cha, Y.K., Johnson, L.T., Confesor, R., Richards, R.P., 2015. Long-term and seasonal trend decomposition of Maumee River nutrient inputs to western Lake Erie. Environ. Sci. Technol. 49, 3392–3400.

Štrojsová, A., Vrba, J., Nedoma, J., Šimek, K., 2005. Extracellular phosphatase activity of freshwater phytoplankton exposed to different in situ phosphorus concentrations. Mar. Freshw. Res. 56, 417–424.

Stucken, K., John, U., Cembella, A., Murillo, A.A., Soto-Liebe, K., Fuentes-Valdés, J.J., Friedel, M., Plominsky, Á.M., Vásquez, M., Glöckner, G., 2010. The smallest known genomes of multicellular and toxic cyanobacteria: comparison, minimal gene sets for linked traits and the evolutionary implications. PLoS ONE 5 (2), e9235, http://dx.doi.org/10.1371/journal.pone.0009235.

Stucken, K., John, U., Cembella, A., Soto-Liebe, K., Vásquez, M., 2014. Impact of nitrogen sources on gene expression and toxin production in the diazotroph *Cylindrospermopsis raciborskii* CS-505 and non-diazotroph *Raphidiopsis brookii* D9. Toxins 6, 1896–1915.

Stumpf, R.P., Wynne, T.T., Baker, D.B., Fahnenstiel, G.L., 2012. Interannual variability of cyanobacterial blooms in Lake Erie. PLOS ONE 7 (8), e42444, http://dx.doi.org/10.1371/journal.pone.0042444.

Sunda, W.G., Granéli, E., Gobler, C.J., 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. J. Phycol. 42, 963–974.

