

Minireview

Duelling ‘CyanoHABs’: unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N₂-fixing harmful cyanobacteria

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Summary

Eutrophication often manifests itself by increased frequencies and magnitudes of cyanobacterial harmful algal blooms (CyanoHABs) in freshwater systems. It is generally assumed that nitrogen-fixing cyanobacteria will dominate when nitrogen (N) is limiting and non-N₂ fixers dominate when N is present in excess. However, this is rarely observed in temperate lakes, where N₂ fixers often bloom when N is replete, and non-fixers (e.g. *Microcystis*) dominate when N concentrations are lowest. This review integrates observations from previous studies with insights into the environmental factors that select for CyanoHAB groups. This information may be used to predict how nutrient reduction strategies targeting N, phosphorus (P) or both N and P may alter cyanobacterial community composition. One underexplored concern is that as N inputs are reduced, CyanoHABs may switch from non-N₂ fixing to diazotrophic taxa, with no net improvement in water quality. However, monitoring and experimental observations indicate that in eutrophic systems, minimizing both N and P loading will lead to the most significant reductions in total phytoplankton biomass without this shift occurring, because successional patterns appear to be strongly driven by physical factors, including temperature, irradiance and hydrology. Notably, water temperature is a primary driver of cyanobacterial community succession, with warming favouring non-diazotrophic taxa.

Introduction

A global proliferation of harmful (toxin-producing, hypoxia-generating, food web disrupting and otherwise noxious) cyanobacterial blooms (CyanoHABs) is underway in response to anthropogenic nutrient over-enrichment and climatic changes (Huisman *et al.*, 2005; Paerl and Huisman, 2008; 2009; O’Neil *et al.*, 2012; Paerl and Paul, 2012), including warming and increased frequency of extreme hydrologic events (storms, floods and droughts) (Trenberth, 2005; IPCC, 2012). This course of events is of great concern with regard to providing access to safe drinking, fishing and recreational waters for a burgeoning human population, and ensuring biodiversity and sustainability of impacted aquatic ecosystems (Paerl and Otten, 2013a,b).

While most CyanoHABs produce cyanotoxins, the human health risks associated with individual taxa can be quite variable. Although the unicellular and colonial genus *Microcystis* is one of the most common sources of surface blooms, the only cyanotoxin it has been found to produce is microcystin (Otten and Paerl, 2015). In contrast, most of the filamentous N₂-fixing and non-diazotrophic genera are capable of producing any combination of the four major groups of cyanotoxins, namely anatoxin-a, cylindrospermopsin, microcystin and saxitoxin (Otten and Paerl, 2015). Filamentous genera are also the most common producers of the taste-and-odor compounds geosmin and 2-methylisoborneol (MIB) in surface waters (Jüttner and Watson, 2007). Thus, from a water quality management perspective, filamentous cyanobacteria may be considered a greater nuisance than *Microcystis* blooms because for each event they necessitate analysis for multiple classes of cyanotoxins – often at a considerable expense.

From both ecological and societal perspectives, there is a pressing need to control and ultimately reverse the troubling trend of cyanobacterial expansion. We cannot alter the effects of climate change in the short term; therefore, reducing nutrient inputs is the principal means for controlling CyanoHAB proliferation (Paerl, 2008).

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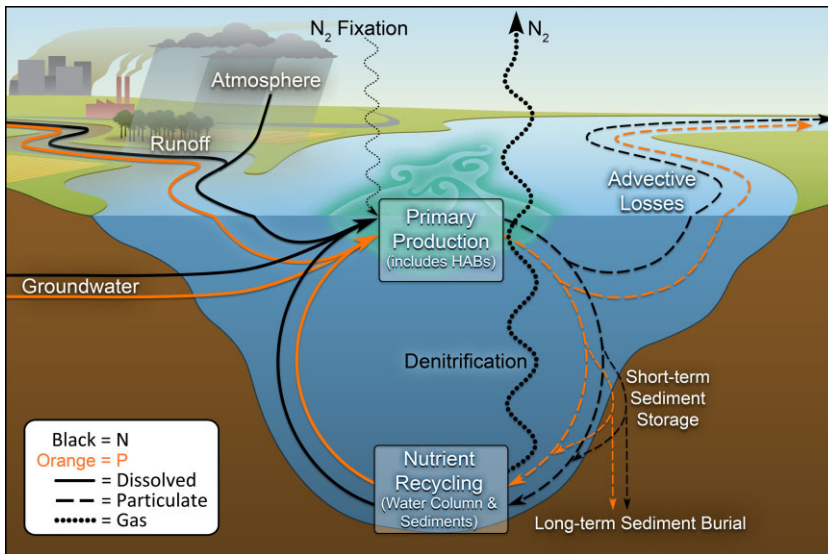


