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Viewpoint

Environmental Studies of Cyanobacterial Harmful Algal Blooms Should Include Interactions with the Dynamic Microbiome

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Biology is complicated. Nowhere might this be more true than in aquatic systems. Lakes, especially those in temperate regions, commonly undergo seasonal dynamics in the background of constant anthropogenic insult. Among the ecosystem level responses are cyanobacterial blooms (cHABs), which render water bodies unusable and potentially toxic. High-profile interruptions of access to potable water affecting >400 000 residents of Toledo, OH in 2014 and more than >2 000 000 residents of Wuxi, China in 2007 highlight this problem.[1] Indeed, global-scale observations report an increase in the size and frequency of cHABs on six of the seven continents.[2] While eutrophication is clearly a primary driving force, climate change, and invasive species are also factors. Ultimately, research into the specific drivers of cHABs continues to provide unclear, and often contradictory mechanisms of bloom formation: an example of this is the ongoing debate on the roles of nitrogen and phosphorus as bloom promoters.[3] Meanwhile, cyanobacteria continue to dominate large freshwater systems despite decades of nutrient control, albeit these controls have been largely phosphorus-focused. There is also tremendous focus on both the physiology and ecology of key cyanobacteria genera (e.g., *Microcystis* and *Planktothrix*) which produce the toxic secondary metabolite microcystin, a compound originally known as "*Fast Death Factor*".[4] However, despite all efforts and tremendous progress, the picture remains complicated, with contradictions, for example, on the roles of pH, temperature, and viruses in constraining or promoting cyanobacterial harmful algal blooms or their production of

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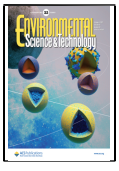

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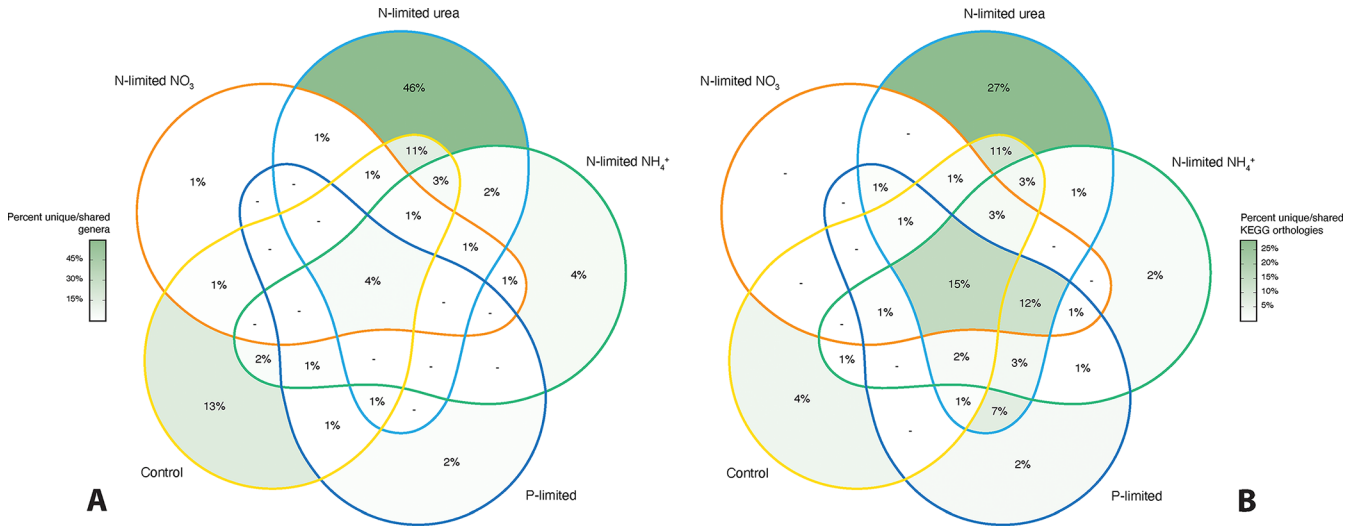

Figure 1. Nutrient availability shifts microbiomes. *Microcystis aeurginosa* NIES-843 cultures started from the same stock were transferred for three generations to unique nitrogen (N) and phosphorus (P) conditions to determine how the cyanobacterium would respond (Steffen et al. 2014). We re-examined metatranscriptomes (via Pound et al. 2021, protocols.io, doi: 10.17504/protocols.io.buvbnw2n) to demonstrate shifts in the (A) community richness (*rpoB* phylogenies), and (B) functional richness (unique KEGG orthology assignments) of the co-occurring microbiome. Results demonstrate that only small components of community (4%) and function (15%) remained common after three generations. Note that unions denoted with "−" are for transcripts when phylogeny (<2) and function (<10) were <1%.

toxin(s).[3] In addition, an important potential cause of variability in both lab and field experiments is frequently overlooked: the co-occurring microbes which numerically represent a majority of the microbial community.