Takamura, N., Iwakuma, T., Yasuno, M., 1987. Uptake of ^{13}C and ^{15}N (ammonium, nitrate and urea) by *Microcystis* in Lake Kasumigaura. J. Plankton Res. 9, 151–165.
Tapia, M.I., deAlda, J., Llama, M.J., Serra, J.L., 1996. Changes in intracellular amino acids and organic acids induced by nitrogen starvation and nitrate or ammonium resupply in the cyanobacterium *Phormidium laminosum*. Planta 198, 526–531.
Tillett, D., Dittmann, E., Erhard, M., von Dohren, H., Borner, T., Neilan, B.A., 2000. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide–polyketide synthetase system. Chem. Biol. 7, 753–764.
Trimbee, A.M., Prepas, E.E., 1987. Evaluation of total phosphorus as a predictor of the relative biomass of blue-green-algae with emphasis on Alberta lakes. Can. J. Fish. Aquat. Sci. 44, 1337–1342.
Turner, R.E., Rabalais, N.N., Justic', D., Dortch, Q., 2003. Global patterns of dissolved N, P and Si in large rivers. Biogeochemistry 64, 297–317.
United States Environmental Protection Agency (U.S. EPA), 2000. Nutrient Criteria Technical Guidance Manual, Rivers and Streams. Report EPA-822-B-00-002. Office of Water, U.S. EPA, Washington, DC. Available at: http://www.epa.gov/waterscience/criteria/nutrient/guidance/rivers (accessed 11/15).
United States National Academy of Sciences (USNAS), 1969. Eutrophication: causes, consequences, correctives. In: Proceedings of a Symposium. National Academy of Sciences Publication 1700. National Academy Press, Washington, DC.
United States Environmental Protection Agency (U.S. EPA), 2014. State development of numeric criteria for nitrogen and phosphorus pollution. Office of Water, U.S. EPA, Washington, DC. Available at: http://cfpub.epa.gov/wqsits/nnc-development/ (accessed November 2015).
United States Environmental Protection Agency (U.S. EPA), 2015. Preventing eutrophication: scientific support for dual nutrient criteria. Office of Water, U.S. EPA, Washington, DC.
Van de Waal, D.B., Verspagen, J.M.H., Lurling, M., Van Donk, E., Visser, P.M., Huisman, J., 2009. The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. Ecol. Lett. 12, 1326–1335.
Van de Waal, D.B., Ferreruela, G., Tonk, L., Van Donk, E., Huisman, J., Visser, P.M., Matthijs, H.C.P., 2010. Pulsed nitrogen supply induces dynamic changes in the amino acid composition and microcystin production of the harmful cyanobacterium *Planktothrix agardhii*. FEMS Microbiol. Ecol. 74, 430–438.
Van de Waal, D.B., Tillmann, U., Zhu, M., Koch, B.P., Rost, B., John, U., 2013. Nutrient pulse induces dynamic changes in cellular C:N:P, amino acids, and paralytic shellfish poisoning toxins in *Alexandrium tamarense*. Mar. Ecol. Prog. Ser. 493, 57–69.
Van de Waal, D.B., Smith, V.H., Declerck, S.A.J., Stam, E.C.M., Elser, J.J., 2014. Stoichiometric regulation of phytoplankton toxins. Ecol. Lett. 17, 736–742.
Van Mooy, B.A.S., Rocap, G., Fredricks, H.F., Evans, C.T., Devol, A.H., 2006. Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. Proc. Nat. Acad. Sci. U. S. A. 103, 8607–8612.
Verspagen, J.M.H., Snelder, E.O.F.M., Visser, P.M., Huisman, J., Mur, L.R., Ibelings, B.W., 2004. Recruitment of benthic *Microcystis* (Cyanophyceae) to the water column: internal buoyancy changes or resuspension? J. Phycol. 40, 260–270.
Vézie, C., Rapala, J., Vaitomaa, J., Seitsonen, J., Sivonen, K., 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations. Microb. Ecol. 43, 443–454.
Visser, P.M., Passarge, J., Mur, L.R., 1997. Modelling vertical migration of the cyanobacterium *Microcystis*. Hydrobiologia 349, 99–109.
Watanabe, M.F., Oishi, S., 1985. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. Appl. Environ. Microbiol. 49, 1342–1344.
Watson, S.B., McCauley, E., Downing, J.A., 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnol. Oceanogr. 42, 487–495.
Welker, M., von Dohren, H., Tauscher, H., Steinberg, C.E.W., Erhard, M., 2003. Toxic *Microcystis* in shallow Lake Muggelsee (Germany) – dynamics, distribution, diversity. Arch. Hydrobiol. 157, 227–248.
Welker, M., von Döhren, H., 2006. Cyanobacterial peptides: nature's own combinatorial biosynthesis. FEMS Microbiol. Rev. 30, 530–563.
Welker, M., Šejnohová, L., Némethová, D., von Döhren, H., Jarkovský, J., Maršálek, B., 2007. Seasonal shifts in chemotype composition of *Microcystis* sp. communities in the pelagial and the sediment of a shallow reservoir. Limnol. Oceanogr. 52, 609–619.
Wetzel, R.G., 1983. Limnology, 2nd edition. W.B. Saunders, Fort Worth, TX.
Wetzel, R.G., 2001. Limnology: Lake and River Ecosystems, 3rd edition. Academic Press, San Diego, CA.
Whitton, B.A., Grainger, S.L.J., Hawley, G.R.W., Simon, J.W., 1991. Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb. Ecol. 21, 85–98.
Wilhelm, S.W., BeBruyn, J.M., Gillor, O., Twiss, M.R., Livingston, K., Bourbonniere, R.A., Pickell, L.D., Trick, C.G., Dean, A.L., McKay, R.M., 2003. Effect of phosphorus amendments on present day plankton communities in pelagic Lake Erie. Aquat. Microb. Ecol. 32, 275–285.
World Health Organization (WHO), 2011. Management of cyanobacteria in drinking-water supplies: Information for regulators and water suppliers. WHO, Geneva, Switzerland. Available at: http://www.who.int/water_sanitation_health/dwq/cyanobacteria_in_drinking-water.pdf (accessed November 2015).
Wynne, T.T., Stumpf, R.P., 2015. Spatial and temporal patterns in the seasonal distribution of toxic cyanobacteria in western Lake Erie from 2002–2014. Toxins (Basel) 7, 1649–1663.
Xie, P., 2006. Biological mechanisms driving the seasonal changes in the internal loading of phosphorus in shallow lakes. Sci. China Ser. D: Earth Sci. 49, 14–27.
Xu, H., Paerl, H.W., Qin, B.Q., Zhu, G.W., Gao, G., 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnol. Oceanogr. 55, 420–432.
Yuan, L.L., Pollard, A.I., Pather, S., Oliver, J.L., D'Anglada, L., 2014. Managing microcystin: identifying national-scale thresholds for total nitrogen and chlorophyll *a*. Freshw. Biol. 59, 1970–1981.
Zhang, H., Culver, D.A., Boegman, L., 2008. A two-dimensional ecological model of Lake Erie: application to estimate dreissenid impacts on large lake plankton populations. Ecol. Model. 214, 219–241.
Zilliges, Y., Kehr, J.-C., Meissner, S., Ishida, K., Mikkat, S., Hagemann, M., Kaplan, A., Boerner, T., Dittmann, E., 2011. The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. PLoS ONE 6 (3), e17615, http://dx.doi.org/10.1371/journal.pone.0017615.
Zurawell, R.W., Chen, H., Burke, J.M., Prepas, E.E., 2005. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J. Toxicol. Environ. Health B 8, 1–37.