Fig. 1. Conceptual diagram, illustrating nitrogen (N) and phosphorus (P) inputs, outputs and storage in aquatic ecosystems. Note that only N has gaseous forms (dotted lines) that can exchange with the atmosphere. Figure adapted from Paerl (2014).

Moving beyond the phosphorus limitation paradigm

Traditional approaches for CyanoHAB control have focused on reducing phosphorus (P) inputs to impaired freshwater systems, based on the fact that excessive P relative to N inputs (or low N : P ratios) were linked to a tendency for systems to be dominated by cyanobacteria (Schindler, 1975; Smith, 1983; 1990; Paerl, 2008). In part, this relationship was based on the fact that some common CyanoHAB genera (e.g. *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia*) can fix atmospheric N₂ into biologically available NH₃, which could support the N requirements of bloom populations (Schindler *et al.*, 2008).

Indeed, the introduction of P input constraints in the 1970s as a means of controlling eutrophication proved to be a scientific and environmental success (Sterner, 2008). However, much has changed with regard to nutrient sources since then. While efforts to reduce freshwater P inputs (e.g. P detergent bans, improved wastewater P treatment) were underway, N loads were increasing dramatically in watersheds and airsheds globally, spurred on by the accelerating use of synthetic N fertilizers, increasing amounts of N-enriched urban and rural wastewaters, atmospheric N deposition from fossil fuel combustion, and slash and burn agriculture (Galloway and Cowling, 2002; Paerl, 2008). CyanoHABs have responded with increasing incidence and magnitudes of non-N₂-fixing (i.e. requiring exogenous N) genera, principally including *Microcystis*, *Planktothrix*, *Oscillatoria*, *Lyngbya* and *Pseudanabaena* (cf. Paerl and Otten, 2013a,b). Given the observed increases in non-diazotrophic CyanoHABs, and increasing evidence of N-limited and N&P co-limited freshwater systems worldwide (Elser *et al.*, 2007; Paerl, 2014), both N and P reductions are being advanced as

best management practices to stem eutrophication and CyanoHAB proliferation (Lewis and Wurtsbaugh, 2008; Conley *et al.*, 2009; Xu *et al.*, 2010; Lewis *et al.*, 2011; Paerl and Paul, 2012; Paerl, 2014; US EPA, 2015).

Instituting N input reduction strategies for CyanoHAB control has been questioned by some as being an unnecessary and costly endeavour, based on the assumption that microbial community N requirements are met by endemic N₂-fixing cyanobacteria (Schindler *et al.*, 2008; Schindler, 2012). However, this assumption has been challenged by Scott and McCarthy (2010) and more recently by others (cf. Lewis *et al.*, 2011; Paerl *et al.*, 2014), who have shown that aquatic microbial N₂ fixation is not only controlled by P inputs, but also by the energetic requirements needed to power this metabolically expensive process. Nitrogen fixation requires an adequate supply of bioavailable iron and trace metals requisite for the enzymatic reaction and undersaturated dissolved oxygen conditions, which will otherwise inhibit nitrogenase activity (Paerl, 1990). These factors control how much N₂ can be fixed to meet the ecosystem-level demands; the percentages appear to generally be less than 50% and often closer to 25% of ecosystem needs (Howarth *et al.*, 1988; Larsson *et al.*, 2001; Paerl and Scott, 2010). Furthermore, in aquatic ecosystems in which both N₂ fixation (a biologically-mediated N source) and denitrification (a biologically mediated N sink) have been assessed, denitrification often is the dominant N flux process (i.e. net loss of N) (Paerl and Scott, 2010) (Fig. 1).