In nature, heterotrophic bacteria persist in fresh waters at concentrations ranging from 10^5 to 10^9 per L of water. Yet in the laboratory, scientists researching cyanobacteria have worked for decades to reduce or even remove these microbial "contaminants" and create axenic cultures. It is clear, however, that without the presence of microbiomes, processes that occur in nature, and the relationship cyanobacteria have to these processes, are limited or halted. A further complication is that while coculture experiments of bacteria alongside algae may seem more ecologically relevant, there is no guarantee that the composition or metabolic activity of microbiomes in laboratory samples remain representative of what one finds under field conditions. As an example, we re-examined the efforts of Steffen et al.[5] which compared transcripts from *Microcystis aeruginosa* NIES-843 cultures in light-limited (control), N-reduced (with different N sources), and P-reduced conditions in samples cultivated with an initially identical accompanying microbiome for three cycles of growth and transfer. Looking beyond the *Microcystis* cells, changes in both the identity and activity of the microbiome in each treatment were unique (Figure 1). This raises many questions. For example, how do changing microbiomes influence biogeochemical processes (carbon and nutrient pools, metabolites, etc.) and consequently the activity of the bloom-causing organism itself? Are experimental results an indirect or direct result of this co-occurring community that may share metabolites and afford protection from environmental stressors, among other functions? To what degree can a microbe's microbiome influence gene expression in the individual or the subsequent production of compounds in complex natural systems? What scientists observe as a biological pattern in nature is a combination of the physics and chemistry at the level of the individual cell interacting with the physics and chemistry of every other organism, all within a changing environment.[6]

Without acknowledging that cyanobacteria are not alone, we cannot move forward with a clearer and more comprehensive understanding of how cyanobacteria blooms are constrained.

We propose a paradigm shift to address the interactions between ecosystem-threatening freshwater cyanobacteria and "the others" that coexist. We know that heterotrophic bacteria are constant companions of algae in lakes around the world[7] and may serve to facilitate[8] or repress[9] cyanobacterial growth, and that bacterial functions during blooms vary in time and space.[10] It is also possible that the metabolic processes of co-occurring microbes are potentially exchanging primary or secondary metabolites with cyanobacteria to mollify or amplify environmental insults. Additionally, it is not just bacteria that may influence cyanobacteria: viruses[11] and fungal chytrids[12] may also play a role in both the repression and success of cyanobacterial blooms.

In summary, we see an urgent need for researchers to report not only on the toxin-producing phototroph of interest, but also the diversity (i.e., richness and evenness) of the co-occurring microbial community during experimental research. Deep sequencing of rRNA genes and entire metagenomes/metatranscriptomes has become a standard laboratory and field tool in microbial ecology.[13] The scientific community should strive to report the identity and function of the entire microbial consortia present in both laboratory cultures and during field research efforts. Researchers are encouraged to consider the functional potential of co-occurring organisms and how these roles might influence cyanobacterial growth dynamics. Moreover, beyond the scope of their initial project, when researchers provide data on the identity or function (RNA)/functional potential (DNA) of a microbiome, they provide informaticians and modelers an opportunity to address microbiome effects in subsequent data analyses. The interactions within the microbial consortia, and effects resulting from environmental impacts are what drive the phenotypes we observe and must be considered during the interpretation of experiments. Furthermore, as microbiome analysis tools continue to improve, including shotgun sequencing efforts, insight into

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the metabolic functions of these microbes will improve our ability to decipher the underlying drivers of undesirable cyanobacterial blooms. What remains certain is that future studies across ecology should no longer ignore the identities and actions of other members of experimental systems, and should document shifts in community structure and function as they occur in laboratory manipulations.

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Author Contributions

Names are presented in approximate order of ascending academic rank.

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Notes

The authors declare no competing financial interest.

Biography

Helena Pound is a 4th year graduate student in the Department of Microbiology at the University of Tennessee-Knoxville. Her research examines microbial communities (with a focus on toxic cyanobacteria) and viruses in aquatic systems, using state-of-the-art RNA sequencing, metabolomics, and computational approaches. She holds a B. S. in Wildlife & Fisheries Sciences (2014) from the University of Tennessee and M.S. in Marine Biology (2017) from the College of Charleston. Her research has been supported by the Great Lakes Center for Fresh Waters and Human Health. Presently she is serving as a NOAA Sea Grant Knauss Fellow for the Department of Energy.

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