Because N can be transformed into gaseous forms, whereas P has no significant gaseous state, P inputs tend to be retained by receiving waters, while N inputs may be volatilized to the atmosphere (via denitrification and ammonification). This retention of P relative to N

enhances N limitation when neither nutrient is saturating. In addition, high N loads interfere with P burial in sediments, resulting in enhanced internal P loading (Smolders *et al.*, 2010). This imbalance further perpetuates N limitation. Hence, external N inputs, by augmenting (and exceeding) ecosystem needs, play a central and continuous role in controlling eutrophication and CyanoHAB potentials (Fig. 1).

Environmental factors influencing CyanoHAB dominance and population succession

Most productive temperate lakes exhibit a seasonal phytoplankton cycle in which diatom blooms during the winter and spring sequester phosphate (PO_4^-) to the point that P becomes the principal growth-limiting nutrient. During this period, spring rains and runoff often result in a surplus of N entering the lake. Even though N may be abundant at the onset of the summer's first cyanobacterial blooms, a typical pattern is for diazotrophs, such as *Aphanizomenon* or *Anabaena*, to occur first followed by non- N_2 -fixing genera – most notably *Microcystis* sp. (McDonald and Lehman, 2013; Wu *et al.*, 2015). This succession pattern was also observed during a high frequency sampling survey of several US Midwestern lakes (Miller *et al.*, 2013). It was concluded that *Aphanizomenon* and *Microcystis* are significantly anti-correlated with one another and that the former was more likely to occur earlier in the season when nutrient concentrations (both dissolved inorganic N; DIN and soluble reactive P; SRP) were higher (Miller *et al.*, 2013). However, this pattern is counterintuitive, since N_2 fixers are expected to dominate when DIN concentrations are lowest (Schindler, 2012).

It has been suggested that following the period of *Aphanizomenon* dominance, a pulse of bioavailable N and P is released that fuels the subsequent rise of non-diazotrophs, such as *Microcystis* (e.g. Beversdorf *et al.*, 2013). This scenario implies that a significant percentage of the standing stock of cyanobacteria is lysed by an unclear mechanism. It is well established that in unfavourable conditions, N_2 fixers of the order Nostocales can form specialized resting cells, or akinetes, that remain vegetative until conditions are favourable for them to recruit back into the water column and form future blooms (Rücker *et al.*, 2009). However, since only a fraction of the cells within a trichome form akinetes, the rest presumably undergo lysis, although the fate of these non-differentiated cells has not been reported to our knowledge. It is possible they are lysed by competing, allelopathic bacteria, consumed by zooplankton, lysed by cyanophages, or undergo programmed cell death (Bayles, 2014), the latter possibly driven by the mosaic array of toxin–antitoxin systems harboured within most CyanoHAB genomes.

If a major component of the cyanobacterial community were to lyse and release a large pulse of N and P compounds into the water column, there is no certainty that non-diazotrophic cyanobacteria would garner a significant portion of these nutrients. The nitrogen cycle is complex, and labile organic and inorganic N compounds from a bloom collapse would be rapidly transformed and uptaken by myriad nitrifying and denitrifying bacteria that are also competing with phytoplankton for this resource (Fig. 1). *Microcystis* is adept at sequestering both dissolved inorganic forms of nitrogen (DIN; as NO_3^- and NH_3^+) and organic forms, including urea, amino acids and other high molecular weight organic compounds (Blomqvist *et al.*, 1994; Hyenstrand *et al.*, 1998; Davis *et al.*, 2010), and specific algal decomposition byproducts (Wang *et al.*, 2010). The observation that *Microcystis* often ends up the dominant phytoplankton in mesocosm experiments in which no nutrients are added (Berman and Chava, 1999; Wang *et al.*, 2010) attests to its competitiveness under nutrient-limiting (both N and P) conditions. This scenario has been investigated in reservoirs throughout the Klamath River system (Oregon/California), where dense early-summer blooms of *Aphanizomenon* often yield to *Microcystis* blooms in late summer. However, in this system, $\delta^{15}\text{N}$ tracer studies suggest the *Microcystis* population is likely sustained by sources of N other than from N_2 fixers (Moisander *et al.*, 2009), including sediment- and water-column regeneration of previously supplied N.

Another potential driver of cyanobacterial succession, and especially the *Aphanizomenon* to *Microcystis* co-dominance pattern, may be temperature. Culture-based experiments indicate that *Microcystis* has higher maximum growth rates at warmer temperatures than *Aphanizomenon flos-aquae* (Paerl *et al.*, 2011a; Fig. 2A). In order to test if temperature shifts favour one genus over another, we analysed a long-term water quality and phylogenetic dataset from two reservoirs along the Klamath River, CA, USA (Copco Reservoir and Iron Gate Reservoir), which exhibited oscillating dominance between *Aphanizomenon flos-aquae* and *Microcystis* sp. The dataset spanned 14 years (2001–2014), comprised 192 discrete samples (0.5 m depth) and was collected as part of the Klamath Hydroelectric Settlement Agreement (KHSA, 2010; details regarding sample collection and processing are available online: KBGAWG, 2009). An analysis of water temperature relative to average cell abundance for each genus corroborated the culture-based observations; the *Aphanizomenon* population in the Klamath system tended to reach peak dominance at water temperatures of $\sim 24^\circ\text{C}$ before abating, whereas the *Microcystis* population showed little indication of peaking at the maximum water temperature observed (26.5°C) over the study period. Relative to water

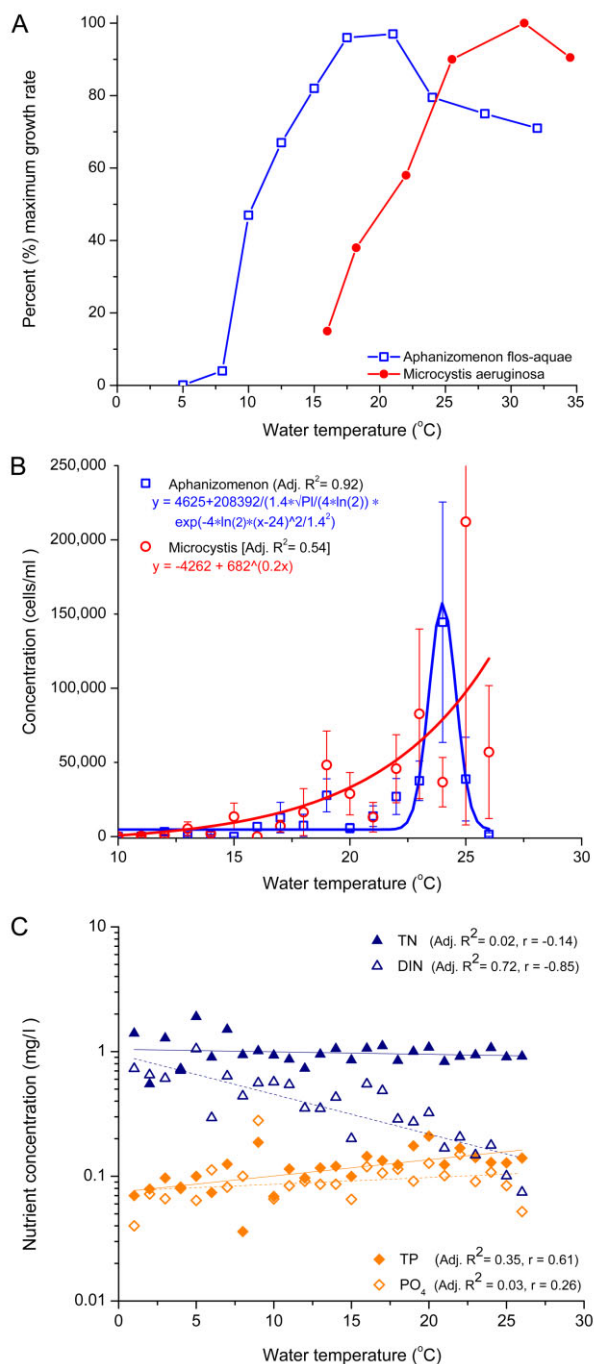


Fig. 2. (A) Estimated maximum growth rates relative to water temperature for *Microcystis aeruginosa* (red) and *Aphanizomenon flos-aquae* (blue) based on culture experiments. Adapted from Paerl *et al.* (2011b). (B) Comparison of mean abundance (cells/ml) of *Aphanizomenon flos-aquae* (blue) and *Microcystis* sp. (red) from Copco and Iron Gate reservoirs on the Klamath River (CA, USA) over a 14-year period. (C) Mean nutrient concentrations (TN – total nitrogen; DIN – dissolved inorganic nitrogen; TP – total phosphorus; PO₄ – orthophosphate as P) relative to water temperature for the Klamath River reservoirs over the study period.

temperature, *Aphanizomenon* abundance exhibited a Gaussian distribution, whereas the *Microcystis* population was best fit with an exponential growth equation (Fig. 2B), suggesting that warmer water temperatures would further favour its growth over *Aphanizomenon*.

This analysis also addressed potential nutrient drivers of this co-dominance pattern (Fig. 2C). As reported elsewhere (Miller *et al.*, 2013), *Aphanizomenon* was dominant at slightly higher DIN concentrations than non-N₂-fixing *Microcystis*, and both were abundant when DIN : PO₄ (as mg/l N or P) was less than 2. The lack of a detectable increase in total nitrogen before, during or after the *Aphanizomenon* blooms suggests that this N₂ fixer is not significantly augmenting the nitrogen pool in this system, and the competitive dominance of *Microcystis* later in the season is most likely explained by its superior nutrient uptake capability and/or its preference for warmer temperatures.

To gain more insight into the putative role of temperature and the dominance of specific cyanobacterial genera, an analysis of phylogenetic and temperature data from 1252 lake samples collected from 1028 lakes during the 2007 US EPA National Lakes Assessment (http://water.epa.gov/type/lakes/NLA_data.cfm) was performed. Cell abundance was compared relative to surface water temperature for six of the most common CyanoHAB genera, including the N₂ fixers *Anabaena* and *Aphanizomenon* and the non-diazotrophs *Lyngbya*, *Microcystis*, *Oscillatoria* and *Phormidium* (Fig. 3). This analysis reinforced the stereotypical moniker for the 'usual suspects' involved in cyanobacterial blooms: 'Annie, Fannie and Mike'. *Anabaena* and *Microcystis* were the most commonly observed CyanoHAB taxa, identified in 77% and 62% of samples, respectively, with the latter reaching the highest cell concentrations of any cyanobacteria in the dataset. The third most abundant taxa were *Aphanizomenon* and *Oscillatoria*, which exhibited similar incidence (36%), followed by *Lyngbya* (25%) and *Pseudanabaena* (10%). Applying a logistic regression trend line to each dataset revealed a weak, inverse association between cell abundance and temperature for the two N₂ fixers, whereas all of the non-diazotrophic genera exhibited a weak, positive association with increasing temperature (Fig. 3).

Combined, these analyses show that cyanobacterial proliferations may occur across a range of water temperatures and that significant competitive advantages are likely only exploited at the upper and lower bounds of a given strain's tolerance. That a number of samples exhibited moderately high *Aphanizomenon* cell concentrations at temperatures well above 24°C – the observed temperature maxima in the Klamath River dataset – suggests that regional ecotypes with differential sensitivities to temperature likely exist.

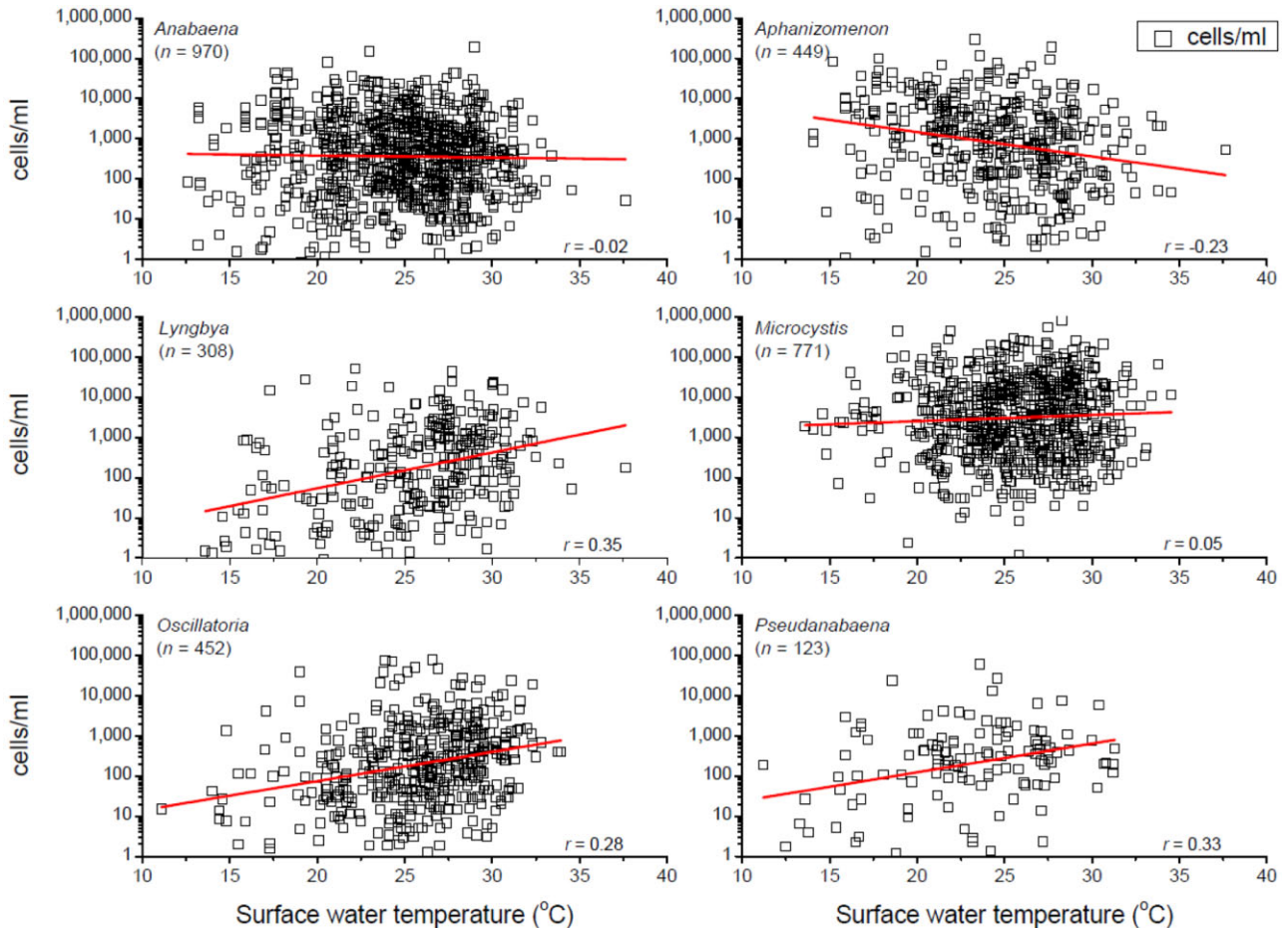


Fig. 3. Comparison of cyanobacterial cell abundance relative to temperature for six common CyanoHAB genera. Data from the 2007 EPA National Lakes Assessment ($n = 1252$).

CyanoHAB responses to environmental change

Bioassays or mesocosm studies have long been used to assess how physical or chemical manipulations influence phytoplankton community structure and function. Contemporary studies have begun to focus on identifying the competitive factors that select for toxic or non-toxic strains of the same cyanobacterial genus. For example, experimental increases in temperature and P have been shown to promote growth of toxigenic (microcystin) strains of *Microcystis* over non-toxic variants (Davis *et al.*, 2009); increased CO₂ concentrations have been found to favour toxigenic *Microcystis* over non-toxic variants (Van de Waal *et al.*, 2011); and toxic *Microcystis* have been demonstrated to exhibit competitive dominance over non-toxic variants during periods of oxidative stress (Zilliges *et al.*, 2011). In order to compare inter-genus community succession patterns, a series of large mesocosm containers (1000 l) deployed in Lake Taihu, China, demonstrated that periods of low N and high P concentrations could stimulate growth of *Anabaena*, but N₂ fixation rates remained

low over the course of the study (up to a month) and *Anabaena* biomass never exceeded that of *Microcystis* (Paerl *et al.*, 2014). However, in that study, neither toxicity nor toxigenicity was evaluated. While highly informative, the use of such controlled experiments carries the expectation that bottle effects can occur and that not all constituents comprising the community will respond similarly under confined conditions relative to a natural setting (Drenner and Mazumder, 1999). Even so, these types of experiments lay the foundation for identifying which interactions/outcomes may be relevant at the ecosystem level, and therefore help to guide future lines of enquiry.

Few investigations to date have attempted to characterize cyanobacterial community succession profiles at the substrain level, although the rise of high-throughput sequencing now makes this a possibility. What is presently recognized is that in addition to common macro-scale patterns of genus succession (e.g. *Aphanizomenon* → *Microcystis*), for most water bodies each bloom-forming genus is actually comprised multiple species or strains. Most of the genetic data supporting this

conclusion come from quantitative polymerase chain reaction (QPCR)-directed investigations profiling the percentage of toxigenic versus total cells present over time (Al-Tebrineh *et al.*, 2010; Orr *et al.*, 2010; Ostermaier and Kurmayer, 2010; Otten *et al.*, 2012). Additionally, microscopic cell counting has identified the presence of co-occurring morphospecies, which could indicate multiple strains, although in general morphology is a poor indicator of genetic diversity (Otsuka *et al.*, 2001; Otten and Paerl, 2011). The rise and fall of these subspecies/strains can occur over intervals as short as days or as long as months (Bozarth *et al.*, 2010; Otten *et al.*, 2012; Miller *et al.*, 2013; Singh *et al.*, 2015), and the primary driver(s) of strain turnover remain elusive, although top-down controls (e.g. cyanophage, predatory bacteria, chytrid fungi or zooplankton) should clearly be further investigated. An added benefit of molecular enquiries is that by identifying the boundary conditions present when one strain is usurped by another seemingly closely related species, one can better scrutinize routine water quality monitoring datasets in an effort to deduce environmental drivers of strain succession.

Predicting community succession patterns: will nutrient reductions today favour N₂ fixers in the future?

The high temperature tolerance of *Microcystis* could explain why in tropical and subtropical systems, such as China's Lake Taihu, it often blooms first and remains generally dominant throughout the entire spring, summer and fall (Deng *et al.*, 2014; Paerl *et al.*, 2014). In the absence of targeted nutrient reduction strategies, N-impacted, temperate systems in the future experiencing augmented temperatures may exhibit larger and more persistent blooms of non-diazotrophic cyanobacteria, such as *Microcystis*, *Oscillatoria* or *Lyngbya*, due to the combined selection pressure of warm water temperatures and excessive N inputs. Due to the trend of ever accelerating anthropogenic stressors, we collectively have few case studies to draw upon to provide a roadmap of restoration options. The phosphate detergent ban in the Great Lakes in 1960s led to the partial restoration of Lake Erie, at the time reversing the widespread proliferation of *Cladophora* (chlorophyte) (Allinger and Reavie, 2013). Even though the phosphate ban remained in place, during the ensuing decades the introduction of invasive species (Dreissenid mussels), the expansion of 'big agriculture' in the region and a change in farming practices, such as a switch from cover crops to no till and from inorganic to organic nitrogen fertilizers, have led to a present-day situation of *Microcystis* and *Planktothrix* dominance – with *Cladophora* still abundant along shoreline margins (Smith *et al.*, 2015).

In Lake Taihu, cyanobacteria were the dominant phytoplankton group from the late-1980s through 1995 (Chen *et al.*, 2003), and similar to Lake Erie, when P concentrations reached their all-time highs in the lake the community composition became dominated by blooms of green algae (primarily *Pediastrum* sp.). Notably, when the P concentrations were moderately reduced 2 years later, *Microcystis* blooms once again resumed their dominance and remain so today. These studies suggest that at extreme P concentrations (> 150 mg.m⁻³), subsequent reductions in P may actually shift the phytoplankton community structure towards cyanobacterial dominance.

Even though outright bans on N inputs have not occurred, the implementation of nitrogen total maximum daily loads in many systems has helped achieve meaningful reductions in phytoplankton biomass without significant shifts from non-diazotrophic to diazotrophic CyanoHABs. Examples include North Carolina's (USA) Neuse River Estuary, and the Potomac River (Washington DC and Virginia/Maryland), in which water quality was plagued by toxic *Microcystis* blooms in the 1980s, which have since been greatly reduced by dual nutrient (N and P) input reductions without a shift to N₂ fixers (Paerl *et al.*, 2004; Harding *et al.*, 2014). A parallel European example is Himmerfjärden, a fjord that drains treated (for N and P) wastewater from the urban region of Stockholm, Sweden, in which persistent eutrophication and blooms (including cyanobacteria) have been arrested with a dual nutrient reduction strategy without a major shift to N₂ fixers (Franzén *et al.*, 2011).

The answer to the cyanobacterial succession question likely pertains to the ecological strategies that CyanoHABs employ in order to compete and maintain dominance under eutrophic conditions. Their key characteristics – which all pelagic genera exhibit – are the ability to form colonies that increase buoyancy (Reynolds *et al.*, 1987) and decrease grazing pressure (Lampert, 1987), highly effective inorganic carbon (CO₂, HCO₃⁻) uptake and concentrating mechanisms (Giordano *et al.*, 2005; Holland *et al.*, 2012; Burnap *et al.*, 2015), and tolerance to high irradiance and UV (Paerl *et al.*, 1985). As such, nutrient concentrations drive phytoplankton biomass, whereas other physical factors, specifically temperature, light availability and water stability/residence time, are key factors that select for specific cyanobacterial genera at different times (Roelke *et al.*, 2004; Soares *et al.*, 2009). By lowering nutrient inventories, the magnitude to which blooms can accrue will be ameliorated, although this alone will likely have little influence on the cyanobacterial community composition itself for the reasons listed above.

As such, there is little reason to expect nutrient reductions to create unfavourable shifts in a lake's cyanobacterial community composition; instead, total primary productivity is expected to decline concomitantly

with nutrient stores with minimal change in cyanobacterial community structure itself. When remediating nutrient-impaired systems, in addition to cutting out P inputs, biologically available N (ammonium, nitrite/nitrate, organic N compounds) inputs should be reduced if the objective is to reduce overall community biomass. Then, after protracted N input reductions (years to decades), it may be feasible to control CyanoHABs with more exclusive P input reduction strategy as long as N inputs are controlled. We caution, however, that in many instances where unabated N enrichment has been taking place for many years to decades, the period of 'recovery' to a more exclusive P-limited situation could also take decades (cf. Paerl *et al.*, 2014).

Acknowledgements

We appreciate the technical assistance and input of A. Joyner. This work was partially supported by the National Science Foundation (DEB 9815495; OCE 9905723; CBET 0826819, 1230543; and Dimensions of Biodiversity 1240851), U.S. EPA STAR Projects R82-5243-010 and R82867701, the North Carolina Department of Natural Resources and Community Development/UNC Water Resources Research Institute, and the USGS Award No. 2012OR127G.

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